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SOYBEAN SEED-BORNE FUNGI IN THE VOJVODINA PROVINCE

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Abstract

The presence and significance of phytopathogenic fungi on and within soybean seeds originating from Vojvodina province was assessed. 75 seed lots from 2001 and 2002 were investigated and seed inhabiting fungi were identified, basing on both morphological and cultural characteristics. A considerable amount of seeds was not infected with parasitic fungi. Fungi from the genera *Diaporthe/Phomopsis* and *Fusarium* were the most often isolated pathogens in both years, from all localities. *Peronospora manshurica* was also found in 2001 and 2002 in the whole country. Facultative parasites from the genera of *Alternaria, Aspergillus* and *Penicillium* were found in more than a half seed lots.

Key words: *Glycine max*, seed-borne pathogens, *Diaporthe/Phomopsis*, *Fusarium*

Introduction

More than 40 species of phytopathogenic fungi, bacteria and viruses may infest soybean seed causing various diseases, out of which 15 can result in significant economical losses, reducing yield and deteriorating quality of seed crop. Infected seed can provide primary inoculum for infestation of new crop and seed-borne pathogens may be dispersed for long distances with it (Hartman et al. 1999).

Soybean seed-borne diseases occur in the Vojvodina province, too (Vidić et al. 2003, Ignjatov et al. 2006, Medić-Pap 2007). Recently soybean becomes one of the most important crops in the province. That is why this work, aiming at the assessment of seed infection level in the most often grown cultivars was carried out.

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Material and methods

Seed samples were taken from six seed companies in the Vojvodina province. Cultivars of different maturity groups were investigated: 'Afrodita' – 0 maturity group, 'Ravnica' and 'Balkan' – group I and 'Vojvoðanka' – group II. The seed sampling procedure was performed in accordance with the ISTA rules (International... 1999). From seeds harvested in 2001 and 2002, 39 and 36 seed lots were tested, respectively. 100 seeds were taken from each sample, randomly. Following visual determination of the *Peronospora manshurica* presence (Pathak et al. 1978), seeds were prepared for pathogenic fungi isolation. Seeds were sterilized in 1% NaOCl and incubated on APDA medium (acidified potato dextrose agar) for seven days at 25°C in the dark (Machado et al. 2002). Pathogenic fungi found in seeds were isolated into pure cultures and identified. Isolates representing the *Diaporthe/Phomopsis* complex were identified on the basis of characteristics described by Hobbs et al. (1985), Jasnić and Vidić (1983), and Vidić and Jasnić (1998). *Fusarium* spp. were isolated on carnation leaf medium (CLA; Fisher et al. 1982) and identification of the isolates was performed according to Nelson et al. (1983) and Burgess et al. (1994). The seed health was determined basing on percentage of fungi present in seeds.

Results

The degree of the soybean seed infestation with the pathogenic fungi was relatively low in both years. The fungi were not isolated of 11 out of 75 studied seed lots. In the majority of samples not more than 1–2% of infected seeds were found. The level of infection exceeded 3% in several samples only. The degree of soybean seed infection was uniform at different localities in the Vojvodina province. Fungi from genera *Diaporthe/Phomopsis* (41% of samples in 2001 and 25% in 2002) and *Fusarium* (51% and 42%, respectively) were isolated from seeds of all four cultivars, originating from various localities. *Peronospora manshurica* was detected in 69% of seeds in 2001 and in 56% in 2002. In majority of the samples the pathogens were present in 1–5% of seeds. Beside the pathogenic fungi, three species of facultative parasites were found, also able cause disease under favourable laboratory conditions: *Alternaria* spp., *Aspergillus* spp. and *Penicillium* spp. Fungi from the genus *Alternaria* were detected in 95% of samples collected in the first year of investigation, and in 92% of samples from the second year (Fig. 1).

