J. Agr. Sci. Tech. (2014) Vol. 16: 517-526

Effect of Cobalt Application on Seed Production in Red Clover (*Trifolium pratense* L.)

D. Tomić[1]*, V. Stevović [1], D. Đurović [1], and R. Stanisavljević[2]

ABSTRACT

A field experiment involving red clover varieties K-39, K-17, Una and Viola was established on an acidic (pH_{H2O} 4.8) soil to evaluate the effects of foliar application of cobalt (a beneficial nutrient for efficient nitrogen fixation) on seed yield, and on seed yield components. The foliar spray of the crop was carried out using cobalt nitrate [$Co(NO_3)_2$] with the treatments: one application at the intensive growth phase during the first growth, while two others during the second growth within the second year of cultivation. Seed yield and yield components were recorded from the second growth within the second year of the study. Regardless of foliar cobalt application, the varieties produced a significantly higher seed yield in 2011, when the rainfall received from the onset of flowering until seed maturation was recorded as lower than that in 2010. The foliar treatment with cobalt was in general accompanied by a positive effect on seed yield and seed yield components in all the varieties. As compared with control, a significant increase in seed yield, in the cobalt applied treatment, was obtained only in Viola, mostly due to the significant increase in flower number i.e. seed number per inflorescence. The more favorable response of Viola to foliar cobalt application may have been attributed to a greater percentage of foliage cover during the treatment, as compared with the other varieties. This suggests that foliar cobalt treatment in future studies should be performed at the early stages of development i.e. during intensive growth throughout the first and second cuts, in order to stimulate nodulation and have greater nitrogen fixation in a needed timely manner.

Keywords: Cobalt, Foliar spray, Moisture regime, Red clover, Seed yield, Yield components.

INTRODUCTION

In Southeast Europe, red clover (*Trifolium pratense* L.) seed crop is commonly established on acidic soils where certain macro- and micro-nutrients are less available to plants (Dear and Lipsett, 1987). Plant growth and metabolism, particularly on acidic soils, is greatly dependent upon cobalt (Co) concentrations in the soil rhizosphere (Palit *et al.*, 1994); and Co availability (Taylor and Quesenberry, 1996). Cobalt is considered to be a beneficial element to higher plants (Vyrodova, 1981). In legumes, cobalt is essential for the microorganisms' fixing of atmospheric nitrogen (Young, 1983). A sufficient supply of Co is important in a number of physiological responses in the crop during the photosynthetic process (Lipskaya, 1972), respiration (Palit *et al.*, 1994; Aleshin *et al.*, 1987), and cell growth as well (Lipskaya, 1970), thereby positively affecting plant organs' growth (Ahmed and Evans, 1960; Mathur *et al.*, 2006; Jayakumar *et al.*, 2007; Jayakumar and Jallel, 2009). Also, a good Co supply induces an increase in chlorophyll

[1] Department of Field and Vegetable Crops, Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, +38132000 Čačak, Serbia.
* Corresponding autor; e-mail: dalibor@tfc.kg.ac.rs
[2] Laboratory for Seed, Institute for Plant Protection and Environment, Teodora Drajzera 9, Belgrade, Serbia.

content (Lipskaya, 1972; Palit *et al.*, 1994), leads to a more developed paliseed tissue, and as well an increase in chloroplast number and size (Lipskaya, 1972).

Symbiotic nitrogen fixation in legumes is dependent on both the content and availability of essential micro-nutrients in the soil. Cobalt supply enhances nitrogen fixation in all *Rhizobim* species and hence, promotes legume growth (Collins and Kinsela, 2011). Cobalt is a component of vitamin B12 which is a component of enzymes and co-enzymes involved in nitrogen fixation in legume nodules (Bond and Hewitt, 1962; Kasimova *et al.*, 1971; Palit *et al.*, 1994; Mathur *et al.*, 2006).

Dilworth *et al.* (1979) reported a direct dependence of bacteroid number and leghemoglobin level in blue lupine *(Lupinus angustifolius* L.*)* nodules on cobalamin content, and a dependence of nitrogen fixation activity on both Co status and cobalamin content. The authors also observed a significant reduction in lupine crown nodulation under cobalt deficiency, most likely due to decreased nodule initiation, with the normal nitrogenase activity in nodules being greatly reduced at sub-critical levels of Co.

