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# EFFECT OF PRECEDING CROPS NITROGEN FERTILIZATION AND COBALT AND MOLYBDENUM APPLICATION ON YIELD AND QUALITY OF SOYBEAN GRAIN

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## ABSTRACT

**The three-year study was conducted on a calcareous chernozem experimental plot of the Institute of Field and Vegetable Crops. The experimental field trial was designed as corn – soybean – wheat crop rotation and performed in four replications. The aim of this study was to determine the effects of soybean seed inoculation with microbiological fertilizer, seed treatment with cobalt and molybdenum, as well as the effect of preceding maize crop fertilization with different dosages of nitrogen, on soybean yield and its protein and oil contents. This would allow defining optimal seed treatment that would enable production of high and stable yield of quality soybeans with the rationalization of the use of mineral nitrogen fertilizers. The application of the largest dose of 250 kg N ha$^{-1}$ was responsible for significantly (by 12.11%) higher yields compared with the control. Grain yield was not affected by the application of cobalt and molybdenum. No significant yield increase was found when seed that was treated only with Nitragin. The contents of protein and oil were statistically very significantly different between the three experimental years because the investigated qualitative properties of grain are highly dependent on hydrothermal conditions. The protein content in the soybean increased very significantly following the increasing amounts of nitrogen. Very significant effect of seed treatment on protein and oil content was found. The two-field system of maize and wheat cultivation should be upgraded to the three-field system: maize – soybean – wheat. The use of cobalt and molybdenum in slightly alkaline and alkaline soils did not contribute to the increase of grain yield or protein content in the grain. However, it caused very significant increase in soybean oil content on slightly alkaline soils. The effect of the increase was 1.77%.**

**Key words:** cobalt and molybdenum, fertilization, Nitragin, protein and oil content, soybean, yield.

## INTRODUCTION

Soybean has great economic importance, primarily due to the chemical composition of the grain, which has high protein (about 40%) and oil contents (about 20%). It is used in food industry, as in many others. Soybean is a "top product" on the world market. The entire soybean trade is very important, and especially soybean products, like flour, soybean oil, etc. (Cvijanovic and Cvijanovic, 1988; Popovic, 2010). Therefore, it is particularly important to achieve cooperation between primary agricultural soybean production and industry (Cvijanovic and Cvijanovic, 1989). The price of soybeans and soybean oil are increasing in the world markets (http://www.servisinfo.com). In order to achieve high soybean yields, soil must have favourable physical properties such as mechanical composition, structure and capillarity (Krmpotic et al., 2003). If stem and pod remains are ploughed after the harvest, soil is enriched with quality organic matter with close C:N ratio, which is beneficial.

Nitrogen is a key element of yield and is most often a limiting factor in achieving high yields (Dixon and Wheeler, 1986; Molnar, 1995). Nitrogen fertilization has its own peculiarities, because the mineral nitrogen, which is the form available for plants, is susceptible to losses due to leaching and mobility in the soil as well as to denitrification processes. On the other hand its content in the soil can increase due to mineralization of organic matter (Malesevic et al., 2005, Glamoclija et al, 2015). In order to obtain the

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full effect of nitrogen fertilization, as of any other agro-technical procedure, quality timely performance is of crucial importance (Crnobarac et al., 2000; Crnobarac et al., 2008) in optimal environmental conditions. The amount of mineral nitrogen depends on the soil type, exploitation mode, processing systems, temperature, humidity and water content in the soil (Starcevic et al., 2003).

*Bradyrhizobium japonicum, Bradyrhizobium elkani and Sinorhizobium fredi* live in symbiosis with soybean and create root nitrogen fixing nodules (Martinez-Romero and Caballero-Mellado, 1996). The abundance of the genus *Bradyrhizobium* in agricultural soils is often small; therefore in case of soybean production it has to be added to the soil in form of bacterial preparation. The use of microbial fertilizers is very important in the production of soybeans, significantly affecting growth, development and productivity. Trace elements cobalt and molybdenum are considered to increase the efficiency of nitrogen-fixing bacteria. The role of molybdenum in the life of plants was established in 1930 when it was found to be compulsory element for binding of atmospheric nitrogen by *Azotobacter* (Bortels, 1930). It reduces total number of nodules while increasing their dimensions and physiological activity (Anderson, 1956) resulting in greater nitrogen fixation. The role of cobalt in biological fixation of molecular nitrogen is specific, so it can not be replaced by any other microelements (Ahmed and Evans, 1960; Hallsworth et al., 1960; Reisenauer, 1960; Kastori, 1983; Hrustic et al., 1998).