The percentage of seeds infected by *P. manshurica* ranged from 1 to 7% in 2001 and from 1 to 5% in 2002. Fungi from the *Diaporthe/Phomopsis* complex were present in 8% of individual samples from the first year of investigation and only in 1–2% in the second year, with the exception of two samples of 'Ravnica' cv. (with 10% and 11%, respectively). *Fusarium* spp. were present in 7% of samples from the first year and in 1–4% of samples from the second year of investigation. Fungi from the genus *Alternaria* were abundant in some seed samples from both years, with an

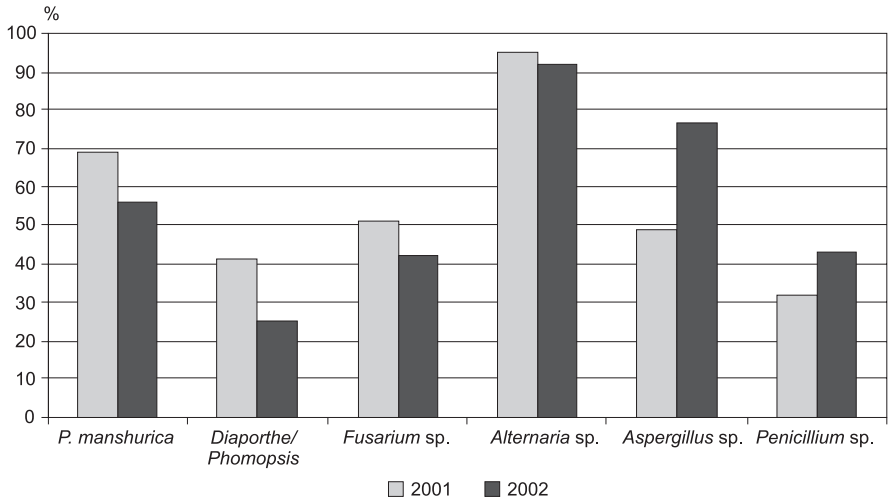

Fig. 1. Percentage of samples infected with fungi in 2001 and 2002

Table 1

Percentage of seeds infested by fungi per tested seed lot (sample) in 2001 and 2002

Fungus	2001	2002
Peronospora manshurica	1–7	1–5
Diaporthe/Phomopsis complex	1–8	1–2
Fusarium spp.	1–7	1–4
Alternaria spp.	3–42	1–46
Aspergillus spp.	1–9	1–19

incidence of over 40%. The level of seed infestation by *Aspergillus* spp. and *Penicillium* spp. varied from 1 to 20% and it was somewhat higher in 2002 than in 2001 (Table 1).

A comparative analysis of parasitic fungi in seeds between cultivars in 2001 elucidated the presence of *P. manshurica* and fungi from *Fusarium* genus in all samples. The highest level of infestation by *P. manshurica* was found in 'Ravnica', while 'Afrodita', 'Balkan' and 'Vojvođanka' were less infested. Seed infestation by *Fusarium* spp. did not vary significantly between cultivars. Fungi from the *Diaporthe/Phomopsis* group were most abundant in 'Balkan', followed by 'Ravnica' and 'Vojvođanka', while in 'Afrodita' they were not detected (Fig. 2).

In 2001 the highest degree of infection by *Diaporthe/Phomopsis* was found in 'Balkan' cultivar, while in 2002 this relation was considerably changed. Cultivar 'Ravnica' was the most infected by fungi from all three genera due to samples collected in the region of Novi Sad. A high degree of infection by *P. manshurica* and *Fusarium* spp. was observed in cultivars 'Balkan' and 'Vojvođanka' (Fig. 3).

