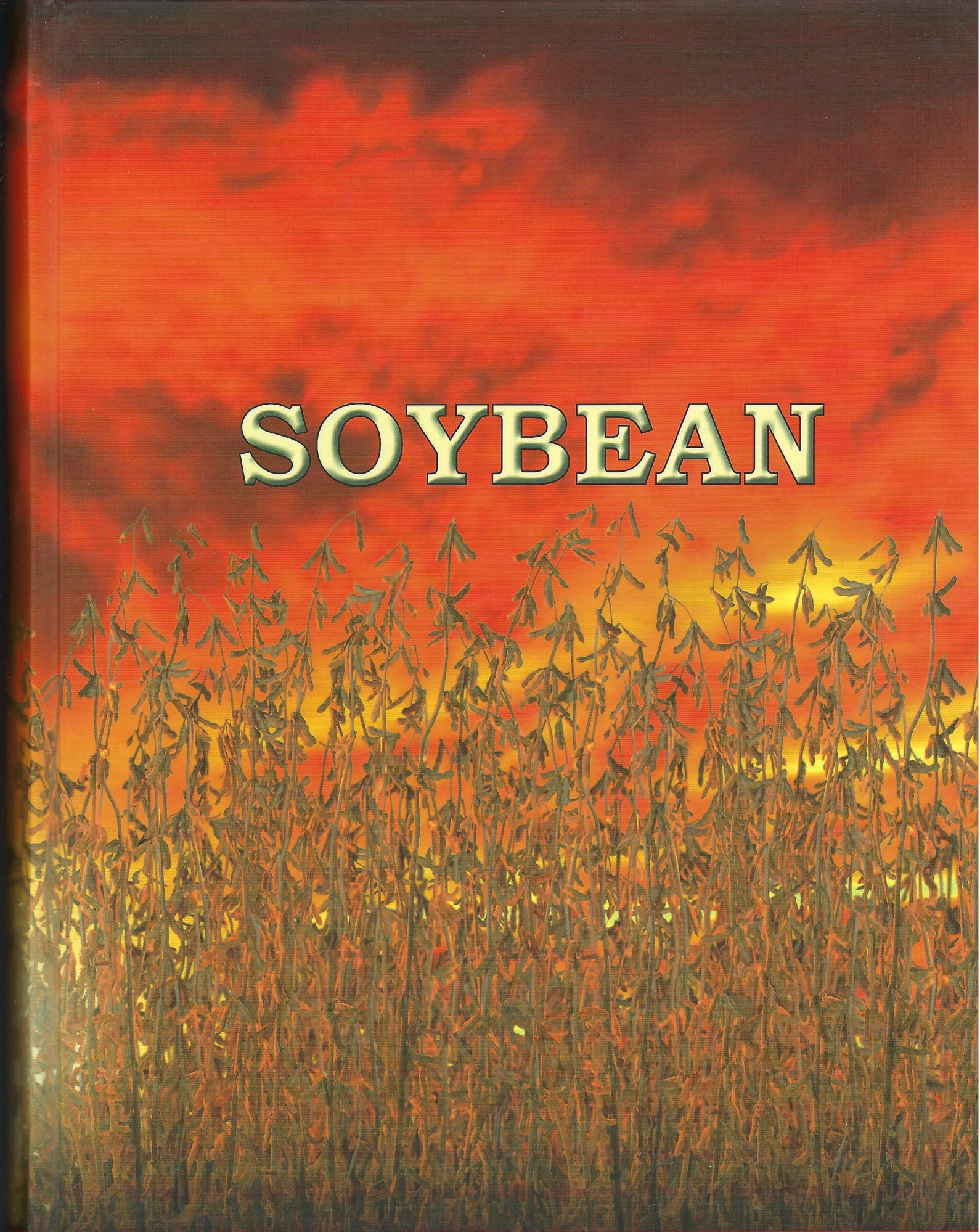
SOYBEAN



Jegor Miladinović • Milica Hrustić • Miloš Vidić

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Institute of Field and Vegetable Crops, Novi Sad
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FOREWORD

This is a translated edition of the book titled “Soja” published in Serbian language in 2008, which displayed the accumulated knowledge on fundamental and applied soybean research, as well as soybean breeding efforts at Institute of Field and Vegetable Crops in Novi Sad, Serbia.

Global importance of soybean is continually growing, with soybean planted areas reaching almost 99 million ha in 2009 and production of soybean grain exceeding 222 million tons. According to these indicators, soybean is the most important industrial plant worldwide, both as a basic source of protein nutrients for cattle, poultry and fish and as the most important source of plant oil.

Simultaneously with increased interest in growing this plant species, scientific research on soybean has also been enhanced, especially regarding fundamental research. For the most part, this book deals with achievements of Serbian researchers. Chapters dealing with soybean morphology and its requirements during growth and development have been updated and revised. Content of chapters dealing with production technology, seed production and importance, as well as chapters giving an overview of diseases and pests affecting soybean is somewhat characteristic for Serbian climate and soil. Therefore, the authors would particularly recommend to foreign readership the chapters dealing with quantitative and qualitative genetics of soybean prepared for this edition by leading experts and professors from University of North Carolina and University of Iowa. Outline of the most recent achievements in the field of soybean breeding has also been prepared by scientists from the USA, where such research is most developed.

Chapter dealing with soybean importance and origin gives a chronological survey of soybean breeding results at Institute of Field and Vegetable Crops. The previous decade was outstandingly dynamic and successful in this area, witnessed by impressive results. The number of soybean varieties developed at Institute of Field and Vegetable Crops released in Serbia has doubled, while there was a ten-fold increase in the number of varieties released abroad - from four varieties released in 2000 to 49 released so far.

Hence we believe that this edition will be useful to everyone involved in soybean production, especially to students and scientists conducting research on soybean.

Authors would like to thank all who have participated in preparation of this book in any way, and especially to reviewers whose efforts and pieces of advice largely contributed to the form and content of this book.

Special gratitude is extended to the Ministry of Science and Technological Development of the Republic of Serbia for financially aiding the printing of this book.

In Novi Sad, in February 2011.

Authors.

IMPORTANCE, ORIGIN AND EXPANSION OF SOYBEAN

Milica Hrustić, Jegor Miladinović

IMPORTANCE OF SOYBEAN

What is the first thing that comes to mind at the mention of the word soybean? An average Chinese person would probably think of all the different dishes and beverages that have been sustaining their and their ancestors' lives for countless generations. An average crop farmer would think of the profit the plant could generate and the beneficial effects soybean imparts on soil it has been grown on. A livestock farmer would associate the word with soybean meal, an essential component of quality animal feed. A nutritionist would immediately think of the essential amino acids found in soybean protein, while an industrialist's attention would be focused on all the processed products that can be made from the plant. The owner of a trucking company might calculate the price of transporting large quantities of soybean beans and processed products, and a merchant would contemplate how much profit buying and selling all those goods might bring. The soybean plant has many varied uses and its importance is multifaceted.

Soybean's importance comes first and foremost from the chemical composition of its grain, which is about 40% protein and around 20% oil. This adds up to soybean having over 60% of different nutrients that can be used for various purposes. Because soybeans can be used whole or can be processed to obtain oil or protein, the plant is used widely and extensively not only in the food sector but in various other industries as well. In more recent times, it has been gaining increasing importance in international commerce too. Soybean meal is an indispensable source of protein in the nutrition of livestock, poultry and fish. Although soybean is an important protein source for the ever-growing human population, soybean protein is still not used as much as it should be in the human diet. There are several reasons for this. In the developed world, there is an abundance of traditional protein sources (meat, milk, eggs)

and soybean is used primarily in special diets and in Asian cuisine. The developing countries, on the other hand, are deficient in high-protein foods but do not have the industrial capabilities needed to process soybean for human consumption.

Soybeans are also a major source of vegetable oil and are used to produce one third of all such oil in the world. Thus far, soybean oil has been primarily used in the food industry for purposes such as cooking and preparation of ready-to-eat meals, mayonnaise, margarine, and so on. There is, however, a growing trend towards using the crop for various other industrial purposes, such as the manufacture of soaps, detergents, paints and varnishes. One of the most common products based on soybean oil is newspaper printing ink, which has an advantage over the traditionally used ink in that it does not rub off. Soybean oil is used increasingly as the carrier of the active ingredient in pesticides, where it reduces the amount water needed for the aerial application of the chemical. One important component of soybean oil is lecithin, which is used in the manufacture of baking and confectionery products as well as in medicine and the textile and chemical industries. Soybean production and processing are constantly on the rise, and because soybean-producing and soybean-consuming regions do not fully overlap, the importance of the crop is increasing both in the processing industry and international commerce and transport.

We must not forget the role of soybean in field crop production either. Being a legume, soybean has the ability to fix atmospheric nitrogen and provide itself with sufficient amounts of readily available nitrogen, thus reducing the need for nitrogen fertilizer application. This makes this plant a very good fit for crop rotations.

HISTORY AND SPREAD OF SOYBEAN

Birthplace of soybean

The earliest history of soybean has been lost in the mists of time. According to ancient Chinese texts, soybean had been grown and highly valued as food for centuries before the first written records appeared. The first written mention of the crop is found in the book Materia Medica by the Chinese Emperor Shennong, dating back to 2838 BCE (Morse et al., 1949). As per ancient Chinese literature, soybean's original name was "shu". This word appears in the Book of Songs (Shi Jing), one of the five classics of Chinese literature, which covers the period from the 11th century to the year 771 BCE (end of the Zhou dynasty). Among the writings of the Shang dynasty (16th to 11th century BCE) a character has been found that has been identified as the original form of the word "shu". Having been in cultivation for several thousand years, soybean, along with rice, wheat, barley, and millet, has been one of the five sacred crops essential to the survival of the Chinese civilization (Gutschy, 1950; Morse, 1950).

For a great many years, soybean did not spread much beyond its native land of northeastern China, which is regarded as the primary place of the plant's genetic origin. By the start of the Common Era, soybean had likely reached central and southern China and the Korean Peninsula. The crop expanded into Japan between 200 BCE and the 3rd century CE (Caldwell, 1973), and the first written records of it in the country date back to the 7th century CE. Between the start of the Common Era and the 15th and 16th centuries, soybean was introduced to Indonesia, the Philippines, Vietnam, Thailand, Malaysia, Burma, Nepal, and northern India. There, different landraces of the crop developed, so this part of the world is considered the secondary point of soybean origin (Hymowitz, 1988).

Global expansion of soybean

Soybean first appeared in the botanical and home gardens of Europe and America only as late as in the 18th century, when the development of marine traffic enabled better communication between distant parts of the world. People of Europe first learned of soybean from the German botanist Engelbert Kaempfer, who visited Japan in 1691-1692 and later wrote a book entitled "Amoenitatum exoticarum politico-physico-medicarum". The book was published in 1712 and included a detailed description of the soybean plant as well as recipes for preparing various soybean-based dishes and beverages (Gutschy, 1950; Hymowitz, 1988). Soybean descriptions subsequently appeared in several other books, the first of which was "Musaeum Zeylanicum" by Paulus Herman (1726). In his 1737 book "Hortus Califfortianus", Charles Linne mentions soybean under the name Phaseolus max., while another book by the same author, a 1753 title called "Species Plantarum", refers to soybean as Dolichos soja. Konrad Moench gave soybean the name of Soja hispida and Maksimovič (1873) that of Glycine hispida.

The written record shows that in the 18th century soybean was grown in Europe only in a handful of botanical and house gardens. Hymowitz (1988) argues that the practice of soybean growing in Holland predates the year 1737, basing his conclusion on a description of plants grown in a garden in Hartekamp that was published by Linne in that year. Soybean seeds sent back to France by missionaries stationed in China were planted at Jardin des Plantes in Paris in 1739. Soybean was grown in the Royal Botanic Gardens at Kew, England in 1790 and in the Dubrovnik area in 1804. The cultivation of soybean in all these and similar cases was motivated either by sheer curiosity or by the desire to determine the plant's taxonomic affiliation, as the crop's uses were still unknown to Europeans.

In Yugoslavia, according to Hymowitz (1988), soybean was cooked after the harvest and then mixed with cereals and fed to poultry in order to increase the production of eggs.

Soybean expansion and popularization in Europe began with the Austrian botanist Friedrich Haberlandt, who in an 1875 book titled "Die Sojabohne" reported detailed results on the study of about 20 soybean varieties from several European countries. Although the results of these trials were promising, it would still take quite a while (except for some parts of Romania, Czechoslovakia, and Austria) before soybean would become a major crop on the European continent.

Due to the absence of adequate cultural practice and a lack of knowledge of the plant's great usefulness, soybean accounted for only a small fraction of the overall agricultural output in 19th century Europe. The failure of the cotton crop in the U.S. in 1908 resulted in a shortage of vegetable oil in England, so soybean was imported into the country from Japan. The oil obtained by crushing the imported soybeans received a great reception and was an outstanding success. Importation of soybean into Europe continued in the subsequent years, and some time later major-scale soybean-growing operations were established in several European countries, most notably Romania, Bulgaria, Czechoslovakia, Austria, Yugoslavia, and Hungary (Gutschy, 1950; Morse, 1949).

During the 18th century, soybeans were grown from time to time on some US farms as well. Henry Jonge is regarded as the first grower of soybean in America. He began cultivating the crop in 1765 using plants he had received from Samuel Bowen in London via China. From 1766 onwards Jonge grew and processed soybean regularly on his estate in Georgia and he even patented some of the processed products made from the plant. After his death in 1777, however, this work was discontinued. Another attempt at growing soybean in what was soon to become the United States occurred in 1770 in the garden of the Philadelphia botanist John Burton. Soybean was probably grown in various locations in the US during the first half of the 19th century as well, but there are no surviving records of this, as the reports were printed in local papers or were presented at local meetings. In the mid-19th century, soybean was tested on a large number of farms across the U.S. and was considered a new crop species. It was accepted by many farmers, who used it as animal feed, hay or silage, either on its own or in combination with other crops.

The scientific approach in evaluating soybean genotypes began to be used in the late 19th century with the introduction of agricultural experiment stations. As early as the start of the 20th century, a great effort was made to collect as many soybean genotypes as possible, which were then studied extensively. Up until that time, only a small number of varieties of the crop had been grown, and they were mostly used for silage. It was only in the 1940s that the portion of the soybean crop harvested for grain reached the one half mark, with 4,000,000 ha being the total area planted. The use of soybean as a source of vegetable oil in Europe between 1900 and 1910 resulted in an increased interest in soybean utilization in the U.S. (Smith and Huyser, 1987). According to Howell (1982), the increase in US soybean acreage was precipitated by the simultaneous emergence of several factors. Perhaps the most important among these was the mechanization of agriculture, which reduced the need for draft animals

and vacated millions of hectares of land that had up to that point been used to produce animal feed. Also, synthetic fibers replaced cotton, which had previously occupied large areas. Another important influence was a large population growth, which brought about significant food shortages. The confluence of these factors created a favorable climate for a plant such as soybean, which had a good balance of oil and protein and was known to have been used in China at the time for obtaining oil and flour. The plant had thus been able to meet all the production and market requirements and had found its place in agricultural, processing and food industries.

SOYBEAN PRODUCTION WORLDWIDE

In the early 1900s soybean was a little-known plant that was grown in only a handful of countries, but by the end of the 20th century it had developed into a leading field crop on the global scale. In the mid-20th century, soybean was grown internationally on about 15 million hectares but had not yet become a major crop in many countries. It was only in the 1950s that a sharp increase occurred in global soybean acreage. In recent years, the crop has been grown on nearly 100 million hectares annually. Aside from the staple cereal grains such as wheat, rice and maize, this is the largest total area sown to any field crop on a global scale. In the mid-1970s, the total annual production of soybean in the world ranged from 50 to 60 million tons. In the next two decades, the global annual output of the crop doubled to over 120 million tons, and in 2009 more than 222 million tons were produced. These increases are not only due to the increased acreage but came as a result of increased yield per unit area as well. Nowadays, soybean is grown in most countries to a greater or lesser extent, but in recent years about 90% of the world's production have been concentrated in only several countries.

For the past few decades, the US has been the world leader in producing, processing and trading in soybean and its processed products. Up until the 1940s, soybeans were grown in the US primarily for the purposes of silage. It was at some point during that decade that half of the annual US crop was for the first time harvested for the beans. After that, areas sown to the crop kept increasing until the 1980s, when they reached 28 million hectares, which is the level they have been at ever since, with some minor fluctuations. For quite some time, the average yields were at around 2 t/ha, and then between 1980 and 2000 they increased to 2.3-2.5 t/ha. In the last couple of years, the yields have again increased significantly, averaging 2.8 t/ha in 2004, 2.9 t/ha in 2005, and 3.0 t/ha in 2006. The total soybean production in the US varies from 80 to 90 million tons per year, which accounts for one third of the world's total output.

Brazil and Argentina do not have a long tradition of soybean growing, but it has not taken long for both these countries to become major soybean producers. Soybean growing began in Brazil in the 1960s and the acreage was initially negligible. The areas planted to soybean rose sharply in the 70s and began to exceed 10 million hectares in the 80s. In the last ten years or so, the country's soybean acreage has doubled and is now exceeding 20 million hectares. The average yields vary a lot, as does the overall production, but in recent years Brazil is producing over 50 million tones of soybean grain, which is almost a quarter of all the soybean produced in the world. Argentina has increased its soybean acreage from several hundred thousand hectares in the 1970s to its present output of more than 16 million hectares and growing. The 40 million tons of soybean grain produced in Argentina every year make this country one of the top soybean growers in the world (Table 1.1).

In the early 20th century, China was the only country that cultivated soybean on areas measured in millions of hectares. The annual hectarage averaged four to five million before 1950 and seven to nine million afterwards, the only notable exception being the year 1955, when the plant was sown on around 12 million hectares. In the last three years, China has grown between nine and ten million hectares of soybean per annum. The average yields have been low – they hovered around 1 t/ha until the 1980s and then began to increase slowly until they reached their present-day levels of 1.6-1.8 t/ha. Over the same period, the annual production of the crop increased as well. For a long time, China produced between seven and nine million tons of soybean a year, with a record 11 million tons in 1938 (Ma, 1982). Since the mid-1980s, Chinese soybean production has been increasing slowly but steadily – in 2005, for instance, 17 million tons of the grain were produced. Before the 1950s, the country accounted for about 50% of global soybean production, but this percentage has decreased since. In the last decade, China has contributed less than 10% to total soybean production in the world. India, the only other major grower of the crop on the Asian continent, cultivates soybean on 6-7 million hectares, with an average yield of around 1 t/ha.

Contribution of Europe to world soybean production ranges from 1 to 2% and the plant is important in only some countries of the continent (this will be discussed in more detail a little later in the chapter).

Baker (1970) argues that soybean has never become popular in Africa, but many African countries have introduced soybean as a commercial crop since the 1970s and now have their own programs aimed at increasing the acreage sown to the plant. In 2008, for example, soybean was grown in Africa on more than one million hectares. There are, however, a number of factors hampering soybean growing on the African continent. These include insufficient knowledge of the growing technologies, a lack of an adequate selection of cultivars and inoculants, lack of processing facilities, and loss of seed germinability during storage (Jackai et al., 1984). The largest producers of soybean on the continent are Nigeria, Zimbabwe, Egypt and Zambia.

World soybean production has been increasing year after year and the crop has spread to almost every part of the globe. Overall, the crop is grown the most on the American continent, followed by Asia, while in Europe, Africa and Australia the volume of soybean production is considerably smaller (Table 1.1).

Table 1.1

Area harvested, yield and production quantity of soybean in the world from 2004 to 2009

	2004			2005			2006			2007			2008			2009		
	000 ha	t/ha	000 t	000 ha	t/ha	000 t	000 ha	t/ha	000 t	000 ha	t/ha	000 t	000 ha	t/ha	000 t	000 ha	t/ha	000 t
WORLD	91,189	2.2	206,461	91,418	2.3	214,347	92,988	2.4	221,501	90,199	2.4	220,532	96,870	2.4	230,952	98,827	2.2	222,269
N. America	31,107	2.8	88,060	30,037	2.8	86,997	30,280	3.0	91,202	27,131	2.8	75,556	31,401	2.7	83,871	32,288	2.9	94,921
USA	29,930	2.8	85,012	28,879	2.9	83,998	28,983	3.0	87,670	25,960	2.8	72,860	30,206	2.7	80,535	30,907	2.9	91,417
Canada	1,177	2.5	3,048	1,158	2.5	2,998	1,225	2.9	3,533	1,171	2.3	2,695	1,195	2.8	3,335	1,382	2.5	3,504
S. America	38,967	2.2	87,104	40,137	2.4	96,874	40,595	2.4	98,884	40,393	2.8	113,747	41,812	2.8	115,505	42,771	2.2	94,561
Brazil	21,538	2.3	49,793	22,895	2.3	52,700	22,006	2.4	52,356	20,565	2.8	57,857	21,271	2.8	59,916	21,760	2.6	56,961
Argentina	14,320	2.1	31,500	14,037	2.7	38,300	15,097	2.7	40,467	15,981	2.9	47,482	16,380	2.8	46,232	16,768	1.8	30,993
Paraguay	1,870	1.9	3,583	1,935	1.8	3,513	2,200	1.7	3,800	2,429	2.4	5,856	2,645	2.6	6,808	2,570	1.5	3,855
Asia	18,445	1.4	27,481	18,323	1.4	26,546	18,633	1.4	26,334	19,479	1.4	27,183	20,600	1.3	27,218	20,362	1.3	27,596
China	9,700	1.8	17,600	9,500	1.8	17,400	9,100	1.7	15,500	8,900	1.5	13,800	9,127	1.7	15,545	8,800	1.6	14,500
India	6,900	1.0	7,500	6,900	0.9	6,600	7,710	1.0	8,270	8,880	1.2	10,968	9,600	0.9	9,045	9,600	1.0	10,217
Europe	1,396	1.7	2,480	1,636	1.5	2,531	2,305	1.5	3,607	1,893	1.4	2,630	1,702	1.6	2,743	1,963	1.7	3,353
Africa	1,133	0.9	1,080	1,150	0.9	1,133	1,221	1.1	1,417	1,210	1.0	1,253	1,241	1.1	1,381	1,316	1.2	9,512
Oceania	33	2.2	74	26	2.1	56	24	2.3	55	13	2.4	32	17,5	2.0	35	42	1.9	80

Source: FAOSTAT, FAO Statistic Division 2010, http://faostat.fao.org/

SOYBEAN PRODUCTION IN EUROPE

The first attempts at growing soybean in Europe were recorded in Holland, France, and England, and, later, in Austria and Germany as well. To this day, however, the crop has never managed to take root and spread to major areas in any of these countries with the exception of France. Up until the 1980s, soybean was grown in Europe mostly in Romania, Bulgaria, Hungary and Yugoslavia and the total acreage was less than 500,000 ha. A major expansion of the crop on the continent began in 1985, primarily in Italy and France, and the acreage reached one million hectares in 1990. With minor fluctuations, the soybean acreage in Europe remained at this level until the last few years, when soybean areas began to increase in Russia and Ukraine (in 2005, for instance, the European area in soybean totaled 1.6 million hectares) (Table 1.2).

Table 1.2

Area harvested (000 ha) and yield (t/ha) of soybean in Europe from 1996 to 2009

	Europe		France		Italy		Romania		Serbia		Croatia		Russia		Ukraine	
Year	000 ha	t/ha	000 ha	t/ha	000 ha	t/ha	000 ha	t/ha	000 ha	t/ha	000 ha	t/ha	000 ha	t/ha	000 ha	t/ha
1996	1,039	1.6	86	2.6	223	3.6	80	1.4	72	2.1	16	2.1	485	0.5	16	0.9
1997	923	2.3	98	2.7	301	3.8	63	1.9	61	2.5	16	2.4	317	0.8	14	1.2
1998	1,214	2.0	111	2.5	351	3.5	147	1.3	82	2.0	34	2.2	377	0.7	31	1.1
1999	1,137	2.0	98	2.6	246	3.5	99	1.8	108	2.9	46	2.5	404	0.8	42	1.0
2000	1,105	1.7	77	2.5	252	3.5	117	0.5	141	1.3	47	1.3	337	1.0	60	1.0
2001	1,039	2.0	120	2.5	234	3.8	44	1.6	87	2.5	41	2.2	371	0.9	73	1.0
2002	977	2.0	74	2.7	152	3.7	69	2.0	100	2.5	47	2.7	362	1.1	98	1.2
2003	1,219	1.5	80	1.8	152	2.5	122	1.8	131	1.8	49	1.6	399	0.9	189	1.2
2004	1,396	1.7	58	2.5	150	3.4	120	2.4	117	2.7	37	2.1	555	0.9	256	1.4
2005	1,636	1.5	57	2.4	152	3.6	136	1.8	130	2.8	50	2.2	690	0.8	310	1.0
2006	2,305	1.5	45	2.7	177	3.1	177	1.9	156	2.7	62	2.8	810	1.0	725	1.2
2007	1,893	1.4	37	2.7	132	3.3	109	1.2	147	2.0	46	1.9	709	0.9	583	1.2
2008	1,702	1.6	22	2.9	108	3.2	46	1.9	144	2.4	36	3.0	712	1.0	538	1.5
2009	1,936	1.7	43	2.5	134	3.5	48	1.7	144	2.4	44	2.6	794	1.2	622	1.6

Source: FAOSTAT, FAO Statistic Division 2010, http://faostat.fao.org/

The case of Italy and its soybean industry is especially interesting. Till the year 1980, according to official statistics, not a single hectare of soybean was planted in the country. In 1981, the first 3,000 ha were sown and the yields were high (3 t/ha). The area in soybean continued to increase until the late 1990s, when it exceeded 350,000 hectares, after which it dropped to about 150,000 ha, despite the fact that the yields were high and stable (over 3.5 t/ha). The decrease in soybean acreage had been caused by reduced soybean subsidies on the part of the European Union, which translated into reduced profit margins for the growers in spite of their record yields on a worldwide scale. A similar thing has happened in France, where the soybean acreage has dropped from 100,000 to about 40,000 ha in recent years.

European soybean production is thus moving east, to countries of the former Soviet Union. In the former USSR, soybean was grown since ancient times in the Asian part of the country as well as in the southern portion of the European part of Russia and in North Caucasus. Following the Second World War, the acreage fluctuated between 200,000 and 400,000 ha, then increased, and then settled finally at around 800,000 ha in the decade preceding the breakup of the Soviet Union. As of 1992, separate data is available for soybean acreage in each of the post-Soviet states. The single largest producer is Russia with 500,000-800,000 ha and an average yield of around 1 t/ha.

In the last 10 years, soybean areas in Ukraine have increased sharply, from several tens of thousands of hectares a decade ago to over 700,000 ha in 2006. Still, just like in Russia, the average yield is low, and in past few years it is slightly over 1 t/ha.

Apart from the former USSR, Romania is the only country in Europe which planted more than 100,000 ha of soybean back in 1939. In the post WWII period, there was a growing trend towards increased soybean growing, and from 1979 on the acreage started to exceed 300,000 ha. From 1991 onwards, the area planted to the crop dropped below 100,000 ha, but in the last few years, as European soybean production began to move to the east of the continent, the country's soybean acreage has slowly begun to rise again. Although Romania has a long tradition of soybean growing, the yields are not high – about 1.5 t/ha.

During the 1984-1993 period, the former Yugoslavia contributed 0.12 and 6.67% to the global and European soybean production, respectively (Božidarević and Vlahović, 1995). After the breakup of Yugoslavia and a period of reduced soybean production, the acreage in the crop increased again in both Serbia and Croatia thanks to the favorable growing conditions in the regions of Vojvodina and Mačva in the former and Slavonija and Baranja in the latter former republic. Serbian acreage in soybean has surpassed 100,000 ha in each of the past few years and the yields have averaged about 2.2 t/ha.

SOYBEAN PRODUCTION IN THE FORMER YUGOSLAVIA AND SERBIA

Soybean has been present in Yugoslav and Serbian field crops production since the start of the 20th century, but the area sown to the crop has varied a lot. In an article entitled "Soybean Production and Its Potentials in Serbia Proper", Bošković (1966) reported that soybean had been introduced to the region 50-60 years prior to that time and noted that the local growers' had a relatively poor knowledge of the value of soybean and its products, which was the reason why the plant could not become a major crop in the country.

An extensive effort to promote soybean growing in the former Yugoslavia was made by Stjepan Čmelik in 1921 in the town of Virovitica (Heneberg, 1966). Further evidence that soybean was not an unknown plant in the country in the early decades of the 20th century can be found in the "Lexicon of Goods in Commerce and Economy", written by Milutin Urbani and published in Zagreb in 1925. The lexicon states: "Soybeans are the seeds of the plant Dolichos soja or Soja (Glycine) hispida; the beans are like those of the round bean and are brown or black. Soybean is cultivated in Japan (Daidsu), China, and East Ind., and some is grown in Germany and even in our country as well. Soybean is used to prepare dishes like Shoyu, Miso Yuba, Tofu (in Japan and China) and bean cheese (Bohnen Käs, Natto). Different kinds of soybean are grown in Germany to be used as a surrogate for coffee or for forage, etc." According to Gutschy (1950), the first larger-scale campaign to promote soybean growing in the country was conducted in 1934 by the oil crushing plant in Zagreb. The campaign did not produce the expected results due to a lack of proper organization, but it did have some positive effects in that soybean growing took root on some farms and became a regular practice.

The official records on soybean growing date back to 1934. Between 1934 and 1939, soybean was grown on 600-3,500 ha in the then Yugoslavia and the yields averaged 0.93-1.26 t./ha (Table 1.3). According to foreign sources (Gutschy, 1950), soybean was cultivated in Yugoslavia on 12,000 ha in 1940 and 17,000 ha in 1941, with the yield averaging about 1.2 t/ha. Such relatively large areas were the result of a large demand for soybean in Germany.

Table 1.3

Production quantity of soybean in Yugoslavia from 1934 to 1939

Year	Acreage (ha)	Production (t)	Yield (t/ha)
1934	600	708	1.18
1935	1,060	986	0.93
1936	644	599	0.93
1937	1,160	1,462	1.26
1938	3,520	3,802	1.08
1939	3,240	2,819	0.87

In the post-WWII statistical records, soybean appears again in 1947 (Table 1.4). In 1949, according to the record, the plant was grown on 15,500 ha, but the areas decreased sharply in the ensuing couple of years, reaching 1,296 ha in 1954. In the 1960s, another attempt was made to try to popularize soybean growing in Yugoslavia. In the year 1960, 20.800 ha of the crop were sown, which was up until that time the largest area planted to this species in the country. This was followed by another decline, and in 1970 the areas dropped to as little as 3,770 ha. For the most part, these large variations concerned the public sector. The private sector's contribution to total soybean production varied from 11 to 70%, but in absolute terms the soybean acreage planted by private-owned enterprises remained more or less constant, ranging from 2,500 to 3,500 ha (Popović, 1966). The largest areas in the private sector were cultivated in Serbia proper and Bosnia and Herzegovina. According to Bošković (1966), the reasons for the lack of soybean expansion in the public sector lay in the low yields, economic policies that were not conducive to the advancement of soybean production, and a lack of processing capacities necessary to obtain profitable, high-quality soybean products. Generally speaking, the acreage in soybean remained small for a number of reasons, including a lack of soybean-growing tradition, i.e. insufficient knowledge of soybean production and uses; not enough economic incentive to grow the plant; and uncertainty with regard to the placement of the crop on the market. Soybean areas in Yugoslavia began to increase markedly in 1975, when a broad public campaign was launched to introduce soybean growing to Yugoslav agriculture on a larger scale. The sowing action plan designed in those years would have probably ended up as another unsuccessful attempt at large-scale soybean growing in the country, had it not been for the fact that it was accompanied by a complementary plan for industrial soybean processing too. The largest annual soybean areas – around 100,000 ha on average – were planted between 1981 and 1990, and the average production output was nearly 200,000 t per year.

Table 1.4

Acreage and yield of soybean in Vojvodina and SFRY from 1947 to 1990

	Vojvodina		SFRY	
Year	Acreage (ha)	Yield (t/ha)	Acreage (ha)	Yield (t/ha)
1947	-	-	1,605	0.64
1948	-	-	5,126	0.65
1949	1,758	0.60	15,507	0.62
1950	1,738	0.31	13,138	0.31
1951	855	0.54	7,044	0.61
1952	41	0.37	1,968	0.56
1953	160	1.09	1,536	0.96
1954	274	0.71	1,296	1.01
1955	258	1.15	2,784	1.21
1956	890	1.00	2,340	0.85
1957	1,620	1.21	6,090	1.32
1958	3,210	0.98	8,140	0.86
1959	4,850	1.72	10,100	1.66
1960	9,680	1.26	20,800	1.25
1961	7,530	0.80	12,800	0.77
1962	476	1.13	7,620	1.04
1963	250	0.90	5,370	1.30
1964	129	1.89	5,720	1.67
1965	124	0.79	8,040	1.19
1966	980	1.54	6,330	1.71
1967	160	1.33	6,740	1.32
1968	207	1.28	4,550	0.65
1969	71	1.01	4,326	1.27
1970	119	1.17	3,770	1.30
1971	244	1.10	4,848	0.87
1972	62	1.99	3,553	1.61
1973	491	1.19	9,449	1.35
1974	1,251	1.76	8,678	1.58
1975	7,944	2.22	14,475	2.07
1976	22,268	1.61	31,293	1.54
1977	23,344	2.16	31,967	2.09
1978	25,913	1.95	34,237	1.84
1979	31,084	2.23	34,358	2.15
1980	13,575	2.08	17,289	1.97
1981	38,296	2.03	47,756	1.94
1982	63,217	2.67	77,391	2.56
1983	82,574	1.94	107,220	1.96
1984	82,722	1.98	114,380	2.00
1985	69,489	1.67	101,233	1.73
1986	62,035	2.33	95,645	2.35
1987	63,537	2.34	105,030	2.26
1988	67,300	1.61	110,214	1.63

1989	50,094	2.30	87,893	2.38
1990	51,258	1.52	93,275	1.68

Source: 1947-1965 period - Popović (1966)

Source: 1966-1990 period – Statistical Yearbook of the S.F.R. Yugoslavia

From the year 1991 on, the soybean acreage in the Federal Republic of Yugoslavia and Serbia has been increasing steadily and in 2006 reached a record 156,680 ha (Table 1.5). During this period, the average soybean yields were around 2.2 t/ha, which is on a par with the average global yields of the crop. The yield of 3.15 t/ha from 2010 shows that not only are there favorable natural conditions for soybean growing in the country, but also that the domestic growers have managed to familiarize themselves with the growing technologies used in soybean cultivation. Despite the fact that an occasional dry year may still cause significant losses resulting in yields well below the long-term average (1992, 1993, 2000) and a reduced soybean acreage in the following year, it is safe to say that soybean has become a major crop in the country and that the areas sown to it will in all likelihood continue to increase in the years to come.

Table 1.5

Acreage and yield of soybean in Vojvodina and Serbia from 1991 to 2010

	Vojvodina		Serbia	
Year	Area (ha)	Yield (t/ha)	Area (ha)	Yield (t/ha)
1991	38,333	2.69	43,530	2.65
1992	58,738	1.31	67,797	1.32
1993	49,463	1.38	55,843	1.38
1994	43,568	1.67	49,621	1.68
1995	46,047	2.08	52,123	2.06
1996	65,496	2.11	72,757	2.10
1997	55,326	2.52	61,014	2.50
1998	75,503	1.97	82,409	1.94
1999	100,712	2.75	108,163	2.72
2000	133,868	1.22	141,559	1.20
2001	80,936	2.39	87,382	2.37
2002	93,044	2.47	100,047	2.44
2003	123,639	1.71	131,403	1.72
2004	109,687	2.72	117,270	2.71
2005	123,054	2.83	130,936	2.81
2006	146,291	2.77	156,680	2.74
2007	136,623	2.10	146,988	2.07
2008	132,762	2.46	143,684	2.44
2009	133,514	2.41	144,386	2.42
2010			155.150	3.15

Source: Statistical Office of the Republic of Serbia http://webrzs.stat.gov.rs/axd/en/index.php

SOYBEAN IN VOJVODINA

Soybean was known in Vojvodina as early as the early 19th century, as evidenced by the reports submitted to the High Command by the Šajkaš battalion, which are cited in the book "History of the town of Šajkaš" by Avram Đukić (1975). One of the book's chapters, "Agriculture in the Šajkaš Area", reports as follows: "In the year 1817, two lots of Persian soybean were sown for trial purposes and produced a seed yield of five pounds and 25 lots. Two years later, the plant was sown again, and on a much larger acreage at that, and the yield was 368 pounds and eight lots. Due to a severe drought, the planting done the following year (1820) using the previous year's crop yielded only eight pounds and four lots of bean. This poor yield performance notwithstanding, the Persian soybean has been shown to produce less ample flour yields than domestic soybeans. A decision was thus made to abandon any further attempts at promoting intensive cultivation of this plant and to leave it to the discretion of the frontiersmen themselves whether to grow the crop or not". This report shows that by 1817 soybean had already become a domestic plant in Vojvodina. As indicated by the report, however, soybean growing was apparently discontinued in the province soon thereafter, as the crop disappeared from the written records for a long while. The plant emerges again in the region only in the 1930s, when it was noted that Yugoslavia had very favorable conditions for soybean growing, especially in the regions of Srem, Posavina, Podunavlje, Mačva and Metohija (Gutschy, 1950).

Vojvodina was the main soybean-producing region in the former Yugoslavia and is also, and even more so, the main soybean-growing area in today's Serbia, where the only soybean production outside the province exists in the region of Mačva in the northwest of Serbia proper (around 10,000 hectares). From the end of the Second World War until 1974, the areas in soybean in Vojvodina varied from several tens to several thousands of hectares, and the yields were around 1 t/ha until the 1970s. After 1975, the acreage began to increase markedly, until more than 82,000 ha of soybean were planted in each 1983 and 1984. After that, the soybean areas started to decrease and then stabilized at around 50,000 ha in the 1990s. In the last ten years or so, the acreage in the crop has been on a steady rise in the province and has reached a maximum of over 120,000 hectares in both 2000 and 2003.

Serbia's soybean output has thus far been unable to meet the domestic demand for this crop. The country's soybean production could be increased not only by increasing the area sown to the crop but also by increasing yield per unit area. This would require higher-yielding varieties, better cultural practices, and more complete protection from weeds. Another way to improve yield levels would be to use double cropping to obtain two harvests per year. This option has not been sufficiently explored in Serbia as of yet.

SOYBEAN PROGRAM OF THE INSTITUTE OF FIELD AND VEGETABLE CROPS IN NOVI SAD

Successful crop production and management of problems associated with crop growing cannot be carried out without the support of good and versatile research work. In the early days of soybean growing in Yugoslavia, the small and highly variable acreage sown to this plant and the uncertain outlook for the crop on the market did not provide enough incentive for a large number of researchers to devote their time and effort to the issues of soybean production. Nevertheless, there were some scientists who did carry out work on identifying and trying to resolve problems occurring in the cultivation of soybean. Nearly all agricultural research centers in the country assembled collections of foreign and domesticated varieties of the crop and studied their agronomic traits. This was first done at agricultural institutes, and then, starting in 1952, at agricultural stations as well. The Institute of Field and Vegetable Crops in Novi Sad is the oldest research institution in Vojvodina, founded in 1938 to serve as an agricultural, trial and control station. After the Second World War, the Institute began collecting material from various industrial crops in order to study and improve it. This included plant species such as sunflower, hemp, soybean, flax, castor bean and others (Stojković, 1963). By 1954, work on soybean selection had been done by Dr Lazar Stojković, Dr Relja Savić and Dr Dušan Dimitrijević, and in 1954 Dr Bogdan Belić began working on soybean breeding as well.

In the mid-1970s, an action plan on soybean was prepared that was the most comprehensive such plan in Yugoslavia up until that point. It included plans for the introduction of soybean into commercial production as well as a plan for the construction of processing facilities for the crop. This created a market for the placement of the plant and gave the growers the assurance that their product would be purchased after the harvest, thus fulfilling the second major precondition for the spread of soybean in Yugoslav agriculture.

As there were no domestic varieties of soybean available in the country at the time, foreign cultivars had to be imported (Hrustić et al., 1998a). The imported varieties were the best of their time (mostly US ones) and were chosen based on their suitability for the domestic growing conditions. Most were from Maturity Group I (Hodgson, Rampage, Chippewa, Traverse and Hark), because it was established that this particular group was best suited for the growing conditions in Yugoslavia. Of cultivars from Maturity Group II, Corsoy, Amsoy and Wells were imported. The early varieties Swift and Evans (Maturity Group 0) and the very early ones Clay and Morsoy (Maturity Group 00) were also introduced. At the same time these varieties were being introduced to commercial production, they were also tested in a series of large-plot trials set up across the province of Vojvodina. These trials had a double purpose. The first one was to investigate the adaptability and stability of the cultivars being introduced in the domestic growing conditions and to match them with the regions most suitable for their cultivation. The second goal was to popularize soybean as a crop

and to allow as many growers as possible to become familiar with the plant. Extension efforts on the advancement and popularization of soybean growing continued after this period as well.

The results of the large-plot trials showed that the adaptability and yield stability of some of the varieties tested was unsatisfactory in Yugoslav conditions and that there was a lot of variability in yield among the different locations and years. This resulted in a reduced number of introduced varieties and only the best continued to be grown commercially. These were the cultivars Evans, Hodgson, Hark, Amsoy, and Corsoy, which were grown in the country for many years.

However, because intensive production cannot be based on introduced varieties developed for completely different growing conditions, it became necessary to develop a set of high-yielding domestic varieties.

Genetic resources

Because soybean is almost exclusively a self-pollinated plant with a very small percentage of cross-pollination, the starting variability is obtained by crossing different genotypes. Choosing genotypes to be used in the crosses as parents is not an easy task, especially in the case of soybean, whose genetic base is very narrow. Modern-day US varieties, which make up more than 80% of the crop's current genetic basis, originate from only ten or so genotypes introduced from China at the start of the 20th century. Modern breeding of soybean began in the U.S. in the 1920s, and the newly developed cultivars spread from there to South America, Europe, India, and even China, the place of the crop's origin. Around the world, these new cultivars then went through the processes of introduction, hybridization and selection.

It is, therefore, not an easy task for a soybean breeder to pick their starting material for selection from a large number of genotypes with a similar genetic background, because only crosses among genetically divergent genotypes result in transgressive segregation for various traits, including those that are economically important. For this reason, germplasm collections are an essential resource in the development, advancement and improvement of soybean cultivars. Soybean breeding at the Institute of Field and Vegetable Crops in Novi Sad was started by using a rich collection of soybean genotypes that had been assembled over time by Prof. Bogdan Belić from various parts of the world. Over the years, the collection has been expanded and organized and today represents the largest assemblage of soybean germplasm in this part of Europe (Vasić et al., 2007).

The Institute's soybean collection is made up of more than 800 cultivars and lines originating from America, Asia, and Europe. Most of the genotypes belong to Maturity Groups 0 and I, but the collection includes genotypes ranging from MG 000 to MG V.

The genetic backgrounds of Novi Sad soybean cultivars consist primarily of American genotypes from the northern germplasm collection. Gizlice et al. (1994) studied the pedigrees of 256 North American varieties developed between 1947 and 1988 and found that 80% of the genetic base of American soybean cultivars originate from only 13 genotypes. It is important to note that the authors of the study analyzed complete pedigrees of the cultivars (not just the parental components) and arrived at a set of 35 ancestral genotypes, whose contribution to the genetic background of American soybean cultivars exceeds 95%. Since some of the most widely used US cultivars (Hodgson, Evans, S1347) are also the parental components of the most widely spread Novi Sad varieties (Afrodita, Balkan, Vojvođanka), and given the fact that the coefficient of parentage between the varieties Hodgson and Corsoy is 0.566 and that between Evans and Corsoy 0.484 (Allen and Bhardwaj, 1987), it is apparent that the list of ancestral genotypes of NS soybean cultivars is much shorter. An analysis was made of the parental components of the NS varieties of soybean (without conducting detailed pedigree analysis and identifying all ancestral genotypes) and 60 different genotypes were found. Eighteen of those 60 accounted for 70% of the genetic makeup of the cultivars, while the remaining 42 were represented with less than 1% each (Table 1.6). The greatest contributors to NS soybean germplasm are the varieties Hodgson, Evans, S-1346, and Corsoy, while a certain smaller proportion originates from European varieties, most notably ISz10, Fiskeby and Four.

Table 1.6

Parental components of NS soybean cultivars and their percentage contribution to the NS germplasm of the crop

No.	Parent	Origin	Percentage	Cumulative perc.
1.	Hodgson	USA	13.85	13.85
2.	Evans	USA	10.96	24.81
3.	S1346	USA	10.00	34.80
4.	Fiskeby	SWE	4.04	38.84
5.	ISz 10	HUN	3.84	42.69
6.	Corsoy	USA	3.46	46.14
7.	Afrodita	YUG	2.88	49.03
8.	Vojvođanka	YUG	2.50	51.52
9.	Balkan	YUG	2.50	54.02
10.	L-16	YUG	2.31	56.33
11.	Krajina	YUG	2.30	58.63
12.	Gema	USA	1.73	60.36
13.	Wells	USA	1.54	61.90
14.	Weber	USA	1.54	63.44
15.	Resnik	USA	1.54	64.98
16.	NS-L-MM	YUG	1.54	66.52
17.	Hawkeye 66	USA	1.54	68.05
18.	Gadir	FRA	1.54	69.59

Methods and directions of breeding

Modern-day soybean breeding involving hybridization began in the 1920s in the U.S. and China and over 3,500 soybean cultivars have been developed globally since (Carter et al., 2004). Soybean breeding and selection is a continual process by which yield levels are increased and resistance to abiotic and biotic stresses is improved. Improved cultural practice and increasing yield potential and atmospheric CO2 concentration have been the contributing factors to an increase in soybean yield levels and soybean growing productivity. Specht et al. (1999) reported an annual yield increase 23 kg in the U.S.

Each cycle of breeding begins with the selection of parental pairs to be used for obtaining new genetic variability. Choosing parental pairs is the first crucial moment in breeding, because it determines the success of the future selection process. Generally, elite parental lines of different origin have the greatest chance of producing superior progeny (Burton, 1997; Miladinović et al., 1999). The choice of pairs to be used in crosses depends on many factors – the traits one is trying to improve, the relative importance of other traits in relation to yield, the origin of the lines being used, and the resources the breeder has available to them. The most commonly used method of choosing the parents is evaluation of varieties and genotypes per se. This is also the most economical method, because the data from small- and large-plot trials are readily available to the breeder. St. Martin et al. (1996) have also developed a test cross method for identifying potential parents in soybean. Test crosses have proven more useful in identifying parents than the method that uses heterosis for the same purpose (Lewers et al., 1998). Another method that has proven very useful in predicting superior combinations is the BLUP (best linear unbiased prediction) one. Although this technique has often been used in animal breeding in the past, its use in plant breeding is of more recent date (Panter and Allen, 1995). An increasing number of researchers are turning their attention to molecular markers, so techniques based on these markers can also be used in the selection of parental components (Helms et al., 1997; Manjarrez-Sandoval et al., 1997; Kiasha et al., 1997).

Once hybridization is performed and genetic variability is obtained, the potentially superior progeny must be turned into homozygous lines.

The choice of the selection method depends on the goal of the breeding program as well as on other important factors, such as the variability one has at their disposal, the availability of agricultural machinery and greenhouses, the number of personnel and their level of training, and so on. Soybean breeding makes use of methods that are used in the selection of other self-pollinated crop species as well. These include: pedigree selection, single seed descent, the bulk method, the early generation testing procedure, and backcrossing. With the discovery of genetic male sterility, recurrent selection has become another useful tool for developing new soybean varieties (Hrustić et al., 1997; Wilcox, 1998).

The backcross method is most often used in cases when a certain trait, such as resistance to a disease, needs to be incorporated into a good standard variety that is widely grown commercially in order for said variety to be able to maintain its share on the market. The goal, therefore, is not to develop a new cultivar but to improve an already existing one that is of good quality (Borojević, 1992).

Similar is the case with early generation testing. This method is used when a cultivar containing a particular trait needs to be developed fast. Testing is conducted as early as the F2 generation and a large number of potentially good genotypes are discarded early (Cooper, 1990).

The bulk method is the most economical way of obtaining homozygous lines after hybridization. This technique is also known as the bulk population method and was first introduced by Nilson Ehle in Sweden in the early 20th century. The theoretical development of the method was first carried out by Harlan and Martini (1938) in their work on barley. The method involves obtaining the next generation by planting a large number of seeds, harvesting the plants in bulk, and planting a sample of the seed the following year. The advantage of this approach is that it enables a larger number of crosses to be grown without the need for a lot of labor, observation, and selection. As the hybrid mixture of the populations contains different genotypes with differing levels of productivity and different interaction with the environment, natural selection occurs, which may result in a loss of valuable genotypes. Still, the theoretical basis of this method relies on the proposition that an increase of yield occurs in the process of selection, because natural selection favors high-yielding genotypes (Suneson, 1956). To make bulk selection more effective, the hybrid generations are grown in bulk only up to the F4 generation instead of up to F6, and this is the major characteristic of the method. Individual selection of the best plants begins in the F4 generation and is followed by the selection of new lines in the later generations by the pedigree method. This is considered a modified and improved version of the bulk method. Two major disadvantages of this type of selection are the loss of genetic variability in each subsequent generation due to the use of an inadequate sample and the possibility that natural selection occurring in the population may take an undesirable turn (Empig and Fehr, 1971).

The pedigree method of soybean selection consists in growing progenies of crosses through generations of self-pollination by growing rows of progenies of plants selected in each generation based on their phenotypic traits, with the pedigree of each line being maintained in the subsequent generations. This was the predominant method of soybean breeding in the U.S. up until the mid-1960s and was effective in developing varieties with an increased grain yield and resistance to lodging. It was also used in the early days of soybean breeding at the Institute of Field and Vegetable Crops. The method is useful for evaluating progenies of crosses between phenotypically different parents, since it makes it possible to identify and discard a large number of undesirable progenies in the early generations, leaving a high frequency of superior lines for final selection in the later generations. Unlike bulk selection, the

pedigree method reduces competition among different genotypes to a minimum and makes it irrelevant for the success of the selection process. The greatest disadvantage of the method is that it is labor-intensive and requires a lot of manpower. It involves individual selection of the plants, their threshing, planting, and marking, and the recording of a large amount of data, all for the purpose of maintaining the pedigree of the lines in successive generations. This makes the method highly unsuitable for handling a larger number of crosses. In addition, pedigree selection involves constant selection of a certain number of heterozygotes that would have become homozygous in the later generations even without the breeder's intervention.

The single seed descent method was proposed by Brim (1966) and the procedure has been the predominant method of soybean selection in the U.S. since. Single seed descent makes it possible to produce three generations of self-pollination in a single year using winter nurseries or greenhouses, thus accelerating the development of homozygous lines for the testing of yield in replicated trials. The method is used in the soybean program of the Institute of Field and Vegetable Crops in Novi Sad as well. However, due to the unavailability of a winter nursery and a lack of sufficient greenhouse space that would accommodate all of the breeding materials, the Institute has had to adapt the method to make it suitable for such conditions and is making use of only those aspects that involve the reduction of space and labor while at the same time maintaining a satisfactory level of variability up to the F5 generation (Miladinović, 1999).

The single seed descent method is usually not applied until a certain level of homozygosity is reached in the F4 or F5 generation. Selection in the earlier generations can still be done, but on a smaller scale, i.e. it is performed in the sense that pods are not taken from plants that are diseased or lodged or prone to pod splitting and so on.

Single seed descent requires that only the most basic data be taken down, such as the designation of the cross and what generation it is in. Also, minimal space is required to grow successive generations of individual plants when compared to the rows of progenies characteristic of pedigree selection. Another advantage of the single seed descent method is the presence of full variability in each generation. With no selection in the early generations, the amount of variability present among the F5 plants is similar to that found in the F2 generation. Finally, the number of recessive homozygotes increases in successive generations – with the postponement of selection for a recessive trait until the F5 generation, nearly 47% of the plants will be homozygous for such a trait (Wilcox, 1998).

Perhaps the biggest disadvantage of single seed descent consists in the irreversible loss of identity of superior plants from the earlier generations. Besides that, a superior plant observed in the F2 generation will be represented by no more than a single plant in the subsequent generations, making it impossible to select a larger number of lines from superior plants. Moreover, plants that would otherwise be discarded will remain in the population up until the F5 generation.

Each method, therefore, has its advantages and drawbacks. The challenge is for the breeder to choose and make use of the most effective method for the achievement of their breeding goals. Comparisons among the different selection procedures (Miladinović, 1999; Miladinović et al., 2000) have shown that the modified version of the single seed descent method that is in use in soybean breeding at the Novi Sad Institute is more effective than the other methods. The best evidence of this is the success of the Institute's soybean program.

Beginnings of soybean breeding at the Institute

During the campaign for the spread of soybean growing in the country in the 1970s, a soybean team was formed at the Institute of Field and Vegetable Crops in Novi Sad. The team was headed by Prof. Dr Bogdan Belić and was composed of plant breeders, cultural practice specialists, phytopathologists and seedsmen. The team's primary goal was to develop the first high-yielding domestic soybean varieties that will be suited to the local growing conditions, thus creating the conditions for the establishment of stable production and the replacement of foreign cultivars on the domestic market. The concept of the program was to develop varieties with different growth periods (Maturity Groups 0, I and II) that could be grown in all the different soil and climatic conditions of the country. Later on, the program's goals were expanded to include the development of cultivars with very short growth periods (MGs 00 and 000) that would enable soybean to be used in double cropping or grown as a stubble crop. Another objective was to develop soybean varieties suitable not only for intensive farming but also for extensive agriculture conditions that involved soils having unfavorable composition or an inadequate nutrient supply, areas in which proper tillage was not possible, and so on. The new varieties also had to be resistant to lodging, pod splitting, and major diseases.

Soybean breeding is a complex and difficult task that takes a long time. It takes six years for a genotype to progress from the initial cross to a line that can be considered pure. Another three years are needed for preliminary and comparative trials. Then, even if no multi-site or large-plot trials are carried out, three more years are needed for the official variety trials. Therefore, it takes a minimum of 12 years for a soybean variety to be developed. Of course, testing can also be done in the early phase of breeding and it is still perfectly possible for an insufficiently tested variety to be put through the official trials and even outperform the standard variety, especially if the standard is an introduced cultivar. In the first 12 years of the Institute's soybean program, seven new varieties were developed based on the existing material and new crosses (Table 1.7). Three of those, NS-6, NS-9 and NS-10, have found commercial success.

Table 1.7

NS soybean varieties released between 1975 and 1987

No.	Variety	Maturity Group	Year of release
1	NS Kasna	III	1979
2	NS – 11	II	1980
3	NS – 6	0	1982
4	NS – 9	I	1983
5	NS – 10	I	1985
6	Hy – 12	00	1986
7	NS – 13	I	1987

Soybean breeding for productivity and other traits

The first soybean cultivars of the NS Institute that had gone through an entire breeding cycle were released in 1988 (Table 1.8).

Table 1.8

NS soybean varieties released between 1988 and 1992

No.	Variety	Maturity Group	Year of release
8	NS – 16	I	1988
9	NS – 17	I	1988
10	NS – 18	I	1988
11	NS – 20	II	1988
12	NS – 21	II	1988
13	NS – 102	I	1989
14	NS – 104	I	1989
15	NS – 105	I	1989
16	NS – 201	II	1989
17	NS – 202	II	1989
18	Dunav	0	1990
19	Kolubara	0	1990
20	Bačka	0	1992
21	Banat	0	1992
22	Sremica	0	1992

Two of the five cultivars registered that year (NS-16 from MG I and NS-21 from MG II) began to be grown commercially on a wide scale. In the four years that followed, a number of varieties, mostly early ones, were released. The best among them, Kolubara and Bačka, spread across the fields of Serbia, gradually replacing the foreign, introduced varieties.

In parallel with soybean breeding, the Institute also worked on all the other problems connected with soybean production. Since soybean was for the most part an unknown crop to Serbian growers, soybean growing technology became the subject of many studies by the Institute's research staff (Belić, 1966; Belić and Molnar, 1977; Hrustić, 1983; Jocić and Sarić, 1984; Relić, 1996; Miladinović et al., 1998; Tatić et al., 2006). The results of this research were immediately transferred to actual agricultural practice, and this undoubtedly contributed to the increase of soybean yields in the country.

Studies dealing with mineral nutrition and nitrogen fixation have shown that soybean growing does not require mineral fertilizer incorporation, provided nodule bacteria are present in the soil. No such bacteria are found in Serbian soils, however, so soybean seeds need to be inoculated before being planted, and this is done using a preparation called Nitragin. Nitragin is a biofertilizer composed of an optimally balanced mixture of the most productive strains of nitrogen-fixing bacteria identified by research conducted at the Institute of Field and Vegetable Crops (Mrkovački et al., 1989; 1992; Milić, 1990; Milić et al., 1991; Marinković et al., 2004).

Many studies have also been conducted to determine the optimum irrigation rates and timing for soybean (Bošnjak, 1978; 1987; Vučić et al., 1981; Pejić, 1993; Dragović, 1994; Miladinović et al., 1997a). Their results have shown that grain fill is the most critical stage of soybean growing and a time when the plant's water requirements are at their highest. These studies have also shown that soybean can be grown as a second or stubble crop in irrigated conditions.

Research in the field of soybean phytopathology has also been of great importance (Jasnić and Vidić, 1981; 1985; 1986; Vidić, 1982; 1987; Jasnić, 1984; Vidić and Jasnić, 1998; Vidić et al., 1998). These studies have consisted in determining the racial composition of pathogens and identification of sources of resistance to the most economically important soybean pathogens in the country, such as Peronospora manshurica (downy mildew), Pseudomona syringae pv. glycinea (bacterial spot), Diaporthe phaseolorum var. caulivora (stem canker), Sclerotinia sclerotiorum (Sclerotinia stem rot) and Macrophomina phaseolina (charcoal rot). Work on breeding for resistance to diseases performed in the Institute's soybean program involves the incorporation of genes for resistance to the dominant races of the above pathogens into commercial soybean cultivars.

All the above research efforts have significantly helped the NS soybean program and have enabled it to focus on the development of varieties best suited for the growing conditions in the country.

The years 1993 and 1994 can be regarded as the golden years of NS soybean breeding, because in those two years alone 12 new NS cultivars of soybean were released that have completely replaced foreign cultivars on the domestic market thanks to their superior characteristics, most notably their high potential for yield.

Table 1.9

NS varieties released in 1993 and 1994

No..	Variety	Maturity Group	Year of release
23	Krajina	00	1993
24	Panonka	0	1993
25	Mačvanka	II	1993
26	Tamiš	II	1993
27	Jelica	00	1994
28	Afrodita	0	1994
29	Ravnica	I	1994
30	Balkan	I	1994
31	Vojvođanka	II	1994
32	Nizija	II	1994
33	Simonida SP	II	1994
34	Šumadija	II	1994

The 12 cultivars included the very early varieties Krajina and Jelica, which are suitable for planting as a second or stubble crop. Krajina is also the standard variety in the national variety trials. There is also a large interest in these two cultivars in the European countries that lie at more northerly latitudes, in which the two varieties can be grown as a full-season crop. Jelica has been released in Russia and Krajina in Russia as well as Hungary.

Together with the cultivar Bačka, which had been registered a little earlier, the varieties Panonka and Afrodita were the mainstays of the early-maturing cultivar range in the country for several years after their release. Afrodita is the standard for Maturity Group 0 in the national variety trials and has also been released in the European Union.

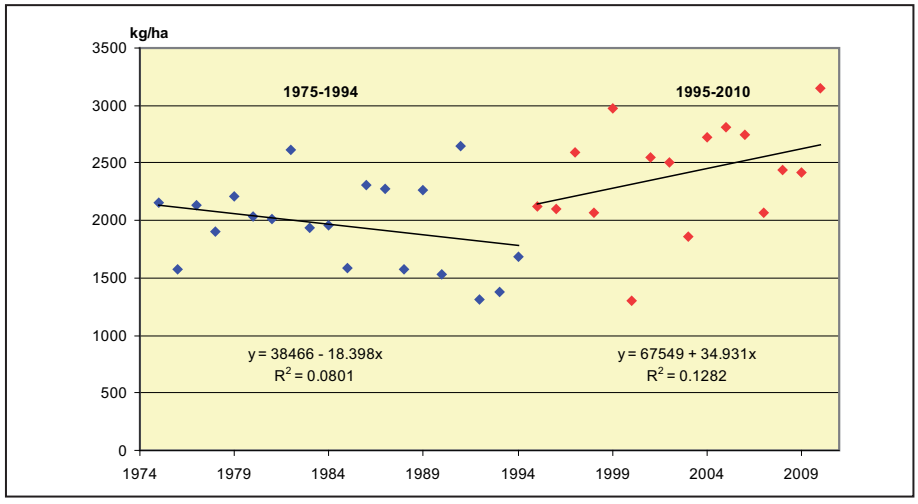
The cultivars Balkan and Ravnica are the most widely grown soybean varieties in the country, because they are medium-maturing and are thus ideal for the growing conditions in Serbia. Thanks to its adaptability and ability to produce satisfactory yields even in unfavorable years and locations, Balkan is still the most sought-after NS variety of soybean on the domestic market and has been released in Romania and Bulgaria as well.

The late-maturing cultivar Vojvođanka managed to supplant from the domestic market the introduced variety Corsoy, which was the last remaining foreign introduction on the Serbian soybean market. Thanks to its extremely high yield, Vojvođanka is one of the most widely grown NS cultivars of soybean. Like Afrodita, it is a registered variety in the European Union.

The year 1995 was the first year in which no foreign cultivars of soybean were grown in the country, and the period between 1995 and 2007 saw an increase of an average soybean yield in Serbia of 44 kg/ha per year (Fig. 1.1). By comparison, from 1975, when foreign cultivars of the crop were first introduced to Serbia, until 1994, the year in which they were fully supplanted by the domestic varieties, the yields decreased by an average of 18 kg/ha per annum, which is a very strong argument in favor of continuing to develop and replenish the domestic stock of soybean germplasm.

Figure 1.1

Soybean yield trends during the 1975-1994 and 1995-2010 periods in Serbia



In each of the first few years following 1995, several new NS cultivars of soybean were released (Table 1.10), but these were either never grown commercially or were sooner or later withdrawn from the market. This was not because they were not good - these were outstanding varieties – but because they did not outperform the already existing cultivars by a significant margin, and the Institute is not in the habit of replacing its cultivar range just for the sake of doing so. The cultivars Danica, Vera, Srbobranka and Indijana, all released during this time, were grown commercially for a while, but their growing was then discontinued, mostly because they were somewhat less adaptable to different growing conditions.

Table 1.10

NS varieties released between 1995 and 1998

No.	Variety	Maturity Group	Year of release
35	Danica	000	1995
36	Pobeda	0	1995

37	Biserka	0	1995
38	Maja	0	1995
39	Košava	II	1995
40	Avala	II	1995
41	Ranka	00	1996
42	Belka	0	1996
43	Vera	I	1996
44	NS – Nada	II	1996
45	Srbobranka	I	1997
46	Indijana	II	1997
47	Gordana	II	1997
48	Jelena	II	1998

Soybean breeding for special traits

Soybean selection at the Institute of Field and Vegetable Crops has so far focused the most on the increase of yield (Miladinović et al., 1997b; 2000; Miladinović, 1999) and its stability and on developing varieties adaptable to different growing conditions (Hrustić et al. 2003; 2004; Miladinović et al., 2003; 2006). However, the Institute's soybean program also makes sure to take into account the preferences of its customers and the processing industry and to adapt to the demands of the market. This is reflected most notably in our work on increasing the protein content of our cultivars by conducting studies in field (Miladinović et al., 1996b; 2001; 2004) and laboratory conditions.

We also do research on nitrogen metabolism in soybean (Miladinović et al., 1996a; Malenčić et al., 2005; Kereši et al., 2007), increasing the quality of soybean oil (Miladinović et al., 1996c; Hrustić et al., 1998b), and studying the antioxidative properties of soybean (Malenčić et al., 2007; 2008). In addition to this, the discerning market of the West has a preference for a good balance between the oil and protein contents in order to use soybean for manufacturing products for human nutrition as well as for certain levels of amino acids containing sulfur, a balance between the levels of oligosaccharides and polysaccharides for the purposes of fish food production (Vucelić-Radović et al., 2005; Hollung et al., 2005), and improved nutritional and medicinal properties of soybean (Cvejić et al., 2009).

The first soybean varieties with increased protein levels developed and commercialized in Serbia were Novosađanka and Proteinka. Another standout in this regard is the NS cultivar, which is capable of having a grain protein content of up to 44% (Table 1.11).

Table 1.11

NS varieties released from 2000 to 2011

No.	Variety	Maturity Group	Year of release
49	Bojana	0	2000
50	Novosađanka	I	2000
51	Milana	III	2000
52	Nađa	0	2001
53	Proteinka	0	2001
54	Tisa	I	2001
55	Morava	III	2001
56	Sanja	0	2002
57	Lasta	0	2002
58	Venera	I	2002
59	Posavka	I	2002
60	Ivana	III	2002
61	Fortuna	00	2003
62	Lara	0	2003
63	Valjevka	0	2003
64	Ana	I	2003
65	Melodija	I	2003
66	Branislava	I	2003
67	Meli	00	2004
68	Bečejka	0	2004
69	Tara	0	2004
70	Zvezda	I	2004
71	Glorija	I	2004
72	Tea	I	2004
73	Sava	I	2004
74	Šapčanka	I	2004
75	Drina	I	2004
76	Mima	II	2004
77	Bistrica	II	2004
78	Alisa	0	2005
79	Iva	0	2005
80	Rita	0	2005
81	Duga	II	2005
82	Senka	II	2005
83	Gracia	000	2006
84	Galina	0	2006
85	Vesna	II	2006
86	Julija	00	2007
87	Diva	I	2007
88	Prima	00	2008
89	Merkur	00	2008
90	Marta	II	2008
91	Idila	II	2008
92	Rubin	II	2008

93	Neda	0	2009
94	Victoria	I	2009
95	Iskra	I	2009
96	Trijumf	II	2009
97	Favorit	000	2010
98	Emina	00	2010
99	Frajla	00	2010
100	Tajfun	00	2010
101	Zlata	I	2010
102	Aleksandra	I	2010
103	Kinđa	I	2010
104	NS Alfa	00	2011
105	NS Virtus	00	2011
106	NS Zenit	0	2011
107	NS Maximus	0	2011

An increased protein content is of particular importance for the purposes of processing, so these cultivars have a variety of special uses in the processing industry. Besides their increased protein levels, these varieties also have a high genetic potential for yield, resistance to lodging, and a high degree of field resistance to the economically important diseases, so the acreage on which they are grown can be expected to increase more and more with time. This projection is further supported by the fact that both Proteinka and Novosađanka have been released in Croatia and Romania, Proteinka is also registered in Ukraine, while Novosađanka has been registered in Hungary and Italy as well. Soybean breeding for an increased grain protein content is a complex and difficult task. High-protein varieties of soybean must also be good performers with respect to the other agronomic ally important traits, most importantly yield, which makes their selection difficult, since high protein content and yield are negatively correlated. It is even more difficult to develop varieties with a protein content of over 45% and a yield performance on a par with that of the commercially grown varieties.

Soybean storage proteins are divided into three large groups based on the sedimentation constant. The dominant fractions are glycinin (11S fraction) and conglycinin (9S fraction), while the 2S fraction contains protease inhibitors. Some breeding programs have it as their goal to reduce the activity of protease inhibitors in the bean in order to save the energy needed to thermally deactivate these inhibitors during the processing. The Institute has no such program. There are two main reasons for that. Firstly, protease inhibitors have a favorable amino acid composition, most importantly they are rich in amino acids containing sulfur. The other protein fractions of soybean (methionine, cystine) are deficient in such acids, so the reduction of this fraction would have a detrimental effect on the favorable amino acid composition of the soybean bean (Pešić, 2003). Secondly, cultivars with reduced inhibitor activity have a decreased total protein content of the seed. Testing of isogenic lines for the Kunitz trypsin inhibitor has shown a significant reduction of the total protein content of the soybean grain, with the oil content remaining unchanged (Vollmann et al., 2002).

The total fatty acid content and composition are another essential component of soybean breeding for a modified chemical composition of the grain. The dominant fatty acid fraction comprises linoleic acid (18:2) with about 55% contribution and oleic acid (18:1) with about 20%. Linolenic (18:3, approx. 8%), palmitic (16:0, approx. 10%) and stearic (18:0, approx. 4%) are also present. The general trend in breeding for fatty acid composition is to reduce the levels of polyunsaturated fatty acids and increase the oleic acid content. Reducing the levels of polyunsaturated fatty acids increases the oxidative stability of soybean oil and also reduces the need for the catalytic hydrogenation of polyunsaturated lipids during the processing of soybean oil. The use of soybean cultivars with an altered fatty acid composition is not only advantageous from the technological point of view but is also beneficial health-wise. Oleic acid is known to be the most desirable fatty acid from the point of view of human nutrition, so the increase of oleic acid levels has a positive effect on the quality of products obtained from high-oleic cultivars. Another benefit comes from the fact that the catalytic hydrogenation of polyunsaturated fatty acids produces not only cis isomers but also trans isomers, which have a proven negative effect on human health.

Among the currently grown NS cultivars of soybean, the variety Venera has an especially high oil content of the grain and another high-oil cultivar, Mima, will also begin to be grown on a large-scale soon. This cultivar takes a little longer to reach maturity than the variety Vojvođanka and hence has to be planted sooner. Although Venera performs the best yield-wise in optimal growing conditions, it also produces stable yields in unfavorable, droughty conditions, which is not typical of a genotype with a long growth period. Venera has been released in Serbia as well as Romania and Bulgaria.

The cultivar Lasta, released in 2002, has a well-balanced fatty acid composition of the oil and a very high oleic acid content. It is not grown commercially at present, because the Serbian market has no special need for high-quality soybean oil as of yet.

Carbohydrates are not abundant in soybean grains but can be a limiting factor in the nutrition of certain animals. The amount of stachyose and raffinose in soybean beans and products limits the digestibility and usability of soybean oil and protein in nonruminants. Soybean breeding for grain carbohydrate composition is aimed at reducing the levels of stachyose and raffinose and increasing the sucrose content of soybean grain. Breeding programs on this are still in the early stages, so it is too early to talk about their results.

For certain uses, the ratio between oil and protein in the soybean beans is important as well. This is the case with soybean milk, for example, because this ratio is of importance for obtaining of a high-quality final product when such milk is manufactured.

In the last few years, the NS soybean program has produced a number of high-yielding cultivars, which are expected to become market leaders in the next several years. These include Tajfun and Merkur from MG 00, Valjevka and Galina from MG 0, Sava and Victoria from MG I, and Venera and Rubin from MG II.

NS soybean cultivars abroad

The registration and release of a cultivar are the end results of the selection process and are therefore an important indicator of a breeding program's success. The release of a cultivar on a foreign market is of special importance, because it is an indication of a wider recognition and a sign that one's work has transcended local boundaries. The NS soybean program has thus far released 49 cultivars abroad (Table 1.12).

Table 1.12

NS soybean varieties released abroad

Country	Variety	Year of release
Hungary	Davodi 2016	1993
	Anita 66	1994
	Bacskun	2002
	Alisa	2003
	Meli	2004
Ukraine	Bojana	2005
	Proteinka	2005
	Ravnica	2005
	Lara	2005
	Sedmica	2005
	Ina	2008
	Tavria	2008
	Poema	2009
	Poltava	2009
	Irina	2009
	Galina	2009
	Larisa	2011
	Fortuna	2011
Italy	Avila	1996
	Condor	1996
	Neoplanta	2005
	Po	2005
	Fortezza	2007
	Tea	2007
Romania	Proteinka	2002
	Balkan	2003
	Venera	2005
	Neoplanta	2005
Bulgaria	Avila	2001
	Balkan	2001
	Zora	2005
	Venera	2005
Croatia	Proteinka	2006
	Neoplanta	2006
	Alisa	2007
	NS Ana	2007
	Galina	2008
	Tea	2008
	Julija	2009
	Merkur	2009

Russian Federatio	Jelica Volga Irina Duga Tavria	2001 2001 2009 2009 2009
Moldova	Tihana Meli Alisa Galina	2010 2010 2010 2010

The Institute has 13 of its soybean cultivars released in Ukraine, eight in Croatia, six in Italy, five each in Hungary and Russia, and four each in Romania, Bulgaria, and Moldova. In 2005 alone, 11 new cultivars were registered abroad. As many as 43 of the total 49 releases occurred after the year 2001, which shows that the registration of NS soybean varieties on foreign markets is of fairly recent date and that NS germplasm of this crop can still be expected to make a major impact on the international market in the years to come

SUMMARY

Despite the centuries of traditional growing in its region of origin, the Far East, it was only in the forties that soybean transformed from a minor crop serving as ensiled feed into a major crop and globally important source of food. For several decades, the United States with about 30,000,000 hectares have been the leading country in soybean production, processing, and trade. In last few decades, Brazil and Argentina, with the acreages of about twenty and fifteen million hectares, respectively, became important soybean producers on the global scale. Attempts to grown soybean have been made in a number of countries. Most countries in the world are presently growing soybean but on a limited acreage.

The soybean was introduced in our country in the 19th century but it remained a minor crop until three decades ago. The global trend of increasing the soybean acreage has been felt in our country too. Aglthough the acreage fluctuated in dependence of yields achieved and economic incentives offered, the soybean should nevertheless be considered a major field crop. The largest soybean acreage is located in the Vojvodina Province. As a result of an intensive research work, the introduced foreign varieties have been replaced by domestic ones and a number of problems in the field of cultural practices has been solved. The presently grown soybean varieties have been developed in our agroecological conditions and the cultural practices have been modified to fit the pravailing climatic and soil factors.

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SOYBEAN MORPHOLOGY AND STAGES OF DEVELOPMENT

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MORPHOLOGY

Root system

Soybean is characterized by a taproot system, typical for the class *Dicotyledonae*. However, when lateral roots emerge during later stages of development, most often the taproot cannot be distinguished from them, which is why soybean root system would best be described as diffuse (Lersten and Carlson, 1987). Soybean root system consists of a taproot, lateral roots, and adventitious roots. According to the time of occurence, root system could be distributed as primary, secondary, tertiary and higher order roots.

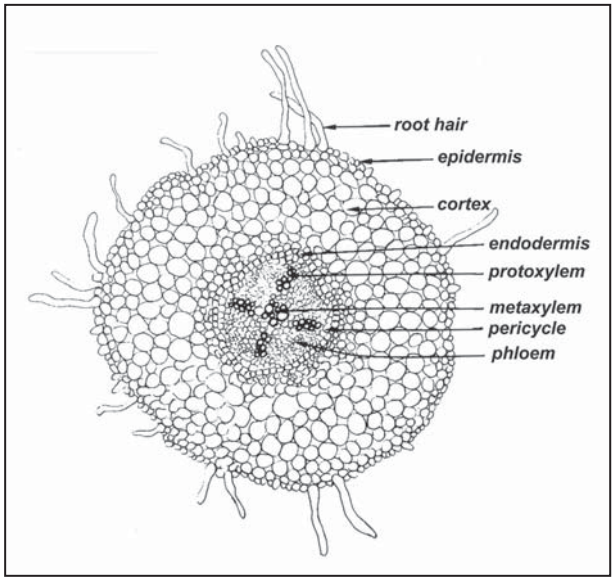
Formation of root nodules is influenced by the activity of nitrogen-fixing bacteria *Bradyrhizobium japonicum*. Root system growth and development are conditioned both by variety and external factors (climate and soil). Number of authors obtained significant differences in growth rate, diameter and number of roots and root dry matter accumulation among genotypes grown in the same environmental conditions (Mitchell and Russell, 1971; Kaspar et al., 1978; Zobel, 1980). On the other hand, Stone and Taylor (1983) and Kaspar et al. (1981) found that growth and development of soybean root system are significantly affected by environmental conditions, especially soil temperature.

Root anatomy

Transverse section of the primary root shows three different parts – the epidermis (rhyzodermis), the primary cortex and the stele (Figure 2.1).

Figure 2.1

Transection of primary root in root hair zone (from Lersten and Carlson, 1987)



The rhyzodermis consists of tabular cells radially elongated along the root axis. These cells are of simple structure, with thin walls that allow easy adoption of water and nutrients. In search of the nutrients, individual cells of rhyzodermis elongate and form root hairs, thus significantly increasing the overall root absorbing surface. The precise increase cannot be determined with certainty, but it is considered that root hairs comprise about 85% of the total area of the entire root system absorbing surface (Carlson, 1969). According to the study by Dittmer (1940, as cited in Lersten and Carlson, 1987) root hairs can be found on all roots, except the taproot where secondary growth removes the epidermis (Table 2.1).

Table 2.1

Data on roots and root hairs of mature, field grown Ilini soybean (from Dittmer, 1940)

Root type	Average root diameter	Average root hair length x diameter	Number of root hairs
	mm	µm	mm-1 root length
Primary root*	2.50	-	-
Secondary roots	0.65	110 x 17	606
Tertiary roots	0.31	90 x 14	210
Quaternary roots	0.23	90 x 14	170

** – Secondary growth caused loss of most epidermis and root hairs*

Cortex is a fundamental tissue that consists of parenchyma cells, located between the rhyzodermis and the stele. In the primary root it consists of 8 to 11 layers of slightly elongated cells with much intercellular space, while in branch roots the narrower cortex has 4 to 9 layers (Lersten and Carlson, 1987). Cortical cells close to the rhyzodermis are considerably smaller than intervening cortical cells and form the exodermis. The innermost cortical cell layer forms endodermis with a continuous suberized casparian strip encircling its radial walls.

The stele consists of radially structured vascular bundle and pericycle. The pericycle is a cylinder of parenchyma that has the capacity to produce new cells and plays a key role in formation of the lateral roots and secondary dermal tissue during secondary thickening. Lateral roots arise in the pericycle at loci directly opposite the ridges of the tetrarch xylem and develop acropetally at approximately 90-degree intervals in four longitudinal rows on the taproot (Mitchell and Russell, 1971). This symmetry is, however, hardly noticable due to the twisting of the main root during growth in soil. Lateral roots are tetrarch or triarch in structure, while tertiary, quarternary, and successive orders of smaller branch roots may be triarch or diarch, and depending on the structure they grow in two, three or four rows around the axis of the primary root (Carlson, 1973).

The most part of soybean root system consists of four to seven heavily branched major lateral roots that emerge from the base of the taproot. Those roots, called basal, are larger in diameter than those growing from the lower parts of the taproot (Zobel, 1980). According to Mitchel and Russell (1971), after lateral growth of 20 cm to 36 cm, major lateral roots abruptly turn downwards and rapidly grow to a depth of almost 2 m under favorable conditions.

Higher order roots are formed whenever conditions are favorable, but the active life span of feeder rootlets is between 10 to 20 days. Afterwards, roots continually dry and die off as they exhaust the resources available in their immediate vicinity, while parental root remains and initiates growth of new rootlets (Huck and Davis, 1976).

Not much is known about adventitious roots. According to Tanaka (1977), they develop from the underground part of the hypocotyl, with growth and function similar to the major lateral roots whose length and diameter they are able to reach. As with other parts of the root system, their growth and development are significantly affected by soil conditions, especially temperature (Stone and Taylor, 1983).

Growth and Development of the Root System

Since growth and development of soybean root system during vegetation period is rather uneven, number of authors tried to trace the precise outlines of root development (Mitchell and Russell, 1971; Sanders and Brown, 1979; Mason et al., 1980). The most accurate determination was suggested by Mason et al. (1980), for they connected the growth and development of soybean root system with the stages of development of above ground plant parts proposed by Fehr and Caviness (1977).

According to this determination, there are five stages of soybean root system development: early vegetative growth (VE - V6), pre-flowering period (V6 - R1), flowering (R1 - R3), pod formation and growth (R3 - R4) and seed growth and maturity (to R6).

Early vegetative growth

Development of root system onsets with seed germination and radicle emergence out of which the taproot forms. During this period the root grows much faster than above ground plant parts reaching daily growth of 2.5 cm to 5 cm given the favorable soil moisture (Mitchell and Russell, 1971), only to reach depths of 0.8 m to 1 m at the end of this stage (Sivakumar et al., 1977). Lateral roots start growing horizontally three to seven days after germination, often reaching length of 25 cm to 30 cm at the end of the phase, and can be found at 3 cm from surface at the most, depending on temperature and soil moisture (Mitchell and Russell, 1971). Secondary and tertiary roots also start growing in the layer of 0 cm to 15 cm, where major mass of soybean root is often found. The percentage of root size found in this layer primarily depends on soil moisture. At the end of vegetation period in dry cultivation, Mayaki et al. (1976) measured 51% total plant root mass in this layer, while this percentage was 67% in irrigated plants. At the end of this phase, when the plant reaches stage V6, ratio of above ground plant dry weight to root dry weight is approximately 3.8 (Sivakumar et al., 1977).

Pre-flowering period

In this stage the growth of the main root slows down, while the lateral roots reach maximum horizontal length. Higher order roots start growing along the whole length of the root while total root dry weight increases. Ratio of above ground plant weight to root reaches the value of 6.8 until stage R1 and inreases during vegetation period due to rapid growth during the reproductive phase (Kaspar, 1985).

Flowering

In the flowering stage, above surface plant parts grow rapidly again, as well as the root. In this period, Mason et al. (1980) determined the increase of total root dry weight in comparison to the previous growth stage by 84% and increase in length by 165%, being the highest increase of all the stages in vegetation period, which is in accordance to the results of Kaspar et al. (1978). Nonetheless, the root growth is slower than the growth of above surface plant parts and ratio between them during R2 stage reaches the value of 9 (Sivakumar et al., 1977). Further increase in length is mostly into deeper soil layers, while the most part of dry weight is still in the layer up to 30 cm. Lateral roots had by this time stopped growing horizontally and started growing rapidly downwards, which can be explained by higher temperature and lower soil moisture closer to the surface (Mitchell and Russell, 1971).

Pod formation and growth

Root is still growing, but slowlier than in the flowering stage. If the soil is well provided with moisture, new root growth is visible in the layer up to 30 cm. Since this occurs rarely in this stage, most often there is only the downward elongation.

Seed growth and maturity

In this stage root growth is significantly slowed as compared to the previous stage, since the plant uses almost all organic matter created in the process of photosynthesis for seed formation and filling. Dry matter increase in soil layer up to 15 cm is mostly connected to the secondary thickening of the taproot and lateral roots (Kaspar, 1985), while growth into depth is mostly finished before seeds start filling. Total root dry weight reduces due to older parts of the root system dying off, while the ratio of above ground plant dry weight to root dry weight reaches the value of 10 (Sanders and Brown, 1976).

Root nodules

Root nodules are an important part of the soybean root system. Similarly to other legumes, soybean plant enters into a symbiotic relationship with nitrogen-fixing *Bradyrhizobium* bacteria living in root nodules, whence their name nodule bacteria. These bacteria take carbo-hydrates from the plant providing it with nitrogen by converting inorganic nitrogen (N2) from the atmosphere into ammonia (NH3) which is readily usable by plants. Typical for soybean is the species *Bradyrhizobium japonicum*, gram-negative, rod shaped bacteria, capable of penetrating thin rhyzodermis cell walls or root hairs, progressing to the primary cortex. Visible 7 to 9 days after infection, nodules are formed by intensive division of bacterial cells and desintegration of host cells. During the third week from infection, the infected root tissue produces leghemoglobin intensivelly coloring the nodules pink, which remain thus colored while active. When leghemoglobin forms, bacteria cease division and nitrogen-fixation commences (Bergersen, 1963).

During the fourth week from the infection, nodules reach their full size (3 to 6 mm) most often being oval shaped, although they could be of irregular shape. A subtle increase of nodule number and size is observed during vegetation period (Zobel, 1980). Nodules actively fix nitrogen for 50 to 60 days, when they dry off and die. One plant can host as many as few hundreds of nodules, mostly concentrated in the shallow soil layer of up to 20 cm, but these could also be found at depths over 1 m (Grubinger et al., 1982). Nodule number is conditioned both by internal factors (genetic and physiological) and external factors (soil nitrogen level) (Gibson and Harper, 1985; Harper, 1987). At least 40 genes are directly involved in the process of nodulation and nitrogen-fixation, from regulation of a certain soybean variety`s compatibility towards a certain *B. japonicum* strain, via infection intensity, to nodule development (Rolfe and Gresshoff, 1988).

Stem

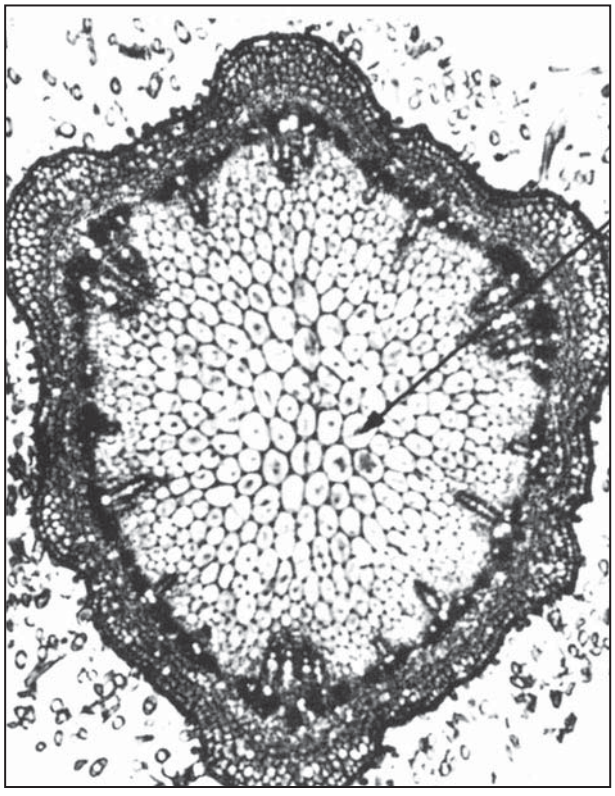
There are two basic types of the soybean stem – the prostratum, with vining growth habit, and the erect type. The first is typical for wild soybean species and can reach heights of 2 to 3 m. Commercial varieties, however, have an erect stem, the height of which, affected by variety and environmental conditions may be 30 to 130 cm. Soybean stem is green, due to the presence of chlorophyll in the parenchyma cells, covered in hairs and mostly branched. On stem there are joints or nodes, clearly visible thickenings which carry leaves; a portion of the stem between two nodes is called an internode. The first node carries the cotyledons, the second node carries the first pair of leaves (prophylli), while other nodes carry alternated trifoliolate leaves.

Stem anatomy

Soybean stem transection clearly shows layers of the stele, primary cortex and epidermis (Figure 2.2).

Figure 2.2

Stem transection (according to Curry, 1982; as cited in Lersten and Carlson, 1987)



The middle of the stem is occupied by a voluminous parenchyma pith tissue made up of large cells without chlorophyll. During the first part of the vegetation period, this tissue serves as storage, while later, due to secondary thickening, the cells move away from each other leaving the stem hollow. Pith is surrounded by a zone of vascular bundles intersected by parenchyma tissue cells, which connects the pith to the primary cortex, thus comprising the network of individual veins, or eustele.

The zone of vascular bundles consists of xylem, positioned towards the pith, and phloem that transports the assimilates, positioned towards the exterior. Xylem and phloem are separated by the rest of undifferentiated cambium. Primary xylem comprises protoxylem and metaxylem. Owing to their non-thickened wall parts, first set protoxylem elements are capable of following the elongation process during stem growth. As elongation growth finishes, protoxylem loses its function, which is taken over by a later differentiated metaxylem whose coiled structure enables an additional slight elongation. Similarly, the phloem is also divided into protophloem and metaphloem, which in addition to sieve-tube cells also has companion cells. During secondary stem growth, the secondary xylem and phloem are created to form a complete cylinder at lower plant parts (where the secondary thickening lasts longest) (Cumbie, 1960).

In the internodal stem parts there are vascular bundles (veins) thicker than usual, from which collateral veins extend and are traceable towards the base as traces of leaf vascular bundles. At each node, three such traces part from the stele and merge into a leaf vascular vein.

The primary cortex is separated from the stele by a layer of endodermis cells, made up of a green assimilation storage parenchyma tissue, externally enveloped by collenchyma. In some soybean varieties, this layer stores the pigment anthocyanin whose presence affects flower color. Varieties that have anthocyanin display purple flower color, while varieties lacking anthocyanin have white flowers.

On the surface, stem ends in epidermal cell layer lacking chlorophyll with a cutinized external wall. Typical for soybean is that epidermal cells elongate into hairs, whose color, density and position are a significant feature in commercial varieties. Varieties with a dominant *Pd1* gene have a larger number of hairs per unit of epidermal area (Bernard and Singh, 1969; Bernard and Weiss, 1973). Hairs are white to dark brown, positioned most often erect to the stem, although they can be appressed. There are even varieties lacking hairs.

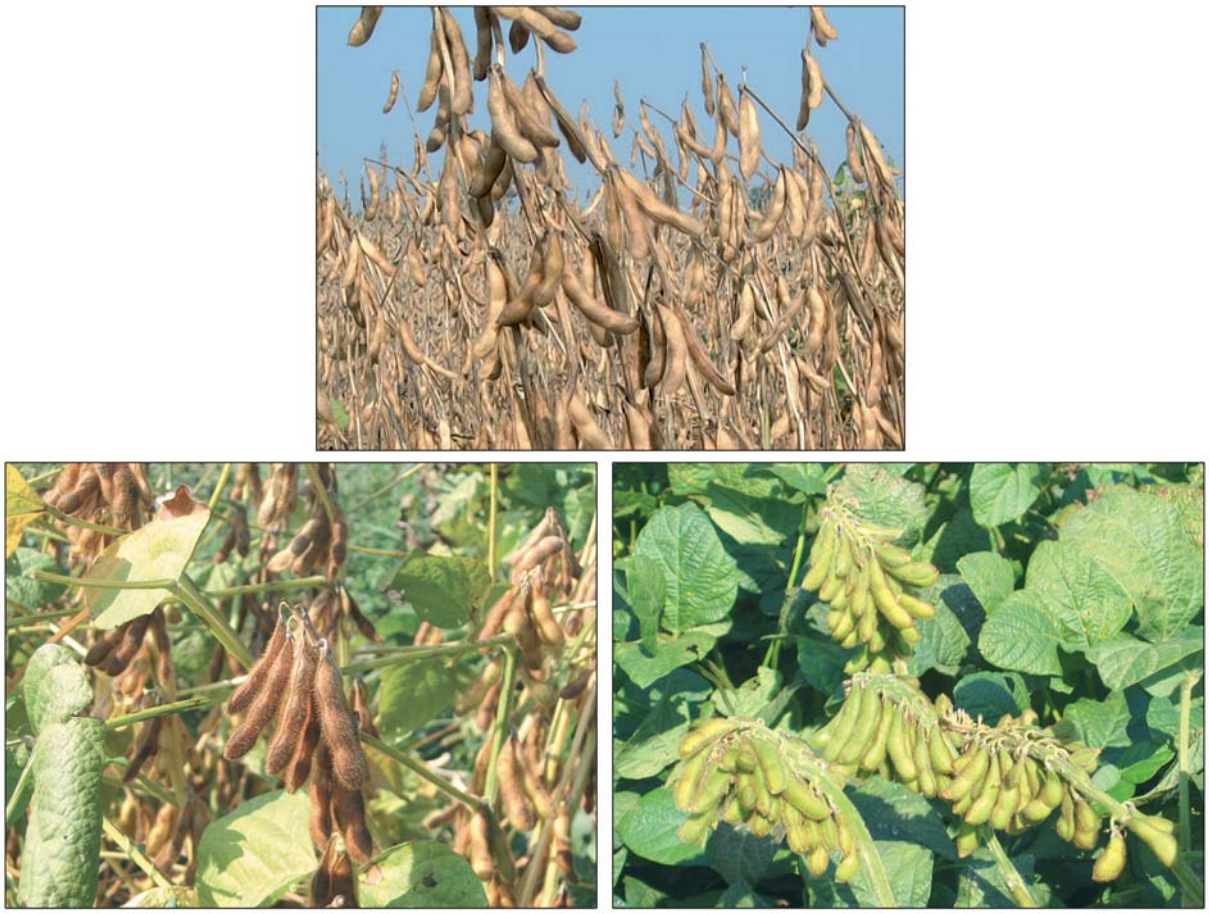
Stem growth and development

Stem development begins with shoot (hypocotyl) emerging above the soil surface. Two cotyledons and a plumule (shoot bud) are observable on the shoot, which grows straight upwards in the erect habit plant. The first stem node carries the cotyledons. Above cotyledons, at the second node, there is a pair of the opposite first unifoliolate leaves.

The third and all other nodes carry typical trifoliolate leaves. In each leaf axil there is a bud which may develop into a branch, a flower or remain as an undeveloped, i.e. dormant bud (Vratarić, 1986). Depending on variety and environmental factors, soybean plant is more or less brached, with common primary branches, while the secondary branching is rare in soybean plants (Dzikowski, 1936). Further stem development is affected by growth type. There are three growth types of soybean plants: indeterminate, determinate and fasciated (Figure 2.3).

Figure. 2.3

Stem growth types (original) *(photo: V. Đorđević)*



Description: indeterminate (top), determinate (left) and fasciated (right)

Varieties of indeterminate (unlimited) growth type have a vegetative cone at stem top. Terminal leaf is often smaller than lower leaves. Until beginning bloom stage they form around 67% of total dry matter. Stem growth and vegetative weight formation prolongs even after beginning bloom stage. Indeterminate varieties are more susceptible to lodging than determinate ones.

Varieties of determinate (limited) growth type finish stem with an inflorescence. After beginning bloom plants cease growing, adversely affecting yield in drought con-

ditions. These varieties form around 80% of vegetative mass until beginning bloom (Lin and Nelson, 1988). Terminal leaf is of the same size as lower leaves. Determinate varieties branch more and are more resistant to lodging than indeterminate ones. Varieties grown here are indeterminate. After formation of the first few leaves, vegetative cone of plants with a fasciated stem divides and forms several merged stems, resulting in two to three leaves at one node. Such plants form a long inflorescence at stem top resulting in a dense pod cluster. As of yet, there are no such commercial varieties developed to display such a stem type.

Leaf

There are three different types of soybean leaves: the first pair of cotyledon (seed) leaves, the second pair of primary (unifoliolate) leaves and true (triofoliate) leaves. The cotyledons are parts of the embryo, attached to the embryonic stem, and they emerge to the soil surface by germination. Round in shape and enveloped in epidermis with stomata, they can be yellow or green in color. Their function is of a food and photosynthesis storage until the plant fully acquires autothrophic nutrition, following which they dry off and drop.

In each cotyledon axis there is a bud from which one unifoliolate (primary) leaf shoots, meaning that each unifoliolate leaf has its node, but these two are for practical reasons regarded as one (Figure 2.4). These leaves are positioned opposite each other at petioles measuring 1 to 2 cm in length, while the leaflet is oval shaped and 2 to 5 cm long.

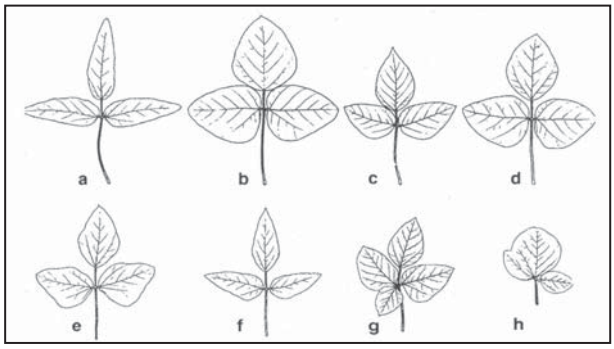
Figure 2.4

The first pair of leaves - prophylli *(photo: G. Mulić)*

All other nodes carry trifoliolate leaves, which are typical for soybean, and alternate up the main stem. Trifoliolate leaves are composed of three leaflets and a petiole. Leaflets may be oval or spear shaped (Figure 2.5). Sometimes it might happen that the true leaf is composed of four, or even seven leaflets (Figure 2.5g), or that lateral leaflets merge with the terminal one (Figure 2.5h). A leaflet is 4 to 20 cm long and 3 to 10 cm wide, light to dark green in color. Leaflet color and size are varietal properties, the same as degree of attachment between petiole and stem. During vegetation period leaves turn yellow and are shed, although there are varieties that do not shed leaves even in maturity.

Figure 2.5

Different leaflet shapes (Dzikowski, 1936, as cited in Lersten and Carlson, 1987)



At the point of petiole attachment to the stem, a pair of opposite lateral bracts or stipules are visible. On these leaf-like outgrowths seven main nerves accompanied with several smaller ones can be observed.

At the petiole base, apart from stipules, a certain thickening might also be observed (*pulvinus*, Dzikowski, 1937, as cited in Lersten and Carlson, 1987). The second smaller thickening is found where leaflets are attached to the petiole. These thickenings, also known as stipels, function as joints since they enable a change of leaflet orientation during day and night due to changes in osmotic pressure.

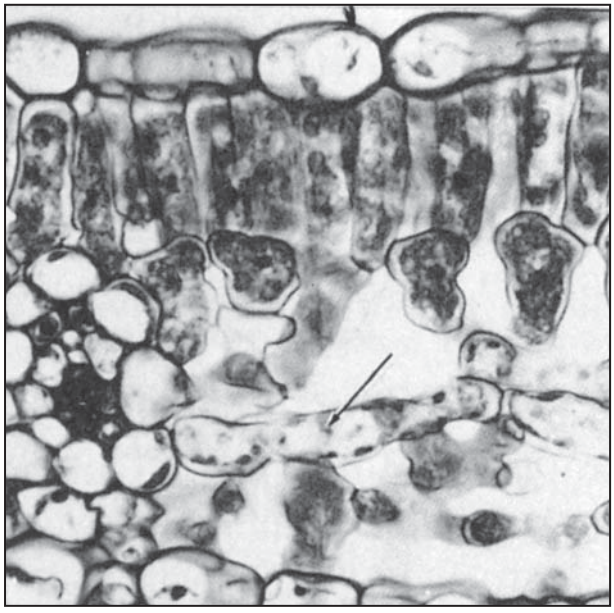
Leaf anatomy

As stated in the stem anatomy paragraph, leaf vascular tissue is made from three separate vascular bundles which are already merged into a larger bundle at basal petiole thickening. As soon as the stem vascular bundles merge, vascular tissue is differentiated into the eustele. Vascular tissues of all leaf parts are created by branching of petiole eustele.

The most important part of the leaf is a leaflet. True soybean leaf is made of three leaflets of identical composition – there are two lateral leaflets and one terminal (Figure 2.6). Leaflet composition is dorsoventral and adapted to its basic functions – photosynthesis and transpiration.

Figure 2.6

Transection of a leaflet (from Lersten and Carlson, 1987)

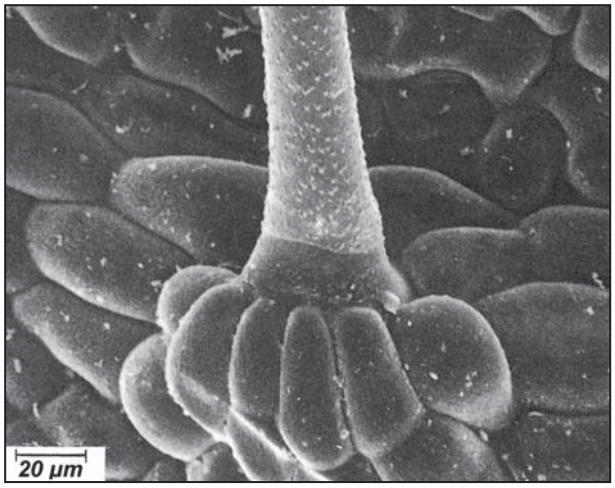


On both leaflet surfaces, epidermal cells are covered with a thin cuticle from which epicuticular wax forms protecting the leaf from heat and excessive water vapor. Continual string of these cells is interrupted by the stomata. Stoma complex comprises a pair of kidney-shaped cells (guard cells) which enclose a pore, the neighbouring epidermis cells (subsidiary cells) and one layer of mesophyll cells. Gas exchange is regulated between the plant and environment through the pore of the stoma complex. Stomata number is affected by light, heat, and moisture, as well as variety. Based on their research on 43 soybean varieties, Ciha and Brun (1975) reached a conclusion on significant differences in stomata number among varieties, as well as the connection between stomata number and drought resistance. The authors found that there is an important difference in stomata number on upper and lower leaf surfaces. Upper side has 81 to 174 stomata per mm^2, and lower 242 to 345 stomata per mm^2.

Typical for soybean leaf are hairs (trichomes), comprising two cells – a short basal one, surrounded by epidermis cells, and a terminal one 0.5 to 1.5 mm long (Figure 2.7).

Figure 2.7

Lower part of a leaf hair (Flores and Espinoza, 1977, as cited in Lersten and Carlson, 1987)



According to the research of Woolley (1964), leaf hair length in the variety Hawkeye is around 1 mm, distanced from each other also 1 mm, and they comprise around 10% of the total leaf area. The same author established that leaf hairs reduce wind blow on the leaf area up to 40%. Plants with denser hairs have physiological and agronomical advantages, since they are less exposed to the pest attacks (Johnson and Hollowell, 1935; Singh et al., 1971; Levin, 1973), water loss through transpiration under high temperatures and drought conditions is reduced (Weigand, 1910; Ghorashy et al., 1971; Ehleringer et al., 1976; Ehleringer and Mooney, 1978) and they have a higher capability of sun radiation reflection as compared to the plants with fewer hairs (Gausman and Cardenas, 1973; Nielsen et al., 1984).

Between upper and lower parts of the epidermis there is the mesophyll, made up of two layers of vertically distributed elongated cells towards the upper leaf surface. It is called the palisade parenchyma because of its characteristical cross section appearance. Palisade parenchyma is also called the assimilation tissue, since these cells are rich in chloroplasts. Lugg and Sinclair (1980) determined that upper, more sun-lit leaves can even form the third layer of assimilation parenchyma cells. Towards the lower leaf surface there are two to three layers of spongy parenchyma cells containing fewer chloroplasts. This mesophyll part contains cells of irregular shape with distinctly wide intercellular spaces, related to a higher stomata number at the lower leaf surface. Such composition of the spongy parenchyma enables uninterrupted gas exchange between the plant and environment.

Leaf blade vascular tissue is situated between the palisade and the spongy parenchyma, while its internal structure is identical to the structure of the stem vascular tissue.

Flower

Soybean flower (Figure 2.8) forms successively at leaf axil on stems and branches from the base upwards.

Figure 2.8

Soybean flower *(photo: G. Mulić)*

The first flowers are often initiated on the fifth, sixth or higher nodes, rarely or never being initiated on lower nodes (Carlson and Lersten, 1987). Flower ranges from 3 to 8 mm in size and white or various shades of purple in color, depending on the presence or absence of anthocyanin, purple being dominant over white. Flowers are borne in the axillary raceme cluster, which mostly comprise 3 to 5 flowers.

Stem in determinate varieties ends in the terminal inflorescence in which up to 35 flowers may be initiated. For varieties of this growth type it is characteristic that growth ceases with beginning of flowering. In indeterminate varieties, however, growth and development are simultaneous – plants keep growing even after flower initiation. These varieties also have the terminal inflorescence, with two to three axillary inflorescences tightly initiated due to short internodes at stem top.

As previously mentioned, soybean plant initiates flowers successively, thus on one plant there could simultaneously be found flower buds, open flowers and pods in seed-filling stage. Affected by variety, planting date and environmental conditions, soybean flowering period in this area lasts from end of May through mid August.

Typical for soybean is a high rate of flower abortion, i.e. more flowers are initiated than pods. This phenomenon is yet to be satisfactorily explained. According to Van Shaik and Probst (1958), the long inflorescence, higher number of flowers and high rate of flower abortion are features inherited quantitatively, with dominant and complementary gene effect, while heritability for flower abortion percentage ranges from 29 to 93%. The same authors concluded that it is difficult to develop a variety with a high flower initiation capacity and a low flower abortion rate, for they found a positive correlation between flower abortion and flower number per node. Pod number per node is an important yield component that affects yield more than seed weight (Heindl and Brun, 1984), which is in agreement with conclusions of Wiebold et al. (1981) who stated that soybean yield can be increased by reducing flower abortion, i.e. by increasing pod number. Probability for flower abortion for flowers found lower on the inflorescence is often less than 10%, while it is over 50% for higher positioned flowers (Wiebold and Panciera, 1990). For this reason it is recommended to breed for lower flower abortion rate, especially of those on inflorescence top, according to Sharma et al. (1990).

Flower anatomy

Soybean has a bisexual, typically papilionaceous flower characteristical for *Papilionoidae*, consisting of tube-like corolla, composed of five uneven separate petals covered in hairs, and a five-pieced calyx. The largest most outward petal more or less envelops the flower and is called vexillum. Two lateral much smaller petals enclosing the pistil from the sides are called alae, while two front petals are merged comprising a naviculum. Flower is zygomorphic, i.e. it can be divided by only a single plane of symmetry.

Inside the calyx there are ten stamina, nine of which are positioned on filaments comprising a whorl around the pistil, while the remaining one is free and positioned below the stigma.

The pistil is monocarpic, with one to four seed embryos. Development of the seed embryo is of *Polygonum* type. The style leans backwards towards the free stamen. Similarly to the corolla, the pistil is covered in hairs, which are lacking from sepals and stamina. At the base, between pistil and stamen there are nectaries.

Stamina compose a whorl around the pistil and consist of a supporting stalk (filament) and an anther which opens and releases the pollen onto the stigma, most often a day before the flower opens, thus greatly reducing the percentage of cross-pollination (< 0.5%). A large number of pollen grains are dropped on the stigma, whence

they germinate into a pollen tube and progress through the style finally reaching the ovary. More than 90% of pollen tubes atrophy even before reaching the ovary, leaving only a small number apt for fertilization (Carlson and Lersten, 1987).

Protruding into the embryonic sac, the tip of the pollen tube bursts and releases its content of two sperm cells. One sperm cell unites with the ovule forming a diploid zygot, the first cell of the future embryo, while the second sperm cell unites with the secondary nucleus of the embryonic sac, forming a triploid nucleus of the endosperm, which in soybean does not develop further. The proembryo is formed as a result of zygote division, from whose cells positioned towards the interior of the embryonic sac embryo is later created. Other proembryonic cells become basal cells (suspensors), which push the embryo into the secondary endosperm, the nutritious tissue formed out of endosperm nucleus and the rest of embryonic sac plasm. With embryo division, the radicle emerges from the end facing the micropyle, while cotyledons emerge from the end facing chalaze, with an apical shoot meristem between them.

Pod

Soybean fruit is the pod, whose number ranges from two to more than twenty in one inflorescence, and up to 400 on a mature soybean plant (Carlson and Lersten, 1987). Owing to a high percentage of soybean flower abortion, this number is ofter much lower.

Pod shape and size vary significantly among varieties, and even among pods on one plant under the influence of environmental factors. Soybean pods can be straight, slightly bended or sickle shaped, ranging 2 cm in length in wild soybean to 7 cm in some cultivated soybean varieties (Figure 2.9). Depending on the seed number, pod length is most often between 4 and 6 cm (Frank and Fehr, 1981).

Figure 2.9

Pod *(photo: G. Mulić)*

Pod color can be light yellow, brown or black, including all shades and transitions among these three colors, which depends on the presence of carotene and xanthophyll, color of hairs, and presence of anthocyanin (Dzikowski, 1936). Darker pod color is dominant over lighter pod color.

There could be one to five seeds in a pod, which depends on varieties and environmental conditions. Varieties grown here there are most often 2 to 3 seeds in a pod (Hrustić, 1984) (Figure 2.10).

Figure 2.10

Pod with mature seeds *(photo: G. Kuzmanović)*

Mature wild soybean pods split and shatter seeds, which is very disadvantageous in commercial varieties from an agronomic standpoint. Modern commercial varieties, developed by soybean breeding, have firm pods that split only under stress (Miladinović et al., 1996).

Pod internal structure

The first pods on the soybean plant are visible around two weeks after initiation of the first flowers. Owing to the successive soybean flowering, on the same plant there could simultaneously be just initiated pods and pods bearing green seed. Pod develops from the ovary, directly following fertilization as the stigma and the style dry and fall off. Pod development is slow at first, becoming faster after flowering is over. Flower corolla is visible at the base of the pod and remains until end of maturity.

As in other plants from this family, pod is made of one carpel which involutes and merges its margins by a ventral seam. The main nerve of the former leaflet resembles dorsal seam. The main part of both seams consists of vascular bundles, created from the former leaf, one being on the dorsal seam, and two on the ventral seam.

The epidermis above the vascular bundles of both seams involutes, forming clearly observable ducts which extend into the layer of parenchyma cells.

In the earlier developmental stages, the pod is covered with a layer of epidermal cells which sporadically form hairs. Below this layer, there is a wide zone of parenchyma tissue embossed with the vascular system and a thin layer of inner parenchyma from which membranous endocarp develops (Carlson and Lersten, 1987). With maturity, the walls of epidermis cells thicken and are externally covered with cuticle. A layer of short fibers forms below the epidermis cells, while vascular tissue connecting main seam bundles is placed inside the following parenchyma layer. Below parenchyma, there is a thin layer of sclerenchyma fibers, responsible for pod shattering (Carlson and Lersten, 1987). The cited authors state that inner sclerenchyma cells, whose fibers are parallel to the vertical cell axis, shorten while drying more than cells of the external sclerenchyma layer, which displays cross orientation of the fibers, causing the pod to twist around the vertical axis and split at seams.

The last layer of flat parenchyma cells is called the endocarp. According to Krul (1978, as cited in Yaklich and Cregan, 1981) this layer regulates seed moisture in mature pods.

Seed

Seed of most commercial soybean varieties is oval shaped, but can include all shapes between round and elongated, almost linear shape. Thousand seed weight ranges from 20 g in wild soybean to over 500 g in some cultivated soybean varieties. Commercial varieties most often have middle-sized seeds of 150 to 190 g thousand seed weight (Hrustić, 1984: Relić, 1996: Miladinović, 1997).

As with most legumes, soybean seed does not include endosperm but is comprised of an embryo enclosed by the seed coat. Mature embryo comprises two large cotyledons, plumules, with two primary leaves enclosing leaf primordia, epicotyl, hypocotyl and radicle.

The part of the seed where it was attached to the pod is called the hilum. Hilum shape, color and size are varietal features. Hilum shape varies from linear to oval, and color may be black, brown, yellow or green, including all shades of these colors, and may or may not differ from the color of seed coat. At one hilum end there is a little channel, chalaza, and at the other end there is micropyle, a small opening between the tips of integuments, through which the radicle emerges. Gas exchange between the embryo and environment occurs mostly through micropyle, due to cutinized walls of outer cell layers in seed coat epidermis. In some varieties, when seed separates from pod, hilum epidermal cell layer lingers on funiculus, causing a white scar along the middle of hilum (Dzikowski, 1936).

Seed coat comprises three parts: epidermis, hipodermis and internal parenchyma. It can be smooth or wrinkled, glossy or matte as affected by variety.

Epidermis is made of a layer of cells shaped like palisade, with walls externally cutinized. In colored seeds, this layer harbors pigments such as anthocyanin in vacuoles, chlorophyll in plastids and different pigment decomposition products (Carlson and Lersten, 1987). Soybean seed can be yellow, green, brown or black, including all shades and transitions among these colors, but it may also be bicolored (Figure 2.11).

Figure 2.11

Soybean seed *(photo: J. Miladinović)*

Tully et al. (1981) established that black seeds are more resistant to low temperatures, which can be explained through reduced water permeability of the pigmented seed. This is in accordance with the results of Dickson (1971) who analysed another legume – common bean (*Phaseolus vulgaris* L.).

Below the epidermis there is the hypodermis, a layer of pillar cells with large intercellular spaces due to uneven thickness of the cell walls.

The tissue of inner parenchyma is composed of 6 to 8 layers of flat thin-walled cells. This tissue is uniform through the whole seed coat, except on hilum which distinguishes three layers - outer, which leans on hypodermis and may contain pigments giving hilum a more intensive color; middle layer composed of thin flat cells and bundles of spiral vessels branching around hilum, and inner layer, mostly typical parenchymal one (Dzikowski, 1936).

Cotyledons make up the largest portion of total soybean seed weight and volume. Each cotyledon is more or less crescent and covered by epidermis. Stomata are present on both sides of the cotyledons.

On the inner, flat side of cotyledon, mesophyll cells are more tightly linked and arranged in two or three palisade layers, whereas on the other side these layers are not visible. Interior of cotyledon consists of spongy parenchyma cells filled with aleurone granules and oil droplets. Calcium oxalate crystals are dispersed through the whole cotyledon inside.

Most soybean genotypes have yellow cotyledons (Williams, 1950), while some can also be green. Together with the different combinations of seed coat pigments, they provide soybean seed with a wide color spectrum.

Plumule is about 2 mm long with two opposite primary leaves, each with a pair of basal stipules. Embryonic stem comprises epicotyl and hypocotyl, made up of epidermis, cortex and pith. It is often around 5 mm long, depending on seed size, and ends in a radicle

Seed chemical composition

Based on dry weight, mature soybean seed regularly contains around 40% proteins, 20% oil, 17% cellulose and hemicellulose, 7% sugar, 5% fiber and around 6% ash (Rubel et al., 1972). The importance of soybean in food and feed production primarily comes from the high contents of seed protein and oil.

Depending on varieties and environmental conditions, seed protein content varies from 30% to 53%, while commercial varieties most often contain 39 % to 42%. Based on the sedimentation constant, reserve proteins of soybean seed are divided into three large groups: 2S (α-conglycinin) comprising mostly protease inhibitors, 7S (β-conglycinin) and 11S (glycinin) (Clarke and Wiseman, 2000).

Soybean proteins contain almost all essential amino acids and are most similar to proteins from animal sources. Amino acids present in soybean seed are lysine (6% to 7%), histidine (3%), arginine (12% to 13%), threonine (4% to 5%), phenylalanine (5%), tryptophan (2%), serine (5% to 6%), glutamine (20%), proline (4% to 5%), glycine (4%), leucine (8%), tyrosine (4%), alanine (5%), valine (4% to 5%), methionine (1%), cysteine (1%), isoleucine (5%), and some 400 free amino acids aside (Leáenko et al., 1987)

Depending on variety and environmental conditions, seed oil content varies from 12% to 24%, and in commercial varieties from 19% to 22%. Soybean oil contains around 10% palmitine (16:0), 3% stearine (18:0), 20% oleine (18:1), 55% linoleic (18:2) and 7% to 8% linolenic (18:3) acid (Swern, 1972). Owing to especially high content of linolenic acid, soybean oil lacks favorable technological features for human consumption as compared to sunflower oil.

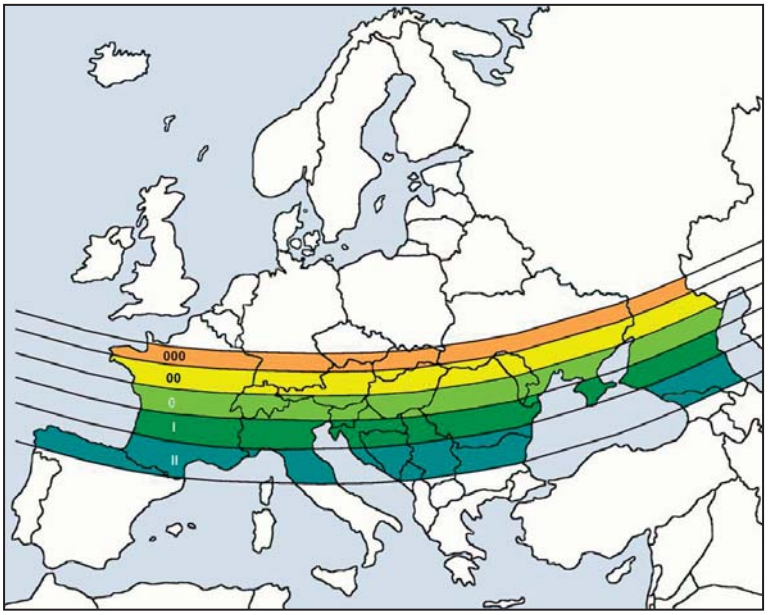
MATURITY GROUPS

Soybean plant is sensitive to photoperiods, meaning that the point when plants enter vegetative and reproductive stages depends directly on the day length. This dependence has caused the division of soybean varieties into 13 maturity groups (Hartwig, 1973). Marks for maturity groups are 000, 00, 0 and Roman numerals from I to X. Soybean varieties designated with 000 are adapted to the conditions of longer days and have a long critical photoperiod, i.e. they are photoperiodically insensitive and are successfully grown at higher latitudes, while varieties marked with X are adapted to the conditions of shorter days and are successfully grown at lower latitudes (Criswell and Hume, 1972). Differences among maturity groups are conditioned by photoperiodical demands of the varieties and if grown at the same latitude, differences in maturity are on average 10 to 18 days. Critical photoperiod progressively decreases from higher to lower latitudes. Requests for photoperiod thus limit the distribution of varieties to a narrow latitudinal belt to which a certain variety is adapted (approximately 200 km, Scott and Aldrich, 1983; Zhang et al., 2007). If a variety is grown at latitude higher than the one it is adapted to, it will flower and mature later or it might not even reach full maturity until the first frost appears. A variety grown at lower latitude in relation to the area it is adapted to will flower earlier, have decreased vegetative weight and mature earlier, consequently causing decreased yield.

For each soybean growing area there is an "optimal" maturity group, meaning that varieties belonging to a previous group are early for that area, and those belonging to a following group are late for that area. In our conditions varieties belonging to maturity group I are the basic varieties, those belonging to maturity group 0 are early, and those belonging to maturity group II are late (Figure 2.12). Under agro-ecological conditions common for our area and optimal planting date in mid April (Rajičić, 1987), vegetation period (from emergence through maturity) for varieties belonging to maturity group I is 120 to 135 days. Varieties belonging to maturity group 0 end their vegetation period in 110 to 120, while varieties belonging to maturity group II take 135 to 145 days for full vegetation period. Due to stress conditions, such as unusually high or low temperatures, pertaining drought, etc., and interaction between variety and environment (Jocković et al., 1994; Miladinović, 1997) vegetation period can be shorter or longer than the stated one.

Figure 2.12

Maturity groups of soybean varieties grown in Europe *(J. Miladinović)*



Phases of growth and development

Soybean development is a continual process which begins with seed emergence, and ends with mature seed and harvest-ripe soybean. Plant development during vegetation period could be divided in two phases – vegetative and reproductive, which could also be subdivided into several stages, or phenophases. Even though few such determinations have been proposed in both Soviet and US research communities, nowadays the determination and alpha-numeral marks proposed by Fehr and Caviness (1977) are widely accepted.

Aim of describing developmental phases

Depending on variety, maturity group, planting date, environmental factors, and applied agronomy, plant development can be decreased or increased. If there were no unique terminology, this would hinder the communication among experts, representatives of agro-industry and a wide range of producers. For example, if a herbicide producer recommends a its application when plants reach developmental stage of 6 leaves, and during application it is not known which leaves should be included into the identification of that particular stage, the herbicide would probably be applied inadequately (Fehr and Caviness, 1977). Stages of soybean development described by Fehr and Caviness can be used for any soybean variety grown at any location, and the descriptions can be used to identify the stage of a single plant or a whole field of soybeans.

Objectiveness and preciseness of the descriptions reduce the risk of different evaluation by persons identifying the stage of a plant to the minimum, which is the main reason why these descriptions are widely accepted in our country.