Soybeans have high protein content hence very high nitrogen requirements. The largest part of nitrogen is provided by nitrogen fixation. Instable quality of soybeans is a common problem in animal feed industry, since protein and oil contents largely depend on the interaction between the genotype and environment (Westgate et al., 2000). According to Varga et al. (1988) plant food rich in nitrogen, may increase soybean protein content, but also reduces its oil content. The same authors suggested that the increase of the applied dose of nitrogen from 37.5 to 225 kg ha$^{-1}$ reduced the oil content by 4.75%. According to Pajkovic (1985) nitrogen fertilization will certainly affect grain protein content but not the oil content (Schmitt et al., 2001). Barker and Sawyer (2005) on the other hand, found no change in protein and oil contents with or without additional nitrogen.

The aim of this study was to determine the effects of soybean seed inoculation with microbiological fertilizer, seed treatment with cobalt and molybdenum, as well as the effect of preceding maize crop fertilization with different dosages of nitrogen, on soybean yield and its protein and oil content. This would allow defining the optimal seed treatment and facilitate production of high and stable yield of quality soybeans with the rationalization of the use of mineral nitrogen fertilizers.

## MATERIAL AND METHODS

Proteinka soybean variety, created at the Institute of Field and Vegetable Crops in Novi Sad, was chosen for the study. Proteinka is an early variety, belonging to 0 maturing group. The study was conducted during the three years, 2006, 2007 and 2008 on a calcareous chernozem experimental plot of the Institute of Field and Vegetable Crops. The field experiment was planned as corn-soybean-wheat crop rotation, performed in four replications. The basic plot size was 18 m2. Plants were spaced at 50 x 3.5 cm (total plant density 571 430 plants ha$^{-1}$). During the growing season common technology for optimal growth and development of soybean crops was applied.

The following factors were studied. The first factor (main plot): mineral fertilizers applied under the preceding maize crop in 8 variants, six of which included ploughing-down crop residua (CR) and two with no crop residues (CR). The control variant excluded the use of nitrogen fertilizers:

1. 0 kg N ha$^{-1}$ + CR + 50 kg N ha$^{-1}$ after wheat;
2. 50 kg N ha$^{-1}$ + CR + 50 kg N ha$^{-1}$ after wheat;
3. 100 kg N ha$^{-1}$ + CR + 50 kg N ha$^{-1}$ after wheat;

4. 150 kg N ha$^{-1}$ + CR + 50 kg N ha$^{-1}$ after wheat;
5. 200 kg N ha$^{-1}$ + CR + 50 kg N ha$^{-1}$ after wheat;
6. 250 kg N ha-1 + CR + 50 kg N ha$^{-1}$ after wheat;
7. 0 kg N ha$^{-1}$ (control);
8. 100 kg N ha$^{-1}$;
9. 200 kg N ha$^{-1}$.

The amounts of phosphorus and potassium in all the variants were 80 kg ha$^{-1}$ $P_2O_5$ and $K_2O$.

The entire volume of mineral fertilizers was added under the preceding crop corn. Total phosphorus (superphosphate 18% $P_2O_5$), potassium salt (48-52% $K_2O$), and half the amount of nitrogen (KAN 27% N) were applied before primary tillage for corn. The remaining amount of nitrogen (KAN 27% N), depending on the variant was applied before the pre-sowing treatment for corn.

In variants 1-6 the crop residua were ploughed-down, and 50 kg N ha$^{-1}$ (KAN 27% N) added immediately after wheat harvesting.

The second factor (subplots, two variants):

1. soybean seed inoculation with microbiological fertilizer NITRAGIN;
2. soybean seed inoculation with microbiological fertilizer NITRAGIN + soybean seed treatment with molybdenum and cobalt (active substances: 16.5% Mo, 1.65% Co).

The four central rows were harvested mechanically, using Wintersteiger combine harvester for soy experiments and grain yield determined (kg ha$^{-1}$). About 200 g of grain were taken from each variant for the determination of protein and oil contents (%). Chemical analysis was performed using the DA-7000 FLEXI-MODE NIR/VIS spectro-photometer (Balesevic Tubic et al., 2007).

Experimental data were analysed by descriptive and analytical statistics using the statistics software package STATISTICA 12 for Windows. The significance of differences among the mean values of the different factors studied in the paper (year, fertilization, seed treatment) was tested using three-way ANOVA (Maletic, 2005):

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_l + (\alpha\beta)_{ij} + (\alpha\gamma)_{il} + (\beta\gamma)_{jl} + \varepsilon_{ijlk}$$

i=1, 2 j=1, 2 l=1, 2, 3, 4 k=3

All evaluations of significance were made on the basis of the LSD test at 5% and 1% significance levels.

The results are presented in tabular and graphic form.

**Weather conditions**

The temperature and rainfall data were collected from the weather station at Rimski Sancevi, near Novi Sad, Serbia.

Relative humidity was at its highest in 2008 (73%) and lowest in 2006 (65%) as compared with the multi-year average of 69% (Table 1).