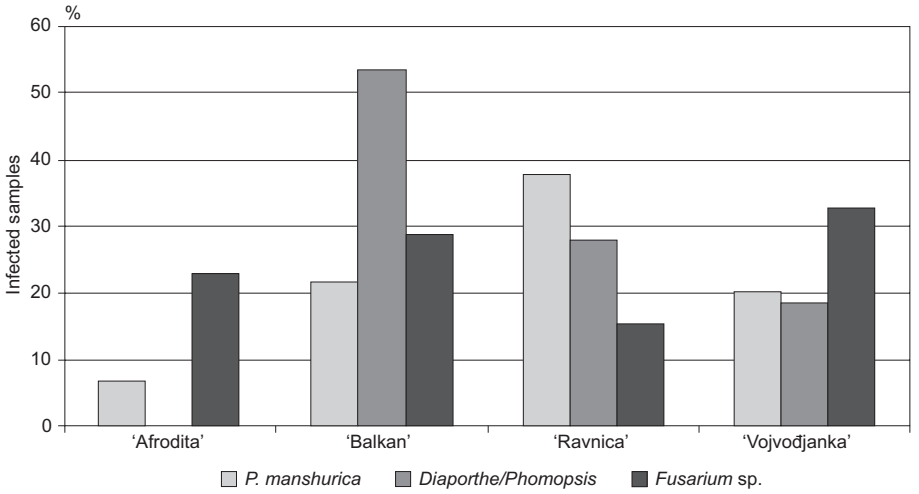

Fig. 2. Occurrence of pathogenic fungi in seed samples of different soybean cultivars in 2001

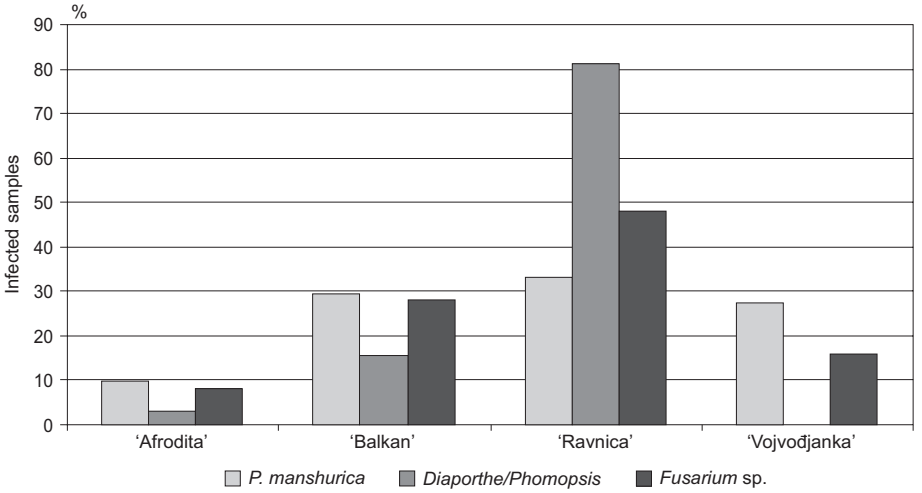

Fig. 3. Occurrence of pathogenic fungi in seed samples of different soybean cultivars in 2002

The increasing order of the infection level in cultivars in both years was as follows: 'Afrodita' < 'Vojvoðanka' < 'Balkan' and 'Ravnica'.

Fungi from the genus *Diaporthe/Phomopsis*

Symptoms caused by certain isolates from the genera *Diaporthe/Phomopsis* on soybean seed can not be visually differentiated. The symptoms are characteristic and easily distinguishable by severe infection only. Seeds are usually flattened,

elongated and deformed with wrinkled and cracked seed coat, partly or totally overlaid with floury whitish cover (Phot. 1).

Phot. 1. Soybean seeds infested with fungi of *Diaporthe/Phomopsis* complex (orig.) (photo by S. Medić-Pap)

Three species from the genera *Diaporthe/Phomopsis* were isolated from the soybean seeds investigated. The dominant species was *Phomopsis longicolla* present in more than 60% of infected seeds, followed by *Diaporthe phaseolorum* var. *caulivora* (nearly 30% in both years) and *P. sojae*, while teleomorph *D. phaseolorum* var. *sojae* was the least represented one (about 10% in both years; Fig. 4).

Seeds infected by *Fusarium* spp. were often symptomless. The grain filling was poor and seeds appeared smaller and shrivelled when severely infected. Seeds infected with *F. graminearum* had a red-pink discolouration.

Six species from the genus *Fusarium* were found in the investigated soybean seed lots: *F. graminearum*, *F. acuminatum*, *F. sporotrichioides*, *F. semitectum*, *F. proliferatum* and *F. equiseti*. In both years *F. semitectum* was the most frequently isolated species. *Fusarium semitectum* and *F. graminearum* were present in a considerable number of seed samples, while the occurrence of the other species was negligible (Fig. 5).