Separate descriptions have been used for identification of vegetative (V) and reproductive (R) phases of development, so that differences in relations between these two phases with different growth types do not influence the procedure of stage identification.

Vegetative growth

Vegetative development of soybean plants begins when cotyledons appear (i.e. emerge) above soil surface and this stage is designated with a VE, where V stands for vegetative phase, and E for emergence. Soybean development depends on temperature, day length, variety and other factors, meaning that there could be significant differences in days needed for the plant to reach next phase. The main factor influencing vegetative development is temperature. Low temperatures decrease seed germination and leaf development, while high temperatures increase them. Thus, depending on the temeprature, days from planting through emergence (VE) could vary from 5 to 15.

Soon after emergence (3 to 10 days), the first pair of unifoliolate leaves appear above cotyledons. When unifoliolate leaves unroll (two edges of each leaflet are not touching), the plant is in VC (cotyledons) stage.

For further determination of vegetative stages, nodes with fully-developed leaves are taken into account. A leaf is considered fully developed (nodes are counted) when leaf at the first upper node is unrolled sufficiently such that the two edges of each leaflet are no longer touching. The first node counted is the one of the unifoliolate leaves. When first trifoliolate leaves unroll, formed at the node above unifoliolate leaves, the plant enters stage V1. It may be 3 to 10 days between stages VC and V1 depending on the environmental conditions. Further vegetative stages are designated with a combination of the letter V and a number (1, 2, 3, to n) denoting number of nodes with fully developed leaves. It usually takes 3 to 8 days for the plant to reach the next stage, from V2 onwards.

Varieties grown here have an indeterminate stem growth type, which means that vegetative stage of development lasts until the end of vegetation period. When the first flower appears, vegetative and reproductive developments of the plant overlap. Depending on maturity groups and environmental conditions, the first flower appears at stages V4 to V6.

It should be pointed out that only nodes on the stem are counted, disregarding those on the branches

Reproductive development

Reproductive stages, designated with the letter R (reproductive) and a numeral, encompass flowering, pod and seed development, and plant maturation.

As with vegetative stages, determination of reproductive stages considers only the stem, for if it is broken or otherwise damaged, reproductive development on the newly-formed branches will be late, which is why branches are not taken into account. The same factors as with vegetative stages influence number of days needed for the plant to reach the next reproductive stage. High temperatures and short days enhance reproductive development, while low temperatures and long days reduce it.

One open flower at any stem node marks the beginning bloom stage, which is designated with R1. As a rule of thumb, R1 and R2 appear simultaneously in determinate types, while period between R1 and R2 in indeterminate types takes 3 days. Full bloom stage (R2) means one open flower at one of upper two nodes on the main stem with fully developed leaves. Full bloom may last from 5 to 15 days.

Plant reaches stage R3 (beginning pod) when the pod is 5 mm long at one of four uppermost nodes on the main stem with fully developed leaves. As the previous stage, this stage can also last from 5 to 15 days.

Pod 2 cm long at one of four nodes with fully developed leaves marks the full pod stage R4. Depending on weather conditions and maturity group, this stage may last for 4 to 16 days.

Beginning seed stage R5 means that 3 mm long seed forms inside pod at one of uppermost nodes with fully developed leaves. When pod at one of these nodes contains green seed which fully fills pod cavity, the plant enters the stage R6. The period of these two developmental stages is mostly affected by moisture available to the plant. Plant remains in stage R5 for 7 to 21 days, and in stage R6 from 9 to 30 days.

When one normal pod reaches mature color, the plant enters beginning maturity stage R7 lasting often 7 to 18 days.

When 95% of pods are mature color, the plant has reached full maturity stage R8. Seeds contain 15% moisture and it takes few more days of dry weather before harvest-ripe fit for combining.

Descriptions of vegetative and reproductive stages represent development of the individual plants. Average stage of the crop is the one in which are 50% of the plants in a field.

SUMMARY

Soybean is an erect annual plant with a hairy stem reaching 30 cm to 130 cm in height depending on environmental factors. Soybean root system is diffuse, with a taproot usually indistinguishable from the lateral roots. Root system is characterised by root nodules whose creation results from a symbiotic relationship between the soybean plant and nitrogen–fixing Bradyrhizobium bacteria. Trifoliolate leaves are typical for soybean, while the flower is bisexual and typically papilionaceus, purple or white in color. Pod contains one to five seeds as affected by the environmental factors. The most important components of soybean seed, proteins (about 40%) and oil (about 20%) are the main reason for soybean cultivation. Due to photoperiod sensitivity, soybean varieties are divided into 13 maturity groups, from 000 for varieties grown at higher latitudes, to X for varieties grown at lower latitudes. Plant development could be divided into two phases – vegetative (V) and reproductive (R), which are in turn further subdivided into more phenophases, designated with numbers.

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SOYBEAN GENETICS

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Soybean genetics encompasses all aspects of genetics within the genus Glycine. Recent reviews of soybean genetics include Speciation and cytogenetics (Hymowitz, 2004), Qualitative genetics (Palmer et al., 2004), Soybean genomics (Shoemaker et al., 2004), Genetic improvement: Conventional and molecular-based strategies (Orf et al., 2004), and Soybean: genetics and breeding (Palmer and Hymowitz, 2004). This chapter will summarize existing information and highlight new information on soybean genetics, from a historical perspective, with an emphasis on data published since the first edition (1998) of this book.

Germplasm

Major Glycine germplasm collections exist in Australia, Brazil, China, Germany, India, Indonesia, Japan, Russia, Republic of Korea, Taiwan, Ukraine, and the United States. Smaller, but important collections exist throughout Asia and Europe. Glycine max collections are given in Table 3.1, the annual G. soja collections (Table 3.2), and the Glycine perennial collections (Table 3.3).

Within the genus Glycine subgenus Glycine, there are 24 recognized wild perennial species, and within the subgenus Soja are two annual species (Table 3.4). The perennial species are diverse morphologically, cytologically, and genomically, and most are endemic to Australia (Table 3.4).

At present world collections contain more than 165,000 G. max accessions, more than 10,000 G. soja accessions, and more than 4,000 perennial Glycine accessions.

The United States Department of Agriculture Germplasm Collection (Curator, Dr. R. L. Nelson, USDA ARS, National Soybean Research Laboratory, Urbana, Illinois 61801 USA) contains more than 17,000 G. max accessions (Table 3.5, number of accessions by maturity group; Table 6, number of accessions by origin), more than 1,100 G. soja accessions, and more than 900 perennial Glycine accessions. All annual accessions in the USDA Soybean Germplasm Collection are assigned to a maturity group which provides a general indication of the area of adaptation. There are 13 designations: 000, 00, 0, and Roman numerals I to X (Table 3.5). Maturity Group 000 is adapted to very high latitudes, generally greater than 50°, while Maturity Group X is adapted to very low latitudes, generally less than 10°.

The USDA Germplasm Genetic Collection consists of the Type Collection, the Isoline Collection, and the Crop Science Society of America registered germplasm releases. The purpose of the Type Collection is to preserve mutations and variants not available in any other germplasm accession. The most common phenotypic classes are represented by reaction to pests, chlorophyll mutations, sterility mutations, seed pigment variants, leaflet form variants, and seed fatty acid mutations. The Isoline Collection contains more than 500 near-isogenic lines developed from 11 recurrent parents and involves more than 80 genes. The most common recurrent parents are cultivars Clark, Harosoy, and Williams. Recently the Genetic Collection has preserved all germplasm releases registered in Crop Science. The Type Collection and the Isoline Collection are included in the Soybean Monograph Qualitative Genetics chapter (Palmer et al., 2004) and on an irregular basis in the Soybean Genetics Newsletter which was established in 1974 as a means of communication at the international level on topics related to genetics and breeding of soybean and immediate relatives. You can contact the editor at soygenetics@missouri.edu

The Soybean Genetics Committee, established in 1955, carries out the following functions: (i) maintains a Genetic Type Collection; (ii) establishes guidelines and rules for assigning gene symbols and designations for molecular probe detected loci; and (iii) acts as a review committee for manuscripts concerning qualitative genetic interpretation and gene symbols in the genus Glycine. For additional information, use http://www.soygenetics.org/rules.htm.

Qualitative Genetics

A comprehensive tabulation of qualitative genetic traits with gene symbols, phenotypic description, source of mutant, and references was presented in the Soybean Monograph Qualitative Genetics chapter (Palmer et al., 2004). A list of gene symbols, with phenotype and references that have been approved by the Soybean Genetics Committee, is presented in Tables 7 to 18 in this chapter. These data illustrate that since 1998, emphasis in soybean genetics has been on disease resistance, seed components (especially fatty acids), and isoenzymes and proteins.

Loci affecting seed pigmentation are of great interest to seed producers, breeders, and in seed technology. Five qualitative trait loci have major effects on seed pigmentation. A summary of the genes controlling hilum color and seed coat color is presented (Table 3.19). A diagram outlining the influence of these loci on seed pigmentation is presented (Table 3.20).

Traditional (Classical) Linkage Map

Soybean is expected to have 20 linkage groups (2n = 2x = 40 chromosomes); 20 linkage groups have been identified. Several linkage groups have only two loci, and two linkage groups have nine loci. Because many of the linkage groups have only two or three loci, two or more linkage groups may in fact be the same linkage group. Studies with primary trisomics are being used to assign linkage groups to their respective chromosomes. Inversions have not been used in linkage studies in soybean to date. Chromosome interchanges (translocations) have been used to determine gene order for mutants of linkage groups 6 and 8 (Mahama and Palmer, 2003). The traditional (classical) genetic map is given in Figure 3.1. The assignment of Molecular Linkage Groups to chromosome is given in Table 3.21.

The Soybean Genome

The genome of the soybean was estimated to contain approximately 1.29 x $l0^9$ bp (Gurley et al., 1979) to 1.81 x $l0^9$ bp (Goldberg, 1978) for 1 n DNA content, with approximately 40 60% repetitive sequences (Gurley et al., 1979; Goldberg, 1978). Renaturation kinetic studies indicate that 65 to 70% of single-copy sequences have a short period interspersion with single-copy sequences of 1.1 1.4 kb alternating with repetitive sequence elements of 0.3 0.4 kb (Gurley et al., 1979). Cytogenetic analysis of pachytene chromosomes has shown that over 35% of the soybean genome is made up of heterochromatin. The short arms of six of the 20 bivalents are completely heterochromatic (Singh and Hymowitz, 1988). A high percentage of soybean DNA, as is true for most plants, is modified by methylation of the cytosine base (Adams and Burdon, 1985).

Recent Fluorescent In Situ Hybridization (FISH) analyses have provided more information about the makeup of the genome. Consistent with early renaturation kinetic studies, it was shown that euchromatin and heterochromatin are relatively well-partitioned from each other (Lin et al., 2005). The relative distribution of heterochromatin to euchromatin is a very important issue in genomic studies because it defines 'gene space'. Mudge et al. (2004) used the Poisson distribution of BACs identified with RFLP probes, from a pool of more than 100,000 BAC clones, to estimate that soybean genes may be restricted to as little as 24% of the genome.

This was possible to estimate because the RFLP probes used in construction of the first molecular genetic map of soybean were generated using methylation-sensitive enzymes, and thus, probably represented genes (Keim et al., 1988).

With the completion of the soybean genome, we have confirmation of many of the earlier predictions. The genome is now predicted to contain 1.115 x $l0^9$ bp, of which 57% is heterochromatic. The genomic sequence also revealed 46,430 genes based on full-length complementary DNAs, expressed sequence tags, homology, and ab initio methods (Schmutz et al., 2010). The soybean genome can be accessed at the Soybase website (Grant et al., 2010, http://soybase.org/gbrowse/cgi-bin/gbrowse/gmax1.01/). In the near future, the utilization of next generation sequencing technologies will result in new methods for identifying methylated sequence on the genomic level that will improve our limited understanding of the soybean methylome (Cokus et al., 2008).

Comparative Mapping

An early legume comparative map study was performed by Boutin et al. (1995). This study compared RFLP maps of mungbean (Vigna radiata, 2n=22), common bean (Phaseolus vulgaris, 2n=22), and soybean (Glycine max, 2n=40). Mungbean and common bean genomes were composed primarily of conserved linkage blocks. When comparing soybean to mungbean and common bean, however, only short and dispersed linkage blocks were conserved (Boutin et al., 1995). Grant et al. (2000) showed that some synteny could be detected between soybean and the model plant Arabidopsis, athough synteny was limited between these two evolutionarily distant species. However, using Arabidopsis as a bridging species, it was revealed that homoeologous segments of soybean chromosomes showed a higher degree of synteny with chromosomes of common bean and mungbean than previously thought (Lee et al., 2001).

The ongoing genome sequencing of Medicago truncatula, a cool season legume, has generated a number of comparative mapping papers in legumes. An in depth analysis of macrosynteny in legumes was conducted using a common set of putatively orthologous markers. These markers were mapped in M. truncatula, M. sativa, Pisum sativum, and Vigna radiata (Choi et al., 2004a; 2004b). Sixty additional molecular markers based on homology to mapped RFLP markers of Glycine max were then mapped in M. truncatula. As expected, the degree of synteny was correlated with phylogenetic distance between species (Choi et al., 2004a). Twenty-three of the 60 mapped markers identified 11 syntenic blocks between M. truncatula and soybean. Synteny seemed to be limited only to small genetic intervals. It was proposed that duplication (polyploidization) followed by gene loss and segmental reshuffling (diploidization) made it difficult to identify lengthy stretches of syntenic chromosome segments between soybean and related legumes (Zhu et al., 2004).

A moderate level of microsynteny between Medicago and Glycine also has been identified. Using hybridizations with BAC clones, about 54% of soybean contigs were shown to possess some level of microsynteny with M. truncatula (Yan et al., 2003). Analysis of regions around supposedly orthologous apyrase genes between Medicago and Glycine indicated at least 6 genes in common over 70 kb (Cannon et al., 2003). Similarly, surrounding the rgh1 locus of soybean and the putatively orthologous region of Medicago, a total of 14 out of 29 genes identified in either Medicago or soybean were in common between the two genomes (Choi et al., 2004b). Surprisingly, some regions appear to be hypersyntenic, between Glycine and Arabidopsis with large numbers of genes in common, over extensive regions (Mudge et al., 2005). A more recent study explores the fractionation of synteny due to gene loss/addition between tandemly duplicated N-hydroxycinnamoyl/benzoyl transferase genes in Glycine, Medicago, and Arabidopsis (Schlueter et al., 2008). However, most of these types of microsynteny analyses have been based on comparisons of a limited number of specific regions. Therefore, it is difficult to draw global level conclusions because genome microstructure is highly dynamic and the level of conservation varies with different parts of a genome.

Genome Duplication and Gene Space

Polyploidization results in a duplication of the entire genome, but duplications of only a chromosomal region or segment can occur as well. Both polyploidization and regional or segmental duplications result in 'paralogous' regions of chromosomes (regions of chromosomes that, after having been duplicated, contain homoeologous loci). Discovery and analysis of paralogous genomic regions in a diploidizing tetraploid can provide a wealth of information about the sequence divergence and structural rearrangements associated with the evolutionary dynamics of a tetraploid genome.

Genetic evidence (Buttery and Buzzell, 1975; Palmer and Kilen, 1987), evolutionary studies, and haploid genome analysis suggest that soybean is an ancient tetraploid (Hadley and Hymowitz, 1973). This hypothesis is supported by the presence of many examples of duplicated qualitative genes (Palmer and Kilen, 1987) and the finding that >90% of random genomic fragments used as probes in the construction of soybean RFLP maps detect two or more fragments (Shoemaker et al., 1996).

Previous studies have shown that within soybean multi gene families (actins, leghemoglobins, and seed storage proteins), pairs of more closely related genes can be identified (Grandbastien et al., 1986; Lee and Verma, 1984; Nielsen et al., 1989). Some members of gene pairs exhibited significant conservation of flanking sequences (Grandbastien et al., 1986; Lee and Verma, 1984; Nielsen et al., 1989). Unpublished sequencing data from BAC-sized paralogous regions show that sequence conservation often drops off precipitously outside of the genic coding region (Schlueter, Shoffler and Shoemaker, unpublished).

Associations of RFLP fragments to mapped loci, and band counting experiments by using multiple enzymes have suggested that more than 90% of nonrepetitive soybean sequences may be present in two or more copies (Shoemaker et al., 1996). The observation that approximately 60% of RFLP probes detect three or more fragments, and therefore, probably three or more loci in soybean, suggests that this higher level of duplication is not simply due to greater conservation of duplicated loci from ancestral genomes, but that much of the genome has undergone genome duplications in addition to the original tetraploidization event.

Early RFLP mapping analysis of the soybean genome detected collinear duplicated loci and suggested that duplicated and paralogous regions of the genome were being detected (Keim et al., 1992). But, at that time, only one region of extensive paralogy was detected and it was by only a few duplicate loci. Putative homoeologous chromosomal regions have now been clearly identified by genetic mapping (Shoemaker et al., 1996; Lee et al., 1999; Lee et al., 2001). Recently, Fluorescent In Situ Hybridization (FISH) analyses permitted visualization of genome duplications at the chromosomal level. Pagel et al. (2004) used BAC clones that were genetically anchored to the ends of molecular linkage group E to identify corresponding duplicated regions on two different chromosomes.

Many linkage groups contained duplicate loci from many other linkage groups. These results suggested that the soybean genome may have undergone intense rearrangement and reorganization following tetraploidization and may not retain significant collinear paralogous regions as seen in other tetraploids. Thanks to the recent genome sequencing of soybean, we can estimate that 30,000 of the 46,430 genes exist as paralogues. The sequence also suggests that 61.4% of the homologous genes are in syntenic blocks on only two chromosomes and 21.53% are in syntenic blocks of four chromosomes. The remaining homologous genes vary in the number of chromosomes in which the syntenic blocks span due to genome fractionization (Schmutz et al., 2010).

When did the Soybean Genome Duplication(s) Occur?

That the soybean genome contains numerous regions that have undergone some sort of duplication event is now well established. It is possible to crudely estimate the relative times at which genes duplicated. Duplicated genes accumulate synonymous substitutions (silent) in a roughly clocklike fashion, and this can be measured by the Ks value. The Ks values provide an estimate of relative times of duplication. A large-scale duplication results in the presence of large number of paralogous gene pairs showing similar levels of divergence from one another (similar Ks values). This will show up as a peak against the background of the birth/death curve for simple duplications. EST collections provide the large number of genes needed to identify such peaks.

Recently, two studies of ESTs using slightly different methods identified such Ks peaks in diverse plant species, including soybean and M. truncatula.

As expected, soybean possessed two peaks whose median Ks values corresponded to ages of approximately 14 and 44 million years ago (MYA) Schlueter et al. (2004). Blanc and Wolfe (2004) used a different calibration, suggesting younger ages. The Schlueter et al. (2004) dates agree better with divergence dates for legumes. Medicago also possessed two Ks peaks, a dispersed early peak suggestive of an accumulation of small regional duplications and a more ancient peak at ~58 MYA. The disparity in the timing of the older duplication (44 MYA vs. 58 MYA) raises the question of whether the two clades underwent distinct duplications independent of each other, or whether a single duplication event occurred. Phylogenetic tree topologies for 39 Glycine genes for which three or four copies exist supported the hypothesis that Glycine and Medicago underwent a single large-scale duplication event in their history, prior to divergence (Pfeil et al., 2005). Interestingly, this means that the rate of synonymous substitution in Medicago may be ~25 - 30% greater than in soybean.

Zhu et al. (1994) estimated that about one out of four duplicated genes has been lost since the last genome duplication event in soybean. An analysis of ESTs from the cultivar Williams 82 indicated that on average, each gene family comprised 3.1 copies; fewer than expected if all copies from two rounds of whole genome duplication were retained and expressed (Shoemaker et al., unpublished). Thus about 25% of soybean gene duplicates have been silenced or lost.

In the past couple of years we have witnessed logarithmic advances in sequencing technologies (Mortazavi et al., 2008; Ansorge et al., 2009). The organization of the soybean genome, once thought to be so complex as to defy analysis, is now beginning to be resolved into an interesting genome to study. RNA-Sequencing (RNA-Seq) and related next generation sequencing methodologies that have emerged from these advances are providing insights into soybean gene expression (Severin et al., 2010), evolution, and development. In combination with the sequence of the soybean genome, we are entering a new era in soybean genomics and plant improvement.

Table 3.1

The major *Glycine max* germplasm collections

Institution	Country	No. of accessions
Institute of Crop Germplasm Resources, CAASa	China	23,578
USDA Soybean Germplasm Collection	USA	18,405
Soybean Research Institute, Nanjing Agricultural Univ.	China	13,000
Asian Vegetable Research and Development Centre (AVRDC)	Taiwan	12,508
Department of Genetic Resources I, National Institute of Agrobiological Resources	Japan	8630
Institute of Agroecology and Biotechnology	Ukraine	7000
N.I. Vavilov Research Institute of Plant Industry	Russia	6126
Centro Nacional de Pesquisa de Recursos Genéticos e Biotec. (CENARGEN)	Brazil	4693
Soybean Research Institute, Jilin Academy of Agricultural Science	China	4200
All India Coordinated Research Project on Soybean, G. B. Pant. University	India	4015
Centro Nacional de Pesquisa de Soja (CNPSO) EMBRAPAb	Brazil	4000
Genetic Resources Management Section, NIAR (MAFF)	Japan	3741
Crop Experiment Stn. Upland Crops Research Div.	Korea, Rep.	3678
Australian Tropical Crops Genetic Res. Centre	Australia	3144
Genebank, Institute for Plant Genetics and Crop Plant Res. (IPK)	Germany	3063
Regional Station National Bureau of Plant Genetic Resources (NBPGR)	India	2808
Taiwan Agricultural Research Institute (TARI)	Taiwan	2699
National Research Centre for Soybean	India	2500
Crop Breeding Institute DR and SSc	Zimbabwe	2236
Sukamandi Research Institute for Food Crops (SURIF)	Indonesia	2194
Instituto Agronômico de Campinas (I.A.C.)	Brazil	2000
International Institute of Tropical Agriculture	Nigeria	1812
National Plant Genetic Resources Lab. IPB/UPLBd	Philippines	1764
CSIRO, Division of Tropical Crops and Pasturese	Australia	1600
Genetic Resources Department-Research Institute for Cereals and Ind. Crops	Romania	1600
G.I.E. Amelioration Fourragere	France	1582
Soybean Research Institute, Heilongjiang Academy of Agricultural Science	China	1558
Institute of Oil Crops Research, CAASa	China	1529
Institute of Plant Breeding, College of Agriculture, UPlBf	Philippines	1508
Instituto Nacional de Investigaciones Agricolas, Station de Iguala	Mexico	1500
Stat. De Genetique et Amelioration des Plantes INRA C.R. Montpellierg	France	1404
Kariwano Lab., Tohoku National Agricultural Experiment Station	Japan	1400
Hokkaido Agricultural Experiment Station	Japan	1383
Centro de Investigación La Selva (CORPOICA)	Columbia	1219
Institute of Crop Breeding and Cultivation, CAASa	China	1200
Institute of Field and Vegetable Crops	Serbia	1200
Institute of Industrial Crops, Jiangsu Academy of Agric. Sci.	China	1199
Corporacion Colombian de Investigacion Agropecuaria (CORPOICA)	Colombia	1170
Genebank Cereal and Oil Crops Inst., Hebei Academy of Agricultural Science	China	1154
Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias *INIFAP)	Mexico	1124
Maharashtra Association for the Cultivation of Science	India	1081
Research Institute for Cereals and Technical Plants Fundulea	Romania	1024
Total		165.397

[a] CAAS = Chinese Academy of Agricultural Science.

[b] EMBRAPA = Empresa Brasileira de Pesquisa Agropecuaria.

[c] DR and SS = Department of Research and Specialist Services.

[d] IPB/UPLB = Institute of Plant Breeding/University of the Philippines at Los Baños.

[e] CSIRO = Commonwealth Scientific and Industrial Research Organization.

[f] UPLB = University of the Philippines at Los Baños.

[g] INRA = Institut National de la Recherche Agronomique.

The data in this table were gathered from the database maintained by the International Plant Genetic Resources Institute (http://www.ipgri.org/) (verified 16 Dec. 2005). Some numbers were updated via direct contacts with the holding institutions. Number of accessions is reported by country and in some cases there may be more than one collection per country.

Table 3.2

The major *Glycine soja* germplasm collections

Institution	Country	No. of accessions
Institute of Crop Germplasm Resources, CAASa	China	6172
USDA Soybean Germplasm Collection	United States	1118
Soybean Research Institute, Nanjing Agricultural Univ.	China	1000
Soybean Research Institute, Jilin Academy of Agric. Sci.	China	600
Soybean Research Institute, Heilongjiang Academy of Agricultural Science	China	400
Crop Experiment Station Upland Crops Research Division	Korea, Rep.	342
Asian Vegetable Research and Development Centre (AVRDC)	Taiwan	339
N.I. Vavilov Research Institute of Plant Industry	Russia	310
Breeding Laboratory Facility of Agriculture, Iwate University	Japan	151
CSIRO, Division of Tropical Crops and Pasturesb	Australia	60
Taiwan Agricultural Research Institute (TARI)	Taiwan	46
Hunan Academy of Agriculture Science	China	45
Tieling District Agricultural Research Institute	China	29
Department of Agronomy, National Chung Hsing University	Taiwan	20
Eastern Cereal and Oilseed Research Centre, Saskatoon Research Centre	Canada	18
Soybean Breeding Laboratory, Tokac. Agricultural Experiment Station	Japan	15
Australian Tropical Crops & Forages Genetic Resources Centre	Australia	10
Grassland Research Institute, Chinese Academy of Agric. Science	China	10
Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP)	Mexico	9
All India Coordinated Res. Project on Soybean G. B. Pant. University	India	7
Maharashtra Association for the Cultivation of Science	India	6
Sukamandi Research Institute for Food Crops (SURIF)	Indonesia	4
Research Institute for Food Crops Biotechnology (RIFCB)	Indonesia	4
Kariwano Laboratory Tohoku National Agricultural Experiment Station	Japan	3
Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)	Costa Rica	3
Genebank Institute for Plant Genetics and Crop Plant Research (IPK)	Germany	2
S.K. University of Agricultural and Technology	India	1
Total		10.724

[a] CAAS = Chinese Academy of Agricultural Science.

[b] CSIRO = Commonwealth Scientific and Industrial Research Organization.

The data in this table were gathered from the database maintained by the International Plant Genetic Resources Institute (http://www.ipgri.org/) (verified 16 Dec. 2005). Some numbers were updated via direct contacts with the holding institutions. Number of accessions is reported by country and in some cases there may be more than one collection per country.

Table 3.3

The major perennial *Glycine* collections

	Country							
Species	Australia	USA	South Africa	Taiwan	Russia	Japan	United Kingdom	Total
G. albicans	5							5
G. aphyonota	1							1
G. arenaria	6	3						9
G. argyrea	17	14						31
G. canescens	298	123	1	2	3		1	428
G. clandestina	541	130	7	3	6	5		692
G. curvata	9	7			1			17
G. cyrtoloba	53	46			1			100
G. falcata	72	28		2		1		103
G. hirticaulis	12							12
G. lactovirens	10							10
G. latifolia	148	42	21		2			213
G. latrobeana	30	12			1			43
G. microphylla	212	20	50					282
G. peratosa	1							1
G. pindanica	5	2						7
G. pullenii	4							4
G. rubiginosa	53							53
G. stenophita	42							42
G. tabacina	536	240	111	4	13	15		919
G. tomentella	634	297	113	5	4	6		1059
Glycine spp.	27	2	1	53				83
Total	2716	966	304	69	31	27	1	4114

The data in this table were gathered from the database maintained by the International Plant Genetic Resources Institute (http://www.ipgi.org/) (verified 16 Dec. 2005). Some numbers were updated via direct contacts with the holding institutions. Number of accessions is reported by country.

Table 3.4

The genus *Glycine*, 3-letter code, 2n number, genone, and distribution[a,b]

	Subgenus Glycine	Code	2n	Genome	Geographic distribution
1.	G. albicans Tind. and Craven	ALB	40	I	Australia
2.	G. aphyonota B.E. Pfeil	APH	40	?	Australia
3.	G. arenaria Tind.	ARE	40	H	Australia
4.	G. argyrea Tind.	ARG	40	A	Australia
5.	G. canescens F.J. Herman	CAN	40	A	Australia
6.	G. clandestina Wendl.	CLA	40	A	Australia
7.	G. curvata Tind.	CUR	40	C	Australia
8.	G. cyrtoloba Tind.	CYR	40	C	Australia
9.	G. falcata Benth.	FAL	40	F	Australia
10.	G. gracei B.E. Pfeil and Craven	GRA	40	A	Australia
11.	G. hirticaulis Tind. and Craven	HIR	40	H	Australia
			80	?	Australia
12.	G. lactovirens Tind. and Craven	LAC	40	I	Australia
13.	G. latifolia (Benth.) Newell and Hymowitz	LAT	40	B	Australia
14.	G. latrobeana (Meissn.) Benth.	LTR	40	A	Australia
15.	G. microphylla (Benth.) Tind.	MIC	40	B	Australia
16	G. montis-douglas B.E. Pfeil and Craven	MON	40	?	Australia
17.	G. peratosa B.E. Pfeil and Tind.	PER	40	A	Australia
18.	G. pindanica Tind. and Craven	PIN	40	H	Australia
19.	G. pullenii B.E Pfeil, Tind. and Craven	PUL	?	?	Australia
20.	G. rubiginosa Tind. and B.E. Pfeil	RUB	40	A	Australia
21.	G. stenophita B.E. Pfeil and Tind.	STE	40	B	Australia
22.	G. syndetika B.E. Pfeil and Craven	SYN	40	A	Australia
23.	G. tabacina (Labill.)Benth.	TAB	40	B	Australia
			80	Complex	Australia, W.C. and S. Pacific Islands
24.	G. tomentella Hayata	TOM	38	E	Australia
			40	D	Australia, PNG
			78	Complex	Australia, PNG
			80	Complex	Australia, PNG, Indonesia, Philippines, Taiwan
	Subgenus Soja (Moench) F.J. Herman				
25.	G. soja Sieb. and Zucc.	SOJ	40	G	China, Japan, Korea, Russia, Taiwan (wild soybean)
26.	G. max (L.) Merr.	MAX	40	G	Cultigen (soybean)

[a] Adapted from Hymowitz (2004) and Pfeil et al. (2006).

[b] Two species designations are questioned:

1. G. dolichocarpa Tateishi and Ohashi (DOL). This described species (2n=80), found on Taiwan, appears to be a member of the G. tomentella complex.

2. G. pescadrensis Hayata (PES). This described species (2n=80), found in Australia, Japan, and Taiwan, appears to be a member of the G. tabacina complex (lacking adventitious roots).

Table 3.5

Number of accessions of *Glycine max* in the USDA Soybean Germplasm Collection by maturity group

Maturity group	Number of accessions
0	1072
00	477
000	135
I	1585
II	1776
III	1672
IV	3837
V	2435
VI	1459
VII	888
VIII	936
IX	747
X	109
Total	17,168*

* Includes 40 accessions that have not yet been assigned a maturity group.

The data in this table were received 12 February 2010 from Dr. R. L. Nelson, USDA ARS, Urbana, IL, USA

Table 3.6

Country (or region) and number of accessions of *Glycine max* in the USDA Soybean Germplasm Collection.

Country or region	Number of accessions
China	6288
Japan	2981
Korea	3621
Russia	693
Other Asian countries	1819
Europe	1109
Africa	166

Americas	324
Australia	10
Unknown	148
Total	17,168*

* Includes 40 accessions that have not yet been assigned a maturity group.

The data in this table were received 12 February 2010 from Dr. R. L. Nelson, USDA ARS, Urbana, IL USA.

Table 3.7

Genes affecting pest reaction in soybean

Gene	Phenotypea	Reference
	1 Alfalfa mosaic virus	
Rav1	Resistant	Kopisch-Obuch et al. (2008)
rav1	Susceptible	
	2. Bacterial blight	
Rpg1	Resistant, race 1	Mukherjee et al. (1966)
rpg1	Susceptible, race 1	
Rpg2	Resistant, race 4 avrA	Keen and Buzzell (1991)
rpg2	Susceptible, race 4 avrA	
Rpg3	Resistant, race 4 avrC	Keen and Buzzell (1991)
rpg3	Susceptible, race 4 avrC	
Rpg4	Resistant, race 4 avrD	Keen and Buzzell (1991)
rpg4	Susceptible, race 4 avrD	
	3. Bacterial pustule	
Rxp	Susceptible	Hartwig and Lehman (1951), Feaster (1951), Bernard and Weiss (1973)
rxp	Resistant	
	4. Brown stem rot	
Rbs1	Resistant	Hanson et al. (1988)
rbs1	Susceptible	
Rbs2	Resistant	Hanson et al. (1988)
rbs2	Susceptible	
Rbs3	Resistant	Willmot and Nickell (1989); Missaoui et al. (2007)
rbs3	Susceptible	
	5. Frogeye leaf spot	
Rcs1	Resistant, race 1	Athow and Probst (1952, as Cs), symbol by Probst et al. (1965)
rcs1	Susceptible, race 1	
Rcs2	Resistant, race 2	Probst et al. (1965)
rcs2	Susceptible, race 2	
Rcs3	Resistant, races 2 and 5	Boerma and Phillips (1983)

rcs3	Susceptible, races 2 and 5	
	6. Downy mildew	
Rpm1	Resistant, race 2	Bernard and Cremeens (1972)
rpm1	Susceptible, race 2	
Rpm2	Resistant, races 2 and 33	Lim (1989)
rpm2	Susceptible, race 33	
	7. Powdery mildew	
Rmd	Resistant (adult plant)	Buzzell and Haas (1978)
rmd	Susceptible (all stages)	
Rmd-c	Resistant (all stages)	Lohnes and Bernard (1992)
rmd	Susceptible	
	8. Phytophthora root rot	
Rps1-a	Resistant, races 1, 2, 10, 11, 13-20, 24, 26, 27	Bernard et al. (1957, as Ps), Lam-Sanchez et al. (1968), Moots et al. (1983), Schmitthenner et al. (1994)
rps1	Susceptible	
Rps1-b	Resistant, races 1, 3-9, 13, 15, 18, 21, 22	Hartwig et al. (1968) (as rps2), Mueller et al. (1978), Laviolette and Athow (1983), Schmitthenner et al. (1994)
rps1	Susceptible	
Rps1-c	Resistant, races 1-3, 6-11, 13-15, 17, 21, 23, 24, 26	Mueller et al. (1978), Laviolette and Athow (1983), Schmitthenner et al. (1994)
rps1	Susceptible	
Rps1-d	Resistant, races 1-7, 9-11, 13-16, 18, 21, 22, 24, 25	Buzzell and Anderson (1992)
rps1	Susceptible	
Rps1-k	Resistant, races 1-11, 13-15, 17, 18, 21-24, 26	Bernard and Cremeens (1981), Laviolette and Athow (1983), Schmitthenner et al. (1994)
rps1	Susceptible	
Rps2	Resistant, races 1, 2, 10, 12	Kilen et al. (1974)
rps2	Susceptible	
Rps3-a	Resistant, races 1-5, 8, 9, 11, 13, 14, 16, 18, 23, 25	Mueller et al. (1978), Laviolette and Athow (1983)
rps3	Susceptible	
Rps3-b	Resistant, races 1-5, 7, 9-12, 16	Ploper et al. (1985)
rps3	Susceptible	
Rps3-c	Resistant, races 1-4, 12, 13	Athow et al. (1986)
rps3	Susceptible	
Rps4	Resistant, races 1-4, 10, 12-16	Athow et al. (1980)
rps4	Susceptible	
Rps5	Resistant, races 1-5, 8, 9, 11, 13, 14, 16	Buzzell and Anderson (1981)
rps5	Susceptible	
Rps6	Resistant, races 1-4, 10, 12, 14-16, 18-21	Athow and Laviolette (1982), Laviolette and Athow (1983)
rps6	Susceptible	
Rps7	Resistant, races 12, 16, 18, 19	Anderson and Buzzell (1992)
rps7	Susceptible	
Rps8	Resistant, races 1-4, 6-9, 13, 15 18, 21, 22, 25, 27, 28, 31, 45	Burnham et al. (2003); Gordon et al. (2006)
rps8	Susceptible	

	9. Pythium	
Rpa1	Resistant	Rosso et al. (2008)
rpa1	Susceptible	
	10. Stem canker	
Rdc1	Resistant	Kilen and Hartwig (1987)
rdc1	Susceptible	
Rdc2	Resistant	Kilen and Hartwig (1987)
rdc2	Susceptible	
Rdc3	Resistant	Bowers et al. (1993)
rdc3	Susceptible	
Rdc4	Resistant	Bowers et al. (1993)
rdc4	Susceptible	
	11. Sudden death syndrome	
Rfs	Resistant	Stephens et al. (1993)
rfs	Susceptible	
	12. Soybean rust	
Rpp1	Resistant	McLean and Byth (1980)
Rpp1-b	Resistant	Chakraborty et al. (2009)
rpp1	Susceptible	
Rpp2	Resistant	Bromfield and Hartwig (1980); Hartwig and Bromfield (1983)
rpp2	Susceptible	
Rpp3	Resistant	Hartwig and Bromfield (1983)
rpp3	Susceptible	
Rpp4	Resistant	Hartwig (1986)
rpp4	Susceptible	
Rpp5	Resistant	Garcia et al. (2008)
rpp5	Susceptible	
Rpp? (Hyuuga)	Resistant	Monteros et al. (2007)
rpp? (Hyuuga)	Susceptible	
	13. Soybean mosaic virus	
Rsv1	Resistant, SMV-1, SMV-1-B, G1 through G6	Kiihl and Hartwig (1979); Zheng and Gergerich (2006)
rsv1	Susceptible	Kiihl and Hartwig (1979)
Rsv1-t	Resistant, SMV-1; Susceptible, SMV-1-B, G1, G2, G4, G5, G6	Chen et al. (1991)
Rsv1-y	Resistant, G1, G2, G3	Chen et al. (1991)
Rsv1-m	Resistant, G1, G4, G5, G7	Chen et al. (1991)
Rsv1-k	Resistant, G1, G2, G3, G4	Chen et al. (1991)
Rsv1-n	Necrotic, G1	Ma et al. (1994), Ma et al. (2003)
Rsv1-s	Resistant, G1, G2, G3, G4, G7	Ma et al. (1995)
Rsv1-r	Resistant, G1, G2, G3, G4, G7	Chen et al. (2001)
Rsv1-h	Resistant, G1 through G7	Chen et al. (2002)
Rsv3	Resistant, G5, G6, G7	Buzzell and Tu (1989)

Rsv3-?	Resistant, G5, G6, G7	Buss et al. (1999)
rsv3	Susceptible	Buss et al. (1999)
Rsv4	Resistant, G1 through G7	Ma et al. (1995), Gunduz (2000)
rsv4	Susceptible	Gunduz (2000)
	14. Peanut mottle virus	
Rpv1	Resistant	Boerma and Kuhn (1976)
rpv1	Susceptible	
rpv2	Resistant	Shipe et al. (1979)
Rpv2	Susceptible	
	15. Cowpea chlorotic mottle virus	
Rcv	Resistant	Boerma et al. (1975)
rcv	Susceptible	
	16. Cyst nematode	
rhg1	Resistant	Caldwell et al. (1960)
with rhg2 rhg3		
Rhg1, Rhg2 or Rhg3	Susceptible	
Rhg4	Resistant	Matson and Williams (1965)
with rhg1 rh2 rhg3		
rhg4	Susceptible	
Rhg5	Resistant	Rao-Arelli et al. (1992), Rao-Arelli (1994)
rhg5	Susceptible	
	17. Reniform nematode	
rrn	Resistant	Williams et al. (1981)
Rrn	Susceptible	
	18. Root-knot nematode	
Rmi1	Resistant	Luzzi et al. (1994)
rmi1	Susceptible	
	19. Soybean aphid	
Rag1	Resistant	Hill et al. (2006)
rag1	Susceptible	
Rag2	Resistant	Kang et al. (2008); Mian et al. (2008); Hill et al. (2009)
rag1	Susceptible	
Rag3	Resistant	Zhang et al. (2010)
rag3	Susceptible	
Rag4	Resistant	Zhang et al. (2009)
rag4	Susceptible	

[a] A susceptible phenotype, when specific races are not identified, indicates that the strain was susceptible to races used to identify the resistance allele at that locus by authors of the first reference.

Table 3.8

Genes affecting herbicide reaction in soybean

Gene	Phenotype	Reference
Als1	Semidominant for resistance to sulfonylurea herbicides	Sebastian et al. (1989)
als1	Sensitive	
Hb	Tolerant to bentazon	Bernard and Wax (1975)
hb	Sensitive to bentazon	
Hm	Tolerant to metribuzin	Edwards et al. (1976), Hartwig et al. (1980), Hanson and Nickell (1986), Kilen and He (1992)
hm	Sensitive to metribuzin	
Hs1	Sensitive to sulfonylurea herbicides	Sebastian and Chaleff (1987)
hs1	Enhanced tolerance	
Hs2	Sensitive to sulfonylurea herbicides	Sebastian and Chaleff (1987)
hs2	Enhanced tolerance	
Hs3	Sensitive to sulfonylurea herbicides	Sebastian and Chaleff (1987)
hs3	Enhanced tolerance	

Table 3.9

Genes affecting *Bradyrhizobium* or *Rhizobium*

Gene	Phenotype	Reference
Rfg1	Ineffective by strain 205	Devine and Kuykendall (1994)
rfg1	Effective	
Rj1	Nodulating	Williams and Lynch (1954) (as no); symbol by Caldwell (1966)
rj1	Nonnodulating	
Rj2	Ineffective by strains b7, b14, and b122	Caldwell (1966)
rj2	Effective	
Rj3	Ineffective by strain 33	Vest (1970)
rj3	Effective	
Rj4	Ineffective by strain 61	Vest and Caldwell (1972)
rj4	Effective	
Rj5	Nodulating	Pracht et al. (1993)
rj5	Nonnodulating	
Rj6	Nodulating	Pracht et al. (1993)
rj6	Nonnodulating	
Rj7	Nodulating	Kokubun and Akao (1994), Harper and Nickell (1995), Vuong et al. (1996), Vuong et al. (2000)
rj7	Hypernodulating	

Table 3.10

Genes affecting root response in soybean

Gene	Phenotype	Reference
Fr1	Fluorescent in UV light	Fehr and Giese (1971)
fr1	Nonfluorescent	
Fr2	Fluorescent in UV light	Delannay and Palmer (1982b)
fr2	Nonfluorescent	
Fr3	Nonfluorescent	Delannay and Palmer (1982b)
fr3	Fluorescent in UV light	
Fr4	Fluorescent in UV light	Delannay and Palmer (1982b)
fr4	Nonfluorescent	
Fr5	Fluorescent in UV light	Sawada and Palmer (1987)
fr5	Nonfluorescent	
Rn (Ames 1)	Normal	Kosslak et al. (1996); Palmer et al. (2008)
rn (Ames 1)	Necrotic root	
Rn (Ames 2)	Normal	Kosslak et al. (1996); Palmer et al. (2008)
rn (Ames 2)	Necrotic root	
Rn (Ames 3)	Normal	Kosslak et al. (1996); Palmer et al. (2008)
rn (Ames 3)	Necrotic root	

Table 3.11

Genes affecting growth and morphology in soybean

Gene	**Phenotype**	**Reference**
	1. Time of flowering and maturity	
E1	Late	Owen (1927b), Bernard (1971)
el	Early	
E2	Late	Bernard (1971)
e2	Early	
E3	Late and sensitive to fluorescent light	Buzzell (1971), Kilen and Hartwig (1971)
e3	Early and insensitive to fluorescent light	
E4	Late and sensitive to long daylength	Buzzell and Voldeng (1980)
e4	Early and insensitive to long daylength	
E5	Late	McBlain and Bernard (1987)
e5	Early	
E6	Early	Bonato and Vello (1999)
e6	Late	
E7	Late	Cober and Voldeng (2001)
e7	Early	
J	Normal	Ray et al. (1995)
j	Long juvenile trait	

	2. Growth of stem, petiole, and inflorescence	
Br1 Br2	Branches originating from upper as well as lower nodes	Nelson (1996)
br1 br2	Few branches only from lower nodes	Nelson (1996)
Dt1	Indeterminate stem	Woodworth (1932, 1933), Bernard (1972)
dt1-t	Tall determinate stem	Thompson et al. (1997)
dt1	Determinate stem	
Dt2	Semideterminate stem	Bernard (1972)
dt2	Indeterminate stem	
F	Normal stem	Nagai (1926), Takagi (1929), symbol by Woodworth (1932, 1933), Matsuura (1933), Albertsen et al. (1983)
f	Fasciated stem	
Lps1	Normal petiole	Kilen (1983)
lps1	Short petiole	
Lps2	Normal petiole	You et al. (1998)
lps2	Short petiole, abnormal pulvinus	
S	Short, internode length decreased	Bernard (1975a)
s	Normal	
s-t	Tall, internode length increased	
Se	Pedunculate inflorescence	VanSchaik and Probst (1958)
se	Subsessile inflorescence	
	3. Dwarfness	
Df2	Normal	Porter and Weiss (1948), symbol by Byth and Weber (1969)
df2	Dwarf	
Df3	Normal	Byth and Weber (1969)
df3	Dwarf	
Df4	Normal	Fehr (1972a)
df4	Dwarf	
Df5	Normal	Palmer (1984a)
df5	Dwarf	
Df6	Normal	Werner et al. (1987)
df6	Dwarf	
Df7 or Df8	Normal	Soybean Genetics Committee (1995)
df7 df8	Dwarf	
Mn	Normal	Delannay and Palmer (1984)
mn	Miniature plant	
Pm	Normal	Probst (1950)
pm	Dwarf, crinkled leaves, sterile	
Sb1 or Sb2	Normal	Kilen and Hartwig (1975), Kilen (1977), Boerma and Jones (1978)
sb1 sb2	Brachytic stem	
	4. Leaf form	
Ab	Abscission at maturity	Probst (1950)

ab	Delayed abscission	
Dlm	Normal	Chung et al. (1998)
dlm	Necrotic spots with chlorotic halo	
Lc Lc	Normal petiolule	Cary and Nickell (1999)
Lc lc	Intermediate petiolule	
lc lc	Short petiolule	
Lf1	5-foliolate	Takahashi and Fukuyama (1919), symbol by Fehr (1972b)
lf1	3-foliolate	
Lf2	3-foliolate	Fehr (1972b)
lf2	7-foliolate	
Lmn	Normal	Yu and Kiang (1993a)
lmn	Leaf margin necrosis	
Ln	Ovate leaflet	Takahashi and Fukuyama (1919), Woodworth (1932, 1933), Takahashi (1934), Domingo (1945), symbol by Bernard and Weiss (1973)
ln	Narrow leaflet, 4-seeded pods	
Lnr	Normal	Wilcox and Abney (1991)
lnr	Narrow rugose leaf	
Lo	Ovate leaflet	Domingo (1945)
lo	Oval leaflet, few-seeded pods	
Lw1 Lw2	Nonwavy leaf	Rode and Bernard (1975b)
Lw1 lw2	Nonwavy leaf	
lw1 Lw2	Nonwavy leaf	
lw1 lw2	Wavy leaf	
Lb1 Lb2	Nonbullate leaf	Rode and Bernard (1975c)
Lb1 lb2	Nonbullate leaf	
lb1 Lb2	Nonbullate leaf	
lb1 lb2	Bullate leaf	
	5. Pubescence type	
Pa1 Pa2	Erect	Karasawa (1936), Ting (1946), symbol by Bernard (1975d)
Pa1 pa2	Erect	
pa1 Pa2	Semiappressed	
pa1 pa2	Appressed	
P1	Glabrous	Nagai and Saito (1923)
p1	Pubescent	
P2	Normal	Stewart and Wentz (1926)
p2	Puberulent	
Pb	Sharp hair tip	Ting (1946)
pb	Blunt hair tip	
Pc	Normal	Bernard and Singh (1969)
pc	Curly (deciduous)	
Pd1	Dense	Bernard and Singh (1969)
pd1	Normal	
Pd2	Dense	Bernard (unpublished)
pd2	Normal	
Pd1 Pd2	Extra-dense	Gunashinghe et al. (1988)

Ps	Sparse	Bernard and Singh (1969), Bernard (1975c)
Ps-s	Semisparse	
ps	Normal	
	6. Seed-coat structure	
B1 B2 B3	Bloom on seed coat	Woodworth (1932, 1933), Tang and Tai (1962)
b1, b2, or b3	No bloom	
N	Normal hilum abscission	Owen (1928)
n	Lack of abscission layer	

Table 3.12

Genes affecting physiology in soybean

Gene	Phenotype	Reference
	1. Reaction to nutritional factors	
Fe	Efficient Fe utilization	Weiss (1943)
fe	Inefficient	
Np	Phosphorus tolerant	Bernard and Howell (1964)
np	Sensitive to high phosphorus level	
Ncl1	Chloride excluding	Abel (1969)
Ncl1	Chloride accumulating	
Ncl2	Cloride excluding	Lee et al. (2009)
ncl2	Cloride accumulating	
Nr	Constitutive nitrate reductase present	Ryan et al. (1983a, 1983b)
nr	Constitutive nitrate reductase absent	
	2. Flavonol glycosides of leaves	
T	Quercetin and kaempferol present	Buttery and Buzzell (1973), (also see Table 13)
T	Quercetin absent, kaempferol present	
Wm	Glycosides present	Buzzell et al. (1977), (also see Table 13)
wm	Glycosides absent	
Fg1	β(1-6)-glucoside present	Buttery and Buzzell (1975)
fg1	β(1-6)-glucoside absent	
Fg2-a	Normal kaempferol rutinoside	Buzzell and Buttery (1992)
Fg2-b	Less kaempferol rutinoside	
fg2	α(1-6)-rhamnoside absent	Buttery and Buzzell (1975)
Fg3	β(1-2)-glucoside present	Buttery and Buzzell (1975)
fg3	β(1-2)-glucoside absent	
Fg4	α(1-2)-rhamnoside present	Buttery and Buzzell (1975)

fg4	α(1-2)-rhamnoside absent	
	3. Seed	
Shr	Normal	Honeycutt et al. (1989)
shr	Shriveled	

Table 3.13

Genes affecting pigmentation in soybean

Gene[a]	Phenotype	Reference
	1. Flower	
W1	Purple	Takahashi and Fukuyama (1919), Woodworth (1923)
w1-1p	Light purple	(Ryoji Takahashi, personal communication 21 Jan. 2010)
w1	White	
W2 (with W1)	Purple	Takahashi et al. (2008)
w2 (with W1)	Purple-blue	
W3 w4	Dilute purple	Hartwig and Hinson (1962)
w3 W4	Purple	
W3W4	Dark purple	
w3 w4	Near white	
w4-dp	Dilute purple	Palmer and Groose (1993)
w4-m	Mutable flower	Palmer et al. (1990a)
Wm	Purple (glycosides present)	Buzzell et al. (1977)
wm	Magenta (glycosides absent)	
Wp	Purple	Stephens and Nickell (1992)
wp	Pink flower	
wp-m	Mutable flower	Johnson et al. (1998)
	2. Pubescence	
T	Tawny (brown); quercetin and kaempferol present	Piper and Morse (1910), Nagai (1921) (as C c)
T	Gray; quercetin absent, kaempferol present	Woodworth (1921), Williams (1950), Buttery and Buzzell (1973)
t-r	Red-buff seed coat	Seo et al. (1993)
Td	Tawny (brown); flavonol present	Buttery and Buzzell (1973), Bernard (1975b)
td	Light tawny (near-gray); flavonol absent	
	3. Seed	
I	Light hilum	Nagai (1921), Nagai and Saito (1923), Owen (1928), Woodworth (1932, 1933), Mahmud and Probst (1953)
i-i	Dark hilum	
i-k	Saddle pattern	
I	Self dark seed coat	
Im	Nonmottled seed	Cooper (1966)

im	Dark mottled seed (with SMV infection)	
K1	Nonsaddle	Takagi (1929, 1930), Williams (1958)
k1	Dark saddle on seed coat	
K2	Yellow seed coat	Rode and Bernard (1975a), Palmer (1984b)
k2 (Urbana 1)	Tan saddle on seed coat	
k2 (Columbia 2)	Tan saddle on seed coat	Rode and Bernard (1975a)
k2 (Urbana 1) [Mdh1-n (Urbana 1) y20 (Urbana 1)]	Tan saddle on seed coat	Chen and Palmer (1996)
k2 (Columbia 1) [Mdh1-n (Columbia 1)]	Tan saddle on seed coat	Chen and Palmer (1996)
k2 (Urbana 1) [Mdh1-n (Ames 7) y20 (Ames 5)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 8) y20 (Ames 6)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 9) y20 (Ames 7)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 10) y20 (Ames 8)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 11) y20 (Ames 9)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 12) y20 (Ames 10)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Columbia 1) [Mdh1-n (Columbia 1) y20 (Ames 11)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 13) y20 (Ames 12)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 14) y20 (Ames 13)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 15) y20 (Ames 14)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 16) y20 (Ames 15)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 17) y20 (Ames 16)]	Tan saddle on seed coat	Chen and Palmer (1998)
k2 (Urbana 1) [Mdh1-n (Ames 6) y20 (Ames 18)]	Tan saddle on seed coat	Chen et al. (1999)
k2 (Urbana 1) [Mdh1-n (Ames 6) y20 (Ames 19)]	Tan saddle on seed coat	Chen et al. (1999)
k2 (Urbana 1) [Mdh1-n (Ames 6) y20 (Ames 20)]	Tan saddle on seed coat	Chen et al. (1999)
k2 (Urbana 1) [Mdh1-n (Ames 6) y20 (Ames 21)]	Tan saddle on seed coat	Chen et al. (1999)
k2 (Urbana 1) [Mdh1-n (Ames 20) y20 (Ames 22)]	Tan saddle on seed coat	Chen et al. (1999)
K3	Nonsaddle	Bernard and Weiss (1973)
k3	Dark saddle on seed coat	
O	Brown seed coat	Nagai (1921), Weiss (1970b)
O	Reddish-brown seed coat	
R	Black seed coat	Nagai (1921), Woodworth (1921), Stewart (1930), Williams (1952)
r-m	Black stripes on brown seed	Nagai and Saito (1923), Weiss (1970b)
R	Brown seed coat	
	4. Pod	

L1 L2	Black pod	Bernard (1967)
L1 l2	Black pod	
l1 L2	Brown pod	
l1 l2	Tan pod	

[a] Genes for secondary traits of interest are listed in brackets.

Table 3.14

Genes affecting fertility-sterility in soybean

Gene	Phenotype	Reference
Fs1 or Fs2	Fertile	Johns and Palmer (1982)
fs1 fs2	Structural sterile	
Ft	Fertile	Singh and Jha (1978)
Ft	Structural sterile	
Ms1	Fertile	
ms1 (North Carolina)	Male sterile	Brim and Young (1971)
ms1 (Urbana)	Male sterile	Boerma and Cooper (1978)
ms1 (Tonica)	Male sterile	Palmer et al. (1978)
ms1 (Ames 1)	Male sterile	Palmer et al. (1978)
ms1 (Ames 2)	Male sterile	Skorupska and Palmer (1990)
ms1 (Danbury)	Male sterile	Skorupska and Palmer (1990)
Ms2	Fertile	
ms2 (Eldorado)	Male sterile	Bernard and Cremeens (1975), Graybosch et al. (1984)
ms2 (Ames 1)	Male sterile	Palmer (2000)
ms2 (Ames 2)	Male sterile	Cervantes-Martinez (2005)
Ms3	Fertile	
ms3 (Washington)	Male sterile	Palmer et al. (1980), Graybosch and Palmer (1987)
ms3 (Flanagan)	Male sterile	Chaudhari and Davis (1977), Graybosch and Palmer (1987)
ms3 (Plainview)	Male sterile	Skorupska and Palmer (1990)
Ms4	Fertile	
ms4 (Ames)	Male sterile	Delannay and Palmer (1982a)
ms4 (Fisher)	Male sterile	Skorupska and Palmer (1990)
Ms5	Fertile	
ms5	Male sterile	Buss (1983)
Ms6	Fertile	
ms6 (Ames 1)	Male sterile	Palmer and Skorupska (1990), Skorupska and Palmer (1989)
ms6 (Ames 2)	Male sterile	Ilarslan et al. (1999)
Ms7	Fertile	
ms7	Male sterile	Palmer (2000)
Ms8	Fertile	
ms8	Male sterile	Palmer (2000)
Ms9	Fertile	
ms9	Male sterile	Palmer (2000)
Msp	Fertile	Stelly and Palmer (1980a, 1980b)

msp	Partial male sterile	
St2	Fertile	Hadley and Starnes (1964)
st2	Asynaptic sterile	
St3	Fertile	Hadley and Starnes (1964)
st3	Asynaptic sterile	
St4	Fertile	Palmer (1974)
st4	Desynaptic sterile	
St5	Fertile	Palmer and Kaul (1983)
st5	Desynaptic sterile	
St6 St7	Fertile	Ilarslan et al. (1997)
st6 st7	Male sterile, female sterile	
St8	Fertile	Palmer and Horner (2000); Palmer et al. (2008)
st8	Desynaptic sterile	

Table 3.15

Genes controlling inheritance of isoenzymes and protein variants in soybean

Gene[a]	**Phenotype**	**Reference**
Ap-a	Acid phosphatase mobility variant	Gorman and Kiang (1977), Hildebrand et al. (1980)
Ap-b	Acid phosphatase mobility variant	
Ap-c	Acid phosphatase mobility variant	
Aco1-a	Aconitase mobility variant	Griffin and Palmer (1987a), Kiang and Bult (1991)
Aco1-b	Aconitase mobility variant	
aco1-n	Aconitase null	
Aco2-a	Aconitase mobility variant	Doong and Kiang (1987b), Rennie et al. (1987a)
Aco2-b	Aconitase mobility variant	
Aco2-bn	Aconitase null	Amberger et al. (1992)
Aco2-c	Aconitase mobility variant	Kiang and Bult (1991)
Aco3-a	Aconitase mobility variant	Griffin and Palmer (1987a)
Aco3-b	Aconitase mobility variant	
Aco4-a	Aconitase mobility variant	Griffin and Palmer (1987a)
Aco4-b	Aconitase mobility variant	
Aco4-c	Aconitase mobility variant	
Aco4-d	Aconitase mobility variant	
Aco5-a	Aconitase mobility variant	Kiang and Bult (1991)
Aco5-b	Aconitase mobility variant	
aco5-n	Aconitase null	
Adh1	Alcohol dehydrogenase present	Gorman and Kiang (1978), Kiang and Gorman (1983)
adh1	Alcohol dehydrogenase absent	
Adh2	Alcohol dehydrogenase present	Yu and Kiang (1993b)
adh2		
Alcohol dehydrogenase absent		
Adh3		
Alcohol dehydrogenase present	Yu and Kiang (1993b)	
adh3		

Alcohol dehydrogenase absent		
Amy1	α-amylase band 1 present	Gorman and Kiang (1977, 1978), Kiang (1981)
amy1	α-amylase band 1 absent	
Amy2	α-amylase band 2 present	
amy2	α-amylase band 2 absent	
Sp1-a	β-amylase mobility variant	Larsen (1967), Larsen and Caldwell (1968), Orf and Hymowitz (1976), Gorman and Kiang (1977, 1978), Hymowitz et al. (1979), Hildebrand and Hymowitz (1980a, 1980b), Kiang (1981)
Sp1-b	β-amylase mobility variant	Orf and Hymowitz (1976)
Sp1-c	β-amylase mobility variant	Gorman and Kiang (1977, 1978), Hymowitz et al. (1979)
Sp1-an	Seed protein band present, β-amylase activity weak or absent	
sp1	Seed protein band absent, β-amylase activity weak or absent	Griffin and Palmer (1986)
Cgy1	β-conglycinin subunit α' present	Kitamura et al. (1984)
cgy1	β-conglycinin subunit α' absent	
Cgy2-a	α subunit of β-conglycinin produced	Davies et al. (1985)
Cgy2-b	α subunit of β-conglycinin mobility variant	
Cgy3	β' subunit of β-conglycinin produced	
cgy3	β' subunit of β-conglycinin absent	
Dia1-a	Diaphorase mobility variant	Gorman et al. (1983), Kiang and Gorman (1983)
Dia1-b	Diaphorase mobility variant	
Dia2-a	Diaphorase mobility variant	Liao and Palmer (1997)
Dia2-b	Diaphorase mobility variant	
dia2-n	Diaphorase band absent	
Dia3	Diaphorase band present	
dia3	Diaphorase band absent	
Enp-a	Endopeptidase mobility variant	Doong and Kiang (1987a), Griffin and Palmer (1987a), Rennie et al. (1987b)
Enp-b	Endopeptidase mobility variant	
Enp-c	Endopeptidase mobility variant	
Ep	High peroxidase activity	Buzzell and Buttery (1969)
ep	Low peroxidase activity	
Est1-a	Esterase mobility variant	Bult and Kiang (1989)
Est1-b	Esterase mobility variant	
Eu1-a	Urease-embryo-specific mobility variant	Buttery and Buzzell (1971)
Eu1-b	Urease-embryo-specific mobility variant	Kloth and Hymowitz (1985), Holland et al. (1987)
eu1-sun	Embryo-specific urease absent	Kloth et al. (1987)
eu1-n4	Urease null - no mRNA	
eu1-n6	Urease – mRNA present, 5% of normal protein	Meyer-Bothling et al. (1987)
eu1-n7	Urease null - no mRNA	Polacco et al. (1989)
eu1-n8	Urease – mRNA present, 0.5% of normal protein	
Eu2	Urease - normal levels	

eu2	No ubiquitous urease, 0.6% embryo-specific urease	
Eu3	Urease - normal levels	
eu3-e1	Lacks both urease types	
Eu3-e3	Reduced levels of both urease types	
Eu4	Urease - normal levels	
eu4	Normal embryo urease, no ubiquitous urease	
Fle	Fluorescent esterase present	Doong and Kiang (1988)
fle	Fluorescent esterase absent	
Got-a	Glutamate oxaloacetate transaminase mobility variant	Kiang et al. (1987)
Got-b	Glutamate oxaloacetate transaminase mobility variant	
Got-c	Glutamate oxaloacetate transaminase mobility variant	
Gpd	Glucose-6-phosphate dehydrogenase present	Gorman et al. (1983), Kiang and Gorman (1983)
gpd	Glucose-6-phosphate dehydrogenase (weak)	
Gy1	Glycinin subunit G1 produced	Nielson et al. (1989)
Gy2	Glycinin subunit G2 produced	
Gy3	Glycinin subunit G3 produced	
Gy4-a	G4 subunit of glycinin present	Kitamura et al. (1984)
Gy4-b	Mobility variant of glycinin G4 subunit	Diers et al. (1994)
gy4	G4 subunit of glycinin absent	Kitamura et al. (1984)
Gy5	Glycinin subunit G5 produced	Nielsen et al. (1989)
Idh1-a	Isocitrate dehydrogenase mobility variant	Yong et al. (1981, 1982), Gorman et al. (1983), Kiang and Gorman (1983, 1985)
Idh1-b	Isocitrate dehydrogenase mobility variant	
Idh2-a	Isocitrate dehydrogenase mobility variant	
Idh2-b	Isocitrate dehydrogenase mobility variant	
Idh3-a	Isocitrate dehydrogenase mobility variant	
Idh3-b	Isocitrate dehydrogenase mobility variant	
Lap1-a	Leucine aminopeptidase mobility variant	Gorman et al. (1982a, 1982b, 1983)
Lap1-b	Leucine aminopeptidase mobility variant	
Lap2	Leucine aminopeptidase present	Kiang et al. (1984)
lap2	Leucine aminopeptidase absent	
Le	Seed lectin present	Orf et al. (1978), Pull et al. (1978), Stahlhut and Hymowitz (1980)
le	Seed lectin absent	
Lx1-a	Lipoxygenase 1 pI 5.85	Hildebrand and Hymowitz (1981, 1982)
Lx1-b	Lipoxygenase 1 pI 5.79	Pfeiffer et al. (1993)
lx1	Lipoxygenase 1 absent	
Lx2	Lipoxygenase 2 present	Davies and Nielsen (1986, 1987)

lx2	Lipoxygenase 2 absent	
Lx3	Lipoxygenase 3 present	Kitamura et al. (1983)
lx3	Lipoxygenase 3 absent	
Mdh1-a	Malate dehydrogenase present	Amberger et al. (1992)
Mdh1-n (Columbia 1) [k2(Columbia 1)]	Malate dehydrogenase absent	Chen and Palmer (1996)
Mdh1-n (Urbana 1) [y20(Urbana 1) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1996)
Mdh1-n (Ames 1) [y20(Ames 1)]	Malate dehydrogenase absent	Amberger et al. (1992)
Mdh1-n (Ames 2) [y20(Ames 2)]	Malate dehydrogenase absent	Hedges and Palmer (1992)
Mdh1-n (Ames 3) [y20(Ames 3)]	Malate dehydrogenase absent	Hedges and Palmer (1992)
Mdh1-n (Ames 4) [y20(Ames 4)]	Malate dehydrogenase absent	Hedges and Palmer (1992)
Mdh1-n (Ames 5)	Malate dehydrogenase absent	Chen and Palmer (1996)
Mdh1-n (Ames 6)	Malate dehydrogenase absent	Chen and Palmer (1996)
Mdh1-n (Ames 7) [y20(Ames 5) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 8) [y20(Ames 6) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 9) [y20(Ames 7) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 10) [y20(Ames 8) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 11) [y20(Ames 9) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 12) [y20(Ames 10) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Columbia 1) [y20(Ames 11) k2(Columbia 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 13) [y20(Ames 12) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 14) [y20(Ames 13) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 15) [y20(Ames 14) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 16) [y20(Ames 15) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 17) [y20(Ames 16) k2(Urbana 1)]	Malate dehydrogenase absent	Chen and Palmer (1998)
Mdh1-n (Ames 18)	Malate dehydrogenase absent	Chen et al. (1999)
Mdh1-n (Ames 19) [y20(Ames 17)]	Malate dehydrogenase absent	Chen et al. (1999)
Mdh1-n (Ames 6) [y20(Ames 18) k2(Urbana 1)]	Malate dehydrogenase absent	Chen et al. (1999)
Mdh1-n (Ames 6) [y20(Ames 19) k2(Urbana 1)]	Malate dehydrogenase absent	Chen et al. (1999)

Mdh1-n (Ames 6) [y20(Ames 20) k2(Urbana 1)]	Malate dehydrogenase absent	Chen et al. (1999)
Mdh1-n (Ames 6) [y20(Ames 21) k2(Urbana 1)]	Malate dehydrogenase absent	Chen et al. (1999)
Mdh1-n (Ames 20) [y20(Ames 22) k2(Urbana 1)]	Malate dehydrogenase absent	Chen et al. (1999)
Mdh1-n (Ames 21) [y20(Ames 23)]	Malate dehydrogenase absent	Chen et al. (1999)
Mdh1-n (Ames 22) [y20(Ames 24)]	Malate dehydrogenase absent	Palmer et al. (2000)
Mips1	Normal	Sebastian et al. (2000); Chappell et al. (2006)
mips1	Reduced levels of seed raffinose, stachyose, and phytic acid	
Mips2	Normal level	Hegeman et al. (2001); Hitz et al. (2002); Chappell et al. (2006); Nunes et al. (2006); Chiera and Grabau (2007)
mips2	Reduced levels of seed raffinose, stacyose, and phytic acid	
Mips3	Normal level	Hegeman et al. (2001); Hitz et al. (2002); Chappell et al. (2006); Nunes et al. (2006); Chiera and Grabau (2007)
mips3	Reduced levels of seed raffinose, stacyose, and phytic acid	
Mips4	Normal level	Hegeman et al. (2001); Hitz et al. (2002); Chappell et al. (2006); Nunes et al. (2006); Chiera and Grabau (2007)
mips4	Reduced levels of seed raffinose, stacyose, and phytic acid	
Stc1a	High levels of seed raffinose and stachyose	Kerr and Sebastain (2000); Skoneczka et al. (2009)
stc1a	Low levels	Kerr and Sebastain (2000); Skonecka et al. (2009)
Mpi-a	Mannose-6- phosphate isomerase mobility variant	Gorman et al. (1983), Kiang and Gorman (1983), Chiang and Kiang (1988)
Mpi-b	Mannose-6- phosphate isomerase mobility variant	
Mpi-c	Mannose-6- phosphate isomerase mobility variant	
Mpi-d	Mannose-6- phosphate isomerase mobility variant	
Mpi-e	Mannose-6- phosphate isomerase mobility variant	Yu and Kiang (1993b)
mpi	Mannose-6-phosphate isomerase absent	Chiang and Kiang (1988)
Pgd1-a	Phosphogluconate dehydrogenase mobility variant	Gorman et al. (1983), Kiang and Gorman (1983), Chiang and Kiang (1987)
Pgd1-b	Phosphogluconate dehydrogenase mobility variant	
Pgd1-c	Phosphogluconate dehydrogenase mobility variant	
pgd1	Phosphogluconate dehydrogenase absent	
Pgd2-a	Phosphogluconate dehydrogenase mobility variant	
Pgd2-b	Phosphogluconate dehydrogenase mobility variant	
Pgd2-c	Phosphogluconate dehydrogenase mobility variant	
Pgd3-a	Phosphogluconate dehydrogenase mobility variant	

Pgd3-b	Phosphogluconate dehydrogenase mobility variant	
Pgi1-a	Phosphoglucose isomerase mobility variant	Chiang et al. (1987)
Pgi1-b	Phosphoglucose isomerase mobility variant	
pgi1	Phosphoglucose isomerase band absent	
Pgi2	Phosphoglucose isomerase mobility variant	Chiang et al. (1987)
pgi2	Phosphoglucose isomerase band absent	
Pgi3-a	Phosphoglucose isomerase mobility variant	Chiang et al. (1987)
Pgi3-b	Phosphoglucose isomerase mobility variant	
Pgm1-a	Phosphoglucomutase mobility variant	Gorman et al. (1983), Kiang and Gorman (1983)
Pgm1-b	Phosphoglucomutase mobility variant	
Pgm2-a	Phosphoglucomutase mobility variant	Yu and Kiang (1993b)
Pgm2-b	Phosphoglucomutase mobility variant	
Pgm2-c	Phosphoglucomutase mobility variant	Yu and Kiang (1993b)
Pgm2-d	Phosphoglucomutase mobility variant	
Pgm3	Phosphoglucomutase mobility variant	
pgm3	Phosphoglucomutase band absent	
Pha1 or Pha2	High seed phytate	Oltmans et al. (2004)
pha1 pha2	Low seed phytate	
Pi1	Trypsin inhibitor present	Kollipara et al. (1996)
pi1	Trypsin inhibitor absent	
Pi2	Trypsin inhibitor present	Kollipara et al. (1996)
pi2	Trypsin inhibitor absent	
Pi3	Bowman-Birk inhibitor band BBI' present	Kollipara et al. (1996)
pi3	Bowman-Birk inhibitor band BBI' absent	
Sdh-a	Shikimate dehydrogenase mobility variant	Yu and Kiang (1993b)
Sdh-b	Shikimate dehydrogenase mobility variant	
Sod1	Superoxide dismutase bands 4 and 5 present	Gorman and Kiang (1978), Gorman et al. (1982b, 1984), Griffin and Palmer (1984, 1989)
sod1	Superoxide dismutase bands 4 and 5 absent	
Sod2-a	Superoxide dismutase mobility variant	Griffin and Palmer (1989)
Sod2-b	Superoxide dismutase mobility variant	
Tia	Kunitz trypsin inhibitor mobility variant	
Tia-a1	Kunitz trypsin inhibitor mobility variant	

Tia-a2	Kunitz trypsin inhibitor mobility variant	
Tia-b1	Intermediate transitional type between Tia and Tib	
Tib	Kunitz trypsin inhibitor mobility variant	
Tib-i5	Kunitz trypsin inhibitor mobility variant	Wang et al. (2005)
Tic	Kunitz trypsin inhibitor mobility variants	Hymowitz (1973)
Tid	Kunitz trypsin inhibitor mobility variants	Zhao and Wang (1992)
Tie	Kunitz trypsin inhibitor mobility variants	Wang et al. (1996; 2001)
Tif	Kunitz trypsin inhibitor mobility variants	Wang et al. (2004)
Tig	Kunitz trypsin inhibitor mobility variants	
Tix	Kunitz trypsin inhibitor mobility variants	Zhao et al. (1995)
ti	Kunitz trypsin inhibitor absent	

[a] Genes for secondary traits of interest are listed in brackets.

Table 3.16

Nuclear genes affecting chlorophyll deficiency or retention in soybean

Genea	**Phenotype**	**Reference**
	1. Chlorophyll deficiency	
V1	Normal	Woodworth (1932, 1933)
v1	Variegated leaves	
V2	Normal	Honeycutt et al. (1990)
v2	Variegated leaves	
Y3	Normal (y3 G1 is also normal)	Nagai (1926), Takagi (1929, 1930), Terao and Nakatomi (1929), symbol by Morse and Cartter (1937)
y3 (with g1)	Green seedling, becoming yellow	
Y4	Normal	Symbol by Morse and Cartter
y4	Greenish-yellow leaves, weak plant	(1937), Woodworth and Williams (1938) (as y5 by error)
Y5	Normal	Symbol by Morse and Cartter
y5	Greenish-yellow leaves	(1937), Woodworth and Williams (1938) (as y4 by error)
Y6	Normal	Symbol by Morse and Cartter
y6	Pale green leaves	(1937), Woodworth and Williams (1938)
Y7 or Y8	Normal	Morse and Cartter (1937),
y7 y8	Yellow growth in cool weather	Probst (1950) (as y8), Williams (1950)
Y9	Normal	Probst (1950)
y9	Bright greenish-yellow leaves	
Y10	Normal	Probst (1950)

y10	Greenish-yellow seedling	
Y11	Normal	Weber and Weiss (1959)
Y11 y11	Bright greenish-yellow leaves	
y11	Lethal yellow	
Y12	Normal	Weiss (1970a)
y12	Whitish primary leaves, yellowish-green leaves	
Y13	Normal	Weiss (1970b)
y13	Whitish-green seedling, greenish-yellow leaves	
Y14	Normal	Nissly et al. (1976)
y14	Light green leaves	
Y15b	Normal	Nissly et al. (1976),
y15b	Yellowish-green leaves	Chen et al. (1999)
Y16	Normal	Wilcox and Probst (1969)
y16	Nearly white lethal	
Y17	Normal	Nissly et al. (1981)
y17	Light yellowish-green leaves	
Y18	Normal	Peterson and Weber (1969)
Y18-m	Unstable allele resulting in chlorophyll chimera	
y18 (Urbana)	Near-lethal yellow	Palmer (1987)
Y18-m	Unstable allele resulting in chlorophyll chimera	
y18 (Ames 1)	Near-lethal yellow	Sheridan and Palmer (1975)
y18 (Ames 2)	Near-lethal yellow	Palmer et al. (2000)
Y19	Normal	Palmer et al. (1990b)
y19	Delayed albino	
Y20 [Mdh1 K2]	Normal	Palmer (1984b)
y20 (Ames 23) [Mdh1-n (Ames 21)]	Yellowish-green leaves	Chen et al. (1999)
y20 (Urbana 1) [Mdh1-n (Urbana 1) k2 (Urbana 1)]	Yellowish-green leaves	Palmer (1984b)
y20 (Ames 1) [Mdh1-n (Ames 1)]	Yellowish-green leaves	Amberger et al. (1992)
y20 (Ames 2) [Mdh1-n (Ames 2)]	Yellowish-green leaves	Hedges and Palmer (1992)
y20 (Ames 3) [Mdh1-n (Ames 3)]	Yellowish-green leaves	Hedges and Palmer (1992)
y20 (Ames 4) [Mdh1-n (Ames 4)]	Yellowish-green leaves	Hedges and Palmer (1992)
y20 (Ames 5) [Mdh1-n (Ames 7) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 6) [Mdh1-n (Ames 8) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 7) [Mdh1-n (Ames 9) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 8) [Mdh1-n (Ames 10) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 9) [Mdh1-n (Ames 11) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 10) [Mdh1-n (Ames 12) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)

y20 (Ames 11) [Mdh1-n (Columbia 1) k2 (Columbia 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 12) [Mdh1-n (Ames 13) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 13) [Mdh1-n (Ames 14) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 14) [Mdh1-n (Ames 15) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 15) [Mdh1-n (Ames 16) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 16) [Mdh1-n (Ames 17) k2 (Urbana 1)]	Yellowish-green leaves	Chen and Palmer (1998)
y20 (Ames 17) [Mdh1-n (Ames 19)]	Yellowish-green leaves	Chen et al. (1999)
y20 (Ames 18) [Mdh1-n (Ames 6) k2 (Urbana 1)]	Yellowish-green leaves	Chen et al. (1999)
y20 (Ames 19) [Mdh1-n (Ames 6) k2 (Urbana 1)]	Yellowish-green leaves	Chen et al. (1999)
y20 (Ames 20) [Mdh1-n (Ames 6) k2 (Urbana 1)]	Yellowish-green leaves	Chen et al. (1999)
y20 (Ames 21) [Mdh1-n (Ames 6) k2 (Urbana 1)]	Yellowish-green leaves	Chen et al. (1999)
y20 (Ames 22) [Mdh1-n (Ames 20) k2 (Urbana 1)]	Yellowish-green leaves	Chen et al. (1999)
y20 (Ames 24) [Mdh1-n (Ames 22)]	Yellowish-green leaves	Palmer et al. (2000)
Y21	Normal	Yee et al. (1986)
y21	Lethal yellow	
Y22	Normal	Palmer et al. (1990b)
y22	Greenish-yellow leaves	
Y23	Normal	Palmer et al. (1990b)
y23	Leaves becoming yellow-white and necrotic	
	2. Chlorophyll retention	
D1 or D2	*Yellow seed embryo*	*Woodworth (1921), Owen (1927a), Veatch and Woodworth (1930)*
d1 d2	*Green seed embryo*	
G1	*Green seed coat*	*Terao (1918), Takahashi and Fukuyama (1919), Nagai (1921), Woodworth (1921)*
g1	Yellow seed coat	
G2	Green seed coat	Reese and Boerma (1989)
g2	Yellow seed coat	
G3	*Yellow seed coat*	Reese and Boerma (1989)
g3	*Green seed coat*	

[a] Genes for secondary traits of interest are listed in brackets.

[b] T234 is allelic to T325 (y20 y20). The gene symbol y15 has been deleted. T234 is now y20 (Ames 23) [Mdh1-n (Ames 21)] (Chen et al., 1999).

Table 3.17

Cytoplasmic factors affecting chlorophyll deficiency, retention, or morphology in soybean

Gene	Phenotype	Reference
	1. Chlorophyll deficiency	
cyt-G2	Normal	Palmer and Mascia (1980)
cyt-Y2	Yellow leaves, becoming yellowish-green	
cyt-G3	Normal	Shoemaker et al. (1985)
cyt-Y3	Yellow leaves, very weak plant, (mutable plants are chlorophyll chimeras)	
cyt-G4	Normal	Cianzio and Palmer (1992)
cyt-Y4	Yellow leaves	
cyt-G5	Normal	Cianzio and Palmer (1992)
cyt-Y5	Green-yellow leaves	
cyt-G6	Normal	Cianzio and Palmer (1992)
cyt-Y6	Yellow leaves, vigorous	
cyt-G7	Normal	Cianzio and Palmer (1992)
cyt-Y7	Yellow leaves, weak	
cyt-G8	Normal	Cianzio and Palmer (1992)
cyt-Y8	Green-yellow leaves	
	2. Chlorophyll retention	
cyt-G1	Green seed embryo	Terao (1918), Veatch and Woodworth (1930)
cyt-Y1	Yellow seed embryo	
	3. Morphology	
cyt-W1	Wrinkled leaves	Stephens et al. (1991)

Table 3.18

Genes affecting seed fatty acid composition in soybean

Gene	Phenotype	Reference
	1. Palmitate	
Fap1	Normal palmitic acid level	Erickson et al. (1988), Wilcox and Cavins (1990)
fap1	Reduced palmitic acid level	
Fap2	Normal palmitic acid level	Erickson et al. (1988), Wilcox and Cavins (1990)
fap2	Elevated palmitic acid level	
fap2	Reduced palmitic acid level	Rahman et al. (1999)
fap2-b	Elevated palmitic acid level	Fehr et al. (1991b), Schnebly et al. (1994)
Fap3	Normal palmitic acid level	Fehr et al. (1991a), Schnebly et al. (1994)
fap3	Reduced palmitic acid level	
fap3-nc	Reduced palmitic acid level	Burton et al. (1994), Wilson et al. (2001)
Fap4	Normal palmitic acid level	Fehr et al. (1991b), Schnebly et al. (1994)
fap4	Elevated palmitic acid level	
Fap5	Normal palmitic acid level	Stoltzfus et al. (2000a)

fap5	Elevated palmitic acid level	
Fap6	Normal palmitic acid level	Narvel et al. (2000)
fap6	Elevated palmitic acid level	
Fap7	Normal palmitic acid level	Stoltzfus et al. (2000b)
fap7	Elevated palmitic acid level	
fapx	Reduced palmitic acid level	Stojšin et al. (1998)
fapx	Reduced palmitic acid level	Rahman et al. (1999)
fap?	Reduced palmitic acid level	Takagi et al. (1995)
fap?	Reduced palmitic acid level	Primomo et al. (2002)
	2. Stearate	
Fas	Normal stearic acid level	Graef et al. (1985), Hammond and Fehr (1983b)
fas	Elevated stearic acid level	
fas-a	Elevated stearic acid level	
fas-b	Elevated stearic acid level	
St1	Normal stearic acid level	Rahman et al. (1997)
st1	Elevated stearic acid level	
St2	Normal stearic acid level	Rahman et al. (1997)
st2	Elevated stearic acid level	
	3. Oleate	
Ol	Normal oleic acid level	Rahman et al. (1996b), Takagi and Rahman (1996)
ol	Elevated oleic acid level	
ol-a	Elevated oleic acid level	
	4. Linolenate	
Fan1	Normal linolenic acid level	
fan1	Reduced linolenic acid level	Rennie and Tanner (1989a)
fan1	Reduced linolenic acid level	Hammond and Fehr(1983a)
fan1	Reduced linolenic acid level	Wilcox and Cavins (1985, 1987)
fan1	Reduced linolenic acid level	Rennie et al. (1988)
fan1	Reduced linolenic acid level	Rahman et al. (1996a)
fan1-b	Reduced linolenic acid level	Stojšin et al., (1998)
Fan2	Normal linolenic acid level	Fehr et al. (1992), Fehr and Hammond (1996)
fan2	Reduced linolenic acid level	
Fan3	Normal linolenic acid level	Ross (1999), Ross et al. (2000)
fan3	Reduced linolenic acid level	
fanx	Reduced linolenic acid level	Rahman et al. (1996a), Rahman and Takagi (1997)
fanx-a	Reduced linolenic acid level	Rahman et al. (1998)

Table 3.19

Seed coat and hilum color phenotypes in soybean controlled by different combinations of genes[a]

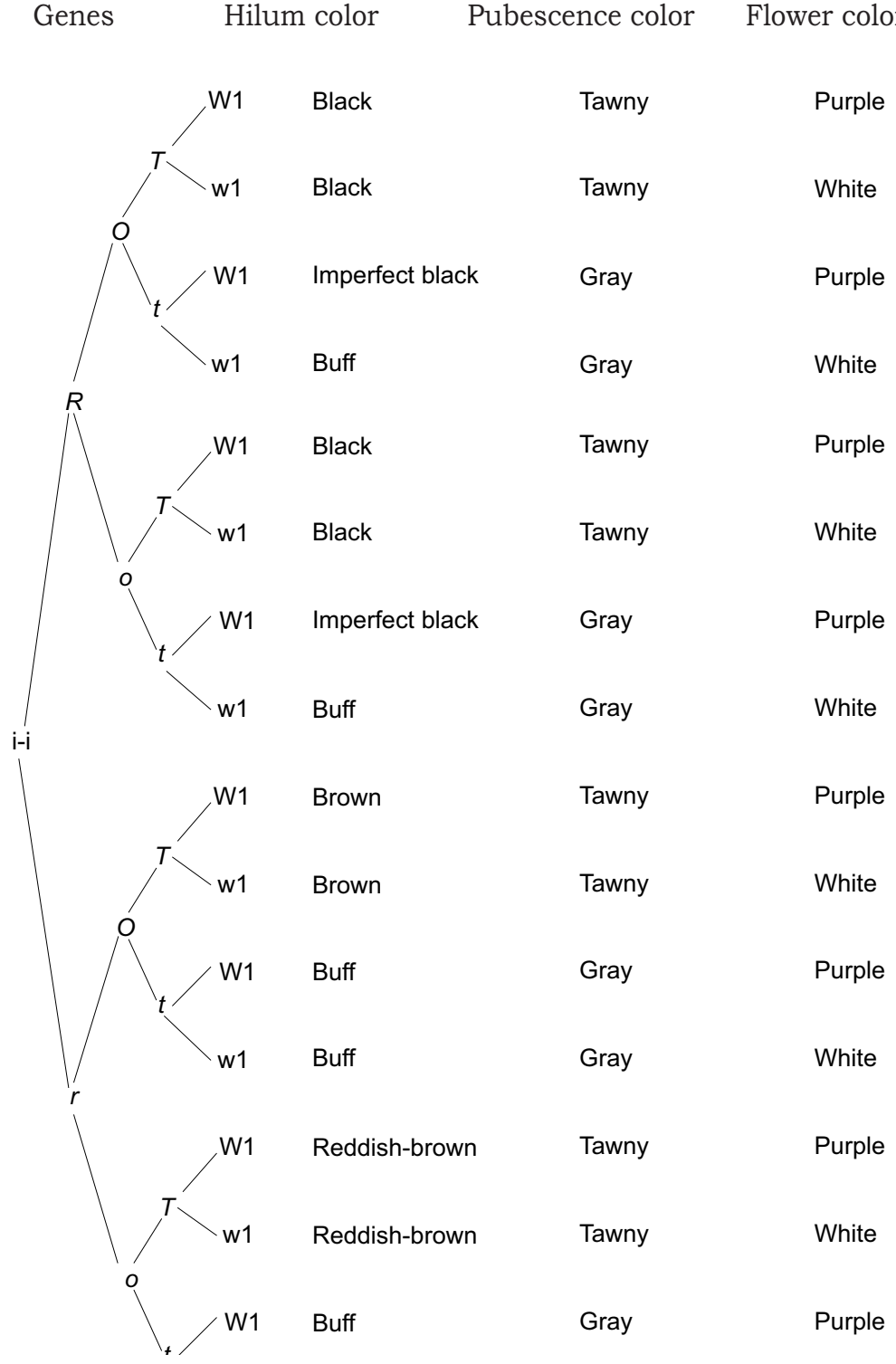
	Seed coat and hilum self-color	Saddle and hilum color	Hilum color	Hilum color
Genes	*i*	*i-k*	*i-i*	*l*
TR	black	black	black	gray
TrO	brown	brown	brown	yellow[b]
Tro	red brown	red brown	red brown	yellow[b]
tRW1	imperfect black	imperfect black	imperfect black	gray
tRw1	buff	buff	buff	yellow
tr	buff	buff	buff	yellow

[a] Modified from Specht and Williams (1978) and Palmer and Stelly (1979).

[b] Sometimes called imperfect yellow (Cober et al., 1998).

Figure 3.1

Inheritance of hilum color in soybean seeds with yellow seed coat[a]



The genes *I, R*, and *O* control the distribution and color of pigmentation in the seed. The genes for pubescence (*T, t*) and flower color (*W1, w1*) also are part of the genetic system controlling hilum color (Buzzell et al., 1987)

Table 3.21

Assignment of unassigned soybean linkage groups to chromosomes

Chromo-some number	Molecular linkage group	cM length (new 5514 marker map)	Status
1	D1a	98.41	Previously assigned
2	D1b	140.63	Assigned based on LG length
3	N	99.51	Previously assigned
4	C1	112.32	Previously assigned
5	A1	86.75	Previously assigned
6	C2	136.51	Assigned based on LG length
7	M	135.15	Assigned based on LG length
8	A2	146.67	Previously assigned
9	K	99.60	Previously assigned
10	O	132.89	Assigned based on LG length
11	B1	124.24	Assigned based on LG length
12	H	120.50	Assigned based on LG length
13	F	120.03	Previously assigned
14	B2	108.18	Assigned based on LG length
15	E	99.88	Assigned based on LG length
16	J	92.27	Assigned based on LG length
17	D2	119.19	Previously assigned
18	G	105.00	Previously assigned
19	L	101.14	Previously assigned
20	I	112.77	Previously assigned

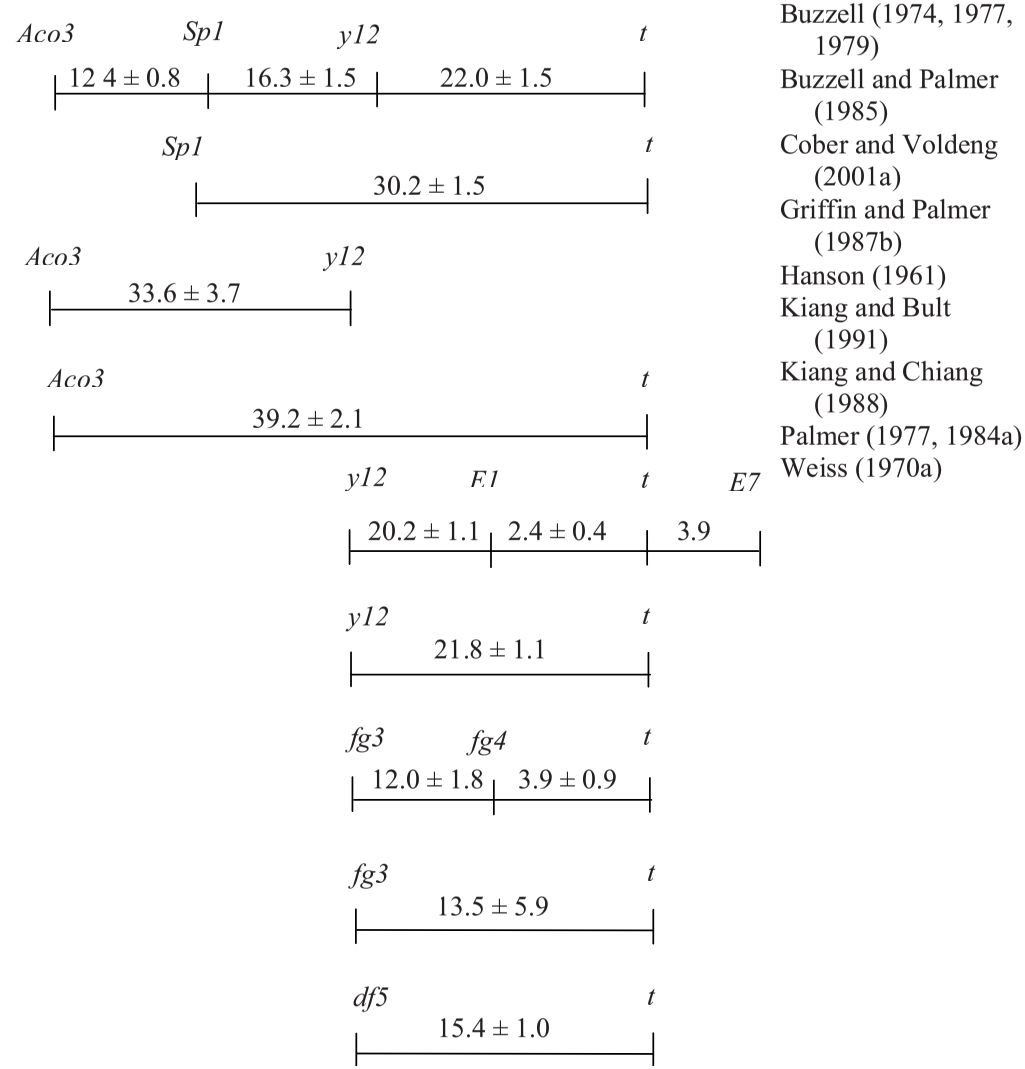
Source: http://soybase.org/LG2Xsome.php

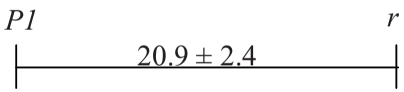
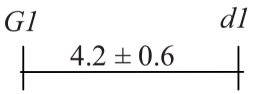
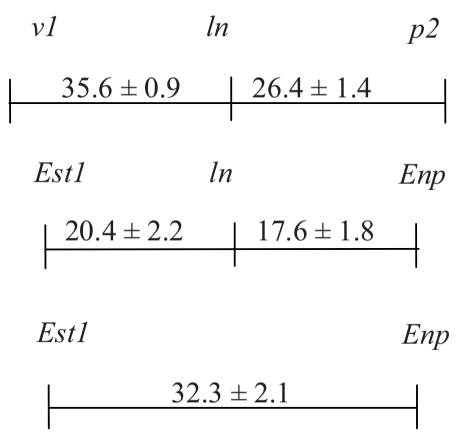
Figure 3.2 *

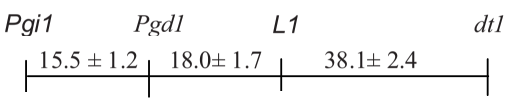
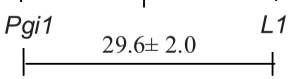
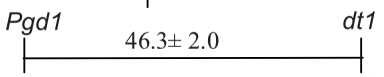
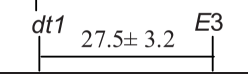
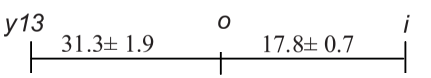
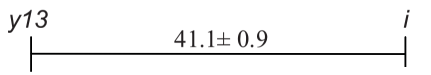
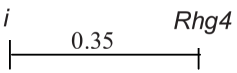
Traditional (classical) linkage map in soybean[a]

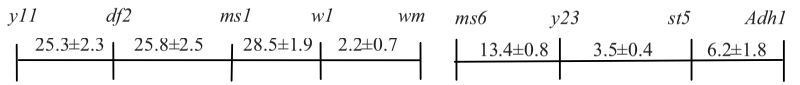
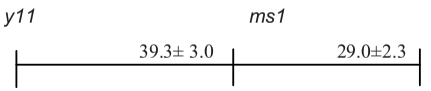
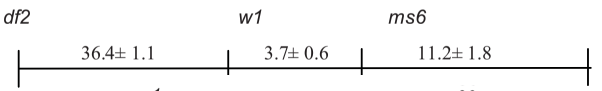
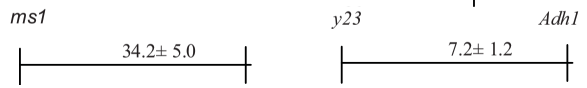
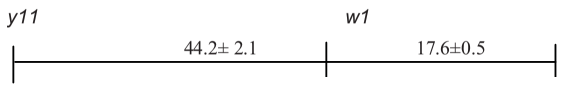
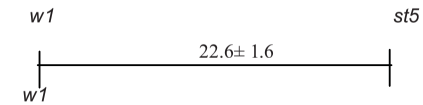
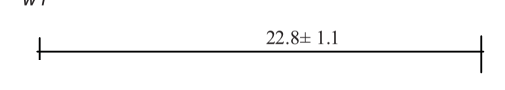
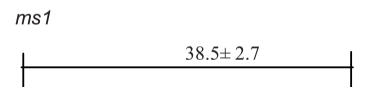
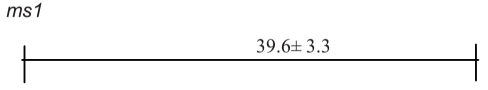
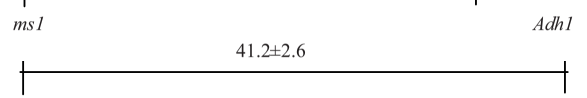
** (Figure starts on the next page)*

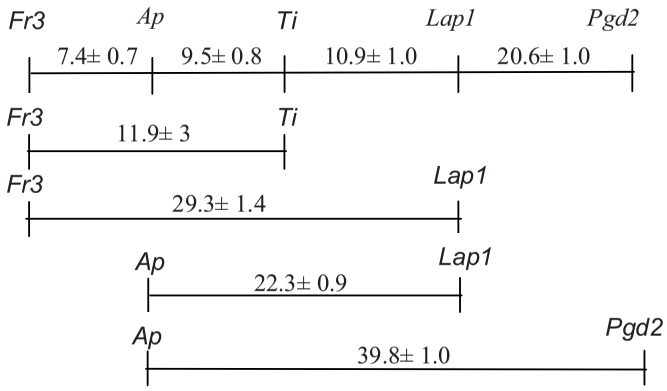
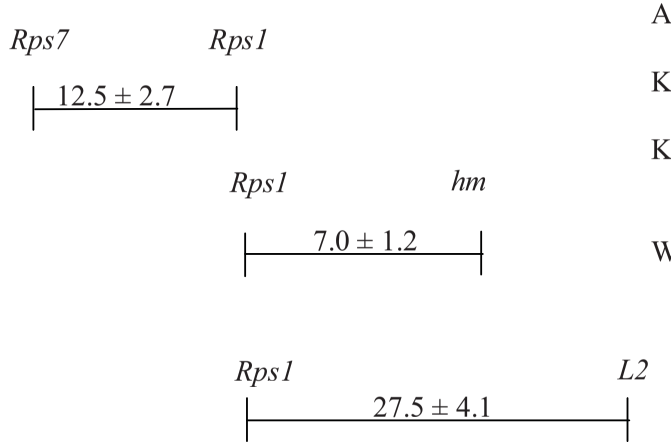
Linkage group		Linked genes	Linkage intensity map[b]	References
1	*Aco3*	Aconitase		Buzzell (1974, 1977, 1979)
	df5	Dwarf		Buzzell and Palmer (1985)
	E1	Late flowering and maturity		Cober and Voldeng (2001a)
	E7	Late flowering and maturity		Griffin and Palmer (1987b)
	fg3	Flavonol glycoside absent		Hanson (1961)
	fg4	Flavonol glycoside absent		Kiang and Bult (1991)
	Sp1	β-amylase		Kiang and Chiang (1988)
	t	Gray pubescence		Palmer (1977, 1984a)
	y12	Chlorophyll deficient		Weiss (1970a)

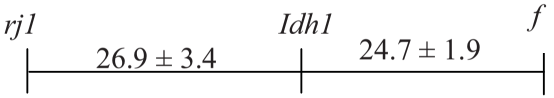
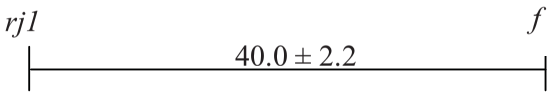
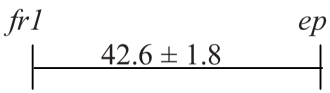
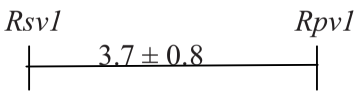


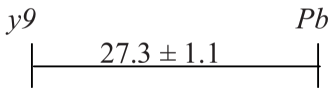
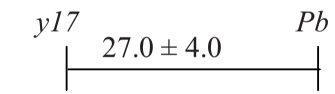
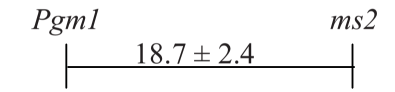
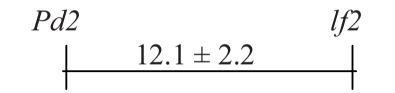
Linkage group		Linked genes	Linkage intensity map[b]	References
2	*P1*	Glabrous		Weiss(1970b)
	r	Brown seed		
3	*G1*	Green seed coat		
	d1	Green seed embryo		Weiss (1970b)
4	*Enp*	Endopeptidase		Abe et al. (1997)
	Est1	Esterase		Muehlbauer et al. (1989)
	ln	Narrow-leaflet, four-seeded pods		Weiss (1970c)
	p2	Puberulent		
	vl	Variegated leaves		

Linkage group		Linked genes	Linkage intensity map[b]	References
5	*dt1*	Determinate stem		Cober and Voldeng (1996)
	E3	Late and sensitive to fluorescent light		Kiang (1990a)
	L1	Black pod		Weiss (1970d)
	Pgd1	Phosphogluconate dehydrogenase		
	Pgi1	Phosphoglucose isomerase		
7	*i*	Self dark seed		Matson and Williams (1965)
	o	Reddish brown seed		Weiss (1970e)
	Rhg4	Cyst nematode resistance (with *rhg1*, *rhg2*, and *rhg3*)		
				
	y13	Chlorophyll deficient		

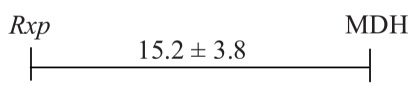
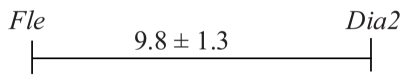
Linkage group		Linked genes	Linkage intensity map[b]	References
8	*Adh1*	Alcohol dehydrogenase		Bult et al. (1989)
	df2	Dwarf		Buzzell (1975, 1976)
	ms1	Male sterile		Buzzell et al. (1977)
	ms6	Male sterile		Ilarslan et al. (1999)
	st5	Male sterile, female sterile		Kiang (1990b)
	w1	White flower		Kiang et al. (1987)
	wm	Magenta flower		Lewers and Palmer (1993)
	y11	Chlorophyll deficient		Mahama et al. (2002)
	y23	Chlorophyll deficient		Mahama and Palmer (1998, 2003)
				Palmer (1976, 1985)
				Palmer and Chen (1998b)
				Palmer and Kaul (1983)
				Palmer et al. (1990b)
				Sadanaga (1983) Sadanaga and Grindeland (1984) Weiss (1970d)

Linkage group		Linked genes	Linkage intensity map[b]	References
9	*Ap*	Acid phosphatase		Chiang and Kiang (1987)
	Fr3	Nonfluorescent root in UV light		Hildebrand et al. (1980)
	Lap1	Leucine aminopeptidase		Kiang et al. (1985)
	Pgd2	Phosphogluconate dehydrogenase		Palmer and Chen (1998a)
	Ti	Kunitz trypsin inhibitor		
10	*hm*	Metribuzin sensitive		Anderson and Buzzell (1992)
	L2	Pod color		Kilen and Barrentine (1983)
	Rps1	Resistant to phytophthora root rot		Kilen and Tyler (1993)
	Rps7	Resistant to phytophthora root rot		Weng et al. (2001)

Linkage group		Linked genes	Linkage intensity map[b]	References
11	*f*	Fasciated stem		Devine et al. (1983)
	Idh1	Isocitrate dehydrogenase		Hedges et al. (1990)
	rj1	Nonnodulating		
12	*ep*	Low seedcoat peroxidase level		Griffin et al. (1989)
	fr1	Nonfluorescent root in UV light		
13	*Rpv1*	Resistant to peanut mottle virus		Roane et al. (1983)
	Rsv1	Resistant to soybean mosaic virus		

Linkage group		Linked genes	Linkage intensity map[b]	References
14	*Pb*	Sharp pubescent tip		Devine (1998) Thorson et al. (1989)
	y9	Chlorophyll deficient		
	y17	Chlorophyll deficient		
15	*ms2*	Male sterile		Sneller et al. (1992)
	Pgm1	Phosphoglucomutase		
16	*lf2*	Seven foliolate		Devine (2003)
	Pd2	Dense pubescence		

Linkage group		Linked genes	Linkage intensity map[b]	References
17	*Idh2*	Isocitrate dehydrogenase	*Idh2* *Fan* *Fas* 27.2 ± 2.0 21.6 ± 1.7	Rennie and Tanner (1989b)
	Fan	Seed linolenic acid		Rennie et al. (1988)
	Fas	Seed stearic acid	*Idh2* *Fas* 37.0 ± 2.7	
18	*Dt2*	Semideterminate stem		Muehlbauer et al. (1989)
	Mpi	Mannose-6-phosphate isomerase	*Mpi* *Dt2* 16.1 ± 6.4	
19	*Aco2*	Aconitase	*Aco2* *Rj2* 44.7 ± 2.0	Devine et al. (1991a, 1991b)
	Rj2	Ineffective nodulation		Lohnes et al. (1993)
	Rmd	Resistant to powdery mildew	*Rj2* *Rmd* *Rps2* 1.9 ± 0.6 2.3 ± 0.7	
	Rps2	Resistant to phytophthora root rot	*Rj2* *Rps2* 3.6 ± 0.9	

Linkage group		Linked genes	Linkage intensity map[b]	References
20	MDH	Malate dehydrogenase		Palmer et al. (1992)
	Rxp	Bacterial pustule resistance		
21	*Dia2*	Diaphorase		Yu and Kiang (1993b)
	Fle	Fluorescent esterase		

[a] Linkage Group (LG) 15 was reported by Kloth et al. (1987), but additional information indicated a more complex relationship. The LG 15 designation was withdrawn. LG 15 was reassigned by the Soybean Genetics Committee for genes *Ms2* and *Pgm1* reported by Sneller et al. (1992). Linkage Group 16 included genes *Pgd1* and *Pgi1*. Because *Pdg1* was shown to be linked to *L1* (Kiang, 1990a), LG 16 has become part of LG 5. The LG 16 designation was withdrawn. LG 16 was reassigned by the Soybean Genetics Committee for genes *Lf2* and *Pd2* reported by Devine (2003). Linkage Group 6 included genes *Df2* and *Y11*. Because chromosome translocations indicated that LG 6 and LG 8 were the same linkage group (same chromosome) (Mahama and Palmer, 2003), LG 6 has become part of LG 8. Thus LG 6 currently is unassigned. The LG 19 linkage relationship between *Aco2* and *Rj2* is putative and is depicted with a dashed line.

[b] Linkage intensity map given as percentage recombination with standard error.

SUMMARY

This chapter summarize existing information and highlight new information on soybean genetics with an emphasis on data published since the first edition (1998) of this book. Soybean genetics encompasses all aspects of genetics within the genus Glycine. Presented is a listing of gene symbols, with phenotype and references that have been approved by the Soybean Genetics Committee. Additionally, the data necessary for suitable phenotype description and mutant genetics are given. Presented are traditional linkage map, methods for development of linkage maps, discussed integration of classical and molecular markers as well as basic concepts of comparative mapping. Described are problems of genomic duplications, homeology in soybean genome and pedigree-based map analysis.

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QUANTITATIVE GENETIC: RESULTS IN SOYBEAN BREEDING

Joe W. Burton

An important feature of modern agriculture is the use of productive cultivars and hybrids, that are resistant to diseases, pests and other environmental stresses. These cultivars and hybrids have been primarily developed by plant breeding methods based on selection of desirable genotypes, genetic recombination of the selections through intermating, and then reselection. Selection techniques and intermating systems are numerous and differ depending on plant species and breeding goals.

Many of the crop characteristics which are of economic importance, such as productivity and quality, are metric traits. In general, metric traits are quantitatively inherited (i.e. polygenic) and are influenced by environment. Thus, when a plant population exhibits phenotypic variation in a quantitative trait, the variability is ascribed to genetic differences among plants and/or differing environmental influences. Statistical analysis of the variation among plants which are systematically related (in families) provides insight into the quantity of variance due to genotypic versus environmental difference. Other analyses can provide information about various types of genotypic variation (e.g. additive, dominance, and epitasis) and variation due to interaction between environment and genotype. Quantitative genetic research which provides these results is the basis for plant breeding practice. These results provide insight into the nature of genotypic variation in plant populations and aid in devising efficient methods for manipulation of that variation. Reviews of quantitative genetic research with soybean (Glycine max L. Merr.) have previously been published (Johnson and Bernard, 1963; Brim, 1973; Burton, 1987; Burton, 1998). This review is a revision of the 1998 monograph chapter and includes a summary of important quantitative genetic results since 1998. In addition to classical quantitative genetic studies in soybean, the last 10 years have seen an increase in molecular genetic research with soybean. While much of it has been concentrated on the development of transgenic plants that carry a new specific trait (e.g. glyphosate tolerance), efforts have been made to identify quantitative trait loci (QTL's) with much emphasis on molecular marker discovery and marker assisted selection. Thus, a review of QTL research in soybeans will also be included in this chapter as it pertains to quantitative genetic variation and plant breeding practice. Marker discovery, mapping and linkage to genes of interest will be treated in another chapter.

PARTITIONING OF HEREDITARY VARIANCE

Soybean is a naturally self-fertilizing species. Because of this, nearly all soybean cultivars in agricultural use are "pure-lines". These may actually be derived from an F_4 or more inbred generation, or they may be a bulk of uniform inbred sister lines. Breeding populations are usually derived from biparental or triparental cross-matings. Progeny of these cross-matings are rapidly inbred through three or more generations of self-fertilization. Selection may or may not be practiced during this inbreeding, depending on plant breeder preference and goals.

A single cross-pollination with soybean produces between one and four seeds. Because soybean cross-pollinations are difficult and time consuming to do, large amounts of F_1 hybrid seed cannot be produced with hand-pollination. One or two generations of self-fertilization are needed to obtain enough seeds to field-test materials on a scale that could be related to performance under typical farm conditions. Thus, genotypic variances have been most frequently estimated in sets of inbred lines where most of the variance is due to additive and additive by additive types of epistatic effects. Controlled mating designs when they have been used with soybean, have either been nested self-fertilization designs or diallel (complete or partial) designs.

Nested self-fertilization designs

Families in nested self-fertilization designs are derived through two or more generations of self-fertilization, the line of descent tracing back to a single plant in a previous generation. Relationships among families are equated with components of variance and covariance among generations. Least squares estimates of genetic variance components due to additivity, dominance, epistasis and linkage can be obtained depending on the reference population and the model specified. This methodology is described in detail by Horner & Weber (1956) and Cockerham (1963). Later, Cockerham (1983) modified procedures for interpreting covariances of self-fertilization relatives by using several identity by descent measures in addition to the inbreeding coefficient (F). These covariances can be used in genetic models with additive and dominance (no epistasis) effects that are general for all gene frequencies. To my knowledge, there are only 6 published soybean studies in which nested self-fertilization designs were used. The last appeared in 1971 (Croissant & Torrie, 1971). Because these studies provide the best information about hereditary variance partitioning in soybean, they will all be reviewed here.

In the first study, the reference population was 94 random F_2 plants derived from the cross of two cultivars, Adams and Hawkeye (Horner & Weber, 1956). Generations F_3 through F_7 were grown in successive years by randomly choosing two progenies which traced back to a single family in the previous generation and ultimately back to a single F_2 plant. Thus, 188 progenies were grown in the F_4-F_7 generations.

Three genetic models, comprised of all additive effects only, additive plus dominance, and additive plus dominance plus additive by additive epistasis, were fit to maturity data (number of days after August 31). Coefficients for each variance component were estimated by least squares procedures. Results of the analysis showed that the all additive model explained 96% of the variation and provided as good a fit to the data as the more complex models. In further analysis of this data by Gates et al. (1960), flowering date and days from flowering to maturity showed dominance variance to be larger than additive variance and for plant height, additive and dominance variances were similar. The genetic model they used for analysis included terms for estimation of linkage effects. Only flowering date, height, and yield showed effects due to linkage. Repulsion linkages predominated for yield and flowering date and coupling linkages predominated for plant height. Again using the data from the same experiment, Hanson & Weber (1961) analyzed homozygous line variability and thus fit models that contained only additive and additive x additive components. In these analyses, 70% of the genetic variance for yield was due to additive x additive epistasis. Epistasis accounted for 19% of the genetic variance for maturity, 32% on seed weight, and 52% on percent oil. Both plant height and lodging had all-additive genetic variance. There were large errors associated with component estimates, and they may have been inflated by failure to consider genotype x environment interactions and bias due to dominance.

Brim and Cockerham (1961) derived and tested 120 each of $F_{2:3}$, $F_{2:4}$, and $F_{2:5}$ lines from two biparental crosses. In addition, they tested 60 families derived through pair matings of the 120 $F_{2:3}$ lines. The genetic models for analysis of data included additive, dominance, and additive x additive effects taken singly, doubly and all together. The all additive model accounted for 97.5 to 99.6 of the genetic variation, depending on the trait. In the model which included all three components, the additive variance was positive for all traits measured in both populations, fruiting period, maturity, height, lodging, unthreshed weight, seed weight, yield, percent protein and percent oil. Most estimates of dominance variance were smaller than the additive component and also negative in some cases. Only unthreshed weight (in population II) had dominance and additive variances that were similar.

Hanson et al. (1967) produced nested families through self-fertilization of progenies derived in two generations of intermating an eight parent diallel. Using a model that included additive and additive x additive effects, they found that additive x additive epistasis accounted for 61%, 55%, 20% and 17% of yield, maturity, lodging, and height genotypic variance. Variance due to epistasis was not found for either protein or oil.

Croissant and Torrie (1971) generated nested sets of $F_{3:4}$, $F_{4:5}$, $F_{5:6}$, and $F_{6:7}$ lines which descended from the F_2 generation of two biparental crosses. These lines were tested in two years. Genotypic variance was partitioned into components due to additive, dominance and linkage effects.

In both populations, additive variance predominated for all traits with the exceptions of plant height in the first population, and lodging and seed weight in the second. Linkage effects were significant for plant height and seed weight in both populations and significant for date of flowering and lodging in only the second population. Most of these were coupling linkages with the exception of seed weight in the second population.

Diallel designs

Unlike self-fertilization designs, diallel designs continue to be used by plant breeders. Usually they are employed to characterize the inheritance of new traits or to investigate the breeding value of new and often exotic germplasm. Because of assumption failures in standard diallel analyses, partitions of genotypic variance are difficult to interpret (Baker, 1978; Sokol & Baker, 1977). Estimates of variance due to general combining ability (GCA) may include variation due to dominance and epistasis as well as additive, and estimates of variance due to specific combining ability may include dominance and epistasis. Also, diallel crosses usually involve a fixed set of inbred lines which limits inferences to that selected set. Often, diallel studies only qualify the presence or absence of genetic effects and focus on the combining ability of particular parents. Burton (1987) surveyed those studies in which variances due to GCA and SCA were estimated. The results of that survey along with more recent results are presented in Table 4.1 as ratios of GCA to SCA. Only those ratios are presented for traits where SCA (nonadditive) sources of variation were significant. It should be noted that diallel parents in all of the studies cited were G. max cultivars or breeding lines.

It is clear that the presence or absence of significant variation due to SCA for a particular trait is diallel cross specific. No trait that has been studied which showed significant SCA in every diallel cross. Yield showed significant SCA in five of the seven diallel crosses in which it was measured (Table 4.1), and seed size (g /100 seeds), a component of yield, showed significant SCA in six crosses. Plant height and maturity showed significant variation due to SCA in four diallels, and SCA was significant for protein and oil in the only experiment where seed composition was measured. The GCA/SCA ratios ranged from 1.1 to 37.5. Thus, as with self-fertilization designs, the preponderance of genetic variation observed in diallel crosses seems to be due to additive effects.

Another type of cross-fertilization design, the North Carolina Design I, has been used in two soybean studies. In one of these, Pushpendra and Ram (1987), used two biparental F_2 populations as their reference populations, and in the other, Priadi (1993) used an insect facilitated random-mating population as a reference. In both studies, standard errors of genetic variance component estimates were large which made interpretation of the results difficult.

Implications for soybean breeding

The foregoing estimates of genetic variance components must be carefully interpreted for the following reasons: 1) errors associated with variance component estimates are large. 2) estimates can only be related to a specific reference set of genotypes (in the case of diallel crosses, this reference set is usually small (10 or less) and fixed), 3) when genetically heterogeneous lines are used (e.g. F_2, F_3, F_4 lines) to estimate genotypic variance, bias due to intergenotypic competition may affect the results (Burton, 1987), and 4) linkage disequilibrium among progenies derived by self-fertilization from biparental crosses can increase epistatic components of variance (Cockerham, 1963). Nevertheless, when properly interpreted, estimates of genetic variance components can influence soybean breeding practice.

Because soybean cultivars are pure-lines, dominance variance is generally considered to be unimportant unless early generation selection is being practiced. Burton and Brownie (2006) reported significant inbreeding depression for seed yield in inbred generations of a single cross between two high yielding cultivars. The genetic causes of the inbreeding depression are not known. Because soybean is self pollinating, we can assume the genetic load to be negligible. So, deleterious genes with large effects are probably not a factor. Molecular studies have now demonstrated that soybean is an ancient polyploidy (paleopolyploid) as a result of one and probably two genome duplications (Lee, et al., 1999; Schlueter et al., 2004). Thus the genome is highly redundant with independent genes encoding different isoforms of the same protein, and there is the intriguing possibility that duplicate loci may mimic or function as heterozygous loci in F_1 hybrids, (Mackey, J., 1970). This may mean that more attention should be given to developing methods for early generation selection. Recurrent half-sib selection with a tester could be useful in this regard (Feng et al., 1004). Variability due to dominance might be more useful if a method were devised for economically producing large amounts of F_1 hybrid soybean seed.

Additive x additive types of epistatic are fixed in homozygous lines. Thus, variation due to additive x additive epistasis in a population should not greatly change breeding procedures (Brim, 1973). On the other hand, breeding value of a parental line based on its mean performance might be overestimated and early generation selection would likely be less effective if epistasis were important (Burton, 1987).

HETEROSIS

In general, heterosis has not been a factor in the improvement of self-pollinated crop plants through plant breeding. Hybrid wheat and rice are the two exceptions. In the U.S., heterosis in wheat has been used in hybrid breeding.

In China, rice hybrid yields above those of standard pure-line cultivars has fueled a renewed interest in the development of F_1 hybrids in soybean. The partitions of genetic variance described in the previous section suggest that non-additive sources of variance are of minor importance in soybean compared with additive sources. However, only a small number of breeding populations were characterized in those studies and in some instances, non-additive variance was present. Because nonadditive gene action (either dominance and/or epistasis) may result in heterosis, investigation of heterosis in soybean is warranted.

Burton (1987) reviewed nine studies in which heterosis had been estimated. In six of these studies, yield heterosis was determined from yield comparisons of spaced single F_1 and parental plants. In the results, 238 different F_1 hybrids averaged 21.4% higher yields than the midparent and 11.1% higher yields than the highest yielding parent (Table 4.2). In the three studies where F_1 and parental plants were grown in bordered row plots that more nearly represented normal farm production practice, 47 hybrid combinations were evaluated. In those experiments, yield results showed 9.6% average midparent heterosis and 4.5% average high-parent heterosis. In six spaced plant evaluations of F_1 performance, average midparent per-plant yield heterosis ranged from 24.6% to 90.1%. High-parent heterosis ranged from 9.4% to 57.8% in four of those studies (Table 4.2). Two other studies where F_1 hybrids were tested in bordered row plots found midparent yield heterosis between 2.5% and 9.3% in 6 crosses (Lewers et al., 1998) and high-parent yield heterosis of 16% and 5% in two crosses (Burton and Brownie, 2006).