The total Co level in the soil includes Co bound in insoluble forms with minerals or locked within stable crystalline structures, and is unavailable to plants (Collins and Kinsela, 2011). In their analysis of an extensive literature on a wide range of soil types and Co levels, the authors found no definite relationships between cobalt uptake and soil pH. All previous studies indicate that a range of soil properties may affect Co uptake, considering also the chemical interactions with the solid and solution soil phase. The soil fertilization with cobalt is dependent upon the rate and physico-chemical form of fertilizer (Sherrel, 1990), and as well on the interactions of Co with the soil (Li *et al.*, 2004; Rosbrook *et al.*, 1992; Sherrel *et al.*, 1990). A substantial amount of the cobalt added to the soil is mostly adsorbed on magnesium-containing minerals in the soil (Mc Kenzie, 1967). Given the low cobalt level of mobility in the plant (Austenfeld, 1979) and its faster movement from aboveground parts to the root, rather than in the opposite direction (Danilova *et al.*, 1970; Palit *et al.*, 1994), foliar treatment is assumed to lead to a more efficient and optimal status of cobalt in the root and in the nodules, in comparison with soil application.

The objective followed this study was to evaluate the effects of foliar Co application on seed yield and yield components in red clover varieties, primarily its indirect effect through nodulation and/or nitrogen fixation.

MATERIALS AND METHODS

Soil Conditions

A field experiment was set up at the Veterinary Extension Service in Čačak (43°54'39.06" N, 20°19'10.21" E, 246 m asl) in 2009-2011. The trial was established on an alluvial soil (pH 4.8), which contained 3.18% organic matter, 0% $CaCO_3$, 22.08 mg extractable P 100 g^{-1} soil and 30.0 mg K 100 g^{-1} soil (Gupta, 2000). The preceding crop consisted of natural meadow. In autumn, prior to seeding, 45 kg ha^{-1} N, 45 kg ha^{-1} P_2O_5, and 45 kg ha^{-1} K_2O were incorporated into the soil through primary tillage.

Weather Conditions

Data on mean monthly temperatures and rainfall were recorded throughout the experiment, at a weather station located near the field site. As compared with the long-term average (1992-2002), the mean annual temperature was 1.34, 0.6, and 0.4ºC higher in 2009, 2010 and 2011, respectively. Monthly rainfall (Figure 1) largely varied across the experimental seasons, particularly during the growing season (April-September).

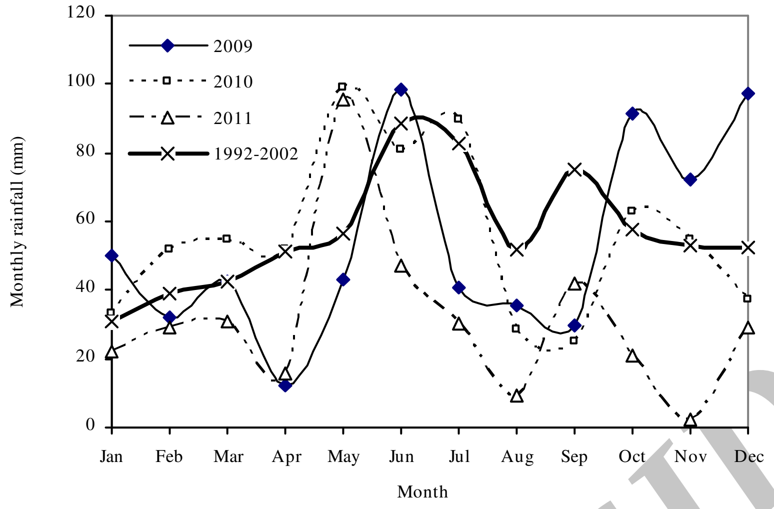

Figure 1. Monthly rainfall distribution for the experimental years *vs.* 10-year (1992-2002) averages.

Experimental Design

The experiment involving four red clover varieties and two treatments with cobalt (Co) (control, foliar Co) was laid out in a randomized block design of four replications and with a plot size of 5 m^2 (5×1 m). Red clover varieties K-17, K-39 (Institute of Forage Crops Krusevac, Serbia), Una (Institute of Field and Vegetable Crops Novi Sad, Serbia) and Viola (a Polish variety) were planted at a row spacing of 20 cm and a seeding rate of 18 kg seed ha^{-1}. Seeding was performed on 3 April 2009 and 27 March 2010. The crop was foliarly treated with Co (as $Co(NO_3)_2$), at a concentration of 0.033 g L^{-1} and a solution rate of 1,000 L ha^{-1}, as follows: one application at the intensive growth phase during the first growth, *vs.* two applications during the second growth in the second year of cultivation (the first being during intensive growth while the second before the onset of flowering). Mechanical weed control was performed twice during the first year of the study. No irrigation was employed.