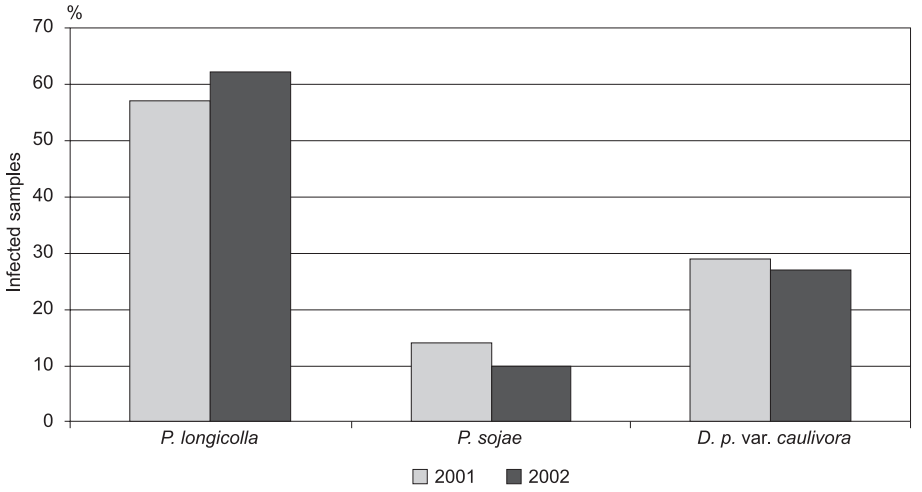

Fig. 4. Occurrence of species from the genera *Diaporthe/Phomopsis* in soybean seeds

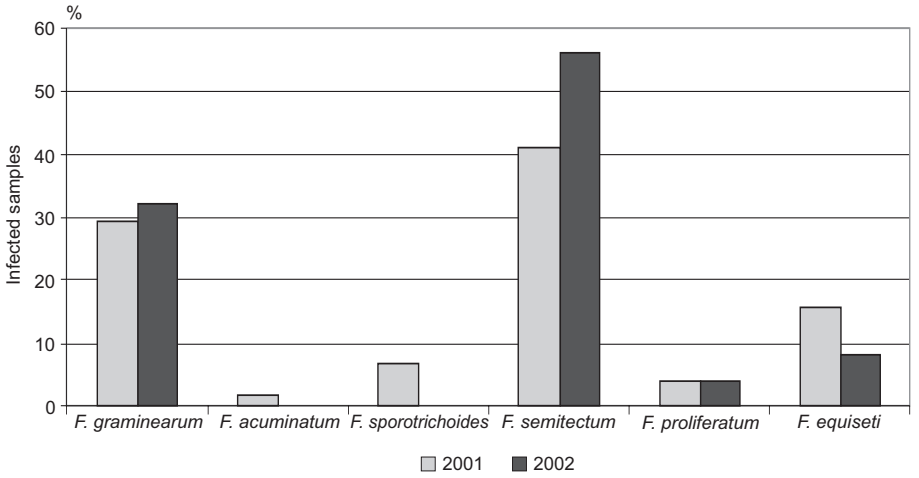

Fig. 5. Occurrence of *Fusarium* species in soybean seed lots

Discussion

Pathogenic fungi in soybean seeds were studied by many authors throughout the world (McGee 1992, Braccini and Dhingra 1996, Avilla et al. 2003, etc.) and recently also in Vojvodina (Komnenić 1991, Vidić et al. 2003, Medić-Pap et al. 2006). The authors have related the occurrence of the fungi and differences in seed infection level to the cultivation region, genotype, environmental factors, time of sowing and harvesting, phenophase and soybean physiology (Kmetz et al. 1978, Balducchi and McGee 1987, Ploper et al. 1989, Avilla et al. 2003).

Ploper and co-workers (1989) have studied the effect of genotype and growing region on soybean seed quality in 15 cultivars grown at different locations in Northwestern Argentina. The prevalent pathogens isolated from pods and seeds were: *Diaporthe/Phomopsis*, *Cercospora kikuchii*, *Alternaria* spp., *Fusarium* spp. and *Colletotrichum* spp. The authors correlated increased level of seed infection by *Diaporthe/Phomopsis* and *Fusarium* in the second year of survey with high precipitation rates at the time of grain filling and maturation. According to numerous authors, this is the most important factor effecting the infection level. The authors reported greater influence of environmental factors, especially humidity during maturation, than the effect of genotype or group of maturation on seed infection level. Higher incidence of fungi from the genera *Diaporthe/Phomopsis* and *Fusarium* reported for more humid years by other authors is in accordance with our results. In Vojvodina province, air humidity and temperatures above the average in August and September of 2001 must have resulted in greater incidence and spread of pathogens which attack seeds through pods.

