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The influence of long-term application of mineral fertilizers on the effects of inoculation with *Bradyrhizobium japonicum*

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Abstract: The influence of long-term application of mineral fertilizers (25 years) on the effectiveness of inoculation and soybean yields was studied in the course of 1991, 1992 and 1993. Trials with the soybean cultivar Kolubara were established at Rimski Šančevi. Indicators used in monitoring the effect of the long-term fertilizer application (a - no fertilization, b - N_{70} P_{60} K_{40}, c - N_{110} P_{90} K_{60}, d - N_{150} P_{120} K_{80}) were the dry matter mass and nitrogen (N) content as well as yields and the number of nodules per root. The greatest dry matter mass and the highest N content and seed yield were achieved in the inoculated treatments in the first year of the study. The effects of the long-term fertilizer application and inoculation were on the decline in the second year of the study, only to get completely lost in the third. At the conclusion of the third year, the greatest dry matter mass and the highest N content were observed in the treatment with the lowest N rate (70 kg Nha^{-1}) as well as in the inoculated treatments with no N applied.

Key words: *B. japonicum*, dry matter mass, N content, soybean cultivar, yield

Introduction

All field crops require sufficient amounts of N for growth and higher seed yields. In contrast to the rest of field crops, the soybean plant satisfies 80 % of its N requirements by symbiotic N_2 fixation. The amounts of mineral N fertilizers incorporated should be such as to merely maintain the level of nutrients in the soil, i.e. supply the soil with sufficent N amounts before sowing. The plant will utilize this N for the formation of its vegetative organs from early growth to nodule formation - in other words, until the establisment of a symbiotic association between nodule bacteria and itself (Eaglesham, 1987). In contrast to small amounts of N

fertilizers, the addition of larger amounts before sowing does lower the level of N_2 fixation (Smith and Hume, 1985; La Favre and Eaglesham, 1987; Senaratne et al., 1987).

The objective of the study was to investigate the effect of seed inoculation and long-term application of various rates of N-P-K fertilizers (25 years) on the efficiency of symbiotic associations and the seed yield.

Materials and methods

Between 1991 and 1993, a field trial with the soybean cultivar Kolubara was established at the Experiment field of the Institute of Field and Vegetable Crops at Rimski Šančevi.

The plot that was used had in the previous 25 years (1965-1990) been the site of an experiment with various rates of mineral fertilizers (a - no fertilization, b - N_{70} P_{60} K_{40}, c - N_{110} P_{90} K_{60}, d - N_{150} P_{120} K_{80}) investigating the effect fertilizer rates have on the yield of winter wheat and maize grown in a double-crop rotation system (the tillage had included disking at 10-12 cm).

Just before sowing, each of the fertilization variants was subjected to two treatments without and with the commercial microbiological preparation Nitragin, which contained highly effective strains of *B. japonicum*.

Winter wheat was the previous crop in the first year of the study and soybean in the second and third, since the trial was conducted in the same plot in all three years. The basic plot size was 200 m^2 (4 x 50 m) and the spacing between rows 0.5 m. The trial was established in a randomized block design with four replications. The soybean was grown under production conditions with no irrigation.

In all three years, sowing was done in the first ten-day period of April and sampling (5 plants per treatment) at flowering, when the plants were about 40 days old. Subsequently, the effect of seed inoculation and long-term fertilization on the dry matter mass, N content and the number of nodules per root was determined. The plants from four inner rows (100 m^2) were used to determine the seed yield ($kgha^{-1}$).

Results and discussion

The inoculation of seeds with the nodule bacteria (*B. japonicum*) has a possitive effect on nodulation, i.e. the nodule number, mass and N content in all variants of N fertilization (Kucey et al., 1989).