Data Collection

Seed yield and yield components of the seed were determined starting with the second growth during the second year of cultivation (in 2010 and 2011). The following yield components were field determined: stem number m^{-2} and inflorescence number m^{-2} (by counting within an area of 0.2 m^2 per plot) and inflorescence number per stem (by counting in a sample of ten randomly selected middle-row stems per plot) (Oliva *et al.*, 1994). Laboratory work included a determination of the number of flowers per inflorescence, the number of seeds per inflorescence (in a sample of ten inflorescences per plot), and thousand seed weight (based on the weight of 5×100 seeds extracted from the same sample and pods). Fertility was assessed and expressed as a percentage of the total number of flowers and seeds per inflorescence. The actual seed yield was determined using yield components (number of inflorescences per unit area, number of seeds per inflorescence, thousand seed weight) and converted to seed yield in kg ha^{-1} (Oliva *et al.*, 1994)

The results obtained were subjected to a mixed-design analysis of variance model (year as a random effect, varieties and foliar fertilization as fixed effects) employing SPSS (SPSS, 1995). Differences between means were tested through LSD test.

RESULTS

Regardless of foliar Co application, the varieties yielded a significantly higher number of inflorescences m^{-2}, inflorescences per stem, flowers per inflorescence, seeds per inflorescence, and higher fertility as well as total seed yield in 2011, as compared with year 2010, in which the rainfall level, starting from the second growth until seed maturation was lower (Table 1).

Stem number m^{-2} was significantly higher in K-17 than in K-39 and in Viola in 2011 (year x variety interaction). The value was higher in the treatment with cobalt than that in the untreated control in both years and for all the varieties. The year×variety×foliar Co treatment for stem number m^{-2} (Figure 2) was the result of a positive response of Viola to foliar treatment with Co in 2010, *vs.* the response of K-39 and K-17 to spraying in 2011.

Inflorescence number m^{-2} in 2010 was found to be significantly higher for K-39 than for Una, whereas in 2011 Viola produced a significantly lower number of inflorescences m^{-2} as compared with the other varieties (year×variety interaction). A significant increase in inflorescence number m^{-2} in the foliar treatment with Co was observed only for the K-17 (variety×foliar treatment interaction) in two seasons.

A significantly higher number of inflorescences per stem in 2010 were produced only by K-39, as compared with Una (year×variety interaction). Foliar Co application did not significantly affect the inflorescence number per stem in any of the varieties tested during either of the two years.

Viola yielded a significantly lower number of flowers per inflorescence, as compared with the other varieties, and a significantly lower seed number per inflorescence, as compared with Una, in two years and in all treatments. Also, K-17 and K-39 produced a lower number of seeds per inflorescence, as compared with Una, in both years and both treatments. Foliar Co application induced a significant increase in flower number per inflorescence in Viola in 2010 and seed number per inflorescence in the same variety for both years (variety×foliar treatment and year×foliar treatment interactions).

In 2010, Una exhibited significantly higher fertility than did the other varieties, regardless of foliar treatment, whereas in 2011 no differences were observed among the varieties.

In all varieties, thousand seed weight in the two studied years and seed yield in 2010 were not significantly affected by foliar Co application. The year×variety interaction was the result of a significantly lower seed yield of Viola as compared with the other varieties in 2011. All varieties produced a significantly higher seed yield in 2011 than in 2010, with Viola having lower seed yield during the two studied seasons, as compared with the other varieties.

DISCUSSION

The content and availability of essential macro- and micro-nutrients in the soil play an important role in nodulation (Powrie, 1964; Vrany, 1978; Mathur *et al.*, 2006; Argaw, 2012), and biological nitrogen fixation (Dilworth *et al.*, 1979; Palit *et al.*, 1994; Keneni *et al.*, 2010; Collins and Kinsela, 2011). Powrie (1964) in alfalfa (*Medicago sativa* L.) and Ozanne *et al.* (1963) in subterranean clover *(Trifolium subterraneum* L.*)* observed that a good soil supply of Co significantly increased biomass and the level of nitrogen fixed. Dilworth *et al.* (1979) reported a decline in nodule weight per lupine plant to an extent of 50-80% under cobalt deficiency. Foliar Co application in a study by Vrany (1978) gave a significant increase in dry matter content

Table 1. Seed yield components in red clover varieties as affected by foliar treatment with cobalt (Control: O, Cobalt: Co).[a]