In 2002, unusually long drought period at grain maturation and harvest considerably reduced seed infection by pathogenic fungi. According to the data obtained in humid years, the percentage of soybean seeds infected by the fungi in experi-

ments conducted in Argentina was considerably higher (17.9% and 24.8%) than in our country (max. 8%). Linear correlation was observed between the infection level and precipitation rates. Two samples of the 'Ravnica' cultivar originating from the region around Novi Sad were separated from others by markedly higher percentage of seeds infected by fungi from the genus *Diaporthe/Phomopsis* (10% and 11%). The results could be explained by simultaneous effect of several factors such as weather, crop rotation, seed health, season of harvest, etc.

Avilla and co-workers (2003) examined the effect of sowing time on soybean seed health in Brazil. The most frequent pathogens isolated from seeds were *Aspergillus* sp., *Cercospora kikuchii*, *Colletotrichum dematium*, *Fusarium semitectum* and *Phomopsis* sp. Comparison of the incidence of *F. semitectum*, the most frequently found pathogen in our work, with the results of these authors, revealed lower incidence of the species in our country. These findings could be explained by greater precipitation in Brazil during the period of seed maturation than in our region.

Braccini and Dhingra (1996) identified fungi from soybean seed in Brazil, using different methods for identification. They found domination of the genera *Phomopsis* and *Fusarium* (18–30% and 17–38%, respectively). The results of our work seem to be in accordance with theirs, but the infection level was considerably lower due to different climate in our region.

Komnenić (1991) identified parasitic fungi associated with soybean seed in Serbia in 1991. *Peronospora manshurica* and fungi from genera *Alternaria* and *Fusarium* were prevalent on seeds of 11 cultivars. Fungi from *Phomopsis* genus were also present in the seeds (up to 3.3%), while *Sclerotinia sclerotiorum* and *Rhizoctonia solani* occurred sporadically. Moreover, the author isolated *F. graminearum* and *F. oxysporum*. In our study six species from the genus were isolated from soybean seed. According to the results of Komnenić (1991) and ours, *Fusarium* spp. caused higher level of infection in more humid year. The incidence of *P. manshurica* was the same in different cultivars in both years of investigation.

In our work the percentage of seed contamination by fungi from the genera *Diaporthe/Phomopsis* was considerably higher than that reported 16 years ago by Komnenić (1991). Higher incidence of these fungi in seeds could have been associated with the spread of soybean production resulting in dissemination of the pathogens. Optimum conditions for the occurrence and spread of parasitic fungi must result also from the increase of soybean cultivation area (Vidić and Jasnić 1998).

Diaporthe phaseolorum var. *caulivora* was detected for the first time in our country during harvest at experimental fields in the region of Novi Sad in 1980, when massive drying and withering of soybean plants was noticed (Jasnić and Vidić 1981). Serbian authors became interested in fungi from the genera *Diaporthe/Phomopsis*, because of increased incidence of diseases which they caused (Jasnić and Vidić 1983, Vidić and Jasnić 1994, 1998, Vidić et al. 1999, 2002).

The decay of soybean, known as Phomopsis seed decay, is primarily caused by fungi from the *Diaporthe/Phomopsis* complex (Kmetz et al. 1978). Causal agents of the disease were *D. phaseolorum* var. *caulivora*, *P. sojae* (teleomorph *D. phaseolorum* var. *sojae*) and an unidentified species of *Phomopsis*. Hobbs et al. (1985) identified

a new species *P. longicolla*. 9 years later Vidić and Jasnić (1994) confirmed the presence of *P. longicolla* in our country which was the first report confirming the presence of the species in Europe.