In the 1991 part of the trial, inoculation increased the dry matter mass and N content in all the fertilization variants. In the uninoculated treatments, the fertilization variant with the highest rate of fertilizer N (d) was also shown to be the one with the greatest dry matter mass and the highest N content per plant. No nodulation was observed in the control variants without inoculation, whereas the largest number of nodules was present in the variant where there was no N and the seeds had been inoculated (a). In the inoculated treatments, the greatest mass

and N content in the plant as a whole was detected in the fertilization variant with 110 kg Nha^{-1} (c) and in the control variant with no fertilization (a) (Table 1).

Tab. 1. Effect of *Bradyrhizobium japonicum* inoculation and fertilizers additions on soybean dry matter mass, nitrogen content and number of nodules in 1991 year

Fertilization variant	Dry matter mass (g/plant)				Nitrogen content (mg/plant)				Number of nodule per plant	
	whole plant		root nodule		whole plant		root nodule			
	+N	-N	+N	-N	+N	-N	+N	-N	+N	-N
a	6.151	3.815	0.591	0	193.66	107.91	28.15	0	28.6	0
b	3.799	2.504	0.339	0	144.16	56.13	15.74	0	14.0	0
c	6.804	3.813	0.501	0	240.92	101.24	22.97	0	18.8	0
d	5.168	5.615	0.138	0	159.23	155.27	6.13	0	11.6	0

In the second and third year of the study (1992 and 1993), however, there occured a change in the effect of long-term mineral fertilizer application as well as in the efficiency of the nodule bacteria in the inoculated treatments.

In 1992, nodules were formed in uninoculated treatments in all the fertilization variants. The dry matter mass and N content were the greatest in the variant with 110 kg Nha^{-1}. The seed inoculation produced the greatest dry matter mass and N content in the variant with the highest N rate (d) and in the control variant with no fertilization and seed inoculation (a) (Table 2).

Tab. 2. Effect of *Bradyrhizobium japonicum* inoculation and fertilizers additions on soybean dry matter mass, nitrogen content and number of nodules in 1992 year

Fertilization variant	Dry matter mass (g/plant)				Nitrogen content (mg/plant)				Number of nodule per plant	
	whole plant		root nodule		whole plant		root nodule			
	+N	-N	+N	-N	+N	-N	+N	-N	+N	-N
a	12.290	8.447	0.210	0.088	452.54	256.09	8.45	3.55	40.4	36.4
b	8.145	12.753	0.190	0.242	372.71	526.97	5.93	4.16	34.0	33.6
c	8.380	10.017	0.146	0.251	375.06	350.27	9.81	9.92	34.0	36.4
d	13.287	12.261	0.242	0.153	459.61	469.43	8.48	5.92	56.2	32.8

Next year (1993), well-developed nodules were also formed in uninoculated treatments in all the fertilization variants, only this time, on average, the number of nodules in the inoculated treatments turned out to be somewhat larger. The greatest dry matter mass and N content in the plant as a whole were achieved in the control variant with no fertilization and seed inoculation (a) and in the variant with 70 kg Nha^{-1} (b) (Tab. 3). The pace of nodulation in the variants with larg-

er N rates was slower, which confirms the results of Rajii et al. (1994) and Mili et al. (1994).

Tab. 3. Effect of *Bradyrhizobium japonicum* inoculation and fertilizers additions on soybean dry matter mass, nitrogen content and number of nodules in 1993 year

Fertilization variant	Dry matter mass (g/plant)				Nitrogen content (mg/plant)				Number of nodule per plant	
	whole plant		root nodule		whole plant		root nodule			
	+N	-N	+N	-N	+N	-N	+N	1N	+N	-N
a	9.056	9.716	0.166	0.209	289.272	284.177	11.496	14.512	42.4	37.4
b	9.034	9.198	0.188	0.141	271.599	262.483	14.494	10.485	34.6	33.8
c	7.476	8.576	0.143	0.217	232.689	277.672	11.241	16.856	33.8	29.0
d	7.719	7.499	0.237	0.105	262.210	242.966	18.189	7.669	36.2	30.0

a - without fertilizers
b - N_{70} P_{60} K_{40}
c - N_{110} P_{90} K_{60}
d - N_{150} P_{120} K_{80}
+N - inoculated plant
-N - uninoculated plant