		SNM	INM	INS	FNI	SNI	F	TSW	SY
Year	2010	354.2[b]	614b	1.71b	66.1b	33.0b	49.5b	1.32	251b
	2011	363.0	891a	2.44a	93.3a	52.9a	56.5a	1.61	638a
Variety	K-39	352.8ab	775a	2.21	80.9a	42.1b	50.9b	1.37	454a
	K-17	367.5a	818a	2.19	84.4a	42.4b	49.0b	1.39	498a
	Una	378.4a	787a	2.04	80.6a	49.4a	61.3a	1.36	537a
	Viola	335.6b	630b	1.86	73.0b	37.9b	50.9b	1.75	290b
Foliar t.	O	331.7b	675b	2.02	76.9b	40.3b	51.4	1.62	385b
	Co	385.5a	830a	2.13	82.5a	45.6a	54.6	1.31	504a
2010	K-39	355.6a-d	712b	2.04bcd	66.1	29.5	44.6cd	1.34	255bc
	K-17	340.6cd	631bc	1.80cde	69.8	30.5	43.2d	1.48	265bc
	Una	368.1abc	553c	1.45e	68.7	41.6	60.5a	1.36	291bc
	Viola	352.5bcd	561bc	1.55de	59.9	30.6	49.8bcd	1.11	194c
2011	K-39	350.0bcd	838a	2.39ab	95.8	54.8	57.1ab	1.40	652a
	K-17	394.4a	1006a	2.57a	99.0	54.2	54.9abc	1.31	731a
	Una	388.8ab	1021a	2.64a	92.4	57.2	62.1a	1.35	784a
	Viola	318.8d	698b	2.16abc	86.1	45.3	51.9a-d	2.39	385b
2010	O	330.3	550	1.64	61.1c	30.5	48.8	1.35	220c
	Co	378.1	679	1.77	71.1b	35.6	50.3	1.29	282c
2011	O	333.1	799	2.39	92.8a	50.2	54.1	1.89	549b
	Co	392.8	892	2.49	93.9a	55.6	58.9	1.33	727a
K-39	O	323.8	682bc	2.12	80.3a	40.1bcd	49.0	1.43	398b
	Co	381.9	867ab	2.30	81.6a	44.2abc	52.7	1.31	509ab
K-17	O	340.6	710bc	2.04	83.9a	39.5cd	45.9	1.45	424ab
	Co	394.4	926a	2.34	85.0a	45.2abc	52.2	1.34	572a
Una	O	356.9	767ab	2.14	79.9a	49.6a	61.7	1.32	508ab
	Co	400.0	807ab	1.95	81.2a	49.2ab	60.9	1.39	566a
Viola	O	305.6	539c	1.76	63.7b	32.2d	49.2	2.28	209c
	Co	365.6	720bc	1.95	82.3a	43.7abc	52.5	1.21	370b
Year (A)		ns	**	**	**	**	*	ns	**
Variety (B)		*	*	ns	**	*	**	ns	**
Foliar t (C)		**	**	ns	*	*	ns	ns	**
A×B		*	*	*	ns	ns	*	ns	*
A×C		ns	ns	ns	*	ns	ns	ns	*
B×C		ns	*	ns	*	*	ns	ns	*
A×B×C		*	ns	ns	ns	ns	ns	ns	ns

[a] SNM: Stem Number M^{-2}; INM: Inflorescence Number M^{-2}; INS: Inflorescence Number per Stem; FNI: Flower Number per Inflorescence; SNI: Seed Number per Inflorescence; F (%): Fertility; TSW (g): Thousand Seed Weight, SY (kg ha^{-1}): Seed Yield.

[b] Values followed by different small letters within columns are significantly different (P< 0.05) according to the LSD test.

[*] *F* test significant at $P \leq 0.05$; [**] *F* test significant at $P \leq 0.01$, ns: Non-significant.

of tops, root length and total dry matter in red clover; there being a significant increase in nodulation (22% as compared with the non-treated plants) and the quantity of nitrogen fixed.

Given the low downward mobility of Co in the plant, its rapid movement from aboveground parts to the root rather than in the opposite direction, and the insufficient uptake through the root system (regardless of its soil content), Co in this study was applied foliarly. Cobalt application had in general positive effects on yield component values and on seed yield in all the varieties.