Among the species from the *Diaporthe/Phomopsis* complex, *P. longicolla* is the most frequent and the most harmful pathogen causing soybean seed decay (McGee 1992, Vidić et al. 2002). Hartman et al. (1999) consider soybean seed decay a disease primarily caused by *P. longicolla*, but other species from the genus can also be included in the process. McGee (1986) isolated following fungi from the *Diaporthe/Phomopsis* genera: *D. phaseolorum* var. *caulivora* 25.8%, *P. longicolla* 39%, and *D. phaseolorum* var. *sojae* 1.6% from soybean seed sampled in 15 fields in Iowa. In their studies related to the effect of soybean harvest delay on seed rot, Vidić et al. (2002) confirmed that *P. longicolla* was the dominant species found on seeds (above 70% of total infected seeds), followed by *D. phaseolorum* var. *caulivora* (up to 21%) and *P. sojae* (8%). Our studies revealed a similar tendency, since the most frequent pathogen was *P. longicolla*, with approximately 60% of total infected seeds, followed by *D. phaseolorum* var. *caulivora,* as confirmed by the above mentioned authors, while *P. sojae* occurred sporadically.

Fusarium spp. cause three types of disease: decay of pods, decay of stem base and roots and Fusarium wilt (McGee 1992). The following causal agents can be found on seeds: *F. moniliforme, F. oxysporum, F. rigiusculum, F. semitectum* and *F. solani*. Yet, it has not been proved whether they are transmitted by the seed to the next crop. The data on incidence and harmfulness of *Fusarium* spp. to soybean in our country are insufficient, since these fungi occur sporadically and the infection intensity strongly depends on weather conditions during vegetation season.

Discussing the results of other authors related to the highest degree of soybean infection caused by *Fusarium* spp., McGee (1992) reported that the percentage of infected seeds was up to 36% in humid tropical regions, while in moderate climate it was much lower (mostly below 10%). In our study, the maximum level of seed infection was 7%, but in majority of the samples significantly lower percentage of infected seeds was registered (approx. 1–3%).

A number of authors (Gally et al. 1998, Roy and Ratnayake 1997, Yasem de Romero et al. 2002, Pioli et al. 2004, Martinelli et al. 2002, Jasnić et al. 2005) reported a decreased growth rate of *Fusarium* spp. and variously manifested symptoms of plant infection (rot and decay of seed and seedlings) under laboratory conditions. Similar results were obtained in our experiments, too. However, studies under field conditions could reveal the significance of these fungi for both soybean plants themselves and for the inoculum survival in soil, which is extremely important in the crop rotation.

Among factors which affect the incidence of soybean seed fungi are genotype and maturity group (TeKrony et al. 1984, Ploper and Abney 1986, Vidić et al. 1999, 2002). Vidić and co-workers (2002) have studied susceptibility of soybean cultivars 'Afrodita', 'Balkan' and 'Vojvođanka' to pathogens from the genera *Diaporthe/Phomopsis* causing seed decay under field conditions for three years in Vojvodina province. According to the authors the percentage of infected seeds of

tested cultivars was not related to the duration of the vegetation season. The results obtained in our study are in agreement with the above mentioned results.

Conclusion

Soybean seed was slightly infected in Vojvodina. Although the significant level of infection was not found during the two years of investigation, it should be monitored continuously. Special attention should be paid to *P. manshurica* and fungi from the genera *Diaporthe/Phomopsis* and *Fusarium* that could reduce seed vigour and germination capacity under certain field conditions.

Streszczenie

GRZYBY ZASIEDLAJĄCE NASIONA SOI W WOJWODINIE

W trakcie badań nad występowaniem i szkodliwością chorobotwórczych grzybów na nasionach soi w Wojwodinie przebadano 75 partii nasion z lat 2001 i 2002. Wyizolowane z nich grzyby zidentyfikowano na podstawie wyglądu kultur i cech morfologicznych. Znaczna część nasion była wolna od pasożytniczych grzybów. Z zasiedlonych nasion z obu lat i wszystkich miejscowości najczęściej izolowano grzyby rodzajów *Diaporthe/Phomopsis* i *Fusarium*. W latach 2001 i 2002 w całym kraju również zanotowano *Peronospora manshurica*. Pasożyty fakultatywne rodzajów *Alternaria, Aspergillus* i *Penicillium* występowały w ponad połowie badanych partii nasion.

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