## RESULTS AND DISCUSSION

The analysis of weather conditions during the three years of research showed that these were most favourable during 2008, regarding the demands of soybean plants at different growth and development stages for temperature, precipitation, and relative humidity. Although the precipitation sum was lowest, for the soybeans as for many other plant species, the distribution is often more important than the total amount. The relative humidity during the vegetation period is also significant (Figure 1 and Table 1).

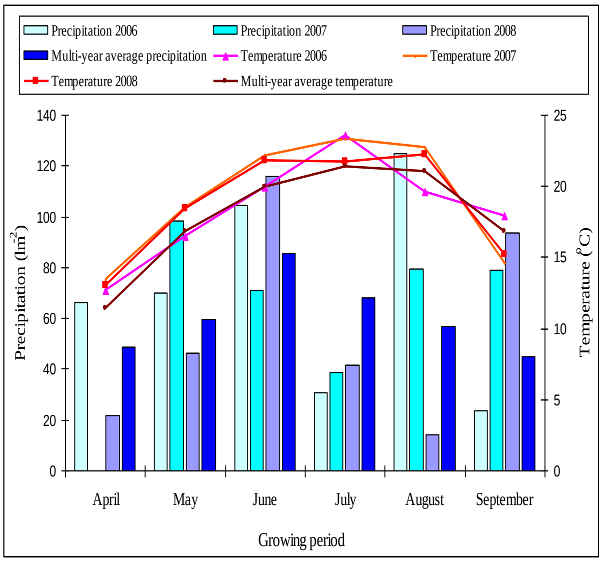


*Figure 1.* Average monthly temperature (°C) and precipitation sum (mm m$^{-2}$), Novi Sad, 2006-2008

*Table 1.* Relative humidity during the vegetation period, Novi Sad (Rimski Sancevi), Serbia (%)

| Month | Relative humidity (%) | | | Multi-year average |
|---|---|---|---|---|
| | 2006 | 2007 | 2008 | |
| April | 63 | 78 | 77 | 70 |
| May | 71 | 71 | 75 | 69 |
| June | 66 | 73 | 76 | 68 |
| July | 55 | 66 | 68 | 66 |
| August | 55 | 66 | 68 | 67 |
| September | 78 | 61 | 75 | 73 |
| Average | 65 | 69 | 73 | 69 |

Similar findings were shown by Cvijanovic et al. (2014), Dozet (2006, 2009), Djukic (2009), Popovic (2010), Popovic et al. (2011, 2013, 2015) and Dozet et al. (2014). Soybean is sensitive to low relative humidity, because it hinders fertilization and may lead to discarding of young pods (Sekulic and Kurjacki, 2008).

**Yield of soybean**

Statistical analysis of grain yield per unit area showed very significant effects for the year (factor A), and the year x seed treatment interaction (AxC), as well as fertilization (factor B). Effect of seed treatments (C) and other interactions between the studied factors were not statistically significant for grain yield per hectare (Table 2).

*Table 2.* Analysis of variance for grain yield per hectare

| Sources of variation | d.f | s.s | m.s. | F-ratio (calculated) | F pr. |
|---|---|---|---|---|---|
| Year (A) | 2 | 6463707 | 3231854 | 16.52 | <.001$^{**}$ |
| Repetition | 9 | 18169929 | 2018881 | 10.32 | <.001 |
| Fertilization (B) | 8 | 3170481 | 396310 | 2.03 | 0.050$^{*}$ |
| Interaction (AxB) | 16 | 3236083 | 202255 | 1.03 | 0.433$^{ns}$ |
| Residual (a) | 72 | 14086432 | 195645 | | |
| Seed treatment (C) | 1 | 20847 | 20847 | 0.26 | 0.610$^{ns}$ |
| Interaction (AxC) | 2 | 806278 | 403139 | 5.08 | <.001$^{**}$ |
| Interaction (BxC) | 8 | 381496 | 47687 | 0.60 | 0.774$^{ns}$ |
| Interaction (AxBxC) | 16 | 481936 | 30121 | 0.38 | 0.984$^{ns}$ |
| Residual (b) | 81 | 6422458 | 79290 | | |
| Total | 215 | 53239647 | | | |

$^{ns}$non significant; $^{*}$significant at 0.05; $^{**}$ significant at 0.01.

During all three years, the average yield was 3362 kg ha$^{-1}$ (Table 3). Very significantly higher yield was obtained in 2008, compared with 2006 (11.36%) and 2007 (11.29%).