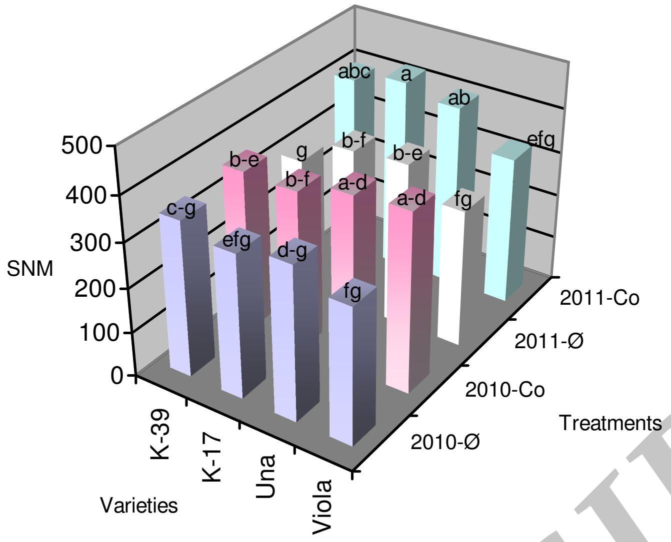

Figure 2. The average number of stems m^{-2} (SNM) of red clover varieties (K-39, K-17, Una, Viola) in different foliar treatments (Ø: Control, Co: Cobalt), in 2010 and 2011. The columns labeled with different small letters are significantly different (P< 0.05) according to the LSD test.

The indirect effect of Co on seed yield and on yield components was likely through increased nodulation and/or increased nitrogen fixation, and while there were differences observed among varieties across years. The differential effects of foliar Co application on certain yield components in the test varieties, notably inflorescence number m^{-2}, can be associated with differences in plant development and percentage foliage cover during the treatment. A significant increase in stem number m^{-2} in Viola in 2011 is partly attributed to plant development at intensive growth stage at the first cut when foliar Co treatment was applied; as compared with the other varieties, Viola had less vigorous stems and a greater percentage of foliage cover.

Inflorescence number per stem was substantially lower than that reported by Valiljević *et al.* (2010) for Una at an inter-row spacing of 60 cm, given the far higher number of plants per unit area throughout the study.

In the present study, the number of flowers per inflorescence in all varieties was lower than the average number in variety Kenland (101 to 142) obtained by Oliva *et al.* (1994), partly due to a higher plant density and less favorable growing conditions. The observance of a significantly lower number of flowers per inflorescence in Viola (compared with the other varieties in the untreated control), along with the results reported by the above mentioned authors, are in agreement with the reports by Julen (1956) and Miladinović (1978) as regards large differences in flower number per inflorescence among red clover varieties.

Weather conditions during the stage of flowering strongly affected fertility, i.e. seed number per inflorescence and hence, seed yield in all varieties, regardless of foliar Co application. This led to a significantly higher seed yield in 2011, when the level of rainfall during the flowering stage was considerably lower than that in 2010. These results are in agreement with those reported by Wilczek and Ćwintal (2008) who found variance in red clover fertility from 51.2 to 69.8% across years; and suggested that high rainfall amounts during flowering can significantly reduce fertility and the harvest index, as regards relation to the potential seed yield. Oliva *et al.* (1994) also reported higher values for fertility in red clover varieties (76-99%). In a study by Wilczek and Ćwintal (2008), seed number per inflorescence in Parada ranged from 61 to 74. A significantly higher number of seeds per inflorescence were reported by Đukić *et al.* (2010) in Una (105.9), at an interrow spacing of 60 cm. In this study, seed number per inflorescence was lower in all varieties, regardless of the positive response to foliar Co application, mostly due to growing conditions and plant density. Jevtić *et al.* (2007) and Wilczek and Ćwintal (2008) indicated a positive effect of insect pollinators on seed number per inflorescence, also directly correlated with weather conditions at the flowering stage.

Although foliar Co application generally had a positive effect on seed yield in all varieties, a significant increase in seed yield was obtained only in Viola in 2011, mostly resulting from an increase in the number of stems m^{-2}, flowers per inflorescence and seed number per inflorescence. Reddy and Raj (1975) obtained better nodulation and increased seed yields in soybean following foliar Co application. Cowpea *(Vigna sinensis* L.*)* seed treatment with cobalt nitrate led to a significant increase in the number of nodules, number of effective nodules per plant, nodule weight and hence, yield component values and total seed yield (Mathur *et al.*, 2006). Cobalt applied as a fertilizer increased the yield in peanut *(Arachis hypogaea* L.*)* (Joshi *et al.*, 1987) and peas *(Pisum sativum* L.*)* (Danilova *et al.*, 1969), mostly due to an increase in nodule number per plant. Jayakumar and Jallel (2009) reported a positive effect of Co applied as a fertilizer up to a certain rate (50 mg kg^{-1} soil) on soybean *(Glycine hispida* Max.*)* growth and nutrient adsorption. Pattanayak *et al.* (2000) also reported a significant positive effect of Co applied at a rate of less than 50 mg kg^{-1} on the number of nodules per plant, number of effective nodules per plant, weight of effective nodules per plant, dry matter accumulation, pod number per plant and seed yield per hectare in cowpea.