Heterosis has also been estimated in comparisons of parents and their F_2 progeny (Loiselle et al., 1990; Gizlice et al., 1993). In soybeans, these studies have an obvious advantage over studies utilizing the F_1 because F_2 seed supply is not limiting. On the other hand, intergenotypic competition within plots may bias the estimate of heterosis. This would be likely in F_2 generations which segregated for maturity and large differences in plant height. Assuming the F_1 heterosis is due to dominance, F_2 heterosis would equal one-half F1 heterosis. Loiselle et al. (1990) evaluated 55 F_2 populations generated in an 11 parent diallel. The F_2 populations and the parents were grown at 3 locations, in three replications of bordered two-row plots. Midparent yield heterosis averaged over the 3 locations was 10.8%. The lowest average heterosis was found at the highest yielding location, which suggests that heterotic response may not be stable across environments. Gizlice et al. (1993) evaluated F_2 bulk populations generated from a 5 parent diallel of ancestral soybean strains and found average yield heterosis to be 9.3%. In the study, F_2 heterosis was compared to genetic similarity measures. One similarity measure was estimated from genetic variation for ten traits among the five parents measured in a controlled growth chamber. The correlation between this measure and heterosis was significant and negative (-0.55) which suggests that more dissimilar genotypes are more likely to exhibit higher heterosis. Even so, two of the F_2 populations with very low estimates of similarity (0.00 and 0.03) showed only 6% and 1% heterosis.

Heterosis for seed composition traits, oil and protein concentration, was generally found to be nonsignificant in most studies where it was investigated (Leffel & Weiss, 1958; Brim & Cockerham, 1961; Weber et al., 1970; Nelson & Bernard, 1984; Dayde´ et al., 1989; Loiselle et al., 1990; Gizlice, et al., 1993). Only midparent heterosis for oil concentration was occasionally found to be significant, and in those instances, the heterosis was negative (Leffel & Weiss, 1958; Weber et al., 1970; and Loiselle et al., 1990). Lewers et al. (1998) found significant midparent heterosis for oil content from -2.2 to 2.2% and for protein content from -1.4 to 0.

The above results are good evidence that particular hybrid combinations in soybean can produce significant high-parent yield heterosis. In addition, this increase in yield is not accompanied by a significant change in seed composition. It is not known what proportion of the yield heterosis is due to dominance and dominance types of epistasis and what proportion is due to additive by additive epistasis. Results of Burton and Brownie (2006) provide evidence for dominance but not dominance types of epistasis. They summarized various genetic causes of dominance and epistatic variation and conclude that all "can be fixed in a pure-line cultivar with the exception of over dominance at single loci." Thus, a pure-line could presumably be selected which would be equivalent in yield to the F_1 hybrid. The progress in genetic yield improvement over the last 50 years has been primarily a result of pure-line selection and recombination which is testimony to the importance of additive gene action and possibly additive x additive types of epistasis (Boerma 1979; Wilcox et al. 1979; Specht and Williams, 1984; Specht et al., 1999). Even so, high-parent F_1 heterosis as a rapid means to significant yield improvement is a tantalizing possibility.

Experimental evidence which clearly demonstrates the superiority of F_1 hybrids relative to modern pure-line cultivars is needed. Methods for producing quantities of F_1 hybrid seeds are needed which permit widespread side-by-side testing of hybrids and cultivars. The most promising methods devised to date involve insect facilitated cross-pollination of nuclear male-sterile plants. Nelson and Bernard (1984) mixed a "female" line which segregated for ms2ms2 nuclear male-sterility with a normal "male" line. Hybrid seed production blocks were arranged so that seeds of the female line (either ms2ms2 or Ms2ms2) were planted 10 m apart in every third male row. This minimized cross-pollination of a male-sterile "female" plant with pollen from a fertile (Ms2ms2) sibling and eliminated the need for roguing the male-fertile segregants. Burton and Carter (1983) suggested using male-sterile maintainer lines which have the green cotyledon trait (d1d1d2d2) as female parents. Any yellow seeded cultivar can serve as the male parent. Hybrid seeds will have yellow cotyledons and can be distinguished from green cotyledons (those due to sib mating). A third method has been described by Lewers et al. (1996) which takes advantage of the close linkage between the W1 flower color locus and the ms6 nuclear male-sterility locus. With this method, termed cosegregation, purple-flowered male-fertile plants (W1W1MS6ms6) can be distinguished from their white flowered male-sterile siblings (w1w1ms6ms6) at the seedling stage. Thus, male-sterile plants can be rogued from the plots as seedlings.

This latter method would also permit a comparison of F_1, F_2 and later generations so that a good estimate of inbreeding depression could be obtained. This would help to resolve the issue of whether dominance or additive x additive epistasis is responsible for heterosis in soybean. Inbreeding depression is a more reliable indicator of dominance than heterosis (Compton, 1977). There are a few experimental estimates of inbreeding depression in soybean. Brim and Cockerham (1961) Lewers et al., 1998; Rohangdale & Raut (2002) and Burton and Brownie (2006) have all reported significant inbreeding depression in some of the populations they investigated. Thus as with heterosis, the frequency and extent of inbreeding depression in soybeans has yet to be determined.

Cytoplasmic male-sterility has been discovered in soybean (Sun, 1999). If a reliable maintainer-fertility restorer system can be devised, this male-sterility could become an important tool for studying heterosis. It also may have utility for producing hybrid seeds on a commercial scale provided the cytoplasmic source does not adversely affect yielding ability.

HERITABILITY

Heritability of a trait is usually defined as the proportion of phenotypic variance for that trait that is due to genetic variation. Heritability estimates for particular traits of a plant population provide information about the strength of environmental influence on the traits relative to genetic influences. Also, heritability estimates are used to predict response to selection (Hanson, 1963). Because soybean breeding is essentially a process of plant population development and management followed by derivation, testing, and selection of inbred lines, heritabilities are usually estimated with data obtained from designed field or greenhouse tests of lines at various levels of inbreeding. Phenotypic and genotypic variances are derived from an analysis of variance (Johnson et al., 1955a). Other heritability estimation methods, the difference method and parent-offspring regression, often involve single-plant evaluation. In the difference method, non-segregating generation variance (parents and/or F_1's) is subtracted from segregating generation (F_2, F_3, etc.) variance (Weber & Moorthy, 1952) to get an estimate of genetic variance. Usually with parent-offspring regression, the F_n family means obtained in one growing season are regressed on F_{n-1} single plant values obtained the previous growing season. (Nyquist, 1991). Or F_n families are regressed on F_{n-1} families where each pair is derived from a different single plant ancestor. A fourth method, realized heritability estimation, is based on the ratio of selection response to selection differential (Falconer, 1960).

Estimates of heritability relate specifically to the particular reference population of genotypes for which they were estimated (Dudley and Moll, 1969). They also

relate only to the specific set of environments and experimental conditions in which the genotypes were tested. The type of experimental unit (e.g. single plant, plot, entry mean) should be noted for proper interpretation and be consistent with the intended use of the heritability estimate. Estimates may also be biased. Parent offspring estimates may be biased by phenotypic family differences when parents and offspring are tested in separate environments (Nyquist, 1991). The difference method may be biased by genotype-environment interactions (Nyquist, 1991). All single plant methods provide unreliable estimates for traits, such as yield, which are affected by intergenotypic competition because single-plant performance is not equivalent to performance under high plant densities of typical farm production systems (Hanson, 1963).

Predicting response to selection

In plant breeding the primary value of a heritability estimate is for use in predicting selection response. Nyquist (1991) gives the selection response equation as:

$$\Delta G = [Cov(X,Y)/\sigma_x^2]S$$

Where: ΔG = selection response ie. change in the population mean,

Cov(XY) = covariance between the selection unit, X, and individual Y which is derived from X either by self-fertilization or by mating with another selection X',

S = selection differential.

The Cov(XY) is equivalent to the product of the genetic variance of selection units, σ_g^2, and a coefficient c which represents the control of parentage in the intermating process (Nyquist, 1991). The coefficient, c, may be either 1/2 or 1, or 2. Thus, $Cov(XY)/\sigma_x^2$ is equivalent to $c\sigma_g^2/\sigma_x^2$ or c times the heritability, ch^2. In self-pollinated species where selection is practiced in generation g and progress accrued in a later generation g′ , c=1 and $Cov(XY)/\sigma_x^2$ is equivalent to the heritability, h^2.

In soybean, meaningful evaluation of most traits requires testing of lines (families) in more than one environment. This requires self-pollination which creates inbred relatives. Genetic variation among inbred relatives includes variance sources other than additive, dominance, and epistasis. Weir and Cockerham (1977) derived the covariance of relatives for loci in linkage equilibrium that is general for any level of inbreeding, any gene frequency and any number of alleles. In addition to additive (σ_A^2) and dominance (σ_D^2) variances, four more terms were needed specifically for inbreeding. These were D_1, the covariance of additive and dominance affects of homozygotes; D_2, the variance of dominance affects of homozygotes; and H* which is the sum of squares of h_i, the inbreeding depression at locus i, (mean of the inbred population minus the mean of the non-inbred population at the i^{th} locus). Inbreeding depression for the i^{th} locus is h_i and the total inbreeding depression is the sum h_i over all segregating loci (Nyquist, 1991).

For self-pollinated species like soybeans, the covariance of interest is that for inbred relatives. The covariance for two inbred relatives, X in generation g and Y in g′, with their last common ancestor in generation t (where t ≤g≤g′) is a follows:

$$Cov(X,Y \mid t;g,g') = Ctgg' = (1+F_t)\sigma_A^2 + [(1-F_g)(1-F_{g'})/(1-F_t)]\sigma_D^2 + (F_g + F_{g'} + 2F_t)D_1 + [F_t + (F_g-F_t)(F_{g'}-F_t)/2(1-F_t)]D_2 + [F_t(1-F_g)(1-F_{g'})/(1-F_t)]H^* + (1+F_t)^2\,\sigma_{AA}^2$$

In biparental populations with only 2 alleles at segregating loci, $H^* = \sigma_D^2$ and assuming inbred parents, gene frequencies at those loci are all ½, so that both D_1 and $D_2 = 0$. For those populations:

$$Cov(X,Y) \mid t;\, g,g') = (1+Ft)\sigma_A^2 + [(1+F_t)(1-F_g)(1-F_{g'})/(1-F_t)]\sigma_D^2 + (1+F_t)^2\sigma_{AA}^2$$

The coefficients for σ_A^2, σ_D^2, and σ_{AA}^2 are presented in Table 4.3 (following Nyquist, 1991) for inbred lines derived from the F_2, F_3, F_4, or F_5 generations. From these, it is clear that estimates of heritability obtained from evaluation of lines derived in early generations would include a portion of dominance variance in the numerator. Estimates of selection response based on these estimates of heritability would be biased upwards if dominance were a significant part of the genetic variation.

All other inbred populations in which allele frequencies cannot be assumed to be ½ would have covariances of relatives which would include D_1, D_2 and H*. If there were no dominance variance, then D_1, D_2 and H* would all be zero.

In considering response to selection among inbred lines of a biparental population, the covariance of interest is Ctgg′ where t is the generation in which the line is derived, g is the generation in which it is tested, and g′ is the generation in which genetic gain is finally obtained. When pure-line cultivars are the eventual selection product, it is reasonable that g′ should equal infinity, when all dominance variance has dissipated. In a typical soybean breeding program, biparental progenies would be advanced by single seed descent to the F_4 generation (t=2), testing and selection would occur in the next generation (g=3) and ultimate gain assessed in a later generation (g´=∞ for convenience). Thus, gain from selection would be:

$$\Delta G = kCtgg'/\sigma_x = kC_{23\,\infty}/\sigma_x = k(1.75\sigma_A^2 + 3.06\sigma_{AA}^2)/\,\sigma_x$$

where σ_x^2 is the phenotypic variance for F_4 derived lines tested in the F_5 generation. Prediction of response in experiments where selected families or individuals are intermated and genetic gain is measured in the new out-crossed population has been discussed by Burton and Carver, (1993).

Empirical estimates

Estimates of yield heritability ranged from 0 to 74% in 25 different soybean breeding populations tested in 9 different studies (Table 4.4). The overall average heritability for yield was 63%. In general, all yield related traits had higher heritabilities than yield. The average variance component heritability estimate for seed weight was 81%. However, realized estimates were much lower, ranging from 32% to 12%. Heritabilities for other yield related traits, plant height, height of first pod, lodging, days to flower, days to full pod, days to maturity, averaged over population estimates were all greater than 50%. Tukamuhabwa et al. (2002) in a half diallel of breeding lines that were resistant, susceptible or intermediate for shattering estimated heritability for shattering resistance to be 70%. These estimates confirm what soybean breeders know from practice, that genotypic yielding ability is more difficult to determine than most other traits because it is so heavily influenced by environmental effects.

Heritabilities for seed composition are generally high. Variance component estimates of protein heritability for 25 biparental populations ranged from 51 to 96 (Table 4.5). Realized estimates for two recurrent selection populations were 29 and 34. Estimates for sets of half-sib families from 3 random mating populations were 65%, 78%, and 82%. Similar results were obtained for other seed components, percent oil and percent sugar, and fatty acid composition of oil. Heritability for another trait, termed cookability, which is likely dependent on seed composition was estimated at 70 for 2 populations.

Heritability estimates for physiological traits, averaged over the populations in which they were measured, were all greater than 50, except some estimates for canopy temperature, seed filling period, and iron efficiency (Table 4.6). Traits related to photosynthesis, harvest index, canopy photosynthesis and canopy temperature, ranged from 0 to 82. Shibles and Sundberg (1998) found that total leaf nitrogen at R5 and reproductive duration were associated with yield. But failure of the traits to be consistent from year to year suggested low heritability. Traits related to nitrogen fixation, nodule mass, total N, and fixed N, had heritabilities between 49 and 67.

Because of difficulties involved in measuring resistance to disease and insects (usually related to environmental influence), heritabilities for these traits are often low. Heritabilities for tolerance to soybean cyst nematode, resistance to brown stem rot, and tolerance to Phytophthora ranged from 19 to 88 (Table 4.7).

CORRELATION

Phenotypic and genotypic correlations among quantitative traits are common in plant breeding. For example in soybean populations, it is common to find seed protein and seed oil concentrations to be negatively correlated. Relations such as this between traits are important because they affect the overall outcome of selection. In the above examples, selection for increased protein concentration usually leads to a decrease in oil concentration (Brim and Burton, 1979). Thus a selection improvement (more protein) is offset by an undesirable consequence (less oil). On the other hand, single-trait selection when there is a desirable correlated relation to another trait can produce a favorable selection response in both traits. Correlation between traits may also provide insight into plant biology. For instance, if selection for increased yielding ability resulted in higher rates of photosynthesis, one could infer that increased photosynthetic rates were needed for higher crop yields.

Genotypic correlations between traits may be the result of either gene linkage disequilibrium or pleiotropy. If the former is the cause of the correlation then genetic recombination should eventually dissipate the correlation. Pleiotropic effects are more difficult to deal with and may require special methods to produce a desired breeding outcome. These usually require introduction of novel germplasm into the breeding population and/or various multiple trait selection techniques.

When initiating a breeding program, parental choice decisions are crucial for the long and short-term success. Those decisions can be wise and informed only when there is an understanding of genetic correlation between traits and knowledge of how selection for one trait is likely to affect other traits of agronomic and economic importance.

Correlated response of a trait Y to selection on a trait X ($\Delta GY\ X$) is predicted as follows:

$$\Delta G_{y \cdot x} = k\sigma_{py}h_y h_x r_A$$

Where k is the standardized selection differential, σ_{py} is the phenotypic standard deviation for trait Y, h_y and h_x are square roots of heritabilities for traits Y and X, and r_A is the genetic correlation between trait X and Y. This equation would be equivalent to predicted gain from direct selection on trait Y (ΔG_Y) if $h_X r_A$ were equivalent to h_y. This is the necessary condition if indirect selection for Y by direct selection on X is to be equivalent to direct selection on Y. In soybeans, there are examples where indirect selection for a trait is more efficient or as efficient as direct selection for that trait. Bravo et al. (1980) found that selection for pod width on an individual plant basis was more effective for increasing seed weight that direct selection for increased seed weight. Similarly, LeRoy et al. (1991) found that selection for decreased pod width in three different populations was more or equally as effective in decreasing seed weight as direct selection for small seed size.

This was true whether the selection unit was a single plant, single plot or entry mean. In another example, indirect selection in a population for increased yield by selecting directly for the correlated trait canopy apparent photosynthesis (r_A=0.96) was 1.46 times more efficient than direct selection for yield (Harrison et al., 1981).

Examples like the above are uncommon. Direct selection is usually more efficient than indirect selection (Johnson et al., 1955b; Byth et al., 1969b). Even so, indirect selection may still be desirable when the overall breeding process is considered. For instance, the ease and expense of indirect selection may be more favorable than direct selection. Time may also be a factor. Most cycles of selection for yield in soybeans require at least 3 growing seasons, one for intermating, one for seed increase, and one for yield evaluation. If indirect selection would permit selection in each growing season, genetic gain per year might exceed those for a direct selection. Tinius et al. (1991) found that mass selection for increased weight of seeds from the male-sterile plants of an insect-facilitated random mating population successfully increased average seed weight and seed yield of male-fertile plants in the population. With this procedure, a cycle of mass selection was completed every year and the yearly gain in yield was 47 kg ha^{-1}. By comparison, direct S_1 family selection for yield in the same population, which required 2 years/cycle, produced yield increases of 19 kg ha^{-1} per year (Burton et al., 1990). Li and Burton (2002) found that indirect selection for seed weight and seed density could increase both yield and protein.

Estimates of genotypic and/or phenotypic correlations between yield, and eight other traits (seed weight, height, lodging, days to flower, fruiting period, maturity, protein and oil) are presented in Table 8. In addition to those, Burias and Planchon (1990) found positive phenotypic correlations between the yield of F_4 lines and the traits, N_2 fixation (0.29), nodule volume (0.23) and nodule dry weight (0.20). N_2 fixation in the study was determined at R5 by the acetylene reduction method. Pantalone et al. (1996) found genotypic correlations between fibrous root score and yield of -0.56 and between root score and protein of 0.42.

SELECTION

Population development

In soybean breeding as in all plant breeding, the initial step in cultivar selection is population development. It may be the most critical step because ultimate success will depend on a beginning population that has adequate genetic variation for achievement of a particular breeding goal. Choice of parents is important. Maximum variation for a trait is desirable, but overall agronomic quality of the lines must also be considered.

It is logical to assume that unrelated lines would produce the most genetically variable progeny. Manjarrez-Sandoval et al. (1995) showed that genetic variance among F_6 lines derived from single-crosses was inversely related to the coefficient of parentage between the two parents.

Also, to be considered is the number of favorable independent loci controlling a trait with different alleles in each parent. If this number is similar in both parents of an F_2 population, then there is a high probability that a superior transgressive segregate can be found (Bailey and Comstock, 1976). If there is a large difference in the number of favorable alleles carried by each, then it will be difficult to select a homozygous line with more favorable alleles that the superior parent. Panter and Allen (1995) suggest that performance as well as pedigree information should be used in choosing parents. The information is included in a mixed linear model for calculating the best linear unbiased prediction (BLUP) of breeding values of potential parents. In practice, they found parents chosen with BLUP results produced superior cross combinations compared to parents chosen by midparent value.

Populations are often developed by 2-way, 3-way, or 4-way crosses of cultivars and/or breeding lines. If it is desirable to use unadapted germplasm then at least one backcross to the adapted parent is often used. This is necessary because unadapted germplasm is less productive, and mean population yield performance has been shown to be linearly related to the proportion of exotic germplasm in the population (Vello et al., 1984). A single backcross will likely result in a lower frequency of derived exotic phenotypes, but the frequency of lines with a desirable composite phenotype (high yield and exotic) will likely be higher (Hintz et al., 1987).

Pure line selection

Populations are advanced through generations of inbreeding in several ways, including standard pedigree selection, modified pedigree selection (single seed descent) (Brim, 1966), or bulk selfing. In the latter two, pure-line identification, evaluation, and selection take place in later (F_5-F_7) generations. With pedigree selection, desirable families are selected (often visually) in each generation and one or more plants from within each family are advanced through selfing to the next generation.

Response to selection by any of the above methods is obtained in homozygous lines derived by self-fertilization, without intermating, from the reference population. From previous discussion (Section 3.1), it is apparent that response to selection largely depends on the level of inbreeding of single plants from which lines are derived and the generation in which they are tested. This may differ from the response in the outbred generations which results when selected lines are intermated (Cockerham and Matzinger, 1985).

Selection in early generations can be shown to be desirable provided the genotypic worth of single F_2, plants F_3 and/or F_4 lines can be successfully determined.

With no selection in an early generation, there is a low probability that a line will be isolated that is homozygous for a large number of favorable alleles (Sneep, 1977; Bailey, 1977). With 20 segregating loci in an F_2 population, Bailey (1977) determined that <2 in 10,000 inbred lines developed without selection could be expected to have as many as 18 loci homozygous for the favorable allele. Using computer simulation, Bailey and Comstock (1976) investigated selection among F_2 plants of a biparental population followed by intraline selection in the F_3 and F_4 generations. Lines were advanced to the F_8 generation by single seed descent. In their results, the probability of fixation of favorable alleles was increased by selection and higher intraline heritabilities. Probabilities were also increased by coupling linkages and decreased by repulsion linkages. Other simulation studies have also shown that early generation selection of traits with higher heritabilities is more efficient in producing superior F_6 lines than selection delayed to the F_5 and F_6 generations (Casali and Tigchelarr, 1975; Yonezawa and Yamagata, 1981). At low heritabilities, it is best to delay selection to the F_5 or F_6 generations. Early generation selection of high heritability traits can decrease the genetic variation in the populations for a trait with lower heritability, such as seed yield (Brim, 1973). This problem might be overcome by beginning with a very large F_2 population (Yonezawa and Yamagata, 1981).

Experiments have been conducted which were designed to compare the relative efficiency of pedigree selection (PS), single seed descent (SSD) and early generation testing (EGT). Boerma and Cooper (1975) tested all three simultaneously in four biparental populations with increased yield as the object. They found no consistent differences among the populations either in mean of selected lines or in mean yield of the five highest-yielding lines from each. Based on these results, they recommended the SSD method since it was the least costly of the three. Snape and Riggs (1975), came to a similar conclusion in simulation studies. Cooper (1990) described a modified version of his earlier EGT method which he has found to be both efficient and successful. Molari et al. (1987) tested four different early generation testing procedures and found no positive selection response in yield prior to the F_5 generation. Likewise, Pushpendra and Ram (1987) found selection for yield and harvest index in the F_2, F_3 and F_4 generations to be ineffective. They did find a positive response to early selection for number of pods. St. Martin and Geraldi (2002) compared the effectiveness of selection based on single replication tests of F_1, F_2, and F_3 derived families from two multiparent, intermated populations. Selection intensities were between 25% and 35%. Selected families were evaluated as $F_{1:3}$, $F_{2:4}$, and $F_{3:5}$ and compared with unselected control lines in replicated experiments. Gain in yield for each of the selection methods was about 4% of the mean of the control lines. In an earlier study, St. Martin and Xie (2000) evaluated a multi-stage selection approach where 11% of lines were selected at the F_3 stage, 21% were selected at the F_4 stage, and 18% were selected at the F_6 stage. Genetic gains for yield as a percentage of the mean of common check cultivars were -1.4% in the F_3, 3.7% in the he F_4 and 9.1% in the F_6. The negative gain in the F_3 was probably a result of single replicate evaluation and additional selection for early maturity.

Streit et al. (2001) did selection among F_2 families followed by selection within families based on F_3 derived line performance in yield tests and compared the results with random F_3 derived line selection without regard to family structure. They concluded that the early generation yield tests were useful but that selection based on family performance gave essentially the same results as random line selection. Byron and Orf (1991) successfully selected for earlier maturity in four populations using PS, SSD, or SSD with early generation selection. In addition, the lines selected by each procedure were not different in yield, maturity, height, lodging, seed weight or fill period. Burias and Planchon (1990) were able to select for increased N_2 fixation (acetylene reduction) at R5 in three biparental F_2 populations and indirectly increase yield in F_4 derivatives of the selection.

Finally, it has sometimes been suggested that intermating the F_2 and/or F_3 generations prior to inbred line derivation would be useful. However in simulation studies, Pederson (1974) and Stam (1977) found no benefit was obtained from intermating prior to selection.

Population improvement - single trait selection

Recurrent selection has been used to improve seed yield (Kenworthy and Brim, 1979; Sumarno and Fehr; 1982; Piper and Fehr, 1987; Burton et al., 1990; Rose et al., 1992), seed composition (Brim and Burton, 1979; Burton et al., 1983; oil quality (Burton et al., 1983; Carver, et al., 1986), seed weight (Tinius et al., 1991), date of flowering (Nelson, 1988), iron efficiency (Beeghly and Fehr, 1989), and reproductive period (Hanson, 1992). Even so, most soybean breeding efforts are still concentrated in traditional PS and SSD methodologies, and very few cultivars have been derived from recurrent selection programs. This has been partly due to the laborious nature of random mating selections in each cycle by hand pollination. More importantly, because soybean cultivars are pure-lines, recurrent selection is often perceived by breeders as a circuitous route to such an objective (Burton, 1987). There is also concern that recurrent selection populations are typically closed and efficient ways to bring new improved sources of germplasm into the system are not obvious.

As previously discussed, however, with multigenic traits there is a low probability using either PS or SSD of obtaining a homozygous line with many more favorable alleles than the best parent. Thus, because most of the genetic variation in soybean is additive, recurrent selection should be successful in increasing the frequency of favorable genes. In support of a cyclic selection program with intermating, Bailey and Comstock (1976) used the following argument. If the probability of fixation of favorable alleles for a pair of first cycle lines is $p = 0.7$ (for example), then the probability that both lines will be homozygous for a favorable allele is 0.49, but the probability that both lines will lack the allele is $(1-p)^2 = 0.09$. Generally, if n lines are chosen for intermating then the probability that none of the n lines is carrying the favorable allele is small, $(1-p)^n$.

Various selection methods have been used or proposed for use with soybean. These include mass selection (Burton and Brim, 1981; Tinius et al, 1991), selection among selfed half-sib families (Burton and Carver, 1993) or within half-sib families (Burton et al., 1983; Carver et al., 1986), and S_1 (or S_2) family selection (Kenworthy and Brim, 1979; Brim and Burton, 1979; Sumarno and Fehr, 1982; Rose et al., 1992). The discovery of genetic male sterility (Brim and Young, 1971) provided a means for insect mediated random mating that eliminated the need for hand-pollination (Brim and Stuber, 1973). It has worked well in mass selection programs (Burton and Brim, 1981; Burton et al., 1983; and Tinius et al., 1991). When S_1 family selection is used in a population with genetic male sterility, male-sterile plants segregate in the test population. The male-sterile plants which occur in the plots are nearly barren. This affects plot yield measurement and can make it difficult to obtain good estimates of genotypic yielding ability (Nelson, 1987). St. Martin (1981) has suggested using S_2 or S_3 family testing to lessen this problem. Even so, the yielding ability of S_1 families which segregate for male sterility has been successfully used as a criterion for increasing yield (Burton et al., 1990).

A natural consequence of using genetic male sterility as a random-mating aid, is the creation of half-sib families. All of the seeds produced on a male-sterile plant are the result of random insect pollinations and therefore are half-sibs. With S_1 family testing, each half-sib family is grown for seed increase and one male-fertile S_0 plant is chosen from each family. The seeds from this plant are used for S_1 family evaluation. Because the number of seeds produced on a single plant is often inadequate for testing, selfed half-sib families (SHS) could be used as the selection unit by combining seeds from 2 or more plants in each family. If hand pollinations were used for intermating, selfed full-sib families (SFS) could be derived from the seeds of a single pod. Burton and Carver (1993) compared the expected genetic gain from selection among S_1, SHS and SFS families. Disregarding epistasis (assuming large families (M>10) of half or full-sibs are selfed), the expected gain for each is,

$$\Delta Gs_1 \cong i(\sigma_A^2 + \tfrac{1}{2} D_1)/\sigma_{s1} ,$$

$$\Delta G_{SHS} = i[(1/16M + 7/16)\, \sigma_A^2 + (9/32M + 7/32)\, D_1]/\, \sigma_{SHS} \cong$$

$$i(7/16\, \sigma_A^2 + 7/32\, D_1)/\, \sigma_{SHS},\ i$$

$$\Delta G_{SFS} = i[(1/8M + 7/8)\, \sigma_A^2 + (1/16M + 7/16)\, D_1]/\, \sigma_{SFS} \cong$$

$$i(7/8\, \sigma_A^2 + 7/16\, D_1)/\, \sigma_{SFS} ,$$

where σ_{S1}, σ_{SHS}, and σ_{SFS} are phenotypic standard deviations of family means. Coefficients of the additive variance increase as family size decreases and with a small family size (m=2), gain from selection among SFS families is similar to selection among S_1 families assuming phenotypic variances of the two are similar. In fact, empirical estimates of phenotypic variance for SHS families derived from two random mating soybean populations, tended to be smaller than phenotypic variances for S_1 families.

This also reduced the difference in expected gain between the two selection methods. Relative efficiency of the two, assuming a large family size, would be approximately 2.29 σ_{SHS}/σ_{S1} Based on their estimates of phenotypic variance, selection for yield among S_1 families could be .98 to 1.93 times as efficient as selection among SHS families, and for protein selection, from 1.10 to 2.17 times as efficient. As explained above, this difference in efficiency would be lessened with small families having only 2 or 3 sibs.

The final issue that should be considered is the size of populations to be tested and the proportion to be selected in each cycle. As Rawlings (1980) states, one needs to choose a population size that is large enough to minimize the loss of favorable alleles due to random drift and yet small enough to maximize genetic gain within the constraints of available resources. This problem has been approached by investigating the probability of fixation of an allele, given various initial gene frequencies, numbers of loci and trait heritabilities (Baker and Curnow, 1969; Bailey and Comstock, 1976; Rawlings, 1980). Effective population size is the critical number to be determined. It is approximately, but not necessarily equal to, the number of individuals (selection units) selected in a cycle to serve as parents of the next generation of intermating. Results of the above investigations generally showed that with effective population sizes between 20 and 30, short-term selection progress could be made without seriously limiting long-term selection gain (Burton, 1987). In an empirical look at short-term progress, Brim and Burton (1979) found that the rates of progress in selection for higher protein were similar whether 12 lines or 45 lines were saved in each cycle.

These results and practical experience demonstrate that recurrent selection programs can be designed that are within the resources of most breeding projects. Populations need not be large to realize genetic progress assuming adequate genetic variation exists within the initial breeding population. In soybean, adequate genetic variance is a concern considering that most soybean varietal development populations originated from a relatively small number of plant introductions. In a comprehensive study of the genetic base for 258 North American soybean cultivars released between 1947 and 1988 by public breeding programs, Gizlice et al. (1994) found that 80 ancestors contributed all the foundation germplasm. More than half of this genetic base was contributed by only 6 of the 80 ancestors. The remainder contributed between 3.8% and 0.01%. For cultivars grown in the Northern U.S. (MG≤4), the unknown parents of 'Lincoln', 'Mandarin', 'Richland', 'A.K. Harrow' and 'Mukden' account for 64% of the genetic base. In the southern states (MG≥5), 'CNS' and 'S100' contributed 45% and an additional 21% was contributed by 'Tokyo', PI54610, and 'Roanoke'. Further analysis showed that 75% of the genes in modern cultivars trace to only 17 cultivars released before 1960.

Luedders (1977) suggested that soybean cultivar development in this century has been similar to a recurrent selection program which by now would be in its fourth or fifth cycle. Using coancestry measures, St., Martin (1982) found that the coefficient of parentage among 27 cultivars released between 1976 and 1980 (fourth cycle selections)

was 0.25. Thus the average inbreeding coefficient of plants derived in a diallel cross of the cultivars would be 0.25. Using the formula, $F_t = 1/N + [(N-1)/N]F_{t-1}$ (Hanson et al., 1967), where N is the population size and t is the cycle of selection, he determined the effective population size was 15, assuming t = 4 cycles of selection, and 11, assuming t =3. These results suggest that some genetic variability has been lost from the original breeding populations. While the importance of the lost genes cannot be ascertained, it is reasonable to conclude that long-term progress in soybean cultivar development will be limited by the small effective population size (Burton, 1987).

Population improvement - multiple trait selection

An agronomically acceptable soybean cultivar meets other standards in addition to having good yielding ability. These include resistance to pod shattering, lodging, diseases, and stress, suitable height for mechanical harvest, and industrially acceptable seed composition (Burton, 1987). This multiplicity of breeding goals poses a problem because as Brim (1973) noted, advantage obtained from superiority in one trait must often be balanced against inferiority in other traits. Thus, simultaneous consideration of more than one trait during the selection process becomes necessary not only to improve the total phenotype but also to prevent an unwanted change in phenotype due to correlated responses (Gardner, 1977; Comstock, 1977).

Three methods of simultaneous multiple trait selection include independent culling, tandem selection and index selection. Independent culling or tandem selection are probably the most widely used, but in theory, index selection is usually the most efficient. Young (1961) showed that superiority of index selection increases as the number of traits under selection increases but decreases as heritability increases or as differences in the relative importance of the traits increase. Pesek and Baker (1969) compared index selection in the F_6 and F_7 generations to tandem selection in those generations. Under tests with different heritabilities, environmental variances, and economic values of two traits, index selection was more efficient for all parameters, particularly at low heritabilities. And yet, tandem selection has been successfully used, to improve traits of high heritability in early generations (e.g. protein and maturity) followed by selection for yield in later generations (Sebern and Lambert, 1984; Byron and Orf, 1991).

A selection index can be defined as $\mathbf{I} = \mathbf{x}'\mathbf{b}$, where $\mathbf{I}$ is the selection criterion, $\mathbf{b}$ represents a column vector of index coefficients and $\mathbf{x}'$ represents a row vector of known phenotypic values for a trait (Lin, 1978). In practice, $\mathbf{x}$ values are measured in a population of genotypes, but the index coefficient $\mathbf{b}$ may be determined in several ways. Lin (1978) and Baker (1986) provide comprehensive summaries of methods for calculating $\mathbf{b}$. The $\mathbf{b}$ values are usually some function of the genotypic and phenotypic variances and covariances of the traits under selection and weights for those traits. In the original Smith-Hazel index, the weights were economic values of the traits under selection (Lin, 1978).

For soybeans, applying economic weights is difficult because there are two levels of economic value, the value to the producer of a volume of soybeans and the value to the processor of oil and protein meal derived from a volume of soybean. Both are subject to seasonal market fluctuation and therefore of little use to a plant breeder who needs a strategy that can be sustained over the several years required for cultivar development. To resolve this dilemma, Brim et al. (1959) used a "base index", where total oil (yield x %oil) and total protein (yield x %protein) were given relative economic weights of 1:1, 5:3 or 5:1. However, they found that yield was the overriding factor in the determination of both traits due to genetic variation and the precision of measuring seed composition. Thus selection for yield per se gives similar results as selection for total protein and/or total oil. Orf and Helms (1994) obtained a similar result when they selected for maximum gross value per hectare (GVH). GVH was defined as value per kg seed (V) times grain yield (Y) in kg ha^{-1} at 130 g kg^{-1} moisture. Value was calculated using three different protein-to-oil price ratios. As with Brim's base index, selection only for yield was just as efficient for maximizing value per hectare as selection for increased GVH.

Another way to solve the problem of economic weighting is to give yield a weight of 1 and all other traits a weight of zero. This is a combination of direct and indirect selection. Supposedly, yield estimation is improved by adjusting for variation in other traits. In soybeans, this type of index has been used where yield was the primary trait (weight=1) and combinations of maturity, lodging, height, seed size, protein and oil were secondary traits (weights=0) (Caldwell and Weber, 1965; Byth et al. 1969a). The indexes were applied to segregating generations of biparental populations. In all cases, selection for yield with the index was either no better or only slightly better than selection for yield alone. Pritchard et al. (1973) used the same kind of index with yield as the primary trait and yield components as secondary traits. They applied the index to the F_4 and F_5 generations of biparental populations and found all indexes resulted in greater progress than selection for yield alone. The index was more efficient in the population with low yield heritability. Both Pritchard et al. (1973) and Byth et al. (1969a) concluded that inclusion of other traits in the index is most helpful when heritability for yield is low due to large genotype x environmental variance.

Restriction indices are another type of selection index which does not involve economic weighting. With these indices, the response of one or more traits is restricted arbitrarily. As proposed by Kempthorne and Nordskog (1959), a restricted index may change one trait while holding another constant. Using such an index, Holbrook et al. (1989) increased yield of a soybean population by 7% without significantly changing oil and protein concentrations. Miller and Fehr (1979) used this type index to increase seed protein concentration and prevent a change in maturity. The desired gains index is another version of restricted indices which restricts more than one trait based on a "desired genetic gain" for each trait (Pesek and Baker, 1970). A drawback to the use of these indices is that the gain in all traits can be lessened if the specified desired gain is unreasonable.

In this regard, industrial requirements for crop quality from planting through harvest and processing should be useful guidelines in determining reasonable desired gains (Burton , 1987). Burton (1991) used another approach to develop a desired gains index for protein and yield. He used the absolute value of the predicted correlated response of protein to selection for yield as the desired gain for protein and the absolute value of the predicted correlated response of yield to selection for protein as the desired gain for yield. Using this type of index in three separate populations, average yield and protein of selected lines were all greater than respective population means. In three random mating populations, Li and Burton (2002) found the genotypic correlation between seed density and protein to be positive (0.90, 0.33, 0.24) and the correlation between seed weight and yield to be positive (0.81, 0.96, 0.19). They showed that by using a desired gains index for seed density and seed weight they could increase yield in all three populations by 2.65%, 2.94%, and 1.56% and simultaneously increase protein by 0.94%, 1.08%, and 0.45%.

If applied in early segregating generations, of biparental populations, indexes might make early generation selection more effective. Weber (1982) investigated the use of family indexes which incorporate information from relatives. To select among F_4 families an index would be developed which would include information from the F_2 and/or F_3 generations. Weights for the family data are determined by maximizing the correlation between the index and the expected genotypic value of homozygous lines sampled from selected families. Weber (1982) demonstrated with simulation that the family indexes maximized gain from selection. Use of an index should also be quite effective in selection for or against more than one trait (Matzinger et al., 1977; Holbrook et al. 1989).

Marker assisted selection

A marker is a character that is linked to a gene, but is not the product of that gene (Weissinger and Moss, 1992). It can be useful as a tool for selection of the gene it is linked to if it is easily screened, not affected by environment, and expressed in both homozygous and heterozygous state. If genes which determine quantitative traits (quantitative trait loci or QTLs) could be identified by linked markers, rapid selection of the trait could be achieved by direct selection of the linked markers rather than the trait itself. In addition, a linkage map of these markers provides information about the number of loci involved in expression of the trait, the chromosomal location of these loci, and the relative size of the contribution of individual loci to trait expression (Stuber, 1992). Molecular genetic science has provided methods for identifying markers linked to QTLs. Isozyme loci were used initially as markers (Stuber et al., 1982). Subsequently, research with DNA markers has resulted in the construction of detailed genetic maps of several plant species (Patterson et al., 1991).

In soybean, five types of DNA markers have been used to construct a linkage map which includes 4 isozyme loci, 365 restriction length polymorphisms (RFLPs), 11 random amplified polymorphic DNA (PAPDs), and 40 microsatelite DNA, and single sequence repeats (SSRs) (Shoemaker and Olsen, 1993; Akkaya et al. 1995) and single nucleotide polymorphisms (SNPs) Shoemaker, et al., 2004). These genetic maps can enhance soybean breeding research in several ways including pedigree tracing and genotype identification in backcross breeding. This review will only discuss this research as it affects knowledge and manipulation of quantitative traits.

Much of the DNA marker research in soybean has been done with a population of lines derived from a cross between A81-356022 (G. max) and PI468916 (G. soja Seib. Zucc.), which were chosen because of their genetic diversity (Keim et al., 1989). QTLs have been identified which affect iron efficiency, hard seededness, protein, oil, maturity, height, lodging, days to R1, days to R8, seed filling period, stem diameter, stem length, canopy height, leaf width, and leaf length (Graef et al., 1989; Keim et al., 1990a; Keim et al., 1990b; Diers et al., 1992a; Diers et al., 1992b). Another early study of lines derived from a cross between two G. Max cultivars, Minsoy and Noir 1, Lark et al., (1993) determined linkages of 132 RFLP, isozyme, biochemical, and morphological markers. Mansur et al. (1993a and 1993b) measured 15 quantitative traits including yield, in 69 F_5 lines derived from the Minsoy x Noir 1 cross to determine cosegregation of QTLs with the markers. Genetic variation was found for all traits. In that investigation, RFLP marker linkages to QTLS could be quickly screened using a bulk DNA sample from two lines with extreme phenotypes for a particular trait. When an RFLP marker was linked to a QTL, one parental allele predominated in the bulked DNA. More recent studies and QTL-trait associations are summarized by Orf et al. (2004).

Of all possible uses of molecular markers in plant breeding, the one which remains the most tantalizing is marker assisted selection (MAS) of quantitative traits. While the method is being used for selection of yield in maize (Stuber et al., 1987), which has had extensive molecular mapping, practical applications in soybean have usually involved selection for one or two genes, usually in backcrossing programs. Selection simulation studies provide information about the usefulness of MAS under assumption of various heritabilities, numbers of loci involved, linkage of QTL and markers, and selection schemes (Edwards and Page, 1994; Moreau et al., 2000; Tar'an, et al., 2003; Bernardo and Charcosset, 2006; Breseghello and Sorrells, 2006). In practice, the term efficiency of MAS for quantitative trait selection compared with traditional breeding has yet to be determined. Ultimately a combination of the two is most likely to provide optimum efficiency. In a review of MAS in crop plants, Francia et al., (2005) note recent advances in genotyping technologies and genomics and conclude that "an increased complementariety between molecular technologies and conventional breeding is expected in the near future for a more efficient improvement of the crop plants".

SUMMARY

Soybean breeding in the USA from a historical perspective, can be viewed as a process of cyclical or recurrent selection in which superior cultivars are selected and released, then recombined and reselected (Burton, 1987). Estimates of genetic gain in yield prior to 1977 in maturity groups 00-IV, range between 0.4% and 1.0% per year (Luedders, 1977; Wilcox et al., 1979; Specht and Williams 1984). Yield progress in maturity groups VI to VIII between 1914 and 1973 was estimated to be 0.7% per year (Boerma, 1979). Since 1977, genetic improvements in yield are occurring at 2 to 3 times the pre-1977 rate estimate of 12.5 kg h^{-1} per year (Specht et al., 1999). In the U.S., investment in soybean breeding in the public and private soybean breeding increased dramatically, which may be responsible for the increased rate of yield advance.

Probability arguments and evidence from simulation studies suggest that cyclical procedures of early generation selection followed by recombination should increase the frequency of favorable alleles in populations and result in more rapid progress (Burton, 1987). This is supported by results from recurrent selection experiments where response to selection for yield has been between 0 and 2.7% per year (Kenworthy and Brim, 1979; Sumarno and Fehr, 1982; Burton et al., 1990; Rose et al., 1994).

Choice of initial parents for any selection progress is critical to its success. Methods need to be developed for better identification of superior parental genotypes. In the absence of other criteria, genetically unrelated parents of nearly equal productivity could probably contribute a large number of favorable alleles to a base population (Manjarrez-Sandoval et al., 1995). Molecular techniques are likely to be useful in determining genetic differences of potential parents. Given the narrow genetic base of cultivated soybeans, effective use of exotic plant introductions in cultivar development should be given a high priority. Rapid cyclical breeding procedures should be very useful for integrating exotic germplasm into a practical breeding program. Such cyclical programs can easily be used to augment standard pedigree procedures either as a source of new breeding lines or as a source of pure-lines with direct cultivar potential. Finally, when multiple traits are to be changed, index selection procedures should be seriously considered along with more common tandem selection and independent culling methods. Long term breeding objectives are usually enhanced by appropriate index methodology. Marker assisted selection is likely to become more useful as marker assays become more economically affordable and their proper and efficient application to practical breeding is determined.

GENOTYPE X ENVIRONMENT INTERACTION

In plant populations, variation in the expression of a quantitative trait is due to both genetic and environmental variability and an interaction between the two. Variation due to genotype by environment interaction (GXE) that stems from differences in ranking of genotypes among environments reduces heritability and makes it difficult to obtain good estimates of genotypic breeding value. Given that such interactions occur, the plant breeder is faced with two decisions: which environments should be used for testing and how many are necessary for adequate genotypic evaluation. The two questions are linked because often the number of necessary environments is dependent upon the kind of environments chosen. A related approach to this problem is to study genotype response to environment and in so doing characterize genotypes according to their performance under a given set of environmental conditions.

Environmental variation can be considered as a continuum from predictable to unpredictable (Allard and Bradshaw, 1964). Predictable variation results from those conditions which are controlled in some way (greenhouse, growth chambers, irrigated) or those which have permanent characteristics (photoperiod, soil type, endemic pathogen). Unpredictable variation is usually weather related. If particular environments can be defined, then it may be possible to develop cultivars specifically for good performance in those environments. As a diversity of farming practices have developed with soybean production systems, plant breeders have examined the notion that cultivars can or should be "designed" for specific cultural practices. Practices investigated for cultivar specificity include late planting following a spring crop (Carter and Boerma, 1979; Boerma et al., 1982; Panter and Allen, 1989), narrow and wide row spacings (Beard and Harville, 1992; Hugie and Orf, 1989), irrigated and dryland production (Bowman et al., 1993; Mayers et al., 1991), very early planting of early maturing genotypes (Pfeiffer et al., 1995), and definable high yield conditions (Cooper, 1981). However, it has usually been difficult to show that separate breeding programs are needed for distinct environments.

Cultivars are also bred for tolerance or resistance to disease, pest and stress environments although such environments are sometimes unpredictable. To deal with unpredictable environments, emphasis is placed on development of cultivars which are stable, ie. perform well in many different environments. Most soybean breeders participate in some type of regional testing program for evaluation of lines over a wide geographical area, and statistical analyses have been developed to determine the relative stability of cultivars (Finley and Wilkerson, 1963; Eberhart and Russell, 1966; Perkins and Jinks, 1968; Hanson, 1970; Shukla, 1972; Lin and Binns, 1988; Hühn, 1979; Kang, 1988). Disease and pest resistance and stress tolerance can all be viewed as contributors to stability. Genetically heterogeneous cultivars (blends, F4 lines, etc.) have also been used to improve stability.

Estimates of genotype x environment interaction

Decisions concerning choice of appropriate breeding method for a particular objective require a knowledge of how the genotypic and environmental populations interact. Direct estimates of variance due to genotype x environment can be obtained from tests of genotypes (g) in several locations (l) and years (g). The total variation due to GXE can be partitioned into variance due to genotype x location (σ_{gl}^2), genotype x year (σ_{gy}^2) and genotype x location x year (σ_{gyl}^2). Hanson (1964) showed that considering l locations and y years as ly random environments would give biased estimates of genotypic and GXE variances unless the intraclass correlations

$$\rho_y = \sigma_{g^2y}/(\sigma_{g^2y} + \sigma_{g^2l} + \sigma_{g^2yl}) \text{ and } \rho_l = \sigma_{g^2l}/(\sigma_{g^2y} + \sigma_{g^2l} + \sigma_{g^2yl})$$

were zero. ρ_y and ρ_l were defined as the correlation of GXE effects within and among genotype-year classes and genotype-location classes, respectively. His analysis indicated that the bias was small as long as the sum of ρ_l and ρ_y was < .4. It is apparent from the above that the intraclass correlations will be small if σ_{gy}^2 and σ_{gl}^2 are small relative to σ_{gyl}^2. Published estimates of GXE variances for several traits including seed yield have found σ_{gyl}^2 to be larger or similar to σ_{gy}^2 with σ_{gl}^2 smaller than either (Schutz and Bernard, 1967; Kwon and Torrie, 1964; Garland and Fehr, 1981). Interaction components of variance were smaller for protein, but σ_{gly}^2 and/or σ_{gl}^2 were the largest components (Erickson et al., 1982; Sjahril and Mak, 1987). Thus, it should usually be acceptable to consider year-location as a random environment in the analysis of cultivar trial results.

Other research has shown that in cultivar trials, most of the GXE interaction is due to lower yielding cultivars (Baihaki et al., 1976). If so, testing in multiple environments would not be necessary since the higher yielding lines would always have a high ranking.

Genetically heterogeneous materials have generally been found to reduce variation due to GXE interaction. This has been shown in comparisons of cultivars and mixtures of cultivars (Schutz and Brim, 1971; Walker and Fehr, 1978) and in comparisons of F3:7 and F5:7 lines (Byth and Weber, 1968). In the latter study, Byth and Weber noted that genetic materials which are environmentally stable reduce bias due to specific environmental influence and may require less testing. They cautioned that the heterogeneity of those materials might result in reduced genetic variation among lines which could cause reduced genetic gain.

Analysis of stability

A common type of stability analysis is a partition of GXE variation by linear regression of individual genotype performance in each environment on some environmental index, usually the mean performance of all genotypes in each environment (Finlay and Wilkinson, 1963). This analysis assumes that GXE interaction is a linear function of environmental effects. Eberhart and Russell (1966) defined a stable genotype, under this type of analysis, to be one with a linear regression coefficient of one and zero deviations from regression. Perkins and Jinks (1968), using a different model, developed similar stability parameters. Results have varied when stability of soybean cultivars has been tested with this approach. In two experiments, regression coefficients were homogeneous and not significantly different from 1, with some cultivars showing significant deviations from regression (Schutz and Brim, 1971; Walker and Fehr, 1978; Dashiell, et al., 1994). Regression coefficients were variable in two other experiments (Funnah and Mak, 1980; Beaver and Johnson, 1981). In another study, lines with highest mean yields generally had regression coefficients greater than one and largest deviations from regression (Smith et al., 1967; Whitehead and Allen, 1990).

Mungomery et al. (1974) pointed out that in regression approaches to the analysis of genotypic stability, there is an a priori assumption that the response to environmental variation is either linear or curvilinear. Since genotypic response is often not linear, non-regression methods have been proposed for characterizing the stability of a cultivar (Shukla, 1972; Lin and Binns, 1988; Hühn, 1979; Kang, 1988). Mungomery et al. (1974) proposed the use of a pattern analysis which would group lines according to their response over environments. Lines are clustered by minimizing the Euclidean distance between pairs of lines and groups. In a test of 58 soybean lines and cultivars, 10 groups were identified with minimum within group variance and maximum between-group variance. In cases where there is a large number of test environments, Byth et al. (1976) showed that the pattern analysis could be extended to produce groupings of environments as well as genotypes. Hanson (1994) used pairwise GXE interaction effects for genotypes among sites as measures of comparative stability or for sites among genotypes to calculate distances and also to determine distances between test sites. Using yield data from the 1986 to 1991 Uniform Soybean Trials, Southern States, he was able to group sites that tended to give similar performance results. These did not correspond to five standard geographical regions normally used for grouping. These results provided a rationale for eliminating test sites that tend to duplicate variety performance information.

SUMMARY

The question of which environments to use in a testing program will depend on available economic and environmental resources, breeding objectives and the nature of GXE interaction. In general, significant line x year interactions suggest that unpredictable environments contribute most to the variability. Because of this, breeders either test in as many environments as possible and/or attempt to manage environmental variation (e.g. with irrigation and/or fertilization). Cowley et al. (1981) showed that heritability estimates for yield in populations of F3 soybean lines were higher in irrigated than in non-irrigated environments. They argued that genetic potential was more fully expressed in non-stress environments. Falconer (1952) stated the problem as follows: Will best results be achieved when selection is carried out under conditions in which the organism will be living or under those which allow a greater expression of a character?

Like Cowley et al. (1981), he argued that expectation of greater heritability would favor selection in an environment other than the one in which the organism will live. He suggested that performance in two different environments be treated as two genetically correlated traits. Then index selection could be used to optimize genotypic performance in both, provided appropriate weights could be determined. Van Sanford et al. (1993) using this approach, developed a selection index based on genetic correlations of cultivar performance in two primary Kentucky locations and the target environment (seven other locations). Predicted response to selection based on the index was greater than selection at either primary location alone or selection based on the mean of the primary locations.

Table 4.1

Ratio of average general combining ability variance to average specific combining ability variance (GCA/SCA) from diallel analysis of traits for which SCA was significant (after Burton, 1987)

Character	Leffel &Weiss (1958)	Weber et al. (1970)	Singhet al. (1974)	Paschal &Wilcox(1975)	Kaw & Menon (1980)	Chauhan & Singh (1983)	Singh (1983)	Tawaret al. (1986)
Yield	ns†	1.6	1.6	ns	1.9	---	12.4	---
Seed wt.	4.0	Ns	ns	21.2	37.5	---	27.7	1.6
Height	2.2	2.7	ns	ns	ns	---	8.6	---
Maturity	15.8	6.2	ns	8.8	ns	---	12.7	---
Protein	---	---	---	---	---	5.2	---	---
Oil	---	---	---	---	---	25.0	---	---

†ns= variation due to SCA was non-significant.

Table 4.2

Average yield heterosis expressed as a percent of the midparent and/or as a percent of the high parent.

	F1 performance of spaced plants†	F1 performance in row plots‡	F1 performance in single rows§	F1 performance of spaced plant¶	Taware et al. (1990)#	Loiselle et al. (1990)††	Loiselle et al. (1990)§§	Gizlice et al. (1993)¶¶	Lewers et al. (1998)‡‡	Burton & Brownie (2006)##
Mean midparent heterosis (%)	21.4	9,6	48,2	60,9	---	29,4	10,8	9,3	5,0	---
Mean high parent heterosis (%)	11.1	4,5	---	38,7	33,6	---	---	---	---	10,5
F1's greater than midparent(%)	76.1	93,6	---	100	---	---	---	---	83	100
F1's greater than high parent(%)	52.1	68,1	---	82	77,8	23,6	---	---	---	100

†Average of results reported in 6 experiments, 238 different F1's (Burton, 1987).

‡Average of results reported in 3 experiments, 47 different F1's (Burton, 1987).

§Average results of 2 experiments, 24 F1's, single rows, 1 yr., 1 location, 3 replications, per plant yield reported (Chauhan & Singh, 1982; Rahangdale and Raut, 2002)

¶Average of results reported in 3 experiments, 22 different F1's tested as spaced plants (Mehta et al., 1984; Kunta, et al., 1985; Dayde et al., 1989)

#9 F1's, spaced plants, 1 year, 3 replications, 5 plants/replication

††55 F1's, spaced plants, 1 year, between 1 and 20 plants.

§§ 55 F2's, 3m bordered rows, 1 year, 3 locations, 10 F2's, 5 m bordered rows; 2 yrs., 2 locations.

¶¶10F2's 5m bordered rows, 2 years, 2 locations/year.

‡‡ 6 Averages of 6 F1's derived from crosses of 6 male lines with 3 Clark isoline testers and 3 Harosoy isoline testers.

##2 F1's, 5m bordered rows, 2 years, 3 locations/year

As previously described, several stability analyses have been proposed and tested; and yet, the information has generally not been put to practical use. Stability analyses are not routinely performed in most breeding programs. Three factors probably contribute to this. One is that a large enough data base may not be available on any given set of lines. Another is the uncertainty about which stability analysis to use and then how to interpret the analysis results. Finally, high mean yield over a wide range of environments is generally perceived to be an adequate indicator of line stability. Even so, stability analyses should be helpful in identifying high-yielding genotypes that respond differently to stressful environments. Also Eskridge and Johnson (1991) recommend using "expected utility maximization" which incorporate economic value of a cultivar over environments and some measure of stability. Hanson (1970) has suggested that stability parameters be calculated annually for the elite lines in regional yield tests. Such a practice is well within the computing capabilities of most soybean breeding programs and would give geneticists another criterion for genotypic evaluation.

Table 4.3

Coefficients for σ_A^2, σ_D^2, σ_{AA}^2 for covariances of inbred relatives in biparental populations at various stages of inbreeding (Nyquist, 1991)

Population of lines		Generations		Genetic Parameters		
	t	g	g´	σ_A^2	σ_D^2	σ_{AA}^2
F2†	0	0	0	1	1	1
F2:3	0	1	1	1	1/4	1
*	0	1	2	1	1/8	1
F2:4	0	2	2	1	1/16	1
F2:∞	0	∞	∞	1	0	1
F3:3	1	3	3	3/2	1/2	9/4
*	1	1	2	3/2	1/4	9/4
F3:4	1	2	2	3/2	1/8	9/4
*	1	2	3	3/2	1/16	9/4
F3:5	1	3	3	3/2	1/32	9/4
F3:∞	1	∞	∞	3/2	0	9/4
F4:4	2	2	2	7/4	1/4	49/16
*	2	2	3	7/4	1/8	49/16
F4:5	2	3	3	7/4	1/16	49/16
F4:∞	2	∞	∞	7/4	1/16	49/16
F5:5	3	3	3	15/8	1/8	225/64
*	3	3	4	15/8	1/16	225/64
F5:∞	3	∞	∞	15/8	0	225/64

†Single F2 plants. This is considered the base originating non-inbred (F=0) population.

*Lines derived in the t generation, and bulk selfed 1 generation beyond g, i.e. g´= g + 1.

Table 4.4

Heritability estimates in percent for seed yield and yield related traits

Trait	Number of Populations	Heritability Estimates†			Reference Population	Citation
		Number of Estimates	Average	Range		
Yield	8	11	37	3-57	Biparental cross F3 and/or F4 lines	Brim (1973)
	6	6	33	0-73	Biparental F3 lines in the F4 generation, high protein and high yield parents	Shannon et al. (1972)
	1	1	68		41 cultivars of maturity groups 00-IV	Buzzell and Buttery (1977)

	2	2	54	53-54	40 F5 and F6 Maturity Group II or II lines, in 30 cm rows	Weaver and Wilcox (1982)
	2	2	41	34-47	40 F5 and F6 Maturity Group II or III lines, in 76 cm rows	
	2	2	21	14-28	F4 lines in the F5 generation	Harrison et al. (1981)
	1	3	63	56-74	Biparental F6 lines in the F9 generation	Metz et al. (1985)
	1	3	54	40-64		
	1	4	55	42-62	S1 families in successive recurrent selection test populations	Burton et al (1990)
	1	4	25	18-32		
	1	1	65		76 F5 derived lines high, high protein, and high yield parent	Chung et al. (2003)
	3	3	42	32-50	30 selfed half-sib families from3 random mating populations	Li and Burton (2002)
	4	4	52	35-63	84 F3 derived lines from biparental crosses	Streit et al. (2001)
Seed weight	8	11	80	44-94	Biparental crosses, F3 and/or F5 lines	Brim (1973)
	1	1	71(32)		F3 lines from six two-way and three-way crosses	Bravo et al. (1980)
	3	3	(13)	12-14	Recurrent selection populations	Tinius et al. (1991)
	3	3	89	87-91	40 BC2 lines; max x soja backcrossed to max	LeRoy et al. (1991)
	3	3	75	69-81	30 selfed half-sib families from 3 random mating populations	Li and Burton (2002)
Seed Density	3	3	56	47-67	30 selfed half-sib families from 3 random mating populations	Li and Burton (2002)
Height	8	11	79	66-90	Biparental crosses, F3 and/or F5 lines	Brim (1973)

Height of first pod	3	3	48	29-63	F3 lines in the F3 from indeterminate by determinate parents	Martin and Wilcox (1973)
Lodging	8	11	63	43-75	Biparental crosses, F3 and/or F4 lines	Brim (1973)
Days to flower	5	5	79	65-91	Biparental crosses F3 and/or F4 lines	Brim (1973)
	1	3	29	26-33	Biparental F6 lines in the F9 generation	Metz et al. (1984)
	1	3	27	26-29	140 F4 lines from a recurrent selection population	Hanson (1985)
	1	1	96			
Days to fruiting	5	5	62	46-81	Biparental crosses F3 and/or F5 lines	Brim (1973)
	1	3	65	60-70	Biparental F6 lines in the F9 generation	Metz et al. (1985)
	1	3	63	60-66	140 F4 lines from a recurrent selection population	Hanson (1985)
	1	1	87			
Maturity	1	1	83		140 F4 lines from a recurrent selection population	Hanson (1985)
	8	11	84	81-94	Biparental crosses, F3 and/or F4 lines	Brim (1973)
	1	3	92	94-99	Biparental F6 lines in the F9 generation	Metz et al. (1985)
	1	3	91	86-96	140 F4 lines from a recurrent selection population	Hanson (1985)

† All estimates obtained from variance components except those in parentheses which are realized estimates.

Table 4.5

Heritability estimates in percent for seed protein and seed oil composition and seed quality traits

Character	Number of Populations	Heritability Estimates‡			Reference Populations†	Citation
		Number	Average	Range		
Percent seed protein	4	7	77	51-89	F3 and/or F4 lines	Brim (1973)
	6	6	84	51-96	F3:4 lines, high protein x high yield parents	Shannon et al. (1972)
	2	2	78	70-86	F2:4 lines, (2 reps, 1 env.)	Shorter et al. (1976)
	2	2	783	75-90	F2:3 and F2:4 lines, (2 reps, 1 env.)	Ophenshaw and Hadley (1984)
	2	2	(32)	(29)-(34)	Recurrent S1 family selection population	Brim and Burton (1979)
	1	1	89		76 F5 derived lines, high protein, and high yield parents	Chung et al. (2003)
	3	3	75	65-82	30 selfed half-sib families from 3 random mating populations	Li and Burton (2002)
Percent seed oil	4	7	77	51-89	F3 and or F4 lines	Brim (1973)
	2	2	84	84-83	F2:4 lines (2 reps, 1 env.)	Shorter et al. (1976)
	2	2	82	71-93	F2:3 and F2:4 lines (2 reps, 1 env.)	Openshaw and Hadley (1984)
	1	1	(28)		Recurrent mass selection populations	Burton and Brim (1981)
	1	1	84		76 F5 derived lines, high protein, and high yield parents	Chung et al. (2003)
	3	3	76	66-82	30 selfed half-sib families from 3 random mating populations	Li and Burton (2002)
% Sugar in seeds	2	2	70	67-72	F2:3 and F2:4 lines	Openshaw and Hadley (1984)
%Oleic acid in seed oil	1	1	(21)		Recurrent mass selection populations	Burton et al. (1983)
%Stearic acid in seed oil	2	2	89	85-94	F5:7 low palmitic lines (2 reps, 4 env.)	Rebetzke et al. (1998)
	2	2	92	89-96	F5:7 normal palmitic lines (2 reps, 4 env.)	

%Palmitic acid in seed oil	2	2	84	86-82	F5:7 low palmitic lines, (2 reps, 4 env.)	Rebetzke et al. (1998)
	2	2	87	83-91	F5:7 normal palmitic lines (2 reps, 1 env.)	
Cookability	2	2	70	78-62	F4:5 and F4:6 lines, (3 reps, 1 env.)	Mwandemele et al. (1984)

†All populations are biparental unless otherwise noted.

‡All heritabilities are estimated from variance components on an entry mean basis except those in parentheses which are realized estimates.

Table 4.6

Heritability estimates in percent for various physiological traits

Trait	Number of Populations	Heritability Estimates‡			Reference Populations†	Citation
		Number	Average	Range		
Harvest index	1	1	82		41 cultivars of maturity groups 00 to IV	Buzzel and Buttery (1977)
CAP§	2	2	53	41-65	F4 lines in the F5 generation	Harrison et al. (1981)§
Canopy Temp.	6	6	7	0-19	144 F3 lines in the F4 and F6 generation	McKinney et al. (1989)
Seed filling period	4	4	48	16-63	F2:3 and F2:4 lines, (3 reps,2 years)	Pfeiffer and Egli (1988)
	2	3	73	50-89	F5:9 lines (2 reps, 2 env.)	Metz et al. (1985)
	1	1	83		140 F4 lines from a recurrent selection population (2 reps, 3 env.)	Hanson (1985)
Nodule mass	3	3	60	55-67	20, 46 and 31 random biparental F4 lines (4 reps, 3 env.)	Greder et al. (1986)
Fibrous root score	1	1	39		46 F2:3 lines in year 1 and F2:4 lines in year 2 (2 or 3 reps, 4 env.)	Pantalone et al. (1996)
Total N	2	3	54#	49-61	110 F2 plants (2 env.)	Ronis et al. (1985)
Fixed N	2	3	58#	53-60		
Iron efficiency	1	1	10¶		100 S1 families from cycle 7 of a recurrent selection population (6 reps, 3 yrs.)	Dragonnuk et al. (1989)
	6	6	55	39-68	F3:5 lines (3 reps, 2 env.)	Diers and Fehr (1989)

†All populations are biparental unless otherwise noted

‡All heritabilities are estimated from variance components on entry mean basis except those in parenthesis which are realized estimates.

§Canopy apparent photosynthesis

¶Estimated on a plot basis.

#Broad sense estimates using the difference method.

Table 4.7

Heritability in percent for disease resistance traits

Character	Number of Populations	Heritability Estimates‡			Reference Populations†	Citation
		Number	Average	Range		
Tolerance to Phytophthora	3	3	84	79-87	F6 lines (4 reps, 1 env.)	Walker and Schmitthenner (1984)
Brown stem rot resistance						
leaf symptoms	2	4	47#	-3-88	Single F2 plants	Sebastian et al. (1985)
stem symptoms	2	4	22#	10-44	Single F2 plants	
Tolerance to soybean cyst nematode race 3	3	6	19	-19-40	54 F3 lines per population (3 reps, 4 env.)	Reese et al. (1988)
Resistance to root-knot nematode	2	2	91	91-92	F2:3 lines, (3 reps, 7 plants/plot)	Luzzi et al. (1994)

†All populations are biparental unless otherwise noted

‡All heritabilities are estimated from variance components on entry mean basis unless otherwise noted.

#Estimated by the difference method.

Table 4.8

Estimates of genotypic and phenotypic (in parentheses) correlations of soybean yield with other characters

Character	Johnson and Bernard (1963)†	Anand and Torrie (1963)‡			Kwon and Torrie (1964)§	
		Cross 1	Cross 2	Cross 3	Cross 4	Cross 5
Seed wt.	0.20	-0.27	0.02	-0.16	-0.59	0.22
	---	(0.03)	(-0.03)	(-0.07)	(-0.46)**	(0.20)
Height	0.30	0.65	0.57	0.43	0.82	0.54
	---	(0.41)**	(0.44)**	(0.32)**	(0.69)**	(0.44)**
Lodging	0.00	0.47	0.36	0.72	0.97	0.44
	---	(0.36)**	(0.07)	(0.36)**	(0.76)**	(0.27)*
Days to flower	0.00	0.76	0.26	0.45	0.87	0.69
	---	(0.37)**	(0.11)	(0.31)**	(0.68)**	(0.47)**
Fruiting period	0.20	0.71	-0.27	0.43	0.89	0.15
	---	(0.38)**	(0.05)	(0.13)	(0.71)**	(0.13)
Maturity	0.40	1.05	0.01	0.47	0.95	0.52
	---	(0.48)**	(0.04)	(0.37)**	(0.75)**	(0.37)**
Protein	-0.20	---	---	---	---	-0.58
	---	---	---	---	---	(-0.42)**
Oil	0.10	---	---	---	---	(0.05)

Table 4.8 (*continued horizontally*)

Character	Byth et al. (1969b)				Byth et al. (1969a)††	Simpson and Wilcox (1983)‡‡			
	Cross 7		Cross 8						
	ML	DL	ML	DL		Cross 13	Cross 14	Cross 15	Cross 16
Seed wt.	0.07	0.15	-0.07	0.27	0.26	---	---	---	---
	(0.10)	(0.16)*	(0.01)	(0.21)**	(0.21)	(0.00)	(0.21)*	(0.02)	(0.04)
Height	-0.28	-0.52	-0.15	-0.08	0.32	---	---	---	---
	(-0.13)	(-0.36)**	(-0.04)	(0.02)	(0.26)	(0.43)**	(0.37)**	(0.40)**	(0.35)**
Lodging	-0.14	-0.48	0.15	-0.17	-0.11	---	---	---	---
	(0.21)*	(0.41)**	(0.03)	(-0.20)**	(-0.26)	(0.45)**	(0.22)**	(0.36)**	(0.30)**
Days to flower	---	---	---	---	---	---	---	---	---
	---	---	---	---	---	---	---	---	---
Fruiting period	---	---	---	---	---	---	---	---	---
	---	---	---	---	---	---	---	---	---
Maturity	0.14	0.10	0.06	0.31	0.59	---	---	---	---
	(0.13)	(0.09)	(0.08)	(0.22)**	(0.37)	(0.54)**	(0.48)**	(0.51)**	(0.60)**
Protein	0.35	0.20	-0.55	-0.25	-0.23	0.54	-0.74	-0.40	-0.20
	(0.22)*	(0.13)	(-0.34)**	(-0.17)*	(-0.14)	---	---	---	---
Oil	-0.03	0.10	0.45	0.07	0.11	-0.22	0.20	0.25	-0.27
	(0.01)	(0.09)	(0.26)*	(0.08)	(0.07)	---	---	---	---

Table 4.8 (*continued horizontally*)

Character	Nelson (1986)§§	Smith and Nelson (1987)¶¶		Wehrmann et al. (1987)†††			Chung et al. (2003) ‡‡‡
	Plant Introductions	Cross 17	Cross 18	Cross 19	Cross 20	Cross 21	Cross 22
Seed wt.	---	---	---	---	---	---	---
Height-	---	---	---	---	---	---	---
Lodging	---	---	---	---	---	---	---
Days to Flower	-0.62	---	---	---	---	---	---
Fruiting Period	0.55	-0.22	0.30	---	---	---	---
			(0.38)				
Maturity	-0.27	---	---	---	---	---	---
Protein	---	---	---	-0.86	-0.64	-0.54	-0.70
Oil	---	---	---	0.83	0.70	0.53	+0.46

Table 4.8 (*continued horizontally*)

	Li & Burton		
	Populations		
	II	III	VII
Seed wt.	0.81	0.96	0.19
Height-	---	---	---
Lodging	---	---	---
Days to Flower	---	---	---
Fruiting Period	---	---	---
Maturity	---	---	---
Protein	0.05	-0.75	0.12
Oil	0.31	0.67	-0.21

*,**Exceeds the 5 and 1% levels of probability, respectively.

†Expected genotypic correlations based on data available to 1963.

‡Data based on two replications in two environments.

§Data based on two replications in five environments.

¶Data based on two replications in three environments. ML = maternal lines, DL = daughter lines.

††Data based on eight F3 - F5 populations grown in Iowa or North Carolina. Levels of significance not given.

‡‡Data based on two replications, one location in 2 yrs.

§§Data based on three replications, one location, in 2 years, 28 Group II or Group III plant introductions.

†††Data based on two replications, two locations (95 BC2F3 lines from each cross).

‡‡‡Data based on two replications, 6 irrigation treatments, 2 years (76 F5 derived lines from one cross)

§§§Data based on two replications, three locations, 1 year (30 half-sib families from 3 random mating populations)

Conclusion

Soybean breeding over the past 60 years has produced cultivars with greater genetic yield potential that are adapted to modern cultural practices. Breeding has also protected the crop by incorporating disease and pest resistance into cultivars. Seed quality has also been improved. However, as Brim (1973) noted, "past success does not necessarily provide conclusive proof of the efficiency of present breeding procedures." Evaluating breeding methods within a quantitative genetic context provides a way to compare the efficiencies of different procedures and determine the likelihood that a new procedure will be successful. Future research should continue to focus on ways to improve method efficiency and ways to increase the rate of improvement (Burton, 1987). These include the following: (i) the development of breeding populations, taking into account the genetic origin of the parents, their overall phenotype, and their performance in diverse environments; (ii) the development of single and multiple trait selection schemes which incorporates a more rapid cycling of elite line identification, selection and recombination; (iii) the investigation of the relative importance of dominance and epistasis, particularly as they affect heterosis; (iv) the development of ways to manage genotype x environment interactions so that heritabilities are increased; (v) the allocation of resources with respect to preliminary vs. advanced testing and (vi) cost effective ways to apply molecular genetic technology to quantitative trait improvements.

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METHODS OF SOYBEAN BREEDING

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Genetic improvement in soybean has been accomplished using a number of different breeding methods. These genetic improvements have been made mainly through the use of conventional breeding methods; however molecular-based plant breeding methods and techniques are now being used by most soybean breeders at least for some of their objectives in cultivar and/or germplasm improvement. Recent reports have indicated that soybean yields are improving at a rate of about 23 Kg ha^{-1} yr^{-1} due to improved genetics, changing production practices and higher atmospheric CO_2 levels (Specht, 1999). Undoubtedly conventional breeding methods will continue to play an important role in improving yield, disease and pest resistance and soybean seed quality; however molecular-based plant breeding strategies that may enhance the rate of genetic improvement are currently playing a larger role in both public and private soybean breeding efforts (Orf et al., 2004). This chapter will briefly present both conventional and molecular-based methods being used by soybean breeders and geneticists throughout the world.

CONVENTIONAL BREEDING METHODS

Conventional breeding methods have been very successful in improving the productivity, hazard resistance and quality of soybean. Breeding for direct improvement of yield remains the trait of greatest emphasis by breeders as it is the trait that is of greatest interest by producers. Breeding to improve or protect yield through hazard resistance or breeding for enhanced quality are also important parts of all breeding programs and may require additional or special breeding methodologies. Progress in breeding has been made for many traits including yield, resistance to pathogens, insects and nematodes, tolerance to herbicides and production hazards,

improvement in seed protein, oil and other quality traits as well as other agronomic characteristics such as standability and adaptability.

In general each breeding method that leads to genetic improvement begins with the breeder making choices as to the parents or starting material to be used to create segregating populations. Those populations are then advanced toward homozygosity, without selection or with selection that may involve various techniques, to produce relatively homozygous lines that are then subject to yield and other trait evaluations. The breeding method (or cycle) is complete when the best line(s) are released as improved pure-line cultivar(s) or improved germplasm. The pure-line cultivar is what is grown by the farmer.

There are many different breeding methods that soybean breeders and geneticists use for cultivar and/or germplasm development. Most, if not all, methods have a number of aspects in common. They include the objectives of the breeding/genetics program, selection of parents, type of populations, and selection and inbred line development.

Objectives

The objectives of a breeding program will sometimes dictate which breeding method(s) might be best used. Even though conventional breeding methods will be discussed first, the identification of objectives is equally important (and the same) for molecular-based breeding efforts. Although the objectives for a specific cross or program may be limited, and highly dependent on the individual situation, many breeding/genetics programs at least have some of the following traits or characteristics as consideration for selection.

In almost all cases yield or productivity is the character of greatest importance or at least among the characters of greatest importance. Since yield is a quantitative trait, it is the most challenging trait to breed for in a genetic improvement program. Over the decades considerable progress has been made in improving yield and there is no reason to expect that further yield increases will not be made in the future.

The yield potential of a cultivar or germplasm line will not be realized if it is injured by diseases, insects or nematodes. In almost all programs, breeding for resistance to some pest or pests is part of the objectives. The amount of emphasis placed on pest resistance depends on the regularity and severity with which the particular pest problem(s) occur in the target breeding area and the level of economic loss that can occur from the pest. Protection against economic loss can be provided by specific resistance, general resistance or tolerance. Specific resistance is usually conferred by one or a few major genes and can be easily transferred to susceptible cultivars. The disadvantage of specific resistance is the fact that it may not provide protection to new races of a pest.

General or field resistance (sometimes called field tolerance) is mainly responsible for reduced levels of infection but does not confer immunity like specific resistance. This type of resistance is generally quantitative (conferred by many genes). It provides protection against multiple races of pests, but is much more difficult to transfer from a breeding standpoint. Tolerance to a pest is usually defined as a cultivar or germplasm line that suffers less loss in productivity than a non-tolerant line even though both lines have similar levels of the pest present. Tolerance is a result of even more complex genetics and interactions than general resistance and thus is not frequently used in breeding programs.

Maturity is an important trait for a breeding program for a particular area. Breeders generally work with lines adapted to their target environment, however if parents of unadapted maturities are used, modified techniques may be needed. Also, for crosses of parents of widely differing maturities, the number of adapted segregating progenies in populations may be limited, thus larger populations are required in order to obtain a given number of progeny of the desired maturity. Although maturity is generally considered a quantitative character, several major genes for maturity have been reported (Palmer and Kilen, 1987; Palmer et al., 2004).

Lodging resistance, plant height and stem termination are traits important in cultivar development. Stem termination is controlled by major genes, however the final plant height and lodging resistance of adapted cultivars is considered quantitative and must be selected for using field trials in the target environment.

Shattering resistance is generally present in most improved cultivars, however many plant introductions or germplasm lines may shatter, especially under warm and/or dry conditions. Although there are major genes for shattering resistance, several minor or modifying genes make shattering resistance challenging to select for since the climatic conditions that induce shattering can vary from year to year.

Seed size may be an important characteristic, especially for special purpose or food type cultivars. Large or small seed size can be selected for using methods like mass selection or bulk breeding. The inheritance for seed size is quantitative, but selecting for extremes in populations can result in many lines with the targeted seed size.

Seed quality, that is the appearance of the seed, is a trait measured in some cultivar development programs. Undesirable seed quality may be caused by unfavorable weather conditions and/or certain diseases. Selection for disease resistance can improve seed quality. Germinability is also sometimes part of seed quality. Poor germination tends to be a greater problem in low latitudes. Since seed quality, including germinability, is a complex trait, lines need to be evaluated from field plots (many times with delayed harvest) over several years.

Seed composition is a very important trait in soybean. Since soybean is used for both oil and protein breeders generally try to aim for 40% protein and 20% oil (on a dry matter basis).

In most cases, to date, soybeans have not been marketed on composition, however protein and oil content has been considered in the special purpose/food soybean market for many years. Recently, there have been limited markets for bulk commodity soybeans with specified oil and/or protein levels. Although both oil and protein levels are quantitative traits they can be readily altered by breeding. As technology for rapidly, accurately, and inexpensively measuring oil and protein content becomes available, seed composition will need to be considered by breeders for all cultivars.

Other seed composition traits, besides protein and oil content, have been explored, and to a limited extent, incorporated into commercial cultivars. Among the traits that have been commercialized to date are low linolenic acid, low or no lipoxygenase, low saturated fatty acids, reduced trysin inhibitor (no Kunitz trypsin inhibitor) and higher levels of sulfur-containing amino acids. Several other traits including mid-oleic acid (50%-60%), combinations of altered fatty acids, higher levels of other essential amino acids, higher sucrose content, lower oligiosaccharides, higher isoflavones and other desirable traits for special purpose and/or food soybeans are being selected for in some breeding programs and may become of greater importance in the future. Many, but not all, of these traits are controlled by a few major genes; but most also have modifiers; thus breeders will need to continually assess the levels of the traits in the lines in their breeding programs.

Resistance or tolerance to several different production hazards may also be important traits in some breeding programs. Among the traits that have received attention are tolerance to iron-deficiency chlorosis (high pH), tolerance to acidity (low pH), drought tolerance, manganese tolerance, salt tolerance, flooding tolerance, and high nitrate tolerance, to name a few. Most of these traits are quantitative in nature and require special field and/or greenhouse or laboratory conditions in order to assess the breeding line or cultivars' response to the particular hazard.

In recent years resistance or tolerance to herbicide injury has become a very important objective for cultivar development. Currently tolerance to glyphosate is present in most cultivars in the USA, Argentina and Brazil. This trait was introduced via transformation and is simply inherited. Resistance or tolerance to other herbicides including metribuzin, dicamba and 2,4D have been reported and in some cases tolerant versions of cultivars released. The resistance or tolerance is generally simply inherited and may have been introduced into soybean via transformation.

As biotechnology and transformation techniques continue to improve, there will undoubtly be other traits or characters introduced into soybeans. In the near term most of those traits will be simply inherited making the incorporation of the traits relatively easy regardless of the breeding method used.

Selection of Parents

Selection of parents is an extremely important part of any soybean breeding method. The selection of parents sets in motion the whole cascade of events in succeeding generations of all breeding methods. The parents used to create segregating populations can be from many different sources such as existing cultivars, adapted elite breeding lines, unadapted germplasm with special desired traits, or even exotic germplasm. Generally elite parents of diverse origin are more likely to produce progeny that are superior to either parent (**and** superior to existing cultivars) than parents that are closely related (Burton, 1987). The way parents are selected depends on many factors, including the trait(s) of interest, the purpose of the cross, the relative importance of characters other than yield, the ancestry of the lines, and the resources and time available. Parents may be selected on the basis of comparative evaluation *per se*, by test-cross evaluation, or other methods that may identify germplasm with good combining ability. In many cases, *per se* evaluation data are readily available in the form of breeder-directed or fee-based yield performance tests or from government required tests. However, if the objective is to identify parental germplasm with favorable alleles not presented in existing cultivars, test-cross evaluations may be a better approach. A method for soybean was suggested by Kenworthy (1980). Another test-cross method developed by St. Martin et al. (1996) outlines a procedure for identifying germplasm lines with the potential to contribute favorable alleles for improving pure-line cultivars of soybean. Another method for improving yield suggested by Henderson (1975) and more fully explored by Panter and Allen (1995) is the use of the best linear unbiased predictions.

Selection of parents will continue to be a very challenging but extremely important aspect that determines the success of all breeding procedures for genetic improvement. In many programs where resources are quite limited, use of existing comparative data and/or the best linear unbiased predictions appears to be very useful. If more time and resources are available, test-cross evaluations can be used to identify parental germplasm with favorable alleles not present in current cultivars or breeding lines. With the increased availability of molecular and genomic data on individual cultivars and genotypes, this data will become more valuable in assisting breeders/geneticists in the selection of parents (Orf et al., 2004).

Once the parents have been selected, a cross or crosses are made to initiate populations. Populations can be developed with different numbers of parents and varying percentages of each parent before inbreeding and selection is begun. The majority of soybean cultivars have been selected from populations that resulted from 2- or 3-parent hybridizations involving existing cultivars, breeding lines or other germplasm (Fehr, 1987a). Multiple parent populations (more than two parents) are less common; however, three, four or as many as eight parents have been used to develop breeding populations. Backcross populations involve the use of a non-recurrent parent and the repeated use of the recurrent parent in crossing. Backcross populations

have generally been developed to transfer genes for pest resistance or other simply inherited traits from an agronomically unacceptable parent into an elite cultivar or breeding line. Although some researchers refer to recurrent selection populations, it is probably more appropriate to refer to a population of a specific cycle of recurrent selection. In recurrent selection there are many (sometimes dozens) of parents.

Inbreeding, Selection and Line Evaluation

After a cross or crosses have been made the populations are then advanced through several generations of selfed inbreeding. A number of factors need to be considered during inbreeding. Among them are the method of inbreeding (including possible selection) and the number of generations of self-pollination to allow before lines are derived for potential cultivar evaluation. Lines can be derived from a population in the F_2 or in any of the more advanced generations of inbreeding. Selection can be practiced among plants during early generations of inbreeding before yield tests are initiated or later among lines during yield testing. The amount and effectiveness of selection depends on the heritability of the trait or character and the environment where the population or lines are grown. Visual or easily determined selection is mainly carried out during early generations of inbreeding, while selection based on data from unreplicated or replicated plots is carried out in later generations. At some point in the inbreeding process nearly homozygous lines are created from individually harvested inbred plants. These lines are then extensively evaluated to identify those that are superior in performance to existing cultivars. The methods of inbred line development include pedigree, bulk, single seed descent, mass selection and early generation testing. Other methods or procedures used in soybean cultivar development include backcrossing and population improvement using recurrent selection which may involve a genetic male sterility system.

Pure line method

Although selection for desired plant types has been going on for centuries and did lead to new and better cultivars, it was not until the early 20th century that scientists developed theories and methods that were routinely applied to genetic improvement of soybeans. The first technique or "method" employed by breeders/geneticists was the pure line method of breeding. In this method no artificial hybridization occurs but rather the breeder selects individual plants from an already existing "mixed" cultivar (that is a cultivar that has several different phenotypes (and thus different genotypes)). Since soybean is a self-pollinating species, it is assumed each phenotype is essentially homozygous and true breeding. By selecting individual plants which are then planted out in progeny rows (that is a row of plants that are from the seed of an individual plant), the breeder can observe and select those progeny rows with the desired trait(s) or characteristics for an improved or new cultivar.

Selected progeny rows (which are generally 2-3 meters long) are harvested and the seed from those rows used to plant (in most cases) multi-row, multi-location, replicated yield trials in the area where the breeder works. The yield test includes standard or check cultivars and/or genotypes. The performance of the experimental lines (selected progeny rows) in a test is compared to the performance of the checks and only those that are superior to the checks are saved for further testing in succeeding years. Besides yield, other traits such as maturity, lodging, seed composition and hazard resistance may, in most cases, also be compared with the check genotypes and used as criteria for deciding which experimental lines will be further evaluated. The first year yield trials are mainly used to eliminate the unpromising lines that are inferior to the checks rather than trying to identify the best experimental line or lines. The breeder may evaluate the selected lines from the first year yield trials a second year in "local" yield trials. Again, yield performance would be the trait of greatest interest but each experimental line in the second year's tests would also be rated for other traits (as noted in the first year evaluations) and compared to the check genotypes. The data from the two years would be combined and only those experimental lines that are superior to the check cultivars or genotypes would be saved for further testing. Thus the emphasis (using all the data collected) shifts to identifying and selecting those lines that are truly superior to the checks.

After the best lines have been identified by local testing, the lines are then entered into regional testing (multi-state or multi-country testing). The regional testing involves experimental lines from several breeders as well as check cultivars or lines. In many cases, the regional tests are government sponsored or official government trials. The procedure for regional trials varies but may require two to three years of testing before a line can be considered for release as a cultivar. The final decision on release is generally made by the institution or company that employs the breeder and is usually made by a cultivar release committee or administrative group.

As the experimental line is being evaluated (usually during the regional testing), the line undergoes a purification and seed multiplication process so there are significant quantities of seed available upon release. The purification process involves selecting a single plant or a limited number of plants (20-100) to be grown out in a progeny row(s) for observation of phenotypic and/or molecular characteristics. Seed from the progeny row or a bulk of the uniform rows is increased to form the initial breeder's seed. The breeder's seed is then further multiplied over several generations (with or without the use of winter seed increases) to provide sufficient seed of the new cultivar at time of release for sale to soybean growers.

Since the process of testing, seed multiplication and release has been briefly described, the next sections will only describe the initial few seasons of the various breeding methods in detail. It should be noted that most methods are carried out with some modifications by breeders, depending on the program and the trait(s) that are being selected.

Pedigree

The pedigree method is used during inbreeding following hybridization and is briefly outlined below.

Season 1: make cross; harvest F_1 seed

Season 2: space plant F_1 plants and verify each plant is a hybrid; harvest F_2 seed

Season 3: space plant F_2 seed; select individual F_2 plants based on objectives and traits for the cross

Season 4: plant a single F_3 progeny row from each F_2 plant ($F_{2:3}$ line); first select the best lines and then the best plant(s) within each line (row)

Season 5: plant a single F_4 progeny row from each F_3 plant ($F_{3:4}$) line; select the best lines first then select the best plant(s) within each line (row)

Season 6 (or later): plant a single F_5 progeny row from each F_4 plant ($F_{4:5}$); select the best lines and harvest entire progeny row

Succeeding seasons: evaluate lines in replicated yield tests compared to check cultivars or lines, if superior to current cultivars multiply seed and release

The pedigree method has been used since the rediscovery of Mendel's laws and has led to many successful soybean cultivars. In the pedigree method, the ancestral lineage of each line tracing back to individual F_2 plants is recorded. Thus care must be taken to keep accurate records so each selection can be traced back to the original hybridization. The size of the F_2 population is a subject of considerable discussion among breeders and depends on the resources of the program including personnel, equipment, land area, laboratory space as well as the philosophy of the breeder (does the breeder favor more crosses with few plants selected from each cross or fewer crosses with more plants selected from each cross?). The literature suggests 2,000-5,000 F_2 plants, with 5-10% of those plants selected. The F_2 population should be space planted so the phenotype of each F_2 can be observed. It must be kept in mind that there are factors such as competitiveness, G x E interactions, genetic components (remaining heterosis, epistasis, dominance, etc.) and the interplay of these factors that can influence the phenotype observed in the F_2. Never-the-less the pedigree method allows selected plants (and their progeny) to be observed in additional generations (if selected) so that those plants that are truly desired from a phenotypic standpoint will be continued to the yield testing phase (Fehr, 1987a). There is also some debate as to whether the F_3 progeny row should be space planted or planted at "normal" densities. Since individual F_3 plants need to be selected from the F_3 progeny rows most breeders use a density lower than for commercial production of soybeans (in the range of 50% to 75%).

With each successive generation of inbreeding, the additive genetic variability within lines is reduced and additive genetic variability between lines increases. Thus, in the pedigree method, the idea is to retain the maximum number of lines that trace back to different F_2 plants. In practice, this means that the number of plants selected within lines decreases with each generation of inbreeding (for example, four plants may be selected from an $F_{2:3}$ line while only two plants may be selected from an $F_{4:5}$ line). The number of generations that pedigree selection is practiced also depends on the resources of the breeding program or when a line appears uniform for the trait(s) being selected. Breeders generally harvest lines in bulk to begin replicated yield testing after the F_4, F_5 or F_6 generation and discard any heterogenous lines for the trait(s) under selection.

The pedigree method has been used to develop many soybean cultivars. This method allows the breeder to discard inferior material based on phenotype early in the inbreeding process, allows the breeder to minimize the relationship among retained lines and provides phenotypic observations over several generations in different environments. However, this method requires considerable land, labor and other resources as well as extensive record keeping, usually requires an experienced breeder to make selections, and is not effective in environments where genetic variability for trait(s) is not expressed. This last point means that the pedigree method is not well suited for inbreeding in greenhouses or winter nurseries and thus only one generation per year can be completed and therefore the pedigree method takes a longer time than some other methods before a line can be released. Thus the pedigree method is no longer widely used in soybean breeding especially in production areas where current cultivars are rapidly replaced by newer cultivars.

Bulk

The bulk method of handling populations during inbreeding is outlined below:

Season 1: make cross; harvest F_1 seed

Season 2: space plant F_1 plants and verify each plant is a hybrid; harvest F_2 seed

Season 3: plant all F_2 seed at normal seeding rate; harvest all seed from all plants (usually with a combine) in bulk

Season 4: plant a representative sample of the F_3 seed at normal planting rate; harvest all seed from all plants in bulk

Season 5: plant a representative sample of the F_4 seed at normal (or slightly reduced) planting rate; select and harvest single plants with desired phenotype

Season 6: plant a single F_5 progeny row from each selected F_4 plant; select the best lines and harvest the entire progeny row

Succeeding seasons: evaluate lines in replicated yield tests compared to check cultivars or lines, if superior to current cultivars multiply seed and release

In the bulk method, plants in a segregating population(s) are harvested together each cycle of inbreeding and a sample of the harvested seed used to plant the next generation. When the desired level of inbreeding is reached, single plants are harvested and grown as progeny rows and then selected lines advanced to yield evaluations. In the classical application of the bulk method, the main force acting on the population is natural selection. Natural selection favors those traits or characteristics that increase a plants competitiveness (that is it allows a plant to produce more seed than another) and may include such traits as tall height, late maturity, and resistance to natural hazards (diseases or insects). Generally, bulk populations are planted at "normal" densities; however, some breeders may use a reduced density to encourage less competitive genotypes.

The beginning population(s) for the bulk method can vary considerably from 2-parent to multiple-parent crosses, to crosses of unadapted by adapted parents, to a mixture of many F_2 populations such that there are one or only a few bulks that contain many crosses. The number of generations of bulk harvest can also vary considerably from two or three to as many as eight to ten. The longer number of generations gives natural selection more time to work. Although natural selection is the "classical" way to conduct bulk selection, many breeders will use some combination(s) of natural and "breeder imposed" (mass) selection for their populations. The bulk method can produce lines with the desired traits if the environment where the method is carried out favors those traits. The bulk method is an easy way to maintain populations during inbreeding and should increase the frequency of the desired genotypes in the population(s). Since bulk selection depends on natural selection, the trait or traits that are "selected for" may change from year to year depending on the natural environment. Also, since the breeder depends on natural selection only one generation per year can be accomplished, thus extending the time it takes to develop a cultivar (Sleper and Poehlman, 2006).

Mass Selection

Mass selection in a heterogenous population that is undergoing inbreeding (self-pollination) is one of the oldest methods of breeding. Some breeders consider mass selection a variation of the bulk method; only in the case of mass selection, the breeder does the selection rather than "nature." Mass selection may be done from either a positive or negative approach. Positive selection is when the desired phenotypes are selected and rebulked. Negative selection is when the undesirable phenotypes are culled or removed from the population. Generally mass selection results in a population that has been selected and therefore improved for one or more traits or characteristics. Some examples of traits in soybean that have been subject to mass selection are maturity, seed size, and seed composition. If mass selection is practiced in the classical sense, the resulting cultivar would be heterogenous for some traits since a relatively "large" number of plants would be bulked to form the cultivar.

However, if many traits are mass selected the resulting cultivar (at least phenotypically) may appear uniform. Mass selection may also be used to maintain the purity of cultivars by the rouging of "off-type" plants and/or seed (negative selection). Mass selection can only be used in environments or situations where the trait(s) or character(s) is expressed and its effectiveness depends on the heritability of the character on a plant or seed basis (it does not work well for characters with low heritability). In many soybean breeding programs, some form of mass selection is used at some point in the cultivar development process.

Single Seed Descent (SSD)

Single seed descent is a method to rapidly inbreed a population before beginning evaluation and was initially proposed by Goulden (1941) and more fully described by Brim (1966).

Season 1: make cross, harvest F_1 seed

Season 2: space plant F_1 plants and verify each plant is a hybrid; harvest F_2 seed

Season 3: plant all F_2 seed and advance plants to maturity; harvest 1 seed from each F_2 plant

Season 4: plant all F_3 seed and advance plants to maturity; harvest 1 seed from each F_3 plant

Season 5: plant all F_4 seed and advance plants to maturity; harvest all F_4 plants (or select F_4 plants on some characteristic(s))

Season 6: plant a single F_5 progeny row from each F_4 plant; select the best lines and harvest the entire progeny row

Succeeding seasons: evaluate lines in replicated yield tests compared to check cultivars or lines, if superior to current cultivars multiply seed and release

Single seed descent has been referred to as a modified pedigree method by Brim (1961). In the strict sense, SSD refers to harvesting and planting only one seed from each plant from the F_2 generation on until plant selection is done. This means, due to failure of germination or a plant to reach maturity or produce one seed, that not all F_2 plants will be represented when generation advance is completed. In practice, most breeders harvest one (or two) pod(s), thresh the pods and take a sample that is approximately the same number of seeds as the previous generation. This maintains the population size and also provides a remnant. Thus, not every F_2 plant is represented only once (some are not represented while others may be represented more than once).

This is many times referred to as a modified single seed descent (or some even refer to it as modified bulk). The general idea of SSD is to, as rapidly as possible, with the use of greenhouses, growth chambers, or off-season nurseries, advance the population to a desired level of homozygosity via inbreeding and then begin evaluation of progeny rows.

Single seed descent is currently the most widely used breeding procedure for inbreeding soybean populations. Its popularity is due to the fact that a breeder can obtain 2, or 3 or almost 4 generations per year with the use of greenhouses, growth chambers, or tropical nurseries. Thus a cross can be made and a population initially evaluated in about two years (compared to six years for the pedigree or bulk method). This savings of three or four years means, for example, that a cultivar can be released in six years instead of ten years with the same amount of yield testing. The use of SSD, however, does not allow for the observation of plants or progeny in early selfing generations and does not allow natural or artificial selection. Never-the-less SSD has contributed to the more rapid turnover of soybean cultivars in the last decade and will continue to do so in the future. Single seed descent also requires less resources and especially time (and as people know, "time is money"), so it will continue to be the method of choice for soybean breeding programs where cultivar development is the primary objective.

Early Generation Testing (EGT)

Early generation testing is designed to identify bulk hybrid populations that have the greatest potential to produce superior lines. Two methods of yield testing have been used:

1) testing of bulk populations, or

2) testing of F_2-derived lines that represent the population. The first method may yield test the population (with replication and locations depending on seed supply) in the F_2, F_3 and F_4 generations and only select plants from those populations that are superior to check cultivars (or a certain percentage of all populations tested).

The second method is outlined below:

Season 1: make cross; harvest F_1 seed

Season 2: space plant F_1 plants and verify each plant is a hybrid; harvest F_2 seed

Season 3: space plant all F_2 seed; harvest each F_2 plant

Season 4: plant a representative sample (20-30 lines) of $F_{2:3}$ lines in yield tests (1 replication, 2 locations); harvest yield tests

Season 5: plant $F_{2:4}$ lines in yield tests (bordered, multiple replications, multiple locations); harvest yield tests, select highest yielding populations, select plants from borders of lines from highest yielding populations

Season 6: plant single F_5 progeny row from each $F_{2:4}$ plant selected; select the best lines and harvest entire progeny row

Succeeding seasons: evaluate lines in replicated yield tests compared to check cultivars or lines, if superior to current cultivars, multiply seed and release

The above method is only one of several procedures described for using F_2-derived lines for yield testing. The concept of EGT was described by Immer in 1941. Some variations of EGT suggest selecting lines at different stages (generations) within populations, among populations or among individuals within populations. Other variations include using EGT to identify the superior populations from which to select lines. (Recall all populations were advanced in the pedigree, bulk, or SSD methods.) If lines, rather than the whole population, are yield tested in EGT, the main limitation is the amount of seed from F_2-derived lines for the first yield tests (some breeders grow F_2-derived progeny rows and delay yield testing for one generation). As with the other methods (except SSD), only one generation per year can be grown since yield tests need to be in the target environment. As yield tests are conducted, evaluations for other traits or characters are usually carried out. This method, in addition to being time consuming, is also expensive due to the land and labor costs of yield testing. Several successful soybean cultivars have been developed using early generation testing.

Backcross

The backcross method is used to add a highly heritable characteristic (allele) to a cultivar or line (recurrent parent) for which it is deficient. The term backcrossing, as originally described by Harlan and Pope (1922), and refers to the repeated crossing of hybrid progeny back to the recurrent parent. The simplest type of backcrossing scheme, a dominant allele that can be evaluated on a single plant basis before flowering (using disease resistance (designated RR) in this example), is briefly described below:

Season 1: make a cross between a susceptible recurrent parent (rr) and a resistant donor or nonrecurrent parent (RR); harvest F_1 seeds (all Rr)

Season 2: F_1 plants (Rr) are crossed to recurrent parent (rr); harvest BC_1F_1 seeds (50% Rr, 50% rr)

Season 3: BC_1F_1 plants genotyped before flowering, rr plants eliminated, BC_1F_1 Rr plants crossed to recurrent parent (rr); harvest BC_2F_1 seed (50% Rr, 50% rr)

Season 4: BC_2F_1 plants genotyped before flowering, rr plants eliminated, BC_2F_1 Rr plants crossed to recurrent parent (rr); harvest BC_3F_1 seed (50% Rr, 50% rr)

Season 5: BC_3F_1 plants genotype before flowering, rr plants eliminated, BC_3F_1 Rr plants crossed to recurrent parent (rr); harvest BC_4F_1 seed (50% Rr, 50% rr)

Season 6: BC_4F_1 plants genotyped before flowering, rr plants eliminated, BC_4F_1 Rr plants crossed to recurrent parent (rr); harvest BC_5F_1 seed (50% Rr, 50% rr)

Season 7: BC_5F_1 plants genotyped before flowering, rr plants eliminated, BC_5F_1 Rr plants allowed to self pollinate; harvest BC_5F_2 seed

Season 8: BC_5F_2 plants genotyped before flowering, rr plants (25%) eliminated, resistant plants 50% Rr and 25% RR allowed to self pollinate; seed from each BC_5F_2 resistant plant harvested separately (BC_5F_3 seed)

Season 9: Plant seed (BC_5F_3) from each harvested BC_5F_2 plant in a separate progeny row, observe progeny rows before flowering and eliminate segregating rows (they were Rr as BC_5F_2 plants); harvest BC_5F_4 seed from remaining progeny rows (RR)

Season 10: begin evaluations to confirm BC_5F_4 plants are phenotypically the same as the recurrent parent (except for disease resistance) including some yield testing

The percentages shown above are what occur on average, so several plants must be crossed each generation to be certain the resistant allele is present in the next hybrids. Modifications to the above outline are needed if the trait cannot be evaluated before flowering (extra crosses need to be made) or if the allele is recessive (selfing or progeny tests are need and/or blind backcrosses can be made). The above description shows five backcrosses; however, some breeders may use fewer or more backcrosses. If molecular markers are used F_1 or BCn F_1 plants with a larger percentage than average of the recurrent parent alleles in the genome can be used for the crosses so the recovery of the recurrent parent proceeds more quickly, and fewer backcrosses will be needed to recover the recurrent parent to a given level. Genes closely linked to the allele being transferred may not be eliminated during backcrossing and thus may make the complete recovery of the recurrent parent phenotype difficult. Details on the aspects noted above are shown in many plant breeding textbooks (Fehr, 1987b; Sleper and Poehlman, 2006).

Backcrossing has been used for many years in soybean breeding for various simply inherited traits like disease resistance, leaflet shape and more recently the Roundup Ready gene. It is possible to backcross more than one trait at a time but a larger number of plants are needed and extra evaluations needed to maintain all the desired traits during backcrossing. Since the number of plants used in backcrossing is relatively small and in many cases phenotypic evaluations for the trait are relatively easy, breeders use the greenhouse, growth chamber and/or off season nurseries to get several generations per year (as discussed in the SSD section). Thus backcrossing can be accomplished in about two years and evaluations another two or three years so the "new" backcross version of a cultivar can be released relatively quickly. Despite the shorter development time, it is still very important to choose a recurrent parent that is outstanding in almost all traits except the trait to be incorporated by backcrossing.

Recurrent selection

Recurrent selection is a cyclic method of population improvement but does not directly lead to release of cultivars. The basic steps in a cycle of recurrent selection are intermating, evaluation, and selection. The main challenge in using recurrent selection in soybean has been the difficulty of the intermating step. Since recurrent selection is designed to improve the frequency of favorable alleles (for the trait undergoing selection) in a population for quantitative traits, further breeding efforts are needed in order to release a cultivar from a recurrent selection population. Recent summaries of recurrent selection studies in soybean by Lewers and Palmer (1997) and Orf et al. (2004) discuss traits or characters investigated, selection methods, and intermating methods as well as marker-assisted recurrent selection techniques.

Use of male sterility in soybean breeding

The use of genetic male sterility to facilitate crossing especially in recurrent selection schemes has been used to some extent since the 1970's (Brim and Stuber, 1973; Lewers et al., 1996). Specht and Graaf (1990) described a breeding method called male-sterile-facilitated cyclic breeding (MSFCB) method for cultivar development. The authors suggest this method combines the best aspects of conventional breeding and diallele selective mating as described by Jensen (1970). Briefly, the MSFCB method involves placing annually chosen elite parents in a checkboard row pattern in an isolation nursery containing rows of male sterile parents. Insects transfer the pollen from the elite parents to the male sterile plants. At least one F_1 seed is harvested from each male sterile plant. It is suggested the F_1 plants be grown in a winter nursery and plants threshed in bulk to provide F_2 seed. The majority of the F_2 seed is advanced for cultivar development using single seed descent (male fertile plants). The cyclic part of the method is continued by using a small portion of the F_2 seed for the next year's male sterile plants in the isolation nursery (the male fertile plants are rogued at flowering). A number of high-yielding cultivars have been released using the MSFCB scheme.

Mutation breeding

Mutation breeding has been used to develop improved cultivars of soybean. However, most breeders agree that mutation breeding is best used when a desired trait or character is not found in the germplasm that can be used for crossing. Since the frequency of the desired mutation (genetic change) is usually very low, the breeder needs to screen a large number of plants (10,000's to 100,000's) using a rapid, inexpensive procedure or technique.

There are many aspects to consider in a mutation breeding program beginning with the trait or character, the screening procedure, the choice of line(s) to subject to mutagenesis, the mutagenic agent to use, the type of plant material to treat and the details of the treatment (dose, condition of the plant material, treatment conditions, etc.). Seeds are the most common plant material treated. The treated seed is usually planted in isolation (to prevent inadvertent crossing). The plants that grow from the treated seed are considered the M1 generation and produce M2 seed. The breeding methods used during the selfing generations after mutagenesis are the same as those used for populations developed from crosses (pedigree, bulk, SSD, EGT) with slight modifications for screening for the desired trait or character. Much greater detail on all aspects of mutation breeding can be found in Fehr, 1987b.

Transformation

Soybeans have had a number of traits added to the genome via transformation. The only trait that has been commercialized to date is glyphosate tolerance. The other transformation events are in various stages of testing and/or regulatory approval. From a breeding perspective once a plant has been stably transformed and has been shown to pass the trait on to its progeny the trait can be treated as qualitatively inherited and used as such in any breeding method.

Use of genetic markers in soybean breeding

The use of genetic markers for cultivar development in soybean breeding programs is just beginning. There is a need to develop methods for the widespread application of marker-based techniques in breeding programs. At least, for the time being, when genetic markers are used in soybean breeding programs, the markers will complement rather than replace traditional breeding methods. Some current and potential uses of genetic markers in cultivar development programs include the selection of parents based on genetic markers, the use of markers in backcrossing (for example to speed up the recovery of the recurrent parent (i.e., reduce the number of backcross generations) or to eliminate undesirable linked loci or to aid in genotyping for the trait being incorporated) and for marker-assisted selection (Orf et al., 2004). As more molecular markers and “breeder friendly” markers (such as single nucleotide polymorphisms) become available, marker assisted selection will be used more extensively to complement or supplement the more traditional proven breeding methods for cultivar development.

Hybrid soybean cultivars

Some research suggests that the use of F_1 hybrid seed might improve the productivity or other characteristics in soybean. The growing of F_1 hybrids on a commercial scale has not been possible due to the difficulty of producing large quantities of hybrid seed economically. Progress towards commercial use of F_1 hybrids appears to be occurring but there have not been any reports of widespread large scale testing of hybrids, let alone commercial soybean grain production from hybrids. In their article about hybrid soybeans, Palmer et al. (2001) listed five components that are critical for developing hybrid soybean on a commercial scale. They are 1) parental combinations that produce heterosis levels superior to the best pure line cultivars, 2) a stable male-sterile, female fertile sterility system, 3) a selection system to obtain 100% female (pod parent) plants that set seed normally and can be harvested mechanically, 4) an efficient pollen transfer mechanism from pollen parent to pod parent, and 5) an economical level of seed increase for the seedsmen and growers that ultimately benefits the consumer. Progress is being made with regard to some of the components, but it is not likely F_1 hybrids will be commercialized in the near future.

SUMMARY

Soybean breeding methods for development of commercial cultivars continues to evolve. Although soybean breeders have been successful in producing cultivars using traditional conventional breeding methods, the most widely used methods have shifted in recent decades to wide use of single seed descent, and with the advent of transformation, renewed use of backcrossing. Many breeders are now using molecular-based plant breeding methods and techniques (such as marker-assisted selection) as part of their cultivar development program. The challenge is to efficiently and effectively introgress the new or modified conventional and molecular technologies into existing cultivar development programs so that progress in soybean cultivar development continues in the future.

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SOYBEAN RESPONSE TO ENVIRONMENTAL FACTORS

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SOYBEAN RESPONSE TO CLIMATE

Soybean originated in East Asia, more specifically northeastern China, where it was grown as far back as 5,000 years ago (Jevtić, 1992). In the course of the 15th and 16th centuries, it was introduced into Indonesia, the Philippines, Vietnam, Thailand, Malaysia, Burma, Nepal, and northern India, where different landraces of the crop developed, so this region can be considered the secondary center of soybean's genetic origin (Hymowitz, 1988). That part of the world has a monsoon climate, characterized by a high amount of precipitation and high temperatures.

Outside of its native region, soybean is a fairly new crop. In Europe, U.S.A., and South America, it began to be grown on a significant acreage only in the early 20th century. Consequently, soybeans that had been grown in a monsoon climate were adapted to conditions with higher amounts of precipitation, somewhat higher temperatures, and short day-length. Intensive soybean growing led to the start of soybean breeding and there are now many varieties of this crop that are adapted to different climatic and soil conditions.

Life on Earth is maintained thanks to solar radiation. The rays of sunshine reaching the biosphere are converted into chemical energy. Plants containing chlorophyll are capable of transforming the energy from electromagnetic waves into the latent energy of organic compounds.

The Sun's radiation that reaches the Earth enables the circulation of energy, regulates the heat and water balance, and plays a role in the formation of temperature zones, i.e. the Earth's climate.

Climate has a great effect on plant growth and development, but man has little influence on it, so its elements (air, light, heat, water) are often a limiting factor in crop production.

Light and heat are mostly of cosmic origin, while water and air are atmospheric in nature.

Air

Air as an environmental factor affects plants by its composition and movement.

Air is a mixture of different gases, the composition of which is rather uniform irrespective of latitude. Absolutely dry air has the following composition expressed as volume percentage:

Nitrogen	78.08 %
Oxygen	20.95 %
Argon	0.93 %
Carbon dioxide	0.03 %

The remaining 0.01% are composed of other gases (hydrogen, neon, xenon, radon, krypton, helium, ozone, methane, ammonia, etc) and various foreign substances (powder, ash, spores, bacteria, pollen) and always contain a certain amount of water vapor as well.

The most important components of air for crop production are oxygen, nitrogen, and carbon dioxide.

In its elemental form, **nitrogen** is an inert gas that cannot be utilized by plants. Elemental nitrogen becomes available to plants when electrical discharges in the atmosphere produce nitrogen oxides, which then reach the soil via precipitation. This process enriches the soil with 8-15 kg/ha N in the temperate zone and up to 100 kg/ha N in the torrid zone.

Soybean uses up large amounts of nitrogen and contains 1.5-1.6% N in the above-ground parts and 6.5-7.0% N in the grain.

Leguminous plants, such as soybean, are capable of forming symbiotic associations with bacteria from the genera Rhizobium and Bradyrhizobium, which result in the formation of root nodules in which elemental nitrogen (N2) becomes converted into the ammonium ion (NH4+). This process is of high importance not only for legumes but for all living things on Earth as well.

The symbiotic association between soybean and the bacteria provides the soybean plant with the necessary amounts of carbohydrate that supply the energy by which the bacteria transform inert N2 into the NH4+ ion. The ion then neutralizes the carbonic acids from the Krebs cycle and amino acids are produced. This way the bacteria provide the soybeans with the amounts of nitrogen needed for the plant to synthesize amino acids and produce protein.

Soybean is capable of fixing large quantities of nitrogen through symbiotic N fixation. Enkina (2005) reported that soybean plants fixed an average of 137.7-167.6 kg/ha N in a multi-year trial with different fertilization treatments. Similar results were obtained by Vera Milić et al. (1998), who reported that soybean N fixation produced 151.3 kg/ha N in a trial on chernozem in the Serbian province of Vojvodina. The amounts of N that enter the soil via nonsymbiotic nitrogen fixation are considerably smaller and range between 5 and 20 kg/ha.

Oxygen is an element necessary for plant and animal respiration. Aerobic microorganisms also require it for their growth.

The oxygen content of the air in the atmospheric layers closest to the ground is stable and the above-ground plant parts always have enough oxygen for respiration.

Oxygen deficiency may occur in the soil. Plants require oxygen for their root respiration. When waterlogging occurs and the soil is severely compacted, oxygen levels in the soil air may drop, which may result in plant death due to root suffocation.

Soybean needs a well-aerated soil. The lowest soil air content that soybeans are able to tolerate is 9% vol., while soybean roots are considered well supplied with air when the air content of the soil is 15-22% vol. (Konova and Hristov, 1975). According to the same authors, the best soils in hydro-aerial terms are those with a total porosity of 55-60 % in which the capillary spaces are filled with water and noncapillary ones with air. Air is needed not so much for root respiration as to support the life of symbiotic bacteria and their fixation of atmospheric nitrogen.

Soybean plants can tolerate low soil oxygen levels as well (1.5% vol.). This is due to their ability to absorb oxygen from nitrate ions in the root (Norman et al., 1970, as cited by Baranov, 2005). The same authors note that it is thanks to this ability that soybeans can survive for several days on end with no air in the soil in waterlogged conditions.

Plants need **carbon-dioxide** for photosynthesis. Its percentage in the air is relatively low (only 0.03%), but its importance for plants is huge, because it is their main source for the production of organic matter.

Over the last two and a half centuries, carbon dioxide levels have been on a constant rise due to the combustion of fossil fuels and wood, deforestation, and soil cultivation. According to Keeling et al. (1995), between the start of the industrial revolution (c. 1750) and 1980, the levels of carbon dioxide rose from 0.028 to 0.034%, so this gas has now become an atmospheric pollutant, as it contributes to the greenhouse effect.

The increase in carbon dioxide levels has also made possible an increase in the rate of photosynthesis and increased yields.

Another major pollutant besides carbon dioxide is sulfur dioxide. Fossil fuel combustion, the smelting of ores, wood stove heating, and the burning of forests all lead to the release of a large amount of sulfur dioxide, which binds with the water from the atmosphere and enters the soil via acid rain.

Acid rain changes the pH of the soil and may directly scorch less resistant plants such as conifers or lichens and cause forest deterioration in industrial areas with significant amounts of sulfur-dioxide.

In addition to the above, the atmosphere is full of other, more or less harmful, gases, dust particles, heavy metals, etc. These can also have a negative effect on plants.

The air is constantly moving, and this movement is called the wind. Wind is a result of different air pressures resulting from the different degrees to which different parts of the planet are heated. Areas around the equator receive the most heat and those around the poles the least. Because of this, the warm air from around the equator rises and moves through the upper parts of the atmosphere towards the poles, while the cold, heavier air from the polar regions travels towards the equator through the lower portions of the atmosphere. This constant, balanced movement of air is disrupted by the rotation of the Earth, differences in the warming and cooling of the land and sea, and the distribution of the land and water masses on the planet.

The wind may affect plants directly or indirectly. The direct action of the wind stirs up the air layer around the leaves and thus enables better gas exchange and increased rates of photosynthesis and respiration. Air movement also increases the transpiration rate and by virtue of this the cooling of the plant. The wind facilitates pollination in cross-pollinated species and the dispersal of seeds and fruits.

Light winds blowing at speeds of up to five meters per second are useful for agriculture and are referred to as breeze. Winds faster than five meters per second are usually harmful from the point of view of agriculture.

Soybean does not tolerate heavy winds well, especially if grown in a dense stand. Heavy winds cause the leaves to breake and the flowers and young pods to fall off and may lead to lodging and major yield losses.

In a temperate climate, negative wind effects can be successfully countered by protective forest belts. These can dampen the force of the wind, prevent aeolian erosion, and increase relative humidity, which has a positive influence on cultivated plants.

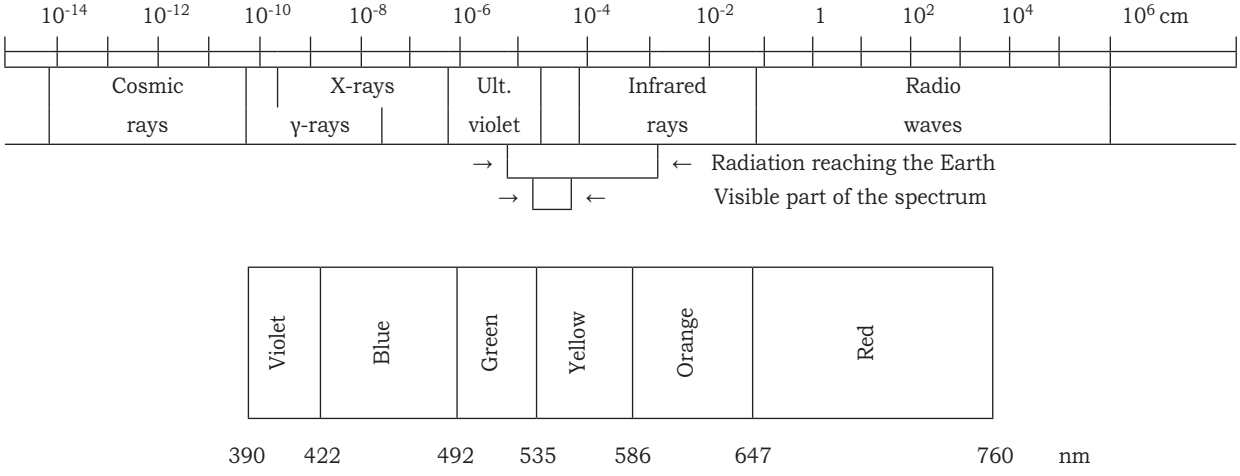
Light

Light is part of electromagnetic radiation that reaches the Earth from the Sun. It is a major vegetative factor, because it affects plant growth and development.

Nuclear reactions release large amounts of energy on the Sun, and some of this energy reaches the Earth in the form of electromagnetic waves. The Sun's electromagnetic radiation is shown in Figure 7.1.

Figure 6.1

The spectrum of solar electromagnetic radiation and a close-up of its visible portion (Stevanović and Janković, 2001)



Soybean leaves are best able to absorb light from the blue/violet part of the spectrum with a wavelength of 400-500 nm (around 95%), followed by the red-orange band, 600-700 nm in wavelength (around 90%). The percent absorption of light of other wavelengths is about 60% (Vratarić and Sudarić, 2007).

Soybean is a C_3 plant in which light saturation occurs at relatively low light intensities depending on atmospheric CO_2 levels and lighting conditions at the different stages of growth (Planchon, 1986). Similar results were reported by Bohning and Burnside (1956), who recorded maximum photosynthesis at a light intensity of 23,672 lux.

According to Molnar (1987), soybean is a plant that needs plenty of light. In a poorly lit phytotron (20,000 lux), the internodes will elongate, the stem will remain slender and prone to lodging, and flowering may not occur. Soybeans will have approximately the same habit of growth as in natural conditions only if the intensity of light is higher (30,000 lux).

When the intensity of sunlight is reduced by 50%, soybean plants will develop a considerably smaller number of nodes, branches, and pods (Mjakuško and Baranova, 1984). To prevent this from happening, care must be taken not to grow soybeans in a stand that is too dense.

Plants respond not only to light intensity and quality but to the duration of lighting as well. Day length varies with season and latitude and is defined as the period between sunrise and sunset. At the equator, it is the same all year round and a day lasts 12 hours. As one moves away from the equator towards the poles, the days are longer than the nights in the summer and shorter than them in the winter. At the latitude of 45º N (where the town of Ruma, Serbia, is located for example), the longest day occurs in June and lasts a little over 15 hours and 34 minutes, while the shortest day length of eight hours and 50 minutes is recorded in December. Plant response to the duration of the day is called photoperiodism. Plants can be divided into three groups based on their photoperiodic response:

Long-day plants (wheat, barley, rye, oat, triticale, sugar beet, alfalfa, red clover, rapeseed, most grasses, flax, broad bean, onion, pea, spinach, lettuce, cabbage, carrot, poppy…)

Short-day plants (soybean, maize, hemp, rye, cotton, millet, pepper, coffee, pineapple…)

Day-neutral plants (sunflower, buckwheat, vetch, raspberry, tomato, cucumber, bean…)

Long-day plants need the length of day to exceed a certain critical point in order to make the transition from the vegetative to the reproductive stage. This critical day length varies with plant species and cultivar and is usually 12-14 hours. The vegetative phase consists in the development of plant vegetative organs (roots, stems, and leaves), while the reproductive one involves the formation of the generative organs (flowers, fruits and seeds).

Short-day plants require the day to be shorter than the critical day length in order to transition from the vegetative to the reproductive stage, while day-neutral plants do not respond to the length of day at all.

There are two types of photoperiodic response. The obligate type is found in plants that cannot make the passage from the vegetative stage to the generative one if the length of day is inadequate. The facultative response is when plants are capable of making the said transition even if day length is not adequate but will prolong their growing season in such a case.