Grain yield was lower at the variants where the crop residua were not ploughed-down (control, 100 kg N ha$^{-1}$ and 200 kg N ha$^{-1}$), compared with those at which they were. Statistically significantly lower yield was found in the control variant (3165 kg ha$^{-1}$) compared to the yield achieved by fertilization with 250 kg N ha$^{-1}$ (3519 kg ha$^{-1}$) and 150 kg N ha$^{-1}$ + CR (3517 kg ha$^{-1}$). The application of the largest N dosage (250 kg N ha$^{-1}$) resulted in significantly higher yields (12.11%), compared to the control. This is explained by the highest amount of total mineral nitrogen determined before sowing (98.80 kg ha$^{-1}$) at the depth of 0 to 90 cm, in the variant with nitrogen 250 kg ha$^{-1}$, and the lowest total content of mineral nitrogen (66.05 kg ha$^{-1}$) in the variant without nitrogen addition or ploughing-down crop residues. Determination of the amount of mineral nitrogen in the soil in spring before sowing, would provide the information on the necessity of nitrogen application and the appropriate quantity, in order to achieve high yield with maximum rationalization of the use of mineral fertilizers. In any case, it is necessary to take into account the type and characteristics of the soil, as they are decisive for the amount of residual nitrogen after the preceding crop (maize).

In 2007, a statistically significant higher yield (3326 kg ha$^{-1}$) without the use of cobalt and molybdenum was obtained. It was 5.39% higher compared to the yield from the variant where Nitragin and concentrated suspension of cobalt and molybdenum were applied to the grains before sowing (3156 kg ha$^{-1}$).

*Table 3.* Effect of cover crops and tillage on weed infestation of field after winter (Average for years 2010-2012)

| Year (A) | Fertilization (kg ha$^{-1}$) (B) | Seed treatment (C) | | $\bar{x}$ AB | $\bar{x}$ A |
|---|---|---|---|---|---|
| | Variant | Nitragin | Nitragin + Co and Mo | | |
| 2006 | 1 (0 kg N ha$^{-1}$ + CR) | 3409 | 3158 | 3283 | 3239 |
| | 2 (50 kg N ha$^{-1}$ + CR) | 3292 | 3422 | 3357 | |
| | 3 (100 kg N ha$^{-1}$ + CR) | 3156 | 3166 | 3161 | |
| | 4 (150 kg N ha$^{-1}$ + CR) | 3081 | 3324 | 3202 | |
| | 5 (200 kg N ha$^{-1}$ + CR) | 3298 | 3267 | 3282 | |
| | 6 (250 kg N ha$^{-1}$ + CR) | 3251 | 3184 | 3217 | |
| | 7 (control) | 3138 | 3182 | 3160 | |
| | 8 (100 kg N ha$^{-1}$) | 3207 | 3148 | 3177 | |
| | 9 (200 kg N ha$^{-1}$) | 3401 | 3228 | 3314 | |
| | $\bar{x}$ AC | 3248 | 3231 | | |
| 2007 | 1 (0 kg N ha$^{-1}$ + CR) | 3330 | 3172 | 3251 | 3241 |
| | 2 (50 kg N ha$^{-1}$ + CR) | 3146 | 2956 | 3051 | |
| | 3 (100 kg N ha$^{-1}$ + CR) | 3433 | 3177 | 3305 | |
| | 4 (150 kg N ha$^{-1}$ + CR) | 3691 | 3739 | 3715 | |
| | 5 (200 kg N ha$^{-1}$ + CR) | 3314 | 3196 | 3255 | |
| | 6 (250 kg N ha$^{-1}$ + CR) | 3573 | 3413 | 3493 | |
| | 7 (control) | 3083 | 2743 | 2913 | |
| | 8 (100 kg N ha$^{-1}$) | 3126 | 2903 | 3014 | |
| | 9 (200 kg N ha$^{-1}$) | 3240 | 3102 | 3171 | |
| | $\bar{x}$ AC | 3326 | 3156 | | |
| 2008 | 1 (0 kg N ha$^{-1}$ + CR) | 3712 | 3807 | 3760 | 3607 |
| | 2 (50 kg N ha$^{-1}$ + CR) | 3768 | 3775 | 3771 | |
| | 3 (100 kg N ha$^{-1}$ + CR) | 3572 | 3761 | 3666 | |
| | 4 (150 kg N ha$^{-1}$ + CR) | 3552 | 3713 | 3632 | |
| | 5 (200 kg N ha$^{-1}$ + CR) | 3483 | 3798 | 3641 | |
| | 6 (250 kg N ha$^{-1}$ + CR) | 3712 | 3981 | 3847 | |
| | 7 (control) | 3304 | 3541 | 3422 | |
| | 8 (100 kg N ha$^{-1}$) | 3361 | 3363 | 3362 | |
| | 9 (200 kg N ha$^{-1}$) | 3422 | 3305 | 3363 | |
| | $\bar{x}$ AC | 3543 | 3671 | $\bar{x}$B | |
| $\bar{x}$ BC | 1 (0 kg N ha$^{-1}$ + CR) | 3483 | 3379 | 3431 | |
| | 2 (50 kg N ha$^{-1}$ + CR) | 3402 | 3384 | 3393 | |
| | 3 (100 kg N ha$^{-1}$ + CR) | 3387 | 3368 | 3377 | |
| | 4 (150 kg N ha$^{-1}$ + CR) | 3441 | 3592 | 3517 | |
| | 5 (200 kg N ha$^{-1}$ + CR) | 3365 | 3420 | 3393 | |
| | 6 (250 kg N ha$^{-1}$ + CR) | 3512 | 3526 | 3519 | |
| | 7 (control) | 3175 | 3155 | 3165 | |
| | 8 (100 kg N ha$^{-1}$) | 3231 | 3138 | 3184 | |
| | 9 (200 kg N ha$^{-1}$) | 3354 | 3211 | 3283 | |
| | $\bar{x}$ C | 3372 | 3353 | | |
| Average 2006-2008 | | | | 3362 | |