CONCLUSIONS

Regardless of foliar Co application, the varieties provided a significantly higher seed yield in 2011, when rainfall distribution in the first part of the growing season was favorable, and the rainfall received from the onset of flowering until seed maturation was lower as compared with that in 2010. The increased seed yield is directly related to the significantly higher number of inflorescences per stem, inflorescences m^{-2}, flowers per inflorescence, fertility and seed number per inflorescence.

The foliar addition of cobalt had in general a positive effect on yield components and seed yield in all varieties. As compared with control, a significant increase in seed yield in cobalt treatment was obtained only in Viola, mostly due to the significant increase in flower number per inflorescence, i.e. seed number per inflorescence. The more favorable response of Viola to foliar cobalt application may be associated with its less intensive growth and a greater percentage foliage cover during the treatment, as compared with the other varieties. This suggests that foliar application of cobalt in future studies should be performed during early stages of development i.e. during intensive growth in the first and second cuts, to stimulate nodulation and nitrogen fixation in a timely manner.

ACKNOWLEDGEMENTS

This work is part of the research project Ref. No. TR-31016, funded by the Ministry of Science and Technological Development, Republic of Serbia.

REFERENCES

1. Ahmed, S. and Evans, H. 1960. The Essentiality of Cobalt for Soybean Plants Grown under Symbiotic Conditions. In: "Proceedings of the National Academy of

Sciences of the United States of America", (Ed.): Saunders, L. M., November 17, 1960. USA. **47**: 24-36.

2. Argaw, A. 2012. Evaluation of Co-inoculation of *Bradyrhizobium japonicum* and Phosphate Solubilizing *Pseudomonas spp.* Effect on Soybean (*Glycine max* L. (Merr.)) in Assossa Area. *J. Agr. Sci. Tech.,* **14**: 213-224.
3. Aleshin, P., Sheudzhen, K., Doseeva, A. and Rymar, T. 1987. Photosynthetic and Respiratory Activity in Rice Leaves as a Function of Cobalt Supply to the Plants. Dok l. Uses Ordena Lenina Ordena Trud Krasnago Znameni Akad. *S-KH Nauk Lenina,* **0(1):** 15-17.
4. Austenfeld, A. 1979. Effects of Nickel, Cobalt and Chromium on Net Photosynthesis of Primary and Secondary Leaves of *Phaseolus vulgaris* Cultivar *saxa. Photosynthetica,* **13**: 434-438.
5. Bond, G. and Hewitt, J. 1962. Cobalt and the Fixation of Nitrogen by Root Nodules of *Alnus* and *Casuarina*. *Nature,* **195**: 94-95.
6. Collins, R. and Kinsela, A. 2011. Pedogenic Factors and Measurements of the Plant Uptake of Cobalt. *Plant soil* **339**: 499-512.
7. Danilova, A., Tischenko, V. and Demkina, N. 1970. Distribution and Movement of Cobalt in Leguminous Plants. *Agrochimiya,* **2**: 100.
8. Danilova, A., Tishchenko, V. and Demikina E. 1969. Some Characteristic Effects of Cobalt on Peas. *Agrokhimiya,* **1**: 85-89.
9. Dear, B. and Lipsett, J. 1987. The Effect of Boron Supply on the Growth and Seed Production of Subterranean Clover (*Trifolium subterraneum* L.). *Aust. J. .Agr. Res.*, **38**: 537-546.
10. Dilworth, M., Robson, A. and Chatel, D. 1979. Cobalt and Nitrogen Fixation in *Lupinus angustifolius* L. II. Nodule Formation and Function. *New Phytol.,* **83**: 63-79.
11. Đukić, D., Vasiljević, S., Stevović, V., Đurović, D. and Balaban, S. 2010. Uticaj Međurednog Rastojanja na Komponente Prinosa Semena Crvene Deteline *(Trifolium pratense* L.*)*. In: "*XV Savetovanje o biotehnologiji*", (Ed.): Spasojević, M.. Zbornik Radova, Serbia, **15(16):** 111-116.
12. Gupta, P. K. 2000. Soil, Plant, Water and Fertilizer Analysis. *Agrobios.* New Delhi, India, PP. 438.
13. Jayakumar, K. and Jallel, C. 2009. Uptake and Accumulation of Cobalt in Plants: A Study Based on Exogenous Cobalt in Soybean. *Bot. Res. Internat.,* **2(4):** 310-314.
14. Jayakumar, K., Jallel, C. and Vijayarengan, P. 2007. Changes in Growth, Biochemical Constituents and Antioxidant Potentials in Radish *(Raphanus sativus* L.*)* under Cobalt Stress. *Turk. J. Bot.***, 31**: 127-136.
15. Jevtić, G., Radović, J., Lugić, Z., Sokolović, D. and Vasić, T. 2007. Uticaj Medonosne Pčele *(Aphis mellifera* L.*)* i Sećernog Sirupa na Prinos Semena Lucerke i Crvene Deteline. In: "*IX Simpozijum o krmnom bilju Republike Srbije*", (Ed.): Kobiljski, B.. Zbornik Radova, Novi Sad, **44(1):** 99-106.
16. Joshi, K., Bhatia, N. and Kulkarni, H. 1987. Groundnut Root Nodulation as Affected by Micronutrient Application and *Rhizobium* inoculation. *Int. J. Trop. Agric.*, **5**: 199-202.
17. Julen, G. 1956. Practical Aspects of Tetraploid Clover. *Proceedings of the 7th International Grasslands Congress*, Massey Agricultural College, 6-15 November 1956, Palmerston North, New Zeland, PP. 471-478.
18. Kasimova, K., Zamanov, B., Abushev, A. and Safarov, G. 1971. The Effect of Certain Trace Elements Molybdenum, Boron, Manganese and Cobalt on the Background of Mineral Fertilizers on the Biological Activity of Tobacco Rhizosphere. *Ref. Zhurn. Biol.*, **3**: 7-9.
19. Keneni, A., Assefa, F. and Prabu, C. P. 2010. Characterization of Acid and Salt Tolerant Rhizobial Strains Isolated from Faba Bean Fields of Wollo, Northern Ethiopia. *J. Agr. Sci. Tech.*, **12**: 365-376.
20. Li, Z., McLaren, G. and Metherell, K. 2004. The Availability of Native and Applied Soil Cobalt to Ryegrass in Relation to Soil Cobalt and Manganese Status and Other Soil Properties. NZ. *J. Agric. Res.*, **47**(1): 33–43.
21. Lipskaya, A. 1970. Anatomo-cytological Features of Cucumber Leaves in the Presence of Cobalt and Manganese in the Nutrient Mixture. *Fiziol. Rast.*, **17**: 475-981.
22. Lipskaya, A. 1972. Accumulation of Chlorophyll in Chloroplasts of Cucumber Leaves under the Effect of Cobalt and Manganese Applied Separately and Together. *Biol. Nauki*, **15**: 90-94.
23. Mathur, N., Singh, J., Bohra, S., Bohra, A. and Vyas, A. 2006. Effect of Soil Compaction Potassium and Cobalt on