Soybean responds to day length and is a short-day plant that requires long nights to start flowering. It needs two to six short days at the stage of one to three trifoliate leaves in order to enter the reproductive stage. (Baranov, 2005). To make this transition, most soybean cultivars require a day length of less than 13-15 hours (Adamenj et al., 2006). The same authors also note that dependence on day length is inversely proportional to the length of the growth period. Cultivars that need a lot of

days to mature are more sensitive to the absence of short days, i.e. their photoperiodic response is obligate. Cultivars with a short growing season originating from northern regions (Maturity Groups 0-III) are capable of developing generative organs even when there is no night at all (24-hour illumination), so their photoperiodic reaction is facultative. This has enabled soybean to have a wide geographic distribution ranging from 40° South latitude to 60° North latitude, although the crop still performs the best in the northern hemisphere at latitudes between 35 and 45° N.

Heat

Heat affects plant growth and development and is considered a primary vegetative factor. Not all places on Earth receive the same amount of heat. One can differentiate between the horizontal and vertical distribution of heat on the planet.

The horizontal heat distribution depends on the angle at which the sunlight strikes the Earth's surface. According to Haberlandt (1878), there are three major plant zones on Earth: frigid, temperate and torrid (Table 6.1).

Vertically, the temperature drops by an average of 0.6°C for every 100 m of altitude. This is a result of the fact that the air is heated primarily from the Earth's surface up and minimally by the passage of sunlight through the atmosphere.

One of the most commonly used indicators of plant heat requirements is the sum of temperatures. The temperature sum is a figure representing the sum of all mean daily temperatures occurring in the course of a plant's growing season. Since the biological minimum needed for the growth of a crop species is not 0°C, a plant's requirement for heat can be described more accurately by using the sum of all mean daily temperatures that are above the biological minimum for the species concerned. This sum is called the sum of effective temperatures. A temperature of 10°C is most often regarded as the biological minimum for soybean.

Table 6.1

Plant zones (according to Haberlandt)

Plant zones	Latitude	Vegetation
I Frigid, 9% of Earth's surface		
Polar	72°-90°	Mountain herbs, lichens, moss
Arctic	66°-72°	Mountain herbs, shrubs, grassland, birch
Subarctic	58°-66°	Conifers, birch, grassland
II Temperate, 49% of Earth's surface		
Cooler temperate	45°-58°	Decidious trees, beech, oak, meadows, small grains
Warmer temperate	34°-45°	Broadleaf evergreens, maize, vine, millet
III Torrid, 42% of Earth's surface		
Subtropical	23°-34°	Myrtle, laurel, lemon, cotton, tea, sugar cane
Tropical	15°-23°	Fig, arborescent ferns, grasses, palms
Equatorial	0°-15°	

Soybean is a plant that needs relatively high temperatures and its temperature requirements increase with maturity group. According to Enken (1959), the effective temperatures required by particular soybean MGs are as follows:

- very early varieties 1,700-1,900ºC
- early varieties 2,000-2,200ºC
- medium late varieties 2,600-2,750ºC
- very late varieties 3,000-3,200ºC

Table. 6.2

Soybean temperature requirements at different stages of growth and development expressed in ºC (Enken, 1959)

Phenological stage	Biological minimum	Sufficient temperature	Optimal temperature
Germination and emergence	6-7	12-14	20-22
Seedling stage	8-10	15-18	20-22
Branching	16-17	18-19	21-23
Flowering	17-18	19-20	22-25
Grain formation	13-14	18-19	21-23
Maturity	8-9	14-16	19-20

Soybean emerges after 6-7 days at the optimal soil temperature (20-22ºC) and after 20-25 days when the temperature is inadequate (8-10ºC). This is why soybeans should be planted when the temperature of the soil rises above 12-14ºC (Baranov, 2005).

When the temperature sum is calculated using the method introduced by Soldati (1985) (as cited by Rajičić, 1988), the results show that soybeans need a sum of effective temperatures of 80ºC (with 8ºC as the biological minimum) in order to emerge. .

Once soybean emerges, it can withstand short-lasting frosts of -3 to -4ºC (Gutschy, 1950). According to Kurnik (1976), young soybean plants are capable of withstanding short-term frosts of -6 to -7ºC without suffering any major damage, provided the temperature of the air gradually increases after that. Because of this, it is possible to plant soybean in Serbia before maize, since the former crop is more resistant to low temperatures.

Soybean needs temperatures of over 19ºC in order to start flowering. Early varieties may lose their flowers as early as at temperatures of 16-17ºC (Zalotnickij, 1962). This often represents a limiting factor in soybean production at higher altitudes and latitudes.

The minimum temperature for soybean grain formation is 14ºC.

Soybeans mature the best when the temperature is 19-20ºC. The minimum temperature for soybean maturation is 8-9ºC, and short-lasting frosts of -2 do -2.5ºC will not result in yield losses at this stage (Enken, 1959).

Soybeans require higher temperatures and develop well when the temperatures are high, but they are at the same time resistant to brief periods of low temperatures and short-lasting frosts.

Water

Plants need water for their growth and development. In soybean, the water content is the highest in the young leaves (up to 90%) and the lowest in the beans, where it may be as low as 10%. Vučić and Bošnjak studied soybean water requirements in Vojvodina in 1980 and found that the crop's potential evapotranspiration was 460 mm (399-491) for MG 0, 480 mm for MG I (449-534), 500 mm for MG II (461-550).

Different soil types may contain different amounts of water. Depending on how much of it there is in the ground, water is retained in the soil by the action of different forces, so we can distinguish between the following soil water constants:

- Field capacity (FC) is the maximum amount of water a soil can retain in field conditions once gravitational water has drained away. At this level of soil moisture, the water adheres to the soil with a force of 0.33 to 0.50 bars.
- The lentocapillary point is the point that separates the water that is readily available to plants from that which is not so readily available. It is also the boundary between the highly mobile capillary water and the slow-moving water that forms a thin film around the soil particle. The adhesive force between the water and soil particle at this soil moisture level is 6.25 bars.
- The wilting point may refer to the initial wilting point, which is when the first signs of wilting start to appear on plants. It represents the boundary between the soil water whose availability to plants is limited and that whose availability is highly limited. At the initial wilting point, water is retained in the soil by forces of 11 to 19 bars, so an agreement has been made to define the initial wilting point as that point at which the soil moisture tension is at 15 bars.

Besides the initial wilting point, there is also the permanent wilting point, which is the soil moisture threshold at and beyond which the plant irreversibly wilts and dies. This water constant separates the water with highly limited availability to plants from that which is unavailable to the plant.

In an average year, the province of Vojvodina, the largest soybean-growing region in the country, has about 400 mm of rainfall during the growing season. Before planting, the soil may have different plant available water reserves depending on the soil type, the crop grown the previous season, the amount of rainfall in the preceding season, and the amount of winter precipitation.

Prior to sowing, soil moisture down to 1 m depth should be measured, and if the measurement shows that the plant available water reserve is low, plant population per hectare should be reduced. This can then considerably help mitigate the effects of a drought, if one occurs later in the season.

In a chernozem soil, available water reserves down to 1 m depth may be as high as 120 l/m^2 (mm). This means that when the right preceding crop is used, the winter has had a normal amount of precipitation, and the growing season has had an average amount of rainfall, water should not be a limiting factor in producing high soybean yields, provided the distribution of precipitation is favorable. Nevertheless, it still often happens that water becomes a limiting factor in obtaining high yields of this crop. This is is most often the case when soybean is grown after a previous crop that dries out the soil to a great extent and uses up large amounts of water (alfalfa, sugar beet, maize) or when the winter has been dry and the soil has not been able to replenish its reserves of plant available water, especially if there is not enough rainfall during growing season. In a semiarid climate such as that of Vojvodina, soybean needs irrigation in order to produce stable and fail-safe yields, which will be discussed in detail in a separate chapter.

Even when there is enough moisture in the soil, short-time water deficits may sometimes occur during warm and windy days. Plants defend themselves from such deficits by closing up their stomata. This has a negative effect on yields, because the closing of the stomata is very soon followed by the cessation of photosynthesis.

Soybean is sensitive to low relative humidity, especially when accompanied by high temperatures and soil drought. Such conditions greatly impede pollination and may cause soybeans to shed their young pods.

Soybean plants do not always need the same amounts of water. At germination and emergence, soybean seeds need enough water for imbibition and germination, but water consumption in that period is relatively small, only 0.5 mm a day (Reicosky and Heatherly, 1990).

Later on, from emergence to flowering, soybean plants will be resistant to water deficits. When growing soybean in field conditions, water deficits during this period may have a positive effect on yields, because in drought conditions the plant forms less above-ground mass and develop a more robust root system in search of water. In situations when there is an abundance of water, plants form a lot of above-ground biomass and a relatively shallow root system, which reduces plant resistance to drought. If a rainy period in the early stages of plant growth and development is followed by a drought at flowering and grain formation, yield losses will be great.

At flowering, soybean is susceptible to soil moisture deficits as well as to low relative humidity, especially if drought during that period is accompanied by high temperatures. In such conditions, the plants will shed their flowers and pollination will be reduced. If there is a rainy period later on, soybeans may produce an average yield. Soybean consumes the largest amounts of water at pod formation and grain fill.

At these stages, according to Reicosky and Heatherly (1990), soybean plants may consume up to 8 mm of water per day.

At maturity, soybean does not require a lot of water and prolonged periods of rain may negatively impact grain yield and quality as well as the germinability of soybean seed crops. Besides prolonged rainy weather, soybean yields may at this stage also be negatively affected by repeated alternations between rainy and warm days, which may lead to pod cracking and seed shattering in susceptible cultivars.

SOYBEAN RESPONSE TO SOIL TYPE

Soil is the surface portion of the lithosphere, which is under the influence of the biosphere, hydrosphere, and atmosphere and has as a result acquired a new quality – fertility, that is, the ability to provide plants with water, mineral substances, and oxygen (Ćirić, 1986).

The soil has formed by the action of pedogenetic factors (parent material, climate, relief, vegetation, fauna and weather) and their effects on the litosphere. The resultant loose layer is called the pedosphere, or soil (Molnar et al., 2003).

This surface layer of the Earth's crust makes life possible for higher plants thanks to its physical, chemical and biological properties. It is their natural habitat. Plants take root in the soil and use it to obtain nutrients, water and part of their carbon dioxide and oxygen supply.

Today it is possible to grow plants even without soil, and this is most commonly done in a greenhouse. The root systems of plants grown in such a way are anchored in a soil substitute through which a nutrient solution is passed that contains all the elements necessary for plant nutrition. Although the proportion of plants cultivated without soil is growing by the day, the vast majority of the world's crop production (over 98%) still takes place on the soil, which is the reason why soil is of such great importance.

Soil is an open system in which the parent material, once homogenous, changes over time under the influence of climate and the plant and animal world. These changes are stratificational in nature and over a certain period of time, which may be measured in thousands of years, different strata form along the soil profile that we call horizons. These layers differ in their physical and chemical properties and these differences reflect the direction and intensity of pedogenetic processes and serve as the basis for soil classification.

Soil classification

Serbia uses the Classification of Soils of Yugoslavia (Škorić et al., 1985), which categorizes soils based on the way in which they are wetted and the composition of water they are wetted by. The classification distinguishes between four different soil orders:

Automorphic soils

This order is characterized by wetting by atmospheric precipitation only. These soils are water permeable, have no impermeable horizons, and the water is not retained in the impermeable horizon for longer periods of time.

Hydromorphic soils

Hydromorphic soils are characterized by the occurrence of occasional or permanent waterlogging and reduction processes in parts of the profile or across the profile. Waterlogging is defined as a condition in which all soil pores are filled with water and may be caused by the retention of atmospheric precipitation and accumulation of surface or ground water that is neither salinated nor alkalized.

Halomorphic soils

These soils are characterized by additional wetting from ground or (less commonly) surface water that is saline and alkaline. Soils of this order have one or both of the following characteristics:

a) contain at least 1% salt (in the case of chloride-sulfate salinity) or 0.7% salt (in the case of sodium salinity) down to 125 cm depth,

b) have clearly differentiated A and B horizons in the profile both texturally and otherwise, with B being a natric horizon (a special kind of argillic horizon that has all the properties of argillic horizons but is in addition 15% or more sodium saturated and has a columnar or prismatic structure).

Subaqueous soils

These are the soils that form in the subaquatic conditions of shallow standing waters (lakes, ponds, and coastal seas). Pedogenetic processes in them are often intermingled with sedimentary ones.

The soils of Serbia are very diverse when it comes to soil types, subtypes, varieties and forms. This diversity is a result of the fact that they have formed on different types of bedrock. The greatest potential in the country in terms of agricultural production can be found in the province of Vojvodina. The most fertile soil types in the province are chernozems, hydromorphic black soils (humogleys), smonitzas (vertisols), brown forest soils (eutric cambisols), and alluvial soils (fluvisols).

The main factors influencing a soil's value from the point of view agricultural production are as follows: soil type, relief, geological substrate, depth of the humus-accumulating layer, soil mechanical composition, soil reaction, levels of macro- and micronutrients, the soil's position, susceptibility to erosion, and irrigation possibilities (Kovačević, 2003).

Table 6.3

Tabular overview of the Yugoslav soil classification (Škorić et al., 1985)

A. AUTOMORPHIC ORDER

Profile class and structure	Soil type
I Undeveloped or weakly developed soils (A)-C profile	1. Lithosol (Kamenjar) 2. Regosol (Sierozem) 3. Arenosol (Aeolian sand) 4. Colluvium (Colluvial deposit)
II Humus-accumulating soils A-C profile	1. Calco melanosol (Limestone-dolomitic black soil) 2. Rendzina 3. Ranker (Humic-silicate soil) 4. Chernozem 5. Smonitsa (Vertisol)
III Cambic soils A-(B)-C profile	1. Eutric cambisol (Eutric brown soil) 2. Dystric cambisol (Acidic-brown soil) 3. Calco cambisol (Brown soil on limestone and dolomite) 4. Terra rossa (red soil)
IV Eluvial-illuvial soils A-E-B-C profile	1. Luvisol (illimerized soils) 2. Podzol 3. Brunipodzol (Brown podzolic soil)
V Anthropogenic automorphic soils P-C profile	1. Rigosol (deep-tilled soils) 2. Hortisol (garden soils)
VI Technogenic soils P-C profile	1. Deposol (landfill soils) 2. Flotisol 3. Aeroprecipitat

B. HYDROMORPHIC ORDER

Profile class and structure	Soil type
I Pseudogley soils A-E/g-B/g-C profile	1. Pseudogley
II Undeveloped soils (A)-G or (A)-C profile	1. Fluvisol (Recent river deposits)
III Semigley soils A-C-G profile	1. Humofluvisol
IV Gley soils A-G profile	1. Gley soil 2. Humogley-eugley
V Peat soils T-G profile	1. Elevated peat soil 2. Mid-level peat soil 3. Low-lying peat soil
VI Anthropogenic hydromorphic soils P-G or Ap-G profile	1. Deep-tilled peat soil 2. Rice paddy soil 3. Hydromeliorated soil

C. HALOMORPHIC ORDER

Profile class and structure	Soil type
I Acutely salinated soils A_{sa}-G or A_{sa}-CG	1. Solonchak
II Solonetz A-$B_{t,na}$-C or A/E-$B_{t,na}$-C	1. Solonetz

D. SUBAQUEOUS ORDER

Profile class and structure	Soil type
I Undeveloped subhydric soils (A)-C or (A)-G profile	1. Protopedon
II Subhydric soils A-C or A-G profile	1. Gyttja 2. Dy 3. Sapropel
III Drained subhydric soils P-C or P-G profile	

Abundance of particular soil types in serbia and their importance for soybean production

Serbia has a total area of 8,836,100 ha, of which 5,701,173 ha are agricultural land. The total amount of arable land in the country is 4,653,415 ha and the distribution by region is as follows: Serbia proper (without the two provinces) - 2,608,375 ha, the province of Vojvodina - 1,646,294 ha, and the province of Kosovo and Metohija - 398,746 ha (OG RS 1998).

The most important and common soils in Serbia are automorphic and hydromorphic ones. The most important soils for soybean production are those found in the plains, river valleys, and hilly areas.

Soybean roots are firm and robust and their proper development (especially the growth of the nitrogen-fixing root nodule bacteria) requires a soil that is neither acidic nor saline, has good water-air properties, and sufficient amounts of available nutrients (Vratarić and Sudarić, 2007).

Soybean also needs a well-aerated soil. The lowest soil air content that soybeans can tolerate is 9% vol., while 15-22% provide soybean roots with an optimum air supply (Konova and Hristov 1975).

According to the same authors, the best water-air regime is found in soils with a total porosity of 55-60% in which the capillary pores are filled with water and non-capillary ones with air. The air is needed not so much for the respiration of the root as to support the life of symbiotic bacteria (Rhizobium japonicum) and their fixation of atmospheric nitrogen.

According to data provided by the Soil Institute at Topčider (as cited by Kovačević, 2003), the following types of agricultural soil can be found in Serbia proper.

Table 6.4

Areas (ha) in particular types of agricultural soil in Serbia proper

Soil type	Serbia proper
Lithosol	76,500
Arenosol	36,000
Ranker	123,000
Chernozem	35,300
Vertisol	624,500
Eutric cambisol	642,000
Dystric cambisol	907,000
Pseudogley	500,000
Fluvisol	250,000
Humogley and eugley	193,000
Total	3,387,300

In Serbia proper, intensive field crop production without any major limitation can only be conducted on about 500,000 ha. Especially problematic in crop production terms are permanently waterlogged soils (81,000 ha), seasonally waterlogged soils (161,000 ha), and floodplains (250,000 ha). In addition, 80,000 ha have become barren due to erosion and large areas are under the influence of erosion to varying degrees (Kovačević, 2003).

In Serbia proper, soybean can be successfully grown on soils such as chernozem, smonitza (vertisol), ranker, alluvium (fluvisol) and black meadow soil (semigley). When growing soybeans, the quality and timing of tillage are of great importance. This is especially true in the case of smonitza, because good aeration is essential for successful soybean growing and the normal development of root nodule bacteria. Alluvial and black meadow soils can only be used for soybean production if they are not waterlogged or too sandy. Light and sandy soils cannot produce stable soybean yields because of the crop's high water requirement at the critical stages of plant growth and development. Light soils are characterized by poor water retention and are therefore not suitable for soybean growing in non-irrigated conditions.

According to Stevanović et al. (1992), the region of Kosovo and Metohija has the following types of arable agricultural soil:

Table 6.5

Areas (ha) in particular types of arable agricultural soil in Kosovo and Metohija)

Soil type	Arable agricultural soil (ha)
Colluvium	60,800
Rendzina	10,000
Ranker	1,100
Vertisol	76,300
Eutric cambisol	81,200
Dystric cambisol	34,500
Calco cambisol	16,200
Terra rossa	3,500
Pseudogley	32,000
Fluvisol	69,800
Humogley	3,800
Eugley	10,800
Total:	400,000

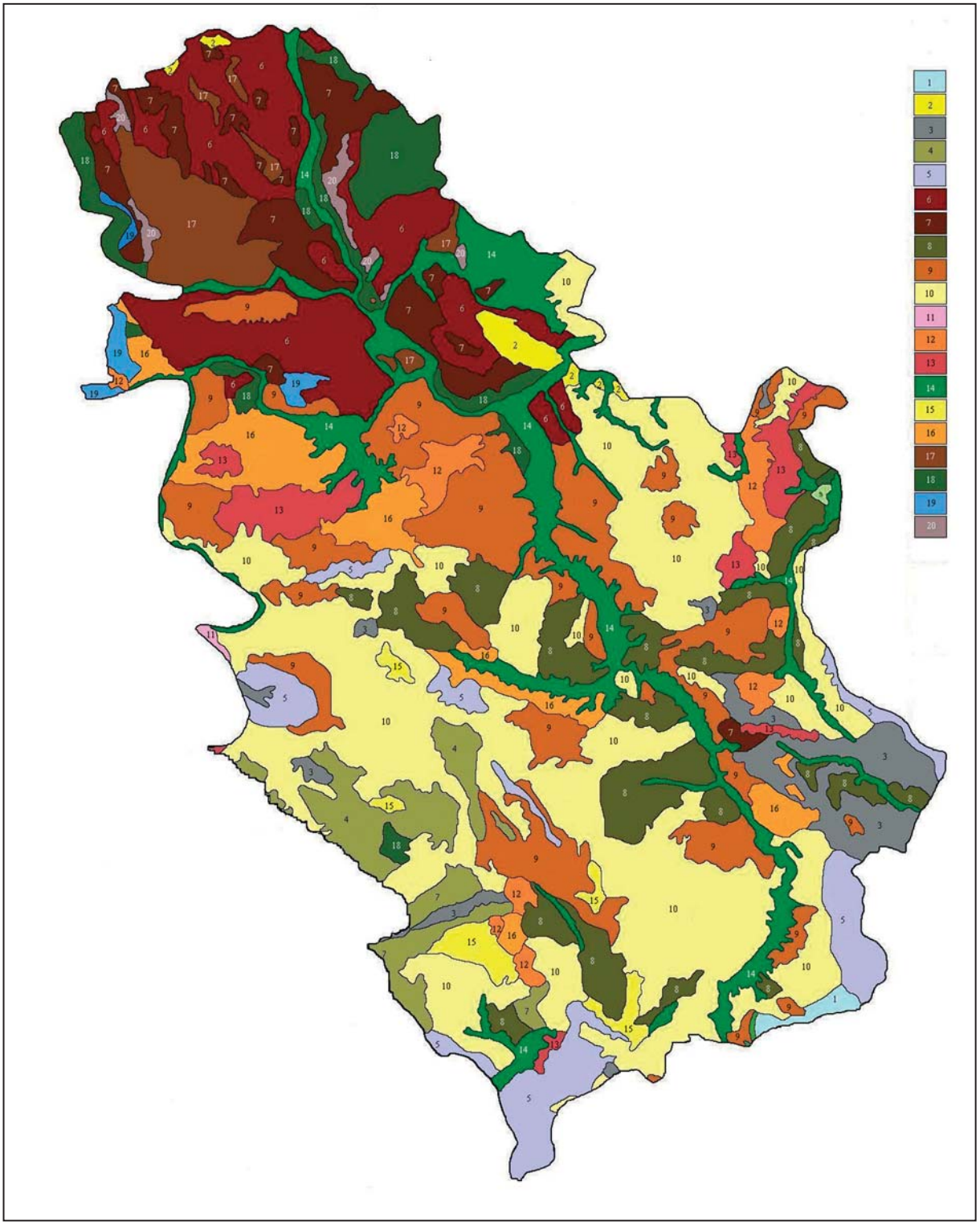
Over 40% of soils in Kosovo and Metohija are naturally acidic or highly acidic (pseudogley, eutric cambisol, dystric cambisol). Stevanović et al. (1992) note that in addition to the naturally acidic soils found in the region, there are also those in which secondary acidification has occurred as a result of intensive soil use, incorrect fertilization practices, and acid rain.

Soybean can be grown on acidic soils as well. In a study by Nenadić et al. (1986), nine different fertilization treatments produced an average yield of 1.22 t/ha with a soil pH of 4.2 in KCl. Nevertheless, soybeans are still best suited for soils with a neutral reaction (pH 6-8), as acidic soils require remediation by liming in order to produce economically viable yields.

Serbia is not a large country in terms of land mass, but it is very diverse thanks to its relief and variable pedogeographic profile and has many different soil types, as can be seen from its soil map (Figure 6.2).

Figure 6.2

Soil map of Serbia, based on the soil map of Yugoslavia (Škorić et al., 1985)



1. *Lithosols on acid rocks and rankers*
2. *Arenosol and eutric brown soil on sand*
3. *Calcareous dolomitic black soil, lithosols and rendzinas*
4. *Calcareous dolomitic black soil, brown soil on limestone and terra rossa*
5. *Rankers and dystric brown soils*
6. *Chernozem on loess*
7. *Chernozem and chernozemic semigley soil*
8. *Smonitzas*
9. *Eutric brown soil*
10. *Dystric brown soil – leached and browns soil on limestone and dolomite*
11. *Leached brown soil and black soil on limestone and dolomite*
12. *Leached and eutric brown soils*
13. *Leached soils*
14. *Fluviate and eugley soils*
15. *Pseudogleys*
16. *Pseudogley and leached pseudogley soils*
17. *Chernozemic semigley soils*
18. *Hydromorphic black soils*
19. *Gley and semigley soils*
20. *Solonchack and solonetz*

Data from the Soil Map of Vojvodina (as cited by Kovačević, 2003) have been used to make an overview of the major soil types in the province. The soil type referred to as the black meadow soil in the old classification is now most often considered a chernozem in the new one.

Table 6.6

Areas (ha) in particular soil types in Vojvodina (ha)

Soil type	Total area (ha)
Regosol	17,054
Rendzina	14,481
Ranker	10
Chernozem	1,323,278
Vertisol	36,159
Eutric cambisol	56,164
Dystric cambisol	1,412
Rigosol	10,510
Pseudogley	20,176
Fluvisol	198,328
Humogley	347,816
Eugley	15,269
Low-lying peat soil	420
Solonchak	19,865
Solonetz	80,333
Protopedon	8,564
Gyttja	
Dy	
Sapropel	
Total:	2,149,839

Vojvodina is the best region for soybean growing in the country. Most of the soils found in the province are fertile (chernozem, smonitza (vertisol), black meadow soil (semigley), alluvial soil (fluvisol), hydromorphic black soil (humogley)) and are the kinds of soil on which soybean can be grown with success..

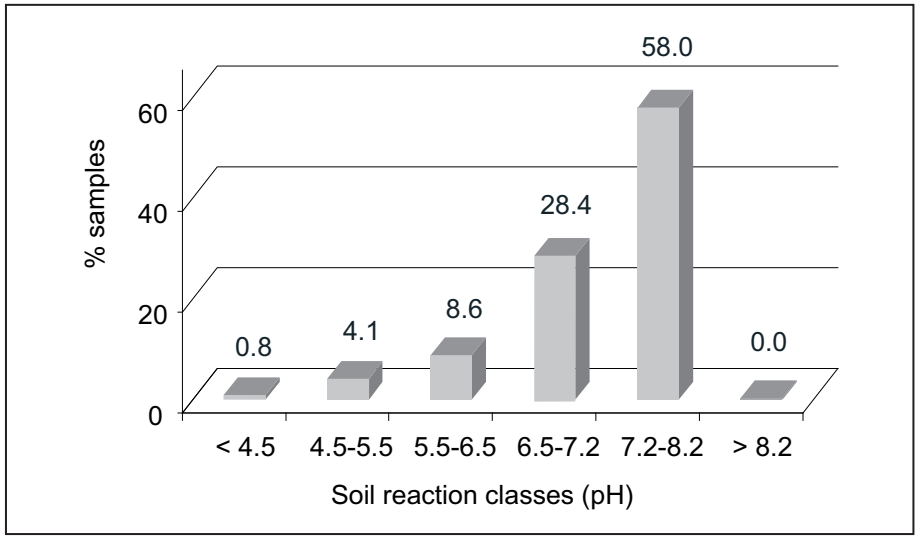
Chernozems and similar soils cover an area of over 1,300,000 ha in Vojvodina and provide optimal soil conditions for soybean growing.

After analyzing more than 90,000 soil samples, it has been determined that the pH values of Vojvodina's plowland vary widely but that most of the soils (86.4%) are somewhere between pH 6.5 and pH 8.2 (Sekulić et al., 2007), which is the optimum range for soybean growing. According to Damenj et al. (2006), the optimum pH values for growing soybeans are those between pH 6.5 and pH 7 and the plant can also be grown with success within the pH 5.5-8.5 range. The same authors also note that soybean growing becomes impossible at pH values below 3.9 and above 9.6.

Thus, if soil pH were to be the only criterion, soybean should not be grown on only 4.9% of the plowland in Vojvodina, as that is the percentage of soils with a pH value of less than 5.5.

Figure 6.3

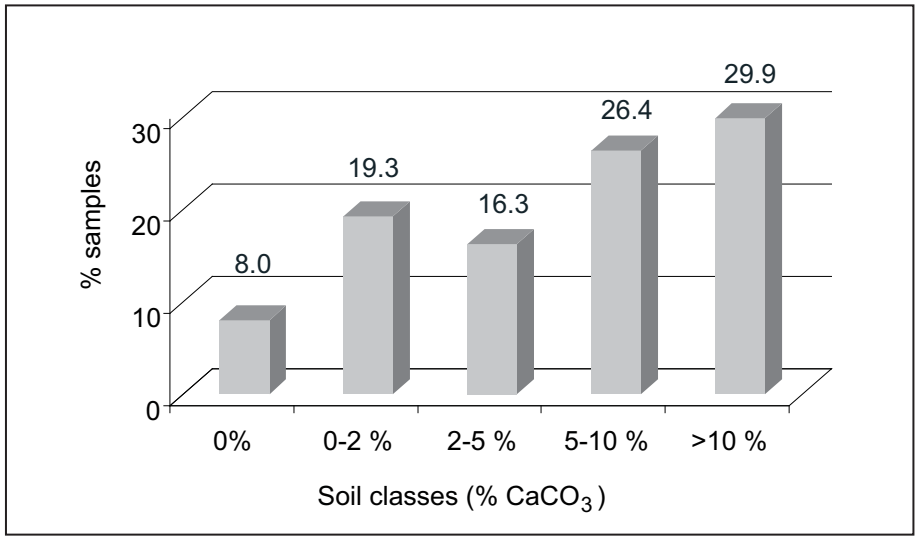
pH values of Vojvodina's plowland (Sekulić et al., 2007)



Soybean is a plant that requires a well-structured, loose soil that is capable of retaining enough water for the plants to be well supplied with moisture during the period of intensive growth and at flowering, pollination and grain fill. The stability of soil structural aggregates is significantly positively influenced by the $CaCO_3$ content. (Figure 6.4).

Figure 6.4

Soil CaCO3 content in Vojvodina (Sekulić et al., 2007)



Soybean plants take up calcium in the form of the Ca^{2+} ion. In Vojvodina, 92% of the plowland have enough calcium and calcium addition by fertilizer is needed on only 8% of the area.

Soybeans are usually grown on fertile and humus-rich soils. Humus has a positive effect on soil structure and on water retention in the soil and thus contributes to the increase of soybean yields. Figure 3 shows humus levels in the soils of Vojvodina.

Only 1.86% of the agricultural acreage in Vojvodina are less than 1% humus. On any of the rest of the areas, humus is not a limiting factor in soybean production. Data shown in Figure 3 show that a little over 10% of the areas in Vojvodina have less than 2% of humus and that 59% are in the 2.5-3.5% range. On all this acreage, careful attention must be paid to the plowing under of crop residues and to the incorporation of organic fertilizer, so as to prevent any further decline in the soil humus content and soil fertility.

In 1951, Nejgebauer reported that the chernozems of Vojvodina had lost over 50% of their humus since the start of cultivation and that their humus content had dropped from 7-8% to 3-5%.

The burning of crop residues is prohibited by law. To our great misfortune, this ban is not enforced to a sufficient degree, so it is not rare that fires can be seen burning at night across Vojvodina once the harvesting of wheat and maize is over. This practice has led to a decrease of soil humus content in the province in the last 50 years. The present-day levels of humus in Vojvodina are in most cases somewhere in the 2.0-3.5% range, and what is even worse, this negative trend seems to be continuing

Figure 6.5

Soil humus content of Vojvodina plowland (Sekulić et al., 2007)

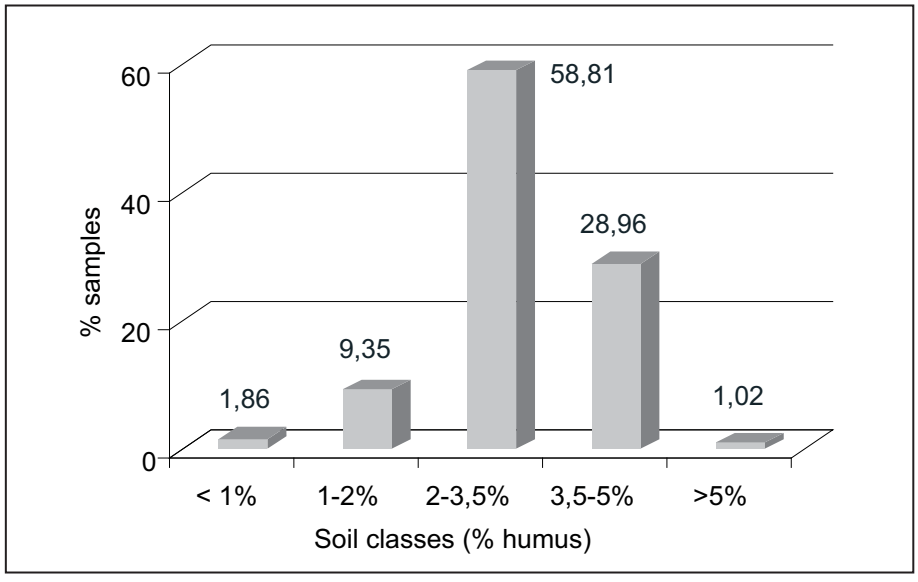
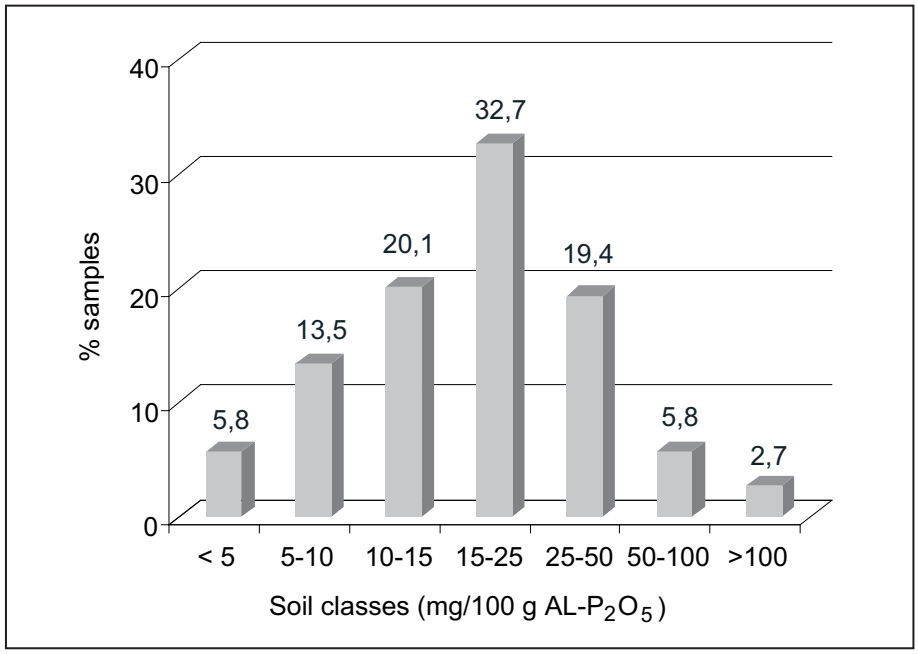


Figure 6.6 shows the levels of readily available phosphorus in the soils of Vojvodina.

Figure 6.6

Readily available phosphorus content of Vojvodina soils (Sekulić et al., 2007)



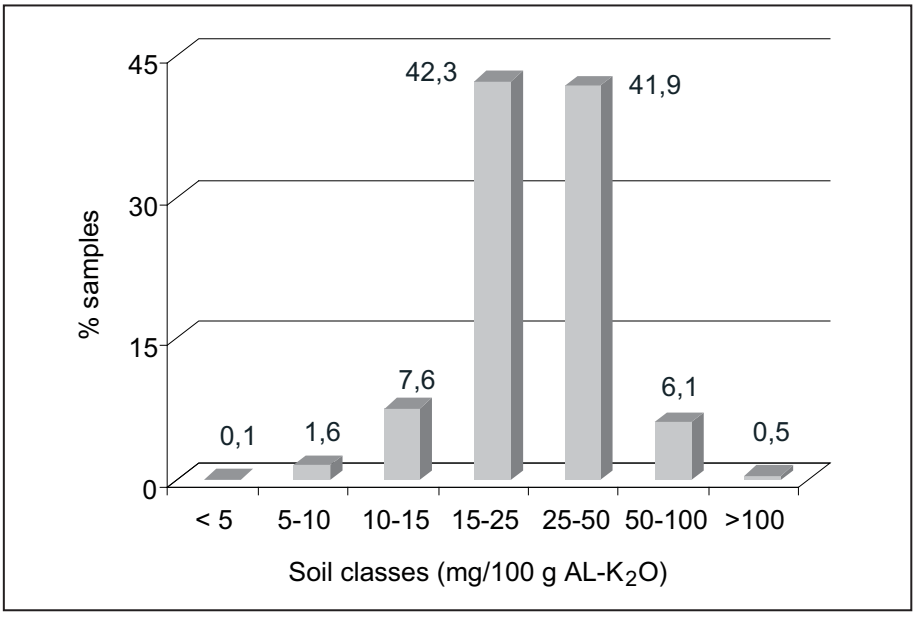
The levels of readily available phosphorus are directly kinked to on human activity.. The figure clearly shows that the distribution curve of the phosphorus content of the Vojvodina soils has the normal shape. The results have shown that phosphorus levels in the province vary a lot and that fertilizer application should take place only after soil samples are taken and analyzed and recommendations are made based on the chemical analysis. Soybean has about 0.65% phosphorus in the grain (Loomis and Connor, 1992), or 0.75% phosphorus expressed as P_2O_5. With yield of 1 ton and the corresponding amount of crop residues, soybeans remove 23-25 kg P_2O_5 from the field.

On 32.7% (15-25 mg P_2O_5/100 g soil.) of the acreage in Vojvodina, it is a sufficient to incorporate only the amount of phosphorus removed from the soil by agricultural yields. On 20.1% (10-15 mg P_2O_5/100 g soil) of the areas, it is necessary to incorporate an amount of phosphorus that is 10-30% higher than that removed by yield. On 13.5% (5-10 mg P_2O_5/100 g soil), the incorporated quantity must be 30-50% higher than the amount removed by yield, while on 5.8% (<5 mg P_2O_5/100 g soil) the fertilization rate must be 100-200% higher than the removed quantity. On 19.4% (25-50 mg P_2O_5/100 g soil), phosphorus fertilization can be reduced by 20-40% compared to the removed amount, while on the remaining 8.5% (>50 mg P_2O_5/100 g soil), phosphorus incorporation can be omitted for a period of one to ten years depending on the results of soil analysis.

Figure 6.7 shows an overview of the readily available potassium levels in the soils of Vojvodina.

Figure 6.7

Readily available potassium content of the soils of Vojvodina (Sekulić et al., 2007)



Soybean grain is 1.82% potassium (Loomis and Connor, 1992), or 2.19% K_2O. With a ton of grain and the appropriate quantity of crop residues, soybean removes 50-60 kg K_2O from the field.

The results show that only 1.7% of the acreage in Serbia (<10 mg K_2O/100 g soil) requires fertilization with potassium amounts equaling those removed by soybeans. On 7.6% of the area (10-15 mg K_2O/100 g soil), 60-70% of the removed phosphorus quantities need to be reintroduced, while 42.3% of the fields (15-25 mg K_2O/100 g soil) require the incorporation of 50-60% of the removed amounts of the element. On 41.9% (25-50 mg K_2O/100 g soil), only 20-40% of potassium are needed, and on 6.6% (>50 mg K_2O/100 g soil) potassium fertilizer application can be completely omitted for a number of years.

SUMMARY

Soybean began to be grown on a large scale relatively recently, bringing about the start of soybean breeding, Today, there is a large number of soybean varieties, which are adapted to different climatic and soil conditions. Soybean varieties originating from the monsoon climate are adapted for cultivation in environments with higher rainfall amounts and somewhat higher temperatures.

The optimum soil air supply for the soybean root is 15-22% vol. When sunlight intensity is reduced by 50%, soybean plants will develop a considerably smaller number of nodes, branches and pods. To avoid this, care must be taken not to have too dense a stand when growing this crop.

Soybean is a short-day plant. In order to make the transition from the vegetative to the generative stage, soybean needs only two to six short days at the one to three compound leaves. The dependence on daylength is inversely proportional to the length of the growing period. Varieties with a longer growing season are more sensitive to the absence of short days, i.e. their photoperiodic response is qualitative in nature. Varieties with a shorter growing period (maturity groups 0-III) can form their generative organs even without night time (with 24- hour illumination), so their photoperiodic response is quantitative.

Soybean has a firm and strong root. The proper development of this root and the nodule (nitrogen-fixing) bacteria developing on it requires a soil that is neither acidic nor saline and has good water-air properties and enough available nutrients.

Soybean is usually grown on fertile, humus-rich soils. Humus has a favorable effect on soil structure and water retention capacity and thus brings about increased soybean yields. This has made it possible for soybean to spread across a belt stretching from 40° S to 60° N. Still, the best results in soybean production are achieved in the northern hemisphere between 35 and 45° N.

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SOYBEAN MINERAL NUTRITION

Novica Petrović, Ivana Maksimović

Mineral elements play diverse roles in the life of plants. They are the constituent elements of organic compounds, their ions catalyze numerous biochemical processes, they play an important role in the regulation of pH and cell osmotic potential, hydration of protoplasm colloids, etc. Owing to these roles, they indirectly or directly affect or participate in all plant life processes, which makes them the essential components of plants.

Due to the specific metabolism of plant species, the importance of individual elements for their growth and development can differ. Plant species feature specific demands for certain elements and capabilities for their utilization. Simultaneously, they are unevenly sensitive to deficiency or excess of certain elements. Due to this, it is very important to be aquainted with specific mineral nutrition for each plant species, even for a certain genotype. Profound importance of the essential elements in the life processes of plants causes the genetic yield potential of a genotype to be fully expressed only under optimal mineral nutrition. Therefore, knowing the specific needs of soybean for the essential elements as well as their physiological effects is an important prerequisite for successful growing of this plant species, especially since soybean features specific needs for mineral nutrition.

This chapter, therefore, shows physiological role and importance, deficiency and excess of the essential macroelements and microelements (Figure 7.1), as well as border values of their content (Table 7.1) in soybean mineral nutrition

THE ROLE OF ESSENTIAL MINERAL ELEMENTS IN SOYBEAN NUTRITION

Nitrogen – physiological role and importance

Primarily, nitrogen occurs naturally in elemental form as a component of air, secondarily as chemically bound in organic matter of plants and animals, and thirdly as salt. Biological fixation of atmospheric nitrogen plays an important role in nitrogen supply for legumes, which is performed owing to the symbiosis between soybean plants and specific bacteria strains from the *Rhizobium* genus. It is considered that, depending on numerous factors, on average 40 to 300 kg N/ha are fixed annually via symbiotic nitrogen fixation (Bethlenfalvay et al., 1990).

Nodule formation and its physiological activity are influenced by many factors, especially by nitrogen concentration in the nutritive medium and its content in plants (Vessey et al., 1988; Franzluebbers et al., 1995).

Legumes are also able to satisfy their nitrogen needs by uptaking it from soil solution in the form of NO_3^- and NH_4^+ ions. Inclusion of nitrate nitrogen into organic compounds after uptake is performed following its reduction to ammonium form. Soybean is capable of reducing nitrates both in the root and in the above-ground plant organs (Wallace, 1986). At lower nitrate concentrations in the environment, procentage of nitrates reduced in the root increases, while it decreases in the above-ground parts, and vice versa, if plants are abundantly supplied with nitrates, nitrate reduction in green organs increases (Wallace, 1986). Nitrate reduction is catalyzed by the enzymes nitrate-reductase (NR) and nitrite-reductase (NiR). Two forms of NR enzyme are found in soybean: NADH-NR and NADPH-NR (Nelson et al., 1984).

Nitrogen is a constituent of many components important for plant life processes, such as structural and catalytic proteins, nucleic acids, compounds participating in energy transfer within cells, etc. Due to this, nitrogen role in plant physiological processes is manyfold. It has been established that nitrogen has favourable effect on the leaf area size and its photosynthetic activity, i.e. the intensity and productivity of photosynthesis, and also the intensity of respiration, water regime in plants, etc.

Nitrogen affects uptake and metabolism of other ions. However, its effect does not depend only on its quantity but also on the form in which it is being uptaken. Generally, it can be said that nitrates stimulate the uptake of K, Ca and Mg in plants, while hindering the uptake of phosphates, chlorides and sulphates, whereas NH_4^+ ion has a contrary effect. Directly or indirectly, nitrogen also affects plant resistance to high and low temperatures, drought, diseases, etc.

Above-ground vegetative organs of soybean contain on average 1.5% to 1.6% nitrogen in dry matter, while soybean grain contains 6.5% to 7.0% nitrogen in dry matter.

Soybean accumulates nitrogen most intensively in the second part of the vegetation period, when the synthesis of organic matter is also most intensive (Schilling, 1983). The quantity of nitrogen outtaken from the soil by soybean yield depends on many internal and external factors. Thus, for example, grain yield of 2.7 t/ha and the corresponding vegetative weight outtakes 240 kg N/ha from the soil.

Nitrogen deficiency

Nitrogen is a very mobile plant nutrient which is quickly transported from older organs into younger ones. Symptoms of its deficiency are thus first visible on the oldest leaves. Due to decreased chlorophyll biosynthesis, the leaves first turn light green, and later greenish-yellow. In the advanced phase of nitrogen deficiency flowers and pods are shed (Streeter, 1978).

Mild nitrogen deficiency in leguminous plants regularly stimulates nodule formation, provided that other necessary conditions are met. The status of soybean plant supply with nitrogen can be determined in different ways, e.g. based on the nodule number and activity, content of total and mineral nitrogen in the leaf, activity of nitrate-reductase, presence of nitrates in the xylem sap, etc. Nitrogen deficiency may be corrected by adding mineral nitrogen fertilizers. These can be applied into the soil or by treating the above-ground organs with a diluted solution of nitrogen compounds. The presence of the enzyme urease in soybean enables a successful application of nitrogen in the form of urea (Oko et al., 2003).

Nitrogen excess

Similarly to nitrogen deficiency, nitrogen excess adversely affects plant productivity. Overabundant nitrogen nutrition supports growth of vegetative organs resulting in overluscious plants. Nitrogen excess also affects root growth so that it becomes shorter and thicker.

At the same time, it should be noted that soybean is the most sensitive of all legumes to excess nitrates. Nitrates in soybean directly inhibit nodule formation and nitrogenase activity (Harper and Gipson, 1984). High nitrate concentration in apoplast increases pH, and consequently immobilizes Fe in it, thus triggering Fe-chlorosis (English et al., 1993).

Nitrogen excess also adversely affects plant resistance to diseases, high and low temperatures, drought, etc. Excess of ammonium and nitrates causes chlorosis and necrosis which at first appear only on leaf edges, and later expand to interveinal area. If nitrogen excess is heavy, it causes leaves to completely dry and later fall off.

Phosphorus - physiological role and importance

Plants uptake phosphorus only in the oxidized state, in the form of orthophosphoric acid ions. Phosphorus salts, such as hypophosphites or phosphites, and PH_3 and P_4 are very harmful to plants even in small concentrations.

Phosphorus plays an important role in the manufacture of a number of cell compounds. For example, phosphoric acid esters have a dominant role in many metabolic processes, such as: photosynthesis, glycolysis, pentose-phosphate pathway, etc. Phosphorus is a constituent element of the nucleic acids, coenzymes involved in many synthetic processes (Slater et al., 1990). Most famous group transmitters are adenosine triphosphate (ATP), coenzyme-A, uridine diphosphate and pyrodoxal phosphate. Phosphorus also enters the composition of coenzymes involved in the transfer of hydrogen: NAD, NADP, FMN, FAD, etc. Compounds, whose constituent is phosphorus, with a special importance are phospholipids and phytin. Phosphorus has a specific effect on the root nodule formation and activity. Optimal supply of phosphorus reduces harmful effects of water deficiency (Gutierrez-Boem and Thomas, 2001).

The optimum phosphorus content in soybean leaf ranges from 0.26% to 0.50% dry matter (Tab. 1). Reproductive organs, especially those of leguminous plants, contain large amounts of phosphorus, about 0.6% dry matter. Phosphorus is intensively uptaken by soybean, especially in the earliest stages of plant growth and development as well as in the period of generative organs formation (Giaquita, 1980). On average, soybean outtakes from 15 to 30 kgP/ha from the soil.

Phosphorus deficiency

The first visible sign of phosphorus deficiency is cessation of growth. Firstly, the leaves turn dark green and cease growing. In time, acute deficiency causes loss of green color, especially in legumes. Simultaneously, the activity of bacteroids in nodules decreases (Cassman et al., 1980) and consequently protein synthesis and chloroplast formation decrease. In the advance stages of phosphorus deficiency, the synthesis of anthocyanin increases, further necrotic spots appear, while leaves gradually dry and fall off. The first symptoms always occur on the oldest leaves. Phosphorus deficiency visibly stunts the stem, which becomes sensitive and susceptible. The root system develops slowly, becoming shorter and less branched.

Phosphorus excess

Phosphorus excess occurs rarely in plants, since its ions in soil quickly bind and become unavailable to plants. However, excessively high concentration of phosphates in the nutritive medium leads to stunting. Under excessive phosphorus, leaves exhibit dark brown spots which spread towards the base. Extremely high concentrations of phosphates trigger premature drying and falling of leaves.

High phosphorus concentration in plant tissues stimulates the process of maturation, consequently shortening the vegetative period and rushing the phases of flowering and fruit formation.

Addition of phosphorus fertilizers in higher doses can decrease iron uptake and transport in plants. Phosphorus has similar adverse effect on plant supply with zinc, manganese, copper and boron. In such cases, chlorosis and other morphological and anatomical alterations are visible on plants, which is characteristic of deficiency in one or more essential microelements.

Sulphur - physiological role and importance

Primary source of sulphur for plants are sulphate ions (SO_4^{2-}) present in the soil solution. Optimal sulphur nutrition is of great importance in soybean production (Lamond et al., 1997, Sexton et al., 1998). It is considered that plants actively uptake sulphur. Apart from the soil, plants can also uptake sulphur in the form of SO_2 via above-ground plant organs.

Plants synthetize a large number of sulphur-containing compounds that play a very important role in the life processes of plants. One of the basic roles of sulphur in protein structure is the formation of disulphide bond between polypeptide chains. Apart from the above mentioned, sulphur also participates in sustaining the balance between the sum of anions and cations, and the formation of osmotic cell potential and oxidation-reduction system.

Sulphur has indirect impact on protein biosynthesis, since the share of amino acids containing sulphur depends on the level of plant supply with this element. In plants, sulphur also creates macroenergetic compounds, such as acetyl CoA and S-adenosyl methionine.

In plant roots, sulphur stimulates the synthesis of phytochelatines, compounds capable of binding heavy metals Pb, Cd, Zn, Cu, etc. and thus disabling their higher accumulation in the above-ground organs (Grill et al., 1990). Application of sulphur into higher pH soils can result in correcting the deficiency of some essential microelements, namely Fe, Zn, Cu, Mn and B.

Plants uptake sulphur in significant quantities. Legumes contain approximately equal amounts of sulphur and phosphorus. Sulphur distribution in plants is specific: it is present mostly in the leaves, less in the seed and the stem, and the least in the root. It is considered that soybean is well provided with sulphur if its content in dry leaves ranges from 0.21% to 0.40% (Table. 7.1).

Sulphur deficiency

Sulphur deficiency stunts plant growth and development. Sulphur deficiency symptoms are similar to those of nitrogen deficiency.

However, the first signs of nitrogen deficiency appear on the oldest leaves, while the first signs of sulphur deficiency appear on the youngest leaves. Under deficient sulphur, leaves turn yellow-greenish, especially young leaves. At first, chlorosis appears at small areas, primarily around leaf veins, and later it spreads to the entire leaf. Older leaves ofter stay green for longer and do not die off. The stem is often shorter and thinner resulting in stunted plants. The root often elongates, while the growth of root hairs in length is particularly striking.

In order to estimate plant supply with sulphur the following are used: content of sulphate ions and total sulphur content in soil and in plants, ratio of N/S, S/Zn contents (Kastori, 1990), and S/Mn in certain plant organs.

Sulphur excess

Sulphur may be found in excess in the atmosphere as a result of fossil fuel combustion. Sulphur also enters the atmosphere naturally due to volcanic activity or its release from geothermal sources. Under excess sulphur, leaves exhibit stunted growth and brown necrotic spots first on the margins and then on interveinal area, gradually spreading towards the main vein. In extreme cases defoliation also occurs. Simultaneously, higher concentrations of sulphates in the soil decrease the uptake of some biogenic elements, namely B, Mo and Se.

Potassium - physiological role and importance

Plants uptake potassium in the form of ion (K^+) from soil solution or sorption complex. From the viewpoint of plant nutrition, especially important influence befalls the ratio of share of potassium and ammonium, sodium, magnesium, calcium and boron in the nutritive medium.

Potassium is highly mobile in plants, always accumulating in the youngest organs and plant parts with intensive cell division, such as the root and stem vegetative cone, or in the organs with intensive transport of materials.

Roles of potassium in plant life processes are diverse. Potassium neutralizes organic acids, stimulates the swelling of protoplasm colloids and affects plant water regime. Potassium stimulates the transformation of light energy into chemical energy and the assimilation of CO_2 (Mauk et al., 1990), as well as the oxidative photophosphorylation. Potassium also affects the metabolism of nitrogen compounds and biosynthesis of proteins. According to Nitsos and Evans (1966), potassium is necessary for the synthesis of the enzyme nitrate-reductase. Its role in this process is specific and cannot be replaced by other monovalent cations.

Plants accumulate potassium in significant quantities. It is considered that soybean is optimally supplied with potassium if fully developed leaves during mid-vegetation contain 1.8% to 3.4% potassium in dry matter (Table 7.1).

Distribution of potassium in plants is specific: in physiologically mature soybean the most of potassium is contained in the leaves, less in the petioles, and the least in the stem (Coale and Grove, 1991).

Potassium deficiency

Soybean plants are considered to be insufficiently supplied with potassium if its content in young fully developed leaves is less than 10 g/kg dry matter (Hallmark et al., 1991). Under potassium deficiency plant growth slows down, and in time completely stops. Due to high mobility of potassium in plants, the first visible symptoms of its deficiency appear on the oldest leaves. The first visual signs in the form of necrosis appear at leaf tip and along leaf margins. In time, the necrotic spots, whose coloration can vary, spread to the interveinal areas towards the middle of leaf. Leaf edges simultaneously fold downward.

Potassium deficiency affects stem composition and growth so that it becomes thinner, susceptible to lodging and with shorter internodes. Potassium deficiency also affects root composition and growth, leaving it short, weakly branched, with decreased number and size of root hairs, loosing its color and easily susceptible to parasites attack.

In case of potassium deficiency plants dry quickly, which makes them susceptible to high temperatures and water deficiency in the soil.

Potassium excess

Potassium excess occurs only very rarely in nature since plants are considerably resistant to a high content of this element. High potassium content in the soil or the application of potassium fertilizers in larger quantites can trigger indirect adverse effects. Potassium excess can lead to deficiency of calcium and magnesium and/or stimulate deficiency of boron, zinc and manganese. During application of potassium fertilizers in higher doses adverse effects could be triggered by the supporting anion (e.g. chlorine).

Calcium - physiological role and importance

Plants uptake calcium in the form of Ca^{2+} ion and/or in the form of complex compounds. Total calcium content in plants ranges on average from 0.5% to 1.0% dry matter (Kastori et al., 1979).

Roles of calcium in plant life processes are many. Recently, its influence has especially been underlined in maintenance of structure and function of cell membranes.

Unlike other cations, calcium activates only a small number of enzymes, often unspecifically, so that its role in enzyme activation can be performed by other cations, e.g. magnesium and manganese.

Calcium stimulates infection by rhizobium, early nodule growth and development (Franco and Munns, 1982), while also affecting cell elongation and differentiation.

Calcium affects swelling of protoplasm colloids by reducing colloid hydration, thus increasing protoplasm viscosity and stability.

The amount of calcium outtaken from the soil through yield primarily depends on yield level and demands of the plant species for this element. Soybean grain yield of 2.7 t/ha and the corresponding vegetative weight outtakes 75 kg Ca/ha from soil. Soybean is considered to be well provided with calcium if dry leaves contain 0.4% to 2% of this element (Table 7.1).

Calcium deficiency

The first visual symptoms of calcium deficiency appear on the youngest organs. The plants adopt bushy appearance and significantly stunt in growth. Leaf cell turgor decreases under calcium deficiency. Leaves start to fold and become phylliform. Signs of chlorosis and necrosis appear first on the youngest leaves, and later on older ones. Necrosis spreads from leaf tip and margins, so that in advance deficiency stages it spreads to the entire leaf. It is characteristical that leaf venation in completely necrotic leaves is always darker in color when compared to the interveinal areas. Calcium affects the stability of chlorophyll-protein complex in photosystem ll (Milivojević and Stojanović, 2003). Under calcium deficiency stem often breaks. Calcium deficiency causes premature flower drop. The number of flowers and pods, if at all formed, is significantly decreased.

Calcium deficiency also affects root growth and structure. Meristematic root tissue looses embryonic features, causing the root to become rudimentary in time.

Calcium excess

Direct effects of calcium excess are not known. Larger amounts of calcium are not toxic for plants, they may even have beneficial effects on their resistance to toxic concentrations of certain elements, especially non-essential ones (Hanson, 1984; Kastori and Petrović, 1987). The presence of larger amounts of calcium in the soil can, however, adversely affect the availability of certain microelements, e.g. Fe (Haleem et al., 1993), B, Mn, Zn and Cu. With the application of larger quantites of calcium salts, harmful effects can be expressed by the supporting anions, such as chloric and sulphate.

Magnesium - physiological role and importance

Unlike calcium, which is mostly passively uptaken, the uptake of magnesium is associated with metabolic processes. Many authors suggest that the uptake of magnesium by soybean is particularly intensive under potassium deficiency. Magnesium content in plants ranges from 0.1% to 1.0% dry matter. Soybean plants which in the above-ground vegetative organs contain 0.5% magnesium in dry matter, are considered to be in optimum supply of this element. Being its integral part, magnesium affects chlorophyll synthesis and stimulates the biosynthesis of other chloroplast pigments, although it is not their constituent. Magnesium is an important activator of many enzymes that participate in the transfer and transformation of energy and it stimulates the activity of many oxidative decarboxylases.

Magnesium also affects the metabolism of nitrogen. Under nitrogen deficiency the ratio of non-protein nitrogen compounds increases at the expense of the proteins. At the same time under these conditions, ribosomes lose their capacity for protein biosynthesis.

Together with other cations, magnesium affects the swelling of protoplasm colloids and owing to this it directly affects plant water regime. Magnesium also participates in the neutralization of organic acids in the cell. The quantities of magnesium outtaken from the soil by yield are very diverse and may depend on many factors. For example, soybean grain yield of 2.7 t/ha and the corresponding vegetative weight outtakes 32 kg Mg/ha from the soil. Soybean is considered to be well provided with magnesium if dry leaves contain from 0.3% to 1.0% magnesium (Table. 7.1).

Magnesium deficiency

If the content of magnesium in fully developed soybean leaves is less than 0.10% dry matter, the plants are considered to be insufficiently provided with this element. The first symptoms of magnesium deficiency typically appear on oldest leaves. If the plants grow quickly, deficiency symptoms can simultaneously appear on younger leaves. Under magnesium deficiency, chlorophyll molecules decompose and chloroplast ultrastructural composition is altered. Chlorosis appears due to the decomposition of chlorophyll molecules first between leaf veins, and consequently the tissue around leaf veins remains green for longer, especially around the main vein. Gradually spreading brown necrotic spots appear on chlorotic areas which are ofter yellow.

Magnesium excess

Magnesium excess rarely occurs in nature. Magnesium is believed to display toxic effect on plants if its content in dry matter is above 1.5%. Adverse effects of high percentage of magnesium are primarily associated with the changed ratio between magnesium and calcium content in plant tissues.

Magnesium excess causes calcium deficiency due to which symptoms resembling calcium deficiency are often visible on the root and the above-ground organs.

Iron - physiological role and importance

Plants can uptake iron through the root or the above-gound organs in the form of Fe^2 + and Fe^3 + ions as well as Fe-chelate. The iron uptake is affected by numerous factors, for example soil pH, content of phosphates, Ca^{2+}, NO_3^-, NH_4^+ in the nutritive medium, etc.

Iron in soybean plants is predominantly or exclusively transported in the form of chelate (Smith, 1984). The mobility of iron in plants is weak so that its retranslocation from older to younger organs is slow.

On average, legume plants contain more iron than grasses. Differences in iron content are also present among genotypes within the same species (Kastori et al., 1978). Optimal iron supply in soybean plants means that dry soybean leaves contain from 44 to 60 mg/kg of this element (Tab. 1). Distribution of iron in soybean organs is as follows: 28% in the leaves, 27% in the stem, 18% in the pods and 27% in the grain. The root of soybean plants also contains significant amounts of iron.

Soybean grain yield of 2.1 t/ha and the corresponding above-ground vegetative organs weight outtakes approximately 1,700 g of iron from the soil.

Iron is involved in numerous processes within plants, whether directly or indirectly. So far, many authors have found a positive correlation between the contents of iron and chlorophyll (Petrovic, 1987). In plants, there are two groups of proteins that contain iron, hemeproteins (cytochromes and the enzymes peroxidase and catalase) and iron-sulfur proteins. Along with Mo, iron is an essential component of the enzyme nitrogenase active site (Terry and Jolly, 1993). The physiologicaly active root nodules contain the protein leghemoglobine which contains iron and the synthesis of which is encoded by the joint action of genes of soybean and rhyzobium.

Iron-sulfur proteins play an important role both in the photosynthesis and the oxidative phosphorylation. The most important metalloprotein from this group is ferredoxin. Iron affects the activity of the enzymes aconitase and RuBPCase (Petrovic, 1987).

Iron also affects protein metabolism. Under its deficiency protein content reduces, while content of soluble nitrogen compounds increases, especially the amino acid arginine, which is considered a characteristical sign of iron deficiency.

Iron deficiency

Iron deficiency is usually observed in alkaline soil, but also in neutral and acid soils. Iron deficiency may sometimes be caused by the iron competition with other heavy metal ions.

Therefore, iron deficiency symptoms and some heavy metals excess symptoms are often similar and sometimes difficult to distinguish. The critical lower limit for iron content in leaf is 60 mg/kg dry matter (Heitholt et al., 2003).

Soybean is one of the plant species that feature greater sensitivity to iron deficiency and a profound genetic specificity regarding iron nutrition (Froehlich and Fehr, 1981). Iron nutrition and its utilization in soybean are very variable among different genotypes. The creation of genotypes tolerant to Fe-chlorosis proved to be the most effective measure to overcome this physiological disease (De Cianzio and Voss, 1994; Goos and Johnson, 2001). SSR markers associated with soybean resistance to Fe-chlorosis were discovered, and their use significantly increases the efficiency of breeding for this trait (Charlson et al., 2003).

Under iron deficiency, the interveinal surfaces of the youngest leaves first turn light yellow to yellow greenish, and later lemon yellow, sometimes even white. Chlorosis on older leaves appears later, often only after the upper younger leaves begin to die and fall off. Chlorotic leaves begin dying when necrotic sports start appearing. Necrosis first occurs on the margins and later on the interveinal surfaces of the leaflets.

Iron deficiency additionally affects the growth and composition of the root system. Iron deficiency has been observed to cause reduction of root elongation and thickening of the root top.

In order to determine the status of plants iron supply, in addition to the usual methods, it has recently been proposed to determine the content of divalent iron and content ratio of P/Fe, K/Ca, K/Mg and N/K.

In the treatment of Fe-chlorosis different iron compounds are applied, as well as agrotechnical, reclamation and other measures. Particularly good results were achieved by the application of chelate iron forms FeEDDHA (Goos and Johnson, 2001).

Inorganic iron salts and Fe-chelates are applied into soil or via foliar application. In the case of above-ground organs treatment, the procedure should be repeated several times. Some authors recommend soaking the seeds in the solution of iron salts prior to planting. In this way the plants can be provided with the necessary amounts of iron only in the early stages of growth and development.

Iron excess

Iron excess is rare in nature. In the event of its excess, the growth of all vegetative organs is inhibited. The leaves turn dark green to blue green, necrotic spots gradually appear, leaflet margins also become necrotic, while the root browns.

Excess of iron can also cause the inactivation of some essential elements, or even deficiency of some essential elements due to the antagonistic action.

It has been noted that soybean genotypes that are able to excessively accumulate iron in the leaves are less resistant to pest attacks and diseases (Elden and Kenworthy, 1994).

Manganese - physiological role and importance

Total manganese content in the soil ranges from 200 to 2,000 mg/kg. Plants uptake manganese in the form of Mn^{2+} ions and Mn-chelate from the soil.

Manganese content in soybean plant dry matter ranges on average from 50 to 120 mg/kg. The critical content is reported to be 15 mg/kg dry matter (Wilson et al., 1982), in case of which manganese deficiency symptoms appear on fully developed soybean leaves.

The roles of managanese in plant life proceses are diverse. Up to now several enzymes containing managanese have been isolated, e.g. superoxide dismutase and acid phosphatase. Manganese affects the activity of numerous enzymes involved into the processes of phosphorilation and oxidation. Manganese is essential for photooxidation of water and CO_2 reduction. A number of researchers have confirmed the beneficial effects of managanese to chloroplast pigments content. According to Ohki (1981) the chlorophyll content in soybean was decreased by 52% under manganese deficiency.

Manganese also participates in the advance stages of nitrates reduction. Flavoproteins, which can reduce nitrates only in the presence of managanese, have been isolated from soybean. Manganese affects not only the reduction of CO_2 and NO_3, but also of sulphate ion. Additionally, manganese plays an important role in the metabolism of amino acids and proteins, as well as lipids (Wilson et al., 1982).

Managanese plays a significant role in disposal of free radicals from cells. Both in the above-ground parts and in the root, soybean was found to contain many isoenzymes that correspond to Mn-SOD (Rodrighes Ferreira et al., 2002).

Manganese stimulates root nodule formation, and consequently the fixation of atmospheric N_2. Manganese is essential for cell division and growth.

Manganese deficiency

Manganese deficiency often appears in alkaline and weakly acid soils, in soils abundant in organic matter or in weakly drained soils.

Manganese deficiency causes chlorosis in plants. This chlorosis does not equally spread over the entire leaf area, but is focused only on the interveinal surfaces. At first, leaves turn light green, and later display brown chlorotic spots dispersed over the entire leaflet giving it a mosaic appearance (Heeman and Campbell, 1980).

Leaflet base, and especially the area around the main vein, remains green and undamaged for long. If managanese deficiency is profoundly expressed, there is necrosis, perforation and eventually total leaf die-off.

In soybean and other legumes, manganese deficiency causes typical changes in the seeds. On the internal surface of the cotyledons dark green areas appear, thought to be caused by the increased content of the soluble organic nitrogen compounds, primarily amino acids. The external surface of such seeds often displays dents (Wilson et al., 1982).

Different managanese compounds and mineral fertilizers fortified with managanese are used in order to correct manganese deficiency, applied into the soil or foliary. Owing to the weak mobility of managanese within plants, foliar treatments should be applied two or three times in 14-day intervals.

In order to supply plants with manganese in the earliest phases of growth and development, it is also recommended to spray the seeds with managanese salts prior to planting, or to immerse them in diluted solution of manganese salts for a short period of time.

Manganese excess

Managanese excess occurs in soils with high content of managanese, low pH and high reduction potential. According to Edward and Asher (1982) critical toxic manganese concentration (which decreases the production of organic matter in the above-ground parts by 10%) in soybean is 600 mg/kg dry matter.

Manganese excess inhibits the synthesis of chlorophyll, causing its content to gradually decrease in plants. It is thought that manganese excess causes the antagonism between it and iron, resulting in secondary iron deficiency. Chlorosis caused by manganese excess differs from the one caused by iron deficiency, primarily in that it does not occur in the same plant parts. Mn-chlorosis first occurs on older leaves, while Fe-chlorosis occurs on the youngest leaves. Chlorosis caused by manganese excess spreads from the leaf tip and margin towards the base, with common folding of the leaflet. After a certain period of time, chlorotic leaves display brown to darkbrown spots and necrosis.

Adverse effect of manganese excess can be mitigated by higher content of K, Na, Mg, and especially Ca in soil. Manganese toxicity in soybean can significantly be reduced by the arbuscular mycorrhizal fungi (Nogueira et al., 2004). Additionally, there were certain efforts to develop soybean genotypes with a higher tolerance to high managanese concentrations in the tissue (Heeman and Campbell, 1981) or a lower capacity for its accumulation (Brown and Devine, 1980).

Zinc - physiological role and importance

Plants uptake zinc in the form of ion (Zn^{2+}) or chelate. The mechanism of its uptake is still not clear. Recently, however, the prevailing opinion is that the uptake and accumulation of zinc in soybean plants is a genetically controlled process (Hartwig et al., 1991).

Zinc belongs to the group of elements whose mobility in plants is not particularly high. White et al. (1981) observed that zinc in the xylem of soybean plants is primarily transported in ion form. In older leaves zinc is weakly mobile, while in younger leaves its mobility is higher.

It is considered that soybean plants are well supplied with zinc if its content at the onset of pod formation in young fully developed leaves ranges from 20 to 50 mg/kg dry matter. If its content is less than 10 mg/kg dry matter, in most cases morphological signs of zinc deficiency are easily visible on plants.

Soybean outtakes 200 to 400 g Zn/ha from the soil depending on yield and other internal and external factors.

Zinc plays a very important role in the transport of materials in plants. It enters the composition of numerous enzymes: carboanhydrase, glautamate and malate dehydrogenase, alkaline phosphatase, superoxide dismutase, proteinase and peptidase. Zinc is also essential for the activation of the following enzymes: isomerase, aldolase, dehydrogenase, RNA and DNA polymerase, etc. The presence of zinc is necessary for the synthesis of auxins, i.e. for plant growth.

Zinc also affects the intensity of photosynthesis and respiration, lipid metabolism and starch formation. It also affects the uptake or metabolism of other ions, e.g. copper, manganese, and especially phosphorus. Zinc increases plants resistance to diseases, viral diseases, drought and low temperatures. Several enzymes were found in the above-ground parts and the root, which correspond to Zn-SOD and neutralize free radicals in cells (Rodrighes Ferreira et al., 2002).

Even when it does not affect yield, zinc content affects nutritional value of soybean products (Jahiruddin et al., 2001).

Zinc deficiency

Sensitivity of individual plant species to zinc deficiency varies, and most sensitive of the field plants are soybean, maize and flax. Typical morphological signs of zinc deficiency are symptoms caused by insufficient quantity of auxins: first of all small, stunt and narrow leaves, shorter internodes and rosette-like appearance due to the lack of apical domination. Besides this, leaves display various deformations, chlorotic spots and necrosis. Most often, symptoms of zinc deficiency first appear on older leaves but can also appear on young leaves. Soybean leaves turn light green to yellow.

During advance stages of zinc deficiency there often occur reduced flowering and fertilization, as well as premature leaf and pod shed. Zinc deficiency in soybean plants can stimulate excessive accumulation of phosphorus, and even cause its toxic effect.

Apart from biological tests, chemical analyses of soil and leaf diagnosis, recently P/Zn share ratio has often been used as a determiner of plant supply with zinc, so that for example under latent zinc deficiency in soybean P/Zn share ratio is 200 to 300. The activity of enzyme RNase is also considered to be a reliable indicator of plant supply with zinc (Johnson and Simons, 1979).

Zinc deficiency can be corrected by its introduction into the soil or by treating the above-ground organs with different inorganic and organic zinc compounds, or mineral fertilizers fortified with this element.

Zinc excess

Resistance of certain plant species and genotypes to zinc excess is very diverse. There is still no unique standpoint on the physiological mechanism of soybean resistance to zinc excess. It is considered that more tolerant soybean genotypes feature large capacity of binding zinc in the root, especially in the cell walls, or of accumulating zinc in the leaves (White et al., 1979).

Critical toxic concentration of zinc in soybean ranges from 400 to 500 mg/kg dry matter. Brown reddish spots appear on the leaves, and necrosis on the leaf margins. Zinc excess symptoms are often very similar to the symptoms of iron and manganese deficiency, differing only in that the former are visible both on younger and older leaves.

In many cases, excessive accumulation of zinc in plants and its harmful effects can be reduced by the increase of soil pH via calcification and application of phosphorus fertilizers in higher doses.

Copper - physiological role and importance

Plants uptake copper in the form of Cu^{2+} ions and as chelate. Copper mobility in plants is intermediate. Copper content in soybean ranges on average from 15 to 35 mg/kg dry matter (Kastori et al., 1978). Copper distribution in plants is specific, and it is considered that out of total copper content in plants approximately 2/3 is found in chloroplasts. Soybean outtakes very small amounts of copper from soil, namely between 100 to 200 g/ha on average.

Copper belongs to the group of polyvalent elements, and this feature largely determines its physiological function. Significant proteins with copper ions in the active site are plastocyanin, cytochrom oxidase, ascorbin oxidase, diphenol oxidase, and enzymes lactase, tyrosinase, hydroxylase and oxygenase.

Enzymes amino oxidase and superoxide dismutase contain copper as the prosthetic group. In soybean above-ground parts and the root several isoenzymes have been found which correspond to Cu-SOD (Rodrighes Ferreira et al., 2002).

Copper affects the stability of the chlorophyll-protein-lipid complex, the metabolism of nitrogen compounds and the synthesis of nucleic acids.

Copper affects polen fertility (Bussler, 1981) and increases plant resistance to low temperatures and diseases.

It has been observed that copper also affects symbiotic binding of molecular nitrogen. However, the mechanism of its effect in this process is not yet clear. It is supposed that copper is included in the synthesis of leghemoglobine. Additionally, it has been observed that the number of nodules is reduced under copper deficiency. Its indirect effect through the metabolism of carbohydrates is also not to be disregarded.

Copper stimulates or inhibits the uptake and physiological activity of numerous elements, e.g. Ca, P, Mn and Fe. There are numerous data on the influence of some heavy metals on the uptake and accumulation of copper in plants.

Copper deficiency

Soybean belongs to plant species which express higher tolerance to copper deficiency. The critical concentration of copper in soybean ranges from 3 to 5 mg/kg dry matter (Robson and Reuter, 1981). Typical signs of copper deficiency in soybean are withering and concave folding of leaves with the youngest organs dying off. Withered leaves gradually dry and die in time. Plastocyanin content and photosystem activity decrease under copper deficiency.

Inorganic salts and chelate copper complexes are used to correct copper deficiency, as well as mineral fertilizers fortified with copper. Their application can be performed both foliary and into the soil.

Some authors state that seed treatment with copper prior to planting stimulates initial plant growth, and in some cases increases yield, especially if soils are insufficiently supplied with this element. To achieve this, it is recommended to immerse the seeds in diluted solution of $CuSO_4$, or to spray the seeds with $CuSO_4$.

Copper excess

The concentration of copper is considered critically high for soybean if its content in leaf dry matter is above 40 mg/kg (Robson and Reuter, 1981). The first signs of excess often appear on older leaves as chlorosis on the leaf tip or the margins. Gradually, leaf turns brown reddish with this coloration spreading from the margins towards the middle and the base of the leaflet. In time necrosis appears and simultaneously both older and younger leaves dry off. At the same time the root browns, shortens and thickens with a small number of lateral roots and root hairs.

In the case of copper excess, it is recommended to use larger amounts of organic fertilizers, calcium and phosphates, and also to treat the above-ground plant organs with diluted solution of $CuSO_4$ or Cu-chelate.

Boron - physiological role and importance

Plants uptake boron in the form of boric acid and hydrated ion. Boron is quickly uptaken, but its translocation from one organ to another is the weakest out of all essential microelements.

Plants accumulate boron most intensively in the phase of intensive leaf area growth, during flowering, fertilization, and seed formation. Boron content in dry matter of dicotyledon plants ranges on average from 20 to 80 mg/kg. It is especially intensively accumulated in the leaf margins and generative organs. Through their biological yield, cultivated plants outtake relatively small amounts of boron per hectare from soil annually, e.g. average amount for soybean is around 100 g.

Boron affects numerous metabolitic proceses directly or indirectly. It affects the activity of the enzymes ribonuclease and ATPase, and consequently the content and the metabolism of nucleic acids, the respiration intensity and phosphorus metabolism.

Boron enters complexes with numerous sugars and owing to this it has beneficial effects on their transport in plants. Primary nitrogen assimilation, i.e. the activity of nitrate reductase, and consequently also the metabolism of proteins largely depend on plant supply with boron. Boron also affects the metabolism of growth materials, primarily the biosynthesis of auxins. Boron deficiency and excess significantly affect the structure of chloroplasts and also consequently photophosphorilation.

Numerous research results point to very favorable effects of boron to pollen germination, growth and stability of pollen tube structure, and the process of fertilization.

Boron content also affects the nutritional value of soybean products (Jahiruddin et al., 2001).

Boron deficiency

Boron deficiency is accompanied by typical morphological, anatomical and physiological alterations. Due to weak mobility and retranslocation of boron in plants, the first deficiency symptoms appear on the youngest leaves and the vegetation points of the above-ground parts and the root. Characteristical anatomical and histological symptom of boron deficiency is the intensive cell division in certain organs and their very slow differentiation.

Chlorotic spots appear on leaves under boron deficiency, the growth of internodes and number of lateral buds decrease, while the mechanical tissue develops slowlier. The root system develops slowly, and the number of lateral roots increases enormously. The root browns in time and acquires a slimy consistency. Flower formation is decreased, while fertilization is often left out or is considerably decreased. Contrary to other dicotyledon plants, soybean weakly reacts to boron nutrition, and often the application of boron has no significant effect on yield level and quality. Border values of boron content in healthy compound leaf at the beginning of flowering ranges from 45 to 60 mg B/kg dry matter.

In case of insufficient boron supply in soil it is recommended to apply mineral fertilizers fortified with boron, and fertilization or foliar treatment with borax.

Boron excess

Similarly to boron deficiency, boron excess causes physiological and morphological alterations in plants. The range between sufficient boron supply in plants and its excess is very small, so that systematic application of boric fertilizers can relatively quickly lead to its excess.

Boron is primarily transported by the transpiration flow in plants, resulting in most accumulated boron on the leaf tip and the margins. In relation to this, the first symptoms of boron excess often appear on the leaf tip and the margins in the form of chlorosis and necrosis, first on older leaves and later on others.

Molybdenum - physiological role and importance

Plants uptake molybdenum in the form of anions, i.e. molybdate ion MoO_4^{2+}. Many factors affect molybdenum uptake from the soil and utilization, so for example, the accumulation of molybdenum in the above-ground soybean organs is especially intensive under soil pH of 5 to 7 (Mortvedr, 1981).

Molybdenum mostly accumulates in the interveinal leaf areas, and significantly less in the stem tissues. If its distribution in the entire plant is considered, it can be concluded that it is mostly present in the leaves. The seed can contain significant amounts of molybdenum, especially the seed of legumes. Soybean seed contains on average from 5 to 6 mg/kg molybdenum in dry matter (Szabó et al., 1987). Molybdenum content is especially high in the nodules of legume plants. Molybdenum accumulation in the nodules can often lead to its decreased concentration in the stem, leaves and seed of the soybean plant (Ishizuka, 1982). Actually, if soybean plants are insufficiently supplied with molybdenum, its content in the nodules is significantly higher than in the leaves, while in case its concentration in the environment is high, the content of molybdenum in the leaves increases more than in the nodules (Franco and Munns, 1981). Soybean plants outtake around 10 g Mo/ha through biological yield of 4 t/ha.

The importance and necessity of molybdenum in the transport of materials in plants have been determined in numerous researches. Based on the research performed with analogue metals, it has been established that the role of molybdenum in the reduction of nitrates is specific and irreplaceable by other elements. Molybdenum plays an important role in N_2 fixation since it enters the composition of the enzyme nitrogenase. Molybdenum also affects nodule development, so that under conditions of optimal supply the number of nodules decreases, while they themselves become larger and physiologically more active. Owing to this, legumes treated with molybdenum fix nitrogen more efficiently, regardless of their often lower nodule weight as compared to the untreated plants. Consequently, it is possible to reduce the use of nitrogen fertilizers on soils insufficiently supplied with available molybdenum through joint application of preparations based on molybdenum and rhizobium.

Molybdenum stimulates the activity of peroxidase, catalase, phenol oxidase, phosphorilation processes, etc. Moreover, molybdenum has beneficial effects on plant resistance to drought and some diseases. Molybdenum is also considered to stimulate cell differentiation.

Molybdenum deficiency

Symptoms of acute molybdenum deficiency in plants rarely occur in nature (Gupta and Lipsett, 1981). Molybdenum deficiency often occurs on acid soils, where there might simultaneously occur insufficient supply of plants with N, Ca, Mg, etc., or manganese excess for example. In all such cases chlorosis appears, which is why it cannot be considered a typical nor reliable sign of molybdenum deficiency. Critical concentration of molybdenum in soybean ranges from 0.1 to 1.0 mg/kg dry matter (Gupta and Lipsett, 1981).

In order to corrrect molybdenum deficiency, different molybdenum compounds can be applied into soil, or as foliar or seed treatment.

Molybdenum excess

Most often, the sign of molybdenum excess is the stunted plant growth. In the case of molybdenum excess, the stem reduces its growth and evidently thickens, while older leaves also thicken. Simultaneously, lateral shoots occur in large numbers. Characteristical sign of molybdenum excess is the appearance of chlorosis, first on the youngest leaves and gradually spreading to other plant parts. Chlorosis is yellow and in time turns brown. In some cases, anthocyanins may appear in leaf and stem epidermis cells.

Molybdenum uptake may be decreased by application of B, Fe, Mn, P, as well as by lowering soil pH. Similarly, molybdenum uptake was observed to decrease through the application of mineral fertilizers containing sulphate, or by the use of gypsum.

Cobalt - physiological role and importance

Plants uptake cobalt in the form of Co^{2+} ion or as Co-chelate. Cobalt content in plant dry matter ranges within wide margins, e.g. from 0.08 to 0.15 mg/kg in the soybean leaf. Distribution of cobalt in plants is specific. It accumulates more in generative than in vegetative organs. In leaves it accumulates primarily in the leaf margins, similarly to boron.

Plants outtake very small amounts of cobalt from the soil. For example, legumes outtake on average 2 to 3 g/ha cobalt through biological yield.

Cobalt enters the composition of the vitamin B_{12}. The content of this vitamin in the soybean nodules is in direct positive correlation to the concentration of cobalt available to plants.

A large number of authors have pointed out the importance of cobalt in the fixation of molecular nitrogen. The role of cobalt in molecular nitrogen fixation is specific, meaning that it cannot be replaced by other elements in this process. Accumulation of cobalt in the nodules shows its importance for the physiological activity of nodules. Cobalt also affects nodule ultrastructure. Its presence stimulates bacterial tissue growth, increases the quantity of cytoplasmic polyribosomes both in plant cells and bacterial cells. The role of cobalt in molecular nitrogen fixation has not fully been explained, despite numerous researches. Its primary role is considered to be the activation of biosynthesis process of nodule proteins, nitrogenase and leghemoglobine.

Cobalt deficiency

Cobalt deficiency symptoms in higher plants are not sufficiently known, except in legumes where nitrogen deficiency signs are observed under insufficient supply with cobalt. Symptoms of cobalt deficiency in plants were first observed by Wilson and Nicholas (1967). According to these authors young leaves display chlorosis under cobalt deficiency.

Plants that are insufficiently supplied with cobalt should be fertilized with this element. Use of cobalt salts and/or Co-helate is recommended. Application of cobalt may be performed into soil or foliary by treating the above-ground organs.

Cobalt excess

Symptoms of cobalt excess have not been observed under normal natural conditions. Cobalt excess is only caused by human activity in the environments contaminated by this element. Cobalt excess causes chlorosis and necrosis, and even dying and dropping of leaves in extreme cases. Signs of cobalt excess are not specific, since similar symptoms are caused by high concentrations of other heavy metals. Besides this, there is antagonism among cobalt, iron and manganese, due to which cobalt excess may cause iron deficiency.

Chlorosis which appears due to cobalt excess is more diffuse than the one due to iron deficiency. Cobalt excess also causes certain morphological alterations of the root giving it a darker color.

Nickel - physiological role and importance

Nickel (Ni) is the chemical element most recently included in the group of essential elements for plants (Gerendás et al., 1997). Up to now, only a limited number of plant species have been determined not to be able to finish their life cycle without the presence of Ni. Those are the plant species in which nitrogen is transported in the form of ureid, such as soybean. Actually, it has been observed that Ni is a constituent element of urease (E.C. 3.5.1.5.), the enzyme from the group of hydrolase, which catalyzes the reaction of urea (carbamide) decomposition to carbon dioxide and ammonia, further involved in the metabolism. Urease has two Ni atoms in the active site and in plants it occurs in two isoenzymatic forms, coded with 4 genes (Polacco et al., 1999). Plants with mutation in one of the genes for urease display symptoms identical to those of Ni deficiency.

The presence of urease has been proven in a large number of living organisms except plants, including also invertebrates, fungi, algae and bacteria. The test for the presence of urease and its activity is one of the parameters for quality assessment of soybean products. High urease activity indicates that soybean grain or flour have not been sufficiently thermally processed, making such products inconvenient for human and animal diet. Thermally unprocessed soybean grains contain protease inhibitors, including also trypsin inhibitors, which in larger amounts lead to pancreatic hypertrophy. It has been established that the presence of an active trypsin inhibitor may indirectly be determined by measuring the activity of urease present in soybean grain. Both these proteins are denaturized and inactivated when heated.

Except nitrogen metabolism, Ni also affects iron metabolism, being its antagonist. Beneficial effects of Ni to seed germination have also been observed. Nickel content is around 0.1 mg/kg dry matter.

Nickel deficiency

Under conditions of Ni deficiency, plant growth slows down, older leaves become chlorotic and plants appear as if they were under nitrogen deficiency (Gerendás and Sattelmacher, 1997). There is loss of urease activity resulting in urea accumulation in the tissue and the creation of necrotic spots on soybean leaf (Krogmeier et al., 1991). Presence of urease inhibitors, such as heavy metal ions, Na^+, K^+, NH_4^+ and thiourea can also cause Ni deficiency.

Nickel excess

Nickel is a heavy metal. Due to the industrial contamination, in agricultural soils nickel may be found in concentrations that are too high for plants. Toxic effects of Ni, if uptaken in excessively high concentrations, are expressed as chlorosis, stunted growth, decreased uptake of water and mineral nutrients, as well as metabolism disorders which lead to reduced yield levels (Poulik, 1999).

Figure 7.1

Schematic of identifying deficiency symptoms of certain essential elements, adapted according to Reddy and Reddi (1997)

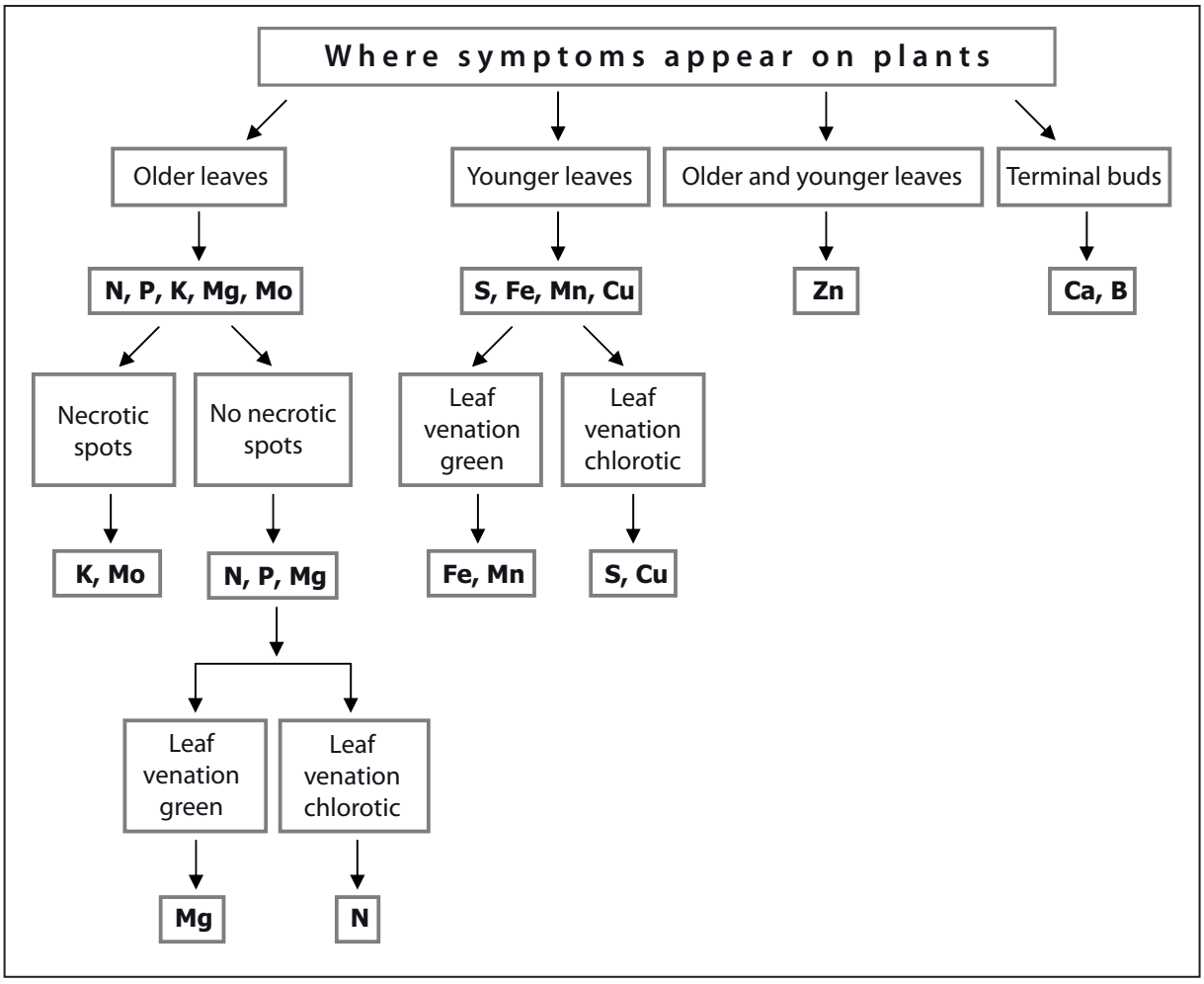

Table 7.1

Border values for soybean supply with essential elements. Composed by Bergmann and Neubert (1976)

Element		Time of sampling for analyses	Plant part analysed	Deficiency	Low content	Satisfactory border concentration	High content	Toxic content
Content of macroelements in mg/kg dry matter	N	Mid vegetation	VPRL*	< 3.00	3.1-4.0	4.1-5.5	5.6-7.0	>7.00
		Before pod formation	GPRL**	< 4.00	4.00-4.24	4.25-5.50	5.51-7.00	>7.00
	P	Mid vegetation	VPRL	< 2.00	0.21-0.25	0.26-0.50	0.51-0.80	>0.80
		Before pod formation	GPRL	< 0.16	0.16-0.25	0.26-0.50	0.50-0.80	>0.80
	K	Mid vegetation	VPRL	< 1.45	1.46-1.80	1.81-3.40	3.41-4.50	>4.50
		Before pod formation	GPRL	< 1.26	1.26-1.70	1.71-2.50	2.51-2.75	>2.75
	Ca	Mid vegetation	VPRL	< 0.30	0.30-0.40	0.41-1.80	>1.80	
		Before pod formation	GPRL	< 2.00	0.21-0.35	0.36-2.00	2.01-3.00	>3.00
	Mg	Mid vegetation	VPRL	< 0.11	0.11-0.30	0.31-1.30	>1.30	
		Before pod formation	GPRL	< 0.11	0.11-0.25	0.26-1.00	1.01-1.50	>1.51
	S	Mid vegetation	VPRL	< 0.16	0.16-0.20	0.21-0.40	>0.40	
Content of microelements in mg/kg dry matter	B	Before pod formation	GPRL	< 10	10-20	21-55	56-80	>80
	Cu	Before pod formation	GPRL	< 5	5-9	10-30	31-50	>50
	Fe	Before pod formation	GPRL	< 38	38-43	44-60	>60	
		34-day-old plants	GPRL	< 31	31-50	51-350	350-500	>500
	Mn	30-day-old plants		< 3	3-13	14-102	103-173	>173
		Before pod formation	GPRL	< 15	15-20	21-100	101-250	>250
	Mo	Before pod formation	GPRL	< 0.4	0.4-0.9	1.0-5.0	5.1-10.0	>10.0
	Zn	Before pod formation	GPRL	< 10	10-20	21-50	51-75	>75
	Al		GPRL	< 16	<11	11-200	201-500	>500
	Co		Leaf			0.01-0.16		

*VPRL – terminal fully developed leaf

**GPRL – top fully developed leaves

SUMMARY

Mineral elements are essential components of plant cells. They play numerous roles in plant metabolism and they indirectly or directly affect all processes in the plant. Mineral elements are constituents of organic compounds, their ions act as catalysts of biochemical reactions, they are regulators of the cell pH and osmotic potential, and they take part in hydratation of cell macromolecules. Although essential elements are vital for all plant species, significant differences between them may be present, with respect to needed amounts and to certain extent metabolic pathways of some elements. Soybean, as a legume, has certain specific features with respect to demands for particular elements and their metabolism and those are pointed out in this chapter. As one of preconditions for full expression of soybean genetic potential for yield and grain quality indeed is well balanced mineral nutrition, special attention here was devoted to metabolic role of essential elements (N, P, S, K, Ca, Mg, Fe, Mn, Zn, Cu, B, Mo, Co and Ni), their threshold values and effects of their insufficient supply and their excess on soybean growth, development and metabolism.

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NITROGEN FIXATION IN SOYBEAN

Nastasija Mrkovački

Nitrogen fixation is an important process for sustaining life on this planet, since it enables conversion of inert gaseous nitrogen (N_2) into ammonium ion (NH_4^+), increasing supply of mineral nitrogen necessary for plant growth and development.

Molecular nitrogen constitutes almost 80% by volume of Earth's atmosphere, but most of the living organisms are unable to assimilate it. Conversion of N_2 involves either a natural process (biological and non-biological) or an industrial production (Haber-Bosch process). A vast amount of energy is needed to break the triple bond between N_2 molecules, whose individual nitrogen atoms bond with hydrogen or oxygen forming compounds (NH_4^+ and NO_3^-) readily available to plants. Biological processes contribute around 65% of total annual nitrogen fixation (17.5×10^7 tons, Burns and Hardy, 1975), while the industrial production contributes around 25% (Newton, 1994).

Only a few genera of prokaryotic organisms are able to fix nitrogen. The largest portion of biological nitrogen in soil is attained by symbiotic nitrogen fixation (9×10^7 tons of nitrogen annually) (Hardy and Holsten, 1972).

Symbiotic nitrogen fixation is a process by which the host plant (macrosymbiont) provides bacteria (microsimbiont) with energy, while bacteria provide the plant with reduced nitrogen from the atmosphere. This process was discovered in pea by Hellriegel and Wilfart as early as 1888. The same year, Beijerinck isolated the pure bacterial culture from nodules of this plant naming them *Bacillus radicicola*. Bacteria from soybean nodules were isolated by Kirchner (1895), and named firstly *Rhizobacterium japonicum* by Buchannan (1926) and subsequently *Bradyrhizobium japonicum* by Jordan (1982).

Nowadays it is known that association between nodule bacteria from six strains of *Rhizobium*, *Bradyrhizobium, Sinorhizobium, Mezorhizobium, Azorhizobium* and *Allorhizobium* and legumes is unique and beneficial to both partners. This association between the plant and bacteria results in nodule formation in the plant root.

Legume crops are independent from nitrogen, which is often a limiting factor for plant growth in soil, owing to the symbiosis with nodule bacteria which fix nitrogen from the atmosphere. According to La Rue and Patterson (1981), soybean crop fixes 60 to180 kg N/ha during vegetation.

Nodule bacteria strains differ primarily in their specificity to form root nodules on only certain legume species. Strains of *Bradyrhizobium japonicum, Bradyrhizobium elkanii* and *Sinorhizobium fredii* (Martinez Romero and Caballero Mellado, 1996) form nodules on soybean (*Glycine max.*). However, there is a possibility that nodules may be formed on soybean root by other species, such as *Bradyrhizobium sp.* and *Rhizobium* species in soil (Sarić, 1960), allowing for vast differences in nitrogen fixation capacity of different symbiotic associations.

Nodulation

Bacteria that form a symbiotic association with legumes enter the plant root through root hairs and trigger nodule formation, whence their name nodule bacteria.

There are three phases of a symbiotic association being established: recognition, infection and nodulation (Prescott et al., 2002).

During the recognition phase, nodule bacteria group on the root surface and a close contact is established between the bacteria cells and root hairs. It is supposed that there are two participants in this close contact: extracellular or capsular polysaccharides of the bacteria on the one hand, and plant lectins of legumes on the other hand. Lectins are a molecular bridge between reactive root antigens and nodule bacteria cells. This part of the recognition phase is called a pre-infection phase and is affected by calcium presence.

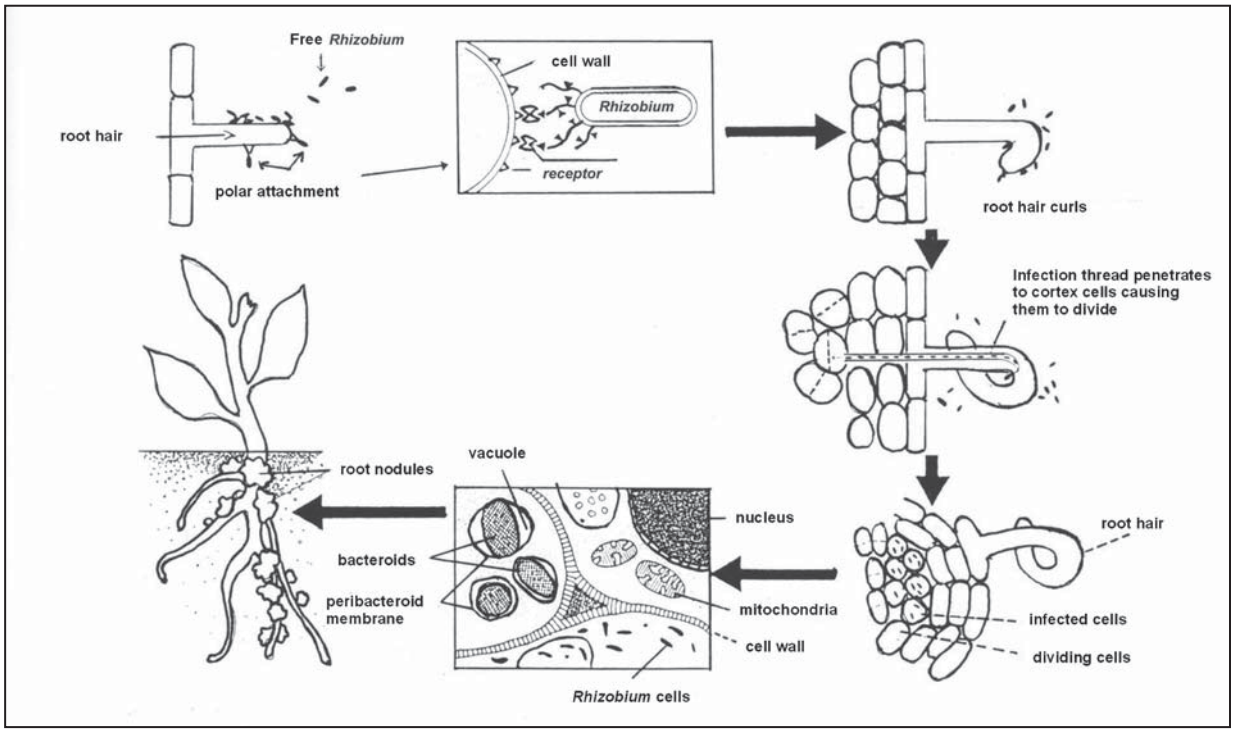
The presence of compatible nodule bacteria in the vicinity of a root hair triggers it to curl, signaling the infection. The infection phase commences with the transformation of tryptophan (from root exudates) into indoleacetic acid which provokes root hairs to curl and deform. There are various views on the role of growth factors in the infection mechanism. Badenoch Jones et al. (1982) concluded that indoleacetic acid (IAA) participates both from the plant and bacteria in the process of root hair curling.

It is considered that bacteria enter through the primary cell wall at the point of root hair curling, causing invagination of the internal cell wall and plasmalemma, following which the infection thread is created. A very small percent of the deformed root hairs form infection threads (0.6% to 3.2%, rarely 8%). Bacterial carbon reserves in the form of polyhydroxybutyrate (PHB) are accumulated in the Rhizobium cells in infection threads (Lodwig et al. 2003, 2005). The formed infection threads form the nodule in a small number (only 1% to 5%) (Mišustin and Šiljnikova, 1968; Crespi and

Galvez, 2000). Free bacteria enter from infection thread into plant cells and plant cells division and nodule formation are stimulated under the influence of plant hormones, marking the nodulation phase. Plant cells are separated from bacterial cells in the nodule by the peribacteroid membrane. Udvardy and Day (1988) showed that peribacteroid membrane controls the type and metabolism of the carbon compounds in the bacteroids. Inside this membrane, the slowly growing nodule bacteria divide several times creating 16 individual cells. These cells are further differentiated morphologically and metabolically to become bacteroids. In one infected root cell there can even be 20,000 individual bacteroids. The necessary enzymes are synthesized in the bacteroids allowing for proper functioning of the symbiotic system (Figure 8.1). Symbionts co-participate in the formation of leghemoglobin (plant-globin, bacteriahem) which protects the enzyme nitrogenase from the oxygen, simultaneously providing sufficient oxygen for the respiratory activity in the bacteroids.

Figure 8.1

Stages in the infection of legume roots by Rhizobia (Ahmadjian and Paracer, 1986)



Several days (6-7) after soybean root inoculation with nodule bacteria, nodulin synthesis commences under the influence of the plant nodulation gene. Among them is the famous N-75, characterized as plant cell wall component (Franssen et al., 1987). During the process of nitrogen fixation and aside from early nodulins, late plant nodulins of the leghemoglobin type are also significant (Graham, 2000), appearing 8-10 days after inoculation in soybean.

Genes for nodulation can be divided into common genes and genes specific for host plant. Nod ABC genes are common genes responsible for the first phase of the nodulation process, i.e. for bacterial cell division, root hair curling and triggering of early nodulins expression. Genes specific for host choice are nod FE, nod H and nod LMN. Genes specific for choosing soybean as host have not been identified. Besides genes for nodulation responsible for nodule formation, genes for nitrogen fixation process are nif and fix genes.

In nodule pure bacteria cultures, only nod D gene was identified out of all genes for nodulation, while all other genes are induced in the presence of plant secretions (Davis et al., 1988; Surin and Downie, 1988). Kosslak et al. (1987) managed to identify isoflavone known as daidzein from plant secretions, which is responsible for induction of nodulation gene in soybean.

Nitrogenase

Nitrogenase is the enzyme responsible for biological nitrogen fixation. Nitrogenase activity is a component of nitrogen metabolism in the nodule, i.e. in bacteroids.

Nitrogenase consists of two proteins: MoFe protein (component I) and Fe protein (component II). Component I – dinitrogenase is a tetramer of molecular mass 200,000-270,000 Daltons, while component II - dinitrogenase reductase is a dimer of molecular mass 55,000-65,000 Daltons. The presence of both proteins is necessary for enzyme activity; for one MoFe protein molecule two Fe protein molecules are needed. Both proteins are extremely instable in the presence of oxygen.

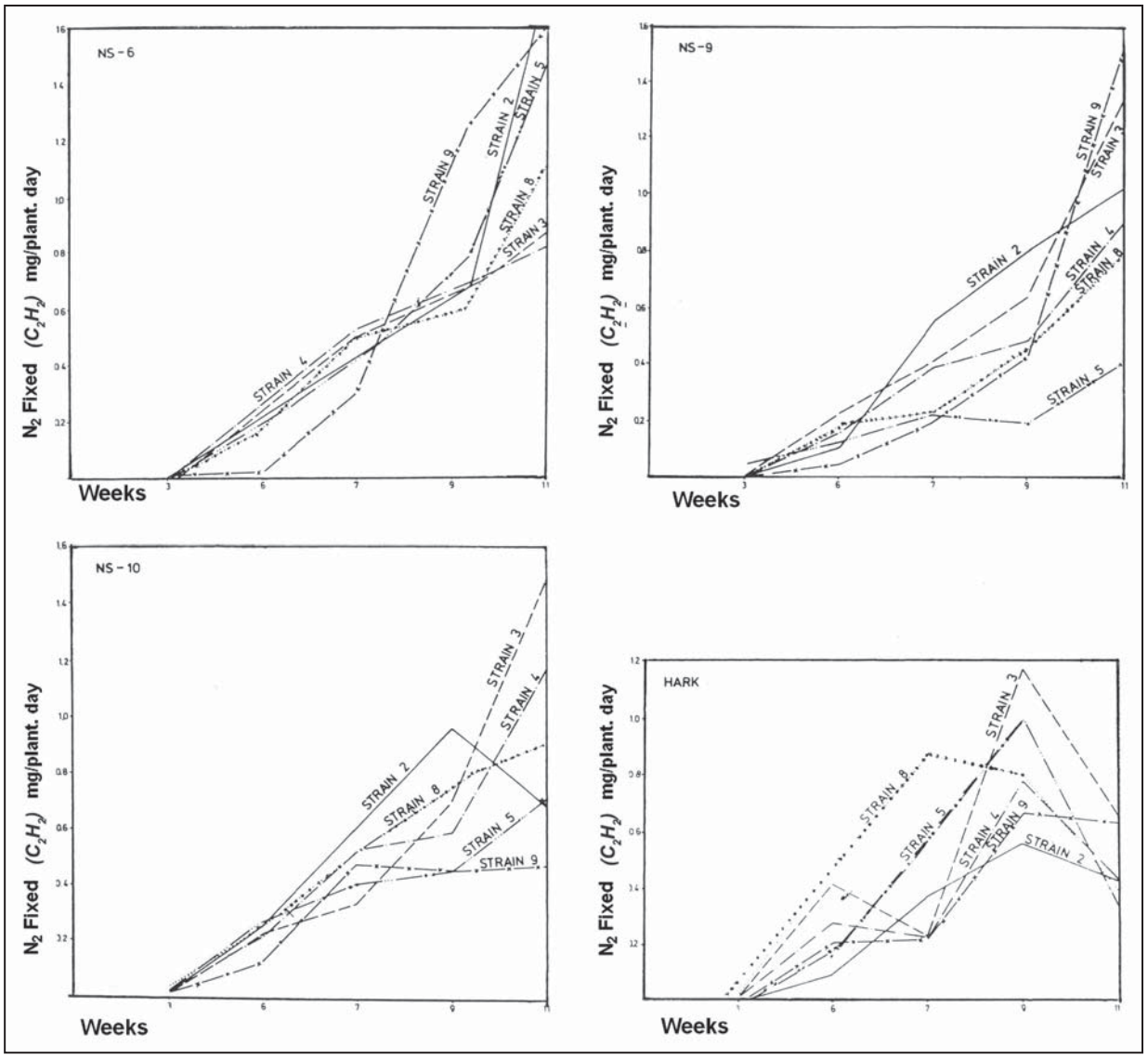
Catalytic reaction of nitrogenase is the reduction of one N_2 molecule to two NH_3 molecules. It is necessary for 6 electrons to be transferred and hydrolized from 10 to 12 ATP molecules for this reaction. Sources of electrons are either ferredoxin or flavodoxin. Electrons are transferred from the donor to Fe protein, and further to MoFe protein.

Nitrogenase activity in soybean was analyzed by many researchers from various aspects: whether soybean nodules reduce acetylene to ethylene, as well as optimal conditions for this reaction, effectiveness of the association between strains of *R. japonicum* and soybean genotypes. Sloger et al. (1975) discovered seasonal and daily variations in nitrogen fixation in field-grown soybean. Activity of nitrogenase in 45 inoculated soybean varieties ranged from 60 to 150 nmol of ethylene per hour (Wacek and Brill, 1976). However, according to other authors, the activity of this enzyme ranged from 700 to 7,500 (Sloger, 1969), from 600 to 5,200 (Kvien et al., 1978), from 657 to 1,640 (Kucey et al., 1989) and from 377 to 1,678 nmol ethylene per hour (Mrkovački, 1990), which shows that nitrogenase intensity is variable and subject to changes depending on the external factors and under the influence of certain processes in the plant. Therefore, its activity should be monitored dynamically for a

longer period, where the beginning, the maximum and the end of the activity would be determined (Sarić et al., 1990; Figure 8.2).

Figure 8.2

Dynamic of Nitrogenase activity in different soybean genotypes (Sarić et al., 1990)



Hydrogenase

It is known that soybean nodules produce H_2, i.e. nitrogenase can irreversibly be reduced to hydrogen protons of molecular H_2 (Hardy et al., 1965). Through hydrogenase, the reduced hydrogen can be included into the oxidation processes (Bothe and Eisbrenner, 1981).

A significant amount of energy is used for H_2 to be formed in nodules, and creation of H_2 can therefore be attributed to the inefficient utilization of photosynthates in the nodules. Nonetheless, Dixon (1968) was the first to find that due to hydrogenase some nodules do not produce H_2. During the nitrogen fixation process, nodules with hydrogenase activity (Hup^+) release some H_2 into the atmosphere, since the created H_2 is included in the hydrogen oxidation system. This research showed for the first time that the recycling of H_2 is simultaneous to nitrogenase activity.

In most agronomically important legumes, including soybean, 30% to 60% of energy is lost in the evolution of H_2. The capacity of bacteroids to recycle H_2 is the main factor in determination of H_2 loss into atmosphere. This standpoint is supported by the results showing that nodules inoculated with Hup^- strains lose on average 32% of the electron flux, while those inoculated with Hup^+ lose on average only 3.8% (Evans et al., 1981).

The amount of the transported nitrogen fixation products from soybean nodules, formed from Hup^+ *B. japonicum* strains is larger than those from Hup^- strains, showing that the process of recycling H_2 influences the balance of carbon utilization and nitrogen compounds assimilation. A number of experiments showed a potential advantage of H_2 recycling, supporting the conclusions that yield and total nitrogen content in legumes can be increased if aided by this ability of nodule bacteria strain. Hup^+ *B. japonicum* strains in soybean plants increase nitrogen content by 26% and dry weight by 16% (Schubert et al., 1977; Albrecht et al., 1979; Ruiz-Arguezo et al., 2001; Baginsky et al., 2002).

NH_4^+ assimilation and nitrogen metabolism in symbiosis

During nitrogen fixation in bacteroids by the reduction process, NH_4^+ ion is formed and later secreted into plant cell cytoplasm. In cytosol NH_4^+ ion participates in the synthesis of organic compounds, primarily of amino acids. Current views in nodule physiology state that nitrogen fixation requests amino acids to circulate between bacteroids and the plant (Prell and Poole, 2006).

There are two pathways, i.e. enzymatic systems of NH_4^+ incorporation and transport in nodules: one is by glutamate dehydrogenase (GDH), and another by glutamate synthase (GOGAT), which also includes glutamine synthetase (GS). These two pathways request different levels of NH_4^+ concentration, namely GDH demands more than 1.5 mM NH_4^+ in solution, and pathway combination of GS and GOGAT demands low levels of NH_4^+.

According to Reynolds et al. (1982) there are two types of legumes, depending on manner of transport and incorporation of NH_4^+: asparagine and ureid producing legume plants. In nodules of both of these plant types, glutamine is the first produced amino acid. Incorporation of NH_4^+ into ureid producing plants onsets when they are grown under atmospheric nitrogen fixation, when the produced nitrogen compound takes the form of ureid - allantoin and allantoic acid.

However, when these plants are provided with other forms of nitrogen, i.e. mineral nitrogen, ureid levels in xylem sap are low and asparagines is the main exported component (Pate et al., 1980). Allantoin and allantoic acid are the ultimate products from *de novo* purine biosynthesis in the nodules. The complete biosynthetic pathway of purine has not been explained, necessitating further research on discovering factors that control transport type – by ureid or amide.

According to McClure et al. (1980) correlation between nitrogen fixation and ureid transport points out that ureid content in xylem sap could be used as an indicator of nitrogen fixation process activity.

Energy balance of nitrogen fixation

Theoretic basis of all energy needs of nitrogen fixation in nodules according to Atkins and Rainbird (1982) encompasses the energy needed for nitrogenase and hydrogenase enzymes functioning, NH_4^+ assimilation, fixed nitrogen transport, growth and sustainance of nodules. Measuring activity efficiency and utilization of the nitrogenase-hydrogenase system shows that 0.66 to 1.38 mol glucose is used for one mol of the fixed nitrogen. The price of NH_4^+ assimilation into organic compounds is around 0.15 mols of glucose per one mol of the fixed nitrogen. Further transport of the fixed nitrogen as well as nodule growth demands additional 0.2 to 0.7 mols of glucose. To sum up, it takes 1.13 to 2.37 mols of glucose per one mol of the fixed nitrogen. If this is viewed in relation to one gram C per one gram nitrogen, it takes 2.9 to 6.1 grams of photosynthetic C per one gram of fixed nitrogen in legume symbiosis.

The amount of energy needed to reduce NO_3^- to NH_4^+, originating from soil or mineral nitrogen fertilizers, does not differ significantly from the one needed to fix atmospheric nitrogen.

Some limiting factors for symbiosis

The capacity of a symbiotic association to fix nitrogen under optimal conditions could be defined as “potential nitrogen fixation” (Lie, 1971). Actual nitrogen fixation is the one performed under less favorable conditions for plant growth, often observed in the field where a limiting factor could prevent full expression of symbiosis. Being an energetically intensive process, nitrogen fixation is significantly influenced by factors controlling photosynthesis level and distribution of photosynthates in the plant. The most important factors influencing nitrogen fixation in field conditions are soil moisture, temperature, light, pH of the environment and plant mineral nutrition (Lie et al., 1980).

Soil moisture.- Legumes are simultaneously intolerant to deficiency and excess of water in soil. This is primarily caused by a high sensitivity of the symbiotic associations. Optimal soil moisture for nodule formation is 60-70% out of full water capacity of the soil (Graham, 1992).

Drought triggers swift inactivation of nitrogen fixation process, but with irrigation the activity is reversible if moisture loss is up to 20% of nodule fresh weight (Sprent, 1971). Plants with limited meristematic growth of the nodules, such as soybean, are more sensitive than plants with elongated nodules which can prolong growth (Engin and Sprent, 1973).

Excessive soil moisture is harmful to nitrogen fixation. A thin layer of water on nodule surface can decrease fixation almost to a zero, which confirms the hypothesis that oxygen presence is a limiting factor for the process of nitrogen fixation.

Temperature.- Temperature significantly influences a symbiotic association and almost all the phases of its development and functioning more or less depend on temperature. Temperature mostly indirectly influences in an unspecific manner via plant metabolic processes such as respiration, photosynthesis and transpiration. Temperature range for a symbiotic system is more limited than the one with plants supplied by nitrogen, and symbiosis ceases when exposed to extreme temperatures.

The effect of temperature to nitrogen fixation is manifested through effects on the root and above ground plant parts. Low soil temperature reduces root hair infection, nodule development and nitrogen fixation. The optimal temperature for the nitrogen fixation process is 14°C do 24°C, which is proven by growing soybean at different temperatures of plant rhizosphere and measuring the quantity of fixed nitrogen. Temperatures higher than 28°C inhibit nitrogen fixation, although this inhibition depends on the host plant and nodule bacteria strain. At higher temperatures *B. japonicum* strain induces two heat-shock proteins (Kishinevsky et al., 1992; Fischer et al., 1993). Lower temperatures increase nodule number, thus compensating for lower specific activity of nitrogen fixation.

Light.- Light affects the symbiosis mostly through the process of photosynthesis by controlling nodule supply with carbohydrates necessary for their development and functioning. Under the influence of high intensity light there is a linear dependence between light intensity, nodulation and nitrogen fixation. Although nodulation and nitrogen fixation can also be sustained in complete absence of light for some time, influence of light on creation of photosynthates presents the key limiting factor for nitrogen fixation in field conditions. Increase of light intensity or CO_2 concentration enhances photosynthesis and also encourages nodulation and nitrogen fixation. In field-grown soybean with CO_2 fortification (0.003-0.012%), nitrogen fixation is enhanced 4 to 5 times, which is conditioned by a large number of the formed nodules, their higher efficiency and prolonged period of nitrogen fixation (Hardy and Havelka, 1976).

During soybean flowering period under reduced light nitrogen fixation is also reduced from 125 to 91 kg of nitrogen per hectare. Similarly, under enhanced light nitrogen fixation is also enhanced up to even 165 kg per hectare, which shows that specific activity of nodules is in correlation with light intensity (Ham et al., 1976).

Soil pH.- Soil pH is probably the most important external factor which influences symbiosis. Soybean plants, as well as some other legumes, grow best on neutral or weak alkaline soil. Low soil pH value reduces soybean supply with nutritional

elements necessary for nodulation. It was proven that low soil pH value inhibits root hair infection, beginning phase of nodulation and survival of nodule bacteria in soil. Nodulation process sensitivity to low pH is under a strong influence of calcium levels, i.e. the demand for calcium increases as pH decreases (Munns and Keyser, 1981; Wood and Cooper, 1984). Oppositely, Ca^{2+} increase in the environment reduces the infection ability of *B. japonicum* strain (Kadreva and Ignatov, 1995). *B. japonicum* cells have a limited ability to regulate intracellular pH if environment pH varies, which is why it is necessary to select *B. japonicum* strains that form nodules and fix nitrogen in sufficient quantity under low soil pH conditions (pH= 4.2) (Mrkovački et al., 1993).

Nitrogen nutrition.- Nitrates present in soil have an inhibitory effect both on the nodulation and nitrogen fixation, i.e. root hair infection, nodule growth and development, nodule number and dry weight (Abaidoo et al., 1990; Rai, 1992), level of nitrogenase activity and they also aid premature nodule senescence (Munns, 1977). Inhibition of nodule development and inhibition of nitrogenase activity are due to lower levels of photosynthates used in NO_3 assimilation. Mobilization of carbohydrates for NO_3 metabolism reduces the quantity of carbohydrates for nodule growth and nitrogen fixation. The other hypothesis states that the first product of nitrates reduction - nitrites can build a complex with leghemoglobin and consequently prevent its regulatory capability in relation to oxygen.

The results of numerous researchers showed that the application of a small quantity of nitrates (20-30 kg/ha) stimulates nodulation in the early phases of plant growth (so called “nitrogen hunger period”). Above these small concentrations, nodule weight is in reverse proportion to the level of nitrates in the environment. Other forms of nitrogen in small amounts also have caused nodulation inhibition. Some sources of nitrogen, such as urea or NH_4^+, caused pH to change in the environment in which the plant grows (Israel and Jackson, 1982), thus the inhibitory effects of these sources could be attributed more to the indirect influence on pH decrease than to the nitrogen supply in plants (Carroll et al., 1985).

Interaction between soybean and *B. japonicum* strain

There are three main possibilities in the interaction between host plant and nodule bacteria strains: nodulating or non-nodulating, effective with efficient nitrogen fixation activity or ineffective with inefficient nitrogen fixation activity.