| LSD | Factors test | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | AxB | AxC | BxC | AxBxC |
| 1% | 195 | 338 | 101 | 585 | 169 | 303 | 525 |
| 5% | 147 | 255 | 76 | 441 | 132 | 229 | 396 |

Grain yield was not affected by the use of cobalt and molybdenum, but there was no significant yield increase when seeds were treated with Nitragin only. The same trend was found by Campo et al. (2009) who explained it as toxic effects of molybdenum on *Bradyrhizobium* bacteria. Similar results of molybdenum fertilization and its weak effect on soybean yields were obtained in earlier studies (Person et al., 1999; Eloir, 2005). The opposite result, showing beneficial effect of molybdenum on the yield of cultivated plants, especially legumes, wee found by De Mooy (1970).

**Protein and oil content**

The contents of protein and oil represent the basic qualitative grain characteristics. The analysis of variance for soybean grain protein content showed that the year (factor A), fertilization (factor B), seed treatment (factor C) and the interaction between year and fertilization (AxB) all had statistically signifycant effect on grain protein content (Tables 4 and 5).

*Table 4.* The influence of testing on grain protein content (%)

| Year (A) | Fertilization (kg $ha^{-1}$) (B) | Seed treatment (C) | | $\bar{x}$ AB | $\bar{x}$ A |
|---|---|---|---|---|---|
| | Variant | Nitragin | Nitragin + Co and Mo | | |
| 2006 | 1 (0 kg N $ha^{-1}$ + CR) | 37.56 | 36.99 | 37.27 | 38.00 |
| | 2 (50 kg N $ha^{-1}$ + CR) | 37.79 | 37.07 | 37.43 | |
| | 3 (100 kg N $ha^{-1}$ + CR) | 37.90 | 37.53 | 37.71 | |
| | 4 (150 kg N $ha^{-1}$ + CR) | 38.10 | 37.39 | 37.74 | |
| | 5 (200 kg N $ha^{-1}$ + CR) | 38.47 | 37.83 | 38.15 | |
| | 6 (250 kg N $ha^{-1}$ + CR) | 38.96 | 37.39 | 38.18 | |
| | 7 (control) | 38.34 | 38.38 | 38.36 | |
| | 8 (100 kg N $ha^{-1}$) | 38.83 | 38.04 | 38.43 | |
| | 9 (200 kg N $ha^{-1}$) | 38.95 | 38.57 | 38.76 | |
| | $\bar{x}$ AC | 38.32 | 37.69 | | |
| 2007 | 1 (0 kg N $ha^{-1}$ + CR) | 35.48 | 34.19 | 34.84 | 35.77 |
| | 2 (50 kg N $ha^{-1}$ + CR) | 34.85 | 34.81 | 34.83 | |
| | 3 (100 kg N $ha^{-1}$ + CR) | 35.21 | 34.06 | 34.63 | |
| | 4 (150 kg N $ha^{-1}$ + CR) | 35.58 | 35.70 | 35.64 | |
| | 5 (200 kg N $ha^{-1}$ + CR) | 37.60 | 37.83 | 37.71 | |
| | 6 (250 kg N $ha^{-1}$ + CR) | 38.14 | 36.40 | 37.27 | |
| | 7 (control) | 33.97 | 33.47 | 33.72 | |
| | 8 (100 kg N $ha^{-1}$) | 35.58 | 35.60 | 35.59 | |
| | 9 (200 kg N $ha^{-1}$) | 37.96 | 37.38 | 37.67 | |
| | $\bar{x}$ AC | 36.04 | 35.49 | | |
| 2008 | 1 (0 kg N $ha^{-1}$ + CR) | 39.24 | 38.75 | 39.00 | 40.05 |
| | 2 (50 kg N $ha^{-1}$ + CR) | 39.13 | 38.92 | 39.03 | |
| | 3 (100 kg N $ha^{-1}$ + CR) | 40.26 | 39.48 | 39.87 | |
| | 4 (150 kg N $ha^{-1}$ + CR) | 40.67 | 39.67 | 40.17 | |
| | 5 (200 kg N $ha^{-1}$ + CR) | 40.48 | 39.97 | 40.22 | |
| | 6 (250 kg N $ha^{-1}$ + CR) | 40.71 | 40.04 | 40.37 | |
| | 7 (control) | 40.22 | 40.22 | 40.22 | |
| | 8 (100 kg N $ha^{-1}$) | 40.75 | 40.26 | 40.50 | |
| | 9 (200 kg N $ha^{-1}$) | 41.19 | 40.99 | 41.09 | |
| | $\bar{x}$ AC | 40.29 | 39.81 | $\bar{x}$B | |
| $\bar{x}$ BC | 1 (0 kg N $ha^{-1}$ + CR) | 37.43 | 36.64 | 37.03 | |
| | 2 (50 kg N $ha^{-1}$ + CR) | 37.26 | 36.93 | 37.09 | |
| | 3 (100 kg N $ha^{-1}$ + CR) | 37.79 | 37.02 | 37.40 | |
| | 4 (150 kg N $ha^{-1}$ + CR) | 38.11 | 37.58 | 37.85 | |
| | 5 (200 kg N $ha^{-1}$ + CR) | 38.85 | 38.54 | 38.69 | |
| | 6 (250 kg N $ha^{-1}$ + CR) | 39.27 | 37.95 | 38.61 | |
| | 7 (control) | 37.51 | 37.36 | 37.43 | |
| | 8 (100 kg N $ha^{-1}$) | 38.38 | 37.97 | 38.18 | |
| | 9 (200 kg N $ha^{-1}$) | 39.37 | 38.98 | 39.17 | |
| | $\bar{x}$ C | 38.22 | 37.66 | | |
| Average 2006-2008 | | | | 37.94 | |