Growth and Yield of Moth Bean. *International J. Soil Sci.,* **1(3):** 269-271.
24. Mc Kenzie, M. 1967. The Sorption of Cobalt by Manganese Minerals in Soils. *Aust. J. Soil Res.*, **5**: 235.
25. Miladinović, M. 1978. Uticaj Načina Setve na Prinos Semena i Vegetativne Mase Crvene Deteline. *Savremena poljoprivreda,* **7(8):** 69-74.
26. Oliva, R., Steiner, J. and Young, W. 1994. Red Clover Seed Production. II. Plant Water Status on Yield and Yield Components. *Crop Sci.*, **34**: 184-192.
27. Ozanne, G., Greenwood, N. and Shaw, C. 1963. The Cobalt Requirement of Subterranean Clover in the Field. *Aust. J. Agr. Res.*, **14**: 39-50.
28. Palit, S., Sharma, A. and Talukder, G. 1994. Effects of Cobalt on Plants. *Bot. Rev.,* **60(2):** 149-173.
29. Pattanayak, K., Dash, D., Jena, M. and Nayak, K. 2000. Seed Treatment of Green Gram with Molybdenum and Cobalt: Effect on Nodulation, Biomass Production and N Uptake in an Arid Soil. *J. Ind. Soc. Soil Sci.*, **48**: 769-773.
30. Powrie, K. 1964. The Effect of Cobalt on the Growth of Young Lucerne on a Siliceous Sand. *Plant Soil*, **21**: 81-93.
31. Reddy, T. and Ray, S. 1975. Cobalt Nutrition of Groundnut in Relation to Growth and Yield. *Plant Soil*, **42**: 145-152.
32. Rosbrook, A., Asher, J. and Bell, C. 1992. The Cobalt Status of Queensland Soils in Relation to Pasture Growth and Cobalt Accumulation. *Trop. Grassl.*, **26**(2): 130–136.
33. Sherrell, G. 1990. Effect of Cobalt Application on the Cobalt Status of Pastures. 3. Comparison of Chelate and Sulfate as Cobalt Sources for Topdressing Deficient Pastures. *NZ. J. Agric. Res.*, **33(2):** 313–317.
34. Sherrell, G., Percival, S. and Gee, M. 1990. Effect of Cobalt Application on the Cobalt Status of Pastures. 1. Pastures with History of Regular Cobalt Application. *NZ. J. Agric. Res.*, **33(2):** 295–304.
35. SPSS. Inc. 1995. STATISTICA for Windows (Computer Program Manual). Tulsa. OK.
36. Taylor, L. and Queseberry, H. 1996. Red Clover Science. *Curent Plant Sci. Biol. Agriculture,* **28**: 44-57.
37. Vasiljević, S., Karagić, Đ., Mihajlović, V., Pataki, I., Milošević, B. and Pejić, B. 2010. Effect of Sowing Method and Seeding Rate on Yield Components and Seed Yields in Red Clover *(Trifolium pratense* L.*). Biotech. Anim. Husbandry,* **26**: 161-165.
38. Vrany, J. 1978. Microbial Changes in Clover Rhizosphere after Foliar and Soil Application of Cobalt. *Folia Microbiol.*, **23**: 236-242.
39. Vyrodova, L. P. 1981. Influence of Soil Properties on the Sorption of Cobalt. *Pochvovedenic Moskova "Nauka",* **7**: 64-73.
40. Wilczek, M. and Ćwintal, M. 2008. Effect of the Methods of Additional Feeding with Microelements (B, Mo) on the Yield Structure and Seed Yield of Red Clover. *Electronic Journal of Polish Agricultural Universities.* Can Be Seen at: http://www.ejpau.media.pl/volume11/issue4/abs-05.html.
41. Young, S. R. 1983. Recent Advances on Cobalt in Human Nutrition. *Micronutrients New,* **3**: 3.