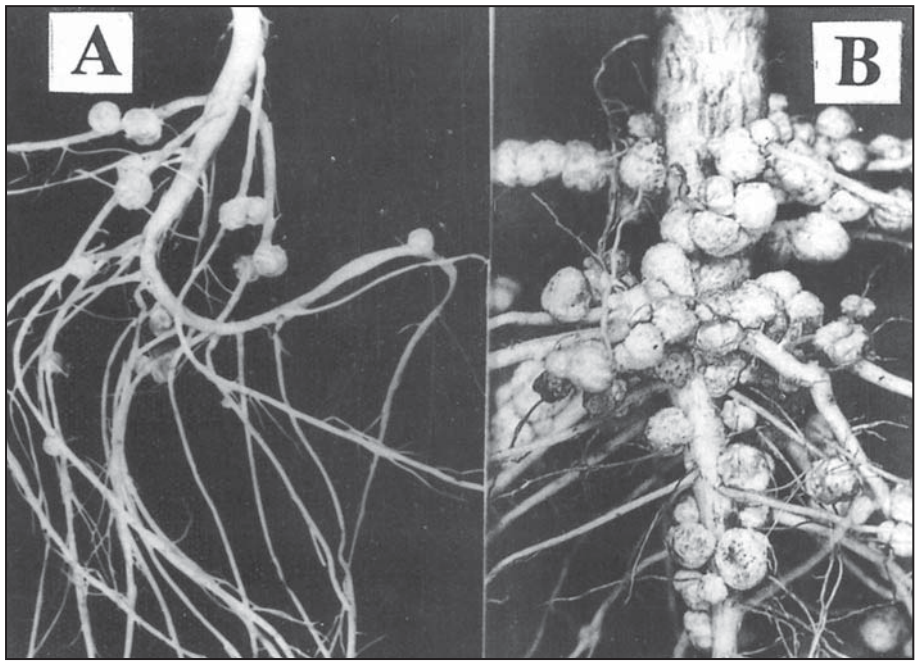
In ineffective nodulation, root nodules are small, white or green at cross section, with the host plant exhibiting symptoms of nitrogen deficiency. In plants which are effectively nodulated, but their nitrogen fixation system does not function efficiently, nodules are normal in appearance, white, green or light pink at cross section, with the host plant exhibiting the same symptoms as the previous one with typical chlorosis.

Effectively nodulated plants with efficient nitrogen fixing bacteria show characteristics of good plant growth with dark green leaves. The taproot hosts a vast number of large nodules that are red or dark red at cross section.

B. japonicum strains form two nodule types on soybean plants: A type – strains which form nodules on lateral roots, and B type – strains which form nodules on the taproot. B type nodules create an effective symbiotic association with nodule bacteria, while A type nodules mostly respond to an ineffective interaction between the host plant and strains (Figure 8.3).

Figure 8.3

Root nodules of Soybean (Mrkovački, 1990)



A - Nodules on lateral roots *B – Nodules on main root*

Soybean breeders are interested in high-yielding varieties with incorporation of favorable traits into new genotypes, but do not pay enough attention to the nitrogen fixing capability of the new genotypes, i.e. their compatibility with highly effective strains. It was shown that there is a significant genetic variability in the quantity of the fixed nitrogen in host plant (Pazdernik et al., 1996; Hungria et al., 2003; Graham et al., 2004). It is possible to develop lines capable of fixing more nitrogen by combining adequate methods for measuring nitrogen fixation with breeding methods.

An important issue of effectiveness is also the adaptability of a *B. japonicum* strain to a certain soybean variety which is the host plant (Sarić and Fawzi, 1983; Sarić et al., 1990; Mrkovački et al., 1989, 1992, 1997). Nitrogen content in nodules and in above ground parts of soybean plant is in correlation to the effectiveness of the

symbiotic association, i.e. with the quantity of nitrogen fixed by *B. japonicum* strains (Table 8.1)

Table. 8.1

Influence of *B. japonicum* strains on nitrogen content in soybean nodules (mg/plant) grown in Jensen solution in glass house (Mrkovački et al., 1992)

Strain	Variety							
	After 36 days							
	Corsoy	NS-16	NS-21	NS-20	NS-9	Amsoy	NS-10	Mean (A)
511	2.68	3.20	3.34	2.77	3.22	3.17	2.70	3.04
D-122	3.04	3.26	3.40	2.48	3.01	3.41	2.17	2.97
D-343	2.74	1.81	2.67	2.05	2.10	2.84	2.18	2.34
518	3.10	3.14	3.66	2.64	3.75	3.44	2.46	3.17
17z	1.28	1.44	1.29	0.84	0.92	1.50	0.91	1.17
1a	2.64	3.24	3.87	2.90	3.60	3.84	2.65	3.25
2/1	2.39	2.69	2.87	2.53	2.97	2.76	2.06	2.61
1003	3.01	3.30	4.03	2.91	3.47	3.35	2.40	3.21
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean (B)	2.63	2.76	3.14	2.39	2.88	3.04	2.19	
LSD 0.05	A - 0.30 B - 0.43 A x B - 0.75							
LSD 0.01	A - 0.39 B - 0.54 A x B - 0.99							
	After 51 days							
511	5.70	5.38	5.04	5.19	6.11	6.00	4.87	5.47
D-122	5.53	5.48	5.90	5.29	5.47	5.26	4.77	5.39
D-343	5.92	4.39	4.99	4.97	4.88	4.90	4.60	4.95
518	6.16	5.43	5.73	5.32	6.33	5.79	4.79	5.65
17z	2.94	3.90	4.20	4.96	3.30	4.66	3.36	3.90
1a	5.71	5.65	6.25	5.39	6.40	5.92	4.99	5.76
2/1	5.73	5.64	6.12	5.74	5.18	5.35	4.98	5.53
1003	5.93	5.76	6.99	6.08	5.56	6.50	4.83	5.95
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean (B)	5.45	5.20	5.65	5.37	5.41	5.55	4.65	
LSD 0.05	A - 0.46 B - 0.70 A x B - 1.20							
LSD 0.01	A - 0.54 B - 0.92 A x B - 1.57							

Competitiveness is the ability of a nodule bacteria strain to induce infection and form nodules on the host plant in the presence of other strains. There are vast differences among strains regarding this trait (Table 8.2). The mechanism which provides a strain with its competitive advantage has not been explained, but there are many factors which point to this trait. The competitive ability of a strain is affected by the ability of a strain to infect the host plant relatively quickly, strain tolerance to climate, pH, and the antagonism with "wild" autochthonous soil strains (May and Bohlool, 1983). This trait is also affected by the compatibility with the variety of the host (Sarić et al., 1987, 1988)

Table 8.2

Competitive ability of *B. japonicum* strains in nodule formation on soybean (Mrkovački et al., 1996)

B. japonicum strain	% of strain participation in nodules inoculated with strain mix*
2b	14.28
2/1	64.28
D-122	21.43

* Strains ratio in the mix: 1 : 1 : 1

The results of Secino et al. (1989) show that nodule bacteria *Rhizobium spp* and *B. japonicum* are directly involved in the nodule formation by producing IAA in culture, i.e. there is a relationship between the plant / *B. japonicum* and IAA biosynthesis. This is confirmed by the results of Milić (1990) and Milić et al. (1991), i.e. via metabolitic processes in the plant the host plant is affected by *B. japonicum* strains production of growth factors of indole type (IAA, indol-3-butyric acid, tryptophan, abscisic acid - ABA), growth factors of phenol type (gallic acid, coumarin and protocatechin acid) and growth factors of gibberellin type (GA_3, GA_7), all of which affect the increase of absorbing surface of the plant root (Table 8.3). This causes an increase of water and nutritive elements uptake and the increase of plant dry matter weight. Also, they affect nitrogen content and the quantity of fixed nitrogen in the plants. Growth factors also affect activity of nitrogenase enzyme.

Table 8.3

Capability of different growth factors production in *B. japonicum* strains (Milić et al., 1991)

B. japonicum strains	Number of separated spots in growth factors (using TLC chromatography)			
	Gibberellins	Indoles	Phenols	Total
2b	7	2	2	11
1b	5	4	4	13
518	4	3	2	9
511	4	2	1	7
1a	3	2	2	7
1	2	1	1	4

Inoculation - Nitraginization

Soon after Hellriegel had discovered that legumes can fix nitrogen, and Beijerinck had isolated nodule bacteria, legume seeds began to be inoculated with nodule bacteria. The first bacterial preparation (Radicin) was made in Germany in 1897 by Nobbe and Hiltner. In Serbia, research on nodule bacteria began with the introduction of soybean into production. A product with nodule bacteria for soybean was applied for the first time in Serbia in 1931 by Konjev. First detailed research on the influence of *B. japonicum* on growth and development of soybean in field conditions was performed by Sarić and Sarić (1959). Nodule bacteria products are widely used today in many countries under various names: N-germ in France, Nitrazon in the Czeck Republic and Slovakia, NS-Nitragin or Azotofiksin in Serbia, and Nitragin or Rizotrofin in Russia (Jemcev and Đukić, 2000; Đukić et al., 2007).

A good microbial product should contain effective nodule bacteria capable to fix as much nitrogen from the atmosphere as possible in a symbiotic association with plant, which necessitates important selection of strains according to their effectiveness. Selection of effective *B. japonicum* strains for soybean as the host plant is the first prerequisite for creation of quality inoculums (Mrkovački, 1990). In addition to the fact that the chosen strains should form nodules efficient to fix nitrogen on plant root and provide the plant with sufficient amount of the fixed nitrogen, the chosen strains in the preparation should also be highly competitive in relation to autochthonous strains. Better nitrogen fixation can be accomplished by strain selection, which besides the capacity for nitrogen fixation must also take into account the competitive ability of a strain in relation to the natural population which is most often inefficient in fixing nitrogen (Table 8.4) (Mrkovački et al., 2002).

Table 8.4

Soybean grain yield (kg/ha)

	1996	% increase	1997	% increase
Control ø	3322.2	100	3525,2	100
Nitragin	3832.1	115.3	4413.5	125.13
	509.9	15.3%	888.3	25.19%

Such superior strains should occupy a significant number of nodules in relation to the soil population (Mrkovački et al., 1997a; Rengel, 2002). Strain's competitiveness for inoculation can be enhanced if genetically engineered to produce substances that are inhibitory for nod gene expression in the natural population (Vlassak and Vanderleyden, 1997). Capability of *B. japonicum* strains to produce growth factors is a strain trait included in selection of highly-effective strains.

Researches show that bacteria are directly responsible for the quantity of the produced IAA in soybean nodules (Hunter, 1986; Milić et al., 1993).

Another important issue is the ability of a strain to sustain itself in a certain type of soil as long as possible, i.e. its adaptability to the physical and chemical soil conditions and to various chemical treatments applied during soybean production (Mrkovački et al., 1992a), as well as its ability to sustain itself well in soil and rhizosphere among numerous soil florae (saprophytic competence) until entering plant roots, in order to subsequently form more nodules and higher activity of nitrogenase and other enzymes in them.

Selection of *B. japonicum* strains leads to the isolation of superior strains and requests laboratory research first, followed by research under controlled conditions in the glass house, and finally research under field production conditions. Only then the most effective strains are used in production, which necessitates continual introduction of new methods (Table 8.5) (Mrkovački and Hrustić, 2003).

Table 8.5

Influence of different inoculation types on soybean grain, above ground parts and yield

	Grain			Above ground parts		Yield	
	Weight	N content	Proteins %	Total weight	% increase	t/ha	% increase
N	13.31	813.51	39.10	7.000	19.06	2846	17.11
N + A	14.68	907.66	39.30	7.032	19.61	2915	19.95
N + N	15.46	943.22	39.54	7.322	24.54	3053	25.63
Ø	10.72	599.09	34.90	5.879	100	2430	100

N – soybean seed inoculated with Nitragin before planting

N+A - soybean seed inoculated with Nitragin and sprayed with Azotobacter

N+N - soybean seed inoculated with Nitragin before planting and 30 days after planting

Ø – non-inoculated variant

Inoculum preparation. - Inoculum is produced on solid or liquid nutritive media. Contemporary inoculum production is performed in fermentors in which optimal aeration, temperature and pH value of the environment are provided in addition to nutritive components. In utilizing this manner of cell growing, a high titration of the preparation is provided, i.e. a large number of active cells applied to the carrier. The field results until now have shown that the most efficient inoculant for soybean should contain at least 10^6 of nodule bacteria cells per soybean seed at the time of application, which is attained by high titration in inoculums production (10^{10} cells/ml).

The most widely-used inoculum carrier in Serbia and abroad is peat. The best peat is the one with a high degree of degradability, with neutral reaction and low

content of alumina. Such peat supplies sufficient nutritional reserves for bacteria to survive for a several months. The basic technological principle in the inoculum production is the application of sterile peat and sterile conditions in all production phases.

Application of a microbial product: - Nowadays, the application of a microbial product is an obligatory measure during soybean planting which is economically justified, since an increase in yield and grain quality can be achieved with small material investments. Significant amounts of organic nitrogen remain in soil after soybean, and such nitrogen is not exposed to leaching which makes this plant species a good previous crop for most field crops.

Microbial products or microbial fertilizers for legumes can be applied in two ways: by seed inoculation or by application to the soil.

However, a microbial product for soybean is not applied to seed. A fine coat of carrier (peat) and inoculum is created on seed pertaining there during mechanical planting. Besides the preparation with peat as carrier, a dry preparation (lyophilizated) is also produced. This preparation can be stored for a longer period of time, but the number of bacterial cells is significantly reduced during lyophilization and storage. Besides the before mentioned preparations which are widely applied, inoculum is currently applied to the seed also by a process of pelleting, i.e. the seed is coated with a liquid inoculum first, and then with a synthetic component. Such product is introduced into the soil together with the seed. In soil pellete protects the inoculum from soil microorganisms and unfavorable environmental conditions, such as acid reaction. On the other hand, pellet slowly decomposes in soil and thus releases the inoculum. Naturally, pellet production is much more expensive and demands special equipment.

Figure 8.4

Microbial product "NS-Nitragin" *(photo: G. Kuzmanović)*

SUMMARY

Nitrogen fixation is an important process for the maintenance of life on Earth. Only a few prokaryotic genera have the capacity to fix nitrogen. Nodular bacteria fall in this group. Association between nodular bacteria from the genera *Rhizobium, Bradyrhizobium, Sinorhizobium, Mezorhizobium, Azorhizobium* and *Allorhizobium* and legumes is unique and beneficial for both partners. As a result of the association, nodules are formed on plant roots. Nodules, i.e., bacteroids, contain nitrogenase, the enzyme responsible for the biological fixation of nitrogen. Reduction process produces the ion NH+ which is exuded into cullular cytoplasm of plants. In cytosol, the ion NH4+ takes part in the synthesis of organic compounds, primarily amioacids. In field conditions, the main factors affecting nitrogen fixation are soil moisture, temperature, light, pH environment, and mineral nutrition of plants. Plants associated with efficient nitrogen-fixing bacteria grow well, their leaf color is dark green, and their main root is overgrown with large nodules which, on average, are red-colored. Nowadays, seed treatment with microbiological preparations is an obligatory practice. It is economically advantageous because it ensures increased yield and improved quality of grain. After harvest, the soybean leaves considerable amounts of organic nitrogen which is not prone to leaching. This makes the soybean a good preceding crop for most field crops.

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SOYBEAN CULTURAL PRACTICE

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The yields of soybean, just as those of other crops, depend on the growing conditions, variety, and the cultural practice implemented, as well as on the investments and know-how of the grower, whose job is to coordinate and harmonize all the crop growing activities.

The method of soybean growing (cultural practice, growing technology) is a chronological series of agronomic practices whose purpose is to adapt the existing growing conditions to soybean's biological requirements in order to maximize the expression of the crop's genetic potential for yield. However, the effects of cultural practices depend significantly on the year, i.e. the moment at which an unfavorable factor affecting the yield appears, how long it lasts, and how severe it is. Because weather conditions cannot be predicted reliably, a recommended technology of growing will be based on average values, which appear with greatest frequency over a number of years. Even with this approach, mistakes will sometimes be made, but their frequency will be much reduced compared to when the technology is changed every year based on previous year's results. A recommended growing technology that is adapted for a particular area with its average weather conditions is an ideal that should be pursued and an effort should be made to implement such recommended practices to as high a standard of quality and as timely as possible. In actual practice, however, growers often have to depart from the recommended growing technology for objective or subjective reasons. This increases the risk of yield losses, which is something the grower must be aware of before taking such a course of action. Therefore, it is also important to know the biology and growing requirements of soybean as a crop species and of the particular soybean variety being grown, because whether or not these requirements have been met during the growing season will often depend on what cultural practices have been applied. In this connection, it is also significant to know and correctly identify the stages of soybean growth and development, so that cultural practices, especially key ones such as the application of herbicides, fertilizer, insecticides, irrigation, crop tending and harvesting, can be implemented in a timely and efficient manner.

When growing any crop, the goal is to obtain high and stable yields of good quality while making a profit and maintaining soil fertility. Agriculture is part of a country's economy and is therefore subject to certain universal economic laws despite having its own distinctive features. Thanks to the vital strategic importance of food production, the government has an obligation to design and implement such agricultural policies (premiums, subsidies, loans, etc) that will create macroeconomic conditions in which agriculture is capable of generating profit margin levels that are on a par with those typifying the rest of the economy. The reason is that from the microeconomic point of view, i.e. from the standpoint of the grower, profit margin is the only economic incentive for growing a particular crop. Yield level and quality play the key role in this.

Based on light absorption and utilization, the theoretical maximum for soybean yields is 7,300 kg/ha (Sinclair, 2004) to 8,000 kg/ha (Sinclair,1998). In practical terms, the genetic potential for yield of a soybean variety is the yield a variety adapted to a particular set of growing conditions produces when there are no water and nutrient deficiencies and effective control is carried out against pests, diseases, weeds, lodging and other stress conditions. In the past five years, the average yield of soybean in Serbia has been 2,485 kg/ha, which is only a third of the crop's genetic potential. The loss of two thirds of the potential yield has been a result of unfavorable weather conditions and plant requirements not being met by the existing growing conditions. To counteract such conditions is the main goal of cultural practices implemented by the growers.

Successful soybean production requires that only those expenses be incurred that are necessary to obtain an optimum amount and quality of the product and generate a profit. Each cultural practice should be considered from the point of view of its economic justifiability, i.e. it should only be opted for if its implementation brings about an improvement of yield that equals or outweighs the cost price of such measure. Included in this are the basic costs of seed, pesticides, fertilizers, labor, machinery, and fuel as well as the expenses of financing and the costs of soil use and irrigation. Cultural practices can be of the type that requires that a decision be made as to whether or not their implementation would be justified (inter-row cultivation, yes or no), but cultural practice also has segments in which the optimal rate of a particular element (seed, fertilizer) needs to be determined. Cultural practice analysis must take into account the specific growing and market conditions, i.e. the relationship between input costs and yield.

Although the countries of the Far East have a long tradition of soybean growing, this plant has become a globally important field crop only in the last few decades. This means that the growers were faced with a completely unknown crop for which growing methods had yet to be developed and honed. They first had to become familiar with the plant and its biological characteristics, needs, and potentials. After that, particular cultural practices had to be chosen and adapted, so that the existing growing conditions could be suited to soybean plant requirements to as high a degree as possible.

Soybean has been grown in Yugoslavia on a significant acreage only since 1975, so it is not a traditional crop in the region as wheat and maize are. For this reason, there was no science-based production technology for it at the start of this period (Hrustić, 1994). There were quite a few cultural practice issues that had yet to be resolved, since foreign experiences in this area could not be transplanted to the local conditions without modification, because each geographic region has its own unique characteristics and these lead to important differences in production technology (Belić et al., 1983). When soybean began to be grown on a large scale in the country, there were still a lot of unknowns (Hrustić et al., 1996). Primary tillage in soybean is the same as in most other spring field crops, but problems started to occur as soon as seedbed preparation had to be performed, because this cultural practice in soybean is quite characteristic. There were also many open questions when it came to fertilizer use, seed inoculation, planting dates, and plant density. In the time since then, however, domestic soybean growers have managed to develop and master a soybean production technology that has been adapted for the country's growing conditions based on average results obtained in multi-year trials and cumulative practical experience.

CHOICE OF VARIETY

The correct choice of variety is an important part of a growing technology aiming to achieve high and stable yields.

When choosing which soybean variety to grow, the following traits should be considered: yield level and quality, days to harvest, resistance to lodging and pod dehiscence, and resistance to major diseases and pests.

Yield potential is certainly the single most important factor in the choice of variety. However, in cases where it is possible that conditions may arise which might limit the potential yield but could be overcome by the use of a genetically resistant variety, other characteristics must also be taken into consideration. In many situations, a trait other than yield potential will have greater importance in the selection of variety. Generally speaking, varieties that take more time to mature produce higher yields, because they make full use of the growing season. In Serbian conditions, however, it has sometimes been the case in dry years that the early varieties produced higher yields than the late ones. Because of their longer growth period, it is possible that the late varieties go through the critical stages of development (grain formation and grain fill) in conditions of lack of rainfall, high temperatures, and low relative humidity (July, August), which negatively affect pod and grain number and grain size. Varieties with a short growth period (Maturity Group 0) go through all the developmental stages faster, so the critical phases of their development occur earlier in the

growing season and they are thus able to avoid the part of the season with the largest water deficits and highest temperatures. In the early varieties, these stress factors affect only grain size, which is why the yields of such genotypes are sometimes higher than those of varieties from MG II (Hrustić et al., 1995; Vidić et al., 1996).

Lodging slows down harvesting and increases yield losses. When lodging occurs prior to the pod formation stage, it reduces the yield by reducing pod number and causing poorer grain fill. Although resistance to lodging is a genetically determined trait, lodging is promoted by factors that stimulate vegetative plant growth such as increased plant density, soil moisture, and soil fertility. For this reason, when growing soybean on an irrigated, fertile soil, varieties with greater genetic resistance to lodging should be used and the seeding rate should be reduced to produce a plant population which is at the lower limit of the optimal stand density range.

In US conditions, the average annual gain in yield attributable to the use of new soybean varieties is about 30 kg/ha (Specht et al., 1999). Every year, therefore, the results of the nearest variety trials should be analyzed and the currently grown varieties should be replaced with the higher-yielding new ones. The best way to pick out the highest yielding variety is look at the average yield across several years and locations. This is because weather conditions cannot be predicted in advance, so a variety that on average produces the best yields in similar locations in dry and wet years is the safest choice. Naturally, the more locations the average is based on, the safer the choice.

Each soybean variety falls into one of 13 maturity groups (000, 00, 0, I, II... X) depending on the geographic area in which it is grown. Since varieties are adapted to a very narrow range of latitudes, the terms early-, medium-, and late-maturing are only relative, that is, they describe the variety's period of maturation in a particular geographic area. Varieties from Maturity Groups II and III can usually be grown with success in any region when planted on standard planting dates. A variety's level of adaptedness in a given area is determined through variety trials. Such trials have shown that the most suitable soybean varieties for the growing conditions in Serbia are those from Maturity Groups 0, I and II. Varieties from MG III take too long to mature, which makes seed maturation problematic in wet years, but such varieties can be recommended when soybeans are grown for silage.

The main goal of crop production is most definitely yield. The choice of the variety, however, also depends on other factors, such as the condition of the plot, planting dates, estimated dates of harvesting, irrigation capabilities, and so on. When deciding which variety to grow, the choice should be made so that it meets as many of the growing requirements as possible. When growing soybean on a larger area, the grower must also take into account all the possible stress conditions that might occur, so in such a case it is advisable that at least two different genotypes be planted. This is because there is no single variety that can withstand the effects of all the limiting environmental factors (Jocković et al., 1994). For this reason, a number of different genotypes should be grown in every growing region (Hrustić et al., 1993).

An even better strategy is to combine varieties from different maturity groups, because in that case, should stress conditions occur, the genotypes will be at different stages of growth and development and the negative effects of stress will thus be reduced or even completely avoided. This approach also make it possible to better organize operations in the field during planting, crop tending, and harvesting, as they become successive rather than concurrent throughout the area (Figure 9.1).

Intensive work on soybean breeding at the Institute of Field and Vegetable Crops has so far produced over 100 varieties. Thanks to the constant advancement of breeding procedures and the simplification of seed production and increase, growers can now choose among several different varieties from each maturity group. Of particular note are the new developed varieties, which are superior to the most widely grown commercial ones.

Among the varieties from MG 0, the highest and most stable yields are produced by the varieties Valjevka and Galina, which have supplanted the long-lived variety Afrodita in commercial production. These varieties produce high yields for their maturity group and are suitable for regular as well as late planting (Table 9.1).

Table 9.1

Recommended soybean varieties, seeding rates, and planting dates for Serbian growing conditions

Maturity Group	II	I	0	00/000
Variety	Venera	Sava	Valjevka	Fortuna
	Rubin	Novosađanka	Galina	Prima
	Trijumf	Victoria		Merkur
	Vojvođanka	Balkan		
No. of viable seeds (000)	350 - 400	400 - 450	around 500	550 - 600
Time of planting	Early April	All of April	All of April	All of spring, or by early July when used in double cropping

The varieties Balkan and Novosađanka were until recently the mainstays of the MG I variety range in the country but are now being replaced with the newly-developed top-quality varieties Sava, and Victoria, which are capable of producing high and stable yields in different growing conditions.

In MG II, the stand-out varieties are Venera, and Rubin. Their best qualities are high yields and resistance to lodging.

Figure 9.1

NS varieties of Soybean *(photo: G. Kuzmanović)*

Soybean can be grown outside the normal planting dates as well, for which purpose varieties with an extremely short growth period (MGs 00/000) are used. In this segment of the Serbian market, the new varieties Fortuna, Julija, and Merkur are gradually replacing the two older varieties of the same type, Jelica and Krajina. All these varieties take about 100 days to mature, which enables them to ripen and produce good yields even when planted late as a second crop in a double cropping system. Trial results and commercial crop production have also shown that early soybean varieties can be successfully grown after harvesting winter barley or winter wheat. When sown in early July and grown under irrigated conditions, such soybean varieties mature in early October and produce yields of 2 to 2.5 t/ha.

SOYBEAN'S PLACE IN CROP ROTATION

Biotic communities are generally characterized by biological balance and self-sustainability due to the coexistence of a large number of species and the uninterrupted circulation of matter. Agrobiological communities, however, are poor in species and often consist of only a single one, and the removal of yield from the plot disrupts their natural biological balance too. It therefore becomes necessary for man to maintain and control this balance using different agronomic, organizational and technical practices. The idea is that a situation where a large number of species live side by side in biotic communities can be partially mimicked in agrobiological communities by the use of crop rotation.

Crop rotation on plowland balances the relationship between field crops production and animal husbandry as well as that among different crops. It makes cultivation and fertilization systems more cost-effective, fulfills cultural practice and biological crop requirements, and addresses the various economic and organizational aspects of crop production. Generally, crop rotation can be defined as an orderly, well thought out plan on how to correctly and cost-effectively utilize the growing environment (most importantly soil and climate) to the maximum. The main goal of crop rotation is to maintain or increase the soil fertility and yield levels of all the crops involved in the rotation. Crop rotation is a planned and predetermined sequence by which crops follow one another in space and time and eventually return to the same field after a while (Molnar. 2004).

In the late 1980s, crop rotation again came to the spotlight globally due to the large impact it has in the field of environmental protection. In conjunction with an appropriate agricultural policy that promotes profitability through the diversification of production, crop rotation makes it possible to reduce the use of chemicals in agriculture and improve soil properties and thus increase the self-sustainability of an agroecosystem. The rotation of crops also increases economic security and cash flow on farms and makes it easier to organize agricultural operations (Heatherly and Elmore, 2004).

Crop rotation reduces the accumulation of crop-specific disease agents and pests as well as weed abundance and adaptability to a particular crop species. Growing a legume crop interrupts the life cycles of diseases in maize and wheat, the two most commonly grown cereals in Serbia. In this sense, crop rotation can be viewed as a preventive, integrated crop management practice that helps protect all the plant species used in the rotation. As a legume, soybean has good quality harvest residues with a low C to N ratio. They increase the soil levels of organic matter rich in organic nitrogen, which is not susceptible to leaching. As an early row crop, soybean fits in very well with the other field crops in rotations. Also, rotating crops with different depths of rooting enables the ascendant biological flow of nutrients and better and more efficient utilization of total nutrients and water from the soil. Ferreira et al. (2000) found that growing soybean in a crop rotation increased nitrogen fixation as compared to when the crop is grown in a monoculture. The alteration of physical soil properties also allows deeper root penetration. All this increases the yields of soybean as well as all those of the other crop species used in a crop rotation without any extra costs.

As the areas in soybean are increasing, this crop is becoming a major constituent of field crops production and an increasing amount of attention is being paid to its place in crop rotations. Soybean is a very good fit for crop rotations, because it is a good previous crop for most of the other field crops grown in Serbia without being very demanding itself when it comes to the crop that precedes it. In Serbian agricultural practice, the most common preceding crops to soybean are wheat, maize, and sugar beet, which are also the most commonly grown crops in the country overall.

Because small grains account for a large proportion of Serbia's crop production, they often precede soybean in the country's fields. Wheat and other small grains are removed from the fields early, which gives the soil plenty of time to rest and regain its properties in a natural way before the next spring crop is planted 8-10 months later. Wheat and winter barley are also becoming increasingly common as a previous crop to soybean when early maturing soybean varieties are used as the second crop in double cropping systems.

Maize is also planted before soybean in Serbia as well as the U.S., the world's top producer of the legume. In the US corn belt (Illinois, Iowa, Minnesota, Indiana, Missouri), over 90% of the agricultural acreage is utilized through a two-crop rotation in which maize and soybean alternate. In Serbian conditions, soybean would be an ideal crop to break up the rotation of maize and wheat (Crnobarac, 2000). Maize is a good preceding crop to soybean, provided the stover is well chopped up and plowed under. One factor that can limit the use of maize before soybean are herbicides based on triazine and sulfonylurea, whose residual effects have a negative impact on soybean.

Opinions vary as to how suitable sugar beet is as the preceding crop to soybean. During the intensification of field crops production, sugar beets were considered a good previous crop for most other field crops. In the last few years, however, sugar beet is increasingly being regarded as an inadequate previous crop. The prolonged dry period in recent years has contributed significantly to this. Without the application of farmyard manure, the biogenicity of the soil will be reduced to the extent that the tillage and fertilizer applied in the course of sugar beet growing will not have the appropriate effect on the crop that follows (Stefanović, 1992). Because of the high fertilizer rates it requires, sugar beet could be a good preceding crop to soybean. The reason that it is not, however, lies in its high water consumption (especially in dry years) and excess moisture during lifting and soil compaction.

Sunflower and rapeseed are high-risk preceding crops to soybean because of many shared diseases. Plots on which sunflowers or soybeans have been infected with white rot should not be used for soybean growing in the next five to six years, because the fungus causing the disease is incorporated into the soil together with harvest residues and remains virulent for a very long time. Soybean should not be grown after other legumes either, not only because of the diseases they have in common but also because the leftover nitrogen is more valuable for other crops.

When it comes to growing soybean as a monoculture, different results can be found in the literature. Johnson (1987) reported a series of results indicating that soybeans give 11- 21% higher yields when rotated with maize or sugarbeet than when grown as a monoculture. According to the same author, maize rotated with soybean also produces higher yields as compared to continuous maize. The prevailing opinion in the literature and the soybean community is that soybeans should not be grown as a monoculture. Growing soybeans repeatedly or, especially, as a monoculture should be avoided due to the accumulation of disease agents and insects, production of allelopathic chemicals during the decomposition of plant residues, and the increasing difficulty of weed control.

The allelochemicals have a negative influence on root growth and the amount of nitrogen fixation in soybean and other legumes. Better use of the soil and better yields are also the reason why soybean's position in a cropping sequence should be carefully chosen. Growing soybean in a crop rotation does not eliminate the problem of diseases, but it does reduce the possibility of infection.

Soybean is a very good fit for crop rotations, because it is an excellent preceding crop to most crop species grown in Serbia. As a legume, soybean also improves soil structure and enriches the soil with nitrogen. Although it produces less harvest residues in terms of mass, soybean has a considerably better C:N ratio than maize and other cereals due to the fact that its nitrogen requirements per unit yield are more than three times higher (Hoeft et al. 2000). Findings on the exact amount of nitrogen that soybean leaves behind on the field vary. Thus, Vanotti and Bundy (1995) reported 45-67 kg/ha, while Mosca et al. (1989) cite 30-60 kg/ha.

FERTILIZATION

Plants need certain amounts of nutrients for their growth and development. The roles individual micro- and macronutrients play in soybean growth and development have been discussed in the chapter Mineral Nutrition of Soybean, so the present chapter will deal only with the plant's needs for the application of particular mineral fertilizers.

The system by which a crop is fertilized will depend on soil and climatic conditions, which means that there can be no universal recommendations in this regard for all crop growing regions without taking into account the specific local growing conditions. Fertilizer application may be the agronomic practice that perhaps benefits the least from experiences from other regions. The results of research on the needs for particular elements are generally similar, but the production conditions vary considerably. Knowledge of the roles individual elements play in the life cycle of a plant and the conditions under which the plant grows will determine the amounts and types of mineral fertilizers that should be applied.

The basic principles of fertilizer application for any crop are applicable to soybean too. Fertilizer use is based on the principle of soil fertility control, i.e. on maintaining the good fertility of fertile soils and improving the poor fertility of less fertile ones while trying to obtain high and stable yields. The basis of the principle is the balance of nutrients at the crop or crop rotation level. The fertilizer rate applied to a crop establishes a balance between the total amount of nutrients the plant needs in order to produce the projected yield and the amount of plant-available nutrients present in the soil. What is also taken into account is that a certain amount of nutrients is removed from the soil by harvesting and that these quantities need to be replenished if soil fertility is to be maintained.

The total nutrient requirements is calculated by multiplying the target yield with the amount of nutrients present in the grains and vegetative plant organs. According to a number of studies, the plant needs 100 kg N, 23-27 kg P_2O_5, and 50-60 kg K_2O in order to produce one ton of grain and the corresponding amount of vegetative biomass. Of these quantities, about 60 kg N, 11-14 kg P_2O_5, and 20-23 kg K_2O are removed with the grain at harvesting, while the remainder of the nutrients that have been taken up by the plants is returned to the soil when harvest residues are plowed under (Franzen and Gerwing, 1997; Johnson, 1992; Cetiom, 1988).

Before determining optimum fertilizer rates that will provide the plant with the right balance of nutrients, one must determine the amount of plant-available nutrient elements present in the soil through soil analysis. Along with information on soil nutrient levels, a soil analysis will usually include a recommendation on the optimum fertilizer rates and time and method of fertilizer application. It has become a common practice to average the results of analysis of several representative soil samples taken from a plot and then apply the recommended fertilizer rate uniformly throughout the entire plot. Fertilizer application provides plants with the minimum amount of nutrients needed to prevent the limitation of yield when optimal growing technologies are used in the average weather conditions of a given area.

It has been a standard practice so far to apply the same fertilizer rate across the whole plot regardless of the variations in fertility observed in different spots within it. This leads to uneven plant development and results in yield losses, as the uniformity of the crop is a key factor in obtaining high yields. Hybrids are as uniform as possible from the genetic point of view, but the phenotypic expression of this potential depends on the uniformity of environments in which individual seeds develop. Because of this, research efforts have been under way to develop and implement an advanced growing technology termed precision agriculture, which makes use of fertilizer rate recommendations pertaining to each individual soil sample. In other words, a precise recommendation is made on how to fertilize each individual part of the plot instead of applying the same, averaged fertilizer rate throughout the plot (Hoeft et al., 2000). In order for this approach to work, soil samples need to be taken at more frequent intervals in order to get a more accurate picture of fertility variation within the plot. Another key part of this system is to pinpoint the exact geographic coordinates of the sampled spot using GPS technology, i.e. to make a map of the plot and a GIS database on a mobile computer. The results of soil tests for each sample with known coordinates are used as the basis for different fertilizer application in different areas of the plot using specially adapted fertilizer spreaders. This is done through the use of a GPS device installed in the tractor that feeds the vehicle's current coordinates into the computer, which then determines optimum fertilizer and pesticide rates based on soil nutrient levels in that particular spot.

The uptake of soil nutrients depends on the stage of plant development. In the early phases of this development, when plants are small, the amounts of nutrients they take up are small as well, but their concentrations in plant tissues are high.

In the latter stages, plant nutrient requirements increase and so do the quantities of micro- and macronutrients taken up by the plant, but nutrient levels in plant tissues become lower due to the dilution effect. The rate of nutrient uptake by a plant is affected not only by the amount of nutrients present in the soil and the form they are found in but by other soil properties as well. If the soil is dry, the uptake of mineral nutrients becomes reduced due to a decrease in soil water availability and uptake. Because the root needs a specific water-air regime in order to perform its activity, nutrient uptake will not be adequate when soil is extremely wet. For this reason, in order to ensure proper plant nutrition, an effort must be made during tillage to provide the future crop with adequate soil water-air and temperature regimes.

Nitrogen deficiency has a similar effect on plant growth and development and yield formation in all cultivated plants. What separates soybean from other field crop when it comes to nitrogen is this plant's ability to meet a considerable proportion of its need for this nutrient through a symbiotic association with nodule bacteria. The proportion of nitrogen originating from nitrogen fixation in the total requirement of soybean for this element ranges between 25 and 70% depending on growing conditions. The remainder of soybean N needs are met by uptake from soil (inorganic N, N produced by mineralization of organic matter, and N left in the soil by the previous crop) (Varco, 1999). Soybeans use soil nitrogen exclusively only in the short period between when they stop using cotyledons for nutrition and the formation of nodules, which, according to most authors, happens within the first two to three weeks of growth and development. This NO_3 form is the predominant source of nitrogen all the way through to the start of pod formation, when its contribution to N nutrition drops sharply (Pedersen, 2004). Later on, as plant N requirements increase, soybeans meet most of their need for N from atmospheric nitrogen. Because the use of fixed nitrogen by the plant predominates from the start of flowering until the grain filling stage (Heoft et al. 2000a), it is important to incorporate root nodule bacteria into soils on which soybeans have not been grown previously. A high amount of nitrate nitrogen prolongs the infection and reduces nodulation, which leads to a decrease in nitrogen fixation. When considering the effects of nitrogen in soybean, therefore, it is always important to indicate whether the plants have been inoculated, and, if so, how successful the nodulation was. Soybeans with well-developed nodules rarely respond to nitrogen fertilizer regardless of the soil type, the time and method of N application, and the amount of N fertilizer applied, so plots with such soybean plants should not be top-dressed (Roth et al., 2003).

In a two-year multi-site trial in Vojvodina, increasing nitrogen rates (with the rates of phosphorus and potassium remaining the same) had a negligible effect on soybean yields, while inoculation had a much greater effect. Other studies found that increasing N rates reduced the number of nodules, so much so that no nodules were found on the roots of soybeans in most locations when the N rate was increased to 90 kg/ha (Belić et al., 1987; Relić, 1988). The same conclusion was reached by Gascho et al. (1989) and Johnson (1992).

Under a certain set of soil conditions (low pH, low organic matter and residual nitrogen contents, a high degree of harvest residue incorporation or soil compaction) soybean plants cannot get enough nitrogen from the soil and N fixation as the sole sources of the element, so the symptoms of nitrogen deficiency appear, in which case the application of nitrogen fertilizer becomes justified, as it leads to a significant increase in yield. Osborn and Riedell (2006) reported that in cooler conditions the incorporation of 16 kg/ha of nitrogen prior to planting resulted in an average seed yield increase of 6% over a three-year period due to faster initial plant growth.

The soybean plant takes up phosphorus and potassium throughout the growing season. The period of peak requirement for phosphorus begins just prior to the start of pod formation and ends when the grains have been fully formed. The uptake of potassium is at its peak during the period of vegetative growth and then slows down at the start of grain formation. The need for the incorporation of these elements into the soil depends on their amounts already present there. According to Ferguson et al. (2006), soybean needs lower soil phosphorus levels than maize and wheat in order to produce maximum yields, which means that this crop, just as the rest of legumes, is well able to utilize the less readily available forms of the element present in the soil. Nevertheless, our research indicates that soil phosphorus and potassium contents that are too high may negatively affect the yields of soybean (Rajičić et al., 1993). High concentrations of readily available phosphorus and potassium in the soil do not harm plants directly. However, due to the possible antagonism between different elements, especially one between phosphorus and other elements, or because of the transformation of other biogenic elements into forms that are not available to plants, a discord may occur in the nutrition of cultivated plants (Bogdanović et al., 1993).

Plants also need micronutrients in order to develop properly. Micronutrient deficiencies are more pronounced in lighter soils, which are prone to leaching, as well as in alkaline and acidic soils. According to Johnson (1992), growers that obtained high yields by providing their crops with sufficient amounts of N, P and K were the first to pay closer attention to the deficiency of micronutrients. The same author also states that an accurate assessment of a micronutrient deficiency requires carefully controlled studies, because the difference between poor, good, and very good micronutrient supply is often minute. If it is determined that a crop is in need of certain micronutrients, they can be supplied by soil or foliar application.

The incorporation of nutrients that are needed by soybeans is mostly done by using mineral fertilizers. The use of organic fertilizers (farmyard manure, green manure, liquid manure, compost) is practically non-existent in soybean production. Soybean is very proficient at making use of the prolonged effects of farmyard manure application and will benefit from it not only when it immediately follows the crop for which the manure was incorporated but also when planted two or three years after the incorporation.

It used to be a common practice in Serbia not to apply mineral fertilizer to soybean, because if this plant was grown on a soil that had good structure and mechanical composition and was naturally fertile, and if the seeds had been properly inoculated, good yields could be expected. In the last couple of years, because of economic difficulties, there has been a drop in the use of fertilizers in all crop species and the soil has been depleted of nutrients as a result. For this reason, when growing soybeans, increased attention should be given to fertilizer application. On a soil well supplied with mineral nutrients, only such nutrient amounts as are removed by harvesting should be returned to the soil in order for its fertility to be maintained. To obtain a grain yield of 3 t/ha on medium fertile soil (10-20 mg P_2O_5 and K_2O per 100 g soil, determined using the Al method), it is recommended to apply 50-60 kg/ha P_2O_5 and 40-50 kg/ha K_2O. If the soil is less fertile naturally, fertilizer rates should be increased. Because soybean yields are less affected by fertilizer application than the yields of other field crops, variety specificity with regard to mineral nutrition has been little studied in this crop. Still, robust, later-maturing soybean varieties require more fertilizer because of their greater need for nutrients. In soybean, fertilization is most commonly done before subsoiling in the autumn using compound fertilizers, while spring preplant application can be used only for incorporating smaller amounts of nitrogen (up to 30 kg/ha). Since any mineral nitrogen incorporated later in the season has a negative effect on nitrogen fixation, no top dressings are applied to soybean crops in the course of the growing season.

It should be borne in mind that no top yields can be expected under conditions of nutrient deficiency. Therefore, decisions concerning fertilizer application in soybean must be made based on knowing the specific conditions in the field in each individual case.

TILLAGE

Tillage is an important factor for the success of soybean production. The tillage systems used differ not only from region to region but also from one field to another, and in some cases the same field will be tilled in a different way depending on the year. Growers should, therefore, make use of the tillage system best suited for the given set of conditions in a particular moment in time (Johnson, 1987). Soybean needs high quality primary tillage and good quality seedbed preparation. Deep tillage activates a larger amount of soil, which promotes the degradation of harvest residues that have been incorporated into the soil and enables the formation of larger moisture reserves, especially those that accumulate during the winter. The purpose of tillage is to provide good soil structure and a favorable water-air and temperature regime and to plow under the harvest residue and destroy weeds.

This promotes uniform emergence, deep rooting, and optimum soybean development throughout the growing season. Tillage should also allow the root system to penetrate into the deeper soil layers and enable better uptake of mineral substances and increased nodule formation and activity. In a compacted soil whose pores are too small, the growth of the root system is limited, which may affect the growth of the above-ground plant biomass and, by virtue of this, yield levels themselves. When growing soybean or any other crop, tillage plays a highly important role in weed control as well. The level of weediness can be reduced by primary tillage and seedbed preparation that are applied in a timely fashion and to a high standard of quality as well as by plowing under weed plants, seedlings, and seeds or by more efficient herbicide application.

Primary tillage

The method and timing of primary tillage in soybean depend largely on soil type and the preceding crop. Soybeans have a great need for good-quality primary tillage applied in a timely manner. Subsoiling must be performed in the autumn, and the best time to implement this agronomic practice in the case of early-maturing preceding crops is late September for heavier soils and no later than the end of October for all other soil types. When a later-maturing preceding crop is used, primary tillage should be performed immediately after harvesting. Applying primary tillage in the spring results in yield losses (Crnobarac, 2002). Spring tillage can be justified only under special circumstances, such as when the terrain is sloping or prone to flooding. Primary tillage in soybean should be at least 25-30 cm deep. Depths shallower than that are acceptable only in the case of light, loose soils. With heavier, compacted soils, however, the plowing depth should not be reduced below the said minimum under any circumstance (Šuput, 1986).

Primary tillage (plowing) is considered to be of good quality when the following requirements have been met: adherence to the recommended plowing depth, making tight tillage passes down the field and tight packing of the overturned furrow slices, good leveling and pulverization of the plowed soil, and burying weed seeds and weed and crop residues down to the full depth of plowing. The quality of plowing during primary tillage determines the quality of subsequent seedbed preparation, planting, inter-row cultivation, and harvesting.

The method of tillage in soybean depends on the preceding crop. If soybean is preceded by a spring or winter small grain (wheat or barley), right after such crop is harvested the remaining stubble is buried into the soil by plowing to a depth of 10-15 cm or the soil is disked using a heavy disc harrow. This results in the incorporation of crop residues into the soil, better moisture retention, and destruction of weeds. It is desirable that the emergence of weeds and volunteer plants be induced during the summer or early autumn, so that these deleterious plants can be destroyed by harrowing or cultivation before they set seed, whereby weed incidence in the next season is reduced. Full-depth plowing (25-30 cm) is performed in late summer/early autumn.

Late harvesting and large amounts of crop residues make tillage after maize somewhat difficult to perform. The first task after the maize harvest is to chop up crop residues, which must be done to a high standard of quality. If this is not done correctly, it will not be possible to properly perform primary tillage either. Crop residue must be chopped and buried in such a way as to enable the seeds to make contact with the soil as opposed to residues of plants. For this reason, full attention should be paid to this agronomic practice. Tillage depth should be up to 30 cm in order to ensure that corn stover is buried in the soil at a depth at which there are favorable conditions for its decomposition.

If sugar beet is the preceding crop, full-depth plowing is carried out right after the sugar beets are lifted, and the field is left unharrowed and exposed to the effects of frost. Plowing depth depends on the condition of the surface on the beet field, soil moisture, and the amount of crop residue. If the conditions for beet lifting have been favorable and the soil has not been compacted to a high degree, plowing can be carried out down to the depths of 20 to 25 cm. Sugar beets are often lifted under very unfavorable conditions, which leaves the soil in a highly compacted state. As a result, the grower cannot take advantage of the deep tillage performed for the previous crop (sugar beet), so the depth of primary tillage for soybean should be 30 cm in such cases.

Before the start of winter, once primary tillage has been carried out, a plow or a disc harrow should be used to smooth out deep dead furrows and tall back furrows in order to make seedbed preparation and planting easier and of better quality. This significantly reduces yield losses, which in soybean can be very high in case this operation is not implemented. A soil that has been plowed in a timely manner and to a high standard of quality should be left to overwinter unharrowed in order that it can freeze better and accumulate more winter precipitation. In Serbian crop production, some growers will harrow the soil lightly in the autumn and close the furrows. This practice is not justified in soybean, because it makes sense only when planting early spring crops that are in need of high-quality seedbed preparation, which is difficult to perform in early spring, because the soil is usually too wet at that time.

Seedbed preparation

Seedbed preparation depends on the soil type and timing and quality of primary tillage and is always performed only when soil moisture is optimal. The goal when preparing the seedbed is to obtain a 5-6 cm layer of fine, warm and wet soil that will facilitate good contact with the seeds and fast and uniform emergence. The rate at which the seed emerges depends on soil moisture and temperature as well as on the closeness of contact between the seed and the soil, because it is through this closeness that soil moisture and temperature are transferred to the seed. Seedbed preparation should also provide uniform planting depth and good seed coverage.

In soybean, as in all other spring crops, the primary goal of seedbed preparation is to level and loosen the soil. Crumbling the soil is not so important, because the soil gets pulverized by frost in the course of the winter. Settling the soil is not a priority either, because the soil settles on its own in the five to six months between the end of primary tillage and the start of spring. With seedbed preparation, the goal is to reduce unnecessary soil water evaporation by increasing soil temperature, because doing so reduces the contact area between the soil and the atmosphere and disturbs the capillary system already established in the soil, thus enabling a more uniform distribution of heat and water in the seedbed (Crnobarac et al., 2003). Loosening the soil introduces air into the surface layer. As air has poor conductivity and a low heat capacity, this allows the seedbed to warm up faster and enables earlier planting and a longer growing season (Figure 9.2).

Figure 9.2

Seedbed preparation *(photo: G. Kuzmanović)*

Seedbed preparation cannot be used to correct errors made during primary tillage, because even though it smoothes out the surface layer of the soil, the deeper layers remain uneven. This results in poorer planting and uneven emergence and makes rooting more difficult, which later affects plant growth and, hence, the yield as well. What is specific about seedbed preparation in soybean is the need to level the soil as well as possible in order to reduce yield losses.

Seedbed preparation is most often implemented in two parts. The first portion is best applied early in the spring, as soon as weather permits, i.e. as soon as the soil dries out, so as to prevent soil from sticking to the implements and to reduce the negative effects of soil compaction and trampling, which worsen soil physical properties, especially where the tire tracks are.Because of this, it is recommended to install double wheels on the tractors and to carry out this operation in as few passes as possible by aggregating several implements or by using combined implements. This operation should not be carried out late either, when the soil dries out too much, because this reduces the uniformity of wetness of the seedbed.

The second part of seedbed preparation is carried out a few days before planting, when the final portion of the preparation is implemented and when the seedlings and emerged weeds are destroyed most easily. This measure can be used to incorporate preplant rates of nitrogen fertilizers and to incorporate herbicides. If done properly, this operation produces finely grained soil structure, which results in more uniform crop emergence as well as better herbicide action. The surface of this layer should be finely lumpy instead of powdery so as to prevent the formation of crust. Below the seedbed, the soil should be loose enough in order to enable easier and deeper rooting and better root aeration. The depth of tilling should be adjusted to the depth of planting, because tillage that is too deep leads to unnecessary water losses and uneven emergence. The disc harrow is used rarely in the spring, except in the case of heavily weed-infested or compacted and heavy soils, because cutting through wet soil creates artificial lumps, which opens up the soil too much and hence increases the evaporation of water from the surface layer.

Preparing the soil for planting a stubble crop

Planting soybean as a stubble crop was not common in Serbia until a few years ago. A reason for this was a lack of appropriate soybean varieties for this kind of cropping, but this obstacle has been overcome in the meantime. Research findings and practical experiences support the increased interest in this form of soybean growing. After wheat or winter barley are harvested, there are enough days without frost left in the season that can be used by very early soybean varieties to reach maturity and produce a certain yield of grain.

The most important thing when preparing the soil for growing soybean as a stubble crop is that the preparation be carried out as soon as possible after harvesting the first crop of the season. Postponing the planting from early July until late July reduces the yield by 53 kg/ha (1.8%) in irrigated conditions and by 19 kg (1.2%) in dry farming with each day of postponement (Van Doren and Reicosky, 1987). The reason for the much greater impact of delayed planting in July than in April is that with each day the planting is delayed in July much more energy (effective temperatures) is lost than when the same happens in April.

As emergence is prolonged, the crop enters grain fill and maturity in late autumn, when the sum of effective temperatures per day is much smaller, which considerably prolongs ripening and hampers harvesting. Since the removal of straw from the field would delay harvesting considerably, it is best that it be chopped with shredders mounted on a combine. In that case, tillage can be performed right after harvesting.

The method of tilling the soil depends on the available machinery. In stubble cropping under irrigated conditions, Vučić (1987) found that minimum tillage - disk harrowing to 10 cm soil depth – very successfully replaces conventional plowing down to 20-25 cm depth. Minimum tillage has the advantage in that it takes less time to perform, and it is also associated with considerably smaller losses of soil moisture, the supply of which is problematic in any case.

In Serbian conditions, stubble cropping can be expected to produce satisfactory yields only in irrigated conditions (this will be discussed in a separate chapter), because in that part of the growing season there is usually not enough moisture in the surface layer for emergence and initial plant development. Plants sown at the start of the season survive thanks to a well developed root system that draws water from the deeper soil layers, whose water content is more stable and originates mostly from winter moisture reserves. The weediness of a stubble crop depends on the previous crop. Part of the weed population can be destroyed by tillage, while herbicides, just as in the case of regular planting, serve to destroy weeds that appear in the course of the season. In conditions where weeds cannot be completely destroyed, the success of growing soybean as a stubble crop is uncertain.

Reduced tillage

Over the last few decades, conventional tillage has been subject to criticism, primarily because it consumes a lot of time and energy. Scientists have begun to develop tillage systems that have been simplified in various ways. This has been done by reducing plowing depth, omitting certain operations, or dispensing with tillage altogether. The main reason for this has been the cost price of tilling, as conventional tillage is the most costly agricultural practice, with plowing accounting for over 50% of the costs. In Serbia, according to Starčević et al. (1995), reduced tillage means tilling the soil without the use of plows and is used primarily when growing winter cereals and, to a lesser extent, row crops. It can be said that reduced tillage reduces the depth of plowing and the number of individual operations (by merging several operations into a single operation or by dispensing with some operations altogether), i.e. certain operations are replaced with simpler and less expensive ones, which decreases production costs and makes work faster and easier to organize. Reasons for reduced tillage can be different: conserving the soil in areas prone to erosion; reducing soil compaction by reducing the number of passes made by heavy machinery; reduction of fuel consumption; or making the production more economical.

Besides having advantages, reduced tillage also has certain disadvantages. These include the necessity of having to purchase specially adapted machines that are relatively expensive, increased weediness, increased difficulty of incorporating mineral fertilizers and crop residues and hence a reduction of soil microbial activity and biochemical processes, increased difficulty of planting, and poorer emergence.

All the other agronomic practices, such as fertilizer application, planting, and crop tending and protection, must be taken into consideration and adjusted, because the reduction of costs must not have a negative effect on the profitability of production. Also, the long-term effects of reduced tillage on soil fertility should also be taken into consideration, in other words, one must monitor and understand the influence this approach has on physical, chemical and biological properties of the soil.

The effects of reduced tillage have been studied in soybean too. According to the findings of Van Doren and Reicosky (1987), reducing tillage depth to 12 cm or less results in yield losses. The losses are especially pronounced if soybeans are grown without tillage (8%), and prolonged growing in this manner reduces the yields by 17.5%. Still, reduced tillage is increasingly used worldwide for growing soybean, especially when this species is used as a stubble crop. In Serbian conditions, Konstatinović and Spasojević (1994) found, soybeans grown in different tillage treatments produced equal yields in the first year of the study, whereas in the second the no-till treatment had 43% lower yield than the other treatments. The authors of the paper concluded that research on this topic should continue. Molnar et al. (1996) studied the effects of tillage method on soybean grain yields and obtained significantly lower yields in the no-till treatment than in the tilled treatments. The reasons for this, according to the authors, lie in the increased weediness of the crop and the inability to attain the desired plant density. No statistically significant differences were observed among the treatments with plowing, chiseling and disking. In Serbian conditions, according to Vučić (1987), no-till farming can only be used to occasionally grow certain crops on good soils, and this approach to tillage should be incorporated into a broader tillage and crop rotation system.

Previous results on the use of reduced tillage are numerous and contradictory and depend on the conditions under which a particular study was carried out, so no general recommendation can be made in this regard. The effects of different kinds of tillage on yield are usually analyzed and compared after a time period of one to two years. In order to properly ascertain the efficacy of reduced tillage, its effects should be monitored in continuity across the entire crop rotation instead of looking at each individual crop separately. In other words, the effects different tillage methods have on yield, weeds, fertilizer use, and soil fertility should be monitored over the long term.

PLANTING

Time of planting

Decision on when to plant spring crops should be governed by the temperature of the seedbed rather than by the calendar. Seedling resistance to late spring frosts should also be taken into account when making the said decision. It is not recommended to plant soybeans before soil temperature stabilizes at 10-12°C and growing, especially if the seed is not of good quality. Planting soybeans too early at a low temperature slows down germination, and cold and wet soil promotes the occurrence of seed and seedling diseases, resulting in a thinner stand and slower initial seedling growth due to seedling exhaustion and reduced seed vigor.

According to Hoeft et al. (2000b), soybean seedlings are relatively resistant to low temperatures, and their frost resistance is greater than that of maize, although the growing point of maize is below the soil surface until the start of the six leaves stage. Temperatures that will completely destroy the above-ground portion of a maize plant are only capable of causing damage to the apical portion of a young soybean. A young soybean plant can withstand short-lasting frosts of -3 to -4°C (Gutschy, 1950), so the threat of winterkill is lower in soybean than in maize. For this reason as well as in order to make organizing the planting easier, it is recommended that soybeans be planted before maize in Serbian conditions. In Serbia, soybean planting is often limited by different factors, ranging from weather conditions to the available machinery. Most often, it is carried out during April, when the planting of maize is in progress as well (Hrustić 1996)

Each stage of soybean growth and development requires a certain amount of growing degree days. In soybean, the sum of temperatures above the baseline temperature of 10°C is constantly at around 100°C, meaning that soybean will emerge once this temperature sum is reached. With earlier planting at a sufficient moisture level, therefore, the period from planting to emergence and early plant growth will last longer. For this reason, planting a month earlier does not mean that harvesting will be a month early as well. In the same maturity group, on average, every two to three days of delayed planting prolong maturation by a day. The later planting mostly reduces the duration of the vegetative stage of growth and development (from emergence to flowering), while the impact on the period from flowering to maturity is considerably smaller. This is a result of the photoperiodic response of soybean, where a crop that has been sown later begins to flower earlier, because it comes under the influence of short-day conditions earlier. Because of this, planting date has less impact on the yields of earlier-maturing varieties, whereas with the later-maturing ones there is a tendency for yields to be lower with later planting. When planting a number of varieties, the rule is to first plant later-maturing ones. If the planting is well passed the optimum dates, the ripening of the soybeans may come into question. (Figure 9.3).

Figure 9.3

Soybean planting *(photo: G. Kuzmanović)*

When planting soybeans at the start of the season in Serbia, varieties with the longest growth period, those from Maturity Group II, should be sown in early April. Early-maturing varieties can be sown later on in April or even in May. However, to obtain yields that are as good and stable as possible, secure a more reliable water supply at emergence, avoid drought during the critical period, and ensure timely harvesting, it is recommended that these varieties too be sown earlier, in the course of April.

Soybean can also be planted beyond the regular planting time early in the season, either as a double crop or as a re-planted crop of soybean. When this is the case, earlier-maturing varieties should be used. Those from MG 0 will manage to mature if sown in May. If planting is done in June or later (early July), varieties from MG 00 or even 000 must be used.

Planting density and seeding rate

One of the main preconditions for obtaining high yields is to attain an optimal plant population per unit area. In each agroecological region, the optimal plant population must be adjusted depending on the variety, planting dates, rainfall amounts, tillage system, available machinery, mineral nutrition, and pest and weed protection.

Soybeans are highly tolerant of different planting densities. The optimal stand density is determined based on the characteristics of a mature plant, bearing in mind that the quick closing of the rows is the key factor of high crop productivity and that first pod height reduces yield losses while making sure that the threat of lodging and diseases is minimized and taking into account the price of the seed being sown. A study by Rigsby and Board (2003) has shown that soybeans are extremely good at compensating for a thin stand and that they do so through increased branching and by forming more pods per plant. According to Markley-Williams (1950) (as cited by Belić, 1964), the axil of each soybean leaf contains a bud that may develop into either a flower or a branch depending on environmental conditions. If the planting density is higher, most of the buds at the lower nodes will develop into flowers. At lower planting densities, on the other hand, most buds at the lower nodes develop into branches. Because of this, according to Roth et al. (2003), decision on re-planting soybean is a very delicate one. The grower must also bear in mind the cost of re-planting and yield losses caused by the later planting. The yield that has been formed will not decline significantly with a minor reduction of plant density, but yield losses at harvesting will increase significantly. According to Nafziger (2002), insufficient plant density will still limit yield levels due to lower leaf area index and maximum leaf area achieved at the start of pod formation, which has as a result lower sunlight utilization. Also, lower plant densities are associated with greater weed abundance and increased competition from weeds, and they also promote branching and the formation of pods closer to the ground level, which increases yield losses at harvesting. (Figure 9.4).

Figure 9.4

Correct planting density *(photo: G. Kuzmanović)*

Planting density has been the subject of studies both in Serbia and worldwide, but the results have differed greatly due to differences in growing conditions and the varieties used. Different genotypes are grown in different parts of the world, each of which has its early, medium, and late varieties which require different plant densities. In view of all the above, it is therefore not surprising that the optimal stand density per hectare may be 200,000 plants in one region and 600,000 or even 800,000 in another.

The number of plants per hectare and their distribution have been the subject of many studies in Serbia. The conclusion of most of the papers is that early varieties should be planted more densely and that late varieties produced the best yields at lower plant densities (Belić, 1964; Hrustić, 1983; Relić, 1996). The main components of yield are plant number and pod number, i.e. grain number per plant and 1000-grain weight. Pod number per plant decreases significantly with an increasing number of plants per unit area. A small number of plants with a large number of pods produce the same yield as a large number of plants with a small number of pods (Rajičić, 1991). Thus, yields obtained at smaller plant densities are comparable to those obtained in stands that are overly dense. Therefore, it is important to determine for each individual variety at which point an increasing plant number per unit area and a declining grain number per plant will produce the best output, i.e. the largest number of grains per m^2 (Rajičić and Jocković, 1990).

The attainment of high yields depends not only on plant density but on proper plant distribution as well. Several small gaps in a row will result in a loss of yield, but the loss will not be as big as when there is one big gap due to poorer yield compensation in the latter case The gaps cause greater damage because of the occurrence of weeds than because of the yield loss per se (Stivers and Swearingin, 1980). Unlike maize, however, soybean is well able to adapt to a lack of plants in the stand, because at a lower plant density it forms a larger leaf area and branches more (Robinson and Conley, 2008).

As a row crop, soybean is planted in wide rows, and the spacing between rows depends on the organization and technical circumstances and biological characteristics. A series of attempts have been made in commercial soybean production to employ different planting methods in order to obtain yields that are as good as possible. Growers tend to attribute good crop performance in a given year to the influence of a single factor, be it the variety or a particular agronomic practice they used, and are not willing to change their ingrained habits easily. However, numerous studies and many years of experience from commercial production have made it possible to develop a certain technology of growing that, more or less modified, can be recommended for most soybean-growing areas in the country.

First of all, it has been determined that 45-50 cm is the most suitable row-to-row spacing for Serbian conditions, both in terms of available machinery and from the point of view of inter-row cultivation and weed control (Tatić et al., 2002; Vignjević, 2006).

Plant-to-plant spacing is a way of regulating plant number per hectare and is dependent on planting date, variety, and seed quality. Thus, the recommended intra-row spacing for the early varieties (MG 0) sown on optimal dates is 4 cm, while the recommended spacings for the medium and late varieties are 4.5-5 cm and 5-5.5 cm, respectively. These spacings produce about 500,000 plants/ha with MG 0 varieties, 400,000-450,000 plants/ha for MG I ones, and 350,000-400,000 plants/ha for those from MG II. Since soybeans are sown with wide-row pneumatic planters, the planting density is determined via the spacing between the seeds being planted, i.e. via seed number per hectare. With narrow-row planters, therefore, it is incorrect to link planting density with seed quantity per hectare, i.e. the seeding rate, because seed size varies a lot depending on the year and variety (Roth, 2003). It is known that germinability varies according to year, which is why it is necessary to adjust the number of planted seeds taking into account the utility of the seed. In each individual case, therefore, it is necessary to calculate the Spacing Between the Planted Seeds In a Row (SBPSIR) using the following formula in order to obtain the recommended number of emerged plants for a given variety (Crnobarac et al., 2001).

$$\text{SBPSIR}\ (cm) = \frac{1000000 \quad x \quad US\ (\%)}{PNPH \quad x \quad IRS\ (cm)} \Rightarrow \frac{1000000 \quad x \quad 88.20}{450000 \quad x \quad 50} = 3.92\ cm$$

Where:

- *US* – Utility of the seed, which represents the percentage weight of the "seed" in a bag that is capable of germination and emergence, i.e. of developing into plants:

$$US\ (\%) = \frac{PURITY\ (\%)\ x\ GERMINABILITY\ (\%)}{100} \Rightarrow \frac{98\ x\ 90}{100} = 88.20\%$$

- *PNPH* – desired plant number per hectare, e.g. 450,000 plants in the variety Sava
- *IRS* – inter-row spacing, usually 50 cm

In ideal conditions, with such an adjustment and with the existing seed quality and the given inter-row spacing, we will practically plant 510,000 seeds in order to obtain the exact desired number of emerged plants per hectare of 450,000.

The most commonly made mistake in commercial production is to plant a considerably larger number of seeds than what is needed "just in case". If the planting is late or the seeds are planted on a poorly prepared plot, the number of seeds planted should be increased. In doing so, however, one must bear in mind the fact that seed number is a not a factor that will compensate for inadequate cultural practice and that increasing the seeding rate alone cannot be expected to result in a top yield.

When extremely early varieties are planted later in the season, because of their smaller habit of growth, it is recommended to use a smaller intra-row spacing and by virtue of this a larger number of plants. Such varieties should be planted with a spacing of 3-3.5 cm, which will produce 550,000-600,000 plants/ha.

Plant number per unit area under irrigated conditions is an issue in its own right. It must be borne in mind that adding water by irrigation creates optimal conditions for plant development and that irrigation is not a reason to increase plant number in soybean. Irrigation is intended to improve plant water supply in an optimal stand and does not necessitate increasing the number of plants. Irrigated conditions may lead to somewhat increased lodging and disease severity, so an inadequate number of plants may have the opposite of the intended effect. In France, the recommended plant populations for each individual maturity group are approximately the same as in Serbia. However, the recommended plant number for irrigated conditions in that country is 10% lower for the early varieties and 20% lower for the late ones (Cetiom, 1996).

The amount of seed used for planting depends on seed quality, most notably seed size. The exact quantity of seed needed for planting can be calculated based on the projected plant density, utility of the seed, and 1000-grain weight.

$$Seed\ quantity\ (kg/ha) = \frac{100\ x\ W1000\,(g)}{SBPSIR\ (cm)\ x\ IRS\,(cm)} \Rightarrow \frac{100\ x\ 140}{3.92\ x\ 50} = 71.41\ \text{kg/ha}$$

Because of a large variation in seed size, the required amount of seed per hectare may vary from 60 kg (500,000 seeds x 120 g) to 100 kg (500,000 seeds x 200 g) for the same stand density. Since a package of seed includes information on its basic characteristics (1000-seed weight, germinability), it is possible to estimate the precise seed quantity required for each specific situation.

According to our own data obtained for a seed crop at Rimski Šančevi, the amounts of seed needed for soybean planting were about 80 kg/ha in the early varieties and about 55 kg/ha in the later-maturing ones, which was due to the variable seed size. Therefore, the rule that 100 kg of seed are needed to plant one hectare of soybean is only provisional. It is only a rough estimate, while the exact quantity in a specific situation will depend on the projected stand, seed size, and seed quality.

Planting depth

Planting depth is important for securing reliable emergence and achieving the desired plant density. While emerging, soybeans elongate their hypocotyls and lift their cotyledons above the ground. After emergence, the arching hypocotyl bearing the cotyledons gradually becomes erect, after which the cotyledons separate. Once

the cotyledons assume a horizontal position, the plant is regarded as having emerged. If the seed has been planted too deep and the soil is also cold, emergence will take long and the seedling may become damaged. If the seed has been planted too shallow, the surface layer of the soil might dry out, which may slow down germination or cause the seeds that have already germinated to desiccate. For these reasons, planting depth must be given due attention. In Serbian conditions, the optimal planting depth for soybeans is 4-5 cm. On wet, heavy soil, i.e. early in the season, and with better seedbed preparation, the seeds should be planted at a shallower depth, and vice versa. If the soil is dry, however, planting depth should not be increased at all costs in order for the planting to reach the moist layer of the soil. It is more important that the seeds are planted at a uniform depth, so that after the rain they can emerge and go through initial growth as evenly as possible, because plant uniformity in a crop is one of the main prerequisites for obtaining high yields per unit area. Good quality planting requires good contact between the seed and the soil, i.e. slight soil compaction in the area around the seed. In wide-row planters, this is achieved by a well-adjusted furrow wheel. All these requirements are much harder to fulfill if the soybeans are sown with a narrow-row planter for cereals.

SEED INOCULATION

Soybean seeds, unlike those of most other field crops, are usually not treated with fungicides prior to planting; instead, they are inoculated with nitrogen-fixing bacteria. Being a legume, soybean has the ability to satisfy its needs for nitrogen by taking up this element from the soil and the atmosphere through symbiotic association with nodule bacteria. Intensive research on this symbiosis was started in the early 20th century from the point of view of the host plant as well as in terms of the study of bacteria taking part in the association (those from the genera *Rhisobium* and *Bradyrhizobium*). As the importance of soybean increased, so did the volume of research on the genetic basis on which this symbiotic association rested and studies were conducted to find out which strains of the bacteria formed the most effective associations with soybean plants. From the agronomic point of view, this meant determining how to utilize the ability of the soybean plant to use up as much atmospheric nitrogen as possible in the formation of biological yield.

Because plants are capable of utilizing different nitrogen sources, it is important to know the conditions under which the plant will opt for a particular source of this element. In the early stages of development, at germination and emergence, the plant uses nitrogen reserves from the cotyledons. These will last until the 20th day after emergence, at which point symptoms of nitrogen deficiency may appear on the soybean seedling in case there is a poor supply of soil nitrogen or nitrogen coming from N-fixation.

The first nodules appear about a week after emergence and are clearly visible, because they grow intensively. After 10-14 days they are capable of meeting most of the plant's nitrogen needs. The nodules will remain active for the next 6-7 weeks, after which their activity will subside. New nodules form later in the season as well, while the period of their death is during pod formation, when nitrogen fixation is at its peak. The number of nodules formed is inversely proportional to the amount of nitrogen present in the soil. According to Abendroth et al. (2006), N_2 fixation consumes more energy than the uptake of soil or fertilizer mineral nitrogen. Because of this, the plant prefers the latter forms of N, which leads to a reduced level of nitrogen fixation.

Since soils on which soybean has not been grown previously do not usually contain strains of soybean nodule bacteria (Sarić et al., 1988), these need to be introduced into the soil together with the seeds. Soils on which soybean has been grown in the past are characterized by a greater abundance and reduced activity of the natural Rhizobium population, so select strains of bacteria are introduced directly with the seeds in order to promote their growth. Unfavorable conditions for the bacteria that result in decreased nodulation include: a low soil pH; high or low temperature; insufficient soil moisture; unfavorable mechanical composition of the soil; and treatment of seeds with fungicides. When using products through which nodule bacteria are delivered to the grower. One must keep in mind that these are living organisms that may lose their vitality under unfavorable conditions. The biofertilizer Nitragin is mostly delivered together with the seed and care must be taken prior to its use to make sure that it is not exposed to temperatures that are too high or too low during storage. The optimum temperature for storing the product is 4°C.

Nitragin is supplied in quantities sufficient for 50 or 100 kg of seed and there is no use in applying quantities larger than that. Exceptionally, in the case of acid soils in which the activity of nodule bacteria is reduced, an increased amount of the product (twice the usual dose) may be used to promote the formation of nodules. Instructions on how to use Nitragin are printed on each bag and must be followed, because the product will be effective only when used as prescribed. Most importantly, one must use the recommended amount of water - up to 0.5 L for 50 kg of seed. When not enough water is added, the product will not mix fully with the seed. Adding too much water, on the other hand, may cause the seeds to imbibe and to be distributed unevenly during planting. It is important to handle the product in the shade, since the bacteria might lose vitality if exposed to direct sunlight. Inoculation must be carried out before planting. When a large amount of seed is treated but not used the same day, it is recommended to repeat inoculation the following day. It must be stressed that Nitragin remaining from a planting campaign cannot be used the following year.

CROP CARE DURING THE SEASON

Soybean production requires that plant growth and development be constantly monitored and that appropriate crop care measures be promptly taken in the course of the growing season. These measures include mechanical and chemical weed control, crop protection from pests and diseases, and irrigation.

Weed control is especially important when growing soybeans. Weeds cause yield losses by competing with soybeans for water and nutrients and by increasing the extent to which the crop is shaded. This results in a situation where genetic yield potentials of soybean varieties are not fully realized. Weeds also hamper soybean harvesting and reduce the quality of the grain, and there is also the threat of them being spread by seeds, all of which makes weed control a necessary agronomic practice in the production of soybean.

Inter-row cultivation is carried out primarily to suppress weeds, and it also serves the purposes of breaking up the soil crust and loosening the surface layer of the soil. The first cultivation can be performed as soon as the rows of soybeans become clearly distinguishable on the field, i.e. at the stage of the first true leaf. The last is performed just before the closing of the rows. There are usually two inter-row cultivations carried out, while the protective zone is cultivated manually. In cases where herbicides for suppressing Johnson grass from rhizomes are used, the grower should wait at least 10 days before performing inter-row cultivation. If the primary soil herbicides fail to achieve the desired effects, it is recommended that a second inter-row cultivation be applied by all means at the stage before the closing of the rows (Figure 9.5).

It is considered that the loose upper layer of the soil acts as a mulch and reduces water evaporation from the deeper soil layers and the appearance of cracks in the soil when there is a severe drought. This enables better retention of water in the soil at the critical stages of flowering and pod formation and also aerates the soil, which stimulates the activity of microorganisms that decompose the plowed soil organic matter and improve the functioning of nodule bacteria, which ultimately leads to increased yields (Crnobarac et al., 2002). When implementing this or, for that matter, any other agronomic practice, care must be taken that it is performed to a high standard of quality, because only then the positive effects of this operation on soybean yield will be fully manifested.

Good quality weed control is achieved by making sure that the weeds do not become overgrown, because that makes powder application more difficult and leads to the displacement of tine weeders and to soybeans being damaged and cut.

Also, the soil should not be too wet in order that it can remain of finely grained structure, because this prevents water evaporation from the deeper soil layers and increases the capability of absorption of growing season precipitation.

Figure 9.5

Inter-row cultivation *(photo: G. Kuzmanović)*

The depth of operation must be uniform (4-6 cm) in order to prevent root injury. After the application of powder, the soil must be as level as possible in order to reduce the evaporation surface area and the extent of deleterious evaporation directly from the soil (furrows up to 3 cm deep are tolerated). The lower layers of the soil must not be brought up to the surface. If the plants are still too small, care must be taken that they are not buried by soil due to excessive operating speed (about 6 km/h being the optimum).

During the first cultivation, the operating parts can be closer to the rows, whereas in the second, due to the development of the root system, which also reaches the surface soil layer, the recommendation is to go for a smaller operating depth and narrower passes in order to avoid root damage. Between the cultivator and a plant not cultivated by it there should be a buffer zone of about 15-20 cm, and the use of stabilizer levers preventing the free movement of the cultivator is mandatory. For an easier and safer tractor ride, crosshairs aiming towards the plant row should be mounted at the front of the tractor and cultivation should be in the same direction as when planting was being carried out. The grower must use a cultivator with the same or half the passing width as that of the planter used to sow the crop in order for the contact rows between two planter passes to coincide with half the outside cultivator sections. The working area of hoes of a cultivator section should overlap at least 3-4 cm, and the hoes should be sharp enough to make sure that any potentially surviving weeds are cut.

The primary way of combating weeds should involve correct crop rotation and crop hygiene as well as all tillage practices, while chemical weed control should be used only as a final additional measure. Herbicides can be used before planting, between planting and emergence, after the emergence of soybeans and weeds, or, in the case of perennial grassy weeds, later on in the season. There are varying opinions about the need for inter-row cultivation in crops in which weeds have been completely destroyed using chemical control measures. Still, most authors think that yields are positively affected by the destruction of the soil crust that often forms between the rows and by the better moisture retention in the early stages, which increases transpiration relative to evaporation. Therefore, at least one inter-row cultivation at an optimum time is recommended.

It should be noted that besides treating the whole field it is also possible to make use of reduced herbicide application in bands at the time of planting, in which case only the protective zone in the plant row that is about 25 cm wide is treated, with the weeds in between the rows being suppressed mechanically. This reduces herbicide use significantly, by about 50%, and is also more acceptable in environmental terms (Sinđić, 1994).

In the 1970s and 1980s, when soybeans were a rarity in Serbian fields, there were no major problems with pests and diseases. As soybeans began to be grown on an increasing acreage and more frequently, however, pest and disease problems began to appear. These will require greater attention in the upcoming period and will be discussed in detail in separate chapters.

Irrigating soybean is mandatory when the plant is grown as a stubble crop and desirable when it is the main crop, especially when grown for seeds. Details on soybean irrigation in stubble and regular cropping will be the subject of a separate chapter.

PLANT REGENERATION

Crop damage may occur in the field for various reasons during the season. In Serbian conditions, herbicides, game and hail are the most common causes of crop damage in the early stages of growth, with damage from late spring frosts also being possible. A newly emerged soybean plant can withstand short-lasting less severe frosts, but long-lasting more severe frosts will cause damage. If the damage affects only the upper parts of a young plant, the plant will recuperate quickly and continue to grow. Some herbicides may also cause damages slowing down the functioning of leaves developed up to that point, especially if not applied and dosed correctly.

Game and hail cause similar types of damage, as they remove a portion of an already formed planted. Game mostly damage young plants above the cotyledons.

As a result, two lateral branches most often appear at the first undamaged node. The damaged part of the crop may also prolong its season. The impact on yield will depend on the size of the damaged area and the overall crop condition.

Hail damage is potentially the most severe. If hail damage occurs in a very young crop and all plants are injured above the cotyledons, no regeneration of any kind is possible. In that case, re-planting is the only option. When re-planting, attention must be given to the herbicides applied previously and varieties that are somewhat earlier-maturing should be used. If hail affects a crop at the stage where there are several leaves present and the lower nodes remain unaffected, regeneration will occur in most cases. The regenerated crop will take somewhat longer to mature than a crop that has not been affected, but the effects of hail damage will be mitigated considerably. Hail may destroy a portion of the leaf biomass or remove part of the stem. In the early stages of growth, leaf damage by hail will have little effect on yield, even when of greater severity. If hail damage occurs later in the season, the effect on yield will not be proportional to the severity of the damage. When up to 50% of plants are damaged by hail before flowering, the yields will drop by 6-14% depending on the initial plant density (USDA, 2007).

The biggest problems occur when hail damages the crop at the reproductive stages of growth, when the plants already bear flowers and the pods have been formed. According to USDA (2007), hail damage affecting 50% of the plants after full flowering reduces the yield by 28-35%. In addition, damage in the plants will cause retrovegetation, i.e. the appearance of new branches and new flowers, which prolongs the growing season significantly.

The issue of re-planting soybean is a very delicate one, because it depends on a number of interconnected factors, such as the stage of plant growth and development, the calendar date, weather and soil conditions, and the relation between the cost price of re-planting and the price of soybean yield (Roth et al., 2003). First of all, one must consider when the maturation of the re-planted crop can be expected. When making the decision on re-planting, the following procedure should be followed: make an estimate of yield from the regular planting at full plant density; determine plant density and distribution in a thinned-out crop; estimate the potential yield of the thinned-out crop; estimate the potential yield of the re-planted crop in conditions under which the new crop would develop (moisture, weeds, etc.); estimate all additional costs of re-planting; and compare the financial gains of the thinned-out and re-planted crops. The decision on whether re-planting would be justified or not should be made only after all this has been taken into consideration.

If the decision is not to go with re-planting, an effort should be made to help the damaged plants regenerate as much as possible. This is done through inter-row cultivation, which breaks up the crust that usually forms under such conditions. At the same time, it also destroys the weeds that have appeared in the meantime.

HARVESTING

Soybean harvesting is carried out at technological or technical maturity, which, according to Roth (2003), usually occurs 7-14 days after physiological maturity (R7). In commercial crops, physiological maturity starts at the point after which no further increases in yield occur. Between physiological and technical maturity, the grain dries out naturally in the field. This drying is a passive process, because the plant is already dead. The rate of drying depends primarily on weather conditions, i.e. temperature and precipitation. According to Hoeftu et al. (2000c), the water content may drop daily by up to 6%, with 3-4% being the usual rate. During drying, as the connection between the plant and its seeds weakens, seed losses occur in the field. These losses increase at harvesting, especially if the harvest is delayed.

Therefore, the yield may become significantly reduced in the field due to harvest losses, which is why harvesting is an important measure in soybean production. Shay et al. (1993) report results of many experiments according to which soybean yield losses at harvesting were up to 12%. According to the same authors, when the right adjustments are made, the losses may be reduced to 5%, which is considered acceptable.

Herbek and Bitzer note that the average yield losses at harvesting are 10%, ranging from 20% to 1-2%. The acceptable level of these losses is 5% or less and can be achieved by the correct adjustment of the combine and other measures. At a yield level of 3 t/ha, a reduction of the loss from 15 to 5% will result in 300 kg more of payable soybean being harvested. The authors have made a rough estimate according to which 16 seeds being found on an area of ¼ m^2 (0.5*0.5 m) translates into a yield loss of about 100 kg/ha. According to a three-year study by Philbrook and Oplinger (1989), delayed harvesting makes carrying out this operation more difficult and increases the loss of yield by 11 kg/ha, or 0.2%, with each day of delay. The average losses were 10%, ranging from 5.5 to 12.7% depending on the year. With timely harvesting, the loss was 6.1%, whereas harvesting delayed by 42 days increased the loss of yield to 13.7%. The percentage loss decreases with increasing yield level. It should be noted that precision adjustment and combining comes at no extra cost, so reducing harvest losses results in a direct increase of profits (Figure 9.6).

Harvesting losses must be borne in mind as early as when the soil is being prepared and the choices of variety and stand density are being made.

Losses at harvesting can be reduced if the dead furrows are closed in the autumn and if the plot is well smoothed out during seedbed preparation, which makes it possible to perform the cutting evenly and close to the ground, leaving as few pods as possible below the cutting height. If several pods, each containing two or three grains, are left unharvested on most plants, it is clear that the harvest losses resulting from this action alone will exceed the amount of seed used for planting several times.

Figure 9.6

Soybean harvesting *(photo: S. Stevanov)*

The correct choice of variety can also help reduce losses at harvesting. Knowing the capacity of the available equipment and the dynamics of the autumn farming operations, such varieties must be chosen that will reach maturity at a time when it will be possible to perform a timely harvest. Planting varieties from different maturity groups extends the duration of harvesting, so that each variety can be harvested at an optimum moisture level, which reduces harvesting losses (Herbek and Bitzer). If a mature crop is left on the field for a long time, pod dehiscence may occur and part of the yield can be lost even before the harvest. According to Hoeft et al. (2000c), it is precisely because of pod dehiscence that the largest losses of yield occur in the field, especially if there are sudden alternations between wet and foggy weather and weather that is warm or characterized by low relative humidity.

Resistance to pod dehiscence is variety-specific and is more common in earlier varieties because of their faster maturation. In varieties that take longer to mature and that become ripe in late autumn, when harvesting conditions are more difficult, it may become necessary to employ desiccation or dry out the moist grains. This nullifies the potential of the late varieties to produce higher yields. Stand density can also affect the quality of harvesting. In a thin stand, there is branching and pod formation at the lower nodes, which most often remain unharvested. In a stand that is too dense, increased lodging may occur, which also hinders harvesting. If provided with sufficient growing space, a soybean plant will form pods at the lowest nodes, which means that the height of the lowest pod will depend on the amount of space in which the plant grew. Among other things, optimum growing space is that space which enables the formation of the lowest pod at a height that will not cause harvesting losses, provided, of course, that the first pod is not formed too high, which would reduce total pod number and result in yield losses. A study has been carried out with 23 Novi Sad varieties and lines of soybean in order to determine the effect of first pod height on total harvest losses in commercial production conditions (Miladinović et al., 1996). The harvesting losses were very small, as low as 3.75%, while losses due unharvested pods were no more than 0.69% of the total yield. Based on this, the authors have concluded that harvesting losses can be reduced to acceptable levels by agricultural practices and that the genotypes involved in the study develop the lowest pods at heights that do not result in major yield losses..

Harvesting should start when the seed water content is at 13-14%. The harvest can also begin earlier, but in that case additional drying is required. Later harvesting increases yield losses and reduces soybean seed quality. According to Hoeft et al. (2000c), harvesting losses and seed damage are minimal at a moisture of 12-15%. Harvesting at moisture levels of over 18% is not recommended because of increased losses in the thresher, seed indentation, and seed coat damage as well as because of the high additional costs of drying (Herbak and Bitzer, 1997). According to Hurburgh (1995), the optimum seed moisture for soybean harvesting is 13-15%. Soybean can be harvested after the grains mature and the leaves fall off. At 18% moisture or more, however, the threshing is more difficult and many grains become dented. If moisture is below 13%, field losses due to lodging or pod dehiscence will increase, as will the losses at harvesting, which may be 10% or more. With each percentage point of decrease of moisture below 11%, the weight of payable soybean decreases by 1.15%. Under favorable weather conditions, grain moisture drops to 13% three to five days after the leaves fall off. This is the optimum grain moisture level for soybean harvesting and storage (Hrustić, 1998).

However, it often happens that, because of stress conditions during the growing season (drought and high temperature), the plant progresses through some stages of growth and development faster and reaches maturity earlier. Thus, it may happen that the leaves will remain on the plant hindering harvesting, although the pods may be mature and the grain of appropriate moisture. It may also happen that an already mature crop cannot be harvested because of rain or excess soil moisture.

Soybean grain is sensitive to impact, since the embryo sits just beneath a thin layer of pericarp and can be easily damaged by mechanical means. This sensitivity is affected by grain moisture content, so a grain with 8-10% moisture is much more sensitive to impact than grains having a moisture content of 11-15%. Damage occurring in the grain may sometimes not be visible, especially with moisture of over 15%, but such damage can significantly reduce germinability nevertheless. Hoeft et al. (2000c) argue that seed soybean should be harvested before seed moisture drops below 12% because of pod dehiscence and pericarp damage. According to Hurburgh (1995), seeds with a moisture content of less than 10% becomes very brittle and splits in half easily during harvesting and handling, so processing such seed soybean reduce germinability too. The new combine harvesters with axial mass flow damage the seeds only half as much as the combines with the classical method of threshing (Herbak and Bitzer; Taylor, 1997).

The reduction of soybean harvesting losses is significantly affected by how well adapted and adjusted the combine is to the plant, i.e. by the specific variety and field conditions. Thus, according to Roth (2003), harvesting losses will be reduced by lower combine speed, appropriate clearance and number of drum revolutions, appropriate adjustment of the sieve and air current, harmonization between peripheral winch speed and combine speed, and cutting that is as low as possible.

The largest harvest losses occur at the header and can reach up to 80% of the total losses, which is why these have to be given due attention. Soybean is easily threshed out of the pod. Its seed size and shape and the fact that the leaves fall off on the plot before harvesting enable easy separation from impurities, but poor adjustment of the combine to the crop and field conditions may significantly increase the losses (Bennett et al., 1999). For this reason, attention should be paid to the following adjustments during combine operation.

Combine speed should not exceed 5 km/ha, because at speeds above that the hoe does not manage to cut the stem; instead, it partially pushes it before cutting, thus increasing harvest losses. Combine speed should be reduced if the cut made by the hoe is too high and uneven and if the losses occurring at the hoe are large. If the crop is weedy, the speed can be less than 3 km/in order to avoid overburdening the combine and enable better threshout and clean up of the seeds.

The floating flexible header with automatic cutting height control makes possible copying the terrain in the direction of and transversely from the direction in which the combine is moving, resulting in lower cutting height, i.e. the collection of even the lowest pods on the stem. Compared with the classical header, the losses with this implement are smaller by 25-30%. According to Hoeft et al. (2000c), the total losses occurring when a well adjusted combine with this kind of header is used do not exceed 4%, as compared to 8-10% for the classical header. The cutting height is usually 5-8 cm, while cutting heights of 10 cm or more will already significantly increase the losses (the lower pods that are unharvested or cut).

A hoe that is sharp enough and well adjusted and narrower hoe points will result in greater cutting width and hence better operation at greater combine speeds.

The peripheral speed of the winch can be higher than the speed of the combine by about 25%, while the winch axis should be about 15-30 cm in front of the hoe.

The drum speed should be reduced to a minimum in order to enable threshing with as little seed damage as possible. Decreasing seed moisture decreases the number of drum revolutions and increases drum and concave clearances as well as the wind (Herbak and Bitzer, 1997). Mature soybean grains absorb and release moisture easily, so grain moisture will vary by up to several percentage points in the course of the day, which is something that must be kept in mind when adjusting the combine. Because of this, Heatherly and Elmore (2004) state that the combine should be adjusted at least twice a day (in the morning/evening and at noon) in line with the fluctuating seed moisture content. According to data provided by Hoeft et al. (2000c), the seed moisture content dropped from 15.3% in the morning to 9.9% at noon, as relative humidity decreased from 82 to 42%.

Harvesting should be completed as quickly as possible, starting at 15% seed moisture. If the seed water content is below 13%, harvesting should be done in conditions in which the pods are more elastic (fog, light rain, high relative humidity).

SUMMARY

High soybean yields require the harmony of all production factors. In practical terms, this means the right choices in selecting the plot and the most suitable crop rotation, then timely apply tillage measures, fertilizer, and, if necessary, weed control, and finally, promptly and efficiently harvest the crop. In addition, one has to know the variety and its needs in terms of plant density, nutrient requirements, and the depth and timing of sowing, so that edaphic, climatic, and genetic factors can all be broughy into harmony with the help of cultural practices. Carefully selected, and through the years proven cultural practices should be used to attain high yields, making sure that the fertility of the soil is maintained as well. Neglecting basic requirements in terms of cultural practices or reducing certain measures will inevitably lead to yield losses.

The failure to do something at a certain point during the prroduction cannot be compensated for later in the process without the loss of yields. If the soil has been badly prepared, one cannot achieve a good stand even if more seeds are sown. The late removal of weeds may make harvesting easier, but the damage has already been done, since the weeds have already destroyed part of the crop. The ommission of NITRAGIN will reduce yields or increase the costs by requiring the application of nitrogen. In order to attain high yields, therefore, due care has to be paid to each and every step in the production process.

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SOYBEAN IRRIGATION IN SINGLE CROP, SECOND CROP AND STUBBLE CROP PLANTING

Đuro Bošnjak

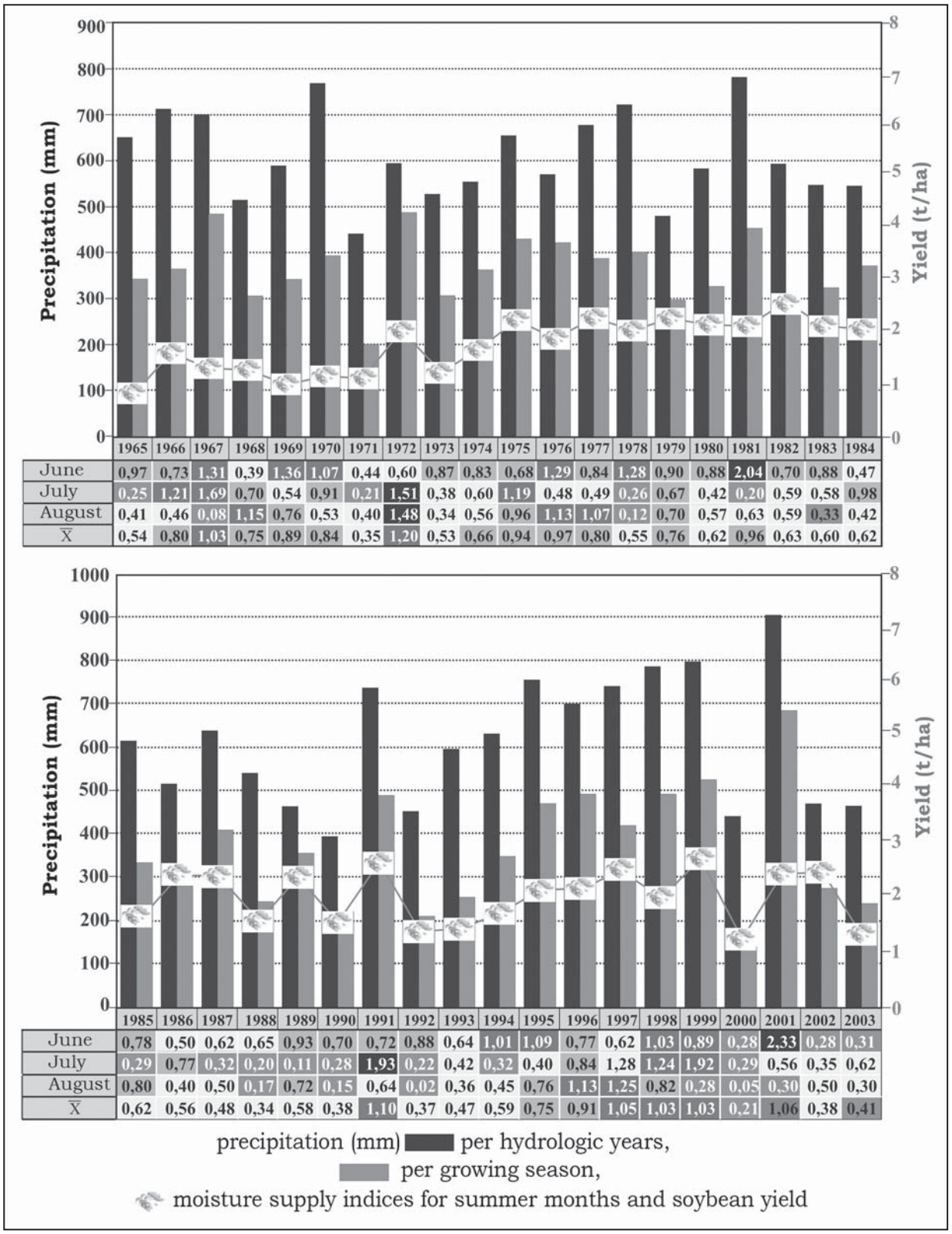
INTRODUCTION

Irrigation is a significant technological measure in soybean production under variable, unstable and unpredictable edaphic and climatic conditions of Serbia, mainly regarding precipitation amounts and distribution. Here drought occurs regularly or occasionally, appearing almost every year, and lasting for shorter or longer periods of time, often seriously decreasing yields in plant production, which is especially evident in soybean. Bošnjak (2004) analysed drought in Vojvodina Province (climatic nomogram in Figure 10.1.) and assessed its relation to the achieved average soybean yields in the last four decades, concluding that soybean yield trends are dependent on the amounts and distribution of precipitation. Yields were proportionally lower in years with higher-intensity longer-lasting droughts. In this period, average soybean yield was 1.86 t/ha (minimum 0.92 t/ha and maximum 2.75 t/ha) with a very high coefficient of variation (27.55). There have been dry years when soybean yield was no higher than a few hundred kilograms in some fields of certain regions of Vojvodina.

Nomograms show monthly moisture supply indices in June, July and August affecting successful plant production; these indices have been calculated according to Hergreaves procedure (1977 and 1992). Months are designated as arid if index values are below 0.33, semiarid between 0.34 and 0.66, semihumid between 0.66 and 0.99, humid between 0.99 and 1.33, and perhumid if index values are above 1.33. According to Hergreaves' recommendation, if one month in a growing season expresses index value below 0.33, that region would be designated as unfavourable for successful unirrigated plant production, meaning that Vojvodina is in fact a region with obligatory irrigation, not supplementary irrigation, as previously considered by our researchers.

Figure 10.1

Climate nomogram of Vojvodina Province: precipitation (mm) per hydrologic years, per growing season, moisture supply indices for summer months and soybean yield



	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984
June	0,97	0,73	1,31	0,39	1,36	1,07	0,44	0,60	0,87	0,83	0,68	1,29	0,84	1,28	0,90	0,88	2,04	0,70	0,88	0,47
July	0,25	1,21	1,69	0,70	0,54	0,91	0,21	1,51	0,38	0,60	1,19	0,48	0,49	0,26	0,67	0,42	0,20	0,59	0,58	0,98
August	0,41	0,46	0,08	1,15	0,76	0,53	0,40	1,48	0,34	0,56	0,96	1,13	1,07	0,12	0,70	0,57	0,63	0,59	0,33	0,42
$\bar{X}$	0,54	0,80	1,03	0,75	0,89	0,84	0,35	1,20	0,53	0,66	0,94	0,97	0,80	0,55	0,76	0,62	0,96	0,63	0,60	0,62

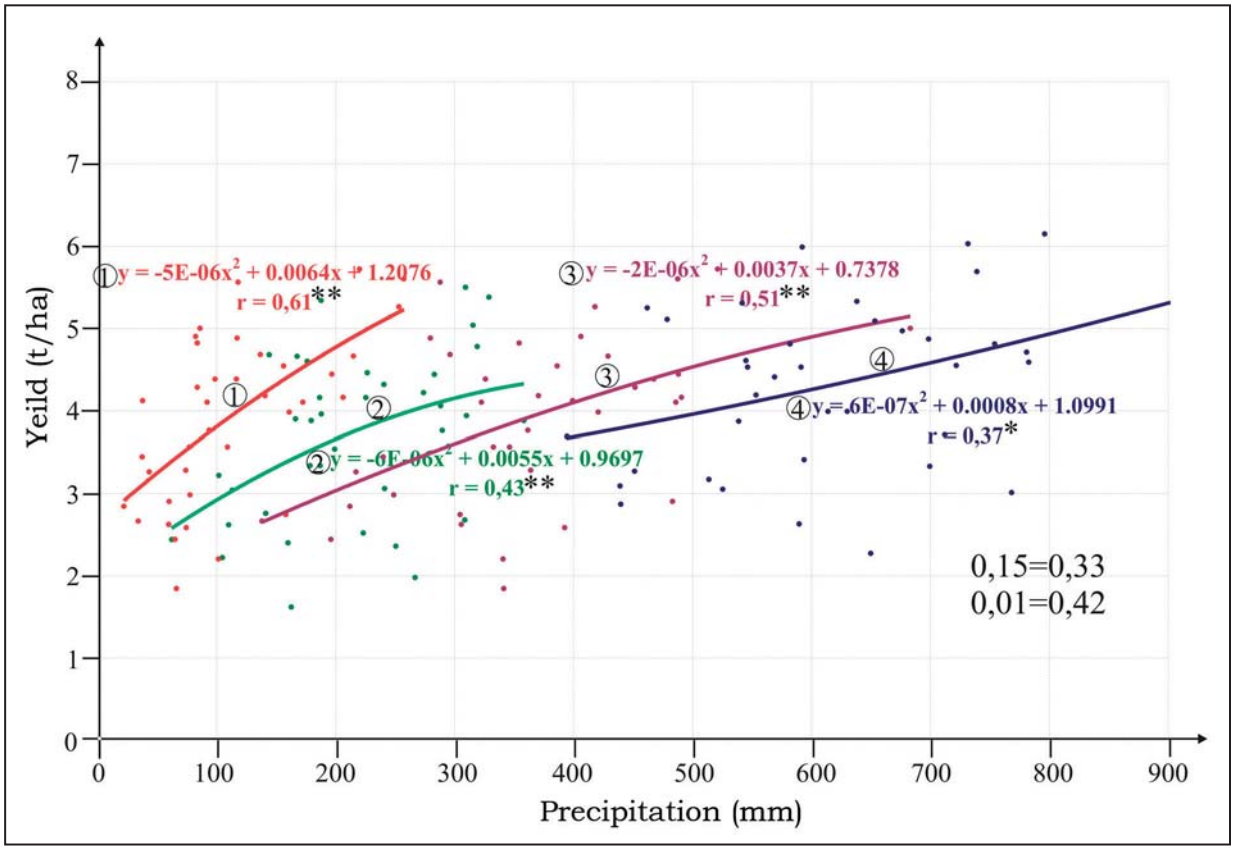
	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
June	0,78	0,50	0,62	0,65	0,93	0,70	0,72	0,88	0,64	1,01	1,09	0,77	0,62	1,03	0,89	0,28	2,33	0,28	0,31
July	0,29	0,77	0,32	0,20	0,11	0,28	1,93	0,22	0,42	0,32	0,40	0,84	1,28	1,24	1,92	0,29	0,56	0,35	0,62
August	0,80	0,40	0,50	0,17	0,72	0,15	0,64	0,02	0,36	0,45	0,76	1,13	1,25	0,82	0,28	0,05	0,30	0,50	0,30
$\bar{X}$	0,62	0,56	0,48	0,34	0,58	0,38	1,10	0,37	0,47	0,59	0,75	0,91	1,05	1,03	1,03	0,21	1,06	0,38	0,41

Period: 1965-1984 (above) and 1985-2010 (below)

Dependence of achieved soybean yields on precipitation was analysed using regression and correlation (Figure 10.2), determining a positively highly significant correlation between soybean yield and precipitation in two hottest months (July and August), three critical summer months (June, July and August) and with precipitation in growing season, while correlation between yield and precipitation was positively significant in hydrologic years.

Figure 10.2

Soybean yield depending on precipitation in: 1. July and August (red), 2. June, July and August (green), 3. growing season (violet) and 4. hydrologic years (blue)



Soybean yields were very low, far from genetic yield potential, and much lower than yields attainable under irrigation. In larger irrigated production, where irrigation eliminates natural water deficit during growing season, high and stable soybean yields are achieved at the level of 4 t/ha, some field plots even achieving above 5 t/ha. Nonetheless, there are far more producers who irrigate and still achieve soybean yields below potential, most often due to irrational irrigation regime.

Soybean irrigation is also significant because it necessitates planned production, especially since soybean is grown on limited areas which should provide stable and high yields. Each yield loss entails lower utilization of processing capacities or demands foreign currency for import of soybean or its products.

Moreover, irrigated soybean production should not be regarded only in the view of increased yields, quality and certainty, but being an annual grain legume, it should also be regarded in the view of the specificity it has in crop rotation on irrigated areas, where soil is utilized intensively, tillaged more often and is exposed to a higher degree of water impact, and consequently deterioration of physical and chemical properties. Being a legume, soybean amends such soil properties, enriching it with nitrogen, all of which favourably affect other crops in rotation. Irrigation is especially important when soybean is grown as a second and stubble crop, produced for grain or fresh matter.

Lately, irrigation has intensively been spreading, larger areas are being irrigated, and larger irrigation systems are being built, owing to new technical and technological solutions in equipment and tools used for irrigating. Soybean fields in Serbia are sprinkler irrigated, using sprinkler lateral on wheels (BK type), or most up-to-date centred or linear wheel-mounted devices with fully mechanized and even automatized irrigation, lifting intensive labour input from irrigation systems. Thus, producers of irrigated soybean can apply current technology and rational irrigation regime using modern and mighty technique, so as to create conditions for best expression of genetic potential in a certain agro-ecological region and achieve high yields at phytoclimatic level.

SOYBEAN WATER REQUIREMENTS

Water requirements are expressed by the term potential evapotranspiration (ETP) that represents water removed by a plant species for achieving highest yields of good quality. Potential evapotranspiration is the basis of all soil reclamation calculations, necessary element of water balance in regards to natural water supply; water deficiency (irrigation requirement) or water excess (drainage requirement) are calculated. In fact, ETP is the starting point of design, building and using hydromelioration systems. Water deficiency triggers irrigation hydro module, while water excess triggers drainage hydro module, which give dimensions to hydro-construction equipment in both cases. When applying water balance, modern principles regard ETP as the basis of irrigation regime, and it can also be used to analyse the success of both irrigated and unirrigated plant production as determined by water supply.

Older research references state that soybean is very much resistant to drought, while later research draws quite the opposite conclusions, stating that soybean is a plant which requires and uses much water, its resistance to drought being deceptive. Soybean performs well in drought until flowering, but if drought prolongs through later phases, it yields low. Soybean has formed under climatic conditions with rainy and warm summers, and has high values of transpiration coefficient.

Several authors (Enken as cited in Vučić, 1976) state that, depending on conditions, soybean transpiration coefficient ranges from 390 to 744, also citing much higher values ranging from 600 to 1000. Regardless of differences in above mentioned values, it is evident that soybean uses significant amounts of water to produce a dry matter unit. Our decade-long research at Experimental Fields Rimski Šančevi of Institute of Field and Vegetable Crops determined that soybean uses between 1100 and 2000 litres of water to produce one kilogram of grain (Bošnjak, 1987).

Soybean is especially sensitive to drought during flowering and grain forming. According to research at Cuban experimental station (Vučić, 1976) drought decreased grain yield by 48-58% in different soybean varieties during flowering phase, and by 41-87% during grain formation. Provided there is optimal soil moisture from the first phases until full flowering, soybean develops luscious mass and forms a large number of pods. If this is followed by drought until the end of growing season, plants shed pods in large numbers, smaller grains form and yields decrease significantly. If drought prolongs until the end of flowering, fewer flowers are fertilized, but if later water supply increases, much higher yields are achieved as compared to drought during grain-filling phase.

Sionit and Kramer (1977) also stated that water stress during growth and pod formation phase decreases soybean yield much severely than during flowering. Doss et al. (1974) determined that pod formation is the most critical period for soybean irrigation. Brown et al. (1985) tested the influence of soil moisture deficit in various reproductive phases of soybean development, and determined various drought stress effects which varied in R2-R3 and R4-R8 phases, showing R4 (ending pod formation and beginning grain filling) as the most critical phase in dry years.

Dragović (1993 and 1994) carried a detailed research on drought affecting soybean yield and quality in different reproductive phases of development (Rl-R8). In relation to optimal soil moisture, drought decreased soybean yield by 2% to 92% depending on the occurrence time and lasting period of drought. Drought decreased soybean yield by 2% in the flowering phase (Rl-R2) lasting for 21 days, by 12% in pod formation phase (R3-R4) lasting for 21 days, and by 21% throughout phases Rl-R4 lasting for 39 days. Drought decreased yield by 35% from grain filling phase through to full maturity (R5-R8) lasting for 52 days, and by 54% throughout phases R3-R8 (period of 73 days), while dry period decreased yield by 92% from flowering to maturity (Rl-R8) lasting for 88 days.

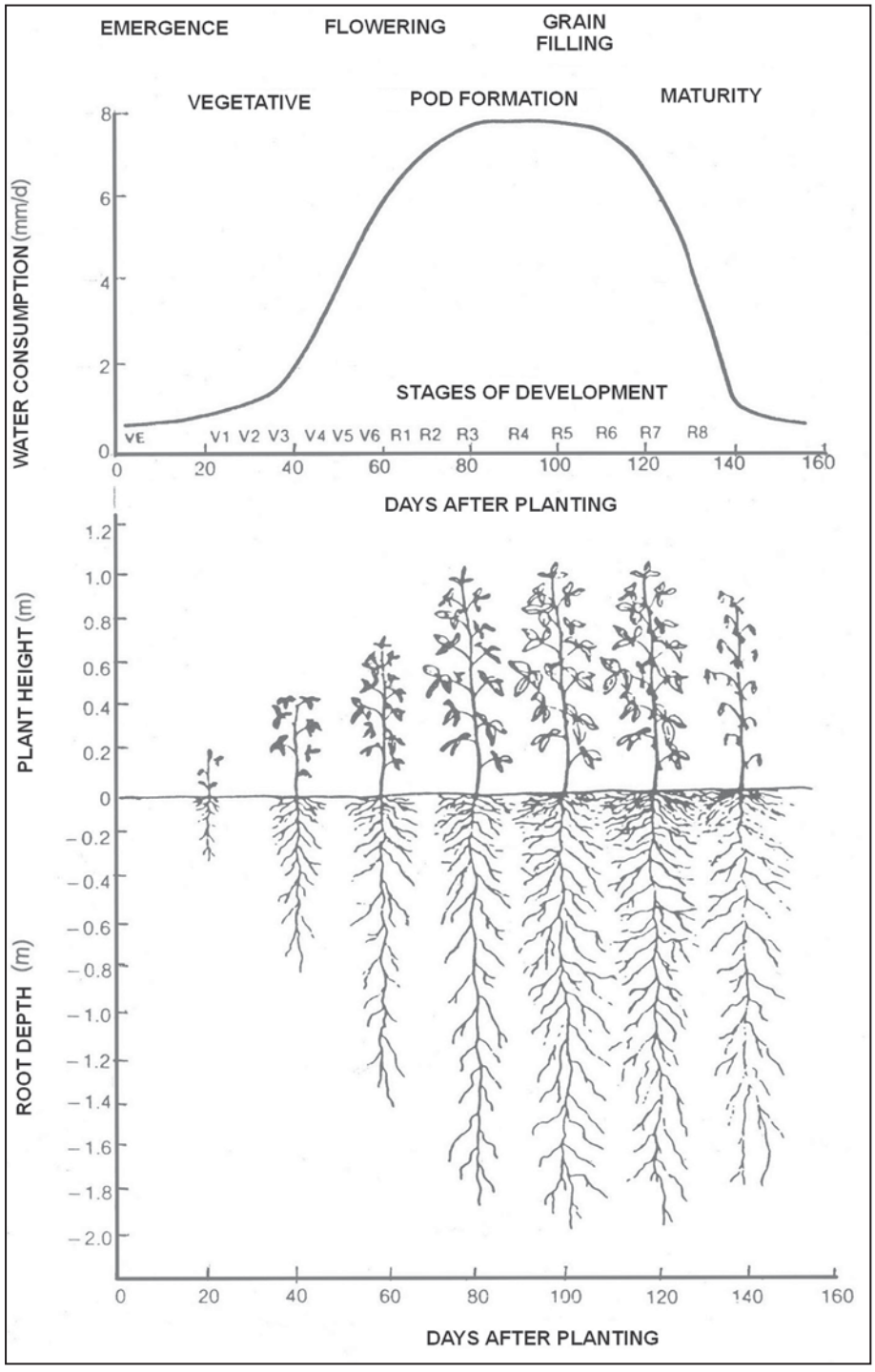
Soybean does not evenly remove water throughout growing season. Water consumption is affected by environmental energy evapotranspiration requirements, i.e. the change in meteorological conditions during growing season and phenophases of plant growth and development. Dynamics of water consumption for evapotranspiration during growing season is shown in figure 10.3.

There is also the illustration of soybean stages of development, vegetative (Vl-V6) and reproductive (Rl-R8) according to Fehr et al. (1971), as well as dynamics of plant upward growth and root downward growth during growing season.

Dynamics of quality water consumption in growing season is also shown, while quantitative values differ in certain agro-ecological regions depending on edaphic and climatic conditions.

Figure 10.3

Schematic overview of soybean evapotranspiration dynamics, stages of development, plant height and root depth during growing season (Reicosky and Heatherly, 1990)



Generally, soybean removes little water by evapotranspiration in the period of emergence and beginning phases of vegetative development, whereas water consumption increases in phases V3 to V6. Soybean removes most water from Rl to R6 phase, when plants are in full growth and have the largest habitus. Soybean evapotranspiration decreases from end of grain filling phase through to beginning of maturity. Daily water consumption depends on various conditions and can be very high. According to data by Hobss and Muendel (1983) and Brun et al. (1985) the highest daily water consumption is during end of July and beginning of August.

Published research articles cite various values of total soybean water requirements - potential evapotranspiration: Timons et al. (1976) for Minnesotta 432-462 mm, Singh and Whitson (1976) for Fort Valley State, USA 392-412 mm, Allison et al. (1958) for North Carolina 706.1-967.7 mm, Herpich (as cited in Henderson, 1967) for Kansas 500-600 mm and Nebraska 450-575 mm. Soybean water requirements in the European part of ex-USSR is 450-500 mm. Vučić (1976) cites several authors which have concluded in different parts of the world that good soybean crop requires 375-750 mm of water. Doorenbos and Pruitt (1977) concluded that total soybean water requirements are 450-825 mm. These data refer to soybean growing seasons which range from 100 to 190 days, depending on temperatures and latitude of the soybean production region.

SOYBEAN WATER REQUIREMENTS IN VOJVODINA

Based on research data, it can be said that soybean water requirements depend on complex influence of edaphic and climatic conditions in a given region. This was the reason for a very intensive research on determination of soybean water requirements in this agro-ecological region during last several decades.

Vučić and Bošnjak (1980) determined soybean potential evapotranspiration for the climatic conditions of Vojvodina: 460 mm for 0 maturity group varieties, represented in the trial by Wilkin and Traverse, 480 mm for I maturity group varieties, represented in the trial by Steel and Hark, and 500 mm for II maturity group varieties, represented in the trial by Corsoy and Wels. Bošnjak (1983) determined that potential evapotranspiration of soybean variety Hodgson from maturity group I is 440 to 450 mm. Monthly water requirements were additionally determined (Table 10.1).

Table 10.1

Soybean potential evapotranspiration (mm) (Bošnjak, 1987)

Year	Month						Total
	V	VI	VII	VIII	IX	X	
1979	25.0	88.4	103.7	104.9	63.6	5.0	390.6
1980	57.2	103.9	115.3	111.6	44.2	12.6	444.8
1981	39.1	117.7	119.8	110.3	55.9	12.3	451.2
Average	39.1	103.3	112.9	108.7	54.6	10.0	428.8

Soybean water requirements increase from planting onwards, peaking in summer months (June, July and August), and decreasing until the end of growing season, influenced by soybean growth, development, yield formation and maturity, as well as changes in meteorological conditions during growing season.

Soybean potential evapotranspiration in 1979 was lower than in other two years and should be specially regarded, since one watering was missed due to technical difficulties; thus it can be said that potential evapotranspiration was 440 to 450 mm.

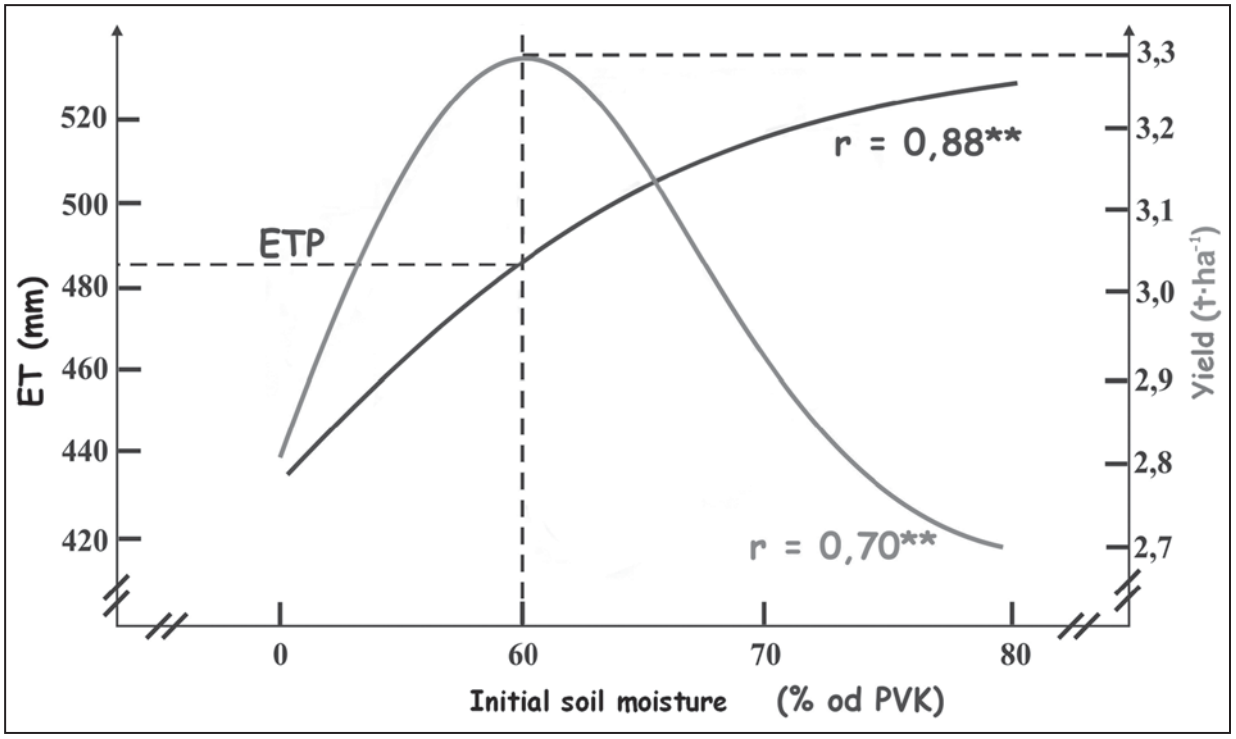
Monthly potential evapotranspiration differed in certain years. Most notable differences were at the beginning of growing season: while the crop was small, and soil surface unshadowed, making soil surface evaporation greater than plant transpiration. At the beginning of growing, potential evapotranspiration depends on meteorological conditions, amounts and distribution of precipitation. In May 1980 precipitation distribution was such that top soil was most often wetted (64.7 mm in 18 rainy days), from where water evaporated easily, which accounts for the highest evapotranspiration in that particular year. Quite the opposite happened in May 1979 when evapotranspiration was the lowest due to low precipitation amounts and few rainy days, while top soil was well turned up, preventing higher evaporation from deeper layers. In 1981 potential evapotranspiration was at the level of a three-year average. It can be concluded that plant water requirements depend on complex activity of several biotic and abiotic factors. In summer, monthly soybean potential evapotranspiration values in certain years differ from the average value much less (15% at most).

Soybean evapotranspiration and yield depend on the level of initial soil moisture (Figure 10.4). As initial soil moisture increased, water consumption for soybean evapotranspiration increased as well, giving a positively highly significant square correlation (Bošnjak, 1988). However, grain yield was the highest at initial soil moisture of 60% field capacity (FC), while yield decreased with further increase of initial moisture. It was even less at initial moisture of 80% FC than in unirrigated control. When moisture supply in soil is plentiful, soybean uses it irrationally, forming abundant vegetation mass, plants lodging and shadowing each other, and mostly becoming photosynthetically inactive. Thus, the created assimilates are used to sustain the luscious vegetation mass instead of forming grain yield.

In 18-year long research period (1976-1994) under irrigation, water removed by soybean for evapotranspiration was determined to be 390-550 mm as affected by irrigation mode, meteorological conditions and variety, i.e. its growing season. Previously mentioned values were the extremes, whereas most common water consumption by evapotranspiration ranged 440-500 mm, or more precisely 450-480 mm in most cases, representing soybean water requirements (potential evapotranspiration) for the climatic conditions of Vojvodina.

Figure 10.4

Soybean yield and evapotranspiration depending on initial soil moisture (Bošnjak, 1988)



Soybean monthly water requirements vary depending on the conditions: in April 10-40 mm, in May 30-60 mm, in June 90-110 mm, in July 100-125 mm, in August 100-120 mm, in September 50-80 and in October to 40 mm. Soybean mean daily water requirements are 1-4 mm, highest values of 3-4 mm being in summer months of June, July and August. The highest soybean daily water consumption by evapotranspiration in the hottest summer days in our conditions reaches 5.5 mm, as determined by balancing water consumption between two waterings.

IRRIGATION REGIME

The basic issue in irrigation practice is determination of irrigation schedule, or the application of a rational irrigation regime to a certain plant species, fully taking into account the climatic and edaphic conditions. Scarcely irrigated plants are insufficiently provided with water and yield less, while abundant and unnecessary irrigation water is used irrationally, more than is needed, causing the soil to overmoisten. This is unfavourable not only to soybean yield, but can also cause undesired consequences of irrigation such as waterlogging, salinisation, leaching of assimilates, etc. Equally harmful are both over-abundant waterings and scarce waterings. Thus, irrigation schedule should be determined with care, especially in changeable climatic conditions with large variations in precipitation quantity and distribution in certain years, such as in Vojvodina.