| LSD | Factors test | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | AxB | AxC | BxC | AxBxC |
| 1% | 0.57 | 0.98 | 0.28 | 1.70 | 0.49 | 0.84 | 1.46 |
| 5% | 0.43 | 0.74 | 0.21 | 1.28 | 0.37 | 0.64 | 1.10 |

*Table 5.* Analysis of variance for protein content in the grain of soybean

| Sources of variation | d.f | s.s | m.s. | F-ratio (calculated) | F pr. |
|---|---|---|---|---|---|
| Year (A) | 2 | 661.4386 | 330.7193 | 199.81 | <.001** |
| Repetition | 9 | 103.9179 | 11.5464 | 6.98 | <.001 |
| Fertilization (B) | 8 | 112.2237 | 14.0280 | 8.48 | <.001** |
| Interaction (AxB) | 16 | 68.0127 | 4.2508 | 2.57 | <.001** |
| Residual (a) | 72 | 119.1771 | 1.6552 | | |
| Seed treatment (C) | 1 | 16.6445 | 16.6445 | 27.17 | <.001** |
| Interaction (AxC) | 2 | 0.2117 | 0.1058 | 0.17 | 0.842 ns |
| Interaction (BxC) | 8 | 6.0513 | 0.7564 | 1.23 | 0.290 ns |
| Interaction (AxBxC) | 16 | 6.4428 | 0.4027 | 0.66 | 0.827 |
| Residual (b) | 81 | 49.6249 | 0.6127 | | |
| Total | 215 | 1143.7450 | | | |

ns non significant; *significant at 0.05; ** significant at 0.01.

*Table 6*. Analysis of variance for oil content in the grain of soybean

| Sources of variation | d.f | s.s | m.s. | F-ratio (calculated) | F pr. |
|---|---|---|---|---|---|
| Year (A) | 2 | 145.7317 | 72.8658 | 118.89 | <.001** |
| Repetition | 9 | 31.4753 | 3.4973 | 5.71 | <.001 |
| Fertilization (B) | 8 | 32.4068 | 4.0509 | 6.61 | <.001** |
| Interaction (AxB) | 16 | 25.2006 | 1.5750 | 2.57 | <.001** |
| Residual (a) | 72 | 44.1258 | 0.6129 | | |
| Seed treatment (C) | 1 | 7.8814 | 7.8814 | 33.30 | <.001** |
| Interaction (AxC) | 2 | 0.0254 | 0.0127 | 0.05 | 0.948 |
| Interaction (BxC) | 8 | 1.0100 | 0.1263 | 0.53 | 0.828 |
| Interaction (AxBxC) | 16 | 1.3000 | 0.0812 | 0.34 | 0.990 |
| Residual (b) | 81 | 19.1685 | 0.2366 | | |
| Total | 215 | 308.3254 | | | |

ns non significant; *significant at 0.05; ** significant at 0.01.