تأثیر محلول‌پاشی کبالت بر روی میزان تولید بذر در شبدر قرمز (*Trifolium Pratense* L.)

د. تومیچ، و. سیتو ویچ، د. دوروویچ ر. ستانیساولویچ

چکیده

آزمایشی، مزرعه‌ای، بر روی واریته‌های K-۳۹ و K-۱۷ و Una و Viola شبدر قرمز در خاکی اسیدی (pH_{H2O} 4.8) صورت گرفت تا تأثیر محلول‌پاشی شبدر با عنصر کبالت (عنصری که در تثبیت مؤثر نیتروژن نقش دارد.) را بر روی مقدار تولید بذر (Seed yield) و همچنین بر اجزاء مداخله گر در تولید بذر (Seed yield components) برآورد گردد. محلول پاشی شبدر با استفاده از نیترات کبالت [$Co(NO_3)_2$] با به کارگیری رفتارهای: یک مرتبه در مرحله رشد فراوان گیاه در چین اول و دو مرتبه در چین دوم دومین سال پس از کاشت محصول انجام شد. میزان محصول بذر و عوامل تأثیرگذار بر میزان تولید بذر در چین دوم دومین سال کاشت محصول رکورد برداری شدند. بدون در نظر گرفتن محلول پاشی (کبالت) محصول، واریته‌ها به طور معنی‌دار بذر بیشتری را در سال۲۰۱۱ (که در آن بارندگی کمتری نسبت به سال ۲۰۱۰ صورت گرفته بود) با مقایسه با ۲۰۱۰ تولید نمودند. محلول پاشی با محلول کبالت بطور کلی تأثیر مثبتی در میزان تولید بذر و عوامل مؤثر در میزان تولید بذر در تمامی واریته‌ها داشت. با مقایسه با کنترل، افزایش معنی‌دار میزان محصول در واریته Viola نسبت به سایر واریته‌ها بیشتر به دلیل افزایش تعداد گل و یا همانا تعداد بذر در این واریته بود. عکس‌العمل مطلوب‌تر واریته‌ی Viola به محلول‌پاشی را می‌توان به درصد بیشتر سطح برگ گیاه بهنگام محلول‌پاشی (نسبت به سایر واریته‌ها) ربط داد. این نکته محققین را برآن وا می‌دارد که در مطالعات آینده (در ارتباط با محلول‌پاشی عنصر کبالت) عملیات پاشش محلول را در مراحل اولیه‌ی رشد گیاه (در چین‌های اول و دوم) مد نظر قرار دهند تا اینکه تشکیل نودول و تثبیت نیتروژن بیشتر در موعدی مناسبتر در گیاه صورت گیرد.