There are several scientifically developed ways of determining irrigation schedule, which are applied into practice. The choice between the manners used is conditioned by human and technical resources available, level of production intensity and natural conditions. Irrigation effect, rational water consumption, energy savings, irrigation cost-effectiveness and soil preservation depend on irrigation regime. Procedures have been developed for all cases, from those lacking adequate experts and equipment for monitoring certain elements of irrigation regime or water balance in soil or plants, to those provided with both at a necessary level. It is always better to apply the simplest irrigation regime based on certain principles, then to approach irrigation schedule without care or on the spur of the moment.

When applying irrigation regime, water supply of the irrigation system should carefully be analysed. If there is not enough water (e.g. accumulation of Telečka plateau etc.), irrigation regime must strive towards maximum plant production per unit of used water, and vice versa, where soil area is limited (small irrigation systems) and water is plentiful, irrigation regime demands maximum plant production per unit area.

Irrigation regime according to critical periods for water

Critical periods for water provide basis for application of irrigation system according to the phases of plant development. For soybean, critical periods start at beginning flowering and last long until ending of grain filling. In this period it is necessary to maintain optimum soil moisture to secure high yields. In our conditions, irrigation should commonly commence at full flowering, since there is enough water in soil at beginning flowering which was accumulated in pre-growing winter season and amended by precipitation, mostly sufficient until end June. Exceptionally, only in extremely dry years with scarce pre-growing moisture supply, irrigation should commence early at beginning flowering phase. This should be done with utmost care,

since increased soil moisture at the onset of growing season results in more luscious and higher plants which are later easily susceptible to lodging, with unfavourable effect on the yield. Often there are production mistakes, because luscious plants are forced by irrigating them early in growing season, while subsequent scarce irrigation does not give desirable irrigation effect.

Having that in mind, our conditions necessitate two to three soybean waterings according to critical periods for water during flowering and grain filling phases. When applying irrigation regime according to critical periods, watering schedules are firm, but should be applied with flexibility, adjusting them to meteorological conditions of the year, primarily precipitation amounts and distribution. It is necessary to analyse pre-growing season winter precipitation, and measure precipitation directly in field by rain gauge during growing season, simultaneously monitoring phases of soybean development and determining irrigation schedule. According to Vučić et al. (1980) there were no significant differences in soybean yield values between irrigating according to critical phases and irrigating according to soil moisture.

Irrigation regime according to soil moisture

It is most reliable to apply irrigation regime according to soil moisture, whereas it is necessary to know technical minimum moisture for soybean (lower limit of optimal soil moisture), monitor soil moisture dynamics during growing season, and have adequate equipment and trained staff. The workload is substantial and heavy, making this method undesirable and not willingly accepted by experts and practitioners, who naturally strive for simplest way of determining irrigation schedule. Due to its reliability, this method has so far been accepted by certain large producers, with an outlook of being more widely accepted, because it facilitates rational water consumption. Soil moisture is measured and once readily available water is removed by plants, i.e. water content reaches the level of technical minimum, irrigation commences.

There are different values of technical moisture minimum for soybean cited in published articles, which is natural since they refer to various edaphic and climatic conditions. For our conditions, Bošnjak (1978; 1983b) and Vučić et al. (1981) determined that technical moisture minimum for soybean is 60% FC. Some references state that the highest soybean yields are achieved by maintaining soil moisture above 80% FC, but this was not achieved in our conditions. Vučić et al. (1981) determined that soybean yield does not increase with initial soil moisture rising above 60% FC (Table 10.2).

Under conditions of southern Bačka region of Vojvodina, on calcareous chernozem soil of the loess terrace, soybean yield decreased with increased initial soil moisture. Opposite was the case in northern Banat region of Vojvodina on marshy black soil (humogley), where soybean yield remained at the same level, or it increased slightly and not significantly with increased initial soil moisture.

Table 10.2

The effect of various levels of initial soil moisture on soybean yield (Vučić et al., 1981)

Site	Variety	60% FC		70% FC		80% FC		Control	
		(t/ha)	%	(t/ha)	%	(t/ha)	%	(t/ha)	%
Rimski Šančevi	Wilkin	3,250	108	3,411	113	3,545	118	3,015	100
	Traverse	3,043	110	3,363	112	3,581	130	2,754	100
	Steele	2,762	101	2,832	104	3,000	110	2,723	100
	Hark	2,649	101	2,564	97	2,769	105	2,627	100
	Corsoy	2,774	92	3,060	101	3,220	107	3,012	100
	Wels	3,382	102	3,556	107	3,765	113	3,315	100
Mean		2,977	102	3,131	113	2,930	100	2,908	100
Kikinda	Wolkin	3,472	137	3,311	131	3,181	128	2,526	100
	Merit	3,257	134	3,202	132	3,329	137	2,431	100
	Traverse	3,283	125	3,245	124	3,198	122	2,621	100
	Steele	3,174	127	2,992	119	3,215	128	2,506	100
	Hark	3,403	112	3,209	106	3,172	105	3,029	100
	Corsoy	3,733	121	3,607	117	3,712	120	3,083	100
Mean		3,387	126	3,261	122	3,301	123	2,699	100

Water balance as the basis of irrigation regime

Lately there has been an option of applying water balance as the basis of irrigation regime, used for daily balancing the content of readily available water in the zone of active rhizosphere from the standpoint of intake and consumption, in order for irrigation dates and distribution to be determined. The method is applicable to variable climatic conditions, characteristic for Vojvodina and other regions of Serbia, since precipitation amounts and distribution vary significantly from year to year. Due to its simplicity, the method is acceptable to users in practice, while its reliability and accuracy have been verified, being at the same level as irrigation regime according to soil moisture (Vučić, 1976; Bošnjak and Dobrenov, 1986). In order to apply water balance as the basis of irrigation regime in irrigation practice, it is necessary to be familiar with its elements, primarily soybean water requirements, not general but daily water consumption at the level of potential evapotranspiration. It is also necessary to know soil capacity for readily available water in the zone of active rhizosphere, to measure precipitation directly on the field, and in case of extremely high precipitation to calculate percolated water. If ground water level is low, its influence on water supply to plants is taken into account.

There are many published methods used to calculate potential evapotranspiration, while most often used are those with simple calculations or formulas based

on meteorological and other elements. Formulas are of great practical value, but are of local importance. Vučić (1971 and 1973) established bioclimatic method for daily calculation of water consumption used for potential evapotranspiration by applying bioclimatic coefficients.

Vučić and Bošnjak (1980) determined that soybean bioclimatic index in regards to temperature, i.e. hydro-phyto-thermic index for our conditions is 0.16 to 0.17. Afterwards, employing detailed analysis, Bošnjak (1983a) determined very high dependence of soybean potential evapotranspiration to air temperature, relative air humidity, deficit in air saturation with water vapour, length of solar insolation and global radiation. These meteorological elements could be a reliable basis for calculating soybean potential evapotranspiration. Therefore, soybean hydro-phyto-meteorological indices have been established in relation to afore mentioned meteorological elements (Table 10.3). The indices show the amount of water (mm) soybean removes by potential evapotranspiration per each unit of mean daily value of a meteorological element.

Table 10.3

Soybean hydro-phyto-meteorological indices

Month	Air temperature	Saturation deficit	Relative air humidity	Solar insolation length	Global radiation
V	0.11	0.33	0.02	0.21	0.49
VI	0.17	0.50	0.05	0.42	1.78
VII	0.18	0.52	0.05	0.46	1.94
VIII	0.17	0.53	0.05	0.43	2.04
IX	0.11	0.39	0.02	0.29	1.53
Average for growing season	0.15	0.45	0.04	0.37	1.65
Average for VI, VII and VIII	0.17	0.52	0.05	0.44	1.93

Good results are achieved by applying one or more meteorological elements in the calculation of potential evapotranspiration. However, the drawback of this procedure is that the total of all environmental activities affecting water consumption by plants cannot be assessed through singular elements. Thus, it has been suggested to use water evaporation from atmometer as the basis for calculating potential evapotranspiration, since it indirectly encompasses the complex effect of all meteorological elements on water consumption by plants in the best possible way.

Even though there are no similarities between atmometers and soil covered in vegetation, a high correlation has been determined between evaporation from atmometers and potential evapotranspiration (Bošnjak, 1983a; 1984). Consequently, adjustment indices for soybean potential evapotranspiration have been established for our conditions in relation to evaporation from Class A, Wild and Pich atmometers located at meteorological station, as well as in relation to Class A atmometers located in the field adjacent to maize, in irrigated and unirrigated soybean crop (Table 10.4).

Table 10.4

Adjustment indices for soybean potential evapotranspiration in relation to evaporation from atmometers

Month	Class A Met. station	Wild Met. station	Pich Met. station	Class A adjacent to maize	Class A in irrigated soybean	Class A in unirrigated soybean
V	0.37	0.77	0.28	0.37	0.37	0.37
VI	0.63	1.55	0.59	0.65	0.65	0.65
VII	0.69	1.47	0.66	0.82	0.86	0.86
VIII	0.64	1.56	0.64	0.94	1.19	1.19
IX	0.50	1.26	0.44	0.67	0.81	0.81
Average for growing season	0.57	1.32	0.51	0.71	0.73	0.73
Average for VI, VII and VIII	0.65	1.53	0.63	0.80	0.90	0.90

By a simple procedure, daily soybean potential evapotranspiration can be calculated by applying established hydro-phyto-termic indices, or adjustment indices for potential evapotranspiration in regards to evaporation from atmometer. It is necessary to know mean daily value of one of the meteorological elements, or evaporation level from atmometer, which can be obtained from the nearest meteorological station or measured directly in field, and by using the formula potential evapotranspiration can be calculated.

$$\mathbf{ETP} = Me \cdot k \quad \text{or} \quad \mathbf{ETP} = Eo \cdot ki$$

ETP = daily potential evapotranspiration (mm)

Me = daily value of a meteorological element

k = hydro-phyto-meteorological index

Eo = daily evaporation from atmometer (mm)

ki = adjustment index for potential evapotranspiration in regards to evaporation from atmometer

There are several possibilities for calculating daily potential evapotranspiration. However, the basic principle is to use the simplest procedure which provides the necessary accuracy. For our conditions it is most acceptable to use hydro-phyto-meteorological indices in relation to temperature, i.e. hydro-phyto-termic indices, which show the amount of water (mm) soybean uses per each degree of mean daily temperature. This method has experimentally been verified in Serbia, and its accuracy is at the level of irrigation regime according to soil moisture. It has also been accepted in practice, not only due to its simplicity, but because mean daily temperature values are easily accessible, whether measured by thermograph directly in field, or supplied by the nearest meteorological station. Moreover, mean daily temperature values do not significantly vary between certain locations in Vojvodina.

Once daily potential evapotranspiration is determined, readily available water content in soil should daily be balanced from the standpoint of intake and consumption in order to determine irrigation schedule. When balancing is employed, readily available water content in soil is measured easily and noted in tables or graphs, and once its supply reaches minimum, irrigation commences (Table 10.5 and Figure 10.5).

When calculating water balance, precipitation is taken into account and added to readily available water supply. If precipitation is higher than the capacity of soil active rhizosphere zone for readily available water (60 cm for soybean), it is necessary to calculate water percolated into deeper layers. High and stable yields are achieved under optimal moisture in the active rhizosphere zone. When this water is used, plants start using less readily available forms and categories of water from active rhizosphere, as well as water from deeper soil layers, resulting in decreased yield.

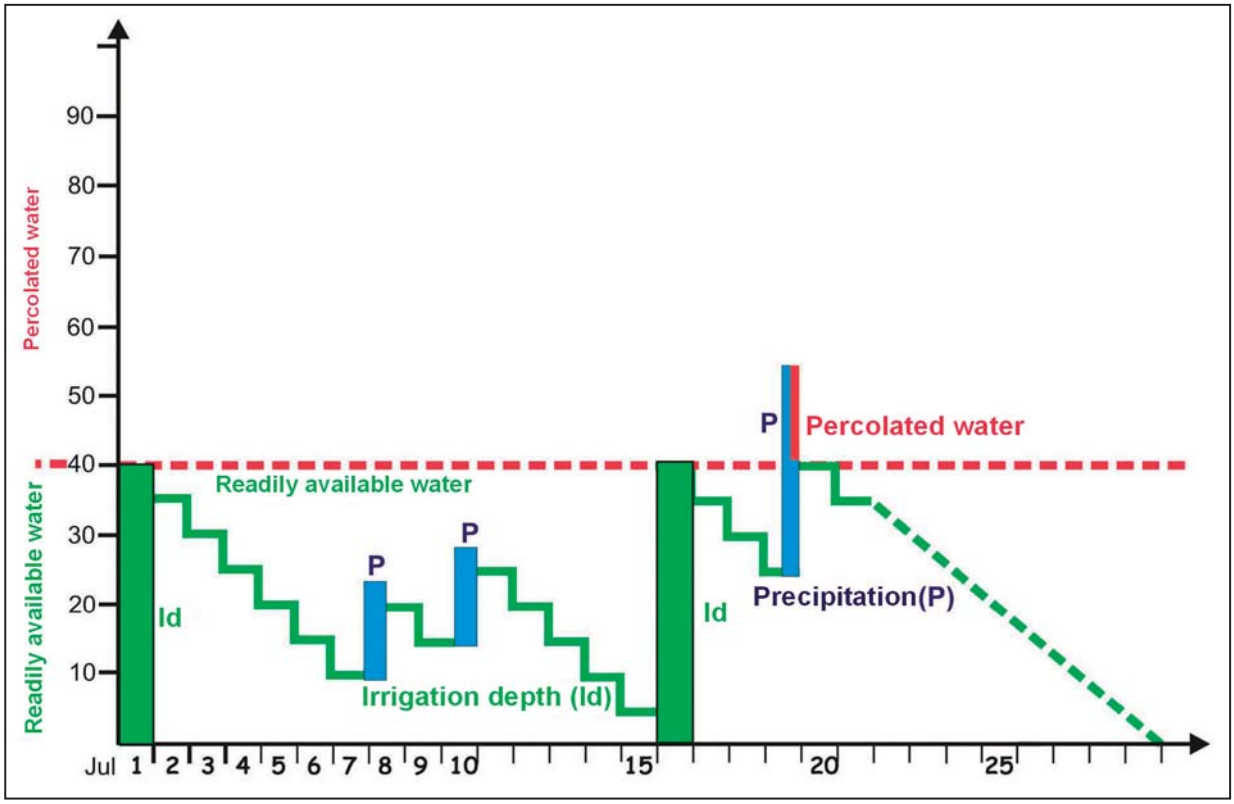
Table 10.5

Example of a table calculation of water balance as the basis of irrigation regime (Bošnjak, 1993)

Date	Temperature (°C)	Daily ETP (mm)	Precipitation, Irrigation (mm)	Readily available water (mm)	Percolated water (mm)
1.VII	23.5	4.2	40.0z	40.0	
2.	24.1	4.3		35.8	
3.	23.2	4.2		31.5	
4.	22.1	4.0		27.3	
5.	24.2	4.3		23.3	
6.	23.1	4.2		19.0	
7.	22.5	4.0		14.8	
8.	20.0	3.6	10.5p	10.8	
9.	21.8	3.9		17.2	
10.	19.5	3.5	12.0p	13.3	
11.	22.1	4.0		21.8	
12.	23.5	4.2		17.8	
13.	22.1	4.1		13.6	
14.	23.4	4.2		9.5	
15.	20.0	3.6		5.3	
16.	22.1	4.0	40.0z	1.7	
17.	23.4	4.2		37.7	
18.	19.9	3.6		33.5	
19.	18.1	3.3	24.0p	29.1	
20.	20.0	3.6		40.0	9.8
				36.4	

Figure 10.5

Graphic representation of water balance as the basis of irrigation regime



THE EFFECT OF IRRIGATION ON SOYBEAN YIELD AND QUALITY

In variable climatic conditions of Vojvodina, crop yields vary from one year to another, and directly depend on the amount and distribution of precipitation. Consequently, irrigation effects on yield increase can also vary significantly, which is the case with soybean (Table 10.6).

Long-term research data were used, and trial included two leading varieties from each of the maturity groups 0, I and II whose yields are shown as general average. The effect of irrigation on yield increase can be viewed in several sub-periods. For the period 1977-1984, irrigation increased soybean yield by 323-720 kg/ha (10.1-29.5%), which is in agreement with many authors cited by Vučić (1976) who state that irrigation in our conditions increases soybean yield by 30%.

Table 10.6

The effect of irrigation on soybean yield increase (Bošnjak. 1996a)

Year	Yield with irrigation (t/ha)	Yield without irrigation (t/ha)	The effect of irrigation	
			(t/ha)	%
1977	3.534	3.211	0.323	10.1
1978	3.527	2.919	0.608	20.8
1979	2.861	2.320	0.541	23.3
1980	3.088	2.704	0.384	14.2
1981	3.115	2.405	0.710	29.5
1982	3.482	3.037	0.445	14.6
1983	3.143	2.698	0.445	16.5
1984	3.863	3.143	0.720	22.9
1985	2.589	1.799	0.790	43.9
1986	3.369	2.742	0.627	22.9
1987	3.865	2.800	1.065	38.0
1988	3.723	2.705	1.018	37.6
1989	3.380	2.510	0.870	34.7
1990	4.164	0.951	3.213	337.8
1991	3.943	4.085	-0.142	-
1992	4.243	2.561	1.682	65.7
1993	4.644	2.820	1.824	64.7
1994	4.840	3.186	1.654	51.9
Average	3.632	2.700	0.932	34.5

Nonetheless, the following period witnessed a significantly greater effect of irrigation on soybean yield increase, since certain years lacked precipitation (much below long-term average). The year 1990 was extremely dry and was proclaimed a natural disaster. The control yielded very low, causing unprecedented fourfold increase in irrigated soybean yield. Quite the opposite was the case of 1991 with sufficient precipitation distributed favourably, supplying plants with readily available water, i.e. soil moisture was above the technical minimum during whole growing season, which made irrigation obsolete and yields very high and equal in both trial combinations. Later years saw very high irrigated soybean yields over 4 t/ha. Yield was increased by 51.9-65.7% in the period 1992-1994.

Irrigation facilitates the improvement of soybean grain quality. The issue of irrigation effect on any product quality, not only in soybean, is constantly tackled. From last watering before harvest, a certain period of time is needed for maturity processes to carry on normally. In variable climatic conditions, precipitation in maturity phase has negative effects on product quality, which is more profound under irrigation, since precipitation following the last watering more significantly deteriorates soybean grain quality. Irrigation also decreases grain oil content, and in our conditions oil content in irrigated soybean can be 1.5% lower than in unirrigated soybean.

However, protein content in irrigated soybean is 2.5% higher, and this is the main reason for soybean growing. Irrigation has a special effect on the increase of 1000 grain weight, which is an important element of soybean quality, i.e. important yield component. In extremely dry years, certain varieties can have an increase of g 80 and even 100 g of 1000 grain weight when irrigated. Seed quality parameters, germination energy and total germinability are of high and stable values in irrigated soybean, regardless of the conditions in a particular year (Bošnjak, 1987).

IRRIGATION METHODS AND ATTAINING A RATIONALIRRIGATION REGIME

Even though mankind has always been irrigating, the issue of attaining a rational irrigation regime in view of irrigation depth is still very much current, not only regarding soybean but other crops as well. This is quite expected, since irrigation modes, techniques and equipment have improved. Each improvement solved many existing problems, while simultaneously creating new challenges.

At the beginning, surface irrigation by flooding was applied, which seemed to wet the soil best. This was replaced by furrows and overflowing, which are still being used at over 80% of irrigated area worldwide. Due to impaired water distribution, irrigation depths were unusually high with low extraction coefficient 0.3 to 0.4 (Maslovarić and Nestorova, as cited in Vučić 1976). Many drawbacks accompanied such irrigation: nutrient leaching, waterlogging, soil salinisation and compaction, infiltration decline, etc.

The problems of excessive irrigation depth are profound in classical systems of surface irrigation, where water is conveyed by open supply, lateral and distribution canals (of I, II and III order). Modern solutions for surface irrigation systems do not include canals. Water is conveyed through underground pipes and distributed by portable irrigation pipeline or light metal pipes. Water is brought into furrows or borders through pipe holes which regulate irrigation depth, discharge and volume of irrigation stream. They are easily portable to another work post, thus overcoming drawbacks of furrow or border length. Most up-to-date surface irrigation systems are equipped with fully-automated irrigation.

Being a broadcast planted crop, soybean is irrigated by furrows made directly prior to setting up rows. Soil should be impeccably flat with a slight one-directional slope. In classic systems where water is conveyed and distributed by open canals, irrigation depth ranges from 80 to 100 mm, since it would be difficult to apply less. With improved technique, lower depths could be applied ranging from 50 to 80 mm.

Furrow irrigation has a set of drawbacks as compared to sprinkler irrigation, which is the only applied method of irrigation on irrigated areas of Vojvodina, but this method has certain drawbacks, too.

Sprinkler irrigation started being used in 1920s. The first devices were wheel-mounted due to heavy iron pipes with stiff couplings. Later developed light aluminium and zinc alloy pipes with quick couplings enabled sprinkler irrigation to widespread. At first, portable sprinkler laterals were used, followed by portable wheel-mounted sprinkler laterals and single far-reaching sprinklers. Most up-to-date equipment is being intensively widespread, considered not to become technically outdated for the following 20 to 30 years. This broad-reaching self-moving central or linear mounted equipment is substantially used in Serbia.

By using portable as well as self-moving BK sprinkler lateral and sprinkler lateral mounted on "Boom" frame, it is possible to determine and achieve irrigation depth with great precision. When active rhizosphere layer (40-60 cm) is moistened, the equipment is moved to another work post. Irrigation depth depends on water-physical features of soil and its capacity to store readily available water. Somewhat complex, but still simple, irrigation depth can be achieved by "Tifon" machine equipped with one far-reaching sprinkler (gun) lately being replaced by ramp (40-60 m) with sprinklers or spray nozzles.

The problem of irrigation regime in regards of irrigation depth is most profound in wide-reaching self-moving machines, which move on moist soil while irrigating. In most cases, these machines are unable to supply the amount of water needed to moisten active rhizosphere zone in one go. Depending on the conditions, 20-30 mm (40 mm at most) of deposit can be applied in one go, which means that such conditions necessitate altering irrigation regime. Therefore, from the standpoint of applying different irrigation depths, including shallow wetting of the soil, a challenge has been set to establish the lowest soil depth which is necessary to maintain optimal moisture by irrigation, under condition that high and stable yields are achieved.

The research was carried out at Experimental fields Rimski Šančevi of Institute of Field and Vegetable Crops, Novi Sad. The trial included three irrigation combinations with irrigation depths of 20, 40 and 60 mm, i.e. the soil layers wetted were 20, 40 and 60 cm. Constant presence of readily available water was maintained in the soil. Irrigation schedule was determined by applying water balance using hydro-phyto-termic index for soybean 0.16. There was a very significant increase in yield of the tested soybean varieties under irrigation, whereas there were no differences between certain irrigation combinations (Table 10.7).

Table 10.7

Soybean grain yield (t/ha) depending on variety and irrigation depth (Bošnjak, 1996b)

Year	Variety	Irrigation depth (mm)				AB	A
		20 mm	40mm	60mm	Control		
1991	Bačka	3.650	4.234	4.145	4.110	4.035	3.893
	Balkan	3.999	4.297	4.046	4.208	4.138	
	Vojvođanka	3.302	3.297	3.490	3.938	3.544	
AC		3.650	3.943	3.894	4.085		
1992	Bačka	3.462	3.414	3.489	2.198	3.141	3.749
	Balkan	4.187	4.352	4.477	2.756	3.943	
	Vojvođanka	4.336	4.964	4.757	2.594	4.163	
AC		3.995	4.243	4.241	2.516		
1993	Bačka	4.368	4.165	4.296	2.651	3.870	4.124
	Balkan	4.295	4.162	4.516	2.632	3.901	
	Vojvođanka	4.891	5.211	5.120	3.176	4.600	
AC		4.518	4.513	4.644	2.820		
1994	Bačka	5.159	4.669	4.761	3.408		4.541
	Balkan	5.232	4.775	4.557	2.956		
	Vojvođanka	5.532	5.046	5.202	3.193		
AC		5.308	4.830	4.840	3.186	B	
BC		4.159	4.121	4.173	3.092	3.886	
		4.428	4.396	4.399	3.138	4.091	
		4.515	4.630	4.642	3.225	4.269	
C		4.368	4.382	4.405	3.152		

LSD	%	A	B	C	AB	AC	BC	ABC
	5	0.208	0.191	0.132	0.381	0.264	0.228	0.457
	1	0.229	0.259	0.175	0.518	0.350	0.303	0.607

For soybean, it is sufficient to maintain optimal soil moisture in 20 cm layer, i.e. it is best to apply irrigation depth which wets ploughing layer of soil. However, in such cases an adequate irrigation regime should be applied, as shown by water consumption by evapotranspiration (Table 10.8). Irrigation depth in all combinations was at the same level in certain years. Nonetheless, irrigation regime differs significantly in terms of number of waterings, and it is inversely proportional to irrigation depth. In fact, irrigation regime must be adjusted to irrigation depth.

Table 10.8

Soybean evapotranspiration (mm) depending on irrigation depth (Bošnjak, 1996b)

Year	Irrigation depth (mm)	Pre-growing supply (mm)	Precipitation (mm)	Irrigation depth (mm)	Number of waterings	Total
1991	20	65.0	427.9	-	-	492.9
	40					
	60					
	Ø					
1992	20	111.1	181.7	160	8	458.8
	40	109.8	181.7	160	4	451.5
	60	121.4	181.7	180	3	483.1
	Ø	225.9	181.7	-		407.6
1993	20	79.0	222.6	200	10	501.6
	40	89.9	222.6	200	5	512.5
	60	66.9	222.6	240	4	529.5
	Ø	221.5	222.6	-		444.1
1994	20	86.9	297.5	140	7	524.4
	40	67.7	297.5	160	4	525.2
	60	49.0	297.5	180	3	526.5
	Ø	166.0	297.5	-		463.5

IRRIGATION OF SOYBEAN AS A SECOND AND STUBBLE CROP

Soybean is a crop with short growing season, and early maturing varieties give extraordinary growing opportunities as a second or stubble crop grown for grain or fresh matter. As a second crop, soybean can be grown after early previous crops are harvested in May and June, such as certain vegetable crops, pea most of all, in large irrigated systems. Also it should be pointed out that in the case of natural disasters (flooding, hail) which destroy spring-sown crops, early maturing soybean varieties planted in May or June could give grain in the same year.

After small grains have been harvested from circa one million ha in Serbia, there are still 70-100 days left until first autumn frosts appear, in some years even longer. Under irrigation, this period can be used to grow stubble crops, and soybean gives good results as such. Farmer's greatest wish is to achieve two grain crops in one year. Apart from favourable soil moisture provided with irrigation, early maturing soybean varieties need temperature sum of over 2050°C (Šiškov, as cited in Vučić 1976). Only bioclimatic temperatures above 10°C or 12°C should be taken into account, which are necessary for soybean to normally mature in autumn.

Biological temperatures above 5°C are not sufficient for maturing process, while temperatures below 0°C (frost) cease soybean growing.

Frost appearance and period of bioclimatic temperatures are irregular in our conditions. The first autumn frosts appear in the second ten-day period of October on average, but there have been years when frosts appeared even on early days of September, effectively ceasing soybean growing. In such cases, not even temperatures above bioclimatic in September and October can affect soybean maturity. However, certain years can have long and warm autumns with first frosts appearing in November, which is highly favourable for soybean maturity process.

If small grains are harvested at the beginning of July, soybeans can be planted by the end of the first ten-day period of July at the earliest. Small grains can be harvested earlier than that in some years, but here we have taken the average conditions so as to determine the sum of bioclimatic temperatures available for growing season of soybean grown as a stubble crop:

July	21 days	21,4°C = 449°C
August	31 days	21,0°C = 651°C
September	30 days	17,2°C = 516°C
October	15 days	12,3°C = 184°C
Total		1800°C

On average, our conditions provide the sum of bioclimatic temperatures of circa 1800°C for soybean grown as a stubble crop. There are certain deviations from the average value, depending on the appearance of the first autumn frosts, so that the sum of bioclimatic temperatures ranges from 1500-1600°C to 2100-2200°C in certain years. This means that depending on the conditions of a given year, soybean can have sufficient sum of temperatures needed for maturity, while in some years the deficit can be very high reaching even 500-600°C.

The first results on successful production of soybean grain planted as a stubble crop were published by Vučić (1981) based on 1979 trial carried out at Experimental fields Rimski Šančevi of Institute of Field and Vegetable Crops (Table 10.9).

Table 10.9

Soybean in stubble crop planting in 1979 (Vučić, 1981)

Variety	Maturity group	No. of plants (000)	Yield (t/ha)	Plant height (cm)	No. of pods	Grain moisture (%)	Oil (%)	Proteins (%)	1000 grain weight (g)
Wilkin	0	853	2,640	51	11	16,9	12,5	42,9	135,4
Šafarova	00	530	1,860	49	12	14,4	13,0	38,0	129,1
Altona	00	657	3,040	50	13	16,3	11,7	42,4	156,7

Soybean was planted on July 13, harvested on October 26, and grain quality elements showed possible maturity, while yields were good and at the level of unirrigated single crop grown soybean. Nonetheless, the following year of 1980 saw significantly lower yields and soybean did not mature since grain moisture was as high as 50% or more, even though it was harvested on November 7. It can be concluded that grain production of soybean grown as stubble crop is uncertain to be successful each year in our conditions. If soybean does not mature fully, it can be used as fresh matter for silage, and it is especially appropriate to be mixed with other forage crops. It improves silage quality due to its protein and oil content. Vučić (1981) concluded that attainable soybean fresh matter yields could over-reach 15 t/ha. Later, based on long-term results, Bošnjak and Dragović (1998) determined that in stubble crop grown soybean in our conditions, fresh matter yields could reach 14.8-29.0 t/ha with the average of 21-22 t/ha.

Soybean is especially interesting for planting in June, when there are high odds of successful grain production. Irrigated soybean is produced as second crop for grain after combine harvested peas and forage mixtures from smaller areas, which are successfully harvested in June, so that soybean is left with lower and higher temperature sum, meaning that varieties should carefully be chosen in order to achieve good results.

According to research data by Pantović (as cited in Vučić, 1981) at a 20 ha production plot of the company PIK Bečej, soybean variety Wilkin planted on June 10, 1975, combine harvested on September 30, yielded 2.264 t/ha with 11.5% grain moisture. Also, the same authors state the results of the second soybean planting performed on July 14 and harvested on October 12. There were eleven varieties and lines from maturity groups I, 0, 00 and 000 and yield ranged from 1.583 to 2.605 t/ha with grain moisture varying between 9.6% and 15.7%. Varieties with longer growing season yielded higher with increased grain moisture, which was at the level of full maturity. It can be concluded that I maturity group soybean varieties can be planted until mid June, and later-maturing varieties until June 25. Soybean varieties with shorter growing season can certainly produce grain, since our conditions provide the sum of bioclimatic temperatures needed for full maturity of soybean, even in most unfavourable years.

In case of some extremely adverse conditions, grain moisture in maturity processes can reach the level of 30-40%, when soybean desiccation can be applied in autumn, which accelerates grain moisture loss and forces grains to mature. Planting at a later date is risky, since it cannot be expected that soybean will surely mature each year. Nowadays there are soybean varieties of shorter growing season, among which highly prominent are those developed by Institute of Field and Vegetable Crops, Novi Sad, with the following growing seasons: 100-110 days (maturity group 0), 90-100 days (00), and 85-90 days (000).

SOYBEAN IRRIGATION REGIME IN SECOND AND STUBBLE CROP PLANTING

Soybean grown as second and stubble crop develops intensively in summer months, when temperatures are high and evapotranspiration requirements of the environment are also high. In order to equalize the environmental energy balance, soybean uses large amounts of water for evapotranspiration. Additionally, summer is regularly low in precipitation necessitating irrigation, especially since soil moisture supply is often at minimum, being used up by previous crop.

Soybean water requirements in second and stubble crop planting are significantly lower, approximately by a third less than in single crop soybean planting. However, irrigation depth is the same, even somewhat higher in certain years. Water requirements range widely from 250 to 350 mm depending on planting date and weather conditions in a particular year. When producing grain in second planting, potential evapotranspiration is 300-350 mm, and in stubble crop planting 250-300 mm.

With second and stubble planted crops, including soybean, irrigation regime is in modified cycles, taking into account precipitation in such a way that precipitation amounts affect a delay of irrigation for a certain number of days. This is applicable in our conditions, since evapotranspiration requirements are high in summer (due to high temperatures), which is a period regularly lacking precipitation. Besides this, the procedure is simple and provides necessary precision, which was experimentally confirmed (Vučić, 1981; Bošnjak, 1999) making it acceptable for experts and practitioners.

The first irrigation is applied before or after planting, depending on the conditions. If soil is dry, and especially if it is of heavy mechanical composition, surface irrigation is applied prior to planting and as needed, by using the furrows and borders of the previous crop. The advantages of irrigation prior to planting are more quality tillage, pre-planting preparation and planting itself. Delayed planting date is a disadvantage of a high irrigation depth and possible precipitation after irrigation.

Irrigation method used in Serbia is sprinkler irrigation, which is performed mostly after planting, since heavy machines can perform violent soil tillage in case

of drought. Irrigation depth is 30 mm, sufficient for swelling, germination and emergence of seed. Subsequent irrigation can be performed in modified cycles with irrigation depth 30-50 mm. Irrigation depths after planting are 30 mm and 50 mm after that, the length of a cycle being 6 to 10 days. Soybean in second and stubble crop planting should be supplied with 5 mm water daily. In case of precipitation, the irrigation is delayed or postponed. Precipitation amounts below 5 mm per day do not disturb the length of a cycle and irrigation is applied as if there had not been any rain. Higher amounts of precipitation delay the following irrigation depth for a day per every 5 mm of rain. If precipitation amount is equal or higher than irrigation depth, the following watering is postponed for one cycle from the day with precipitation, i.e. precipitation counts as irrigation. Extremely high amounts of precipitation are drained down, moistening deeper soil layers. High yields demand optimal moisture to be maintained in the zone of active rhizosphere, as water is removed form that zone by plants in one cycle.

SUMMARY

Soybean irrigation is important in Serbian edaphic and climatic conditions, which can be characterised as variable, unstable and unpredictable, especially when it comes to the amount and distribution of precipitation. Precipitation alone cannot provide sufficient amounts of water to secure high soybean yields. Soybean potential evapotranspiration totals 450-500 mm, with the following monthly values: 40 mm in April, 30-60 mm in May, 90-100 mm in June, 110-125 mm in July, 100-120 mm in August, and 50-80 mm in September.

When growing soybean as a single crop, it is most advisable to apply irrigation regime according to soil moisture, with the technical minimum being 60-65% FC. In practice, irrigation regime is most commonly based on critical periods, with the goal being to maintain soil moisture at optimal levels during flowering and grail filling phases. Lately, there has also been a possibility of using water balance as the basis of irrigation regime, in which case the readily available water content of the active rhizosphere is calculated daily from the standpoint of water inflow and consumption. Irrigation commences when water supply reaches minimum. Daily water consumption by potential evapotranspiration is calculated using a bioclimatic procedure involving hydro-phyto-meteorological indices, which have been established for soybean in Serbian conditions. Evaporation from an open water surface measured by atmometer can also be used, since adjustment indices for evapotranspiration in relation to atmometer have been developed. It is recommended to use mean daily temperatures and hydro-phyto-thermic indices, while the procedure is simple and applicable in practice providing the necessary accuracy.

Soybean yield increase resulting from irrigation may range from modest to very high depending on the year, i.e. primarily on the amount and distribution of precipitation. In fact, irrigation stabilizes yield at a high level and improves soybean grain quality.

When irrigated, soybean becomes very interesting for obtaining two harvests in a year. It is grown as a second or stubble crop either for grain or fresh matter. In relation to single crop soybean, second crop soybean has lower water requirements (by a third less), while irrigation depth is the same, or even higher in some cases. Potential evapotranspiration of second crop soybean is 300-350 mm and of stubble crop soybean 250-300 mm. If needed, the first watering is performed after planting, while subsequent waterings are carried out in modified cycles. Precipitation is taken into account, so that subsequent waterings are delayed by a day per each 5 mm of rain. If substantial rain falls, the subsequent watering is postponed by one cycle at the most, regarding precipitation as irrigation. In fact, soybean takes up readily available water from the active rhizosphere in one cycle, while irrigation is needed to sustain the optimal moisture necessary for high soybean yields.

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SOYBEAN SEED PRODUCTION

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Within the framework of modern agriculture, seed production has become a link between the increasingly successful plant breeding and the development of a more cost-efficient and voluminous plant production. Current and future achievements in the domain of plant breeding cannot be successfully transferred into practice without a thorough knowledge and simultaneous development of seed production (Mirić, 1998). Seed production may be considered as merged activities from the spheres of science, agricultural practices, and biotechnology, that ends by economic aspect, i.e. trade.

The main objectives of soybean seed production as defined by Milošević et al. (1998) are:

- Multiplication of seed of registered varieties. At the moment when a new soybean variety has been officially registered, small quantities of its seed are available and it is necessary to multiply the seed to meet the market demand.
- Maintenance of the morphological, biological and agronomic characteristics of soybean varieties, i.e., cultivar value, seed quality and the genetic yield potential. During the commercial exploitation, a part of cultivar value may be lost which may lead to a decrease of the genetic yield potential.
- Maintenance of soybean seed quality. As the seed is a living entity and the carrier and donor of a large number of genetically controlled traits, all activities during the seed production should be aimed at preserving that quality.
- Organization of soybean seed production. The main objective is to provide production of certified an quality seed in an efficient way, as well as to prevent possible failures during seed production.

ORGANIZATION OF SOYBEAN SEED PRODUCTION

Extensive, well organized and efficient seed production provides sufficient quantities of quality soybean seed. Quality is achieved through a long process that begins with the planting of seed crop, crop care, and close surveilance of seed crop by seed expertss, and ends with harvest, processing, quality testing and certification. Seed production and processing are subject to strict legal regulations. As plant breeding is subject to the regulations pertaining to varietal registration, testing and protection, seed production and marketing are regulated by strict lower and upper limits of quality standards, specified for a large number of seed characteristics. Furthermore, regulations have been established that must be strictly observed by seed producers, processors, inspectors and laboratories involved in seed testing.

In the process of seed production, scientific institutes, seed producers, seed processors and seed trade organization make an integral organizational entity. Production of pre-basic soybean seeds categories is organized at the Institute, under the supervision of breeders, aimed at the maintenance of quality and varietal characteristics of both, newly developed genotypes and varieties already in commercial production. Multiplication of commercial soybean seed is done at agricultural estates, under contract. Agricultural estates engaged in seed production as a specific activity, must meet official requirements that regulate seed production.

Control of seed production is performed by professional services (agricultural institutes, departments, agricultural stations). Control of seed quality is performed by accredited laboratories specialized for such tests. Seed marketing is organized through a distribution network, which covers all activities from seed processing facility to the end user.

Soybean seed categories

Variety is the basic factor of plant production, a prerequisite for the establishment and planning of the production, processing and trade (Miric, 1998). New varieties provide a continual supply of innovations into crop production, so that it can be said that cultivar variety is the main factor of growth and development of plant production.

After release of a new variety, limited amounts of its seed are available, and it is necessary to multiply additional quantities in order to meet the market demand. Seed multiplication is based on a system of a limited number of reproductions, and each reproduction must have a completely known origin. Soybean seed production includes the follwing seed categories:

- pre-basic seed
- basic seed
- certified seed 1st generation
- certified seed 2nd generation

Pre-basic seed is produced in breeding centers in which the variety was created and it is under direct control of the breeder, who applies special management practices and retains only typical plants. The amount of seed is limited, and it is not used for commercial purposes, but serves as basic material for seed reproduction. Soybean basic seed is also produced under supervision of the breeder. Itis also checked for atypical plants, which are removed, and it receives additional management practices specific for seed production. Basic seed is multiplied for the production of certified seed of the first generation. Production of certified seed of the first and second generations is done by agricultural estates that are entered in the register of seed producers. Production of these categories of soybean seed is done under the supervision of the breeder, breeding center and seed certification agency.

To ensure varietal purity and seed quality, a system of supervision of seed production has been designed and soybean seed standards have been established. Supervision includes data of variety multiplication, field inspection of soybean seed crop and control of seed during its preparation for the market.

Within the framework of seed production process, field inspections are made to determine the variety, seed category and origin, cultivation practices applied, the presence of weeds, varietal purity and quantities of harvested and processed seed. The first inspection is done at the flowering stage, to check varietal purity and weed incidence. The second inspection is done at the time to plant maturation, to confirm varietal purity and estimate yield level. A report is made for each inspection and if the crop meets all standards prescribed for seed crop, the produced soybean seed is issued an official certificate.

Soybean seed acreage and quantities per category

The planning of seed production acreage is important for meeting the market demand. Therefore a detailed assessment is needed of all relevant factors, to ensure the necessary quantities of soybean seed per category to supply the needs of the market. Important parameters for the assessment are the interest of farmers in soybean production and economic parameters that make soybean growing attractive to farmers. In addition, soybean seed acreage depends on the estimate of climate conditions during the season, which may greatly affect seed crop performance. Considering all this, we obtain information necessary for determining the acreage for soybean seed production, which should provide the necessary quantities of seed per category.

As stated above, the acreage under commercial soybean dictates the size of the acreage allocated for soybean seed production. Since in recent years the soybean acreage in Serbia has been steadily above 100,000 hectares, the soybean seed acreage and production volume have been proportioned to meet such market demand (Table 11.1).

Table 11.1

Soybean seed acreage and quantities per category

Seed category / Production year	Pre-basic seed / Super elite		Basic seed/ Elite		Certified seed 1st generation/ Original		Certified seed 2nd generation/ 1st reproduction	
	ha	t	ha	t	ha	t	ha	t
1999	12	42	22	81	364	1138	6661	15426
2000	17	26	25	69	567	1500	7743	10763
2001	21	86	42	138	525	1828	8294	15650
2002	20	56	41	108	471	1118	4800	10031
2003	20	50	44	83	365	1090	5000	9470
2004	20	60	45	100	450	1000	6500	13000
2005	20	69	61	241	553	1890	8430	17290
2006	19	59	55	266	630	2110	7736	20044

SPECIFIC CULTIVATION PRACTICES APPLIED IN SOYBEAN SEED PRODUCTION

Using of cultivation practices specific to variety and conditions, makes seed production significantly different from commercial production. Each segment of seed production should be adjusted to seed crop, either by developing specific practices or by using adequate machines. Substantially more expensive and delicate, seed production implies the choice of best plots, better trained and more conscientious workers and technicians, in order to obtain cost-effective production (Mirić, 1998).

Differences in growing conditions can result in limited soybean seed production in some areas. However, high yields and quality of soybean seed indicate that soybean could be successfully grown in many regions of our country with proper regionalization of varieties and use of appropriate agricultural practices..

Selection of plot and crop rotation

In soybean seed production, special attention should be paid to the choice of plot. Deep, fertile soils, with a neutral reaction and favorable water-air properties are most convenient since the degree of soil fertility, depth and structure of the plowed layers have a major impact on yield. Also, it is preferable to choose a plot with irrigation facilities because a long drought during seed filling period may cause significant decrease of yield and quality of soybean seed (Balešević-Tubić et al., 2001). According to legal regulations, an 1 m spatial isolation should be provided for a plot intended for soybean seed production. As the soybean is a self-pollinating plant species with the

percentage of open pollination of about 0.4%, this distance is sufficient to ensure the varietal and genetic purity of seed.

Soybean fits many crop rotations and it is as a good previous crop for most of the field crops. The most frequent previous crops are wheat, corn and sugarbeet. Sunflower and rapeseed are risky previous crops for soybean because these three crops share common diseases. According to the current regulations, soybean seed production must not be organized in plots planted to sunflower, rapeseed or soybean in the previous two years.

Primary tillage and seedbed preparation

For successful production of soybean seed, the primary tillage has to be performed on time, i.e., in the fall. Spring tillage has a negative impact on the seed yield of soybean. Yield reductions are especially high in unfavorable years, although even in favorable years spring tillage tended to reduce soybean seed yield by some 1200 kg/ha compared with fall tillage (Tatić et al., 2006). To preserve the accumulated soil moisture, furrows should be closed and the field leveled in early spring, as soon as weather and soil conditions permit these operations to be performed.

Final seedbed preparation is performed immediately before planting, to the depth of 4-5 cm and driving the machine in planting direction. The purpose of seedbed preparation is to provide conditions for quality planting, uniform emergence and rooting of plants, which is later reflected on plant growth and yield performance. This operation can be combined with herbicide application and weed eradication (Figure 11.1).

Fertilization

When fertilizing a soybean seed crop, it is necessary to take into account soil nutrients status, desired yield level and economic effects. Based on agrochemical analysis of soil and plant requirements, it is decided whether there is or there is no need to apply mineral fertilizers. In this way, a risk of excessive fertilization is avoided while the cost of this practice is effectively controlled. It used to be a common practice in our country not to treat soybean with mineral fertilizers, however, a generally reduced application of fertilizers for all crops has resulted in a notable soil fertility reduction. Therefore, if we wish to achieve high yields and stop further soil impoverishment, fertilization of soybean should be seriously considered. For soils that are well-provided with nutrients, the amounts to be added should corespond to the amounts removed with crop yield, which are typically about 30 kg N, 50-60 kg P and 40-50 kg K. For soils deficient in certain nutrients, the doses of the deficient nutrients should be doubled.

Figure 11.1

Seedbed preparation *(photo: G. Kuzmanović)*

Seed inoculation

Soybean has the ability to utilize different sources of nitrogen. If there are large quantities of accessible nitrogen in the soil, the plant will opt for this source, which has a negative impact on nodulation. The presence of soil nitrogen at the beginning of soybean growth is important even after successful inoculation. However, if during growing season the soybean uses soil nitrogen instead of atmospheric nitrogen, it will not lead to increased yields.

When using Nitragin, one should bear in mind that nodular bacteria are living organisms which lose vitality under unfavorable conditions. Preparation of the biofertilizer should be done in the shade, and seed inoculation immediately before planting. The biofertilizer is packed in bags sufficient for treatment of 50 or 100 kg of seed and is supplied with seed. Bacteria lose their vitality after nine months.

Planting

Time of planting depends on soil temperature and variety. In our conditions, soybean is usually planted in April. Planting may begin when the soil layer at the depth of planting reaches the temperature of 10-12 °C. Late cultivars, from maturity group II, should be planted first, followed by cultivars from maturity groups I and 0. The row spacing of 45-50 cm has been found to be most suitable for the local condi-

tions, considering the available machinery, between-row cultivation requirements, and weed control. Plants spacing in the row depends on planting date and variety, and it can vary from 3 to 5 cm. It is important to follow the recommendation for plant density. It is the most common mistake in soybean production to increase the seed rate, just in case. If planting is performed late or in a poorly prepared plot, one should be aware that an increased seed rate will not compensate for inappropriate cultivation practices. Increased seed rate is not a guarantee for high yield.

Management practices

In order to produce healthy and quality seed it is necessary to apply management practices that reduce or compensate for the negative impact of environmental factors (Hrustić et al., 2004). Management practices may be aimed at regulating and stimulating crop growth and development or crop protection from weeds, plant diseases and pests (Milošević et al., 1998). Between-row cultivation is done to regulate water and air regime of soil, break the crust to reduce the loss of soil moisture, to aerate the soil and to stimulate soil microbial activity. On the other hand, weeds are controled. Cultivation, at the depth of 3-10 cm, is performed twice: first time at the stage of first trifoliate leaf, the second at the stage of row closing. (Figure 11.2).

Figure 11.2

Row closing stage *(photo: G. Mulić)*

In our conditions, chemical measures are applied only to control weeds. In soybean seed production special attention should be paid to the control of weeds which must not be fould in seed plots, i.e., *Xantium* spp. and *Solanum nigrum*. Herbicides may be applied before planting, after planting but before emergence or after emergence of soybean and weeds. In the case of soybean, in our country, fungicides are not applied to control diseases. Seed is not treated before planting nor the soybean crop during growing season. The continual increase in soybean acreage makes the problem of disease increasingly important with us, and it is necessary to keep in mind that proper and timely cultural practices considerably decrease the risk of disease occurrence.

Irrigation

Under changing climatic conditions, such as here, where dry and rainy years alternate, soybean seed production is very risky without the use of irrigation. In dry, and even in rainy years, the distribution of rainfall is very important for unhindered supply of water to plants according to their requirements and needs at a given stage of development. Deficit of rainfall at the stage of grain formation and filling has the greatest negative impact which is manifested by a significant reduction of seed yield and deterioration of soybean seed quality. In our agroecological conditions, the soybean is the most suitably irrigated from mid-July to the end of August, when it passes through critical stages of development. Soybean producers under irrigation conditions remain to provide the necessary elements by modern cultivation practices and rational watering regime, in order to make best use of the genetic potential of the cultivar and climatic conditions of the agroecological region (Bošnjak and Dragović, 1998).

Weeding

In soybean seed production, weeding is a very important measure to maintain varietal purity. Two weedings are mandatory, performed at stages when differences between varieties are most prominent. First weeding is done at full flower, when atypical plants are removed on the basis of flower color and other morphological characteristics. Second weeding is done at full maturity, when it is possible to discern the best all atypical plants (height, hair color, time of maturation), which provides a high level of varietal purity.

Harvest

Quality and timely harvest is a very important moment for the success of soybean seed production and therefore it must be given greater attention in relation to the harvest of commercial crop. Soybean harvest is performed with the wheat combine

adjusted for soybean crop. Particular attention should be paid to the number of drum revolutions and the distance between the drum and the concave because the soybean is very sensitive to shock and can be easily damaged by mechanical action. Moisture content in grain is very important, so that soybean seed should be harvested when moisture content in seed is 12 to 15%. The seed with lower moisture is significantly more sensitive to shocks, and the seed with higher moisture should be dried (Hrustić et al., 2004). In soybean seed production, after harvest of each variety, combines and transport vehicles should be completely cleaned, in order to maintain varietal purity.

SOYBEAN SEED PROCESSING AND STORAGE

Seed processing

Seed processing is one of the three segments of soybean seed production (seed production in the field, processing and marketing). The task of seed processing is to eliminate excess moisture and unwanted dockage, while processed seed is treated, packaged, i.e., prepared for marketing. Seed is processed after approbation, i.e., after professionally controlled harvested seed.

Seed vitality duration in the course of storage depends on the reduction in seed moisture content to a level that is sufficiently low to prevent physiological and pathological damage. High temperatures during seed drying may damage the seed by excessive drying or by an interaction of seed moisture content and accelerated physiological damage. Success in seed drying depends on achieving a balance between costs, damage during drying and destruction of seed stored with high moisture content.

The process of soybean seed processing starts by unloading harvested seed into the receiving bin which is required to have a seed buffer. From the receiving bin, the seed is transported to fans where fine cleaning of seed is performed. After fine cleaning, the seed goes to the spiral separator, where whole seeds are separated. Gravitational separation can also be done, by seed weight. Also, depending on health condition of the seed, it can be treated with fungicides.

The processing is finished by measuring certain quantities of seed and packaging them in bags. A label is sewn on each bag containing the following information: name of the company that has performed seed processing, number of seed lot, category, year of production, name of variety, weight of the package, a note declaring whether the seed was treated and which chemical was used, and the date of label validity expiration (Figure 11.3).

Figure 11.3

Labeled soybean seed *(photo: G. Kuzmanović)*

Seed storage

Storing seeds means saving the environmental capacity of seeds. Solving this problem must begin in the field during seed production and continue after harvest. Seeds can not exclude or compensate for damage, but it accumulates over time.

Research by electrophoretic variation of proteins and enzymes of fresh and aged soybean seeds shows a significant decline in vigor of soybean seeds stored in regular storage conditions. Qualitative changes of proteins and enzymes have been noticed in old soybean seeds. The existence of a large number of different bands in dry aged seeds shows that there exists activation controlled by the genes for initiation of viability, as well as the expression of a variety of other phenotypic characteristics during the aging process. Researches have indicated that aging does not produce only a phenotypic and genotypic variability, but also leads to chromosomal aberrations, different types of chlorophyll and phenotypic mutations in the population (Chauhan et al., 1985).

Fatty acids, measured in seeds, were taken for many years as an index of seed quality. Reduction of the level of semi-unsaturated fatty acids is clearly linked with the reduction of the percentage of germination and vigor of stored soybean seed (Hailstones and Smith, 1988). Spontaneous oxidation of unsaturated fatty acids causes a high reactivity of free radicals, hydroperoxide and secondary products. The same authors state that the soybean seed exposed to natural aging reduced its linolenic

acid by 4.3% and linoleic acid by 6.8%, of the total lipid fraction. In the fraction of polar lipid these differences were small: linolenic acid was reduced only by 1.3%, while linoleic acid showed no visible change.

The main external factors that affect seed damage during storage are temperature, relative humidity, as well as oxygen. Possibility of regulation of these factors is a basis for longer storage of soybean seed. Seed with low vitality perishes first. Seed loses germination energy, then vigor, and eventually becomes completely lifeless.

The internal factors which affect the capacity for seed preservation are moisture content and initial seed quality. The storing seed with moisture content above 12%, at higher relative air humidity, the degree of seed damage is faster. Also, in such conditions, storage fungi become a problem for seed storage. Soybean seed with 12.5% moisture content, was infected with storage fungi (Anderson and Baker, 1983). The moisture content at which an attack of storage fungi is minimal in soybean is around 9-10%.

Information on viability of seed lots is very useful from the aspect of decision on seed storage. Seed lots having the viability of 70% have a lower ability to preserve quality during storage in relation to the lots with the viability of 95%. Usually there is no alternative regarding the preservation of lots with different viability, however, attention should be paid to the length of storage of seed lots with worse viability (Deoluche and Baskin, 1973).

The process of seed aging involves damage to numerous systems within the tissue. As the time passes, the tissue becomes more permeable which indicates that there has occurred a damage of cells' membranes, and there also occur various negative changes in metabolism. The seeds with such changes have a significantly lower vigor. It has also been determined that a large number of characteristics have been disturbed to the ultimate consequence - death of seeds: a reduction of the metabolic activity, increased sensitivity to stress conditions, reduced viability and seedling growth, reduced storage capacity, reduction of yield. The capacity of preserving seed lots depends on the actions applied before storage: weather conditions during vegetation season, time and method of performing the harvest, drying, processing, etc. (Deoluche and Baskin, 1973).

One of the important issues when talking about seed storage is the maintenance of optimal conditions for preservation of seed quality during storage. Water content in seed and storage temperature are very important factors that determine seed viability and the potential for storage. Reduction of viability after six months of storage has been observed in the studied soybean cultivars, depending on storage temperature (0, 25, 35, 45 and 55 °C) and relative air humidity (45, 55, 65, 75 and 84%). The proposed optimal storage conditions, on the basis of these studies were temperature of 25°C and relative air humidity of 55-65%, i.e., lots with good quality, harvested at the moisture of 15% and below, may be successfully stored in these conditions for six months (Nkang and Umoh, 1996).

Seed damage during storage is a cumulative phenomenon: harvest before the optimal maturity, poor conditions during seed maturation, poor drying conditions, storage of seed with high moisture content. Very frequently the damage to seed during storage is not manifested as reduced viability, but as loss of seed vigor. Therefore, the design and implementation of seed storage system must be directed towards the preservation of seed vigor.

TESTING QUALITY OF SOYBEAN SEED

In seed production, one of significant shortcomings is sowing seed that has no capacity to produce well-developed and healthy plants that will provide high and stable yield. Seed testing has been developed exactly to reduce this risk by examining seed quality before planting. Seed testing is a concept composed of several procedures.

Seed sampling

Precision and accuracy of results of seed testing depends on the accuracy of sampling a seed lot. That is why this operation must be performed by a trained sampler, following specific rules (ISTA, 1999). Sampling a soybean seed lot is performed with a Nobbe trier, which has a longitudinal slot. Individual sample is taken from a bag of which an aggregate sample is made. Depending on the size of the lot, number of bags has been determined from a single lot that has to be sampled, in accordance with a sampling scheme (ISTA, 2003). A lot of soybean seed can have a mass of 10,000 to 20,000 kg. An aggregate sample for a soybean seed lot with a mass of 20,000 kg is 1,000 g. The sample which is submitted for laboratory testing of seed must accurately represent the lot from which it was taken. For these reasons, it is very important, prior to sampling, to verify the uniformity of seed lot (ISTA, 1999). The average sample is made from the aggregate sample and for soybeans it is 500 g. In the laboratory, using a seed splitter, a working sample is made from the average sample for certain analyses in testing seed quality.

Seed purity testing

The purpose of testing seed purity is determination of the percentage of components in relation to the mass of the working sample, which serves to draw a conclusion on the components of a seed lot, determination of different types of seeds present in the sample, as well as inert matter. Basically, the working sample of soybean is

divided into three components: pure seed, other seeds, i.e., seeds of other species, and inert matter, determining the percentage of each part by measuring its mass. The term pure seed includes the mature and undamaged seeds of the usual size, a portion of seed larger than half the normal seed size, provided that a part of the seedcoat is present. Other seed includes the seeds of any plant species that does not belong to the pure seed. This category is restrictive for soybean seed, because it should not contain seed of other cultivated species nor weeds. Inert matter are mechanical ingredients (earth, stones, etc.), parts of other plant organs, as well as soybean seed less than half the normal size and seed without seedcoat.

Determination of moisture content

Weight of sample for determination of moisture content in soybean seed is 4-5 g, and taking two independent samples. For soybean seed, grinding is obligatory before drying. Grinding is done so that at least 50% of the ground material passes through a sieve with openings of 4 mm. If the soybean seed that is being tested with a moisture content above 10%, it is necessary to perform previous drying. Two independent samples each of 2.5 g are weighed, dried to moisture below 10% and weighed again to determine the loss of moisture. The work sample of soybean seed is dried by the method of low constant temperature, i.e., at the temperature of 103±2°C for a period of 17±1 hour. Moisture content in seed is expressed in percentage (by the weights of moist seed and seed after drying) with one decimal place. If the seed was subjected to the process of previous drying, moisture content is calculated from the results obtained in the first and second stages of drying.

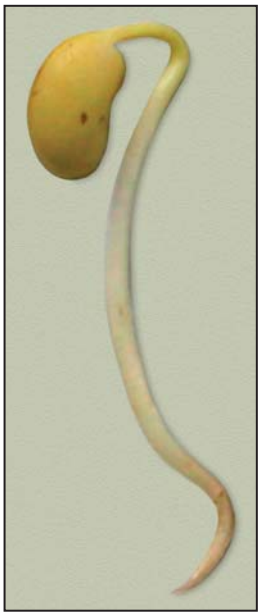
Testing of viability

Determination of viability of soybean seed presents the determination of maximum potential of germinated seeds of a lot. Obtained results can be used for comparisons of quality of different seed lot, they indicate the injuries, diseases and other properties of seed, and they are also used for determination of quantity of seed for planting of soybean. Filter paper or sand can be used as a medium for testing the viability of soybean seed. Sand is used most frequently because it provides a natural growth of the seedling, its easy uprooting without damage, and it also reduces the possibility of secondary infection by harmful microorganisms. Inoculation is done in germination trays at a temperature of 20-30°C or at constant temperature of 25°C. After five days, reading is done of germination energy, and after eight days viability is estimated, i.e., assessment of seedlings is performed. The concept of normal seedling includes three categories: 1) whole, undamaged, healthy seedling, which has a well developed, long, thin primary root, which is usually covered with numerous root hairs and which ends in a thin tip, a developed shoot with erect and elongated hypocotyl,

considering that the soybean has the epigeal type of germination; two cotyledons that are green and similar to the leaf, having characteristic shape and size; the green and well developed primary leaf. 2) The seedlings with slight damage, whose growth does not fall behind the undamaged seedling. 3) The seedling with secondary infections caused by fungi or bacteria, but in which the presence of all basic structures can be observed. Viability is expressed as a percentage of normal seedling, via its number (Figure 11.4).

Figure 11.4

Soybean seedling *(photo: Z. Nikolić)*



Testing the health conditions of seed

Depending on the type of pathogen, different methods are applied (Jovićević and Milošević, 1990) for determination of the health status of soybean seed. *Peronospora manshurica* can be detected by direct examination using a magnifying glass or a stereo microscope, by observing whitish layers on the surface of soybean seed. *Cercospora sojina* is determined by the method of seed germination on filter paper or moist sand, or by insemination of seed on the nutritious PDA (potato-dextrose agar) medium. *Diaporte phaseolorum* var. *soyae* is most frequently determined by the method of nutritious MA (malt agar) or PDA medium. Black-colored stromata form in the culture, and pycnidia with picnospores form on seed. The presence of the fungus *Diaporte phaseolorum* var. *caulivora* is determined by the method of nutritious PDA medium. The colony of the fungus appears in the form of whitish down feathers.

It is possible to find *Phomopsis longicola* in a seed sample by the method of nutritious PDA medium, at 23-25°C for a period of five to seven days. The presence of this parasite may be very significant since seed viability can be greatly reduced in the case of more intensive seed infection by this fungus. *Colletotrichum dematium* var. *truncatum* is determined most frequently by the method of filter paper. Whitish layers appear on the seed, and viability of the infected seed is reduced. For determination of *Fusarium spp.*, either filter paper or nutritious PDA medium are used. For establishment of the fungus *Ascochyta soyaecola*, the method of filter paper (pycnidia of cinnamon-brown color appear on the seed coat) or the nutritious PDA medium are used. Determination of the presence of the bacterium *Pseudomonas syringae* pv. *glycinea* is done by the method of macroscopic examination of intensively infected seed, by observing circular or polygonal spots, light to dark brown in color, on the seed. Soybean mosaic virus is determined by test plants indicators. Plumule extract, individually prepared, serves to inoculate young plants of *Dolichos uniflorus* or *Phaseolus lathiroides*. Symptoms can be observed already after 15 days. Also, to prove the presence of this virus in a seed sample, ELISA (enzyme-linked imunosorbent assey) test can be used.

APPLICATION OF BIOCHEMICAL AND MOLECULAR MARKERS IN SOYBEAN SEED PRODUCTION

Methods of biotechnology have become an integral part of breeding and seed production of soybean. They are used when testing the quality of seed, its genetic purity, resistance to diseases, stressful environmental conditions, as well as many other characteristics.

Identification of varieties and control of genetic purity of seed are very important in soybean seed, both for breeders' rights protection and control of genetic material in production. Analysis of the genetic purity of seed helps seed producers to use high quality seed. Identification of varieties can be observed from several aspects: identification in its basic sense - which variety it is; varietal diversity - whether this variety is different in relation to others; varietal purity - whether in a sample there is more than one variety; description of variety -- is it possible to obtain information that will be used for variety description.

Traditional, morphological markers are used to test distinctness, uniformity and stability of a variety, the so-called DUS tests. UPOV uses standard references in the description of a new variety, especially when it concerns the genetic authenticity of seed for the market. In the case of soybean, genotypes are distinguished most frequently using the following morphological markers: flower color, pubescence color, leaf shape, type of stem growth, length of growing season and, in some cases, the characteristic of resistance to a certain type of pathogen.

However, in modern soybean breeding varieties are being created that do not differ much phenotypically, considering that highly adaptable varieties are created. Similarly, based on morphological markers it is not possible to determine the degree of seed purity because environmental conditions, as well as stress during seed development, may mask specific morphological characteristics. In addition, there are a number of other disadvantages of using field trials to test the genetic purity of soybean seed: the crop must grown in a region to which the variety is well adapted; field trials require a minimum period of six months for all characteristics to be exhibited during the vegetation season; they are expensive and require land, equipment and personnel to perform, as well as permanent controls; morphological characteristics used to describe a variety cannot also be used for identification of all varieties; in the course of testing, subjective assessment of staff performing the tests may appear.

Biochemical markers - Isozymes

Application of biochemical markers has an advantage in relation to morphological, considering the tests that are at the level of the genotype, as opposed to the phenotype, while the effect of environment is minimal. Both proteins and amino acids allow the application of a suitable analytical method for identification of varieties. Proteins are direct products of genes and are therefore very suitable as markers of structural genes. In plants there are proteins that are highly polymorphic, particularly in the reserve proteins in seed but also other proteins in seed and vegetative parts, as well as enzymes.

It can be said that the electrophoresis of proteins and enzymes is used successfully in the breeding, production and marketing of seed, as a source of genetic markers, in the recognition of varieties in DUS tests and breeders' rights protection, in production and certification of seed, in terms of control of identity of the variety and genetic purity, documentation of genetic resources in gene banks and other collections. This technique has been adopted by ISTA as a standard reference method when testing the quality of seed.

Electrophoresis of isozymes, based on different migration of polymorphic enzyme proteins through the gel in an electric field, appeared as a suitable technique for identification of varieties. Seed lots, using isozyme fingerprinting, can be examined and identified in a very short period of time.

Variety-specific isozymic variability in soybean was presented with several enzymes such as α-amylase, peroxidase, urease and isocitrate dehydrogenase. Success in using isozyme fingerprinting for identification of soybean varieties indicates a possibility of combining this technique with the traditional, i.e., with morphological markers. Conducted studies showed an increase in identification with 77% (only isozymes) to 95% (isozymes and morphological markers), which confirms that we should continue to use the traditional method of identification (Cardy and Beversdorf, 1984).

Using morphological markers, out of 36 soybean varieties tested, only six were identified, while the use of isozyme markers was much more efficient because it succeeded in identifying 15 varieties of soybean.

When examining the quality of seed, electrophoretic variation of proteins and enzymes may be used for determination of the degree of damage of seed during aging. An increase has been noted in the number of protein, esterase and glutamate oxaloacetate transaminase bands in dry seed, as the period of seed aging increased. This indicates that qualitative changes of proteins and enzymes take place in old soybean seed (Chauhan et al., 1985)

Laboratory techniques are much faster and cheaper in relation to the field, and they are also more flexible temporally. They found their place when a quick check of new plant genotypes is needed, as well as for detection of changes in the genome in soybean seed that is multiplied or kept in storages.

Molecular markers

Molecular markers represent new opportunities for breeders in finding new sources of variability and detecting genetic factors that control characteristics that are inherited quantitatively.

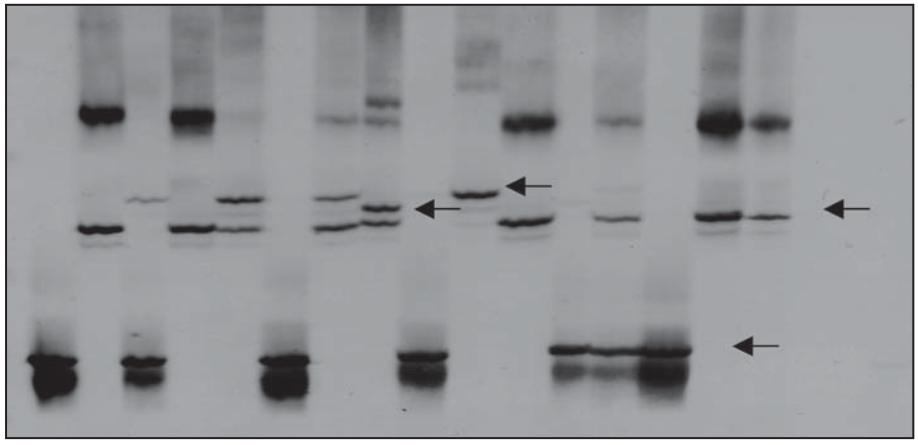
The initial type of DNA markers, which includes the use of a group of bacterial proteins called restriction enzymes (RE) for cutting DNA into fragments was named RFLP (Restriction Fragment Length Polymorphism). RFLP were the first widely used molecular markers in soybean seed production for testing quality and genetic purity. These markers and epistatic interactions between them explain 71% of the variability of characteristics. Using the RFLP technique, two QTL alleles have been marked that control the contents of proteins and oil in soybean seed. It was also found that the QTL allele for high protein content causes a reduction of yield (Sebolt et al., 2000).

In 1990 it was established that a new molecular genetic technique, called Random Amplified Polymorphic DNA (RAPD), can be used to identification of varieties. Environmental conditions, aging of seeds, mushrooms prevalence and origin of production, have no influence on the stability of RAPD in soybean seed, so this technique can be routinely used in seed testing. This is fast, simple and relatively inexpensive methods, many loci can be quickly identified, and the RAPD markers can be successfully used in the identification of soybean cultivars (Jianhua et al., 1996). On the basis of the four primers used, genetic differences were found between varieties of soybean, which can be a way towards their complete identification (Zlokolica et al., 1999). By examining RAPD profiles of artificially and naturally aged soybean seeds, via 188 primers, it was found that six showed polymorphism between artificially aged and fresh seeds, and two showed polymorphism between artificially aged and fresh seeds (Jianhua et al., 1996b).

In recent years microsatellites are extensively studied in plants because they are easy to detect using PCR, they showed significant polymorphism and are codominant in nature. A system of markers such as SSR (Simple Sequence Repeat) have become frequently used markers for genetic mapping (Figure 11.5).

Figure 11.5

SSR polymorphism of soybean genotypes *(photo: Z. Nikolić)*



SSR markers show excellent complementarity with RFLP and RAPD markers for use in molecular biology, genetics and breeding of soybean. The SSR method is essentially more sensitive in relation to the traditional use of morphological and biochemical methods for identification of soybean varieties, and will probably in the future become a standard method for identification of varieties and determination of genetic purity of soybean seed. In addition to quality characteristics of soybean seed, molecular markers are used to facilitate the discovery and use of new genes of resistance to diseases. SSR markers were identified that could be used for the beginning of mapping the alleles for resistance to *Phytophthora sojae* (Burnham et al., 2002).

Diagnostic DNA markers, i.e., the predicting ability of DNA markers, depends on their linkage with resistance genes and phenotypic expression of genes in different conditions of the environment and genetic basis. In the process of identification and approval of soybean varieties, it is necessary to use molecular markers for the detection of differences in the genomes between varieties, as well as the assessment of genetic divergence. Each variety should have its “passport” which would contain descriptors of morphological characteristics and specific combinations or code names of polymorphic molecular markers (Nikolić, 2002).

SUMMARY

In the broadest sense, the term seed production refers to the processes of seed growing, seed processing, and seed trade. Seed production consists of a number of distinct phases, such as the establishment and growing of the seed crop, its inspection and control in the field, harvesting, processing, the testing and determination of seed characteristics, and the distribution and sale of the seeds. Seed production can be regarded as the final stage of plant breeding, as the newly developed production characteristics have a new biological value.

The seed production cycle is organized by a complex comprised of research institutes, seed growers, seed processors, and seed suppliers. High seed categories are produced by the organization that developed the seed and this process is under the direct control of the breeder. Lower seed categories, on the other hand, are produced by agricultural organizations under the supervision of the breeder/research institute and the relevant inspecting authority.

The production of soybean seeds is a responsible job, so the seed production technology for this crop must be devised in a way that will minimize production risks, maximize the economic effects, and meet all the necessary legal requirements. High quality seeds are the first prerequisite of high quality seed production, so, in order to fulfil this goal, the seeds are processed, packaged, tested, and, if found to meet the legally prescribed requirements in terms of quality, sold.

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SOYBEAN DISEASES

Miloš Vidić, Stevan Jasnić

Soybean diseases can significantly affect yield level and stability of that industrial crop. Epiphytotic attacks by some diseases may bring into question the profitability of soybean growing. According to Sinclair and Backman (1989), in 1987, diseases reduced the global yield of soybean by 10.3 million tons.

A large number of phytopathogenic microorganisms are parasites of soybean, and they cause various diseases. More than 100 soybean pathogens have been described worldwide, and about 35 of these are considered capable of causing significant economic damage on soybean (Sinclair and Backman, 1989). Mycoses are most numerous and harmful, followed by bacterial and viral diseases.

Generally, an agroecological region is intensively attacked by a single or a few pathogens, while others either do not occur at all or they occur sporadically. These latter, minor pathogens may intensify their occurrence in some years, when an array of factors happens to coincide.

The increase of soybean acreage in our country has caused diseases to progressively gain importance in soybean production. Simultaneously, study of the major parasites has been intensified and measures for their control have been searched for. A number of soybean parasites have been described, which were new in our country. Their morphological and biological characteristics and their epidemiology under the local agroecological conditions have been studied to gain better knowledge of these parasites and come up with effective measures for their control.

It has been found that the most important parasites were *Peronospora manshurica* (downy mildew) and *Pseudomonas syringae* pv. *glycinea* (bacterial blight) on leaves, *Diaporthe phaseolorum* var. *caulivora* (stem canker) and *Sclerotinia sclerotiorum* (white rot) on stems and *Macrophomina phaseolina* (charcoal rot) on roots. Most dangerous parasites of the seed were fungi of the genus *Diaporthe*/*Phomopsis*.

In this chapter, the above parasites are described in some detail. It also addresses other agents of soybean diseases in our country, as well as those which have not been found in our country, but which cause serious problems in some soybean-growing regions in the world.

As soybean parasites are rarely controlled by chemicals, both in the world and in our country, attention is focused in this text on cultural practices.

BROWN SPOT

Distribution and economic importance

Brown spot was first described on soybean in Japan (Hemmi, 1915; as cited in Sinclair and Shurtleff, 1975), although indications exist that it had been prosent earlier on soybeans in Italy (Anonymous, 1913; as cited in Ammar, 1983). Up till now, brown spot has been reported in a majority of countries of Asia, South and North America and Europe (Sinclair ad Dhingra, 1975; Ammar, 1983; Ivanović, 1992). In Serbia, this disease occurs frequently on soybeans in the Vojvodina Province (Štraser, 1982; Ammar, 1983; Jasnić and Vidić, 1985).

The negative impact of brown spot on soybean yield is caused by premature shedding of infected leaves. An intensive attack of the disease can destroy up to 40% of the foliage (Young and Ross, 1978), which is particularly reflected on the number of pods per plant and grain size, i.e., 1000-grain weight (Almeida, 1980., as cited in Ammar, 1983). Soybean yield can be reduced by 20% by an intensive occurrence of the disease (Whitney, 1978). Inoculation in the field reduces the yield from 12% to 34% depending on the cultivar (Lim, 1980). Studies conducted in our country have shown that brown spot significantly affects the 1000-grain weight and oil and protein contents. Field inoculation with a suspension of conidia is more detrimental and causes larger yield reductions in the late than in the early cultivars (Ammar, 1983). According to Giesler and Weissling (2004), yield reduction ranges from 8% to 15% when premature defoliation occurs on 20% to 50% of soybean plants.

Symptoms

Symptoms of brown spot appear on soybeans in the spring, immediately after emergence. They are present throughout the growing season on all aboveground plant parts.

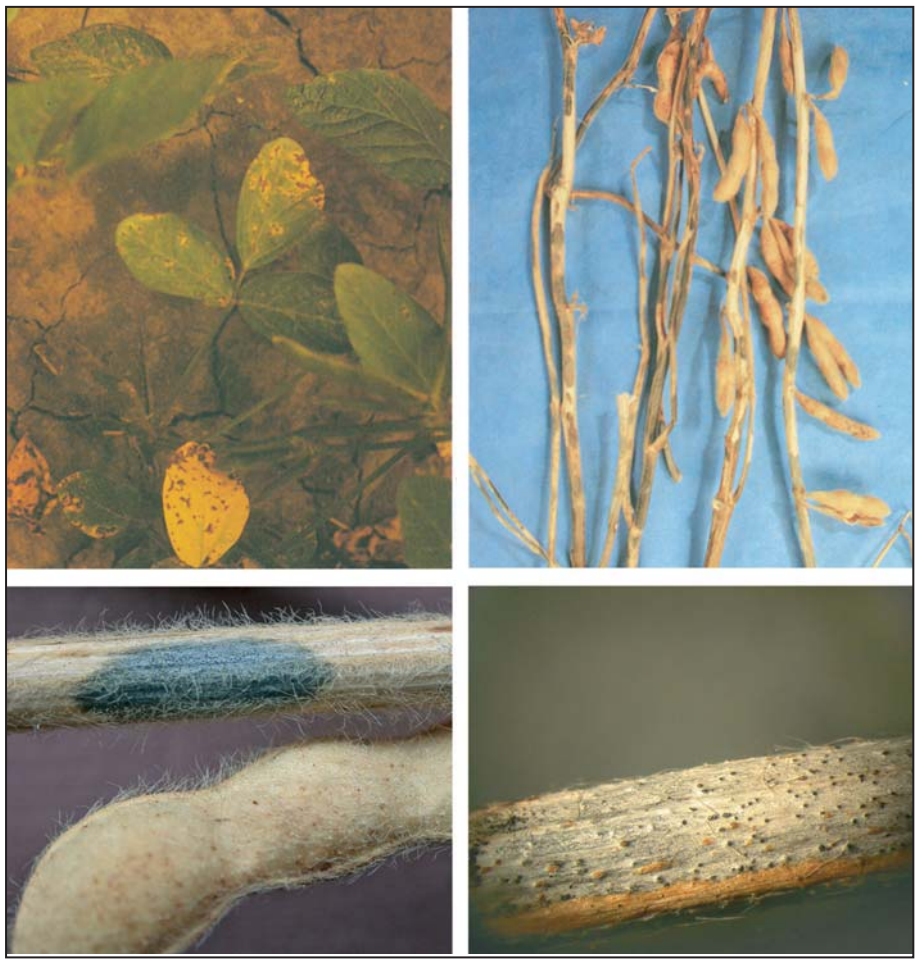
Dark brown to black spots, of irregular shape and different size, can be observed already on soybean cotyledons. Dark-red spots occur on the simple leaves. They are small initially but they increase over time reaching 4 to 5 mm in diameter. Spots are surrounding with leaf nerves, which gives them a characteristic angular shape. Spots are visible on the upper side and the underside of infected leaves (Figure 12.1a). The simple leaves rapidly turn yellow and fall off.

When weather conditions are favorable, the disease spreads to young trifoliate leaves. Numerous, small, light brown spots enlarge and coalesce with each other, creating necrotic surfaces of various size. The infected leaves turn yellow and fall off prematurely. During a strong epiphytotic appearance of the disease retain only a few top leaves.

At the end of the growing season, when leaves turn yellow naturally, symptoms spread to the stem, lateral branches and pods. At the beginning, these spots are oval, wet and brown-colored. As the spots age, they become grayish to dark blue in color. Their size is from several millimeters to several square centimeters. They coalesce, so that in some cases only small portions of the stem, branches and pods remain uninfected (Figure 12.1b, c). Within the spots, there are small black pycnidia immersed in plant tissue (Figure 12.1d).

Figure 12.1

Septoria glycines *(photo: M.Vidić and R. Jevtić)*



a) Spots on a leaf (top left); b) Symptoms on the stem (top right);
c) A spot on the stem (bottom left), d) Pycnidia formed within a spot (bottom right)

Causal organism, biology and epidemiology

The brown spot of soybean is caused by *Septoria glycines* **Hemmi**. This fungus is a highly specialized parasite, which has no other host plants but soybeans (Ammar, 1983).

The fungus grows slowly on nutritive media, forming colonies which are light gray at first and then turn black. The hyphae branch intensively; they are septate and have thick walls. Inside the mycelium, the fungus forms dark pycnidia, spherical or slightly flattened, with thin walls and the ostiole at the top. The pycnidia are 90 to 100 microns indiameter. The conidia are hyaline or pale-green, filiform and curved. They have up to 3 septate that are visible only at the time of germination. They germinate terminally, producing one or several hyphae. At a temperature of 25°C, the hyphae develop in 24 to 48 hours (Ammar, 1983).

S. glycines overwinters in infected harvest residues, rarely in soybean seeds. In plant residues (mostly in stems), pycnidia form in the fall, and conidia develop in them in the spring of the next year. When released, conidia are carried by wind or raindrops to young soybean plants where they produce infection hyphae. The hyphae penetrate the leaf through the stomata without developing appressoria, where they perform the primary infection. The mycelium of the fungus develops intercellularly in leaf tissue causing cell necrosis. After the infected leaves fall off, pycnidia with conidia form in them, and they are responsible for secondary infection during vegetation. Warm and humid weather conditions advance the appearance and distribution of brown spots. Usually, the dry period in July stops the spread of the foliar infection in upper parts of the plant.

There are no soybean genotypes fully resistant to *S. glycines* yet, but significant differences in sensitivity have been noted. The cultivars Kent, Dormon, Lee, Ogdon Raonoke (Baeza, 1971) SRF-150, SRF-200, Kirin 3-11, and Fiskeby V (Ammar, 1983) have shown to be less susceptible. Following inoculation under controlled conditions, not a single soybean genotype has shown resistance to *S. glycines*, however, the cultivars CTS-40, IAS-5, Pi-230975 and Pi-204332 have shown a lower level of infection and slower disease development (Almeida, 2001).

Control

For control of brown spot it is recommended to avoid soybean growing in monoculture, to plow under or burn harvest residues, to use healthy seed for planting and to develop and use in production less susceptible cultivars. The occurrence of brown spot on seed crop can be effectively prevented with two to three fungicide treatments. Treatments should be performed from the beginning of flowering to pod filling, using preparations based on thiophanate methyl.

DOWNY MILDEW

Distribution and economic significance

Downy mildew is counted among the most widespread soybean diseases. It was detected for the first time on soybeans in Kashmir (China) in 1908, and then in the U.S.A. in 1923 (Sinclair and Shurtleff, 1975). Since then, it had spread to most soybean-growing countries where this plant is grown (Aćimović, 1988). In Serbia, it occurred first on soybeans in the region of Mačva and it was described in the early 1950s (Nikolić, 1951a).

There are conflicting or discrepant opinions on the impact of downy mildew on the yield and quality of soybeans. The importance of the disease is either overrated or underrated. However, there are few reliable data on the impact of downy mildew on soybean yield. Athow (1973) claimed that the yield reduction is around 8%. Dunleavy (1987) reported that the average reduction reaches 11.8% and that very sensitive cultivars, in years favorable for the disease, suffer losses up to 25%.

According to our observations (Vidić, 1992), downy mildew is particularly harmful in years with abundant rainfall in the first part of vegetation followed by a long dry period in the second part of vegetation. During the rainy period, intensive infection occurs on the leaves. In the dry period, infected leaves dry up and fall off rapidly, in consequence to a combined action of the parasite and water shortage in the soil. The infected plants mature too early, curtailing the grain filling period which is negatively reflected on soybean yield.

Symptoms

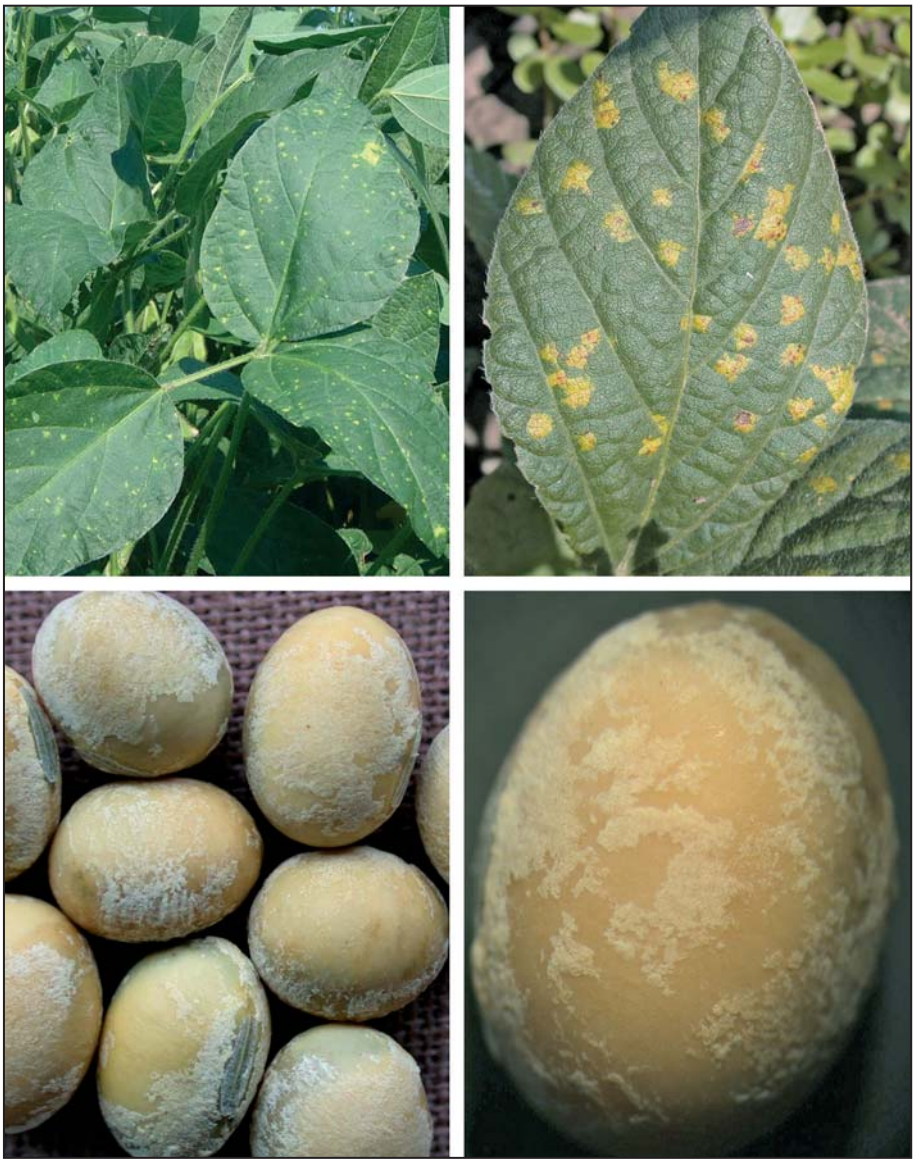
Downy mildew is most common on leaves and seeds, but it may also cause systemic infections of soybean plants. Systemic infections occur on plants developed from infected seeds or when soybean seedlings come in contact with oospores from the soil. First symptoms can be seen at the base of simple leaves, in the form of light green or yellow patches, which fan out along leaf nervure. The parasitic fungus continues to develop in systematically infected plants by penetrating the newly formed trifoliate (true) leaves. The growth of the infected trifoliate leaves is disrupted, and they are mottled with yellow or pale green fanlike spots. The edges of the lamina curl downwards. Conidiophores with conidia emerge through stomata, forming a grayish-brown cover on the underside of the leaves. The growth of the systemically infected plants is retarded and such plants are significantly shorter than the healthy ones. The percentage of infected plants in the field is very low (less than 0.5%).

Conidia from systemically infected plants spread to surrounding plants causing secondary infection, first on leaves and later on pods and seeds.

Secondary infections are most widespread and most damaging to soybeans. First symptoms of the disease are pale green or yellowish spots (Figure 12.2a).

Figure 12.2

Peronospora mashurica *(photo: M.Vidić and R. Jevtić)*



a) Downy mildew symptoms on the leaf (top left), b) Spots on the leaf (top right), c) and d) Symptoms on seed - a seed encrusted with mycelium and oospores (bottom)

They usually occur in the phenophase of two or three true leaves, while their occurrence at the time of development of the first true leaf is much less frequent. The spots expand and enlarge quickly in humid weather, and in some instances they coalesce.

The infected tissue dries up and necroses, gradually becoming darker, from yellow to brown (Figure 12.2b). If a large part of the leaf becomes infected, its margins curl down, it dries up and falls off. Leaf shed is especially intensive during drought, which in our agroecological conditions takes place almost regularly in late July and throughout August. Disease symptoms are also visible on the underside of the leaf, where conidiophores and conidia tend to cover the spots. This cover is initially pale brown, becoming purple with age. This symptom is characteristic for downy mildew, making it easy to distinguish from the other diseases of soybean leaves.

Pods can be infected by downy mildew, however, symptoms are not visible from the outside, but inside the pod and on seeds. The seed coat is partially or completely covered with pale yellow mycelium and oospores. The mycelium usually forms around the hilum, although the whole seed becomes covered in the case of intensive infection (Figure 12.2c). Infected seeds are smaller than healthy ones their germination rate and germination energy are considerably lower.

Causal organism, biology and epidemiology

Downy mildew of soybeans is caused by *Peronospora manshurica* (**Naum**) **Syd. ex Gäum**. (syn: *Peronospora sojae* **Lehman & Wolf**). This phytopathogenic pseudofungus is an obligate, highly specialized parasite, which attacks only soybeans.

The mycelium of *P. manshurica* is composed of a mass of unicellular (nonseptate) hyphae 7 to 10 microns in diameter. The mycelium develops in the intercellular space of the host plant, penetrating the cells by means of the cylindrical, curved and branched haustoria. Conidiophores with conidia emerge through stomatal openings, creating a loose cover on the underside of the leaf. Conidiophores are thin and long (240 to 984 x 5 to 9 µm), grey to pale violet in color. They branch dichotomously (two to ten times), each bearing a pair of sterigmata at the tip. Elliptical, unicellular and hyaline conidia, 19 to 24 µm in diameter develop on the sterigmata (Sinclair and Backman, 1989).

In the course of the sexual reproduction cycle, the male sexual cells antheridia fertilize female cell oogonia and form oospores. The oospores are dark brown in color and spherical in shape, 20 to 45 µm in diameter. They have a solid outer wall, which can be smooth or wrinkled. Oospores form in infected leaves, inside pods and on seeds. According to McKenzie and Wyllie (1971), they can be found in the root tissue, inside the stem and petioles of systemically infected plants.

Oospores are the overwintering form of *P. manshurica*. Recent studies suggest that the fungus is also transmitted by the mycelium as well as seeds (Roongruangsree et al., 1988). At the time of soybean seed germination and emergence, oospores on infected seeds and harvest residues produce germ tubes through which the parasite penetrates the seedlings performing primary infection. The emerged plants are systemically infected.

Soil temperature of 13ºC is optimal for the development of primary infection (Lehman, 1953a) so that highest percentages of systemically infected plants occur on plants from earliest sowing dates (Vidić et al., 1995a).

Secondary infections are caused by conidia from systemically infected plants. Conidia are disseminated by wind reach the surrounding foliage, germinate in the infection hypha and the fungus penetrates the leaf mesophyll through the stoma. Conidia germination takes place in a drop of water or dew, in the temperature interval from 10 to 30ºC. Under favorable conditions, the incubation period lasts 7 to 10 days (Grabe and Dunleavy, 1959). Yellowish spots then occur on the upper side of the leaf, while the fungus' reproductive organs are visible on the underside.

The number and size of spots depend on leaf age and sensitivity of soybean genotype. On young leaves, especially those belonging to sensitive genotypes, spots are large and they cover a large portion of the lamina. Many small spots tend to develop on old leaves. Less susceptible soybean genotypes also react to infection by forming small spots on the leaves.

The spread of downy mildew during growing season is promoted by frequent rains, heavy morning dews, high relative air humidity and moderate temperatures of 18 to 22ºC. Sporulation of this pseudo-fungus is possible within the temperature range from 10 to 30ºC (Aćimović, 1988).

High variation in pathogenicity has been observed within the population of *P. manshurica*. The first three races of the pathogen had been registered by Geesman (1950a) in the U.S.A. After him, Lehman (1953b), Grabe and Dunleavy (1959) and Dunleavy (1971, 1977) identified other 29 physiological races, based on reactions of 14 species differential cultivars. These races were marked by serial numbers, ending with the number 32. The cultivar Union was resistant to all of these races. However, Lim et al. (1984) determined another race, numbered 33, to which Union was susceptible. These authors found the cultivars Pridesoy, Palmetto, Cabot, Ogdon and Acadian to be resistant to race 33.

Geesman (1950b) found that resistance to *P. manshurica* is controlled by one dominant gene (Rpm). Later it was discovered that the cultivars Kanrich and Pine dell Perfection were immune or highly resistant to all known *P. manshurica* races (1 to 32), and the gene responsible for their resistance was marked Rpm (Bernard and Cremeens, 1971). By backcrossing the cultivar Kanrich as a source of resistance, resistance to the parasite was incorporated into several commercial cultivars. Recently, Chowdhury et al. (2002) confirmed that a dominant gene (Rpmx) controls resistance to downy mildew and they identified RAPD markers associated with this gene. Availability of effective resistance sources and knowledge of the nature of resistance greatly facilitate soybean breeding for resistance to this pathogen. However, the large number of physiological races and steady recurrence of new races make soybean breeding a continuous process. Namely, the resistant cultivars during time become more or less sensitive. The research centers involved in soybean breeding in our country pay significant attention to this problem.

Recently developed domestic cultivars Bačka, Afrodita, Ravnica and Vojvodjanka as well as a number of prospective lines had exhibited high resistance to downy mildew. When the racial composition of the parasite changed due to strong selection pressure, these cultivars have lost the vertical (specific) resistance, but they still retained a degree of horizontal resistance, so that there are no downy mildew outbreak even when conditions and quite favorable for its development.

Control

Development and cultivation of resistant cultivars are the most effective and environmentally most acceptable measures of downy mildew control. Most of the recently developed domestic soybean cultivars, which currently dominate the domestic soybean production, had possessed a satisfactory resistance, which has gradually become ineffectual due to changes in the racial composition of the parasite.

Healthy seed should be used for planting or it should be treated with metalaxyl-, oxadixyl- and mancozeb-based fungicides. Fungicides that may adversely affect the nodular bacterium (*Bradyrhizobium japonicum*) should be avoided. Following intensive downy mildew outbreaks, soybean seed crop should be treated with a combination of systemic and protective fungicides. The number and date of treatments should match weather conditions during growing season. According to Dunleavy (1987) soybean treatment at 7-day intervals between phenophases V1 and R7 with preparations based on metalaxyl had significantly increased yield and reduced the development of conidia on leaves and the percentage of infected seed in susceptible soybean cultivars.

Crop rotation is an important measure in downy mildew control because oospores of the fungus remain vital in soil for several years. Plowing under of harvest residues at greater depth also reduces the inoculum, and therefore the intensity of downy mildew occurrence.

ASCOCHYTA LEAF AND POD SPOT

Distribution and economic significance

Ascochyta leaf and pod spot is widespread in most soybean-growing regions of the former Soviet Union (Enken, 1959), where it was detected for the first time. More precisely, it was detected in eastern Siberia (Abbramoff, 1931, as cited in Jasnić, 1984). Later, the disease was observed in China and Japan (Ling, 1948; Kurata, 1960. as cited in Jasnić, 1984).

There are no reports on the occurrence of the disease on soybeans in the USA. In Europe, it was observed in Germany (Frandesen, 1953), former Czechoslovakia (Novakova - Pfeiferova, 1958) and Serbia (Jasnić, 1984).

There are no precise data on the impact of Ascochyta spot on the yield and quality of soybeans. In Serbia, an intensive occurrence of the disease was registered in 1981, in the vicinity of the town of Bečej, but its harmfulness was not estimated (Vidić et al., 1983b).

Symptoms

Ascochyta spot symptoms occur already on seedlings and, in the course of subsequent soybean development, on leaves, leaf petioles, stems, branches and pods. The cotyledons of infected seedlings show necrotic, brown spots, sometimes causing the seedlings to wilt and dry up.

First signs of the disease occurring on the leaves are large (1-2 cm in diameter), brown, more or less round and slightly sunken spots. The spots expand concentrically, forming light and dark rings, light brown in the center. They are usually located near the edge of lamina, fusing together as they grow. Infected leaves dry up prematurely and fall off.

Close to the end of the growing season, lenticular or elliptically elongated spots start to occur on the stem, most frequently near the nodes. Later they occur on branches and pods. Within the spots, small black pycnidia are formed, arranged in irregular concentric rings. In the case of early infections, spots girdle the stem, so that the plant part above the infected spot gradually dries up.

Causal organism, biology and epidemiology

Ascochyta leaf and pod spot on soybeans is caused by *Ascochyta sojaecola* **Abram**. The mycelium is dark brown, well branched and septate. It develops in the intercellular spaces of the host plant. The fungus forms black globular pycnidis with conidia (pycnospores). The conidia are hyaline, cylindrically elongated, with rounded of slightly tapering ends. They may be bicellular or unicellular. The pycnidia and conidia forming on infected soybean parts are larger than those forming in culture media. Jasnić (1984) reported that the size of pycnidia from spontaneously infected soybean stems is 120 to 230 µm in diameter, and of those from potato-dextrose agar 70 to 160 µm. The same author stated that the size of conidia in pycnidia from soybean stems is 3.81 - 6.0 x 7.5 - 12.5 µm, and those from culture medium 2.5 - 5.0 x 3.8 - 7.5 µm. The conidia germinate in drops of water forming one or two primary hyphae.

Under unfavorable conditions, *A. sojaecola* survive in infected plant residues and soybean seeds. Primary infection is caused by conidia from pycnidia formed on plant residues. Infection pathway starts most often from leaves, to which conidia are brought by rain drops, wind or insects. Through the leaf stalk, disease symptoms spread to the stem and branches. Pods and stems can also be infected directly, by air-borne conidia.

Humid and moderately warm weather favors the infection and spread of the parasite.

Control

Use of healthy seed and strict adherence to crop rotation are basic preventive measures in the control of Ascochyta spot. The disease can be effectively controlled with fungicides, which is sometimes recommended for intensive infections of seed crop.

PHYLLOSTICTA LEAF SPOT

The disease is widespread in major soybean-growing regions in the world (Sinclair and Backman, 1989) and it was registered in Serbia (Stojanović and Kostić, 1956; Jasnić et al., 1983). Although it can cause premature drying and loss of leaves, the damage caused by it is not of great economic importance.

Symptoms

Disease symptoms occur in all phenophases of soybean development, primarily on leaves but also on the other above-ground plant parts. Round, irregular or square spots, up to 2 cm in diameter, occur on young leaves. Near the edge of the lamina, spots are V shaped, being bordered by leaf nerves. At the beginning, spots are pale green and chlorotic, with indistinct dark border. As they age, they become light brown, with a reddish or dark brown edge. The tissue within the spot necroses, dries up and falls out, leaving holes that give the leaf a grid-like appearance.

Symptoms spread from the lamina to leaf petioles and then to stems and pods, where elongated spots are formed, light grey or brown in color, bordered by a narrow reddish-purple edge. Soybean seed can be infected via infected pods.

Causal organism, biology and epidemiology

Phyllosticta leaf spot is caused by *Phyllosticta sojaecola* **Massal**. (syn. *P. glycinea* **Tehon** et **Daniels**). Teleomorphic stage of this fungus has been described under the title of *Pleosphaerulina sojaecola* (**Mass**.) **Miura**.

The mycelium of the fungus is grayish-black and septate. Oval or spherical pycnidia 58 to 165 µm in diameter develop within necrotic spots. A large number of unicellular, hyaline, elliptical conidia form inside the pycnidia. The size of the conidia is 2.0 to 3.4 x 4.6 to 7.8 µm (Jasnić et al., 1983).

In unfavorable conditions, the parasite subsists in infected seeds and harvest residues, infecting plants by conidia.

Control

To control the Phyllosticta leaf spot, it is recommended to respect crop rotation, use healthy and quality seeds for planting, as well as the destroy or plow under the infected harvest residues.

FROGEYE LEAF SPOT

Distribution and economic significance

In regions with warm and humid climate, the frogeye leaf spot is one of the most widespread soybean diseases. It had been first described in Japan in 1915, then in the U.S.A. in 1924 (Sinclair and Backman, 1989). Reduction of yield of sensitive cultivars in the United States has been estimated at about 15% (Laviolette et al., 1970). Introduction of resistant cultivars in production significantly reduces yield losses caused by this disease.

Symptoms

The frogeye leaf spot is primarily a foliar disease, although its symptoms can occur on soybean stems, pods and seeds. Spots on the leaf are round or irregular-shapes, 1 to 5 mm in diameter. Small wet spots are first observed on the upper side of the leaf. The spots are light colored in the center, with a reddish-brown edge. Over time, the central part of the spot becomes ash grey to light brown in color.

Spots occur also on the underside of the leaf, where they are initially somewhat darker than the spots on the upper side. At the time of conidiophore and conidia formation, the center of the spots on the underside becomes dark or light grey, bordered by a thin reddish-brown halo. On completion of sporulation, the central part of old spots turns white. Small points - stromata of the parasitic fungus - can be seen inside the spots. The spots coalesce, forming irregularly shaped necrotic areas which cause the leaf to dry up and fall off prematurely.

Symptoms on the stem usually occur at the end of growing season. Their occurrence is considerably less intensive than that on leaves. Spots on the stem are elongated, flat or slightly sunken in the middle. Their edges are bordered by a thin dark brown to black area. As they age, the spots first become brown and then light grey, with numerous black stromata in the central part. Similar symptoms can be seen on the pods.

Spots of different colors and sizes can be seen on the seed coat of infected soybean seeds. Spot size varies considerably, from small to very large. In some cases they may cover the entire seed coat. Their color varies from light grey to dark grey or brown. Sometimes, the differently colored spots merge into one. The larger infected seed coat area causes the lower germination rate and germination energy of seeds.

Causal organism, biology and epidemiology

The frogeye leaf spot of soybeans is caused by *Cercospora sojina* **Hara** (syn. *C. daizu* **Miura**). Inside the spots, fungus mycelium forms stromata. They are thin and dark-colored. Each of them develops 2 to 25 condiophores that emerge in clumps through stomatal openings. Conidiophores are light- to dark brown, their size is 52 to 120 x 4 to 6 µm, and they are divided with one or several septa (Sinclair and Backman, 1989). Scars that are visible near the tip of conidiophores show the places where conidia had been attached. Namely, after maturation, a conidium detaches from the conidiophores that continues to grow, sequentially forming new conidia (up to 11 on a single conidiophores).

Conidia are spindle-shaped, round at the base, with a scar at the point where they had been attached to the conidiophore. They can be unicellular, but they are mainly transversally divided by septa (up to 10 septa). They are hyaline of faintly colored. The size of conidia from soybean leaves is 24 to 108 x 3 to 9 µm (Aćimović, 1988). They germinate in water drops, developing one or several initial hyphae, which typically grow out from the base and top cell.

The fungus survive as a mycelium in seeds or plant residues. Infected soybean seeds have delayed germination and emergence. After germination, the fungus sporulates on the cotyledons, providing inoculum for the infection of neighboring plants. Symptoms on leaves occur after 9 to 12 days, and the formation of conidia start approximately 24 to 48 hours later. These conidia are rain- or wind-borne and under

favorable weather conditions they spread infections to neighboring leaves, stems and pods. Young leaves are more susceptible to parasite's attack than the old ones. Seeds become infected in direct contact with spots on the pod.

Hot and humid weather favors the sporulation of *C. sojina*, outbreak and spread of the disease.

Soybean varieties exhibit different responses to the pathogen, from very sensitive to resistant. The dominant major genes Rcs1, Rcs2 and Rcs3 control the resistance to the most common fungus races 1, 2 and 5 (Sinclair and Backman, 1989).

Control

In regions where the frogeye leaf spot occurs intensively, it is recommended to grow resistant or less susceptible cultivars. Healthy seed should be used for sowing, or it should be pre-treated with thiram and thiabendazole-based fungicides. After harvest it is advised to destroy or deep plow infected harvest residues. Soybeans should be grown in the same plot after at least a two-year interval. Susceptible cultivars should be treated one to three times with preparations based on benomyl, thiabendazole, carbendazim, etc. The first treatment should be performed at the beginning of pod formation, and the other two, if necessary, at 14- to 21-day intervals (Mc Gee, 1992).

PURPLE SEED STAIN

Distribution and economic significance

The purple seed stain had been observed on soybeans already in 1921 in Korea, in 1922 in Japan and in 1924 in the USA (Aćimović, 1988). Nowadays, the disease is widespread in Brazil, Japan, Taiwan, Uganda and all major soybean-growing regions in the U.S. (Sinclair and Backman, 1989). The purple seed stain was registered in the vicinity of Banja Luka, Bosnia (Lušin, 1960). In years with heavy rainfall during the soybean maturation, symptoms of the purple seed stain are often observed on soybean seeds in Serbia (Vidić, unpublished data).

Intensive occurrence of the disease causes considerable damage. In 1978, for example, in the south of the USA (15 federal states), the loss was estimated at 3.5 million tons and in 1979 at 500,000 t. In Brazil, the losses range from 30% in the north to 15% in the central part of the country (Sinclair and Backman, 1989). Infected seeds have poor germination, which adversely affects crop stand.

Symptoms

First symptoms of the disease can be observed at the beginning of seed forming, or during seed filling. They occur on all aboveground parts, but most frequently on soybean leaves. Small, angular or irregularly-shaped spots can be seen on both sides of the infected leaves. Spot size ranges from very small to large, about one centimeter in diameter. They are easy to perceive and clearly distinguished from other types of leaf spot by the characteristic reddish-purple color. Spots gradually grow and coalesce, forming irregularly-shaped necrotic surfaces. Leaf nervure often becomes necrotic. The infected leaves turn yellow and fall off prematurely, starting from the young, upper leaves. In plots intensively infected by the purple seed stain, groups of infected plants can be seen. Their upper leaves are yellow and necrotic, or have already been shed, while the lower leaves are still green.

Symptoms on the stem and leaf petioles are manifested in the form of reddish-purple, slightly sagging spots, a few millimeters in length. The infection of leaf petioles causes the leaf laminas to drop off, while the infected petioles remain on the stem. Round, reddish-purple spots develop on the pods of highly sensitive soybean genotypes, becoming dark purple with age.

Symptoms of soybean seeds are quite distinctive and easily recognizable. The seed coat is partially or entirely colored with shades of pink, from light purple to dark purple color (Figure 12.3), while cotyledons retain their natural color. Seeds may also be infected, but showing no visible external symptoms. Cotyledons on young seedlings become wrinkled and dry, acquiring a dark purple color and falling off prematurely. From cotyledons the infection can spread to the stem, on which necrotic rings are formed. The infected young soybean plants wilt and die. In hot and humid weather conditions, a velvety, grey-whitish cover consisting of mycelia, conidiophores and conidia of the fungus develops on the surface of dead seedlings.

Causal organism, biology and epidemiology

The purple seed stain is caused by *Cercospora kikuchii* (**T. Matsu. & Tomoyasu**) **Gardner** (syn. *Cercosporina kikuchii* **T. Matsu. & Tomoyasu**). The fungus' mycelium is branched and septate. Young hyphae are hyaline, becoming light brown with age. They develop in intracellular space of plant tissues, causing cell necrosis. On soybean stems, petioles, leaves and seeds, in addition to spot symptoms, the parasitic fungus forms a stromatic layer, in which conidiophores and conidia develop. The stroma cover the surface of infected organs and they form below the seedcoat, with conidiophores emerging on seed surface, usually in groups of 3 to 20. Conidiophores are long, non-branched and septate. They are thick and gray-brown at the base, and narrow on the top, slightly curved and hyaline. Conidia are elongated, with curved and pointed tips. They have a large number of septa (up to 49). Their size varies considerably depending on the

climate and plant organ on which they were formed. In Japan, the dimensions of *C. kikuchii* conidia are 4-5 x 70-164 µm, with up to 22 septa, and in North Carolina (USA) they are 1.3-6.1 x 38.8-445 µm, with 2 to 49 septa (Sinclair and Backman, 1989).

C. kikuchii sporulates abundantly in conditions of high relative humidity, at temperatures from 23° to 27°C (minimum 18°C). Conidia germinate in water drops, producing one or more initial hyphae.

Primary inoculum comes from harvest residues from previous year and soybean seed, on which the parasite overvinters after the vegetation period. Next year, the fungus fructifies on infected harvest residues. The primary infection occurres at the time of soybean flowering. Secondary infections are caused by conidia, which successively form through the vegetation on leaves, petioles and stems.

When infected seeds are sown, mycelia of the fungus spread from the seedcoat to cotyledons and synchronize their development with seedling growth. Seedlings usually manage to survive, but cotyledons shrivel, dry up and fall off earlier. The infected seedlings become covered with conidiophores and conidia which subsequently infect soybean plants. The parasite is transmitted from one region to another by infected seeds.

The occurence and expansion of soybean purple staining are stimulated by warm weather (28 to 30°C) and longer periods of high relative air humidity. Significant differences have been observed among soybean genotypes in their sensitivity to the disease. Cultivars resistant to leaf and stem spot do not have to be resistant to seed infection and vice versa.

Figure 12.3

***Cercospora kikuchii* - Purple staining of the seed coat** *(photo: M. Vidić and R. Jevtić)*

Control

Less sensitive and more resistant cultivars are recommended for commercial production. Soybean should not be planted in the same field in two consecutive years or after other legumes. High-quality and healthy seed should be used, or it should be treated before use with appropriate fungicides to prevent the infection of seedlings. Fungicides based on benomyl, captan, thiram and carbendazim are recommended for seed treatment. If necessary, particularly in the case of soybean seed production, fungicides based on benzthiazole should be applied at the time of pod forming. Depending on infection severity, two additional treatments may be performed at 14- and 21-day intervals. After harvest, harvest residues should be destroyed or deep plowed.

POWDERY MILDEW

Distribution and economic significance

The powdery mildew was first detected on soybeans in the early 1920s in Germany (Wahl, 1921, as cited in Aćimović, 1988). The disease has also been found in Brazil, Canada, China, India, Puerto Rico, South Africa and the USA (Sinclair and Backman, 1989). It has not been registered in Serbia so far. It belongs to the group of economically important soybean diseases. Intensive disease attacks on sensitive genotypes could reduce their yields by 10 to 35% (Dunleavy, 1980; Phillips, 1984).

Symptoms

The powdery mildew is primarily a foliar disease, although its symptoms may occur on all aboveground parts of the soybean plant. White, powdery patches can already be seen on cotyledons. Those are the mycelium and conidia of the parasite. On the upper leaf surface, whitish powdery pathes are formed, which merge and cover a large part of the lamina. Later on, the infected area becomes grayish, with small black dots - cleistothecia. Margins of the infected leaf curl; the leaf gradually dries up and falls off prematurely. The symptoms spread from lower to upper leaves, simultaneously infecting the stem, branches and pods, particularly in the case of susceptible soybean genotypes. Less sensitive cultivars exhibit significantly milder symptoms. Their leaves only show small whitish spots, inside which the parasitic fungus has fructified. Chlorotic spots occur along leaf nerves, which in some instances slightly enlarge and become necrotic. Resistant cultivars exhibit a hypersensible reaction, in

the form of tiny chlorotic spots on the leaf and necrosis of leaf nerves. Some soybean cultivars are sensitive to the disease at the seedling stage, while acquiring resistance later on.

Causal organism, biology and epidemiology

The causal fungus is *Microsphaera diffusa* **Cke.** & **Pk.** (syn. *Erysiphae polygoni* **DC**., *E. glycines* **Tai**, *Microsphaera* sp.). Long after the occurrence of the powdery mildew, it was believed to be caused by a fungus from the genus *Erysiphae* (*E. polygoni, E. glycines*). The confusion occurred because immature cleistothecia were used for identification. Namely, only fully mature cleistothecia have branched appendages on their tips, a reliable morphological feature indicating the genus *Microsphaera.*

This fungus belongs to the group of obligate parasites, whose hosts are also the bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), mung bean (*Vigna radiata*, *V. unguiculata*), wild soybean (*Glycine* spp.) and some species of the families *Caprifoliaceae* and *Solanaceae* (Sinclair and Backman, 1989).

On the surface of infected soybean parts, *M. diffusa* forms a septate, well-developed mycelium which is white initially and light gray later on. The fungus penetrates the epidermal cells of the host by means of haustoria. Short, simple conidiophores produce chains of conidia - oidia. They have rounded sides and flat ends (barrel shaped), and their dimensions are 27.7 to 54.1 x 17.1 to 21.1 µm (Sinclair and Backman, 1989). In the telemorphic stage, the parasite forms cleistothecia. Young cleistothecia are light yellow, acquiring a darker brown color with age. Appendages that grow on their surface (about 50) remain unbranched until cleistothecia are fully mature. Mature cleistothecia are completely black, spherical, 85-120 µm in diameter. Apnendages are several times longer than cleistothecium diameter, and on their top they branch dichotomously five to six times. Cleistothecia contain pear-shaped asci, which in their turn usually contain up to six askospores (Paxton and Rogers, 1974).

Ascospores perform the primary infection in the spring, and the fungus spreads by conidia during the vegetation period. Cool weather favors the development of powdery mildew. Resistance to the disease is controlled by a single dominant gene (Rmd), which has been incorporated in several commercial cultivars (Demski and Phillips, 1974).

Control

In regions with intensive attacks of powdery mildew, it is recommended to grow resistant cultivars. If economic viability can be achieved, the disease can be effectively controlled by foliar fungicides on the basis of benomyl, thiabendazol and chlorothalonil.

RUST

Distribution and economic significance

Rust was first observed and described at the beginning of this century on soybeans in Japan (Aćimović, 1988). It is a major soybean disease in the eastern hemisphere. Its area of distribution stretches from Japan in the east to India in the west and from Russia in the north to Australia in the south (Ford and Sinclair, 1977). The disease is also present in some countries: Brazil, Puerto Rico, Colombia (Sinclair and Backman, 1989). So far it has not been detected in Serbia. In recent years, its importance in the USA kept growing steadily.

Damages caused by the disease are considerable, especially in the tropical and subtropical zones. Yield reductions range from up to 40% in Japan, 10 to 50% in southern China, 10 to 40% in Thailand and 23 to 90% in Taiwan (Sinclair and Backman, 1989).

Symptoms

Rust symptoms typically occur on soybean leaves, but they also occur on stems, pods and leaf petioles. First signs of the disease are small, chlorotic, water-soaked spots on leaves. Their color is gray or brown. The spots increase in size, forming polygonal infected surfaces (2 to 5 mm^2) restricted by leaf nerves. As the spots age, their color becomes yellowish-brown to reddish-brown. After that, uredosori form within the spots, more frequently on the upper side than on the underside of leaves. Those are oval protuberances beneath the epidermis, filled with uredospores of the parasite. Uredosori are concentraed in groups, forming clumps which are the most characteristic symptom of the rust, both on the leaf and the other parts of soybean plant. After maturation, lesions appear in the epidermis and uredosori release rust-brown powder, i.e., uredospores of the fungus. Soybean leaves affected by rust turn yellow, wilt and drop off, accelerating plant maturation and adversely affecting the yield.

Causal organism, biology and epidemiology

Soybean rust is caused by *Phacopsora pachyrhizi* **Syd.** & **P. Syd** (syn: *P. sojae* (**P. Henn**.) **Sawada**, *P. vignae* (**Bres**.) **Arth**., *Uredo sojae* **P. Henn**., *Uredo vignae* **Bres**., *Uromyces sojae* (**P. Henn**.) **Syd**. and *Uromyces sojae* **Miura** non **Syd**.). This fungus is an obligate parasite. In addition to soybeans, its hosts are a number of other legumes (Sinclair and Backman, 1989).

P. pachyrhizi regularly passes through the uredostage on soybean plants. Spermagonia and aecidia stages have not been observed, while the teleutostage is encountered rarely. The fungus has no secondary host.

Within the spots on the underside of leaves, the fungus successively forms uredosori for a few weeks. They are located below the epidermis, in small or large groups. They are spherical in shape, 100-200 µm in diameter and have a reddish-brown, rusty color when mature. Uredosori are rarely formed on the upper side of leaves and these are considerably smaller than those on the leaf underside.

Uredospores are oval or egg-shaped, at first hyaline and then yellowish-brown. Their size is highly variable, depending on the host plant and external conditions. The size varies within the range of 18-45 x 13-28 µm (Sinclair and Backman, 1989). They are formed on short stalks, and after maturation are released through a central pore.

Teleutosori have been observed only on several occasions on soybeans in Japan and Taiwan, although they form more frequently on other host plants (Sinclair and Backman, 1989). Teleutosori form between uredosori or along the edge of the spot, usually on the underside of leaves. They are round or irregularly shaped, 150-250 µm in diameter. While young, their color is light brown. As they age, their color turns from dark brown to completely black. Teleutospores are arranged in three to five layers in teleutosori. Teleutospores are unicellular, slightly elongated, with yellowing-brown interior and dark brown cell wall. Although they are capable of germination and can produce basidia with basidiospores, their role in the life cycle and epidemiology of *P. pachyrhizi* is not known.

Warm and humid climatic conditions favor rust occurrence and spread of soybeans. Uredospores germinate in a temperature range from 8 to 28°C (optimum from 15 to 25°C), forming the hypha on which subsequently develop appresoria. The appresorium gives rise to the infection hypha which grows and penetrates the epidermal cells of the leaf. In the leaf epidermis and mesophyll, the fungus grows intercellularly, penetrating adjacent cells by means of haustoria. Under conditions of optimal temperature for the development of infection (20 to 25°C) spots on the leaf appear five days after inoculation. In the subsequent 9 to 10 days, there follows uredosoral differentiation, and in the next 9 to 10 days uredospores start to be released (Sinclair and Backkman, 1989).

There are several physiological races within the *P. pachyrhizi* population. Four genes have been identified (Rpp1, Rpp2, Rpp3 and Rpp4) that control rust resistance. These genes have been incorporated in several commercial cultivars of soybean.

Control

Resistant cultivars are recommended for regions in which the disease occurs intensively. Rust can be effectively suppressed by fungicides. However, several treatments are required forh full protection of soybean crop, which considerably increases the cost of production.

ANTHRACNOSE

Distribution and economic significance

Anthracnose was first described as a disease of soybeans in 1917 in Korea (Hemmi, 1920). It occurs in most soybean-growing countries. It was found in the U.S.A. (Wolf and Lehman, 1924) and Canada (Conners and Savile, 1944). In South America, athracnose was reported on soybeans in Brazil (Machado and Carvalho, 1975), Columbia (Patino, 1967) and Jamaica (Martyn, 1942). In Africa, anthracnose was found on soybeans in Senegal (Girard, 1979) and Cameroon (Bernaux, 1979). In Asia, the disease is widespread in Taiwan (Sawada, 1922), China (Ling, 1948), Malaysia (Wong et al., 1983), Bangladesh (Rahman and Fakir, 1985), India (Lambat et al., 1969) and the far-eastern part of the former Soviet Union (Nelen and Žukavskoja, 1968). It is present in Australia (Pakbery and Lel, 1972).

In Europe, anthracnose occurs in France (Signoret, 1975), Italy and Spain (Érsek, 1979). In our country, it is present in all soybean-growing regions in the Vojvodina Province (Robotić, 1981; Jasnić, 1983). The intensity of infection depends on weather conditions, location and cultivar.

Under favorable weather conditions, anthracnose may cause significant economic damage in soybeans, reducing the yield by 16 to 26% in the U.S.A. (Backman et al., 1982), 30 to 50% in Thailand and 100% in some parts of Brazil. Anthracnose can reduce seed germination by 25% and it may kill up to 80% of emerged seedlings (Nickolson, 1973). The disease is dangerous and harmful in our country too. According to N`Dzi (1994), it reduces plant height by 6%, seed number per plant from 9 to 22%, and yield between 8 and 23%. The same author found a disease intensity of 15% on soybeans grown in the vicinity of Bečej in 1989.