In 1991, seed inoculation increased the seed yield: there were significant differences in yield height between the uninoculated and inoculated variants, which is in congruence with the results of Sari and Sari (1959), Rajii (1993) and Kucey et al. (1989). In 1992 and 1993, by contrast, the differences were insignificant (Table 4). This can be accounted for by the translocation of the nodule-forming bacteria from one plot to another, which was due to the three-year tillage by disking at 10-12 cm and the seedbed preparation by means of a tiller with the pulverizer harrow and crop tending equipment. In consequence, plants in the uninoculated control treatments had well-developed nodules.

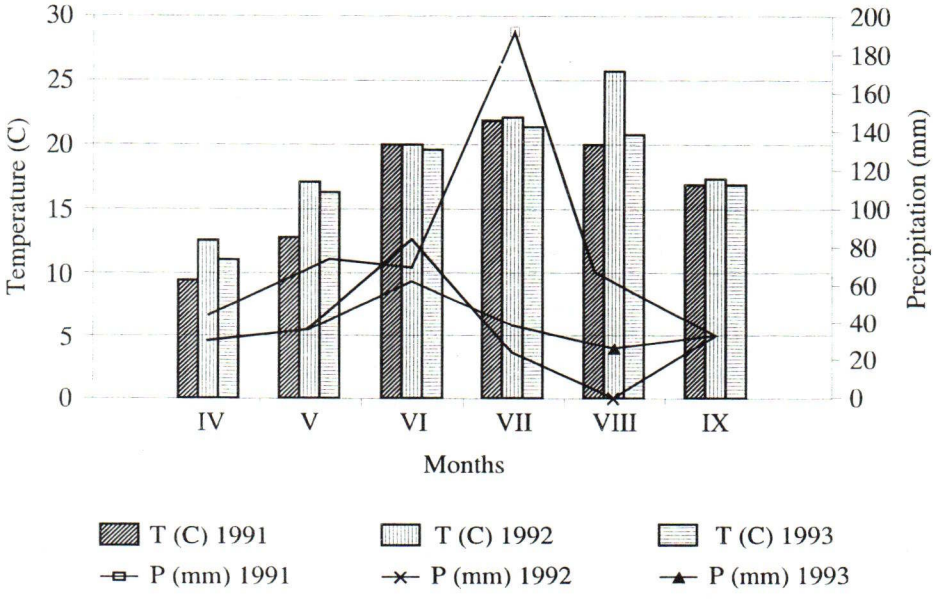
Tab. 4. Effect of *Bradyrhizobium japonicum* inoculation on seed yields

Year	Seed yield $kgha^{-1}$		
	+N	-N	Average
1991	3890	3177	3534
1992	2478	2342	2410
1993	2448	2498	2473
Average	2939	2672	2805

+N - inoculated plant
-N - uninoculated plant

Graph 1 shows mean temperature and precipitation values for the 1991-1993 period. It was in April, May and August of 1992 and 1993 that greater differences in temperature in relation to 1991 were observed. The amount of precipitation in July and August was much lower in 1992 than in 1993. Furthermore, July and August precipitation was much lower in 1992 and 1993 than in 1991. Dry as they were, 1992 and 1993 were unfavorable for soybean, a result of which was a much lower seed yield.

Graph. 1 Temperature and precipitation between 1991 and 1993



The effect of long-term fertilizer application and seed inoculation on the investigated symbiotic association parameters was the greatest in the first year of the study. In the second year, the effect was lesser, only to get completely lost in the third.

Conclusion

The three-year study has led us to the following conclusions:

- The greatest dry matter mass and the highest N content and seed yield were achieved in the inoculated treatments in the first year of the study.
- The effect of inoculation was on the decline in the second year of the study, only to get completely lost in the third.
- In 1993, the pace of nodulation in the variants with larger N-P-K rates was

slowed down. The greatest dry matter mass and N content per plant were achieved in the inoculated treatment with 70 kg Nha^{-1}.