Other interactions were not statistically significant ($p<0.05$, $p<0.01$).

The average protein content for the years studied was 37.94% (Table 4). The highest soybean protein content (40.05%) was found in 2008, which is very significantly higher (5.39%) compared with 2006 (38.00%) and 11.97% compared with 2007 (35.77%). Due to highly statistically significant differences in soybean protein content between the years, we can conclude that the investigated chemical characteristic was strongly dependent on hydrothermal conditions during the production year, which is consistent with the results of Dozet et al. (2008).

The highest percentage of protein in the grain was obtained by preceding crop fertilization with 200 kg $ha^{-1}$ nitrogen without ploughing-down the crop residues (39.17%). It is very significantly higher compared with the control (37.43%).

The seed treatment was found to have very significant effect on protein content. The application of trace elements cobalt and molybdenum with microbiological Nitragin significantly ($p<0.01$) lowered the percentage of protein in the grain (37.66%), compared to the variant without cobalt and molybdenum (38.22%).

Analysis of the years x seed treatment interaction (AxC) in all three years, revealed a statistically significantly lower protein content with the use of cobalt and molybdenum.

Analysis of variance for oil content in soybean grain showed that the year (factor A), fertilization (factor B), seed treatment (factor C) and year x fertilization interaction (AxB) had statistically very significant effects on the investigated grain quality parameter. Other interactions between the studied parameters were not statistically significant (Table 6).

The three-year average soybean oil content was 21.67% (Table 7). The LSD test showed a very significant impact of year on soybean oil content. The highest grain oil content was found in 2007, and the lowest in 2008. All the differences in the oil content were highly significant ($p<0.01$).

*Table 7*. Impact of studied factors on grain oil content (%)

| Year (A) | Fertilization (kg ha$^{-1}$) (B) | Seed treatment (C) | | $\bar{x}$ AB | $\bar{x}$ A |
|---|---|---|---|---|---|
| | Variant | Nitragin | Nitragin + Co and Mo | | |
| 2006 | 1 (0 kg N ha$^{-1}$ + CR) | 21.52 | 22.10 | 21.81 | 21.35 |
| | 2 (50 kg N ha$^{-1}$ + CR) | 21.55 | 21.77 | 21.66 | |
| | 3 (100 kg N ha$^{-1}$ + CR) | 21.30 | 21.58 | 21.44 | |
| | 4 (150 kg N ha$^{-1}$ + CR) | 21.31 | 21.76 | 21.53 | |
| | 5 (200 kg N ha$^{-1}$ + CR) | 21.17 | 21.48 | 21.33 | |
| | 6 (250 kg N ha$^{-1}$ + CR) | 20.93 | 21.67 | 21.30 | |
| | 7 (control) | 20.99 | 21.20 | 21.09 | |
| | 8 (100 kg N ha$^{-1}$) | 20.92 | 21.27 | 21.09 | |
| | 9 (200 kg N ha$^{-1}$) | 20.86 | 20.96 | 20.91 | |
| | $\bar{x}$ AC | 21.17 | 21.53 | | |
| 2007 | 1 (0 kg N ha$^{-1}$ + CR) | 23.12 | 23.53 | 23.32 | 22.80 |
| | 2 (50 kg N ha$^{-1}$ + CR) | 23.40 | 23.41 | 23.40 | |
| | 3 (100 kg N ha$^{-1}$ + CR) | 23.13 | 23.79 | 23.46 | |
| | 4 (150 kg N ha$^{-1}$ + CR) | 22.90 | 22.96 | 22.93 | |
| | 5 (200 kg N ha$^{-1}$ + CR) | 21.78 | 21.93 | 21.85 | |
| | 6 (250 kg N ha$^{-1}$ + CR) | 21.47 | 22.23 | 21.85 | |
| | 7 (control) | 23.56 | 24.33 | 23.94 | |
| | 8 (100 kg N ha$^{-1}$) | 22.64 | 22.84 | 22.74 | |
| | 9 (200 kg N ha$^{-1}$) | 21.49 | 21.90 | 21.69 | |
| | $\bar{x}$ AC | 22.61 | 22.99 | | |
| 2008 | 1 (0 kg N ha$^{-1}$ + CR) | 21.16 | 21.53 | 21.34 | 20.86 |
| | 2 (50 kg N ha$^{-1}$ + CR) | 21.29 | 21.59 | 21.44 | |
| | 3 (100 kg N ha$^{-1}$ + CR) | 20.79 | 21.21 | 21.00 | |
| | 4 (150 kg N ha$^{-1}$ + CR) | 20.52 | 21.19 | 20.85 | |
| | 5 (200 kg N ha$^{-1}$ + CR) | 20.76 | 21.15 | 20.95 | |
| | 6 (250 kg N ha$^{-1}$ + CR) | 20.59 | 21.02 | 20.80 | |
| | 7 (control) | 20.30 | 20.81 | 20.56 | |
| | 8 (100 kg N ha$^{-1}$) | 20.26 | 20.68 | 20.47 | |
| | 9 (200 kg N ha$^{-1}$) | 20.27 | 20.47 | 20.37 | |
| | $\bar{x}$ AC | 20.66 | 21.07 | $\bar{x}$ B | |
| $\bar{x}$ BC | 1 (0 kg N ha$^{-1}$ + CR) | 21.93 | 22.38 | 22.16 | |
| | 2 (50 kg N ha$^{-1}$ + CR) | 22.08 | 22.25 | 22.17 | |
| | 3 (100 kg N ha$^{-1}$ + CR) | 21.74 | 22.19 | 21.96 | |
| | 4 (150 kg N ha$^{-1}$ + CR) | 21.58 | 21.97 | 21.77 | |
| | 5 (200 kg N ha$^{-1}$ + CR) | 21.24 | 21.52 | 21.38 | |
| | 6 (250 kg N ha$^{-1}$ + CR) | 21.00 | 21.64 | 21.32 | |
| | 7 (control) | 21.62 | 22.11 | 21.86 | |
| | 8 (100 kg N ha$^{-1}$) | 21.27 | 21.59 | 21.43 | |
| | 9 (200 kg N ha$^{-1}$) | 20.87 | 21.11 | 20.99 | |
| | $\bar{x}$ C | 21.48 | 21.86 | | |
| Average 2006-2008 | | | | 21.67 | |