Symptoms

Anthracnose symptoms may occur throughout the soybeans growing season, from emergence to maturity, and on all plant parts - seeds, seedlings, stems, leaves and pods. In the local ecological conditions, disease symptoms typically occur in the second part of the season. Symptoms are usually manifested on the stem and lateral branches, and rarely on pods. First symptoms are irregularly shaped, sunken, dark brown spots on the stem and branches. In humid weather, numerous black fruiting bodies (acervuli) form within the spots, covering their entire surface, so that they become black. The acervuli develop numerous minute black spines (setae), which protrude from the acervuli. The spots usually coalesce, forming irregularly-shaped black areas that cover larger part of the surface of the stem and branches (Figure 12.4a,b). On the pods, there occur dark brown, irregularly shaped lesions, which later on become covered with numerous acervuli. In humid weather, the infected pods may rot.

Seeds in heavily infected pods are small, wrinkled, and frequently covered with a gray mycelium, within which acervuli are formed. The infected seeds become rotten. The less infected plants do not show disease symptoms on seeds and pods.

Figure 12.4

***Colletotrichum* spp.** *(photo: M. Vidić and R. Jeftić)*

a) Anthracnose symptoms on the stem and pods, b) stem spot (detail)

Disease symptoms on leaves occur in the form of small or large reddish spots, which expand and coalesce, becoming grayish-brown later on. Inside the spots are formed black acervuli. Similar spots form on leaf petioles. They make a ring around the petiole. Stronger infection causes premature drying and falling off of leaves.

Anthracnose also attacks soybean seeds, reducing their germination capacity. If infected seeds are sown in regions with humid and warm springs, disease symptoms occur on seedlings too. Sagging, dark brown necrotic spots develop on cotyledon leaves. The spots tend to expand to the hypocotyl. When necrotic spots form a ring around the hypocotyl, the seedling dries up and wilts. It happens often that seedlings become light brown, start to rot and die. Numerous blackish acervuli form on infected seedlings. Large-scale destruction of seedlings makes the crop stand too thin.

If infected seedlings survive, plants become systemically infected, often showing no visible disease symptoms. The symptoms become manifest under favorable weather conditions, i.e., at the time of soybean maturation. Infected plants are stunted in growth, maturing too early and forming small and poorly filled grains.

Causal organisms, biology and epidemiology

Anthracnose of soybeans is caused by several fungi from the genus *Colletotrichum*: *Colletotrichum truncatum* (**Schw**.) **Andrus** et **Moore** (syn. *C. dematium* (**Pers** ex **Fr.**) **Grove** var. *truncatum* (**Schw**.) **Arx**., *C. dematium* var. *truncata* (**Schw**.) **Arx** and *C. glycines* **Hori**). The teleomorphic stage has not been observed in these species. Other species from this genus are: *C. destructivum* **O'Gara,** with the teleomorphic stage *Glomerella glycines* (**Hori**) **Lehman** et **Wolf**, *C. gloeosporioides* (**Penz**) **Sacc**., with the teleomorphic stage *G. cingulata* (**Ston**) **Spauld**. et **Schrenk,** and *C. graminicola* (**Ces**.) **Wilson,** whose teleomorphic form is unknown. All species except *C. graminicola* subsist, overwinter and spred via infected seeds.

Of the species listed above, the following occur in our country: *C. dematium* (Jasnić, 1983) and *C. destructivum* (Robotić, 1981). Observations have shown that the species *C. dematium* occurs more intensively and in more soybean-growing regions in the Vojvodina Province than *C. destructivum* (N`Dzi, 1994).

Soybean diseases caused by these two species are difficult to distinguish, because their symptoms do not provide reliable indication about the species of fungus.

The fungus *C. dematium* forms a grayish-white, septate mycelium. Within the mycelium, there collect black tufts of irregular shape, which frequently coalesce into larger groups - stromata. They are rounded, elongated or irregular in shape, with the average size of 150 x 80 µm (Jasnić, 1983). Black-colored fruiting bodies, acervula, are produced in the stromata, often in groups. They are egg-shaped, elongated or hemispherical to truncate conical in shape, with numerous short or long black hair-like, needle-shaped spines - setae. The setae differ in length, pointed at the top, with 3 to 5 transverse septa, the size of 50-155 x 2.5-5 µm. Profuse hyaline conidia develop in the acervuli. The conidia are unicellular, curved, with more or less pointed or rounded tips and a few drops of oil. The size conidia formed in the culture was 18.75-23.75 x 3.75-4.25 µm (Jasnić 1983). Tiffany and Gilman (1954) claimed that the size of conidia of the fungus *C. dematium* formed on soybeans was 17-21 x 3.5-4.5 µm. Conidia germ in one or more initial hyphae, on whose ends there form dark, irregular-shaped, ovoid or rounded appresoria. The fungus does not form the perfect stage.

C. destructivum forms a white mycelium that later turns pink (Robotić, 1981). The fungus produces numerous black fruiting bodies - acervuli. They are oval to semiglobose in shape, their size is 45-80 µm, and they have a large number of setae. The setae are needlelike, light to dark brown, with 1 to 4 transversal septa, of the size of 85-250 x 4.5 to 7.5 µm. A large number of hyaline, unicellular, cylindrical conidia with rounded ends forms in the acervuli. The size of the conidia is 14.5 to 25.3 x 4.1 to 6.3 µm.

The fungus develops the perfect stage, *Glomerella glycines*, which in its turn forms perithecia with asci and ascospores. The perithecia are black, usually pyriform and less frequently spherical in shape, their size ranging from 225 to 280 µm. A number of hyaline, spindle-shaped asci form in the perithecia. Each of them contains

eight unicellular, slightly curving and tapering ascospores. The size of the asci is 83-104 x 1.3-8.5 µm, of ascospores 25.6-31.7 x 4.8-6.7 µm (Robotic, 1981).

The epidemiology of *C. dematium* has been thoroughly studied in our country. Infected soybean harvest residues play a significant role in the overwintering of the parasite and its dispersal during growing season. The fungus overwinters in harvest residues in the form of the mycelium. Mycelia are also seen in soybean seeds.

In the subsequent year, acervuli with conidia form on harvest residues, which then infect soybean plants. In our agroecological conditions, acervuli are formed in the period from late February, during mild winters, to the first 10 days of July. The formation intensity peaks from the first decade of April to the end of May (N`Dzi, 1994). Temperature is not a limiting factor, since the fungus produces acervuli and releases conidia in a wide temperature interval from 10° to 30°C, with the optimum temperature from 25° to 28°C. Conidia germinate at temperatures between 12 and 38°C (N`Dzi, 1994). The only limiting factors for the development of soybean anthracnose in our country are the amount and distribution of rainfall. For infection to be established, high relative air humidity, of at least 70%, is required for a period of 12h. Conidia are released from acervuli passively, in the form of a viscous drop, and they are carried to host plants due by raindrops or insects.

Plants can be infected by the fungus from emergence to maturity, but the period from flowering to grain filling is critical (N`Dzi, 1994). In the local conditions, infection can be induced earlier, when conditions are more favorable for germination of conidia. The mycelium of the fungus is endophytic. The spread of the mycelium is limited in young plants. When plants reach the flowering stage, the mycelium spreads to the stem, leaf petioles, leaves and pods, invading seeds at the stage of seed formation. In systemically infected plants, symptoms are not noticed until maturity, when the infected parts become covered with acervuli. Disease symptoms become evident early only in humid and warm summers. In our country, secondary infection is not important for the distribution of the fungus. Seed infection also has no significance in the dissemination of the fungus, as in regions with warm and humid springs infected seedlings simply deteriorate and die. The rare surviving, systemically infected plants may serve as a source of secondary infection.

Differences exist in the sensitivity of soybean genotypes to *C. dematium*. Investigations conducted in our country have shown that out of a total of 203 cultivars and lines tested under conditions of natural infection, 107 were susceptible and 96 demonstrated no symptoms of anthracnose. Early cultivars and lines where more resistant: NS-1, NS-L-32, NS-L-34, NS-L-59, Altona, NS-13, etc. (N`Dzi, 1994). Tošić et al. (1986) reported that high sensitivity to the parasite was exhibited by the cultivars Zvečka, NS-10, Evans, NS-9, OS-45, Aura, Hodgson and Hark.

The epidemiology of *C. destructivum* is similar to that of the previous species. However, it is characteristic for this fungus that it can infect only old soybean plants (after phenophase R7) (Sinclair and Backman, 1989).

Control

Agrotechnical measures are usually recommended for anthracnose control: use of healthy seed, crop rotation, removal or plowing under of harvest residues, and use of soybean cultivars resistant to the parasites.

Chemical measures are used for treatment of seed crops. A combination of fungicides based on benomyl and mancozeb (0.75 + 3 kg/ha, respectively) at the stages of flowering and the beginning of grain fill provides satisfactory results in soybean protection against anthracnose. Seed treatment with preparations based on TMTD in the amounts of 300-400 g/100 kg of seed brings satisfactory results (Nelen and Zhukovskaya, 1968). Preparations based on benomyl and mancozeb are also efficient. These preparations do not affect significantly the formation of nodular bacteria. According to data from India, seed treatment with preparations based on thiram, captan and difolatan reduces seed infection and increases seed germination rate (Khare and Chacko, 1983).

STEM CANKER

Distribution and economic significance

Soybean stem canker was first described by Welch in 1947, as a distinct disease of soybeans in Iowa, USA. Previously, the symptoms of this disease have been confused with symptoms of stem and pod blight caused by other species of the genus *Diaporthe*. In a few subsequent years, the disease spread to northern and central parts of the United States (Minnesota, Illinois, Indiana and Ohio) and Ontario in Canada (Crall, 1950; Andrews, 1950; Kernkamp and Gilbert, 1951, Hildenbrand, 1951). In the early 1980s, the disease began to occur suddenly in southern states (Maryland, Alabama, Tennessee, Carolina) where it had not been reported previously (Backman et al., 1981; Hilty, 1981; Keeling, 1982; Krause and Fortnum, 1983; Kulik, 1985). In South America (Brazil and Argentina) the southern type of stem canker, caused by *Diaporthe phaseolorum* var. *meridionalis*, was present as the only disease form for a long time, until 1999, when the northern type of stem canker, caused by *Diaporthe phaseolorum* var. *caulivora*, was confirmed for the first time in Argentina (Pioli et al., 2003).

The stem canker has been reported in Europe. In Serbia, it was described for the first time in Vojvodina in 1980 (Jasnić and Vidić, 1981). In the period from 1981 to 1984, it started to spread rapidly and intensively in most soybean-growing regions. In years favorable for the development of the stem canker, the percentage of infected plants in some locations in the Vojvodina Province exceeded 25% (Jasnić and Vidić, 1985).

Stem canker is counted among the economically most important soybean diseases. Its harmfulness is due to the time of disease occurrence - it wilts and dries soybean plants at the beginning of pod formation, so that seeds are either missing or small and poorly filled because of interrupted seed filling. The earlier occurrence of diseases symptoms cause higher and late infections considerably lower damage.

According to Athow and Caldwell (1954), infected plants yield is 60% less than healthy plants, while Frosheiser (1957) estimated the yield reduction at 13%. Grau (2006) reported that an intensive disease attack that occurred in the State of Wisconsin (USA) caused a drastic reduction in soybean yield, between 50 and 80%. Our data indicated that the yield of soybean is reduced by 50 to 62%, depending on the cultivar and when plants were infected at the stage of pod formation; plants with milder symptoms suffered a lower yield reduction, between 9 and 20% (Vidić and Jasnić, 1988a). Late cultivars exhibit higher susceptibility to the disease than early ones, as manifested by more serious symptoms, a higher percentage of infected plants, and considerably higher yield losses. Black spots on the stem do not affect the synthesis of proteins and oil in grain (Vidić and Jasnić, 1988a).

Symptoms

First signs of the disease can be seen on plants at the flowering stage. Under the local climatic conditions, the disease usually occurs late, in the first half of August, although in some years it occurred in the firs decade of July, in the phenophases of pod forming (R3 and R4).

Initially, small, slightly sunken, elliptical or irregularly shaped spots may be observed on one or more basal nodes of the soybean stem. The spots subsequently spread to adjacent internodes, reaching a length of 6 to 10 cm and finally girdling the stem (Figure 12.5a). Stem tissue inside the spots becomes necrotic, the transport of water and nutrients is interrupted, and the infected plants wilt and dry up. At the beginning of disease development, the spots are grayish-brown in the center, with a thin redish brown border. As the infected tissue ages, it becomes darker and darker, from dark red, chocolate brown, to completely black. The black color of the spots is conspicuous against the green stems of surrounding healthy plants. This characteristic symptom clearly distinguishes the stem canker from the other diseases of soybean stem. Similar symptoms can occur on lateral branches. Top leaves of infected plants show chlorosis between veins, followed by a gradual loss of turgor. The leaves wilt, become shriveled and in the end dry up completely. The dry leaves do not fall off immediately but remain hanging on the infected plant, which makes them easily observable (Figure 12.5b). In the case of weak infection, individual infected plants are scattered across the plot. In the case of intensive infection, infected plants make small or large groups. Pods of infected plants dry up and remain empty, or small and insufficiently filled grains manage to be formed.

Figure 12.5

Diaporthe phaseolorum* var. *caulivora *(photo: M. Vidić)*

a) A symptom on the stem. b) Prematurely wilted plants

Causal organism, biology and epidemiology of the parasite

The causal agent of the soybean stem canker is *Diaporthe phaseolorum* (**Cke** et **Ell**.) **Sacc**. var. *caulivora* **Athow** et **Caldwell**. However, disease symptoms exhibited by this parasite had been attributed for a long time to the fungus *Diaporthe phaseolorum* (**Cke** et **Ell**.) **Sacc**. var. *sojae* (**Lehman**) **Wehm**, the causal agent of the pod and stem blight and Phomopsis seed decay.

Studying soybean diseases, Welch and Gilman (1948) found significant differences in the pathogenicity and morphological characteristics between the fungus *D. phaseolorum* var. *sojae*, the causal agent of the pod and stem blight, and *Diaporthe phaseolorum* (**Cke** et **Ell**.) **Sacc**. var *batatatis* (**Harter** et **Fielld**) **Wehm** which, according to these authors, also causes the soybean stem canker. This fungus had been previously known as a parasite of sweet potato (*Ipomoea batatas*). Later, based on comparative tests of soybean and sweet potato isolates, Athow and Caldwell (1954) found that these isolates significantly differed in some morphological and pathogenic characteristics, so they concluded that those were two different varieties of the fungus *D. phaseolorum*. They proposed for the causal agent of the stem cancer to be called *Diaporthe phaseolorum* (**Cke** et **Ell**.) **Sacc**. var *caulivora* **Athow** et **Caldwell**, the name which persisted to date. Recent studies have indicated that there are minor morphological differences between the causal agents of stem cancers from the northern and southern U.S. states.

Therefore, now it is considered that this species has two “formae specialis”: *D. phaseolorum* f. sp. *caulivora* from the north and *D. phaseolorum* f. sp. *meridionalis* from the south (Morgan-Jones, 1989).

When infected parts of a stem are placed in a humid environment, numerous fruiting bodies, perithecia, form within the spots. Perithecia form in the epidermis of the infected stem. Usually they cluster in large aggregations, forming stromatic structures in the shape of small black bumps on the stem. Elongated beaks break through the epidermis, forming a black outgrowth on the surface of the stem, which can be observed by the naked eye.

On potato agar (PDA), the fungus grows rapidly and forms a dense white aerial mycelium. The substrate mycelium is yellowish-white, becoming yellowish-brown with age (Jasnić and Vidić, 1983). Eight to 10 days later, the mycelium starts to produce black, irregularly shaped stromata 1 to 5 mm in size. Numerous perithecia form in the stromata, usually 5 to 8 in each. The number of perithecia per stromatic aggregation differs considerably, from 1 to 25 or 8.2 an average. Individual perithecia can be seen rarely. The perithecia are black, their spherical base embedded in the cortical tissue, with a long beak that protrudes from the mycelium. The size of the spherical part is in the range of 170-450 x 180-500 µm. The beak length is between 320 and 1500 µm, the width at the base 30-130 µm and 35-65 µm at the top (Jasnić and Vidić, 1983).

Asci with ascospores are formed in the perithecia. Ascospores are released through the opening of the beak, forming pink-colored, sticky drops. The asci are elongated elliptical, with thin walls which become thicker at the base of the ascus. The ascus size is 22.5-35.0 x 5.0-8.7 µm. Inside the ascus, eight elliptically elongated, bicellular ascospores are formed. Ascospores are slightly taper in the middle, near the partition wall. Each cell contains two drops of oil. The askospore size is 8.7-12.0 x 2.5-3.7 µm (Jasnić and Vidić, 1983).

When cultivated on acid substrates such as Czapek and ASS (acid synthetic agar), after 10 weeks, the fungus forms, in addition to perithecia, the anamorphic stage, i.e. pycnidia with pycnospores. Pycnidia are spherical in shape, black-colored, with a diameter of 170-230 µm. Only A-conidia (α konidia) form in the pycnidia. They are unicellular, elongated elliptical in shape, with rounded ends, each containing two drops of oil. Konidia size is 6.2-10.0 x 2.5-3.7 µm. B-conidia (β konidia) do not form in the pycnidia (Jasnić and Vidić, 1983). Frosheiser (1957) reported also that *D. phaseolorum* var. *caulivora* forms pycnidia with A-conidia on a clover substrate. However, this author did not report the presence of B-conidia, unlike Timnick et al. (1951) who had found that this fungus produces pycnidia with A- and B-conidia. Pycnidia are rarely produced in nature (Sinclair and Backman, 1989).

The fungus overwinters and survives from one year to another on harvest residues and in soybean seeds. Harvest residues (parts of stems, pods and roots) play an important role in the epidemiology *D. phaseolorum* var. *caulivora*.

They serve as substrate for the formation of perithecia with asci and ascospores which infect soybean plants the subsequent year. The development of perithecia, germination of ascospores and infection of soybean plants require adequate moisture and temperature. Rainfall amount and schedule in the course of growing season determine the time of occurrence of perithecia and the dynamics of release of ascospores. The perithecia develop and release ascospores in the temperature interval from 10 to 27°C, the optimum temperatures ranging from 20 to 25°C. Ascospores germ in infection hyphae at temperatures from 10° to 32.5°C. The optimum temperature for the germination of ascospores is 22.5°C (Vidić and Jasnić, 1988b). Under the local conditions, no formation of perithecia and pycnidia has been observed in the course of the growing season. In exceptional cases, perithecia can be formed on the root and root crown, if excessive rainfall precedes soybean maturation. Formation of pycnidia on soybean plants has been observed in southern states in the U.S., however, they have no importance in the epidemiology of the fungus (Sinclair and Backman, 1989).

Perithecia form on overwintered harvest residues in the spring, usually in late May and the first half of June. Release of ascospores starts 5 to 10 days after the development of perithecial beaks, and it continues throughout the growing season, after heavy rains. Ascospores exit perithecia through an opening in the beak, where they form a viscous drop, and they are carried to soybean plants by rainydrops and wind. Infection is induced through leaf laminae and petioles, as well as through lesions in the stem (Vidić and Jasnić, 1988b). Infection can be induced from emergence till maturity, but soybean plants are most sensitive from the phenophase of flowering to the beginning of grain filling. Depending on maturity group, soybean cultivars reach these phenophases in July and August. Therefore, if heavy rainfalls occur in July and the first half of August, intensive attacks of the disease can be expected (Vidić, 1987).

Soybean seed may also be infected by the fungus (Frosheiser, 1957, Peterson and Strelecki, 1965; Kmetz et al., 1974, 1978, Vidić, 1987; Vidić and Jasnić, 1990), but contradictory opinions are voiced about the role of infected seeds in the transmission of the parasite from year to year. According to Hildebrand (1956) and Sinclair and Schurtleff (1975), infected seeds do not affect the spread and intensity of parasite infection in the field, but they serve as a vehicle for the fungus to travel large distances. However, when the disease began to spread in the southern United States, some researchers started to claim that infected seeds play an important role in the spread of *D. phaseolorum* var. *caulivora* (Hobbs et al., 1981; Backman et al., 1985). According to our results, *D. phaseolorum* var. *caulivora* is capable of infecting soybean seeds only after pod formation of soybean plants. The parasite is not transferred from the stem to seeds in the process of grain filling, but the infection occurs via the pods, by airborne ascospores. Infection of seeds neither affects the emergence and spread of the parasite in the subsequent year, nor it has a significant effect on germination. It only affects negatively the energy of germination. The presence of the parasitic fungus in the seed may cause seed rot after planting and damping off of soybean seedlings. Infected seedlings and rotten seeds act as the inoculum for secondary infections,

because reproductive organs of the fungus develop them during growing season. Investigations have indicated that, in the local agroecological conditions, the seed does not play an important role in the epidemiology of *D. phaseolorum* var. *caulivora* (Vidić and Jasnić, 1990).

A three-year field trial, which included 60 soybean cultivars and lines, has shown that there existed large differences in suscebility to *D. phaseolorum* var. *caulivora* among the tested cultivars and lines (Vidić et al., 1990). Under conditions of natural infection in the field and inoculation with ascospores in the growing shed, the reaction of cultivars and lines to the pathogen depended on their maturity groups. The earliest genotypes were less susceptible to the parasite's attack and exhibited less severe symptoms. Late cultivars exhibited a high sensitivity, manifested by severe symptoms. The lower sensitivity of the early cultivars to the parasite is considered to be due to a discrepancy between the time of ascospores release and the phenophases at which soybean plants are most susceptible to the parasite. Our investigation indicated that soybean plants are most sensitive during the stages of flowering and pod formation (Vidić and Jasnić, 1988c). The early cultivars (maturity group 00) recorded a very low percentage of infected plants, up to 3.7%, and only mild symptoms, without wilting, which indicated that these cultivars possess a high level of resistance. The Chinese cultivar Feng-Show-10 showed highest resistance in this maturity group. In the maturity group 0, there also were no prematurely wilted plants, and the percentage of plants with spots on the stem was low, ranging from 2.5 to 5.8%. In the maturity group I, first wilted plants could be seen. In this group, the cultivars SFR-100 and Mandarin (Ottawa) were distinguished for reduced sensitivity. In the maturity group II, very good field resistance was exhibited by the late cultivars Harosoy-63 and Reiner. All cultivars in the maturity group III proved to be highly susceptible under the local agroecological conditions (Vidić et al., 1990).

Studies of the pathogenicity of a large number of isolates of stem canker from different parts of the United States and Canada showed that there existed significant variability among the tested isolates. Based on the reaction of six soybean cultivars, Keeling (1984) differentiated six physiological races of the fungus. Races 1, 2 and 3 that originated from the State of Mississippi were labeled as southern races and the race 4 from Ohio, 5 from Indiana and 6 from Iowa as northern races. In that study, the cultivar Tracy M exhibited resistance to the southern races and sensitivity to the northern races. Studies of other authors have led to a conclusion that *D. phaseolorum* var. *caulivora* has at least two races, southern and northern (Mc Gee and Bidle, 1987; Higley and Tashibana, 1987).

A study of Morgan-Jones (1989) indicated that the described races differed not only in virulence for different soybean cultivars but also in some morphological characteristics. The author suggested for the northern and southern races to be considered as "formae specials" or two varieties of the fungus, proposing the name *D. phaseolorum* f. sp. *caulivora* for the northern race and *D. phaseolorum* f. sp. *meridionalis* for the southern race.

The cultivars Tracy and Tracy M, which originate from southern United States, have a specific resistance to the southern race (*D. phaseolorum* var. *meridionalis*), which is controlled by two pairs of major genes (Kilen et al., 1985). Later it was found that the resistance to *D. phaseolorum* var. *meridionalis* is controlled by four major genes, Rdc1 and Rdc2, identified in the cultivar Tracy M (Kilen and Hartwig, 1987), and Rdc3 and Rdc4, identified in the cultivars Crockett, Dowling and Hutcheson (Bowers et al., 1993; Tyler, 1996; Pioli et al., 2003). Regarding the northern race (*D. phaseolorum* var. *caulivora*), which has been observed on soybeans in Serbia, there are no resistant genotypes, but differences were established among genotypes in the suscebility to the disease (Vidić et al., 2008).

A variability study of a large number of *Diaporthe phaseolorum* var. *caulivora* isolates has indicated that there existed a considerable variability in virulence and that the isolates originating from the Vojvodina Province were quite close to the northern race, *D. phaseolorum* var. *caulivora*, while they were substantially different from the southern race, indicating that the existence of different races of the fungus in our country cannot not disregarded (Vidić, 1991; Vidić et al., 1994a).

Control

Control of *Diaporthe phaseorum* var. *caulivora*, the causal agent of soybean stem canker, is done with a combination of agrotechnical and chemical measures. Healthy seed should be used for planting. In some years, earlier sowing may help to completely avoid the infection of early cultivars, and reduce the intensity of disease attack on late cultivars (Vidić, 1987). After harvest, plant residues should be destroyed or plowed under, to reduce the amount of inoculum for the next year. Crop rotation is also an important measure that can reduce the intensity of infection. It is recommended to use cultivars that are less susceptible to *Diaporthe phaseologum* var. *caulivora*.

Chemical measures are rarely used in the control of this parasitic fungus, but they could be applied to protect the soybean seed crop. Best results are achieved with a combination of systemic fungicides based on benomyl (Benlate WP-50) and thiophanate (Enovit M) with a protective fungicide based on mancozeb (Dithane M-45). Best protection against soybean stem canker has been achieved with a combination of Benlate WP-50 and Dithane M-45 in a doses of 0.75 + 3.0 kg/ha, respectively, performed between full flowering (R2) and the beginning of grain filling (R5) (Vidić et al., 1986). If disease attack is low to moderate, satisfactory control is achieved with one or two treatments. In the case of a severe infection, full protection of soybeans cannot be achieved even with several treatments.

POD AND STEM BLIGHT

Distribution and economic significance

The pod and stem blight was first described as a distinct disease of soybeans in 1922 in the U.S. (Lehman, 1922), when it was distinguished from the disease complex caused by various species of the genus *Diaporthe/Phomopsis* on soybean stems, pods and seeds.

Today, this disease is widespread in all regions of the world where soybeans are grown. In addition to the U.S., the pod and stem blight occurs in Canada, Brazil, Guyana, India, Japan, Korea, China, Taiwan, in the Far East part of the former Soviet Union and Egypt (Sinclair and Backman, 1989).

The pod and stem blight is also present in Europe, Russia (Vladimirovsk and Moscow regions) (Kozireva et al., 1982), Hungary, France, Croatia (Cvjetković, 1977). In Serbia, the disease was reported in mid-1980s in Vojvodina (Jasnić and Vidić, 1985) and Central Serbia (Tošić et al., 1986).

The pod and stem blight may cause considerable damage during long, hot and humid summers, which are suitable for the development of the disease. The disease causes early maturation of plants, which affects the yield and quality of seed, seed germination and viability. Seed yield may be reduced by as much as 50%. As the intensity of the disease increases, so does the percentage of seed infection and may range from 25% (Nicholson et al., 1972) to 66% (Kmetz et al., 1978). The infected seed has poor germination and quality, and greatly reduced percentages of oil and proteins. Use of infected seed causes a poor crop stand, i.e., a reduced number of plants per unit area. Depending on weather conditions, the intensity of infection may be some high in our country too. In 1981, for example, a high disease intensity was registered for some soybean varieties in the Novi Sad area, from 17.8% in the cultivar Wells to 38.1% in the cultivar Corsoy (Vidić et al., 1983b).

Symptoms

Symptoms of the disease occur on all above-ground parts of plants, but they are considerably less frequent on leaves. First signs of the disease can be seen on seedlings that germinated from infected seeds. Seedlings may be systemically or locally infected. The systemically infected seedlings are stunted, pale green in color and they usually die soon after emergence. In the case of local infection, cotyledons become covered by pale, light red or brown spots which differ in size. The spots may be lense-shaped or very large, in which case they cover the entire cotyledon. On hypocotyls and below soil surface, there occur reddish-brown stripes. Seedling tops may be deformed or completely necrotized.

Disease symptoms on the above-ground parts occur later. During hot and humid summers, the symptoms are manifested early, at the beginning of pod forming. In hot and dry summer, they occur during plant maturation. The infection takes place much earlier, but disease symptoms are not manifested, as the causal agent remains latent in the plant. Presence of the parasites can be easily checked by isolation from apparently healthy plant parts.

First disease symptoms can be seen in the stem or branches, typically occurring around damaged and injured parts of the stem, broken branches and the places from which bottom leaves with petioles had been thorn off. These areas are covered with numerous black dot-like pycnidia which are arranged in parallel lines (Figure 12.6). During long humid periods in the summer, the stem can be completely covered by pycnidia.

On pods, pycnidia occur in the form of scattered black dots. The heavily infected pods contain deformed seeds, which are wrinkled, with cracks on the seedcoat, poorly filled, partially or fully filled with the whitish mycelium. The viability of such seeds are extremely low. During dry and hot summers, the symptoms are milder. Fewer pycnidia are formed, typically on the basal part of the stem or near nodes. Pods usually remain free of pycnidia. Seeds in slightly infected pods appear normal, but they may be contaminated and have inferior viability. Diseased plants ripen prematurely and form a smaller number of pods that contain small seeds.

Figure 12.6

Phomopsis sojae *(photo: M. Vidić and R. Jeftić)*

Pycnidia in parallel lines on the stem

Causal organism, biology and epidemiology

The causal agent of the pod and stem blight of soybeans is *Diaporthe phaseolorum* (**Cke** et **Ell**.) **Sacc** var. *sojae* (**Lehman**) **Wehm**. (syn.: *Diaporthe sojae* **Leh**.), whose conidial (anamorphic) stage is *Phomopsis sojae* **Leh**.

The parasite had been first described by Lehman in 1922, as *P. sojae*. The same author also found the perfect stage of this fungus and named it *D. sojae* (Lehman, 1923). Wehmeyer (1933) found no significant morphological differences between the species *Diaporthe phaseolorum*, the causal agent of the pod blight of beans (*Phaseolus lunatus*) and the species *D. sojae*, so he determined the form occurring on soybeans as a variety and named it *D. phaseolorum* var. *sojae*.

D. phaseolorum var. *sojae* becomes heterothallic at the end of the growing season (Welch and Gilman, 1948), which forms intra- and intercellular mycelia. On soybean stems, just below the epidermis, the fungus forms a large number of pycnidia. Pycnidia form within black stromatic structures and are usually clustered into more or less homogeneous groups - conidiomata. Conidiomata are usually unilocular, sometimes multilocular, with an opening, ostiole, at the top. Pycnidia are lense shaped, without or with a short neck, whose length is less than 200 µm. The dimensions of pycnidia formed on the stem are 112-229 x 92-204 µm, while those formed on the pods are slightly smaller, 76-121 x 74-117 µm (Dimitrijević and Jurković, 1982). Inside the pycnidia, two types of pycnospores form on simple, non-branched conidiophores: A spores and B spores (stylospores). A pycnospores or A conidia are unicellular, elliptical, hyaline, usually containing with two oil drops, 5.0 to 10.0 x 1.4 to 3.1 µm in size. In a drop of water they germinate into short infection hyphae. B pycnospores or B conidia are unicellular, elongated or threadlike, hyaline and usually curved at one end. They are not able to germinate, and their role is unclear. The dimensions of B pycnospores are 13.7 to 19.0 x 1.2 to 1.9 µm (Dimitrijević and Jurković, 1982). Perithecia form rarely on overwintered basal part of soybean stems, usually singly inside stromatic structures. Those are black, spherical bodies immersed in the epidermis, with long and narrow neck, which breaks through the epidermis and protrude above the stem surface. The dimensions of the spherical part are 185-346 x 148-282 µm and the length of the neck is from 0.25 to 1.5 mm (Athow and Caldwell, 1954). Inside perithecia form a large number of asci, each containing 8 ascospores. The asci are hyaline, elongated-elliptical and slightly curved, 38.0-51.2 x 5.0-10.3 µm in size. Ascospores are bicellular, hyaline, elliptical, slightly constricted in the middle, near the transversal septa, with two drops of oil in each cell. Their size is 9.2-13.5 x 3.3-5.6 µm (Athow and Caldwell, 1954).

The fungus develops well on PDA. When cultured on this substrate, it forms a loose whitish mycelium, which turns yellowish to light brown with age. Within the mycelium, individual dark brown to black stromatic structures are formed. They are irregularly scattered over the entire mycelium, containing individual pycnidia or groups of pycnidia. Perithecia form rarely in the stromata, exclusively in cultures several weeks old, which were exhibited to light.

The parasite overwinters as mycelium in soybean harvest residues or seeds. In the spring, pycnidia with A and B conidia form in masses on overwintered residues. The conidia are exuded in the form of sticky droplets. Infection is transmitted by raindrops, which carry conidia over to healthy plants. In some instances, perithecia are formed on infected plant residues at the beginning of summer. Infection by ascospores is effected by raindrops. The fungus is latent in soybean plants and first pycnidia occur during plant maturation, so that secondary infections are of no importance. Seeds are infected only via pods and they are of no great importance in the spread of the parasite in the field. Seeds act as a vehicle that helps the fungus spread to different parts of the world (Mc Gee, 1983).

In addition to soybeans, *D. phaseolorum* var. *sojae* also parasitizes numerous other crops such as beans, garlic and onions, mung bean, peanuts, tomatoes, okra, etc. (Sinclair and Schurtleff, 1975). Overwintered and infected harvest residues of these crops may serve as a source of inoculums, for transmitting the fungus to soybean plants.

Control

The disease is controlled by cultural practices: crop rotation, deep plowing under of harvest residues, as well as the use of healthy seeds or of less susceptible cultivars. Also, it is recommended to sow late cultivars, or to delay the sowing of early and medium maturing cultivars in order to reduce the seed infection rate (Dhingra et al., 1979; Tekrony et al., 1984).

Chemical control is rarely used, mainly in seed crops. It that a single treatment is recommended at the phenophase of the beginning of pod forming, or another treatment can be performed 14 to 21 days later, using fungicides based on chlorothalonil, benomyl and thiophanate methyl. The chemical treatment of soybean seeds by fungicides that do not affect significantly the activity of nodular bacteria (benomyl, thiabendazole, mancozeb, thiram, captan, etc.).

SCLEROTINIA STEM ROT

Distribution and economic significance

Sclerotinia stem rot (white rot) is a widespread disease of soybeans, which occurs in most countries where this industrial crop is grown. It was first detected and described in Taiwan in 1919 (Sawada, 1919, as cited in Dhingra and Sinclair, 1975). In North America, the disease was first detected in Manitoba (Bisby, 1924, as cited in

Sinclair and Shurtleff, 1975), wherefrom it spread to other parts of Canada and most U.S. states (Sinclair and Dhingra, 1975). Its presence has been confirmed in South America, China, Japan and New Zealand (Sinclair and Dhingra, 1975).

In Europe, the white rot of soybeans was first described in Germany (Pape, 1921; Wahl, 1921) and later in different regions of Russia (Abramoff, 1931; Loukyanovitch et al., 1931; Mikailenko, 1965; Ovčinikova and Sabliovskiy, 1973), Poland (Garbowski and Juraszkowna, 1933), Sweden (Lihnell, 1939), Denmark (Buchwald, 1947), Spain (Graasso, 1962), Hungary (Molnar and Vörös, 1963), France and Romania (Perny and Signoret, 1990).

In Serbia, the disease was explained in some detail in the early 1980s (Vidić, 1982), although it had probably been present on soybeans earlier.

The white rot is potentially the most dangerous soybean disease, as it can cause wilt and rot of developing plants. Damage is especially extensive if the infection occurs in the phenophases of flowering and pod forming. The diseased plants wilt rapidly and rot completely, so that the percentage of yield reduction is almost identical to the percentage of infected plants. Vidić (1982) found that the infection of 28.8% of plants lowers the soybean yield by about 22%. Sinclair and Backman (1989) reported that the intensity of infection of 10% reduces the yield by 0.25 t/ha. Late infections cause less damage because the symptoms occur only on some parts of the plant.

Since a long wet period is needed for the outbreak of infection and spread of the disease, white rot generally occurs in humid regions and in irrigated soybeans. According to Sinclair and Backman (1989), the disease is of no great economic importance in the U.S., except for sporadic local epiphytotics. In Brazil, however, the average annual yield losses reach as much as 15%.

In the opinion of other authors, the occurrence of white rot is mainly local, but damages can be substantial. Hine and Wheeler (1970) reported that in Arizona in 1968, some plots suffered yield reductions of 68%. Molnar and Vörös (1963) registered 7 to 14% of infected plants in irrigated plots in Hungary, while Kurnik (1962) claimed that the soybean crop was completely destroyed in some plots. The disease causes significant damage in soybean fields in the Amur region (Russia), where 25% of plants are infected on average (Ovčinikova and Sabliovsky, 1973). Similar disease intensity was reported for Moldova (Ganja, 1981).

In years with frequent and heavy rainfall in the course of summer, white rot is the most dangerous soybean disease in Serbia. In such years, the intensity of infection exceeds 50% in many plots (Jasnić and Vidić, 1985), causing drastic reduction in soybean yields.

Symptoms

The Sclerotinia stem rot occurs on all aboveground plant parts, in all phenophases of soybean development.

Immediately after plant emergence, wet spots develop on the hypocotyls and cotyledons. They spread rapidly and cover the entire seedling, which then completely rots and becomes covered with white mycelium. Infection of seedlings happens rarely. First symptoms of white rot are usually observed at the time of full flower and pod forming, i.e., when the soybean crop begins to close the rows. Apical leaves of infected plants lose turgor, wilt and gradually dry ap. At first they are grayish-green, but with aging they acquire progressively darker shades of brown. Dry leaves remain on the infected plants, which makes them easily perceived (Figure 12.7a). Symptoms on the stem usually occur 10 to 15 cm above the ground.

Oval-shaped, wet spots pale brown in color develop on one or several internodes. The spots spread quickly to adjacent internodes. The infected stem tissue becomes soft, water-soaked and it gradually rots. Due to the interrupted transport of water and nutrients, plant parts above the infected segment wilt and dry up. In the case of humid and rainy weather, the whole plant rots. Symptoms of lateral branches are identical to those on the stem. The infected plant parts become covered with white cottony mycelium (Figure 12.7b).

Figure 12.7

Sclerotinia sclerotiorum *(photo: M. Vidić)*

a) Severe white rot infection in the field; b) White rot of the stem

The disease has been named after this eye-catching symptom. On the surface of stems and branches, inside the stem cavity and pods, the fungus produces black sclerotia differing in shape and size. During harvest, they fall to the ground or mix with seeds.

Different kinds of symptoms occur on pods and seeds, depending on plant phenophase at the time of infection. In the case of early infection, young pods dry up completely before they manage to form the seeds and such plants produce no yield. If the upper, uninfected plant part manages to form seeds before the basal part of the stem became infected, such seeds remain small because of a curtailed filling period. Pods can also be infected directly. In that case they become wet and soft, with white mycelium protruding from them. The seeds in such pods are flattened, with shrunken and wrinkled seedcoats. Sometimes, such seeds rot completely, and sclerotia are formed in their stead.

Causal organism, biology and epidemiology

The Sclerotinia stem rot (white rot) of soybean is caused by *Sclerotinia sclerotiorum* (**Lib**.) **de Bary** (syn: S. *libertiana* **Fuckel**, *Whetzelinia sclerotiorum* (**Lib**.) **Korf and Dumont**). The fungus causing the disease is a broad polyphage that parasitizes over 400 plant species. Most of them are dicots, although many of them are monocot cultivated plants (Bolton et al., 2006). The fungus can be found in different geographical areas (Purdy, 1979). In addition to soybeans, other major crops host this pathogen such as sunflower, rapeseed and many vegetable crops. It also attacks numerous species from the spontaneous flora.

On the surface of infected plant parts, in the stem cavity and pods, the fungus produces sclerotia which vary in shape and size depending on the place of origin. If formed on plant surface, the sclerotia are spherical and slightly flattened from the side adhering to the plant, several millimeters to one centimeter in diameter. The sclerotia formed in the stem cavity are cylindrical-elongated, several centimeters in length. They are made up of tightly packed, intertwined hyphae, black and wrinkled on the surface and white or pale yellow inside.

Sclerotia help the fungus survive unfavorable conditions. They remain vital in the soil for several years, on account of high resistance to fungicides, low and high temperatures (Bedy, 1961, as cited in Sinclair and Shurtleff, 1975). In water-soaked soil, however, the sclerotia rot and perish in 26 to 31 days (Moore, 1949).

Under favorable conditions, sclerotia germinate producing hyphae which can directly penetrate the plant tissue (Bardin and Huang, 2001, as cited in Bolton et al., 2006). Also, one or several apothecia can form on sclerotia. Apothecia consist of a short or long stalk and a cup-shaped enlargement. Their color is pale-yellow to brown. The diameter of the cup ranges from 1 to 10 mm. Apothecia contain a hymenial layer, with asci and paraphyses. Asci are cylindrical, narrow at the bottom and gradually widening towards the top. Their average size is 119.6 x 8.6 µm. Ascospores are arranged in single rows, eight of them in each ascus. They are unicellular, elliptical, and hyaline. Their average size is 12.7 x 7.4 µm (Vidić, 1982).

The optimum temperature for the development of apothecia ranges from 11 to 15°C, with continuously soaking the sclerotium for 10 to 14 days (Abawi and Grogan, 1979; Boland and Hall, 1988). Mature apothecia release ascospores under pressure, in the form of a white mist that reaches the surrounding plants. If caught by wind, they may be carried for several hundred meters (Suzi and Kobayashi, 1972, as cited inAbawi and Grogan, 1979). Ascospores germinate within the temperature range of 0 to 25°C, the optimum temperature ranging from 15 to 20°C. A drop of water for a period of 16 to 24 or more hours is also needed for germination (Sinclair and Backman, 1989). Soybean plants are typically infected in leaf axils, where the ascospore germinates, forms an appresorium while the infection hypha penetrates through the cuticle and epidermis into the host plant (Cline and Jacobsen, 1983). The infection hypha penetrates the cuticle by means of enzymes, by mechanical force of the apressorium, or through stomatal openings (Lumsden, 1979; as cited in Bolton et al., 2006). Recently it was found that *S. sclerotiorum* secretes oxalic acid, which blocks the protective function of the cell and allows the infection hypha to easily pass through the stomatal opening (Guimaraes and Stotz, 2004. as cited in Bolton et al., 2006).

First symptoms of white rot appear on nodes, and then spread to all aboveground plant parts. Secondary spreading of the disease is also possible, when healthy plants come in contact with infected plants, especially in a dense and lodged crop (Vidić, 1982, Boland and Hall, 1988).

Humidity plays an important role in the biology and epidemiology of *S. sclerotiorum*. Occurrence of apothecia, release of ascospores and development of infection take place only if a long rainy period happens to follow the closing of plant rows (Boland and Hall, 1988). The intensity of white rot is high in well-developed and densely-planted crops. Positive correlation was found between the intensity of the disease and lodging of soybean plants (Vidić, 1982).

There are no soybean genotypes resistant to *S. sclerotiorum*, and the situation is similar with the other host plants of this parasite. However, differences have been observed between different soybean cultivars and lines in the sensitivity to the parasite. Several authors have reported the presence of partial resistance in certain genotypes. For example, Arahana et al. (2001) found that the soybean cultivars Dassel, Corsoy 79, DSR137 and S19-90 exhibit partial resistance, which is controlled by several genes. Yang et al. (1999) mention the cultivars Corsoy 79 and S19-90 as partially resistant and Calla et al. (2007) observed partial resistance in PI 194 639.

Under the local agroecological conditions, late soybean genotypes exhibit a high degree of suscebility. When conditions are favorable for the development of the disease, the attack of white rot is very strong. In the case of early cultivars and lines, the disease either does not occur at all, or it is present in traces. However, when inoculated in controlled conditions, the early genotypes too exhibit high sensitivity to the disease. Thus it can be inferred that early genotypes do not possess physiological resistance, but rather avoid the attack of the parasite (disease eascape) in the field (Vidić et al., 1983, Vidić, 1992).

It is assumed that physiological resistance and avoidance mechanisms are responsible for different reactions of soybean cultivars to *S. sclerotiorum*. The avoidance mechanism include early flowering and maturation, lodging resistance, i.e., erect plant that permit air circulation and fast drying inside the crop canopy. It has been proved that one or several of these factors can significantly reduce the intensity of white rot in soybeans (Vidić, 1982; Boland and Hall, 1987; Nelson et al., 1991; Kim et al., 1999). Kim and Diers (2000) found genetic evidence for the existence of avoidance mechanisms and physiological resistance by mapping three loci (quantitative trait loci, QTL) that control soybean resistance to the parasite. Two of the loci control disease avoidance mechanisms that are primarily related to flowering date, plant height and lodging. The third locus most likely controls partial physiological resistance. In view of the results of these authors, it seems that a wider application of molecular markers might make way for the development of commercial soybean cultivars possessing satisfactory levels of resistance to *S. sclerotiorum*.

Control

As fungicide application provides only limited effects in white rot control in soybeans, attention should be focused on agrotechnical measures. Crop rotation is an important measure, which can significantly reduce the intensity of the disease. Soybeans should not be sown in the same field or after another sensitive crop (sunflower, rapeseed, beans, etc.) for an interval of four to six years. This period should be prolonged if the previous susceptible crop had suffered a strong attack of white rot.

Soybean seed should be free of sclerotia of the fungus, which can be achieved by high-quality seed processing. Soybean seeds should also be free of mycelia of the fungus. In regions where the white rot occurs often, it is recommended to use early cultivars, which are less susceptible to the pathogen. Cultivars resistant to lodging should be used for soybean growing under irrigation and in humid regions. The number of plants per unit area should be optimized for each cultivar, i.e., for each maturity group, because too dense a stand tends to cause an early and intensive plant lodging.

Although chemical control of the white rot gives only partial results, foliar treatment is regularly applied in countries where the disease causes significant damage. Preparations based on iprodione, vinclozolin and thiophanate methyl are applied two times, first in the early flowering stage and another 15 days later.

An important measure of chemical protection is soybean treatment with preparations based on benomyl, thiabendazole, captan and thiram, as well as with combinations of these preparations. Biological control has recently been introduced, which involves the application of granulated formulations of spores of the fungus *Coniothyrium ninitans*, which parasitize sclerotia.

CHARCOAL ROT

Distribution and economic significance

The charcoal rot of root and stem is a widespread disease of soybeans. It was first detected and described in 1943 in the United States, wherefrom it subsequently spread to most countries of South America, Asia, Africa, Europe and Australia (Sinclair and Dhingra, 1975). In Serbia, the charcoal rot of soybeans was described in the early 1960s (Aćimović, 1963). Since then, it has occurred regularly but with variable intensity (Jasnić and Vidić, 1985; Tošić et al., 1986; Vidić et al., 1994b).

The disease occurs on soybeans in all phenophases and it can significantly reduce soybean yield. In regions with the warm climate (the tropical and subtropical belt), infections of seedlings and young plants are frequent. In India, Gangopadhyay et al. (1973) registered 32 to 77% of infected seedlings, which considerably reduced the crop stand. In the regions with a moderate climate, the charcoal rot is typically observed in the generative stage of soybeans, especially on plants weakened by drought. In dry years in Serbia, the disease infects 40 to 50% of soybean plants, causing the yield decrease by 20 to 25% (Aćimović, 1988). Our studies have also indicated that a high intensity of disease attack significantly reduced the yield of soybeans. The fungus affects the yield primarily by reducing the numbers of pods and seeds per plant, while its impact on grain size is low (Vidić et al., 1995b).

Symptoms

The charcoal rot is a disease of the root and basal part of the stem, but symptoms can also occur on seedlings. In the latter case, reddish-brown spots can first be seen on hypocotyls. The spots expand and become dark brown to black. In warm and dry weather, infected seedlings dry up and die. If infection is followed by a period of wet and cool weather, the seedlings will survive, but will remain latently infected. Such latently infected seedlings continue to grow, and disease symptoms occur later, in a long dry period accompanied by high temperatures.

In the local agroecological conditions of Serbia, the charcoal rot occurs in the second part of soybean vegetation. The infection first occurs on plant roots. Light brown spots can be noticed at the beginning of infection, which latter spread to the entire root system. The symptoms then spread to the basal part of the stem, lateral branches, and, if conditions are favorable, to a larger part of the plant. Surface tissues of the root and stem become light grey, while numerous black microsclerotia develop underneath the epidermis. The entire depth of the infected tissue is pervaded with microsclerotia, which resemble finely ground particles of coal. Because of the

necrosis of the root system and the plugging of the vascular tubes with microsclerotia, leaves the infected plants turn yellow and wilt. As the infected leaves do not fall off right away, but remain hanging on the stem, the infected plants are readily noticed in the field. The epidermis of the infected parts of the stem is easily detached and it cracks longitudinally, forming narrow strips which are a reliable indication that the plant had been infected by the charcoal rot (Figure 12.8).

Figure 12.8

Macrophomina phaseolina *(photo: M. Vidić)*

Symptoms on the stem

Causal organism, biology and epidemiology

The causal fungus of the charcoal rot of soybean is *Macrophomina phaseolina* (**Tassi**) **Goid**. (syn: *Macrophomina phaseoli* (**Maubl**.) **Ashby**, *Rhizoctonia bataticola* (**Taub**) **Briton-Jones**, *Sclerotium bataticola* **Taub**.). This fungus is a distinct polyphage that parasitize over 400 wild and cultivated species (Dhingra and Sinclair, 1975). In our country, it is most frequent on soybeans, sunflowers, sugarbeet and corn (Marić, 1974; Marić et al., 1988; Babović and Bulajić, 1994).

On potato dextrose agar (PDA), *M. phaseolina* develops rapidly, forming a sparse whitish mycelium. The mycelium is septate and branched. The lateral hyphae typically grow at a right angle from the parent hypha, and they are constricted at the branching point.

After three days of incubation, sclerotia begin to form, first in the center and later on the entire surface of petri dishes. Sclerotia are black, small, globular or elongated. Their size is 57-107 x 50-74 µm (Aćimović, 1988).

In addition to sclerotia, the pathogen also forms pycnidia with conidia on infected bean plants (Luttrell, 1947, as cited in Aćimović, 1988) and dead soybean seedlings (Gangopadhyay et al., 1973). However, the development of pycnidia and conidia is a rare phenomenon.

Under unfavorable conditions, the fungus survives in infected plant parts remaining in the soil and in soybean seeds. In light, sandy soils that dry quickly, sclerotia may remain vital for several years, while in wet conditions they rot in several months.

The mechanism of infection of soybean plants was studied in detail by Ammon et al. (1974). According to these authors, sclerotia germinate in the soil, each producing several initial hyphae. When they come in contact with soybean roots, the initial hyphae form appressoria with infection hyphae which penetrate the root either directly through the cuticulum or through natural openings. The mycelium develops intercellularly, and the enzymes and toxins that it secretes cause necrosis of the surrounding cells. Development of sclerotia is a reliable indication that the infected tissue is dead. The mycelium also penetrates into the xylem, where it also forms sclerotia. Because of the gradual decay of the root system and plugging of vascular tubes by sclerotia, the infected plant begins to wilt.

Infected soybean seeds have poor germination and germination energy. After planting, such seeds either fail to germinate, or their seedlings die in short order, which occurs frequently in regions with high temperatures at the time of planting.

M. phaseolina is extremely thermophilous. The optimum temperature for its development is from 30 to 35°C (Aćimović, 1963). Sclerotia begin to germinate at 28°C, and soybean seedlings are infected only if the soil temperature at the time of emergence is 30°C or higher (Sinclair and Shurtleff, 1975). Soybean plants are prone to infection if they have been weakened by a long-term drought or some other adverse (stress) conditions. Also, mature plants are much more sensitive to the parasite's attack than younger plants (Iliyas and Sinclair, 1974). The results of our study have indicated that the occurrence of charcoal rot intensifies in response to early sowing and in the case of early soybean genotypes (Vidić et al., 1994b). Namely, when in the course of summer conditions become favorable for germination of scleoria and plant infection, the plants planted early are in the final phenophases, they are more sensitive and are faster and more intensively infected than the plants from later planting dates. The increased susceptibility of early soybean varieties is associated with the age of plant tissue at the time of infection.

Although resistant soybean lines are available (good sources of resistance), little attention has been paid to the introduction of resistance in commercial varieties. In field tests conducted in Missouri (USA), the cultivars Asgrow4715, DeltaPineland 3478, Hamilton and Jackson exhibited a moderately resistant reaction to *M. phaseolina* (Smith and Carvil, 1997).

Control

As *M. phaseolina* attacks more severely less vigorous (weakened) plants, the risk of this pathogen's attack is considerably reduced if the soybean crop grows in optimal conditions throughout the vegetation period. It is therefore necessary to apply all recommended cultural practices correctly and timely. Disease intensity is reduced by performing the planting at an optimum time. The seed should be healthy or disinfected before use with fungicides based on mancozeb, thiram or carbendazim. The number of plants per unit area must be optimal for each cultivar, because a dense crop stand produces weak plants that are prone to attack by the parasite. Balanced nutrition increases plant vitality thus reducing the occurrence and spread of disease. Irrigation of soybeans during dry spells is the most effective measure of controlling the charcoal rot.

Adhering to the recommended crop rotation is also important. Soybeans should not be planted in the same field, or after other sensitive crops, for a period of four to six years.

BROWN STEM ROT

Distribution and economic significanc

The disease was first detected in 1944 on soybeans in the USA (Illinois). It is widespread in midwestern and southeastern parts of the United States. It has also been reported in Canada, Egypt, Japan and Mexico (Sinklair and Backman, 1989). In Serbia, the disease was observed on soybeans in 1984, in the vicinity of Župski Aleksandrovac (Tošić and Buturov, 1986) and later in the locations of Vranje, Vladičin Han, Varvarin, Negotin and Požarevac (Tošić et al., 1986; Tošić and Antonijević, 1987).

The brown stem rot is one of the most dangerous diseases of soybeans, especially in the main soybean-growing regions in the U.S. and Canada. The infected plants are forced into an early maturation, causing average yield reductions from 17 to 25% (Sinclair and Backman, 1989). Disease intensity is particularly high, and damage extensive, if pod and grain formation unfold in cold and wet weather and then there occurs a long period of dry and warm weather (Tachibana, 1982). The percentage of infected plants may be high, and the extent of yield reduction depends greatly on the cultivar. Gray and Sinclair (1973) found that the infected plants of the cultivars Beeson and Calland have lower yields than the healthy plants, by 25% and 31%, respectively. In long-term soybean monoculture, the percentage of infected plants goes up to 100%, and the yield is reduced by about 65%. The brown stem rot affects all yield components, primarily on the number of grains per plant and grain size (Dunleavy and Weber, 1967).

Symptoms

The brown stem rot attacks the stem and lateral branch of soybeans, and it may also infect the root system. As the rotting stem gradually reduce the capacity to perform its basic functions, changes start occurring on the leaves.

First symptoms, visible inside the stem, typically occur around the middle of the growing season. Vascular tissues and stem cortex turn dark reddish-brown in color, which expands from the roots up. Nodal tissues acquire the darkest shades of the color. The extent of stem decay is associated with the yield level of infected plants. In very susceptible cultivars, the stem cortex rots along the entire stem length.

On the stem surface, disease symptoms become visible in late phenophases. The basal part of the stem becomes light brown, the color gradually expanding to lateral branches. The tips of the main stem and branches curl and dry up, disrupting further plant growth. Simultaneously, the leaves turn yellow and wilt, but retaining fan-shaped areas of green color along the veins. In dry and hot weather, infected plants shed their leaves 20 to 30 days earlier than healthy ones.

Causal organism, biology and epidemiology

The brown stem rot of soybeans is caused by *Phialophora gregata* (**Allington** & **Chamberlain**) **W. Gams.** (syn. *Cephalosporium gregatum* **Allington** & **Chamberlain**). In addition to soybean, this fungus infects also the red clover (*Trifolium pratense*), the mung bean (*Phaseolus aureus*) (Dunleavy, 1967), and the adzuki bean (*Vigna angularis*) (Sinclair and Backman, 1989).

On infected soybean stems and nutrient mediums, the parasite develops a white mycelium. Hyphae are branched and hyaline, septate or nonseptate, 1.2 to 4.7 µm in diameter. Conidiophores agglomerate on all parts of the hypha. They are usually straight or with slightly curved tops, unicellular or septate, 4-15 µm in length, rarely up to 25 µm long.

Conidia form successively at the tips of conidiophores. After maturation and separation from conidiophores, irregular no catenulate heads which disintegrate upon in contact with water. They can be unicellular, bicellular or multicellular. The unicellular conidia are elliptical-elongated and hyaline. If they form of soybean stems, their dimensions are 3.9 to 4.3 x 6.8 to 9.4 µm, while they are much smaller on artificial mediums. Sometimes the unicellular conidia elongate, forming a constriction and a septum in the middle. The process repeats several times, and the resulting conidium is much longer than the maternal one and divided by septa. These multicellular conidia may form an outgrowth at one end, in a process similar to budding, which subsequently becomes a single-celled conidium. Such unicellular conidia do not divide further. In a drop of water they germ in 24 hours, developing the initial

hypha on one end, rarely on both ends. The optimum temperature for sporulation ranges from 15 to 20°C (Sinclair and Backman, 1989).

P. gregata overwinters in the soil, on infected soybean harvest residues. When conditions become favorable in the spring, the fungus begins to sporulate intensively on harvest residues buried to the depth of 30 cm in the soil. The sporulation continues until all plant remains rot away. The fungus maintains vitality in the soil for several years even without the presence of host plant remains.

The infection of soybean plants starts from the roots, from where the parasite's mycelium spreads to the stem, traveling mainly through vascular tubes of the xylem. The presence of conidia in the xylem has been confirmed, wherefrom they spread to other plant parts. The mycelium of the fungus secretes toxins, gregatin A, C and D, which cause plant wilting (Taylor et al., 1985). The role of soybean seed in the maintenance and spread of the parasite has not been determined yet.

Cool weather encourages the occurrence and distribution of the spread of the brown stem rot on soybean. Disease symptoms develop most intensively in the temperature range from 15 to 27°C. Infection intensity goes down above 27°C and it stops altogether at 32°C (Gray, 1974). Plant sensitivity increases with age and the distribution of disease symptoms accelerates proportionally.

Variability in pathogenicity has been observed in the population of *P. gregata*. There are two pathotypes of the fungus. One is much more harmful, which causes leaf chlorosis, necrosis and wilting in addition to stem rot. The other does not affect the foliage but only the soybean stem (Mengistu and Grau, 1986).

Resistance to *P. gregata* was found in the U.S.A., in several introduced soybean genotypes (Chamberlain and Bernard, 1986, Tachibana and Card, 1972). A high level of field resistance has been incorporated in the commercial cultivars BSR 101, BSR 201, BSR 302 and Chamberlain. Research conducted in our country showed that the cultivar Amsoy is highly susceptiable (Tošić and Antonijević, 1987).

Control

The most effective measure in control the brown stem rot is a multi-year crop rotation. Soybeans should not be planted in the same plot, or after red clover, for at least three to five years. In regions in which the disease occurs regularly and intensively, it is recommended to grown resistant cultivars.

PHYTOPHTHORA ROOT AND STEM ROT

Distribution and economic significance

The disease was first observed in 1948 on soybeans in Indiana, and then in 1951 in Ohio (Suhowecky and Schmitthenner, 1955., as cited in McBlain and Schmitthenner, 1991). It had spread quickly to all major soybean-growing regions in the U.S. and Canada (Sinclair and Backman, 1989). Besides the North American subcontinent, it is present in Australia, Japan, Hungary, Italy and some parts of the former USSR (Sinclair and Backman, 1989). The disease has not been observed on soybeans in Serbia.

Phytophthora root and stem rot is a dangerous soybean disease, especially in the U.S. where it occurs at about 16 million hectares. The Ohio state alone, the damage caused by the diseases is estimated at $ 50 million annually (Schmitthenner, 1985). Later, the same author reported that five million hectares have been infected by the parasite in northern and central parts of the United States, yield losses in some plots ranging up to 100% (Schmitthenner, 1989). Intensive infection reduces the yield of susceptiable genotypes by halved (Sinclair, 1982). Therefore, only resistant or tolerant soybean cultivars should be planted in the heavily infected areas (McBlain and Schmitthenner, 1991). In Australia, the damage usually exceeds 20%, and in certain plots the damage ranges from 50 to 90% (Rose et al., 1982).

Symptoms

As the disease occurs in all phenophases, the symptoms are complex and diverse. Decay of seeds and nonemerged seedlings may occur immediately after planting. Damping-off causes the newly emerged seedlings and young plants to die quickly. This produces large or small patches of bare soil, which occur especially in field depressions, which retain water longer than the other parts of the field. This explains why the symptoms of this disease are often confused with and mistakenly identified as consequences of water stress.

In later phenophases, disease symptoms occur on soybean leaves, stems, branches and roots. First symptoms can be seen on basal leaves, as yellowing between leaf nerves and along the edge of the lamina. After that, top leaves become chlorotic and lose turgor while entire plants wilt and dry up. Dry leaves remain attached to infected plants, which makes them easily observed. Infected plants are typically concentrated in small or large groups along the row. Individual infected plants scattered in the field and rarely seen. The infected plants are easily uprooted and the roots and stems show typical symptoms of *Phytophthora* rot. Tissue of the main root and lateral roots becomes dark brown, soft, rotten and watery. The rot then spreads to the stem and when it infects a dozen nodes, the plant begins to wilt.

The infected part of the stem is light brown at the beginning, and eventually becomes darker. The interior of the stem is dark brown, so that its longitudinal cross section clearly shows the height to which the disease infected the plant.

The described symptoms are typical for sensitive soybean genotypes. These plants quickly wilt and dry, and they fail to bring any yield if infected early in the season. The less susceptible or more tolerant soybean cultivars do not wilt quickly. The infected plants have a slow growth. The leaves become slightly chlorotic, the symptoms resembling those that occur due to the absence of soil nitrogen or due to waterlogging of the production field. In some cases, a necrosis makes the basal part of the stem brown. These plants yield about 40% less than the healthy ones.

Causal organism, biology and epidemiology

The causing fungus of the root and stem rot of soybeans is *Phytophthora sojae* **Kaufman** & **Gerdemann.** The parasite had been first named *P. megasperma* var. *sojae* (Hildebrand, 1959) and later it was renamed into *P. megasperma* fs. *glycinea* (Kuan and Erwin, 1980). This pseudofungus develops a white mycelium, whose hyphae branch at the right angle, and then they usually start to curl. The young hyphae are unicellular, developing septa and characteristic swellings with age.

Sporangiophores are simple and indeterminate. They develop elliptical sporangia, without papillae (42-65 x 32-53 µm). Zoospores differentiate in the sporangia. The sporangia germinate by forming thin membranous vesicles, into which zoospores are extruded. Consequently, the vesicles rapidly expand and ruptured. The released zoospores infect soybean plants. Sometimes, zoospores germinate inside the sporangium, in which case their germtubes break through the sporangial wall. The vacated sporangium often forms new sporangia. Under certain temperature conditions, the sporangium produces directly an infection hypha, i.e., it functions as a conidium.

Zoospores are oval, with straight sides and rounded ends. At each end they have a single flagellum, the front one being several times shorter than the hind one. The optimal temperature for the formation of zoospores is 20°C.

In its life circle, *P. sojae* develops both antherida and oogonia. Antheridia are amphigynous and paragynous. Oogonies are round or slightly flattened, 29-58 µm in diameter, with thin walls. When antheridia fertilize oogonia, the latter give rise to oospores. Those are permanent spores, round, double walled. The interior is filled with finely granulated cytoplasm, with a refractive body in the center. Oospores germinate to produce hyphae, which are capable of causing infection, or which form sporangiophores and sporangia (Sinclair and Backman, 1989).

P. sojae forms masses of oospores on the roots of sensitive and tolerant soybean cultivars. They are persistent, capable of remaining vital for a few years on infected harvest residues in the soil.

When conditions become favorable for germination in the spring, they produce infection hyphae which perform the primary infection of soybean roots. Sporangia with zoospores then appear on the infected tissues. After zoospores are released, water moves them to root hairs, where they cluster to form cysts. Cysts germinate and produce hyphae. When hyphae come in contact with the soil, they form apressoria and infection hyphae, by means of which the pseudofungus penetrates the plant tissue.

Heavy showers can sometimes cause infection of soybean leaves. Zoospores are splashed on leaf laminae when rain drops hit soil surface. If the shower is followed by a long period of humid and cool weather, the foliar form of the disease intensifies and symptoms spread via leaf stalks on branches and the stem.

The mycelium grows intercellularly inside the root tissue, and intracellularly in the hypocotyls. It penetrates the cell by means of ring-shaped or round haustoria.

Seed rot, seedling decay before and after emergence, and root and stem rot occur usually on soybean plants grown on heavy, compacted, clayey, poorly drained soils prone to waterlogging. Intensive infections break out when soybean planting is closely followed by abundant rainfall and a period of low temperatures. Long dry periods combined with high temperatures significantly reduce the intensity of disease occurrence.

High variability exists within the *P. sojae* population. So far, at least 55 physiological races of the pathogen have been identified (Dorrance et al., 2003). Also, a large number of genes has been identified in seven loci (Rps1, Rps1-b,-c Rps1, Rps1-k, Rps2, Rps3, Rps3-b, Rps3-c,-d Rps3, Rps4, Rps5 Rps6 and Rps7), which control the specific resistance to certain physiological races (Athow, 1987; Ploper et al., 1985, Layton et al., 1986; Kilen et al., 1974; Kilen, 1986, Weng et al., 2001). In the U.S., where the root and stem rot is most widespread, development of resistance to this disease is a main goal of soybean breeding. Because of the frequent occurrence of new physiological races, the work on resistance breeding is a continuous activity. The work is focused on the development of cultivars possessing specific resistance to dominant races in particular agroecological region, and on the development of tolerant cultivars.

Control

The most efficient measure of control of this disease is the development and cultivation of resistant and tolerant soybean cultivars. When using tolerant cultivars, it is recommended to treat seed with metalaxyl-based fungicides. These fungicides successfully prevent the infection during seed germination and plant emergence. The fungicides can be applied simultaneously with sowing or later. The intensity of the disease can also be reduced by various agrotechnical practices: crop rotation, quality soil tillage and seedbed preparation, optimum sowing date, waterlogging prevention and other practices.

PHOMOPSIS SEED DECAY

Distribution and economic significance

Already in 1922 in the U.S., Lehman described the occurrence of seed rot and blight of soybean seedlings. He explained these phenomena as results of infection with *Diaporthe phaseolorum* var. *sojae*, and its conidial stage *Phomopsis sojae*.

The disease was registered in several soybean-growing countries, but it is particularly widespread in the U.S. Soybean seed pathogens from the genus *Diaporthe/Phomopsis* are present in most soybean-growing regions in Serbia. In 2002, for example, high percentages of infected seeds (over 65%) were found in various locations, while in the next, 2003, the occurrence of the disease was negligible (Vidić et al. 1995c; Vidić et al., 2006).

Exact data on disease harmfulness are not yet fully established. However, many authors single out the seed rot and seedling decay as the most frequent and most detrimental disease of soybean seed (Mc Gee, 1986; Gleason et al., 1987; Rupe and Ferriss, 1987; Thompson et al., 1988). Infected seeds can be heavily damaged and rotten. Their germination and vitality are considerably reduced, which causes poor emergence, high percentage of blighted seedlings, and reduced plant density in the field. In warm regions, if temperatures are favorable at the time of soybean emergence, the disease can cause large-scale destruction of seedlings and extensive damage (Sinclair and Backman, 1989).

In the agroecological conditions of Serbia, the occurrence of seedling decay has not been observed due to low soil temperatures in the spring, which do not favor the development of the disease.

Symptoms

The symptoms are typically manifested on seeds. In the case of severe infection, seeds are small, nonviable, shrunken, with cracked seedcoats, sometimes covered with the mycelium of the parasite. Such seeds usually rot and perish (Figure 12.9b). The less infected seeds have a normal appearance, without signs of the disease. They have reduced germinability, vitality and quality. Infected seeds produce diseased seedlings. Pale light red to brown spots develop on the cotyledons. They can be very small or cover the entire cotyledons. Reddish-brown stripes can be seen on the hypocotyls of the infected seedlings, at or below the soil surface. Top buds are deformed or they perish. The seedlings are necrotized, rotten and eventually they die.

The surviving adult plants show another set of symptoms at the end of the growing season, which are identical to stem and pod blight. Grayish spots on stems and pods are covered with masses of pycnidia, which form black areas of variable size. The infected pods form a smaller number of seeds, which usually are infected.

Figure 12.9

Phomopsis longicolla *(photo: M. Vidić and R. Jevtić)*

a) Healthy seeds, b) Rotten seeds

Causal organism, biology and epidemiology

Many researchers hold the opinion that the seed decay of soybeans is a complex disease, caused by several fungi from the genera *Diaporthe* and *Phomopsis* species such as *D. phaseolorum* var. *caulivora*, *D. phaseolorum* var. *sojae* and an undetermined species designated as *Phomopsis* sp. (Kmetz et al., 1978; Kulik, 1983 and Mc Gee, 1983). Studying infected seeds, Kmetz et al. (1978) isolated *Phomopsis* sp. about six times more frequently than the other species described as causal agents of seed decay. This finding led Hobbs et al. (1985) to conduct a detailed study of the cultural and morphological characteristics of *Phomopsis* sp. They found the fungus to be a separate species which they named *Phomopsis longicolla* **Hobbs**. From this complex, *Diaporthe phaseolorum* var. *caulivora*, *Diaporthe phaseolorum* var. *sojae* (anamorph *Phomopsis sojae*) and *Phomopsis longicolla* have been described as pathogen of soybean seeds in Serbia. Of the above species, *P. longicolla* is the most common and most harmful.

P. longicolla develops well on potato-dextrose agar, on which it forms a white, woolly-like, dense mycelium. Within the mycelium, there form large, black, joined stromatic structures (Vidić et al., 1996). In the stromata, the fungus forms pycnidial conidiomata, single or combined into large or small groups. The conidiomate are unilocular or multilocular and have very long necks, more than 200 µm. The neck length ranges from 210 to 422 µm, or 337 µm on average (Vidić et al., 1996). The conidiomatal locules are spherical, up to 500 µm wide. A conidia, and very rarely B conidia, form in the locules, on branched and septate conidiophores. A conidia are unicellular, hyaline, elliptical or tapering at the ends, each containing two drops of oil, their dimensions ranging around 5.0-9.0 x 1.5-3.5 µm. (Hobbs et al., 1985). B conidia are unicellular, hyaline, threadlike and curved at one end.

The perfect stage of the fungus, i.e., perithecia with asci and ascospores, has not been observed yet.

The fungus overwinters in infected seeds and harvest remains on which it forms pycnidia. In Serbia, intensive attacks of soybean stem blight (Vidić and Jasnić, 1994) are more frequent than the occurrence of seed infection (Komnenić, 1991). Garzonio (1981) found that infected parts of the stem are the main source of the inoculum for disease development in subsequent year, while infected seeds do not play an important role in the disease epidemiology but serve for parasite distribution from one region of the world to another. According to Kulik (1981), pycnospores are not released before the beginning of May. They are discharged for a long period of time and are carried to uninfected soybean plants by rain drops. Disease symptoms usually occur at the end of the season. The number of infected pods gradually increases from phenophase R3 to R8. Seed infection, however, is quite low between phenophases R3 and R7 and it achieves significant proportions between phenophases R7 and R8 (Kmetz et al., 1978). Warm and humid summers favor the development of the infection and the spread of pathogen in the field. Detailed descriptions of the other agents of pod and stem blight and seed decay from the genus *Diaporthe*/*Phomopsis* have been presented earlier.

Control

The recommended practices for disease control are two- to three-year crop rotation, use of healthy seed, planting of cultivars resistant to the pathogen, destruction and plowing under of harvest residues.

Regarding chemical measures, soybean seed can be treated with preparations based on thiabendazole, usually in combination with thiram and captan, or with preparations based on thiram and captan. Soybean seed plots should be treated from the beginning of pod formation till pod maturity with fungicides based on benomyl, chlorothalonil or thiophanate methyl.

RHIZOCTONIA DISEASES

Distribution and economic significance

This disease produces a variety of symptoms, depending on environmental i.e. environmental conditions. It includes pre- and post emergence, damping – off, root and stem decay and leaf, and bad blight In sub-tropical and tropical regions of the world, symptoms are manifested on the stem, leaves and pods, leading to leaf shedding and plant wilting and drying.

This type of the disease is widespread in the Philippines, China, India, Taiwan, Malaysia, Mexico, Puerto Rico and sub-tropical parts of the USA (Sinclair and Backman, 1989). Another disease type causes the damping off of seedlings, root and basal stem rot and drying of old plants. This disease type occurs in countries with moderate climate, i.e., in some parts of the United States (Crall, 1950), Brazil (Machado et al., 1973), the former Soviet Union (Zhukovskaya, 1977) and Serbia (Jasnić and Vidić, 1986).

The disease may cause significant economic damage. Yield losses in tropical parts of the United States can be as high as 35% (Sinclair and Backman, 1989). The damping of seedlings may reduce the soybean stand by 50% (Sinclair and Backman, 1989) and the yield up to 40% (Tachibana et al., 1971; Machado et al., 1973).

Symptoms

As previously stated, the disease symptoms vary in dependence of ecological factors. Wet and warm weather encourages the development of the disease.

Under the climatic conditions of Serbia, the damping of seedlings and symptoms on the stem occur most frequently. Symptoms on the leaves and pods are quite rare, because the summers are hot and dry, and they do not favor the development of the disease.

First symptoms occur in the spring, under conditions of prolonged warm and humid weather, in the form of delayed growth, lodging and damping off of seedlings. On the infected hypocotyl, usually at soil surface level, there occur elliptical, elongated, sunken, dark red spots with a dark rim. The spots may coalesce, girdling large parts of the stem (hypocotyls) and spreading toward the root. The root necrotizes and perishes, causing the lodging and decay of greater or lesser number of seedlings. On surviving seedlings there develop elongated, elliptical, sunken, dark red spots with still darker edges. The infected plants wilt and dry if the necrosis spreads over a large portion of the stem. The root of the infected plants is underdeveloped and necrotized, bearing a small number of bacterial nodules (Figure 12.10a). The necrosis and decay of soybean plant tissues is caused by toxins produced by the parasite. Strong infections cause the wilting and decay of a variable number of plants in pathes (Figure 12.10b).

During a long period of humid and warm weather, symptoms may occur on stems and later on basal leaves. After that they may spread to pods. Greenish-brown, watery spots can be observed on leaves. Later, the spots first turn dark red, then brown and finally black. The spots vary in size from very small to big, the latter covering large parts of the lamina. The necrotized parts fall out and the leaf becomes riddled with holes. In a very humid weather, the spots become overgrown with a weblike cover in which is imbedded a larger number of small brown or black bodies - sclerotia.

Figure 12.10

Rhizoctonia solani *(photo: M. Vidić)*

a) Root rot, b) Patches of wilted plants

The infected leaves fall off, so the infected plants become completely defoliated. The shed leaves serve as sources of infection for pods and seeds. The symptoms on infected pods are small brown spots or large brown areas. The infected pods either do not contain seeds or, if seeds were formed before pod infection, they are infected.

Causal organism, biology and epidemiology

The root and stem rot, damping off of seedling and plant wilt are caused by *Rhizoctonia solani* **Kühn**, whose perfect stage is *Thanatephorus cucumeris* (**Frank**) **Donk** (syn.: *Hypochnus cucumeris* **Frank**; *H. solani* **Prill** & **Delacr**; *H. filamentosus* **Pat**.). The perfect stage, which develops quite rarely, is represented with basidia and basidiospores. *R. solani* is a polyphagous parasite that in addition to soybeans attacks a variety of plants from different families, vegetable crops, flowers, clover, alfalfa, etc. The fungus is highly variable in cultural characteristics, pathogenicity and the response to environmental changes. The isolates that cause root and stem rot and damping off of seedlings do not cause disease symptoms on soybean leaves. *R. solani* is divided according to the type of hyphal fusion (anastomosis of hyphae) into anastomatic groups (AG groups), and these groups are subdivided into subgroups. For example, there are 13 subgroups in group AG-1. Fungus isolates from soybeans belong predominantly to group AG-4 (Sinclair and Backman, 1989).

On potato dextrose agar, the fungus develops rapidly and forms spherical colonies with a white, weblike, aerial mycelium. The color of the aerial mycelium changes with age into light brown. The substrate mycelium becomes yellowish-brown and it acquires a raylike structure.

After three to four days, the fungus forms small, dark brown or black sclerotia. Young hyphae branch in the direction of growth and they are hyaline. Old hyphae are yellow or brown colored, septate, thick, and they typically branch at a right angle and constrict slightly at the point of junction with the main hypha. The length to width ratio is greater than 5:1. Simple or branched chains of moniliform cells form on old mycelia. The cells are brown colored, cylindrical, pear- or irregularly shaped, and they contain a large number of nuclei (3 to 20). The dimensions of these cells are 17-30 x 25-50 µm (Jasnić and Vidić, 1986). The sclerotia of the fungus are brown to black, small, oval or irregular in shape, consisting of a solid mass of moniliform cells, with a diameter of up to 0.25 mm. Not all isolates of the fungus manage to produce sclerotia.

R. solani is a soil born pathogen and it has excellent saprophytic characteristics. The mycelium of the fungus persists for 3 months in a dry soil or 9 months in a wet soil. It overwinters in the soil, in the form of sclerotia or mycelia, even in the absence of plant residues. The mycelium persists on all types of plant residues (harvest residues, root remains, etc.). The fungus isolates that infect soybean seeds overwinter and are transmitted from year to year by soybean seeds.

Fungus growth in the soil depends on the presence of organic and other nutrients, soil temperature and pH, as well as the presence of antagonistic microorganisms. The fungi population is usually distributed in the topsoil, up to the depth of 10 cm, its numbers decreasing with soil depth. Under favorable conditions (temperatures between 26 and 30°C and water saturation of the soil of over 70%), the parasite infects soybean roots and basal stems. The fungus creates pectolytic and proteolytic enzymes that facilitate the decomposition of plant tissue and penetration of the fungus.

Control

Use of healthy seed is a recommended protective measure. If the seed is infected, it should be treated with a fungicide based on PCNB, benomyl and thiabendazole or combinations of preparations based on carboxyn and thiram, or iprodione and thiram. In a case of a heavy infection, a foliar treatment should be made with systemic fungicides based on benomyl, carbendazim or thiabendazole. Multi-year crop rotation and abstaining from growing soybeans after sensitive crops such as potatoes, beans, tomatoes and sugarbeets may considerably reduce the frequency of occurrence of the disease. Resistant cultivars should be grown. Biological control of the disease includes seed treatment with the fungus *Trichoderma pseudokiningii* or the bacterium *Bacillus subtilis*.

FUSARIUM WILT, ROOT ROT, POD AND COLLAR ROT

Distribution and economic significance

Fusarium wilt and the necrosis of roots pod and collar rot are diseases that are widespread all over the world. The Fusarium wilt of soybean plants was first registered in the United States in 1917 (Cromwell, 1917), and later in Japan (Hara, 1918, as cited in Aćimović, 1988), Canada (Conners and Savile, 1944), India (Marthur, 1954), Brazil (Machado et al., 1973), the former Soviet Union (Lobik, 1930), Germany (Noll, 1939) and other countries. The disease was also found and described in Serbia (Tošić et al., 1986; Aćimović, 1988; Jasnić et al., 2005). Rot root was discovered and described in the U.S. in 1961 (Sinclair and Backman, 1989).

Damages on diseased plants can be significant. Degree of damage depends much on the phenophase in which the infection is induced, percentage of infected plants and the causal fungus from the genus *Fusarium*. In the early infections reduce the crop stand up to 64%, the number of pods formed is reduced by 50%, and the yield can be reduced up to 59% (Sinclair and Backman, 1989). In India, 40% of seeds from infected pods do not germinate while brown spots develop on the cotyledons and hypocotyls of seedlings from infected seeds (60%) (Saharan and Gupta, 1972). Of the plants infected that way, approximately 20% rot and die and the other manage to survive.

Symptoms

Different disease symptoms occur on soybean plants seedlings blight, wilt of young and old plants, necrosis of roots, basal stems and pods. In other words, symptoms may occur on soybean plants from emergence till maturity. The symptoms vary in dependence of plant age at the time of infection, *Fusarium* species causing the infection and environmental conditions.

First signs of the disease can be observed on seedling and young plants if the weather is chilly (14°C). Infected seedlings are slow to emerge, or the germs may perish before emergence. The seedlings that manage to emerge grow slowly. Their cotyledons are chlorotic and later become necrotic. Lower parts of the main and lateral roots are being destroyed by the parasite's attack the cortex and vascular elements. If the soil moisture is low, the seedlings wilt, lodge and dry up. Adventives roots often replace the necrosed and dead parts, so that the plants infected after emergence rarely perish completely. However, they are stunted due to poor root development and their seeds are nonviable and malformed. Pods can also be infected under conditions of prolonged humid weather. Seeds in infected pods are also infected and they transmit the pathogen from one year to another.

Seedling may also show other types of symptoms. Those may be sunken, watery, cream-colored spots on cotyledons and hypocotyls. As the spots age, they become darker, dark brown to black, and they increase in size. Infected hypocotyls become thinner and they soften up. The roots are poorly developed. The pods tend to dry up prematurely. They dry from the tip towards the base, becoming brown to black. The infected pods either do not form seeds or, if they do form, they are infected and brown to black in color. The plants with infected pods usually do not show any other type of disease symptoms.

Symptoms may also occur on older plants, in mid-season, in warm weather conditions (temperatures above 28°C). The typical sign of the disease is the chlorosis of leaves, which wilt and shrivel. Infected plants gradually wilt and dry up. They produce few pods. A weak rot appears on the roots. Cross sections of stems and roots show the necrosis of the vascular system. Conductive vessels of the infected plants become brown to black.

Causal organism, biology and epidemiology

A number of species from the genus *Fusarium* cause seedling blight, wilt, necrosis and rot of roots, basal stems and pods. The most frequent species are *Fusarium oxysporum* **Schlecht**. ex **Fr**., the causal agent of plant wilt and root rot, and *F. semitectum* **Berk** and **Rav**., the causal agent of pod disease, ring necrosis of the stem and rot of the root and basal stem. In Serbia, the following *Fusarium* species have been found on the seeds: *F. graminearum*, *F. acuminatum*, *F. sporotrichoides*, *F. semitectum*, *F. proliferatum* and *F. equizeti* (Medić-Pap, 2006). The following species have been isolated from soybean plants: *F. avenaceum*, *F. equizeti*, *F. oxisporum* and *F. poae* (Jasnić et al., 2005).

F. oxysporum attacks a large number of plant species. It is a highly variable sungus composed of a complex of varieties and physiological races. They differ in host plants they attack and the degree of pathogenicity, but they do not differ in morphological characteristics.

This fungus includes some specialized forms such as *F. oxysporum* f. sp. *tracheiphilum* (**E.F. Smith**) **Snyd.** and **Hans**, whose race 1 (syn. *F. trachephilum* **E.F. Smith**) as well as *F. oxysporum* f. sp. *vasinfectum* **(Atk.) Snyd.** and **Hans**, cause soybean wilt. *F. oxysporum* f. sp. *vasinfectum* race 2 causes cotton wilt, but it also infects soybeans, while *F. oxysporum* f. sp. *glycines* **Armst**. and **Armst**. pathogen soybeans but it is not highly specialized and it attacks other plant species too.

F. oxysporum is a highly variable species. Its aerial mycelium is well developed, septate, white with purple overtones. The substrate mycelium is at first colorless, later becoming deep blue to purple. Conidiophores are unbranched or they branch into monophialids which are typically short. Sporodochia are cream, brown or orange colored. The fungus forms intercalary or terminal chlamidospores which come in

pairs. Microconidia are unicellular, oval or kidney shaped and they form false heads. Macroconidia are hyaline, with 3 to 5 septa, sickle-shaped, with a well-developed foot at the base and a thinned top cell.

F. semitectum cause a pod and collar root. The fungus forms septate, light brown to dark brown aerial mycelium, while the substrate mycelium is pink to dark brown. Sporodochia, if they form, are orange colored. Conidiophores may be unbranched or branched into mono- or polyphialids. The fungus produces terminal and intercalary chlamidospores. Macroconidia are spindle shaped with a papilla on the base cell, while those formed in sporodochia are slightly curved and with a well-developed foot at the base cell. Microconidia are formed rarely.

In addition to the species mentioned above, the following species have been found to cause pathological changes in soybeans: *F. equiseti* (**Corda**) **Sacc**., *F. graminearum* **Schwabe** with the teleomorphic stage *Gibberella zeae* (**Schw**.) **Petch**, *F. moniliforme* (**Scheld**) **Snyd.** and **Hans** with the teleomorphic stage *G. fujikuroi* (**Sawada**) **Ito** and *F. solani* (**App**. and **Wollenw**.) **Snyd.** and **Hans** (Sinclair and Backman, 1989).

Most *Fusarium* species are soilborn microorganisms that inhabit various plant residues in the soil. These fungi overwinter as chlamidospores or mycelia. Some species are transmitted from year to year by infected seeds. The primary inoculum, however, remains in the soil.

Those are facultative parasites that penetrate, i.e., infect plants through injuries, lenticels or directly through epidermal cells. Injuries can be caused by mechanical means or by soil pests and nematodes. Optimal conditions for the development and spread of the pathogens are water-saturated soil and air temperatures between 14 and 23°C. Unfavorable weather conditions and reduced plant vitality facilitate the development and spread of the diseases.

Control

Control of *Fusarium* diseases complex is difficult and rarely successful. Strict adherence to crop rotation is recommended. After an intensive occurrence of a soybean disease in a certain plot, that plot should not be used for soybean growing for at least 5 years. Healthy seed should be used for planting. Planting should be performed under favorable humidity and temperature conditions, i.e., in warm and dry, well-drained soil. Soybean cultivars should be less sensitive or resistant to dominant species of the genus *Fusarium*.

PYTHIUM ROT

Distribution and economic significance

Germ blight and seedling damping off, root rot and drying of soybean plants were first described in the U.S.A. (Koehler, 1931). Later, these kinds of disease were found in all soybean-growing regions of the world (Sinclair and Backman, 1989). The disease was registered in Serbia too (Jasnić et al., 1988).

Seed and germ rot before emergence and seedling blight after emergence cause the occurrence of empty patches in the field.

There are no reliable data on the harmfulness of this disease, but it appears not to cause extensive damage.

Symptoms

Disease symptoms occur from germination and emergence to the middle of the soybean growing season. The symptoms are most frequently manifested on seedlings and young plants, while they occur seldom on old plants.

Seedlings exhibit different types of symptoms, depending on the particular type of parasitic fungus of the genus *Pythium* attacking them. Soft rot occurs on the basal part of hypocotyls of infected seedlings. The infected tissue is initially translucent and moist and later it becomes darker. At the infection court, the infected hypocotyls are constricted and frequently break. The broken plants wilt and die (Figure 12.11.). Symptoms on hypocotyls and cotyledons may also be sunken, dry, reddish-brown spots. The spots coalesce and cover large portions of the cotyledons and the basal part of the stems, spreading to the root including the radicle. In some instances, the disease causes only the thickening of the hypocotyl, without other symptoms, resembling a toxic phenomenon caused by some herbicides. The apical meristem either becomes stunted or dies off, and the two secondary stems growing from the axillas of the cotyledon leaves are typically stunted, short and they bear small leaves.

In the case of older plants, symptoms usually occur from the phase of two true leaves until the middle of the growing season (Jasnić et al., 1988). Greenish-brown sunken spots develop on the basal part of the stem. They gradually coalesce and spread upwards, to several basal internodes and the bases of lateral branches. The tissue inside the spots gradually rots, acquiring a dark red color. The infected plants become stunted and chlorotic; they wilt and dry, and are easily uprooted. Roots of these plants are often necrosed and rotten. The infected bark cracks, peels and falls off, leaving the central woody cylinder exposed. If the necrosis spreads to minor roots or root tips, secondary roots are formed which help the plants to survive.

Figure 12.11

Root rot and damping off of young plants *(photo: M. Vidić)*

Causal organisms, biology and epidemiology

Germ blight and seedling damping off, root and stem rot and drying of soybean plants are caused by 8 species from the genus *Pythium* (Dhingra and Sinclair, 1975). The most frequent species are: *Pythium debaryanum* **Hesse**, *P. ultimum* **Trow**, *P. aphanidermatum* (**Edson**) **Fitz.**, *P. irregulare* **Buisman**, *P. spinosum* **Saw**, *P. mamillatum* **Neurs** and *P. myriotylum* **Drech**. Most of these species cause seed rot, germ burn and damping off of seedlings and young plants, while *P. myriotylum* usually occurs later in the season, causing the necrosis of roots and stems, and wilting and drying of old plants.

All species of the genus *Pythium* that parasitize soybeans can be easily isolated on mediums rich in sugar. On such mediums, all species of this genus grow well, forming a leathery, grayish-white mycelium with fully developed, colorless, aseptate hyphae. The hyphae give rise to the organs for asexual reproduction - sporangia. Sporangia differ in shape and size in different *Pythium* species. They produce either a germ tube or zoospores, each bearing two cilia, develop in them. Zoospores, after developing a membrane, form germ tubes by means of which they infect host plants.

The organs for sexual reproduction are female sex cells – oogonia, and male sex cells - antheridia. Oogonia are spherical, with a thin and smooth wall. They contain egg cells - oospheres. Antheridia are usually cylindrical or cone-shaped and they vary from species to species in size, shape, origin and the number on one oogonia. They can be monoclinous, when they form on the same hypha with oogonia, or diclinous, when they form on different hyphae. The copulation of oogonia and antheridia, i.e., the fertilization of the oosphere, produces a zygote which differentiates into a permanent spore - oospore. Oospores have a thick double wall, which enables them to overwinter and survive in unfavorable environments. After overwintering, they germinate into sporangia, which either give rise to into hyphae or zoospores form in them.

Most pseudofungi from the genus *Pythium* are soilborn microorganisms, which are saprophytes that inhabit plant residues in the soil. They overwinters either as oospores in the soil or on plant residues.

Germ blight and seedling damping off, which are caused by most of the species mentioned above, occur intensively at relatively low temperatures, between 10 and 15°C, and high relative humidity or in the presence of large amounts of water in the soil. The species *P. debarianum* and *P. myriotylum*, however, are most virulent at somewhat higher temperatures, between 26º and 30°C. Soybean plants are infected by sporangia of the *Pythium* species. At high relative air humidity, they produce infection hyphae, but if in water, they produce zoospores. Zoospores move in water until they come into contact with the host plant. After that they become quiescent, cover with a thin membrane around them, and then develop a germ tube by which they infect soybean plants. These pseudofungi survive in the form of permanent spores – oospores, which may retain their vitality in the soil for several years.

Control

The most important measures of control of the disease are cultural practices. A crop rotation of 4 to 5 years is required. The seed for planting should be of good quality, with high germination energy, without cracks and other damages of the seedcoat. Harvest residues of the previous crop should be plowed under several weeks before soybean planting. Planting should be performed in warm weather, at temperatures above 18°C. Irrigation should not be performed in a period of ten days after sowing. Adequate drainage system is needed for wet soils.

Regarding chemical measures, seed treatment is recommended with systemic metalaxyl-based fungicides, or protective fungicides based on thiram, captan and carboxyn, or combinations of these fungicides.

BACTERIAL BLIGHT

Distribution and economic significance

The bacterial spot of soybeans is widespread in many soybean-growing countries. It has been registered in Asia (India, China, Japan, Korea and Taiwan), South America (Venezuela, Brazil, Argentina, Guyana and Cuba), North America (USA and Canada), Africa (South African Republic, Mozambique, Zimbabwe and Ethiopia), Australia and New Zealand. In Europe, the disease was detected on Iceland, in France, Denmark, Sweden, Romania, Hungary, and the former Czechoslovakia and Soviet Union (Sinclair and Dhindra, 1975).

This bacterial disease was found in Serbia already in 1940, in the vicinity of Belgrade (Grujičić and Tomašević, 1956). Later on it was observed in the region of Mačva and in the vicinity of Belgrade (Nikolić, 1951b), in western Serbia (Babović and Numić, 1966), and then in locations of Požarevac, Šabac, Negotin and Vranje (Tošić et al., 1986). In the Vojvodina Province, it occurs with variable intensity (Balaž et al., 1990; Ignjatov, 2007).

The bacterial blight is a important disease of soybeans. It is capable of causing large economic damage, especially in regions with a cool and rainy climate. In our country, it causes large damage when spring and summer are cool and rainy.

Seedlings that germinate from infected seeds tend to dampen off. Infected seeds have much poorer germinability and germination energy than healthy ones. After planting, infected seeds rot and perish, and the crop stand becomes too thin. According to Klikov (1963), seed contamination rate in Ukraine ranged up to 68%, about 50% of young plants perished in Georgia and 71% in Krasnodar (Russia). In older plants, the bacterial blight causes leaf shed resulting in reduced assimilation area. Thin crop stand and reduced assimilation area cause yield loss of more than 50% (Williams and Nyvall, 1980). In the period from 1975 to 1977, this was the most harmful soybean disease in the United States. Annual losses during that period were estimated at 62 million dollars (Kennedy and Alcorn, 1980).

Under the agroecological conditions of the Vojvodina Province, *P. s.* pv. *glycinea* occurs each year on soybean leaves. Damage is caused by leaf drying, which negatively affects yield level and seed quality (Balaž et al., 1995; Balaž and Aćimović, 2008).

Symptoms

The bacterial blight of soybeans attacks all above-ground plants parts: leaves, stems, pods and seeds. The symptoms on infected leaves and pods are most conspicuous.

First symptoms occur on the cotyledons of soybean seedlings that germinated from infected seeds. Water-soaked spots can be seen on cotyledons, usually along their margins. They quickly widen and elongate, covering large or small parts of cotyledons. The tissue within the spots turns light brown and later dark brown and necrotic. The spots, underside of the leaves sink and become covered with a thick, sticky bacterial exudate. The infected seedlings become stunted and they die if the vegetative cone is infected.

The most typical disease symptoms on adult plants occur on the bottom and middle leaves. Those are small, angular, translucent, water-soaked and yellow to light brown spots. Later, the spots become brown and in the end black, with a water-sooked, and bordered by yellowish-green margin. During wet and cool weather, the spots increase in size and coalesce into large, irregularly-shaped necrotic areas (Figure 12.12.). Within the spot, on the underside of the leaf, a dense and sticky bacterial exudate covers the spot giving it a glossy appearance. In the case of old spots, especially during heavy rains and wind, the central part often cracks and drop out, so the leaf becomes ragged. Young leaves, which are very sensitive, become stunted, deformed and chlorotic. Old infected leaves often fall off.

Figure 12.12

Pseudomonas syringae* pv. *glycinea *(photo: M. Vidić)*

Symptoms of bacterial blight on leaves

Chlorotic spots develop on the stem and leaf petioles. Later they become brown and then black. The tissue inside the spot is sunken and filled with bacterial exudates.

On pods, there occur small water-soaked spots, which later on enlarge and coalesce to cover most of the pods. The spots grow darker with age, turning from brown to brownish-black and finally to black. They are sunken are covered with bacterial exudate. Seeds in the diseased pods become infected. The infected seeds are smaller than healthy ones, poorly filled, with a deformed and wrinkled seedcoat, in some instances covered slimy bacterial colonies. Sunken or raiset spots can be seen on infected seeds, whose seedcoat frequently becomes colorless. In the case of a weak infection, seeds show no visible disease symptoms.

Causal organism, biology and epidemiology

The bacterial blight of soybeans is caused by *Pseudomonas syringae* pv. *glycinea* (**Coerper**) **Young**, **Dye**, and **Wilkie** (syn. *P. glycinea* **Coerper**).

White colonies form on the meat-peptone agar, which are bulging, bright and round. The bacteria are rod-shaped, gram-negativne, asporogenous, mobile, with the amphitrichous arrangement of cilia. They cause a green, fluorescent pigmentation of the medium. Inoculated tobacco leaves show the necrosis – hypersensible reaction. The optimal temperature for bacterial growth is between 24 and 26°C, the maximum is about 35°C and the minimum about 2°C (Arsenijević, 1988).

The bacterium secretes several toxins. One of them inhibits the synthesis of chlorophyll, causing chlorosis of young leaves and the formation of chlorotic halos around the spots (Gulya and Dunleavy, 1979). It has been found that *P.s.* pv. *glycinea* has 12 physiological races (Cross et al., 1966; Fett and Sequera, 1981, Thomas and Leary, 1980; Hevesi and Érsek, 1981; Gao, 1998). Soybean cultivars show varying degrees of suscebility to these races. Only race 4 and a specific isolate that exhibits similarities to race 5 have been found so far in Serbia (Balaž et al., 1990). A recent study confirmed that race 4 is dominant on soybeans in Serbia (Ignjatov et al., 2007).

The bacterium usually overwinters in harvest residues and it also can be transmitted from one year to another by infected seeds. Harvest residues that remain on soil surface play an important role in the epidemiology of the bacterium. The bacteria may remain vital in such residues for more than 7 months. Conversely, the harvest remains plowed under at the depth of 15 to 30 cm rot away and the bacteria residing in them perish, so they are of little significance for the spread of infection (Moskovec and Krasnov, 1963).

Infected seeds play an important role in transmitting the bacteria from one year to another. In infected seeds, the bacteria remain vital up to 4 years (Moskovec and Krasnov, 1963). Infected seeds produce diseased seedlings. Primary infection of cotyledons is the main source of inoculum for secondary infections. Bacterial exudate spreads by means of raindrops during windy and rainy weather.

Control

Cultural practices are the most efficient means of crop protection. Healthy seed should be used for planting. Crop rotation should be strictly applied, i.e., soybeans should not be cultivated in fields with a history of intensive occurrence of the bacterial blight. Harvest residues should be plowed under immediately after harvest, to reduce the amount of the inoculum. Delayed planting tends to diminish the intensity of disease occurrence. Soybeans should be harvested in dry and warm weather, at the optimum seed moisture. Soybean harvest in rainy weather increases the infection. In regions favorable for the development and spread of the disease it is desirable to plant cultivars resistant to *P.s.* pv. *glycinea*. Regarding chemical measures of protection, it is recommended to treat seed with fungicides based on thiram and captan (Klikov, 1963), or with antibiotics such as ayeromycin, achromycin, tetracycline and streptomycin (Kaufman and Chamberlen, 1957). Seed treatment with antibiotics based on oxytetracycline and hydrochloride reduced the seed contamination with the bacterium from 50-90% to 0-2% (Leben, 1975). Seed crops found to be infected by this bacterium should be treated with preparations based on copper oxychloride.

BACTERIAL PUSTULE

Distribution and economic significance

The bacterial pustule is widespread in all regions of the world where soybean is grown: USA, Canada, Colombia, Bolivia, Brazil, Nicaragua, Australia, New Zealand, Japan, Malaya, Taiwan, India, Sudan, Nigeria, Zimbabwe and the former Soviet Union. In Serbia, it was registered in 1934 in Ruma and Leskovac, in 1941 in Belgrade and Šabac, in 1946 in the vicinity of Kragujevac (Grujičić and Tomašević, 1956). In the Vojvodina Province, this bacterial disease was observed in the 1960s (Arsenijević and Panić, 1967).

Economic importance of this disease in soybean production has not been exactly determined. The disease causes premature leaf shedding, which may reduce the yield, by reducing the size and number of grains. According to Hartwig and Johson (1953), in the U.S., susceptible cultivars yield 8 to 11% less than the resistant ones.

Symptoms

First disease symptoms are small, pale green spots with raised central parts on both sides of the leaf. Later on, the spots change color and become yellow, reddish-brown and finally brown, with a light yellow margin.

On the upper but more often on the underside of the leaves, raised pustules form in the central part of the spots. These disease symptoms resemble those of rust. In some cases, pustules do not form inside the leaf spots. The tissue inside the spots becomes brittle, it cracks and falls out, giving the infected leaves a regged appearance. The spots do not appear moist and have pustules in their center, which distinguishes this disease from the bacterial blight, caused by *Pseudomonas syringae* pv. *glycinea.*

In conditions favorable for development of the disease, the number of spots increases. The spots expand, coalesce and cover large parts of leaf surface, forming necrotic zones. Intensive infection causes leaf shedding while the infection spreads to other plant parts. Elongated brown pustule can be seen on the leaf petioles and soybean stems. On infected pods, symptoms first occur in the form of small, oval, dark green, slightly raised spots, which later become reddish-brown and then brown. Infected pods transmit the disease to seeds. Infected seeds have lower germination rate and are smaller than healthy one. The seedcoat of the infected seeds is shrunken, often showing yellowish-brown spots.

Causal organism, biology and epidemiology

The bacterial pustule of soybeans is caused by *Xanthomonas campestris* pv. *glycines* (**Nakano**) **Dye** (syn.: *X. phaseoli* (**E.F. Smith**) var. *sojensae* (**Hedges**) **Starr** et **Burkholden**; *X. campestris* pv. **phaseoli** (**Smith**) **Dye** and *X. glycines* (**Nakamo**) **Magron** et **Prevot**).

On meat-peptone agar, the bacteria form yellow, slimy, convex and glossy colonies. The bacteria are rod-shaped, gram-negative, asporogenous, mobile, with 1 or 2 polar cilia. The optimum temperature for bacterial growth is between 30 and 33°C, with the maximum temperature of 38°C and the minimum of 10°C (Arsenijević, 1988).

Infected seeds are the main source of infection, which carry the pathogen from year to another. The bacteria may retain vitality in seeds for over 24 months. Plant debries are not a significant source of infection, as the residing bacteria lose their vitality during winter. In the course of growing season, the bacteria spread by rain and wind, but they can also be transmitted by some insects. Wet and rainy weather is favorable for infection and spread of the bacterium. It penetrates the plant through stomatal openings and injuries, and then it spreads intercellularly.

Control

All control measures applied against the bacterial blight can be used to control this bacterial disease.

WILDFIRE

Distribution and economic significance

This disease has not been registered in Serbia. It has been described as a soybean disease in the U.S. and Brazil (Sinclair, 1982), Australia, Zimbabwe and the former Soviet Union. It occurs most frequently on tobacco.

This bacterial disease is not important for the cultivation of soybeans.

Symptoms

First symptoms of the disease can be seen on the basal leaves. The infected leaves show dark brown spots surrounded with a wide yellow ring. The size and shape of spots are different but large spots are predominant. Spots gradually coalesce and form large necrotic areas on the lamina. The tissue inside the spot becomes brittle, it tears, shreds and falls, giving the leaf a ragged appearance. Strongly infected leaves dry up and fall off.

Causal organism, biology and epidemiology

The wildfire is caused by *Pseudomonas syringae* pv. *tabaci* (**Wolf** et **Foster**) **Young**, **Dye** et **Wilkie** (syn. *P. tabaci* (**Wolf** et **Foster**) **Stevens**).

When placed in nutrient mediums, it forms white, round, slightly bulging colonies, with a translucent margin. The bacterium is rod-shaped, gram-negative, asporogenous, mobile, with several polar cilia. It causes a hypersensitive reaction of tobacco in 3 to 6 days (Arsenijević, 1988). The optimal temperature for bacterial growth is between 24 and 28°C, with the maximum temperature of 38°C and the minimum of about 4°C.

Isolates from soybeans are pathogenic to both tobacco and soybeans, while isolates from tobacco are pathogenic only to tobacco.

The bacterium overwinters and survives in infected seeds as well as the remains of infected plants debries. It is usually transmitted from one year to another by seeds, and in the course of the growing season it is spread by raindrops. Long-term rains facilitate the spread of this bacterial disease.

Control

The wildfire is controlled in the same way as the bacterial blight.

SOYBEAN MOSAIC

Distribution and economic significance

Soybean mosaic is the most common virus disease of soybeans. It occurs in all soybean-growing parts of the world (Sinclair and Backman, 1989). The disease was described for the first time in Serbia in 1964 (Nikolić and Stakić, 1964). The disease is present in all soybean-growing regions of the country.

The soybean mosaic has great economic importance in some countries. In the U.S., soybean yield may be reduced up to 50% in some plots (Sinclair and Backman, 1989). In the former Soviet Union, yield reductions ranged between 24 and 94% (Muravjeva, 1973).

Infected plants remain stunted and form a small number of pods, with fewer seeds. Seeds in infected pods are smaller, with reduced quality and germination compared with healthy pods. Nodules on the roots of infected plants are fewer and smaller. If the number of infected plants is large, the combined effect of the above factors causes a significant yield reduction. Although this viral disease is widespread in Serbia, the damage is limited and insignificant, due to a low intensity of infection.

Symptoms

First disease symptoms can be seen on young plants, emerged from infected seeds. These plants are spindle-shaped, with deformed, wrinkled and curled primary leaves. Their laminae can retain a normal shape or their edges curl downwards. The later, trifoliate leaves exhibit more serious symptoms than primary leaves. They become motled; their laminae are wrinkled, especially around the nerves. They curl asymmetrically, grow slowly and become chlorotic (Figure 12.13).

Disease intensity is highly variable, depending on the cultivar, virus strain, plant age at the time of infection and environmental conditions. In the case of susceptible soybean cultivars, early infections, those occurring before flowering, cause severe symptoms such as stunted growth and extensive deformations of leaves. Typical symptoms on trifoliate leaves are apperiance of translucence and chlorotic nervure. Later manifestations are dark green enations along the main nerve, and downward curling of laminae along the edge. In some cultivars, necrotic spots develop between leaf veins. The necrosis spreads through leaf petiole to the stem, causing premature leaf shedding and wilting of the whole plant.

Early infections of susceptible cultivars may cause the infection of pods. They are curved, stunted, flattened and their hair cover is sparse. The infected pods form a small number of seeds, which are small, nonviable and mottled, dark brown or black markings on the seedcoat (Figure 12.13). Their germination is low.

In addition to the sensitivity of cultivars and virus strain, the intensity of symptoms is also considerably affected by temperature. Strongest symptoms occur at temperatures between 18 and 20°C, lower between 24 and 25°C, while at 30°C the symptoms disappear, i.e., they become masked (Ross, 1970)

Figure 12.13

Soybean mosaic virus - Symptoms on leaves and seeds *(photo: Koenning, 2005)*

Causal organism, biology and epidemiology

The soybean mosaic virus (SMV) is classified in the genus *Potyvirus*, family *Potiviridae* and it has several strains (Cho and Goodman, 1979). This virus is thread-like, 750 nm in length and about 15 nm in with. The virus thermal inactivation point is between 55 and 60°C, dilution end point is 10^{-3}, and longevity "in vitro" is 2-5 days.

The virus is transmitted by seeds. The percentage of transmission may be high, up to 30% and in some cases up to 75%, depending on soybean cultivar, virus strain, and the date of infection. In the case of early infection, before flowering, the percentage of infected seeds is high. Conversely, in the case of late infection, after flowering, the percentage of infected seeds is reduced.

About 30 species of leaf aphids transfer the virus from infected to healthy plants, in a nonpersistent way. The most important species are *Acyrthosiphon pisum*, *Aphis craccivora*, *A. fabae*, *A. glycines*, *A. gossypii*, *Myzus persicae*, *Rhopalosiphum maidis*, *R. padi*, *Schizaphis graminum*, etc.

The virus survives in infected seed, by which it is transmitted from year to year.

Control

Soybean protection includes preventive measures, which prevent the occurrence of infection and its spread. Healthy seed should be used for planting. Seed plots should be planted at least 3 to 5 km away from commercial fields, to prevent the spread of the virus by leaf aphids. It is recommended to grow cultivars resistant to the virus or those whose seeds do not transfer the virus, such as the cultivar Merit and several others. It is advisable to use aphicides in order to control the vectors of the soybean mosaic virus.

BUD BLIGHT

Distribution and economic significance

This virus disease has been registered in the U.S., Canada, China and Australia. It has not been found in Serbia, but as it is likely that the disease is present in all tobacco- and soybean growing countries, its presence in our country cannot be ruled out.

This soybean disease has no high economic importance in the world. The extent of damage caused by it depends on the number of infected plants and time of infection. In heavily infected fields, yield reduction can range from 25 to 100% (Dunleavy, 1957).

Symptoms

First symptoms are observed on young plants, emerged from infected seeds. Infected plants are stunted and with short internodes. The most typical symptom, which occurs later, is the deforming and curving of terminal buds to form a crook. They become brown and necrotic, dry up and fall off. These plants parts are brittle and easy to break. Leaves tend to curl. The lamina is slightly or heavily wrinkled and it becomes bronze-colored.

Heavily infected plants do not form pods, but if they were formed prior to infection they become covered with brown spots. Pods usualy fall off before ripening. The infected pods contain few small seeds, which are infected by the virus.

Causal organism, biology and epidemiology

Bud blight is caused by the tobacco ringspot virus (TRSV). On the basis of its characteristics, it is classified in the Nepovirus group. The virus is polyhedral, with a diameter of 28-30 nm. The thermal inactivation point is between 60 and 65°C, the dilution end point is between 10^{-4} and 10^{-5} and the longevity "in vitro" of the virus is between 6 and 10 days at 25°C. The tobacco ringspot virus has several strains.

The virus has a wide range of host plants. Beside tobacco and soybeans, it is an important parasite of a large number of weeds, vegetables (cucumber, pumpkin, watermelon, melon, spinach, beans, peas, etc.) and flowers (*Gladiolus* spp., *Petunia hybrida*, etc.).

The virus is transmitted by mechanical contact of infected and healthy plants. It is transmitted by nymphs but not by adults of *Thrips tabaci* and grasshopper *Melanoplus differentialis*. In addition to insects and nematodes, *Xiphinema americanum* too is a vector of the virus. All these vectors transmit the virus to soybeans with low efficiency.

Virus transfer by seeds is most important. Seeds play a role in the survival of the virus as well as in its transmittance from year to year. The virus can remain vital in infected seed up to five years. Percentage of seed infection depends on the time of infection. In the case of early infections, before flowering, seeds become infected but their numbers are small or they do not form at all.

Control

Use of healthy seed is a recommended control measure. Weeds shoud be eradicated in soybean plots as well as in surrounding plots, since weed plants are important reservoirs of the virus. It is also advisable to grow resistant cultivars. Virus vectors should also be controlled. Soybean seed production should be organized in fields spatially isolated from commercial fields and other plants susceptible to the tobacco ringspot virus.

SUMMARY

This chapter summarizes the presently accumulated knowledge about the major economic diseases of soybeans. Special attention was given to the economically most important soybean diseases in the Serbia. We also described some diseases that are currently not found in our country, but which are economically important in soybean-growing regions around in the world and which may occur in Serbia.

The soybean diseases mentioned in this chapter were described in the following order: distribution and economic importance, symptoms, causal organism, their biology, epidemiology and disease control.

Soybean crop is attacked by a large number of pathogens (fungi, bacteria, viruses, etc.). The intensity of occurrence and economic importance of soybean diseases varies in dependence of climatic conditions, cultivar grown and location of production.

Downy mildew, caused by *Peronospora manshurica*, bacterial blight (*Pseudomonas syringae* pv. *glycinea*), Sclerotinia stem rot (*Sclerotinia sclerotiorum*), stem canker (*Diaporthe phaseolorum* var. *caulivora*) and charcoal rot (*Macrophomina phaseolina*) are the most widespread and economically most important diseases of soybeans in Serbia. In addition to them, the following diseases have also been observed: brown spot (*Septoria glycines*), ascochyta leaf and pod spot (*Ascochyta sojaecola*), Phyllosticta leaf spot (*Phyllosticta sojaecola*) anthracnose (*Colletotrichum truncatum* and *C. destructivum*), pod and stem blight (*Diaporthe phaseolorum* var. *sojae* - *Phomopsis sojae*), Phomopsis seed decay (*Phomopsis longicolla*), brown stem rot (*Phialophora gregata*) Rhizoctonia disease (*Rhizoctonia solani*), Fusarium wilt, root rot, pod and collar rot (*Fusarium* spp.), Pythium rot (*Pythium* spp.) bacterial blight (*Xanthomonas campestris* pv. *glycines*) and soybean mosaic (Soybean mosaic virus). These diseases, however, occur only periodically.

Descriptions are also given of forgeye (*Cercospora sojina*), purple seed stain (*Cercospora kikuchii*), powdery mildew (*Microsphaera diffusa*), rust (*Phacopsora pachyrhizi*), Phytophthora root and stem rot (*Phytophthora sojae*), wildfire (*Pseudomonas syringae* pv. *tabaci*) and bud blight (tobacco ring spot virus), although these diseases are not present on soybeans in Serbia.

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PESTS OF SOYBEAN

Radosav Sekulić, Tatjana Kereši

The animal pests of soybean have not been studied to a sufficient degree in Serbia, where this crop is a fairly recent introduction. Multiple studies (Tomašević, 1965; Đurkić, 1956; Rajković, 1982; Kereši, 1993; Sekulić et al., 1983, 2004; Jovičić et al., 1986; Simova-Tošić, 1995, etc) have shown that there are about 90 animal species that cause damage to soybean in the country. Of these, more than 83% are insect pests, while the rest are members of other animal groups (Čamprag et al., 1996). Phytophagous species are found throughout the growing season, from planting to harvesting, and can cause damage to any part of the plant, including the root system and root nodules, stem, leaves, flowers, and fruits.

Various species of birds and game (pigeons, rooks, doves, pheasants, and others) can feed on soybean seeds sown in the field. Also, there are several rodent species (hamster, European ground squirrel, common vole, etc.) that destroy soybean seeds planted in the soil and can thus throw the target plant density off kilter. Imbibed seeds and germs of soybean are attacked by the whole range of Diptera species (the so-called seedling flies), some of which, such as the species *Delia platura*, are regarded as major pests of the crop.

The underground parts of the plant and the root nodules, especially in young plants, are damaged by wireworms (larvae of the family *Elateridae*), grubs (larvae of the family *Scarabaeidae*), larvae of the *Sitona spp.*, cutworms, mole crickets, some nematode species (*Meloidogyne spp.* and *Pratylenchus spp.*), and dozens of other pest species.

Seedlings and young plants of soybean are damaged by the imagoes of various Coleoptera species, such as the gray corn weevil, beet leaf weevil, black beet weevil, alfalfa snout beetle, Sitona spp., the darkling beetles *Opatrum sabulosum* and *Pedinus femoralis*, *Maladera holosericea*, and others. In the early stages of development, a soybean crop can also suffer damage from *Acheta deserta*, cutworms, and other harmful species. In addition to this, damage can also be inflicted on germinating seedlings by birds looking for seeds planted in the soil. Pheasants tear off the apical portion of the plant, the first pair of leaves, or the cotyledons, thus slowing down or

preventing their further development. In the early stages of plant growth, the brown hare, hamster, and other rodents may also cause significant damage to soybeans by ripping off the apical parts of the plant or by destroying the plantlets altogether. Hamsters destroy young soybeans in patches of the crop surrounding their burrows.

The leaves of fully developed soybean plants are susceptible to damage caused by a variety of species from different animal groups, such as insects, mites, gastropods, mammals, and others. The most important ones among these are the strawberry spider mite (*Tetranychus atlanticus*), the painted lady butterfly (*Vanessa cardui*), the silver Y moth (*Autographa gamma*), the cabbage moth (*Mamestra brassicae*), the cotton bollworm (*Helicoverpa armigera*), and the beet webworm (*Loxostege sticticalis*). The strawberry spider mite in particular is capable of causing significant damage to soybeans. According to Tomašević (1965), *Tetranychus atlanticus* can cause yield losses of up to 27% in soybean. Soybean leaves are also fed upon by brown hares, pheasants, hamsters, field voles, some species of gastropods, and so on.

Young soybean stems may suffer damage from a number of species that are the pests of seedlings and young plants of this crop. More developed soybean stems are infested and damaged by different species of leaf aphids, cicadas, mites, and game (brown hare, deer, etc.).

The most commonly cited pests of soybean flowers are plant bugs and thrips. Their harmfulness increases in dry, hot weather conditions, especially after prolonged spells of droughty weather. The most prominent among the thrips is the highly polyphagous species *Thrips tabaci*. Szili (1976) reported finding these pests in Hungary on 60- 80% of soybean flowers.

Soybean seeds and pods also get attacked and damaged by dozens of pest species from different animal groups, including mites, mammals (brown hare, hamster, field vole, etc.), bugs (lygus bugs, alfalfa plant bug, etc.), and caterpillars of butterflies (lima bean pod borer, cotton bollworm, marbled clover, beet webworm, and others). The largest amounts of damage are caused by different rodents, bugs, and the lima bean pod borer. The pod borer in particular may cause damage of up to 90% in China. Its young caterpillars bore their way into the seeds, while the adults damage them on the outside, rendering them unsuitable for sowing.

On the global scale, soybean pests, parasites, and weeds cause yield losses of 29.1% (Cramer, 1967, as cited in Čamprag et al., 1996). In some areas in Japan, soybean yields are reduced by 20% by pests. Apart from a few isolated instances, there is no hard data on soybean yield losses due to pest damage in Serbia as of yet.

In the text below, we will discuss in greater detail soybean pests that are economically important or have the potential to be so.

PESTS OF UNDERGROUND PLANT PARTS

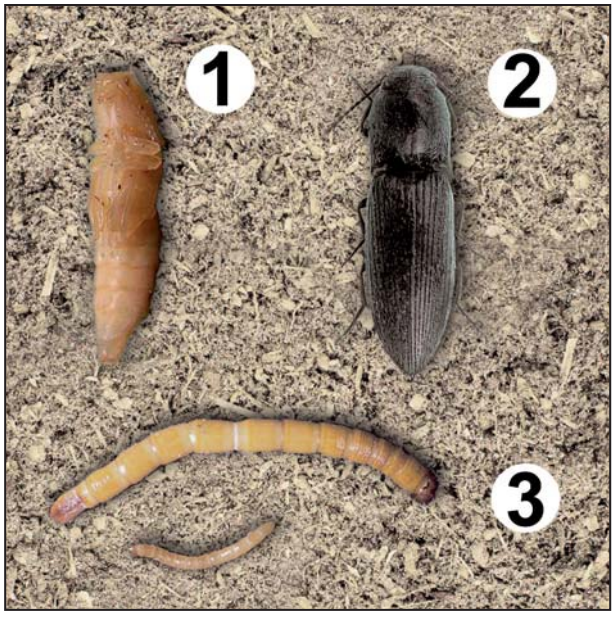
Click beetles (*Elateridae*)

The larvae of the click beetles, the so-called wireworms, are regarded as some of the most economically important pests of underground plant parts, especially in row crops. On chernozem and black meadow soil in northern parts of Serbia, 12 phytophagous insects of the family Elateridae have been recorded, with *A. sputator* L. and, especially, *Agriotes ustulatus* Schall. being the dominant species (Čamprag et al., 1985). In the neighboring countries, with few rare exceptions, the same Elateridae species are dominant (Perju and Mare, 1984; Štrbac, 1983; Szili, 1979; Szarukan, 1992).

Adult click beetles have elongated, flattened bodies and are about 7-10 mm long. Their larvae (wireworms) are hay-colored to reddish and have elongated, cylindrical bodies enveloped in a hard chitinous shell (Figure 13.1).

Figure 13.1

Agriotes ustulatus *(photo: B. Đukanović)*



1. pupa, 2. adult insect, 3. larva

The larva of the insect has a small head and three pairs of short thoracic legs, all of equal length. The larvae of *A. ustulatus* are up to 10 mm long in the first year of life and reach a length of 25 mm in the last. In all the phytophagous species of click beetles, the larvae live in the soil throughout their development. Click beetles develop over a period of three to five years. Most Elateridae species overwinter as larvae in each year of development but the last, when they spend the winter as adults.