- 1992 and 1993 were dry and unfavorable for soybean, so the yields were much lower than in 1991.

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UTICAJ PRODUŽNOG DEJSTVA MINERALNIH ĐUBRIVA NA EFEKAT INOKULACIJE SA *BRADYRHIZOBIUM JAPONICUM*

- originalni naučni rad -

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Rezime

Za rast i veći prinos zrna svih ratarskih kultura potrebno je da se obezbedi dovoljna količina azota. Soja je biljka koja za razliku od svih ratarskih kultura oko 80% svojih potreba za azotom obezbeđuje simbiotskom azotofiksacijom. Dodavanje azotnih djubriva u manjim količinama pre setve soje ne smanjuje nivo azotofiksacije. Medjutim, dodavanjem veće količine azotnih đubriva redukuje se nivo azotofiksacije (Smith and Hume, 1985; La Favre and Eaglesham, 1987; Senaratne et al., 1987).

Cilj rada je bio da se ispita uticaj dugogodišnje (25 godina) primene različitih doza NPK djubriva i uticaj inokulacije semena soje na efikasnost simbiotske zajednice i prinos zrna.

U toku 1991-1993 godine postavljen je ogled sa sortom soje Kolubara na Rimskim Šančevima, na oglednim poljima Instituta za ratarstvo i povrtarsvo u Novom Sadu. Ogled je postavljen na parceli na kojoj je prethodnih 25 godina (1965-1990) bio stacioniran ogled sa razliitim dozama mineralnih djubriva ((N_{70} P_{60} K_{40}; N_{110} P_{90} K_{60} i N_{150} P_{120} K_{80}).

Na svakoj varijanti djubrenja bilo je dva tretmana sa nitraginom i bez nitragina. Neposredno pred setvu seme je inokulisano komercijalnim mikrobiološkim preparatom -nitraginom, koji sadri visoko efektivne sojeve *B. japonicum.* Predusev u prvoj godini je bila ozima pšenica, a u drugoj i trećoj godini soja.

Setva je bila u prvoj dekadi aprila 1991, 1992 i 1993 godine. U fazi cvetanja soje, kada su biljke bile stare oko 40 dana, uzeto je po 5 biljaka sa svake varijante i odredjena je masa suve materije, sadržaj azota po biljci, broj kvrica i prinos zrna.

Najveći pozitivan efekat produžnog dejstva djubriva i inokulacije semena ispoljen je u prvoj godini istraživanja. U drugoj godini se efekat smanjuje, da bi se već u trećoj godini ovaj efekat izgubio.

Rezultati ogleda u 1991 godini pokazuju da je na svim varijantama djubrenja inokulacijom poveana masa suve materije i sadraj azota u biljci.

Na kontrolnim varijantama bez inokulacije nije došlo ni do formiranja kvrica, a najvei broj kvrica formiran je na varijanti bez azota i sa inokulacijom semena (a). Najveća masa i sadržaj azota u biljci ostvarena je na varijanti sa inokulacijom i djubrenjem sa 110 kg Nha^{-1} (c) kao i na kontrolnoj varijanti bez djubrenja (a).

U 1992 i 1993 godini kvrice su formirane i na neinokulisanim (kontrolnim) varijantama. Verovatno je došlo do prenošenja kvrinih bakterija sa inokulisanih parcela na neinokulisane orudjem za osnovnu i predsetvenu obradu kao i orudjem za negu useva.

U 1992 godini najveća masa suve materije i sadržaj azota u biljci dobijen je na varijanti sa najveom dozom azota i inokulacijom (d) kao i na varijanti gde je bila primenjena samo inokulacija (a) (tab. 2).

Sledeće (1993) godine najveća masa suve materije i sadržaj azota u biljci dobijena je na kontrolnoj varijanti samo sa inokulacijom semena (a) i na varijanti sa 70 kg Nha^{-1} (b) (tab. 3.).

Godine 1992 i 1993 su bile sušne godine, nepovoljne za soju i postignut je mnogo manji prinos nego 1991 godine.