| LSD | Factors test | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | AxB | AxC | BxC | AxBxC |
| 1% | 0.35 | 0.60 | 0.17 | 1.04 | 0.30 | 0.52 | 0.91 |
| 5% | 0.26 | 0.45 | 0.13 | 0.78 | 0.23 | 0.40 | 0.68 |

The effect of preceding crop nitrogen fertilization was highly statistically significant. Very significantly higher oil content was determined in the variant 2, compared to variants 1, 4, 5, 6, and 9. Schmitt et al. (2001), Barker and Sawyer (2005), Osborn and Riedell (2006) and Djukic et al. (2008) did not find significant effects of fertilization on soybean oil content.

The oil content was very significantly affected by the pre-sowing seed treatment. In relation to the oil content when only microbiological fertilizer – Nitragin was applied (21.48%), grain treated with Nitragin, cobalt and molybdenum showed a statistically highly significant increase in oil content (21.86%).

By analysing the year x fertilization interaction (AxB) for the three-year study period, the lowest soybean oil content was recorded in the variant 9, (200 kg N ha$^{-1}$ without crop residues).

## CONCLUSION

The results obtained in the present study led to the following conclusions:

The application of the largest dose of nitrogen (250 kg ha$^{-1}$) resulted in significantly higher yields, (by 12.11%) compared to the control. This can be explained by the fact that the highest amount of total mineral nitrogen (98.80 kg ha$^{-1}$) found at the depth between 0 and 90 cm before sowing, was determined in case of 250 kg ha$^{-1}$ nitrogen use, while the lowest (66.05 kg ha$^{-1}$) was determined in the control treatment. Determination of the amount of mineral nitrogen in the soil in spring before sowing would provide the information on the necessity of nitrogen application and the appropriate quantity, in order to achieve high yield with maximum rationalization of the use of mineral fertilizers. In any case, it is necessary to take into account the type and characteristics of the soil, as they are decisive for the amount of residual nitrogen after the preceding crop (maize).

The contents of protein and oil were statistically very significantly different between the experimental years because the investigated qualitative grain properties were highly dependent on hydrothermal conditions.

Preceding crop nitrogen fertilization contributed significantly to the protein content. The protein content in the soybean very significantly increased with increasing amounts of nitrogen. The seed treatment had very significant effect on protein content. The application of trace elements cobalt and molybdenum with microbiological Nitragin significantly ($p<0.01$) lowered the percentage of protein in the grain (37.66%), compared to the variant without cobalt and molybdenum (38.22%).

The two-field system of maize and wheat cultivation should be upgraded to the three-field system: maize - soybean - wheat. Given that the amount of mineral nitrogen depends on the preceding crop, the content of mineral nitrogen in the soil should be determined before sowing soybeans. In case that sufficient amount of mineral nitrogen is found (over 65 kg N ha$^{-1}$ in the soil profile 0 to 90 cm), the application of nitrogen fertilizers can be avoided, rationalizing the use of mineral fertilizers, which is a priority.

The use of cobalt and molybdenum in slightly alkaline and alkaline soils did not contribute to the increase of grain yield or protein content in the grain. However, it caused very significant increase in soybean oil content on slightly alkaline soils. The effect of the increase was 1.77%.

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