An exception to this among the economically important species is *A. ustulatus*, which always overwinters in the larval stage. Following mating, the females lay their eggs (up to 660 per female) primarily in fields planted to wheat, alfalfa, or clover or in other areas with dense vegetation. Oviposition occurs chiefly in the surface layer of the soil, where soil is wet and loose. After hatching, the larvae live in the soil and move about it both vertically and horizontally. Vertical migrations occur especially as a result of changes in soil moisture and temperature, and they also come about as a consequence of the larvae needing to feed or molt. In wetter conditions, the larvae move from the lower soil layers to the upper ones, whereas when the surface layer of the soil dries out, they migrate in the opposite direction. Wireworm activity is favored by temperatures of about 20°C. Horizontal migrations are limited to several meters across and occur mainly when the larvae are in search of food.

Favorable conditions for the reproduction and, hence, harmfulness of click beetles include: the cultivation of a small grain, alfalfa, clover, or a any crop grown in a dense stand on a large area; repeat sowing or monoculture of small grains; reduced mechanical tillage; high or increased presence of weeds in the crop; irrigation; intensive use of insecticides on plowland that destroys not only pests but also numerous beneficial insects, and so on. Soil moisture in particular is considered an especially important limiting factor in the reproduction and development of click beetles. Eggs laid in dry soil lose water quickly, as do the young larvae, which dehydrate and die. The most important natural enemies of click beetles are ground beetles (the family Carabidae), which are very numerous in agrobiocenoses. According to Bobinskaja and Grigoreva (1965), a single adult member of this family of mostly predatory insects is capable of destroying two to four larger-sized wireworms in the course of a single day, while ground beetle larvae destroy 10-15 young wireworms in the first year of life alone. Various bird species (especially crows and starlings) can also significantly reduce wireworm populations. In the stomach of a single rook alone, no less than 350 click beetle larvae have been found.

Wireworms are highly polyphagous and cause damage to a large number of cultivated plants and weeds. There is virtually not a single plant species that is not threatened by these pests. The largest damage occurs in the first part of the growing season in herbaceous plants. The larvae of click beetles pose the greatest threat to the planted seeds, germs, and newly emerged young plants. The preferred food of wireworms are young underground plant parts, and the pests cause the greatest damage to various row crops. Because soybean is a crop with a large number of plants per m^2, this species is less susceptible to wireworm damage than the other row crops (sugar beet, sunflower, maize, etc). A higher incidence of wireworms in soybean will cause the plants to suffer from dwarfism or stunted growth or to become completely destroyed. At the PIK Bečej agricultural estate, wireworms reduced soybean plant density by up to 50% in some years when wheat was used as the preceding crop. The damage occurred in patches, in which wireworm incidence was 10 or more individuals per m^2. When wireworm population in the soil is high, older plants may be destroyed too, especially in years favorable to wireworm activity.

Control measures

Wireworm numbers and damage can be successfully reduced by a combination of cultural practices and chemical and other measures. Agronomic practices are of great importance in limiting the reproduction of click beetles. Especially important among them is well-timed, quality tillage. Crop residues of small grains should be plowed under immediately after harvesting, preferably right away, because this way a large number of Elateridae eggs and young larvae will become exposed to unfavorable weather conditions and numerous natural enemies. Deep summer plowing in the month of August also reduces wireworm abundance in the soil. In addition to tillage, correct crop rotation, the preceding crop, and weed control contribute to this as well. In particular, click beetle reproduction is promoted by the continuous growing of small grains in the same field (for two to three years in a row). Consistent alternation between small grains and row crops, on the other hand, represents a limiting factor to the mass propagation of these insects.

Chemical suppression of soil pests should be carried out only after determining the number of these pests on each plot intended for soybean planting. The threshold of economic damage or the critical number of larvae per m^2 for soybean are not yet known for Serbian conditions. For rough guidance, we can look at a piece of data reported for Hungary, a country whose insect fauna is similar to that of Serbia. In that country, according to Szili (1979), the critical number of wireworms for soybean is 4-8/m^2. Another useful observation has been made by Toth (1992) in the Hungarian part of the region of Baranja. That author reports that the tolerance of soybean crops to being attacked by wireworms and other pests of underground plant parts is two to three times higher than that of the other row crops. Wireworm incidence and the need to suppress these pests in soybean fields are increased when this crop is grown after small grains (primarily when a small grain has been grown in the same field for two years in a row) or after a perennial legume (Čamprag et al., 1996).

If soil sampling shows that wireworms need to be controlled by chemical means, the treatment can be performed in one three ways - by treating the entire area of the field just before sowing; by incorporating granulated or liquid chemicals during planting using a depositor or a sprinkler; or by treating the seeds themselves. The first method of control has now been largely abandoned, because it is economically unviable and environmentally harmful (it increases contamination levels in the field and results in the destruction of the natural enemies of the pests). At present, the most commonly used of the three methods is the incorporation of granulated insecticides into the area of the rows or the application of liquid formulations by spraying the row area at the time of sowing. In Serbia, chemicals based on **terbufos**, **carbofuran**, **chlorpyrifos**, **fenitrotion + malathion**, **foxim**, **carbosulfan**, **bifenthrin**, **tefluthrin,** and others are used the most for this purpose in row crops. However, none of these pesticides is registered for use in soybean (Savčić-Petrić and Sekulić, 2007). In Hungary, pesticides based on **diazinon** and **carbofuran** are used for pest control in fields sown to soybean (Ocskó et al., 2007).

The third method of controlling soil pests, which is regarded as highly promising, is to use seeds previously treated with insecticides for planting. With major row crops such as sugar beet, sunflower or maize, this method is used for wireworm incidence levels of up to five individuals per m^2 (or even higher). Because of soybean plant density and initial plant development, it is thought that this control method could completely replace the other methods of chemical pest control, especially since there are indications that insecticide application in bands during soybean planting has a negative effect on the bacterium *Rhizobium japonicum*. Vratarić (1986) argues that insecticides should be applied a month before planting soybeans. Romanian researchers Barbulescu et al. (1994) studied the compatibility of *Rhizobium* and some fungicides and insecticides for soybean seed treatment in comparison with soybean seeds to which only the bacterium has been added. They found that pesticides based on **carbofuran** and **bifenthrin** exhibit no major incompatibility for root length, plant height, and pod number. On the contrary, the study results showed that in some cases seeds treated with these chemicals performed even better with regard to said traits than the nontreated seeds. In France, soybean seeds are treated with **furathiocarb** (Promet CS-400) dosed at 0.12 mg a.i. per 1 kg seed. Because of its systemic effect, this pesticide is recommended for reducing pest numbers on the above-ground plant parts (small leaf weevils and thrips), (Cluzeau, 1994). According to the latest research carried out in Bulgaria (Nikolova, 2006), the following insecticides used for seed treatment exhibited good efficacy in protecting soybeans from soil and above-ground pests in the initial stages of plant development: **fipronil** (Cosmos 500 FS, 0.75 l/100 kg seed), **thiamethoxam** (Cruiser 350 FS, 0.9 l/100 kg seed), **imidacloprid** (Gaucho FS 600, 1, 2 and 3 l/100 kg seed), and **thiodicarb** (Semevin 375 FS, 3 l/100 kg seed).

When considering insecticides for the control of soil pests, preference should always be given to systemics, the method of application notwithstanding. This reduces the number of pests on the above-ground plant at the start of the season, leading to a reduced number of treatments or their complete absence. In Serbia, there are no registered chemicals for treating soybean seeds as of yet.

Scarabs *(Scarabaeidae)*

A number of species of this family have been reported as pests of underground plant parts in soybean (Szili, 1979; Simova-Tošić et al., 1988; etc.). Among them, the most common pests of field crops in general are the April beetle (*Rhizotrogus aequinoctialis* **Hrbst**.), the summer chafer (*Amphimallon solstitialis* **L**.) and some *Anisoplia* species (*Anisoplia austriaca* **Hrbst**., *A. segetum* **Hrbst**., *A. lata* **Er**., etc). Multi-year studies have shown that among the larvae of the *Anisoplia* species the species *A. austriaca* is dominant. This pest has been found on 76% of the total area under soybean surveyed (Kereši, 1993). Species of the genus *Anomala* may even damage the leaves of soybean (Simova-Tošić et al., 1988) and are widely distributed around the world and considered to be major pests of cultivated plants.

The adult insects are medium- to large-sized and are most often brown. The larvae of scarabs and *Anisoplia* spp. are contorted in the form of the letter C and are known as grubs. They are cylinder-shaped and whitish except for the head, which has a yellow, chestnut-like color. The rear portion of their bodies is flared and has characteristic morphological traits that are used to distinguish one species from another. When fully grown, grubs reach a length of 25 to 65 mm depending on the species. The larvae of the phytophagous species always live in the soil (Figure13.2).

The development of a scarab beetle generation typically lasts two or three years but may sometimes be extended to four to five calendar years. Overwintering is in the form of larvae or larvae/adults. Eclosion of the imagoes takes place in the spring or early summer. Fertilized females lay their eggs into the soil in fields with a dense plant population such as those sown to small grains or alfalfa or on other kinds of noncultivated grassy terrain surrounding plowed field. The hatched larvae live the longest and the older they are, the more harmful they become. Over the year, they are prone to vertical and horizontal migrations, especially under the influence of soil temperature and moisture. In the rhizosphere, they come closest to the soil surface in the spring, early summer, and autumn. The fully grown larvae in the last year of development are found in the surface soil layer, where in chambers they have built up they transform into pupae and, later on, into adult insects.

Figure 13.2

Larvae of scarab beetles *(photo: R. Sekulić)*

Grubs are very polyphagous and attack the underground portion of many cultivated plants and weeds, with row crops being particularly hard hit. The roots of younger plants are often bitten through completely, while those of older plants suffer

smaller or greater bite injuries, which are most often pit-shaped. The injured plants lose turgor and gradually dry out and die or fall behind in their development. The damage in the field occurs on individual plants or on groups of plants forming smaller or larger patches. The greatest damage occurs when the weather is hot and dry and grub incidence in the soil is high. Particularly prone to noticeable damage are soybean crops planted after perennial crops such as alfalfa or clover or after small grains grown in the same field for two or more years.

Control measures

The population of grubs in the soil is reduced by the following: correct crop rotation; removal of stubble; weed control; deeper, more frequent tillage; and so on. The pests should be chemically controlled when the inspection of a soil intended for soybean planting reveals the presence of 4-8 larvae per m^2 (Szili, 1979). The insecticides and methods of their application are the same as in the case of click beetles.

Diptera *(Diptera)*

Soybeans suffer damage from a number of species of the order Diptera, most notably those of the families *Anthomyidae* and *Agromyzidae*. Their larvae can cause injury to imbibed seeds, germs, or seedlings or mine the leaves of soybeans. They have been reported as pests of soybean in many countries in Europe. Generally speaking, damages caused by phytophagous fly species in soybean are minor, except for some rare cases. Several Diptera species that mine the leaves of soybean have been reported worldwide. In Serbia, Simova-Tošić et al. (1988) have recorded a very low incidence of the species *Liriomyza congesta* **Beck**., which attacks soybeans as well as pea, horse bean, alfalfa, and other legumes.

The most important deleterious species of the order Diptera in soybean is ***Delia platura*** Mg., or the seedcorn maggot. This species is regarded as a major pest of soybean in France (Dalbello-Polese., 1990). In the Northern Caucasus region, destruction of 20% of the soybean crop has been recorded in some fields. In Serbia in 1994, 3-5% of soybean plants at the cotyledon stage were destroyed by the seedcorn maggot at the PIK Bečej agricultural estate.

Adult individuals of ***Delia platura*** are black-gray in color and have distinct black stripes on the thorax. They are about 6 mm in size. The larvae are white in color, have no legs, and their bodies taper at the front. When fully grown, they are around 7 mm in length. The seedcorn maggot produces two to three generations per year and overwinters as a pupa in the soil. Eclosion occurs in the spring and the females lay their eggs into the surface layer of the soil following fertilization. The hatched larvae feed on decomposing organic matter, but they damage soybeans as well. The pest has been recorded on more than 30 species of cultivated plants.

It attacks soybean as well as maize, sugar beet, pea, sunflower, and some vegetable species.

During their development, the larvae damage imbibed seeds, germs, and cotyledons, and they also attack the newly emerged plantlets, boring their way into the young stem. As a result of this, the plants fail to develop and either die or, if they manage to survive the attack, start lagging behind in growth. Greater damages occur when the spring is cooler and emergence is prolonged. In such circumstances, the crop may thin out if the larval abundance is high.

Control measures

Damage by ***Delia platura*** is reduced by all agronomic practices that promote germination, emergence, and early plant development. Seeds of good quality should be used, planting should be done on optimal dates and to an optimal depth, and so on. In France, ***Delia platura*** is controlled by insecticides based on **carbofuran**, **chlorpyrifos**, **diazinon**, **chlorfenvinphos,** and **dichlofenthion** (Perny, 1988). **Furathiocarb** (Deltanet; 8 kg/ha) and **benfuracarb** (Oncol 5 G; 12 kg/ha) are also recommended. When using the last two chemicals, caution is advised, because the microgranules might have a toxic effect on the bacterium *Rhizobium japonicum* (Dalbello-Poleze et al., 1990). Romanian researchers Barbulescu (1994) studied how preventive seed treatment with insecticides based on **carbofuran** (Carbodan 35 ST, 20 l/ton), **bifenthrin** (Talstar 20 ST; 4 l/ton), **imidacloprid** (Gaucho 70 W; 10 l/ton), and **furathiocarb** (Promet 400 CS; 20 l/ton) and an insecticide coded as 80415-A (2.5 l/ton seed) affected the percentage of germinated and attacked plants and grain yield at harvesting. Germinated plant percentage increased by an average of 13% when the seed had been treated as opposed to an untreated control. The largest increases were achieved with **imidacloprid** (25%) and **furathiocarb** (22%). The use of these insecticides also resulted in the lowest percentage of infested plants (3.6 and 3.7%, respectively). On the control plots, plant damage levels were three times higher (12.2%). On average, grain yields increased by 35.4% relative to the control. In Serbia, there are no registered insecticides for the control of this pest yet.

Nematodes *(Nematoda)*

Several dozen nematode species have been recorded in soybean fields worldwide. In the former Soviet Union, Truskova (1973) analyzed the rhizosphere and other parts of the soybean plant and found 159 nematode species, whereas in the U.S. over 100 species of nematodes have been identified in soybean (Schmitt and Noel, 1984). In the southern, warmer parts of the United States, the most important nematode pests are those from the genus *Meloidogyne* and the species *Heterodera glycines*. The latter pest, the soybean cyst nematode, has been known in eastern Asia as a problem species since 1915. It is listed as a quarantine pest in Serbia and many other countries and is a potential danger to soybean crops (Krnjajić and Krnjajić, 1987).

In neighboring Hungary, species of the genera *Pratylenchus*, *Rotylenchus* and *Helicotylenchus* (Elekes and Budai, 1979) have been reported in soybean. In eastern Croatia, Ivezić (1980) also found these genera as well as some others, with phytoparasitic *Pratylenchus* species being the most numerous. The abundance of these pests increases in the autumn compared with the spring and summer. In soybean fields in Serbia, Jovičić et al. (1987) also found *Pratylenchus* species most often, and in some of the cases the soybean crops had noticeable damage from these pests. Of the other nematode genera, *Tylenchorhynchus* and *Helicotylenchus* are also found. Polyphagous species of the genus *Meloidogyne* (*M. incognita* Chitw. in particular), which are known pests of soybean (Riggs and Schmitt, 1987), have been reported in Serbia as well (Čamprag et al., 1996).

Although soybean fields have been found to have a very rich nematode fauna, only a relatively small number of these species (around 30 of them) are an actual pest of the crop. These nematodes damage soybean in most soybean-growing regions of the world. The damages are manifested in the fields in the form of patches of plants lagging behind in growth. In the U.S., attacks by different nematodes result in average yield losses of 2% (Le Clerg as cited in Dekker, 1972).

Free nematodes, the *Pratylenchus* species, are highly migratory endoparasites that cause lesions on the roots of soybeans. As a result of their attack, necrosis occurs on the affected plant parts and brown spot appears on the surface of the tissue. Such plants have trouble supplying themselves with nutrients and water and will start falling behind in growth and their leaves will gradually turn yellow. On the older radicles, necrotic rings will appear, as a result of which the underlying parts will deteriorate and new rootlets will form. The injured plants have a reduced root system that, as a rule, contains a multitude of nematodes at different stages of development. The surface of the root that has been damaged in this way is penetrated by the causal agents of different plant diseases and the overall level of damage is thus increased. In Serbia, two species from this genus have been identified, *P. crenatus* and *P. neglectus*.

Species of the genus *Meloidogyne* cause the appearance of galls, or cecidia, on the roots of soybean plants and thus belong in the group of so-called cecidogenic nematodes. They are very polyphagous, attack many cultivated plants, and are endoparasites that exhibit pronounced sexual dimorphism. The males are small (0.8-2 mm long), colorless, and worm-like in shape, while the females are pear-shaped and 0.5-0.8 mm in size. They produce several generations per year or more. At a temperature of 25-3°C, the development of one *Meloidogyne* generation takes three to four weeks. They are mostly pests of tropical and warmer climates (Krnjajić and Krnjajić, 1987).

As the nematodes feed intensively and exude certain secretions in the process, the destruction of cell membranes occurs in parts of the root and a type of giant cell is formed. This results in the tissue becoming hypertrophied and smaller or larger galls, or cecidia, are formed.

The size of the galls depends on the nematode species, severity of the attack, and susceptibility of the soybean cultivar. The symptoms of attack by this group of nematodes include stunted plant growth and plant chlorosis and wilting under stress conditions. When the number of nematodes is high, plants of susceptible cultivars become desiccated and die before reaching maturity and becoming harvest-ready. The galls formed by the nematodes differ from the nodules of the bacterium *Rhizobium japonicum* both in their structure and in their interior color. Galls of the *Meloidogyne* species are an integral part of the root and cannot be separated from it, while the nodules of *Rhizobium japonicum* can be easily detached from the root. Also, the interior of the galls is whitish in color, while the nodules are reddish on the inside.

Control measures

The growing of resistant varieties of soybean is the best way to control nematodes. A number of soybean cultivars are resistant to attacks by *M. incognita*, while the cultivar Santa Roza has been shown to be resistant to the species *M. javanica* in Brasil (Kiihl as cited in Hinson and Hartwig, 1982). Cultivars resistant to the *Meloidogyne* species and *Rotylenchulus reniformis* (Hinson and Hartwig, 1982) have also been found to be resistant to the nematodes of the genus *Pratylenchus*, which are the nematodes most commonly found in Serbia. Šamota and Ivezić (1988, 1989) studied 26 cultivars and lines of soybean in eastern Slavonia and found them to have different levels of susceptibility to the dominant genus *Pratylenchus*.

In addition to growing resistant cultivars, nematode damage can also be reduced by using multi-field crop rotations and excluding the affected crop species as much as possible as well as by a high level of agronomic practice application that creates good conditions for crop development. Special attention needs to be given to top-quality tillage, optimal fertilizer application, weed control, and crop management in general.

PESTS OF ABOVE-GROUND PLANT PARTS

Maize leaf weevil *(Tanymecus dilaticollis* Boh.*)*

This species is primarily found in the steppes and plains of southeastern Europe and Asia Minor (Čamprag, 1977). In Serbia, it is especially common in the province of Vojvodina and in the eastern parts of central Serbia. High populations of this pest are for the most part coincident with areas in which maize is grown on chernozem and black meadow soil (Čamprag and Sekulić, 2002). The maize leaf weevil avoids soils that are heavy, compacted, and extremely wet. It has been reported as a pest in Hungary (Szili, 1979), Romania and Serbia. During April and May, Tomičin (1964) recorded a fairly severe outbreak of this weevil in soybean in the vicinity of Novi Sad. In some of the fields, 20-40% of the leaf mass was damaged. According to the data obtained by Romanian researchers, 8-10 imagoes per m^2 may cause significant damage to soybeans (Radulescu and Paulian, 1973).

The adults of the species are gray and about 7 mm long. The larvae are of similar size and are white in color, apod, and slightly bent. The maize leaf weevil produces one generation per year and overwinters as an imago in the soil, most commonly at a depth of 40-60 cm. Most of weevil population overwinters in fields on which maize was grown and these areas are the main hotspots of the pest. Besides growing continuous maize on large areas, mass reproduction of the maize leaf weevil is also favored by hot and dry springs and moderately wet summers.

The main damage is caused by the imagoes during April and May during the period of complementary feeding. The weevil is very polyphagous and damages a large number of cultivated plants and weeds. It is a dangerous pest of maize, but it causes major damage to other row crops as well (sugar beet, sunflower, etc.). The plants are especially under threat from emergence until the formation of several leaves. During that time, the insect damages the cotyledons and the first leaves or consumes entire plants.

Large damages can be expected in soybeans if they are planted on plots on which maize was grown previously, especially as a perennial monoculture. In such cases, several dozen imagoes can be found per m^2. The outskirts of soybean fields can also be damaged, especially if they are bordering on fields on which maize was grown in previous years.

Similar damages can also be caused to soybeans by other snout beetles, such as *Tanymecus palliatus* F., *Psalidium maxillosum* F., *Otiorrhynchus ligustici* L., and others. For the most part, these beetles damage soybeans at about the same time as the maize leaf weevil does.

Control measures

In soybean as well as in general, damages by the maize leaf weevil will be reduced if growing continuous maize is avoided. Good quality tillage and soil preparation as well as other crop tending measures implemented during early crop development will also reduce the damage by *Tanymecus dilaticollis* and other members of the family Curculionidae. Chemical control of this pest is resorted to if there are 8-10 imagoes per m^2 (Radulescu and Paulian, 1973).

In agricultural practice, *Tanymecus dilaticollis* is most intensively controlled by foliar treatments of sugar beet. The control of the maize leaf weevil and other snout beetles is carried out using insecticides based on **fenthion**, **fenitrotion**, **deltamethrin**, **bifenthrin**, **lambda-cyhalothrin**, or **cypermethrin** or by ready-made combinations based on **monocrotophos** and **cypermethrin**, **chlorpyrifos** and **cypermethrin,** and so on. Practical experience has shown that the best results in controlling this pest are achieved when using **fenthion.** The use of systemic insecticides for the control of soil pests (in the form of granules, liquid formulations, or by seed treatment) would surely reduce the populations of this pest as well.

Small leaf weevils *(Sitona* spp.*)*

Sitona spp. are members of the family Curculionidae and are known pests of leguminous plants. In Serbia, the most common Sitona species are: *Sitona humeralis* **Steph**., *S. lineatus* **L**., *S. crinitus* **Hrbst**. as well as some others. In Serbia and some of the neighboring countries, they are known pests of soybean, and so is the species *S. punctolineatus* **Steph**. in Hungary (Szili, 1979). Among the cultivated plants, they cause damage to alfalfa, red clover, pea, vetch, bean, lentil, soybean, and other legumes.

The adults of the Sitona spp. are grayish in color and 3 to 7 mm long. In some of the species, the elytra have spotted stripes that can be more or less prominent. As in all snout beetles, the larvae are whitish, feetless, and slightly bent in shape. They produce one generation per year and overwinter as imagoes in the soil or underneath plant residues. They appear in early spring and feed intensively, leaving their characteristic semicircular bite marks on the margins of young leaves. The fertilized females lay eggs mostly in the soil. The period of oviposition is very prolonged and can last until well into the summer months. Upon hatching, the larvae descend into the soil and bore their way into the bacterial nodules and proceed to feed on them. When fully grown, the larvae leave the nodules and transform into pupae in the surface layer of the soil. The adult insects of the new generation appear over the summer, feed for a while, and then go on to overwintering.

Damage on the plant is caused by both the adults and the larvae. Visible damage by the imagoes appears especially when the soybean field is adjacent to a crop of freshly overturned perennial legume or a field on which pea has been grown.

The imagoes cause the most damage on the margins of soybean fields. The larvae, living at the expense of bacterial nodules, reduce the amounts of nitrogen in the root and soil, causing the plants to develop at a slower pace. Such plants also become more susceptible to attacks by phytopathogenic microorganisms. A single larva can damage several bacterial nodules during its development. The harmfulness of Sitona is particularly pronounced when the spring is dry and warm and the insect population is high. The plants are especially at risk from the moment of emergence until the formation of several pairs of true leaves.

Control measures

All agronomic practices promoting faster plant growth will reduce damage by the Sitona spp. Special attention must be paid to crops bordering on freshly plowed up fields of perennial legumes (alfalfa and clover). In Hungary, which has a similar climate to the north of Serbia, the critical number for the Sitona spp. in soybean is 8-15 imagoes/m^2 (Szili, 1979). These insects are highly sensitive to insecticide applications. The formulations can be applied in the form of a powder or by spraying. In Serbia, the only registered insecticides for this purpose are **fenitrotion-**based ones (in alfalfa). The other insecticides intended for controlling harmful snout beetles will also have a satisfactory effect when used against the Sitona spp. In France, as already mentioned, seed treatment with the systemic insecticide **furathiocarb** is used for controlling small leaf aphids and thrips.

Leaf aphids *(Aphididae)*

Six species of leaf aphids have so far been found in soybean fields in Serbia and the neighboring countries. In Serbia, Simova-Tošić (1995) identified three species: *Acyrthosiphon pisi* **Har**., *Aphis craccivora* **Koch**. and *Aphis fabae* **Scop**. Hungarian researchers Ovari and Rakk (1990) also reported the first two of the three species as well as *Myzus persicae*. According to their two-year results, *A. craccivora* was the dominant species with 55.6%, followed by *A. pisi* with 44.2% and *M. persicae* with only 0.2%. In Bulgaria, Nikolova (2006) identified five species of leaf aphids in total. The most abundant among them were *Aphis gossypii* **Glov**. (59.7%), *A. fabae* (22.1%) and *Acyrthosiphon pisi* (11.6%), while *Aphis craccivora* and *Therioaphis maculata* **Buckt** combined accounted for the remaining 6.6% of the leaf aphid population.

Leaf aphids are small insects. They are light green to almost black in color and can always be found on plants in smaller or greater numbers. Generally speaking, leaf aphids are especially numerous on herbaceous plants from mid-spring to mid-summer, after which their population declines due to unfavorable weather conditions and an increased presence of natural enemies. In soybean, the aphids are especially abundant from mid-June on, with their numbers peaking in late June and early July (Ovari and Rakk, 1990).

The feed by sucking out plant sap from all the above-ground plant parts (leaves, stem, flower, pod). This causes the leaves to twist and deform, the flowers dry out and fall off prematurely, and the seeds that manages to form is smaller and less capable of germination. Chlorotic spots form in places where the leaf surface has been punctured by the feeding aphid and the entire plant suffers from stunted growth.

The aphids form smaller or larger colonies on the plant. In addition to the direct damage they cause, they are also important as vectors of different causal agents of diseases, most notably viruses. In France, besides the species mentioned above, *Aphis gossypii* and *Rhopalosiphum maydis* are also known as vectors of the soybean mosaic virus (Dalbello-Polese, 1990).

Acyrthosiphon pisi **Har**. – The green pea aphid is one of the largest aphid species in existence. Wingless individuals of *A. pisi* are 3-5 mm long and of green color. Out of the winter eggs deposited on perennial legumes the founder individuals appear in the spring (late April/early May) that then proceed to establish colonies of wingless aphids. During the growing season, winged parthenogenetic females appear that migrate to various herbaceous plants, thus spreading the infestation. In the autumn, sexed individuals appear again and proceed to mate and the females lay eggs on their winter hosts. The insect produces 4-10 generations per year.

The green pea aphid is highly polyphagous and attacks red clover, alfalfa, bean, pea, soybean, vetch, and other plant species. In soybean, it can be found on plants that have developed only a few leaves and is often present in mass numbers from mid-June onwards (Szili, 1979). This aphid is one of the most important vectors of the soybean mosaic virus (Tošić, 1995).

Aphis craccivora **Koch**. – The cowpea aphid is 1.4 to 2.1 mm in size. The color of the wingless females is a very dark grey nearing black, while that of the larvae is even darker, virtually black. This aphid produces about 10 generations per year and overwinters as an egg on perennial legumes, much like the green pea aphid. When the winter is mild, the aphid may overwinter as a parthenogenetic female. In the spring, the first colonies of this pest appear in April and the migrations start as early as May. The colonies develop on the abaxial surface of the leaves and on the youngest, apical parts of the plant. The aphid infests many different plants, mostly notably legumes, and is very common in alfalfa. In soybean, it may appear on young plants that have only two to three leaves, whose growth will often be stunted as a result of the infestation. The cowpea aphid is also important as a virus vector.

Aphis fabae **Scop**. – The black bean aphid is 1.8 to 2.5 mm in size. Its body is in oval in shape and covered in a fine waxy powder. The color of the wingless females is a fuzzy or greenish black, while the larvae are dark green in color. The larvae go through four stages in their development. The black bean aphid overwinters as a winter egg on woody plants, most commonly on spindle (*Euonymus europaeus*). In the spring, two to four founder generation develop on the primary hosts. The first flights of winged, parthenogenetic females from the winter to summer hosts (various herbaceous plants) begin in about mid-April and are particularly intensive in mid-May.

They are found on their summer hosts in mass numbers in May and June. Intensive reproduction of the pest is favored by temperatures of 20 to 25°C and by high relative humidity. The aphid may develop up to 13 to 18 generations per year. At the end of the summer and in early autumn, the winged form of the insect appears again and migrates to the primary hosts, where it produces sexed progeny, after which the fertilized females lay eggs that then overwinter.

The black bean aphid is highly polyphagous and causes damage to about 165 cultivated plant and weeds. It is most often found in sugar beet, fodder beet, sunflower, poppy, vetch, and other species. It is also important as a virus vector.

Myzus persicae **Sulz**.- The green peach aphid is yellow-green in color and up to 2.5 mm in size. The aphid mostly goes through a full life cycle and overwinters as a winter egg, primarily on peaches. It may produce up to 16 generations per year. The winged, parthenogenetic females fly over to their summer hosts from mid-May onwards. Intensive reproduction of the aphid on herbaceous plants occurs in the second part of spring and in early summer.

The green peach aphid has a wide global distribution and is highly polyphagous. It infests over 400 plant species and is very important as a vector of various diseases. According to Heinze (1983), the aphid is known to transmit nearly 200 viruses. As a virus vector, this aphid is ten times more important than the black bean aphid because of its greater mobility (Dixon, 1976). It is also a major vector of the soybean mosaic virus (Simova-Tošić, 1995). The green peach aphid has been reported as a pest of soybean in Hungary (Ovari and Rakk, 1990), and there have been reports of it in Serbia as well (Čamprag et al., 1996).

Leaf aphids have many natural enemies. For soybean, Simova-Tošić (1995) reports the following as being the most numerous in Serbia: the seven-spot ladybird (*Coccinela septempunctata* **L**.); the larvae of hoverflies (*Syrphidae*); the green lacewing (*Chrysopa carnea* **Steph**.); and the aphid midge (*Aphidoletes aphidimyza* **Rond**.). The role of these insects as predators can be very important and they must be taken into consideration as beneficial insects before a decision is made on whether to control leaf aphids by chemical means.

Contorl measures

By plowing under the crop residues on time and by performing deeper tillage after small grains and annual legumes that mature early, unfavorable conditions are created for the maintenance and further reproduction of the leaf aphid population.

In Serbia, there are a number of registered insecticides for controlling leaf aphids and species of the order Homoptera in general (Savčić-Petrić and Sekulić, 2007). Aphid control in sugar beet has shown that the best results are achieved by using insecticides based on **dimeothoate**, **fenitrotion**, **deltamethrin, bifenthrin, pirimiphos-methyl,** and **pirimicarb**.

The last of these chemicals is ecologically selective towards many natural enemies of the aphids (*Coccinellidae* and others.) and should therefore be used as much as possible against leaf aphids as part of an integrated approach to pest management.

In Hungary, the recommended pesticide for combating early attacks by leaf aphids and other pests in soybean is **carbofuran,** an insecticide intended primarily for controlling soil pests (larvae of the families *Elateridae*, *Scarabaeidae* and others). In this case, the systemic effects of this chemical are counted upon.

Thrips *(Thysanoptera)*

The thrips fauna and its deleteriousness in soybean have not been studied enough in Serbian conditions. In Ukrainian fields planted to this industrial crop, as many as 47 species of thrips have been recorded, 37 of which are phytophagous and 10 predatory. In the former Yugoslavia and the neighboring countries, six phytophagous species of thrips were identified, all belonging to the family Thripidae. In Serbia, Simova-Tošić (1995) has found three species, namely *Kakothrips robustus* **Uz**, *Frankliniella intonsa* **Tryb**., and *Aeolothrips fasciatus* **L**. The first two are phytophagous, while the third is a predator of aphids, mites, and other small organisms. In Vojvodina, four species and one genus of thrips have been registered in soybean crops, with the species *Thrips tabaci* **L**. being dominant, accounting for 53.5% of the population (Đurkić et al., as cited in Čamprag et al., 1996). While studying the thrips fauna of Hungary, Ovari and Rakk (1990) identified four species of this order, two of them, *T. tabaci* and *Frankliniella intonsa*, phytophagous. The former of the two species, the onion thrips, was predominant, accounting for 86% of all the individuals collected. The onion thrips are an important pest of soybean in all the major soybean-growing regions of the world (Čamprag et al., 1996).

Thrips are small insects, measuring 0.5-2 mm in size. They have two pairs of narrow, membranous wings that are fringed on the margins. They are common in soybean crops and will remain there throughout the growing season, although their numbers are at their peak in July and August. The reproduction and development of thrips are favored by hot and dry weather and lengthy periods of drought. Thrips are especially common on the youngest parts of the plant (leaf and flower buds, flowers, etc). The plants are damaged by the adults and larvae sucking out plant sap. Thrips are vectors of various viral diseases.

Thrips tabaci **L**. – The onion thrips is 0.8-1 mm in size and light yellow to brown in color. The larvae are always lighter in color than the adults. The species is found throughout the world and produces a number of generations per year. It appears on cultivated plants as early as the beginning of April. Onion thrips are present throughout the growing season and their numbers are especially high during the summer months. This pest is highly polyphagous and is found on more than 100 species of cultivated plants and weeds. It causes great damage to tobacco, onion, cotton, and other crops. Anđus (1996) found the onion thrips to be the most common species in sunflower.

As a soybean pest, the species has been reported in Serbia, Hungary, and a host of other countries. In most of the cases, it was the dominant species in the thrips population of a given soybean crop. It is found on soybeans from early or mid-June until mid-September and its numbers peak in July and early August. This species of thrips is most often found on soybean leaves of different age and is especially common at the time of intensive plant growth. It has also been observed that the highest density of the onion thrips coincides with the flowering stage in soybean.

Kakothrips robustus **Uz**. – The adults of the pea thrips are 1.4 to 1.8 mm in size and are of dark brown, almost black color. The pea thrips has one to two generations a year and overwinters as a larva in the surface soil layer or in other secluded places. The adult insects appear in the spring and this is when they are most commonly found on the buds of different legumes, on which they feed by sucking out plant sap. The females lay their eggs into plant tissue, and the larvae hatch about a week after that.

The pea thrips primarily infests legumes. Both the larvae and the adults suck out plant sap from the leaves, flowers, and pods. When the incidence of the insect is high, the flowers dry out and the pods assume a silverish appearance, similar to pea pods. The pest may cause major damage to pea, vetch, clover, alfalfa, soybean, bean, and other plant species. The pea thrips have been reported as a pest of soybean in Serbia (Simova-Tošić, 1995) and are fairly common as a soybean pest in Hungary as well (Szili, 1979).

Frankliniella intonsa **Tryb**. – The European flower thrips is similar in length to the onion thrips. The females are 0.8 to 1 mm long and dark brown. The species produces several generations per year and overwinters as a larva underneath plant residues. The flower thrips is a highly polyphagous species. In central Europe, it damages around 150 plants, both cultivated and weedy. According to Jenser (1988), European flower thrips are commonly found in Hungary on the flowers of cultivated legumes.

The species has been found in soybean crops in Serbia (Simova-Tošić et al., 1988), and there have been reports of it in Hungary too (Szili, 1979). It appears at the start of soybean flowering. In China and Taiwan, this pest injures not only soybean flowers but soybean leaves as well.

Control measures

Practice has shown that the mass occurrence of onion and other thrips in different crops can be chemically controlled by a number of insecticides. In Serbia, there are a number of insecticides registered for foliar treatments against thrips in tobacco.

These include formulations based on **deltamethrin**, **dimeothoate**, **malathion**, **acetamiprid**, **methomyl**, **pirimiphos-methyl,** and **imidacloprid** (Savčić-Petrić and Sekulić, 2007).

In France, soybean crops are treated with systemic insecticides, with the goal being to reduce the populations of a number of pests including thrips. **Furathiocarb** (Promet CS-400) dosed at 0.02 mg a.i. per 1 kg seed (Cluzeau, 1994) is used for this purpose. This control measure is particularly useful, because it reduces not only the thrips population but a number of other pests as well. The most important among these are the soil pests of the root, but the practice is also effective against many other species appearing at the start of the growing season.

Bugs *(Heteroptera)*

The bug fauna of soybean in Serbia and the neighboring countries is at present comprised of 34 known phytophagous species (Čamprag et al., 1996), most numerously those of the families *Miridae* and *Pentatomidae*. Not all of these pests are equally harmful to soybeans, and some are found only on weeds within soybean crops. Based on a number of studies carried out in Vojvodina over a number of years, Kereši (1992; 1993; 2000; 2001) identified a total of 35 bug pests in the province. Of that number, 27 are phytophagous (albeit some are indifferent to soybean), 6 zoophagous, and 2 mixed feeders. Among the phytophagous species, the dominant ones are *Exolygus rugulipennis* **Popp**., *E. pratensis* **L**., the alfalfa plant (*Adelphocoris lineolatus* **Goeze**), and *Trigonotylus ruficornis* **Geoffr**., while the dominant zoophagous species is *Nabis feroides* **Rem**. In Hungary, according to Szili (1979), bugs infest soybean fields in late May and species of the genera *Lygus*, *Exolygus*, and *Capsodes* and the species *Adelphocoris lineolatus* are commonly found. A total of 17 bug species have been identified in soybean in that country. Nikolova (2006) found 41 bug species on soybeans in Bulgaria, with the most numerous being the phytophagous bugs *L. rugulipennis* (16.7%), *Polymerus cognatus* **Fieber** (15.9%), *A.lineolatus* (11.8%), and *Piezodorus lituratus* **Fabr**. (5.4%). On the global scale, several other bug species have been reported as major pests of soybean, most notably *Nezara viridula*, *Dolycoris baccarum, Lygus pratensis*, and some others (Turnipseed and Kogan, 1987; Colazza and Bin, 1990). The first species on the list is especially abundant in tropical regions, while the other two may occasionally be of importance in Serbia as well.

The body of a bug is more or less flattened and the forewings are partially hardened (chitinized). Bugs vary in size but are most often up to 10 mm long. Their mouthparts are adapted for piercing and sucking. They feed by sucking out plant sap, but many of the species are zoophagous too. Species of the families *Anthocoridae* and *Nabidae* are particularly noteworthy as predators of various harmful insects and other animal organisms. Along with phytophagous bugs, these species are also often very numerous in agricultural biocenoses (they make up to 47% of all bugs found in soybean in Serbia).

Phytophagous bugs are very polyphagous and feed on a large number of cultivated plants and weeds. They attack all of the above-ground plant parts and are particularly damaging to soybean leaf and flower buds, flowers, pods, and grains.

As the soybean pod or seed is punctured or sucked on by a bug, tiny, almost imperceptible dots will appear and some loss of color will occur and the grain may become completely shriveled and desiccated. The severity of the damage depends on the stage of seed development and the number of bugs present. Significant damage in terms of yield losses and seed quality reduction will occur when soybeans are attacked in the earlier reproductive phenophases (RG). With increasing severity of the attack, the percentage of empty seeds increases and seed size, germinability, germination energy, oil content, and storage capacity decrease (Daugherty et al., Todd et al., Thomas et al., as cited in Kereši, 1992). In Serbia, species of the genus *Lygus* (*Exolygus*) are dominant in soybean crops. They are especially abundant after migrations from other mature crops or after the cutting of perennial legumes. Species of this genus are especially damaging to soybean flowers and buds and cause significant reductions in pod number per node and seed number per pod. Despite all this, the harmfulness of bugs to soybean and their effects on soybean yields cannot be regarded as having been studied to a sufficient degree yet. In the future, because of the increasing areas planted to soybeans in Serbia, we can expect that this insect group will adapt even better to soybean as a species and that its importance as a pest of soybean will increase even further in the country.

Exolygus rugulipennis **Popp**. – The adults are pale-green or gray to brown in color and 5 to 6 mm in size. The larvae are 1.2-4.4 mm long (Figure 13.3) and are yellowish green, wingless and oval in shape. As a plant pest, this bug is one of the most common and harmful species of the genus *Exolygus* in Europe (Čamprag et al., 1996). It produces 2 to 3 generations per year and overwinters as an imago in fields or on noncultivated terrain underneath plant residues. It is extremely polyphagous and damages around 100 cultivated and weed species of plants. It attacks all above-ground plant parts, especially flowers and seeds. *E. rugulipennis* appears in mass numbers in small grains, legumes, sunflower, sugar beet, and other cultivated plants. It is also common in soybean fields in Vojvodina. It has been reported as a soybean pest in Romania (Reyes, 1988), Bulgaria, Turkey, and other countries. Mass incidence of this pest (and bugs in general) is promoted by hot and dry weather.

Having overwintered, the imagoes leave their winter habitats when the mean daily temperature reaches 10-16°C. They are very active in their search for fresh, juicy food and can fly over an area of 1 to 2 km or more in a matter of several days. In the beginning, they damage weedy plants around plots and in other noncultivated habitats. Once the weeds become too rough and unsuitable for feeding, the bugs migrate in mass numbers to cultivated plants as well. The form that migrates to soybean crops are imagoes of the first generation, while the second generation adults develop on soybeans. The first maximum of the imagoes occurs in late June and early July, while the second takes place in August. The numbers of second generation larvae peak in early August (Kereši, 1992).

Figure 13.3

Larva of *Lygus* spp. *(photo: T. Kereši)*

Exolygus pratensis **L**. – This species is also common in Europe and is present in Serbia as well. The adults are elongated and oval-shaped and 6 to 7 mm in size. Their color varies from light yellow to greenish or dark brown with reddish stripes. The life cycle and seasonal activity are similar to those of *E. rugulipennis*. The bug is polyphagous and is found on many weeds and cultivated plants. It is a known pest of soybean in northern Bosnia (Vaclav et al., 1970), Hungary (Szili, 1979), Romania (Reyes, 1988), Serbia (Kereši, 2001), and other countries.

Adelphocoris lineolatus **Goeze**. - The alfalfa plant bug is most common in perennial legumes. It produces two generations per year and overwinters as an egg. The bug is polyphagous and can cause damage to over 140 plant species. Low incidence levels of the pest have been reported in soybean crops in northern Bosnia (Vaclav et al., 1970) and Hungary (Szili, 1979). In Serbia and (Kereši, 2001) and Bulgaria (Nikolova, 2006), this insect is one of the more abundant bugs in soybean fields. According to the latter author, the damage caused to soybeans by *A. lineolatus* manifests itself as a decrease in pod number per plant, 1000-grain weight, and biological yield (by 3.9 to 45.2%). The severity of the damage on grains is the highest on the uppermost nodes. The phenol content of the damaged grains decreases and such grains have reduced trypsin inhibitor activity and increased levels of crude protein, total protein, and crude fiber.

Dolycoris baccarum **L**. – Mature individuals of the sloe bug are brown and their antennae and connexivum are banded black and white. They are about 12 mm in size. The bug produces two generations per year and overwinters as an imago in various secluded places. It is a polyphagous pest that primarily damages the generative

organs of the plant. In the spring, it most abundant on weeds at first, following which it flies over to cultivated plants. It is often reported as a major pest of sunflower and is also common in alfalfa and other legumes. It has been found in soybean crops in a number of countries, Serbia included (Simova-Tošić et al., 1988, 1995; Kereši, 1993, 2001).

Nezara viridula **L**. – The southern green stink bug is very numerous in tropical and subtropical regions. It damages many cultivated plants and is an established soybean pest in the southern US states as well as in Brazil, Cuba, Japan, India, and Turkey. The bug is also a frequent pest of soybean in France and Italy. It has been found in Serbia too, albeit in very small numbers (Simova-Tošić, 1995). The southern green stink bug appears during the summer months, as does the sloe bug.

Contorl measures

Bug numbers are reduced by weed control, both in the fields themselves and in the areas surrounding the plots and in other habitats of the pest. This is because the bug populations are maintained primarily on weeds, from where they migrate to cultivated plants. Deeper tillage is also useful, as it destroys the individuals overwintering underneath crop residues after soybean or another crop has been taken off the field.

According to data from Hungary (Szili, 1979), bug control by chemical means becomes necessary in soybean if 15-20 *Exolygus* individuals or 8-10 individuals of the alfalfa plant bug or some other bug species are caught in an insect net after 10 swoops. During the period between pod formation and full seed maturity, the threat from bugs increases. The critical numbers during that time are 10-15 individuals of the genus *Exolygus* or 4-6 individuals of the alfalfa bug or some other bug species.. According to the findings of Bulgarian author Nikolova (2006), the threshold of damage by *A. lineolatus* in soybean is 3-4 individuals/m^2.

In France, the recommended chemical for the control of *Nezara viridula* is **Karate**, dosed at 0.15 l/ha (Dalbello-Polese., 1990). In Serbia, there are no registered insecticides for controlling bugs on soybeans as of yet. However, previous experience with bug control in small grains and some vegetable species has shown that this group of pests can be controlled by insecticides based on **deltamethrin**, **fenitrotion**, **fenthion**, **chlorpyrifos**, **malathion**, **pirimiphos-methyl,** or **trichlorfon**.

Owlet moths *(Noctuidae)*

Owlet moths are nocturnal insects that fly towards sources of light. They are medium-sized, drab in color, and have characteristic markings on the forewings that are used to distinguish among the different species of the family. There are very many Noctuidae species in existence, but relatively few are economically important.

These insects are very polyphagous and injure virtually all plant organs of various cultivated and weedy plants.

Cutworms – The three most well-known cutworm species are *Agrotis segetum* **Schiff**. (turnip moth), *Agrotis ipsilon* **Huf**. (black cutworm), and *Euxoa temera* **Hb**. They are widely distributed across the globe and occasionally occur in mass numbers. These species are very dangerous enemies of various row crops. The moths have brownish grey to dark grey forewings variegated to a smaller or greater degree, while the hind wings are unicolored and of a lighter shade. The adult caterpillars are up to 45 mm in length. Their color is an earth-tone grey and they are somewhat lighter-colored on the underside. They are found in the surface layer of the soil in areas around their food plants. All three species of cutworms mentioned above have been reported as pests of soybean in Serbia and other countries.

At the start of their development, the caterpillars damage the lower leaves of plants by making circular perforations that are for the most part of irregular shape. More mature caterpillars, however, cause injury to plants by chewing through their stems at or slightly above or below the soil line. The damaged plants lag behind in growth, wilt, dry out, and deteriorate. Attacks by these pests either thin out the crop as larger or smaller patches of affected plants appear within it or cause total plant destruction throughout the entire field. In the spring, caterpillars of *E. temera* appear first, followed by those of the black cutworm and then the turnip moth.

Contorl measures

Cutworm damage can be significantly reduced by early sowing, inter-row cultivation, weed control, and other agronomic practices. Chemical control of these pests in row crops can be done using insecticides based on **alpha-cypermethrin**, **cypermethrin**, **deltamethrin**, **lambda-cyhalothrin**, **methidathion,** and others. When applying the treatments, larger amounts of the liquid should be used, at least 300-400 l per ha.

Leaf owlet moths – A number of species of leaf owlet moths have been reported thus far as soybean pests in Serbia and other countries. These pests are hygrophilous and mostly very polyphagous. Among them, the most important and potentially most harmful to soybeans are: *Autographa gamma* **L**. (the silver Y moth), *Helicoverpa armigera* **Hbn**. (the cotton bollworm), *Heliothis maritima* **Grash**. (the shoulder-striped clover), *H. viriplaca* **Huf**. (the marbled clover), and species of the genus *Mamestra* **Hd**.

Autographa gamma **L**.- On their forewings, the silver Y moths have a characteristic marking resembling the letter Y or the Greek letter gamma, which is how they got their name. The hindwings are grayish yellow and unicolored. The caterpillars are greenish in color and reach a length of over 30 mm when fully grown. They have only three pairs of prolegs and move with a characteristic looping gait (Figure 13.4).

Silver Y moths occasionally occur in mass numbers and their gradation usually lasts one to two years. Periods of their low incidence, when only individual members of the species are found, last several years or more. The silver Y moth is a typical migratory species and the caterpillars found on cultivated plants are for the most part progeny of moths that have migrated from the wider Mediterranean region. The moth usually infests wetter habitats and produces two to three generations per year. It can enter the winter in any life stage. In Vojvodina, the caterpillars are the most abundant in late spring and early summer. They cause damage to the leaves of plants and may strip the plant completely bare of leaves at higher incidences. The damage by the caterpillars may also be inflicted on the stem and generative plant organs. The greatest damages are caused by the caterpillars of the later generations. This species can feed on about 390 plant species but most often does so on sugar beet, tobacco, alfalfa, clover, pea, and potato. It is more common in weed-infested crops.

Figure 13.4

Silver Y caterpillars and damage they cause to soybean leaves *(photo: T. Kereši)*

The silver Y moth has been reported as a pest of soybean in a number of countries. In northern Caucasus, it causes considerable damage to this crop from time to time. In 1961, Milatović and Maceljski (1962) recorded damage from the silver Y caterpillars in soybean variety trials in a number of locations in Croatia, northern Bosnia, and Serbia, with the severity being the greatest at the towns of Ćuprija, Bijeljina, Vinkovci, and Beli Manastir. In the north of the region of Banat, the moth is known to have caused great damage in the past as well (Petrik, 1966). In a field located in the area of the town of Požarevac, Simova-Tošić (1995) observed severe silver Y damage in late July of 1987, with the density being 15 caterpillars per 100 plants.

Helicoverpa armigera **Hbn**. – The moths of the cotton bollworm have a wingspan of about 40 mm. Their color varies from an off-yellow to olive grey to brown. The hindwings are always lighter in color. The characteristic Noctuid markings on the forewings are not particularly pronounced. The caterpillars grow up to 40 mm in size. Their color also varies a lot, ranging from nearly black, brown, or green to pale yellow or pink (Figure13.5). The caterpillar of the cotton bollworm has many narrow, wavy stripes of different colors (often black) on the back and a wide light-colored band along the sides of the body. The spiracles are black and the underside of the body is yellow. *H. armigera* is widely distributed around the world but is most common in tropical and subtropical regions. It is also found in southern, warmer parts of Europe. In recent years, this species has been reproducing in great numbers in Serbia and the neighboring countries due to an increase in growing season temperatures in the region.

Figure 13.5

Variations of caterpillar color in cotton bollworm *(photo: T. Kereši)*

The cotton bollworm produces two to three generations per year and overwinters in the soil as a caterpillar or pupa. During the growing season, the flight of the moths lasts from mid-May to mid-October. These insects are more numerous in May/June and August/September. The fertilized females deposit around 500-1000 eggs, primarily on reproductive plant organs. The caterpillars are very polyphagous. They prefer to feed on cotton, tobacco, maize, tomato, pepper, alfalfa, and other plant species and may damage any of the above-ground plant organs but are especially keen on generative ones. Younger caterpillars may strip tobacco plants bare of their leaves. Mass reproduction and development of the species are promoted by high temperatures and rainfall in the spring as well as by high temperatures during the summer. The optimum temperature for this insect is 22 to 28°C.

The cotton bollworm has been reported as a soybean pest in Bulgaria, Russia, Romania, and other countries. In Serbia, the pest was identified on soybeans in the area of the city of Niš by Simova-Tošić (1995). Since 1993, the cotton bollworm has been increasing in importance as a pest of soybean in Serbia. During the extremely warm and dry spring and summer of 2003, mass incidence and overpopulation of this insect were recorded throughout the province of Vojvodina, with the numbers being especially high in northern Banat and Bačka and areas gravitating towards the Tisza river. According the dynamics of moth flight, at least three generations were present. The first developed in late May and in June, the second during July, and the third during August and early September. Significant damage was recorded in maize, sunflower, soybean, tobacco, pepper, tomato, string bean, and other crops. The first damages on soybean leaves were observed in June (Figure 13.6), but these had little impact on further plant development.

Figure 13.6

Damage caused by cotton bollworms to soybean leaves *(photo: R. Sekulić)*

According to data from New Zealand, losses of leaf mass of up to 30% do not cause significant yield losses in soybean, so chemical control of this and similar pests should be used only when damage exceeds that percentage. When the pods appear, the caterpillars will puncture more or less circular holes in the exact spots where the grains have begun to develop or have already formed. A survey of soybean fields carried out in the wider area of the town of Bečej in late July of 2003 showed that pod damage levels ranged from 17.1 to 56.3%, averaging 42.4%.

A month later, in the month of August, the damage levels in stubble crops were 78.8-94.2%, or 85.3% on average. One to four puncture holes could be found on the pods (Figure 13.7), meaning that that many grains were destroyed (Sekulić et al., 2004). In the upcoming period, if predictions about a global rise in air temperatures (by 1°C by the year 2025) come true and droughts become more frequent (Čamprag et al., 2004), we can expect major outbreaks of the cotton bollworm in various cultivated plants, including soybean. *Heliothis maritima* **Grash**. – The shoulder-striped clover has been reported as a soybean pest in Hungary, Bulgaria, and Serbia (Szili, 1979; Simova-Tošić, 1995; Đurkić, unpublished data). A major presence of *H. maritima* caterpillars has been reported in Hungary, whereas in Serbia Simova-Tošić have found individual specimens in the area of the town of Ćuprija, but no significant economic damage has been reported.

The moths of this species are yellow brown and their wingspan reaches 30 to 40 mm. Dark stripes on their forewings form a pattern resembling the letter V. The adult caterpillars are 45-50 mm long and yellow to dark green In the course of the year, the moth produces two generations and most commonly overwinters as a pupa in the soil. The moths can be seen from May to September, while the caterpillars are found from mid-June until the end of the growing season. They are polyphagous and damage mostly yellow sweet clover, alfalfa, maize, sunflower, vetch, bean, and other crops. They feed on leaf mass and may strip the plant bare of leaves. The second generation caterpillars cause injury to leaves as well as flower buds and young pods in leguminous plants, soybean included.

Figure 13.7

Puncture holes made by cotton bollworm on soybean pods *(photo: R. Sekulić)*

Heliothis viriplaca **Huf**.- The marbled clover also feeds on a fair number of plant species. Its food plants include alfalfa as well as soybean, vetch, clover, tobacco, sunflower, maize, cotton, and other plants. This moth has been reported as a pest of soybean in Bulgaria, Ukraine, Russia, Hungary, Serbia, and other countries.

In the summer of 1928 in northern Caucasus, a second generation of this species damaged up to 70% of soybean leaves in some locations. In Serbia in 1994, at the PIK Bečej agricultural estate, a major outbreak of this pest was observed on soybeans planted as a stubble crop and 16 ha of soybean were chemically treated so as to prevent damage from occurring. The marbled clover is mentioned as a soybean pest by Simova-Tošić (1988) as well.

Mamestra spp.- This genus is represented by a number of species in the entomofauna of Serbia. Among them, the most common and damaging to cultivated plants are *Mamestra brassicae* **L**. (the cabbage moth) and *M. oleracea* **L**. (the bright-line brown-eye moth). As a pest of soybean, the two species have been reported in Hungary, Romania, Bosnia, Croatia, Serbia, and other countries (Kurnik, 1970; Milatović and Maceljski; 1962, Barbulescu et al., 1994; Szili, 1979).

The moths are grey to dark brown and have characteristic markings on the forewings. They are about 20 mm in size, while the caterpillars reach a length of 45 mm. The caterpillars are light green to grayish brown and are always somewhat lighter colored on the underside. Both of the species produce two generations per year and have similar life habits. They overwinter in the soil as pupae. The adults are especially numerous in late spring and during April. The first generation caterpillars are seen from the second half of June until mid-July, while the second generation ones are found from mid-August till mid-September.

The caterpillars are very polyphagous and more commonly injure cultivated crucifers, sugar beet, and pea. The second generation caterpillars are several times more numerous than the first generation ones and may often strip plants of their leaves in the infested crops in late August and early September. They are hygrophilous pests and are hence found in larger numbers in wet habitats and irrigated crops.

Control measures

Deep tillage in autumn significantly reduces the mass reproduction of leaf noctuids. This measure can help reduce the number of overwintering pupae of these pests by about 80%. The control of weeds on which supplemental feeding of the imagoes takes place also results in reduced numbers of these insects.

Chemical control of leaf owlet moths is performed at the time when caterpillars of the second and third stages of growth are dominant. During this period, the caterpillars are susceptible to the insecticides being applied and the leaf mass has not suffered any major damage yet. Most of the leaf is destroyed by the older caterpillars. A number of insecticides for the control of leaf owlet moths have been registered in Serbia (Savčić-Petrić and Sekulić, 2007). In sugar beet, a crop in which the control of these pests is most widespread, formulations based on the following chemicals have proven effective: **methidathion, bifenthrin, lambda-cyhalothrin, alpha-cypermethrin**, **cypermethrin**, **monocrotophos** + **cypermethrin**, **methomyl, chlorpyrifos**, **chlorpyrifos + cypermethrin,** and others. The critical number of leaf noctuids for soybean has not been determined yet.

Leaf noctuids can be controlled by biological means too. This is done by releasing wasps of the genus *Trichogramma*, which parasitize the eggs of the owlet moths, at the time when moth flights and mass oviposition of the pests begin, as well as by using control agents based on the bacterium *Bacillus thuringiensi*.

The painted lady *(Vanessa cardui* L.*)*

The painted lady is one of the most common pests of soybean and has been reported as such not only in Serbia but in a host of other countries as well (Croatia, Bosnia, Bulgaria, Hungary, Romania, Ukraine, etc.). A report on the occurrence and harmfulness of this species in the southern part of the Baranja region in Hungary can be found in Toth (1992). The report states that in the summer of 1980 second generation painted ladies were found on 22% of the acreage under soybean and that control measures had to be carried on over 1,400 ha planted to this crop. During 1980 and 1982, as many as 8-10 caterpillars could be found per m^2, and in several cases 80 to 110 caterpillars were recorded. The pest-infested patches on the plots were 30 to 120 m in diameter, and often 40-80% of the plants were damaged, with leaf mass losses of 15-36%. According to data from the Timis County in Romania, there was a significant outbreak of this pest in the area in 1987 and 1988. In some fields, 10 caterpillars per m^2 were recorded. Significant damages by the painted lady were reported in the Bijeljina region in Bosnia in 1962 and 1963 (Vaclav and Batinica, 1964).

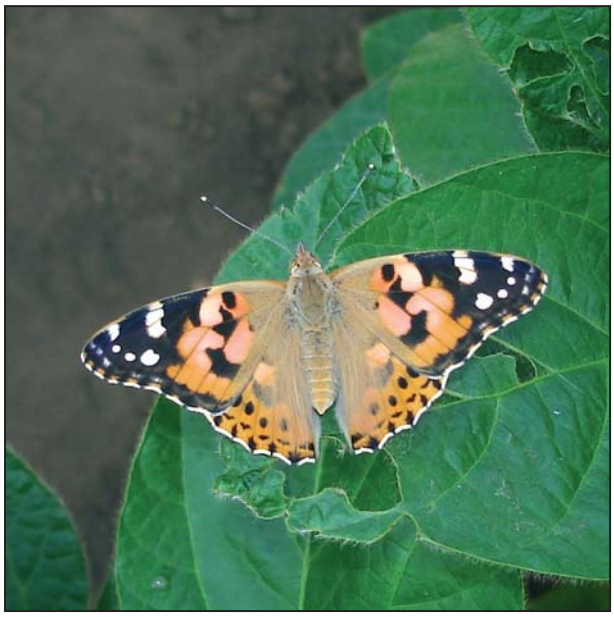
A number of authors have written about the occurrence and harmfulness of the painted lady in Serbia (Petrik, 1964, 1966; Dobrivojević, 1962; Hadžistević, 1962; Sekulić et al., 1983; Simova-Tošić et al., 1988, Simova-Tošić, 1995, and others). The first appearance of the pest was recorded as far back as 1946, and major damages were reported in 1958 and 1962. Hadžistević (1962) notes that soybean fields attacked by this insect in the area of the town of Obrenovac were infested with weeds quite heavily and that the dominant weed species was burdock. Five to seven caterpillars were found on some plants. In the same field, only on creeping thistle, Dobrivojević (1962) found up to 17 caterpillars.

In Vojvodina, in late June of 1980, there were mass outbreaks of first generation painted ladies in a number of locations (Sekulić et al., 1983). In the Inđija area, the hotspots of the infestation had up to 288 caterpillars/m^2, or nine individuals per plant. During the outbreak, over 450 ha of the crop were treated against the pest in the areas of the towns of Sombor and Pančevo. Later reports of the pest in soybean included one in the Ćuprija area in 1987 (Simova-Tošić, 1995) and one in Vojvodina in 1996 (unpublished data by the authors of the present chapter). During the spring and summer of 2006, very heavy infestations by the painted lady of the first and second generations were recorded in soybean crops across Vojvodina.

Unfortunately, due to the absence of a pest reporting and forecasting service at the provincial level and a lack of communication among the regional agricultural services, the real proportions and possible damages caused by this outbreak have never been determined, not even roughly. The abundance of the caterpillars in some locations (in the Temerin area, for example) was such that it required the implementation of chemical protection measures, although it remains a matter of debate whether these measures were necessary and applied in a well-timed manner (Kereši et al., 2007).

Figure 13.8

The painted lady *(photo: G. Kuzmanović)*



The moths of this species have a wingspan of about 55 mm and their body length is 20 mm on average. The wings are extremely variegated and covered in black and white spots, with the base color being that of rust (Figure13.8). The eggs are light green, small, and flattened. When fully grown, the caterpillars are 35-40 mm long and have a multitude of hairs across the back that are forked like spines. The color varies greatly, from nearly black in younger caterpillars to grayish brown in older ones. The caterpillars have a black or brown most commonly dotted stripe along the back of their body and two longitudinal yellow stripes along each side (Figure 13.9). The pupae are around 20 mm long and their color is a shiny silverish white with a grayish green or coppery sheen. They are found on the injured leaves and cling on to the leaf surface with the rear portion of their body hanging upside down.

Figure 13.9

Painted lady caterpillars on soybeans *(photo: G. Kuzmanović)*

The painted lady produces two to three generations per year. The moths appear early in the spring, mate, and then deposit around 500 eggs on the leaves of different plants. The hatched caterpillars first skeletonize the leaves, then chew out irregular-shaped holes in them. If abundant enough, they will strip the plant bare of its leaves. They wrap the affected leaves in cobweb-like strands and form caterpillar nests that may on occasion envelop the whole plant. The caterpillars primarily feed on various weed plants and leaves of creeping thistle, welted thistle, and burdock and then moves on to cultivated plants such as soybean, string bean, bean, sunflower, tobacco, castor bean, and others. They are found in the wild in late spring and in the summer (most abundantly in June and July). While developing, a caterpillar of this species will consume a total of 1.8 grams of soybean leaves, most of it in the second half of its development. The amount of leaf mass consumed by a painted lady caterpillar is about equal to that of one trifoliate leaf. At the time when this pest is present in crops, the leaf area of the plant is at its peak and there are 12-25 trifoliate leaves per plant. Thus, only three or more caterpillars per plant are capable of damaging over 25% of the leaf mass (Čamprag et al., 1996).

The painted lady appears in mass numbers from time to time and can cause significant damage on such occasions. It is a migratory moth that migrates each year from northern Africa and the Mediterranean in varying numbers. These insects sometimes fly in huge, loosely packed swarms to central and northern Europe, sometimes as far north as Finland and Iceland. Return migrations of the moths from north to south have also been recorded in late summer and in autumn.

Control measures

The main method of cultural control is systematic removal of weeds (creeping thistle, welted thistle, burdock, etc) from crops of soybean and other affected species.

The presence of one or two caterpillars per plant cannot cause significant economic damage and can be ignored, especially if the pest is found at the end of flowering, when soybeans usually have the most leaf mass (Sekulić et al., 1983). In Serbia, there are no registered chemicals for the control of this species as of yet. In France, insecticides based on **deltamethrin** have exhibited good efficacy in controlling the caterpillars of the painted lady (Dalbello-Polese et al., 1990). Laboratory trials conducted by Sekulić et al. (1983) in Serbia have shown that formulations based on **etrimfos**, **endosulfan**, **triazophos, and methomyl** (Sekulić et al., 1983) are highly effective in controlling painted lady caterpillars. As this pest occurs only in patches, it is possible to treat only the affected portions of the field.

The lima bean pod borer *(Etiella zinckenella* Tr.*)*

This species is a known soybean pest in a number of countries (Romania, Bulgaria, Hungary, Macedonia, France, China, etc.), and there have been reports of it in Serbia as well. In the Chinese province of Shatung, damages by the lima bean pod borer reached up to 90% between 1940 and 1942. In Romania at present, damage by this pest in soybean and other legumes ranges between 5 and 30% (Perju et al., 1983). In Hungary, according to Manninger (1963), the insect caused pod damage of 7%, while Szili (1978) states that 12-16% of the pods were attacked by the pest in some locations in that country. In Bulgaria in 1934, around 45% of soybean pods were attacked by the borer at the start of one of its gradations, and the infestation levels were as high as 95% in some locations.

There is little data on the occurrence and harmfulness of the lima bean pod borer in Serbia, where this pest has not been studied to a sufficient degree overall. The first major outbreak was recorded in 1960-1962 in the vicinity of the city of Belgrade, and on that occasion the borer almost completely destroyed black locust seeds (Mihajlović, as cited in Simova-Tošić, 1995). Between 1985 and 1988, a survey of soybean crops carried out by Simova-Tošić et al. (1988) revealed that the pest was present in several areas in the country. Although the borer was found at a number of sites, however, its incidence for the most part was not high.

The moths of this pest species are gray and their forewings have an orange band running across the middle. Their wingspan ranges from 24 to 28 mm and their bodies measure about 18 mm in length (Figure 13.10). When fully grown, the caterpillars reach a length of 15 to 22 mm. Their color varies from a greenish yellow to a grayish red. The pupae are brown and 9 to 12 mm long and are always found enveloped in a silky whitish cocoon covered with bits of soil.

Figure 13.10

Lima bean pod borer *(photo: T. Kereši)*

The borer produces 2-7 generations per year depending on climatic conditions present in a given area. In areas with a warmer climate, the pest produces one generation after another without interruption. This insect has a wide ecological amplitude and can adapt to a broad spectrum of environmental conditions. In Romania, it produces two generations per year, whereas in Bulgaria and Hungary it also produces two as well as part of a third, provided warm weather conditions extend well into the autumn. In Serbia, the situation is similar. Because the period of oviposition is extended, the generations cannot be clearly separated from one another. The lima bean pod borer passes the winter as an adult, cocooned caterpillar buried at a shallow depth of 2-3 cm or nestled within fallen leaves on the ground. In the spring, the caterpillars turn into pupae, out of which the adults emerge after several weeks. The imagoes fly intensively during June, July and August. In Hungary, 90% of the moths caught in the course of a growing season were captured during this period (Meszaros and Reichart, 1993). The imagoes first appear in late May and June.

The moths are active during the evening and night. They supplement their diet by feeding on the nectar of different plant species in bloom. The fertilized females lay a total of about 600 eggs on the green pods of pea, soybean, and other legumes. The eggs are deposited individually or in small groups. At 17°C, embryonic development lasts 15 to 16 days. The development of the caterpillars is completed within three to four weeks depending on the temperature. When grown up, the caterpillars leave the pods and turn into pupa in a cocoon at a depth of 3-4 cm in the soil. Dry springs and summers promote the mass reproduction of the lima bean pod borer. Years with increased precipitation, on the other hand, reduce the abundance of this pest. The borer enters gradations from time to time, and periods during which it is found in low numbers are often very long. The intensity with which this insect reproduces is greatly affected by numerous parasitoids and predators.

The lima bean pod borer is a polyphagous pest. The caterpillars of the species cause damage to nearly 80 cultivated and weedy plants, most commonly pea, soybean, vetch, bean, and other species. The hatched caterpillars first penetrate the pod and then bore their way into the seeds, on which they feed while causing damage to it on the inside in the process. The older caterpillars chew on the seeds on the outside and may destroy it completely. The damaged seeds are not suitable for sowing, especially if the damage is located in the part of the seed where the germ is. In such cases, the germination rate is only 6% (Šćegolev, 1995). Inside a pod, a single caterpillar is found that may cause damage to several seeds. Typical symptoms of a pod damaged by the pest are the presence of grains that are partially or completely eaten out, caterpillar droppings, and loose threadlike filaments with which the interior of the pod is webbed. The caterpillars of the first generation develop in pods of the bladder senna, pea, bean, and other legumes. The second generation infests later-maturing legumes, especially soybeans and black locusts (Simova-Tošić, 1995). In Romania, the Danube basin, and Moldova, the first generation damages pea and the second soybean and lupin.

Control measures

The mass reproduction and harmfulness of the lima bean pod borer can be suppressed by growing resistant cultivars and implementing different cultural, biological, and chemical measures. In Brasil, the resistance of 10 soybean cultivars to being attacked by the borer has been studied and significant differences were found among them with regard to the percentage of attacked pods and the percentage of damaged grains. However, no correlation was found to exist between this variability and pod length.

Cultural control practices such as spatial isolation, tillage, and irrigation can significantly reduce the number of these insects. Fields sown to soybean and other annual legumes should be located as far away as possible from black locust groves, where the pest develops unimpeded. In trials carried out by Podkopaj (1964), 15% of soybean pods were attacked by the insect when soybean fields were at a distance of 50 m from a grove of black locusts, but the infestation level was only 2% when the fields were at a distance of 500 m. Spatial isolation should also be provided in relation to fields of pea which were attacked by the borer the previous year. Deeper tillage right after the harvesting of pea, vetch, and other legumes prevents the eclosion of a new generation of imagoes to a very high degree. The irrigation of soybeans and other affected legumes at the time when the caterpillars transform into pupae significantly improves the control of the lima bean pod borer.

Biological control of this pest can also be performed by the release of egg parasitoids (*Trichogramma* spp.) during oviposition (Grigorov, 1976). The use of bioinsecticides based on the bacterium *Bacillus thuringiensis* is also recommended. The incidence of the borer can also be reduced by some biotechnical procedures such as using pheromones (Perju, 1995) or light traps.

Chemical control of the pest should be an option only for fields situated near black locust groves or adjacent to plots on which peas infested by the borer were grown the year before. After soybean flowering, the critical concentration of the insect is two to three eggs per plant, or an infestation level of 5%. There is very little data on the use of chemicals to control this pest in soybean. According to Grigorov (1976), good results can be achieved with formulations based on **methyl parathion** or **trichlorfon**. Two to three treatments are recommended. The first should be carried out as the first pods with fully developed grains are being formed or when the imagoes are flying in mass numbers. In order for chemical control to be as effective and economical as possible, it is recommended to monitor the start of the flight of imagoes and the period of oviposition. The control of the borer is primarily an option for soybean seed crops, because the seeds damaged by the pest are not suitable for planting.

Mites and ticks *(Acarina)*

Phytophagous acarines are the most important pests of soybean in Serbia as well as in numerous other soybean-growing countries of the world. In France, for example, these insects are very common soybean pests, and their last massive outbreaks were recorded in 1985 and 1986 (Dalbello-Polese, 1990). In the main soybean-growing areas in Bulgaria, the Acari are also among the top natural enemies of soybean (Atanasov, 1991; Nikolova, 2006). A similar situation exists in other countries as well. In Croatia in 1987, Vratarić (1988) recorded a high incidence of acarines in many soybean-growing locations in the country and noted that this pest group was becoming an increasing problem in the cultivation of this crop. The economic importance of acarines in soybean is best illustrated by a report from the US state of Illinois according to which over 80,000 ha of soybean had to be chemically treated against these pests (Colwell, as cited in Turnipseed and Kogan, 1987). In Serbia, Čamprag et al. (1996) also concluded that the Acari are the most important pests of soybean in the country.

Soybean crops in Serbia and elsewhere are infested by a number of Acari species. The two most important ones belong to the family *Tetranychidae.* They are *Tetranychus atlanticus* **Mc Gregor** (= *T. turcistanicus* **Ug. et Nik**.) – the strawberry spider mite – and *T. urticae* **Koch**. (= *T. telarius* **Prichard** and **Backer**) – the red spider mite, also known as the two-spotted spider mite.

The strawberry spider mite *(Tetranychus atlanticus* Mc Gregor*)*

The adult females of this species are egg-shaped and 0.5 mm in size. The females of the summer generations are yellow-green, while those of the winter ones are of a reddish color resembling that of brick.

The males are yellowish, somewhat smaller, and tapering at the back (Figure 13.11). Immediately after oviposition, the eggs have a translucent glassy appearance, while later on they become yellowish white in appearance. The eggs are spherical and about 0.14 mm in size. The larvae are yellowish, have three pairs of legs, and are about 0.5 mm long. The second stage of development, the nymph, has four pairs of legs, as do the adults.

Figure 13.11

Tetranychus atlanticus *(photo: B. Đukanović)*

The species is particularly widespread in southeastern Europe. In Serbia, it is present throughout the country. It can produce 10-14 generations per year. The generations merge together, especially during the summer, so individuals representing all stages of development can be found on the plants at the same time.

The fertilized females overwinter in groups in plant residues, the surface soil layer, or other secluded places. They leave their overwintering spots fairly early in the spring and turn from red to yellow-green in color. They first infest weedy plants, feed on them, and then start laying the eggs. Plants of the spontaneous flora are their transitional hosts before their migration to various cultivated plants. Among the weedy plants, the species is most often found on deadnettle, dandelion, jimson weed, field bindweed, and other weeds (Tomašević, 1965).

The females deposit their eggs usually on the abaxial surface of the leaf, near the main veins. At a temperature of 25°C, a single female will lay a total of about 190 eggs (Atanasov, 1991). Under favorable conditions, large colonies of the pest will form in a short time. They are covered by a fine web that protects them from unfavorable weather conditions and natural enemies. Underneath the web, all stages of development can be seen – eggs, larvae, protonymphs, deutonymphs, and imagoes. When the mite population becomes too high, they move on to the adaxial surface of the leaves, and often the whole plant becomes infested.

The pest keeps pace with the growth of the plant, so the colonies are the largest on the youngest, uppermost leaves of soybean (Figure13.12). The colonies also form on young pods (Rajković, 1982).

Figure 13.12

Soybean leaf damaged by the strawberry spider mite *(photo: B. Đukanović)*

How long a generation takes to develop depends on the temperature. At 30°C, the development lasts only 6 days, whereas at 12°C the duration is five times as long – more than 30 days (Dobrivojević and Petanović, 1982).

The optimum temperature for the development of the pest is 25 to 28°C. This species likes warm and dry weather and overpopulation occurs only during the summer months. The Acari population begins to increase on soybeans as early as June and peaks in August. An increased frequency and abundance of rainfall, on the other hand, negatively affect population density in this pest. In soybean cultivars that have less well developed hairs or those in which the hair cover is thinner, the fine web that covers the acarine colony does not provide sufficient protection from unfavorable weather conditions and natural enemies (Čamprag et al., 1996).

In soybean crops in Vojvodina, the natural enemies of phytophagous acarines are several species belonging to the families *Coccinellidae*, *Anthocoridae*, *Chrysopidae* and *Phytoseidae*. The most important among them are the predatory bug *Orius niger* and the green lacewing *Chrysopa carnea* (Rajković, 1992).

According to Atanasov (1970; 1991), the presence of three to four predators for every 100 individuals of the strawberry spider mite can slow down the reproduction of this pest and reduce its numbers. However, this ratio is rarely achieved, and when it is, this usually happens towards the end of the growing season, when the pest can no longer have a major impact on yields.

The strawberry spider mite is a highly polyphagous pest. It feeds on about 90 plant species, especially those of the family *Papilionaceae* (Atanasov, 1970). The mite is found on all cultivated plants but is especially common in soybean, maize, cotton, bean, hop, tomato, eggplant, apple, plum, and other species. In Vojvodina in 1956, the most severely affected crop was maize, followed by annual legumes (soybean and bean) and then small grains (Đurkić, 1956). The species is regularly found throughout Serbia in all cultivated plants (Tomašević, 1965).

The active instars of this mite suck plant sap intensively, as a result of which small silvery spots appear on soybean leaves scattered in an irregular pattern at first. As the attack by the pest intensifies, the spots merge together and spread across entire leaf blades. The affected plants increase their transpiration rate, the rate of photosynthesis decreases, and so on. Plants attacked by the mite were found to have a 34% higher transpiration rate than the healthy plants (Tomašević, 1965). Severely affected leaves gradually turn yellow and dry out. When the mite overpopulates in the summer, the crops change their appearance across entire fields. The severely infested plants complete their season prematurely, lag behind in growth, and have fewer pods, which are smaller, as are the seeds contained in them (Đurkić et al., 1977).

The extent of damage depends on the period when the large mite population has formed on the plants. In a three-year study carried out by Tomašević (1965), the yields of soybeans damaged by the strawberry spider mite were 27% lower than those of healthy plants. According to recent study from Bulgaria (Nikolova, 2006), damage by *Tetranychus atlanticus* on soybean manifested itself in the form of reduced levels of chlorophyll a and b, carotenoids, crude protein, and protein as well as in reduced nitrate reductase activity. The final damage was manifested as a reduction in grain number per plant, 1000-grain weight, and biological yield.

The initial damages in soybean usually appear on the margins of the field, since the mites for the most part pass the winter with success outside the cultivated areas (on weed-infested terrain surrounding the plots, alongside canals and roads, etc.). Then, when the weedy vegetation becomes too coarse and unsuitable to be used as food by the pest or is destroyed by mowing, the mites start injuring soybeans and gradually spread towards the center of the field. In the Srbobran area, Đurkić et al. (1977) recorded a high percentage of injured plants on the margins of soybean fields as early as late June (Figure 13.13).

Figure 13.13

Margins of a soybean field damaged by the strawberry spider mite *(photo: R. Sekulić)*

After that, the authors monitored the dynamics by which the pest spread towards the center of the field. During the first inspection in late July, it was established that the marginal area of the field 50 m deep had 77% of the plants infested, whereas when an area 150 m deep was inspected the infestation level was only 23%. If the inspection had been carried out at the start of the mite attack, the difference would probably have been even greater. The mites spread slowly by active means, mostly by moving across plants that touch each other. Expansion by passive means such as air currents, birds, various animals, or man seems to play an important role in the spread of the pest.

Tetranychus urticae **Koch**. (the red spider mite, or the two-spotted spider mite). This species is considerably less common in Serbian soybean fields than the previous one. This is attributed to its poorer adaptation to having soybean as the host compared with the strawberry spider mite (Rajković, 1992). The red spider mite is a cosmopolitan species and extremely polyphagous. It attacks over 200 cultivated and weedy plants and is very aggressive. According to Tischler (1980), this mite will puncture the leaf with its mouthparts about a 100 times in five minutes, meaning it will destroy that many cells of the epidermis and the palisade parenchyma during that time. Given the right climatic conditions (hot and dry weather), this will quickly result in the necrosis and suberinization of parts of the leaf tissue.

As regards its morphology and life habits, the species is very similar to the strawberry spider mite. One difference between the two species is the morphology of the male's genital organs. Another is that *T. urticae* females of the summer generations are greener in color.

The damage caused by this mite is also similar to that caused by the strawberry spider mite, and its extent depends on the time of attack and incidence of the pest. Most often, the crops will first be infested along the margins of the field. Sometimes the infestation will occur throughout the field.

This only happens if soybean had maize as the previous crop and the corn was attacked by the mite fairly severely with post-harvest tillage being poorly done (Szili, 1979). As for environmental requirements, those of the red spider mite are very similar to those of *T. atlanticus*. The highest incidence is recorded during the summer months and in late August and early September.

In the first part of 2002, due to a lack of rainfall, unusually high temperatures, and low relative humidity, an early outbreak of the two mite species (*T. atlanticus* and *T. urticae*) was recorded in Vojvodina. The infestation began as early as May and June and soybeans as well as other crops were affected (bean, string bean, and maize). During July, the weather conditions favored a further spread of these pests. In the municipalities of Vrbas and Kula, for example, all soybean fields were infested, whereas in the municipalities of Srbobran and Bečej 70 and 30% of the soybean acreage was affected. At the beginning of July, an official edict was issued to start with the control measures and these were carried out on most of the state-owned acreage under soybean, mostly on the margins of soybean fields. A similar situation occurred in 2003 as well (Radonić and Čubranović, 2002; Radonić and Knežević, 2003).

Control measures

The incidence of the mites is reduced by timely tillage performed to a high standard of quality, weed control (especially in uncultivated areas around the fields), and the use of irrigation during the summer. Tilling right after the harvest and deep plowing (quality incorporation of crop residues) destroys the mites by chemical means and incorporates them into the deeper layers of the soil. By destroying weeds in uncultivated areas around the field, from where the mite attacks usually start, unfavorable conditions are created for the maintenance and formation of large populations of these pests that might endanger the crops. Irrigation of soybean during the summer months creates a microclimate within the crop that is characterized by increased humidity and other factors that prevent the mites from reproducing during the most favorable part of the year. This practice also removes the mites from the plant mechanically by washing them off of it.

Chemical control of mites in soybean should only be carried out on the margins of the field and at the start of the formation of colonies. The breadth of the treated area is determined by inspecting each individual plot.

Treating an entire field is neither economically nor environmentally viable. Chemicals should only be used if the critical number of pests or the threshold of economic damage have been exceeded in the infested margins of the field. Treatments should begin if 50% of the plants have been infested regardless of the actual number of mites or if more than five individuals are found per leaf on average (Vratarić, 1986). According to data from Hungary, the critical number of mites is 10 mobile individuals per one leaf of soybean (Toth, 1992). Bulgarian author Nikolova (2006) considers an average of three to four mites per leaf to be the critical number.

In France, **dicofol** dosed at 480 g a.i. per 1 ha (Dalbello-Polese., 1990) used to be the only recommended chemical for the control of the dominant mite species in soybean. Nowadays, besides **dicofol**, formulations based on **chlofentezine** (200 g a.i. per ha) and **propargite** (855 g a.i. per ha) (Cluzeau, 1994) are also used. In the Baranya region in Hungary, good results in controlling mites in soybean have also been achieved using insecticides based on **propargite** (Omite 57 E dosed at 1.5 l/ha) (Toth, 1992). Presently, **propargite** and **cyhexatin** (Ocskó et al., 2007) are also recommended for combating mites in that country. In Serbia, the only officially registered chemicals for controlling this pest group in soybean are those based on **abamectin.** In Bulgaria, the insecticides **bifenthrin** (Talstar 10 EC) and **flufenoxuron** (Cascade 5 EC) have proven to be the most effective chemicals for controlling the strawberry spider mite (Nikolova, 2006).

To successfully control mites, one to two treatments are usually needed with an interval of 8-10 days in between. Since mites first infest the abaxial surface of the leaf, an increased quantity of water and an increased working pressure in the sprayer should be used for the treatment in order to get coverage on both the abaxial and adaxial leaf surfaces. Well-timed chemical control of mites that is performed as infrequently as possible is considered to be the best approach because it spares many species of useful or indifferent fauna (larvae of the green lacewing are voracious predators of mites).

The use of insecticides to control mites in soybean is fully economically justified when a critical number of these pests has been reached. According to a study by Tomašević (1965), insecticide use in one such case resulted in a yield that was about 30% higher compared to the untreated control.

Hamster *(Cricetus cricetus* L.*)*

Hamsters are polyphagous pests that cause damage to many plant species, soybean included. The hamster is a threat to soybean crops from emergence all the way until harvesting. The potential of this species to cause significant damage to soybeans is particularly high in the early stages of plant development and during ripening. When hamsters feed on newly emerged soybeans, the characteristic empty patches containing no plants are created within the crop.

These are more or less circular in shape and 10-70 m in diameter (Figure 13.14). Such damage can occur even when the plants are 20-30 cm tall. The patches are randomly scattered around the plot and their number depends primarily on the density of the hamster population.

Figure 13.14

***Cricetus cricetus* - Damaged soybeans around a hamster's burrow** *(photo: T. Kereši)*

In maturing soybean crops, hamsters can also cause damage by snapping the pods off of plants and by collecting the grains for the winter. Several years ago after small grains were harvested at the site of Nadalj in Vojvodina, the pest infested a nearby soybean field in smaller or larger patches and proceeded to destroy the crop completely (Sekulić, unpublished data). At the PIK Bečej agricultural estate at Bečej, it has been observed that the soil mounds surrounding the hamster burrows hindered the combine-harvesting of soybean and thus contributed to the dispersal of seeds and to an indirect increase in yield losses caused by the pest.

The hamster is a Eurasian species that primarily inhabits steppes. In Serbia, it is especially common in the province of Vojvodina but is also found south of the rivers Sava and Danube, in the Belgrade area and in the lower Pomoravlje region. It is a known soybean pest in other countries as well.

The hamster is one of the larger rodents. The adult individuals are 24-34 cm long and their tail length ranges from 4 to 6 cm. They reach a weight of up to 0.5 kg. The cheeks of a hamster have been transformed by evolution into pouches, which the animal uses to store and transport food. The hamster will also fill these pouches with air in order to produce characteristic sounds by which it scares off its enemies (Figure 13.15). A hamster's legs are short and powerful and the rodent uses them when digging its burrows. The fur is most often yellowish-red dorsally and black on the underside. The sides of the head and the body have white spots on them.

Hamsters live in burrows at a depth of 0.5-1.2 m. Each burrow consists of a chamber lined with grass, in which the hamster lives, and several storage chambers, in which the animal places grains and other foods for the winter. There are usually two tunnels leading out of the main chamber, both of which end up at ground level. They are at a distance from each other. One is placed at an angle and is used by the rodent for exiting the burrow, while the other is almost vertical and enables the hamster to quickly re-enter the burrow. The holes at the surface of the soil are most commonly 6-9 cm in diameter.

Figure 13.15

Hamster – *Cricetus cricetus* *(photo: B. Đukanović)*

The hamster spends the winter hibernating in the burrow. Hibernation stops and is then resumed depending on how the temperature fluctuates. During the pauses in hibernation, the hamsters feeds on the food it has stocked over the summer and autumn. The animal starts waking up from hibernation for good in late February of early March and the process is complete by the end of April or in early May. The species reproduces two to three times a year. The females will give birth to 6-12 pups two to three weeks after mating. Within a month, the young become fully grown and sexually mature. The lifespan of a hamster is 6 to 8 years and the pest appears occasionally in mass numbers.

These mass outbreaks are especially favored by the high reproductive potential of the species, an abundance of food all year long, and favorable weather conditions, in particular by warm and dry autumns. Long and wet winters unfavorably affect hamster reproduction. The many natural enemies of the species (birds of prey, foxes, polecats, and others) may significantly affect hamster population density as well. In Serbian conditions, mass outbreaks or gradations of hamsters occur at four- to five-year intervals and usually last two to three years (Ružić, 1983). The last such occurrence was recorded in the last three years in Vojvodina.

Harmful rodents were a big, if not the biggest, problem in small grains in the autumn of 2004 and 2005 and in row crops in the spring of 2005 and, especially, 2006. This included field mice as well as the hamster. Very severe infestations by these pests were recorded in the areas of Sremska Mitrovica, Kikinda, Pančevo, PKB Bečej, Vrbas, Novi Sad, Ruma, Sombor, Subotica, Bačka Topola, and Zrenjanin. In some cases protection measures were implemented several times in the same fields. Despite all this, major damages were reported in young crops of sugar beet, sunflower, soybean, and alfalfa, so some of the crops had to be replanted, especially in the private sector. The greatest damage by the hamster was recorded in southern and central Bačka (observation by the authors of this chapter). Alfalfa fields (as cumulative habitats) were the most heavily infested. Thus, in the autumn of 2005, there were 8,300 holes made by field mice per one hectare in the Subotica area, over 13,100 and 13,900/ha at Krčedin and Sirig, respectively, and up to 22,300/ha in the Vrbas area. In those same locations and fields, the number of openings dug by hamsters was 0, 13, 25 and 50 per hectare. These incidence levels are considered high and very high (Kereši et al., 2006; 2007).

The hamster is an omnivorous animal. Besides various cultivated and weedy plants, it feeds on different foods of animal origin (earthworms, snails, smaller-sized mice, grubs, and other insects). It damages plants throughout the growing season. In the spring, it snaps off young plants of soybean and other species. During that time, the preferred targets of its attack are alfalfa, clover, soybean, small grains, sunflower, sugar beet, maize, and other crops. In the course of the summer, at the time when the seed matures, the pest migrates to fields sown to small grains, soybean, sunflower, and maize. On those occasions, it feeds on the seeds but also stocks food for the winter in the chambers of its burrow. The chambers will contain 10 to 50 kg of grain of different cultivated and weedy plants. Hamsters are active at dusk and during the night. One individual will usually have a territory measuring 750 to 1000 m^2. The animal can bring back food from a distance of 350 to 400 m (Hamar, as cited in Krsmanović, 1984). The pests has caused significant damage to soybeans in Hungary, in the area east of the river Tisza (Szili, 1976; 1979). Complete devastation of a maturing soybean crop was also reported on a farm in Vojvodina during August and September of 1961 (Čamprag et al., 1996).

Control measures

Hamster overpopulation can be prevented by cultural control and by the use of chemicals. Among the cultural practices, particularly important are prompt and rapid harvesting, reduction of grain dispersal, incorporation of stubble and other crop residues right after harvesting, weed control, and others. Good results in controlling hamsters on smaller areas and at lower incidence levels have been achieved by flushing the pest out with water.

Chemical control of this rodent is at its most effective and economical in the spring, when most of population has finished hibernating. In Borsod county in Hungary, the best time to control this pest is between April 5 and May 10 (Gyurko, 1977). Chemicals or poisoned bait should be used there are two or more occupied burrows per hectare (Benedek, 1984, as cited in Čamprag et al., 1996). The chemicals used for the purpose of hamster control are pesticides based on **aluminium phosphide** and **magnesium phosphide**. One tablet or two to three pellets of one of these rodenticides are deposited into hamster burrows, but only those that are actually occupied by the pest. Once the pesticide is placed in a burrow, the entrance hole to it must be closed. In the spring, freshly prepared bait containing up to 3% **zinc phosphide** can also be used. In Serbia, besides the above rodenticides, formulations based on **bromadiolone** and **cholecalciferol** are also registered for use against the hamster. The larger the area on which it is carried out, the more successful a campaign to control hamsters and other rodents will be. Rodent control and bait preparation will produce the best results if done in coordination with the relevant local extension services.

Common vole *(Microtus arvalis* Pall.*)*

The common vole is one of the most common small rodents in Serbia. It is most often between 9 and 11 cm long and rarely exceeds 15 cm. There are several subspecies of the common vole, which are found throughout Europe and in most of Asia. In Serbia, it is especially common in cereal-growing parts of the country, where it occurs en mass periodically and causes great damage. The pest damages soybean as well as many other crops. The vole lives in burrows it digs up, which can be up to 50 cm deep. It lives in pairs or in groups consisting of one male and several females. The voles produce six to seven litters per year, with 4 to 9 pups per litter.

The reproduction starts in March and the peak numbers are found in June and July. After that, the population gradually declines and is at its lowest in October and November (Straka, 1967). Favorable conditions for the reproduction of this rodent are created when there are large acreages under legumes, growing small grains in monoculture is common, and weed infestation levels are increased in the fields.

The common vole is a polyphagous pest that damages nearly 200 cultivated and weedy plants. It primarily feeds on the green, juicy plant parts but also consumes grainy foods.

Unlike the hamster, which only feeds on the above-ground plant parts, the vole damages the underground portion of the stem as well as the root. Voles feed throughout the year, even during mild winters with high snow cover. The common vole is a known pest of soybean in Serbia, Croatia, Hungary, and other countries. At the start of the growing season, it destroys planted and germinated seeds. The vole feeds on the green plant parts of soybeans after emergence as well as on the pods and seeds of maturing soybeans.

It snaps off the lateral branches and stems of soybean, breaks them into smaller pieces, and takes them to its burrow (Szili, 1979). During periods of mass incidence, the common vole can cause great damage to fields under soybean in Hungary.

Mass outbreaks or gradations of the rodent occur fairly suddenly, but they also recede in the same manner. Dry and hot weather during the growing season and winters with a lot of snow create favorable conditions for the reproduction and development of this pest, while abundant rainfall and heavy showers in the spring are unfavorable to it (Hoffman and Schmutterer, 1983).

Control measures

Cultural control practices such as timely harvests of small grains, maize, sunflower, soybean, and other crops, incorporation of crop residues, and deep tillage can be of great help in the control of this pest and can help suppress its mass reproduction to a great extent. Deeper tillage can destroy as much as 85-90% of the common vole population in a field.

The voles are chemically controlled when there are one to two open burrows per 100 m on average (Benedek, 1974, as cited in Čamprag et al., 1996). To cost-effectively control the common vole and other rodents, control should be carried out primarily in the hotspots of these pests (fields of alfalfa, clover, pastures, grassland, etc.), because they can be found in these habitats even when their populations are low. Under such circumstances, good results are achieved even when less labor and less rodenticide are used for control purposes. The common vole is controlled by using pre-packaged or freshly prepared bait to which an appropriate rodenticide is added. In Serbia, formulations based on **zinc phosphide**, **bromadiolone**, **chlorophacinone**, **cholecalciferol,** and **sodium selenite** are registered for this purpose (Savčić-Petrić and Sekulić, 2007). In actual practice, the most commonly used of the chemicals is **zinc phosphide**. It is used at a concentration of 2-3% in combination with cereal seeds, ground cereals, maize, sunflower, soybean, pea, or vegetable oil.

The European hare *(Lepus europaeus* L.*)*

The European hare is a species of the steppes. It has black-tipped ears whose length exceeds that of the head. The hare feeds on plant foods throughout the year. During the spring and summer, it feeds exclusively on various field and vegetable crops, primarily cereals and leguminous plants.

Soybean is under threat from this species practically from emergence until harvesting. In the beginning, young plants are damaged, then younger and older leaves, followed by green pods, and even mature grains. The hare causes the greatest damage by biting off the growing point when the plants are at the stage of two to four leaves (Figure 13.16).

Damage caused during this period usually results in the deterioration of young plants. Older plants compensate for such damage by resorting to lateral branching. If too much of the plant is destroyed, even the older plants become unable to catch up with the undamaged plants in their development. On smaller-sized soybean plots, the damage is visible throughout the area, whereas on larger-sized ones the damage is more noticeable on the margins of the field or along the paths that the hares use to move about.

Figure 13.16

Apical portion of the plant cut off by the European hare *(photo: G. Kuzmanović)*

The European hare is known as a soybean pest in Serbia, Croatia, and other countries. In Hungary (Somogy county), there have been reports of noticeable damage on soybeans caused by the hare biting off stem apexes at the stage of two to four leaves (Ludven, 1974). The European hare is considered a major pest of soybean in that country. In the Banja Luka are in 1966, Vaclav et al. (1970) observed complete devastation of crops by the pest in some places. Hare damage to young soybeans where the injury is in the form of stem apex removal (typically above the cotyledons) has also been reported in Vojvodina. The extent of damage depends first and foremost on the incidence of the pest.

In Hungary, to eliminate damage caused to soybeans by the European hare, **Dendrocol 17 SK**, dosed at 4 to 6 l per ha, has been used. This formulation is based on natural white tar and copper soap (Szili, 1979). Good efficacy was also obtained when plants were treated with 1% **potassium soap**. This resulted in a repellent effect where the crop was protected over the following 8 to 10 days, until the next rain.

SUMMARY

Soybean is in Serbia impacted with about 90 various pests. Most of them are insects (over 83%), till the rest are other animal pests. Phytophagous species take place during whole vegetation, from planting to harvest, injuring all parts of the plant: root system and root nodules, stem, leaves, flowers, pods, and seeds.

Economic importance has following species: Germinated seeds and root system, especially in the begining of the vegetation are injured by *Elateridae*, *Scarabaeidae*, *Sitona* spp., *Delia platura* **Mg**., *nematodes* (*Pratylenchus* spp. and *Meloidogyne* spp.), various birds (*Aves*), etc. On overhead parts of the plant, from the begining to the end of vegetation, various pests occur, but the most important are the following: *Tanymecus dilaticollis* **Gyll**., *Sitona* spp., *Aphididae*, *Thysanoptera*, *Heteroptera* (*Lygus* spp.), *Pyrameus - Vanessa cardui* **L**., *Autographa gamma* **Hb**., *Mamestra brassicae* **L**., *Loxostege sticticalis* **L**., *Etiella zinckenella* **Tr**., *Helicoverpa armigera* **Hbn**., *Scotia* spp., *Tetranychus* spp., *Cricetus cricetus* **L**., *Microtus arvalis* **Pall**., *Lepus europaeus* **L**., etc.

Special economic importance, so far, have *Tetranychus atlanticus* **Mc Greg**., *Pyrameus cardui* **L**., *Cricetus cricetus* **L**., *Lepus europaeus* **L**. and other rodents.

Tetranychus atlanticus **Mc Greg**. represents the most important soybean pest. *T. urticae* **Koch**. also occur, but in significantly reduced number. The biggest impacts on soybean occur in years with dry summer (2002, 2003).

Yield of the impacted plants could be reduced up to 27%. *Pyrameus cardui* **L**. occasionally could outbreak. The last outbreak of this species was recorded in 2006, when chemical insecticides were used on several hundred hectares. *Cricetus cricetus* **L**. attacks in the begining of the vegetation (mass occurance in 2006), destroying the plants in oasis-like parts of the field. Signifficant injuries could occur in maturation also.

There is no exact data about total losses caused by pests in our country, so far.

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”SOJAPROTEIN” – THE LEADER IN SOYBEAN PROCESSING

THE ROLE OF ”SOJAPROTEIN” IN THE DEVELOPMENT OF SOYBEAN GROWING

The region of Bečej, located in the geographic centre of the province of Vojvodina is one of the most important agricultural regions in Serbia. It is a major producer of crops and industrial plants. Owing to its natural resources and its geographic position, the development plan for the food industry in the eighties included the establishment of a soybean processing factory in Bečej. By the construction of the ”Sojaprotein” factory, the process of soybean processing should meet the local demand for high-value proteins used in food industry, decrease the deficit of edible oil, while the production of proteins for animal feed should support the development of cattle breeding.

Instead of importing, Yugoslavia should become a country that exports the highly in-demand plant proteins and soybean sowing should contribute to a better crop sequence and yield growth in agriculture. The construction of the ”Sojaprotein” factory started in 1977 and regular production started in 1983, while the final constitution was completed in 1985.

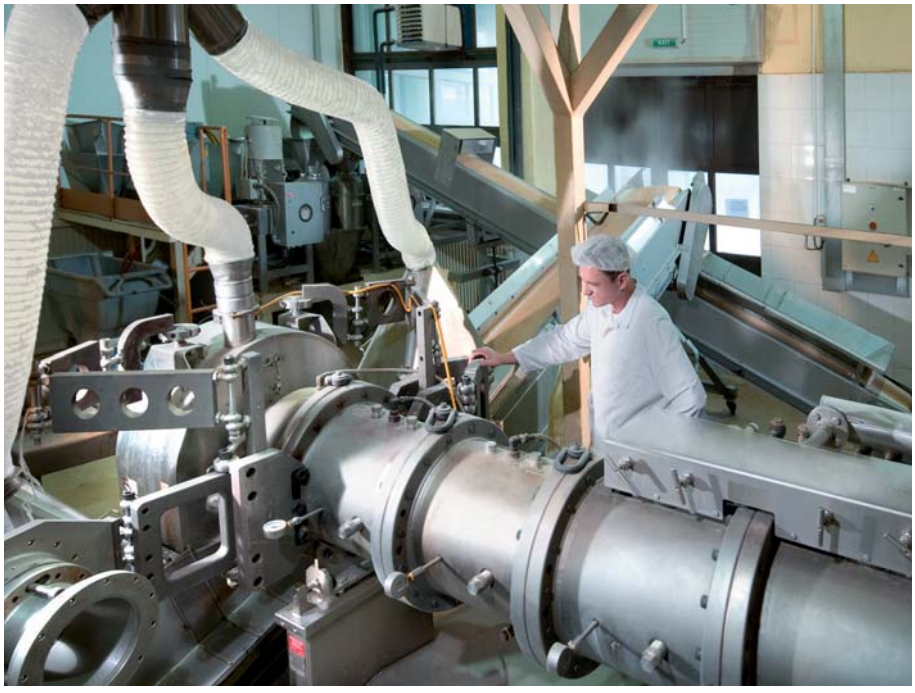
The soybean was an underrepresented crop in Yugoslavia until the establishment of ”Sojaprotein”. Thus, during the period between 1971 and 1980, soybean was sowed on 19,000 hectares on average. The beginning of the construction of the factory in Bečej resulted in a gradual expansion of surfaces containing soybean crops. A more stable raw material base for the work of this factory was established through the considerable financial investments of ”Sojaprotein” in primary agricultural production, as well as by the work of field experts for the purposes of making farmers familiar with an unknown crop.

That local production should be a main raw material base has always been one of viewpoints of ”Sojaprotein”. Since its establishment, the company has encouraged and supported soybean production in different ways and thus the needs for raw materials are nowadays met by locally grown beans.

For 25 years now, ”Sojaprotein” has organized the production of mercantile soybean together with its contract farmers, ensuring the necessary raw material and sowing conditions. Owing to long-term cooperation with soybean producers, partnership with them and advance investments in sowing, soybean production for the needs of the factory’s capacities first reached optimal quantities in Yugoslavia and then in Serbia. The sowing of around 100,000 hectares on average was organized by ”Sojaprotein”, while the record sowing of 112,000 hectares was achieved in 2000, and in 2006 a record quantity of 340,000 was purchased from other producers. ”Sojaprotein” owns a warehousing space for storing 100,000 tons of beans in silos and floor storage, while the annual harvest is temporarily stored in several collection spots in soybean production regions. The holding company ”Victoria Group”, within which the company ”Sojaprotein” operates, consists of 10 companies that are related to agricultural production and sales in their business activities, while one of its members, ”Victoria Logistic” has taken over the procurement of raw material for production.

”SOJAPROTEIN” – A FACTORY KEEPING UP WITH INNOVATIONS

”Sojaprotein” owns modern technological equipment from the renowned European and American producers. Special attention is given to maintaining the technical and technological readiness of the factory by constantly monitoring and by applying innovations in this branch of the food industry. Devices enabling ”Sojaprotein” to keep the pace with the leading soybean producers in the world were installed.



There is a tendency to the maximum utilization of the production capacities, along with the increase in productivity, the elimination of production bottle-necks, the installation of new devices and modern packaging for finished products. Thus the annual capacity of the factory was increased from the projected 160,000 tons to 250,000 tons of processed beans owing to the installation of new equipment.

The investments in human food production equipment have been considerable over the last couple of years. New mills from renowned producers have been installed, with which the production of full-fat soybean flour and defatted soybean flour has doubled. A new line for the production of textured products with a twin-screw extruder with the capacity of 3 tons/hour and accessory equipment has been installed in accordance with all the requirements of the food industry standards, which resulted in an increase in the capacity, quality and portfolio of textured products. The new lines for sack packaging of flour and textured products improved the quality of packaging and the accuracy of product measurement. The automated line for sack palletisation has also improved product packaging. New condensators have been installed in the extraction unit, which is a very important step in the production process.

A new boiler room has been constructed for the biomass, which completely meets the needs of the plant for steam and makes significant energy savings. ”Sojaprotein” makes a considerable contribution to environmental protection by having switched to renewable energy sources.

In its further development, ”Sojaprotein” has moved to a higher level of soybean processing in two phases:

Phase I – the construction of the plant for the production of traditional soybean concentrates with a capacity of 70,000 tons a year. The traditional soybean concentrates are mostly used for the production of high quality livestock feed, as well as additives in the food industry. The investments made during this phase will be completely realized during 2011.

Phase II – the construction of the plant for the production of functional soybean concentrates with the capacity of 30,000 tons a year. The functional concentrates are one of the most important components in the food industry, particularly in meat processing where the sensory and nutritive characteristics of products have been improved.

After these investments, all processed soybean will be finalized in highly profitable products for the food industry and direct human nutrition.

The construction of these plants made Sojaprotein the only producer in Europe that based its production on locally grown soybeans. Processing locally grown soybean from cultivars developed through natural selection, ”Sojaprotein” products are obtained from non - genetically modified beans.

THE APPLICATION OF INTERNATIONAL STANDARDS IN THE PRODUCTION PROCESS AND ON THE QUALITY

The management team of ”Sojaprotein” has been dedicated firstly to meeting the requirements and expectations of its clients, establishing of partnership relations with suppliers along with support and stimulation of the activities of employees, their initiatives and responsibility for quality. Development objectives relate to expanding the existing portfolio of soybean products, as well as to improving their application.

The optimal number of employees with the appropriate professional qualifications with the constant acquisition of new knowledge, monitoring product quality by accredited laboratories and investments in new equipment, its preventive maintenance and working conditions represent the base on which the achievement and maintenance of product quality relies.

In accordance with the policy of the permanent development of the quality system and the adoption of international standards, the ”Sojaprotein” company has developed the Identity Preservation Program, which has been internationally certified. This system defines the process of preserving the genetic pureness of soybean products, starting from seed production, until the delivery of the final product, strictly respecting the procedure of control for all the segments of the production chain and with clearly defined sequence and documentation.

”Sojaprotein” guarantees that the products originate exclusively from non-genetically modified beans of protected origin, produced in Serbia.

”Sojaprotein” has established a quality system in accordance with the requirements of the ISO 9001 standard, the product safety system in accordance with the requirements of HACCP and ISO 22000 and the system of environmental protection in accordance with the requirements of the ISO 14001 standard. A system in accordance with the OHSAS 18001 standards has been established for the purposes of maintenance and continuous improvement of the health and safety management system in the factory. The products of ”Sojaprotein” also meet Kosher and Halal requirements.

By engaging accredited laboratories that perform the control of conditions in the raw material production process, control of the purchase of raw materials, control of the receipt of input materials, the production process, the analysis of the chemical composition, the microbiological characteristics and the control of product safety, ”Sojaprotein” ensures a quality and a safe product, which is a basic precondition for a stable placement on foreign and local markets. By improving its production process in accordance with these standards, ”Sojaprotein” tends to take a significant market position in the EU countries, Russia, CEFTA countries, EFTA countries, Turkey, the Near and Middle East and North Africa in the field of the production of healthy and safe food made of soybean for human and animal nutrition. This is achieved by meeting the highest requirements of the international quality standards, product safety standards and environmental protection standards, professional training and raising the awareness of all employees to act preventively, to constantly improve quality and product safety and to protect the environment they live in.

A CERTIFIED SYSTEM FOR PROCESSING SOYBEANS

The procedure for processing soybeans adopted while designing the factory has been applied since the start of ”Sojaprotein” operations and it has not been changed in its foundations, though it has been modernized and upgraded in certain parts over years.

Soybeans delivered from the fields are initially controlled at the receipt point (moisture, impurities and genetic modification are subject to control). The received beans are first classified according to moisture content. Then gross impurities are removed and they are dried to the optimal moisture content and stored in silos for a longer period of time.

At the beginning of the processing cycle, a bean passes through the phase of rough and fine cleaning once again. In order to hull the beans more easily, they are additionally dried and tempered and transferred to the preparation unit. Beans are crushed, hulled, conditioned (warmed up by steam) and then passed through cylinders that shape them into flakes. The raw material used in the production of full-fat products and the raw material subject to extraction are separated in the preparation plant.

Oil is separated there and the defatted material is subject to hydrothermal processing for the purpose of removing the remaining content of hexane dissolvent and inactivating anti-nutritive components (desolventisation and toasting). Also, lecithin is separated from the raw oil by degumming.

Degummed soybean oil and lecithin from the extraction plant are stored in tanks and sold in a raw state. Depending on the quality of the selected soybean, as well as on the technological parameters applied, defatted flakes are forwarded to the plant for the production of flour and grits for human nutrition or for the production of soybean meal used as animal feed. Full-fat and defatted flours and grits are produced in the flour and grit production plant at different degrees of heat processing and granulation. Defatted tempered toasted soybean flour of a particular granulation is a fundamental raw material for the production of textured products (flakes, particles and slices). Products intended for human nutrition and the food industry are packed in sacks and in the appropriate packaging, while products intended for household use are forwarded to the plant for small packaging.

THE ”SOJAPROTEIN” PRODUCTION PROGRAM

Protein and oil products are obtained through soybean processing in ”Sojaprotein”. According to the technological procedure and their purpose of use, the products are classified into the following groups:

FOOD INDUSTRY PRODUCTS

Full-fat products: flours and grits with an oil content of around 23%

Low-fat products: flour with an oil content of around 8%

Defatted products: flours and grits with an oil content of around 1.5%

Lecithinated products: defatted flour with applied soybean lecithin

Textured soybean products: defatted textured proteins in the shape of flakes, particles and slices – neutral colour or coloured, a protein content of 52%

Functional mixtures of products containing albumen: Sopromix 1 (protein 57%), Sopromix 2 (protein 48%) and Sopromix HE (protein 60%)

Soybean oil and lecithin: raw degummed soybean oil and raw soybean lecithin

PRODUCTS FOR HOUSEHOLD USE

”Soja Vita” products: flour, flakes, particles, slices, roasted soybeans, vegetarian pates, soybean lecithin in jars and the dietetic beverage ”Leci Vita”.

Soybean products are the basic protein component in the preparation of animal feed for intensively reared animals. Through their use, better results are achieved in the production of proteins of animal origin: meat, milk and eggs.

PRODUCTS FOR ANIMAL NUTRITION

Meal: protein content 44%

The ”Sojaprotein” products are used particularly in the vegetable oils and fats industry, the meat processing industry, the confectionery industry, the production of pasta, bakery and the pharmaceutical industry. Owing to the appropriate technological procedure of soybean processing, nutritive and biological values are preserved in these products.

The basic advantages of their use in the food industry include the following:

- increase the total nutritive and biological value and the utilization of the final product
- increase the sensory properties of final products
- the economy of final products production

The ”Soja Vita” program products are intended for household and restaurant preparation of different dishes. They are used individually or in combination with other food products, enabling home or professional cooks to show their ability and adapt the menu to different tastes.

SALE ON LOCAL AND FOREIGN MARKETS

As a tradition, the major part of soybean products are sold in the local market, where ”Sojaprotein” has been acknowledged as a supplier with a long tradition of components for the production of high quality livestock feed, as well as a reliable supplier of different branches of the Serbian food industry. Although it has been comparatively less represented on the international markets, the sales of soybean products on the international markets represents a long-term objective of ”Sojaprotein”.

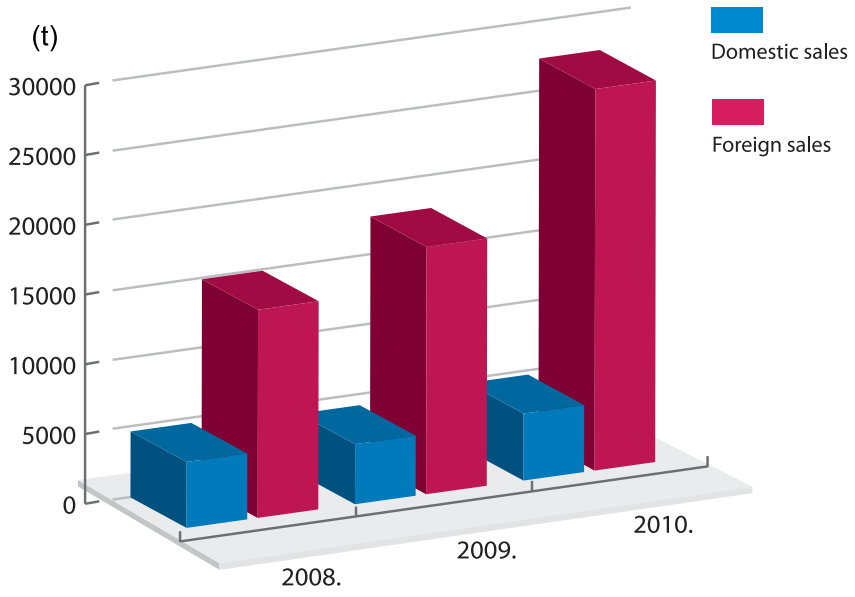
Owing to the fact that soybean products have been produced from NON GMO varieties of locally grown soybean, ”Sojaprotein” has a comparative advantage at the international market and the interest for these products constantly grows, and nowadays soybean products are sold in the 36 countries worldwide.

Since its beginnings, the export activities of the factory have had a significant role in the development of the company, having passed through different phases just like the factory itself. During the eighties of the last century, the export activities have been in the function of cooperation and toll processing of soybean providing for the missing quantity of soybeans for processing. After that, the export activities based on the sales of higher processing phases of NON GMO soybeans have developed, first modestly and more and more in time. During the period after 2000, the export activities of soybean products for different use maintained their development trend in parallel with the expansion in the European markets.

The completion of the privatization process at the end of 2002 was marked as a turning point in the philosophy and export objectives of ”Sojaprotein”. The foundations of export expansion of ”Sojaprotein” that took place in the following years and that lasts until today were established then.

The fact that the factory processed only NON GMO soybeans had a particular impact on the expansion of export activities. Owing to the production of NON GMO soybeans in Serbia under the auspices of ”Sojaprotein” and ”Victoria Logistics”, the conditions for issuing a valid certificate on the preservation of origin, traceability and genetic purity (IP NON GMO) have been established, by which the requirements applied in the European Union and other countries that do not accept GMO products have been met. Even during the years of oscillation of the total exports, ”Sojaprotein” managed to improve the export structure by marketing products subject to a higher degree of processing (textured products, flours and grits).

DOMESTIC AND FOREIGN SALES 2008-2010.
HIGHER PROCESSING PHASES PRODUCTS (t)



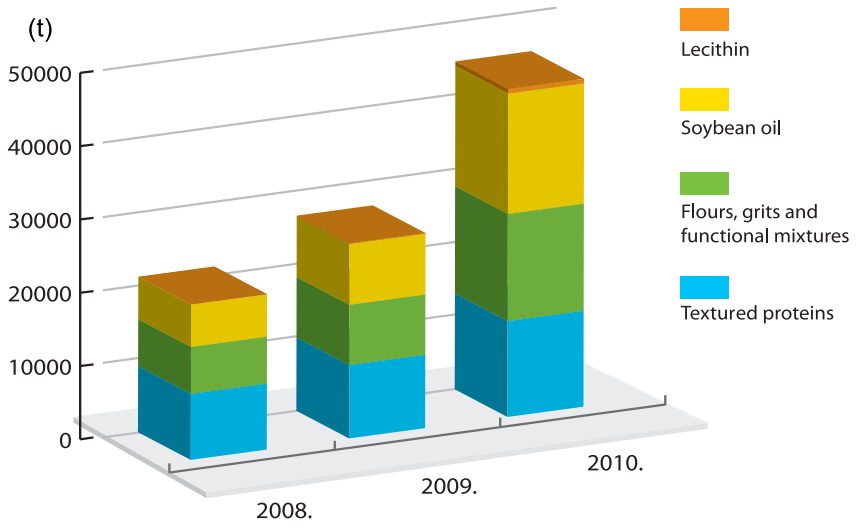
In recent years, the share of textured products in the total exports has been additionally increased with the tendency of further growth as a result of the continuous objective of conquering new markets and of the continuous investment in the state-of-art equipment produced by renowned producers. The export structure of soybean products evenly includes textured products, flours, grits and raw soybean oil. Along with lecithin, these are the main products of ”Sojaprotein” that are exported, and the products used for animal nutrition account for the remaining part of the portfolio.

The regional structure of the exports changes depending on the product structure in particular years. The market of the European Union is the most important

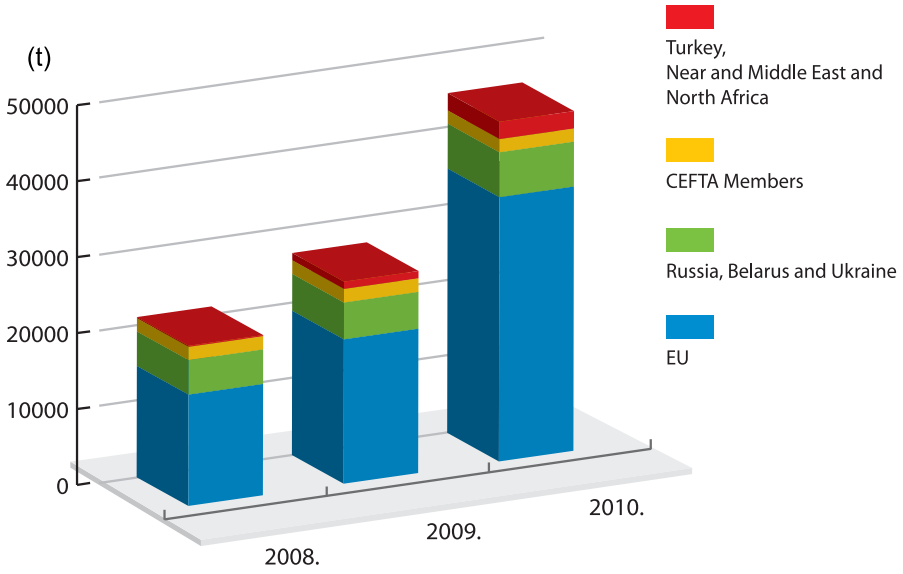
market where the products of ”Sojaprotein” are sold, accounting for over 70% of the total exports, followed by the Russian Federation, as well as by countries that are members of CEFTA and EFTA. As of 2008, these products are sold in Turkey, the countries of the Near and Middle East and North Africa.

The clients include trading companies that further distribute the goods, as well as end users from different branches of the food industry.

FOREIGN SALES 2008-2010.
PRODUSCTS STRUCTURE (t)



FOREIGN SALES 2008-2010.
REGIONAL STRUCTURE (t)



”SOJAPROTEIN” AS A MEMBER OF A SIGNIFICANT HOLDING COMPANY IN THE SECTOR OF AGRICULTURE

The ownership transformation procedure of ”Sojaprotein” started in 2000 when the first cycle was completed. The remaining part of the social capital (in the amount of 39.63%) was sold in the second cycle at a public auction at the Belgrade Stock Exchange in 2002. The local company ”Victoria” was a buyer. The ”Sojaprotein” factory became a part of the closed production system by establishing the company ”Victoria Group” in 2004 with its registered office in Novi Sad. The system consists of 10 companies located throughout Serbia. Having become members of the ”Victoria Group”, some of these companies were reconstructed by significant investments and new technologies were introduced, while completely new factories were constructed for some of those members. As one of the biggest agricultural complexes in Serbia, the ”Victoria Group” secures its leading role in the agricultural sector with its profitable business operations.

SOJAPROTEIN ČLANICA VICTORIA GROUP

FABRIKA MINERALNIH ĐUBRIVA Fertil ČLANICA VICTORIA GROUP

VICTORIAOIL ČLANICA VICTORIA GROUP

VICTORIA LOGISTIC ČLANICA VICTORIA GROUP

Veterinarski Zavod Subotica ČLANICA VICTORIA GROUP

SP LABORATORIJA ČLANICA VICTORIA GROUP

LUKA BAČKA PALANKA ČLANICA VICTORIA GROUP

VICTORIAPHOSPHATE ČLANICA VICTORIA GROUP

VICTORIA ZORKA MINERALNA ĐUBRIVA ŠABAC Članica Victoria Group

VICTORIA BIOEnergy ČLANICA VICTORIA GROUP

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