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The effect of foliar or soil top-dressing of urea on some physiological processes and seed yield of faba bean

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Abstract. This research was conducted to determine the influence of foliar or soil applied urea fertilizer on symbiotic N_2 fixation, intensity of the net photosynthesis, nitrogen accumulation and seed yield of the faba bean cultivar Nadwiślański. A pot experiment was conducted over two years, in an experimental greenhouse at IUNG-Puławy. Feeding faba beans with urea caused a partial restriction of symbiotic nitrogen fixation, reduced root nodule mass and nitrogenase activity. This inhibitory effect had a temporary character and was lower when urea was supplied to the leaves than to the soil. Feeding faba bean with urea increased the intensity of the net photosynthesis in leaves, which in turn gave greater plant nitrogen accumulation and increased faba bean seed yield (an average increase of 14–15% for foliar feeding and 2–4% for soil top dressing in comparison with no top dressing). Foliar feeding with urea was found to be more beneficial than soil top-dressing, irrespective of the basic dose of N.

key words: faba bean, top-dressing of urea, symbiotic N_2 fixation, photosynthesis, yield

INTRODUCTION

The faba bean (*Vicia faba* L. *ssp. minor* Harz) is a plant which can survive in soils which are poor, medium or rich in nitrogen (N) thanks its ability to symbiotically fix N in association with bacteria of the family *Rhizobiaceae*. High soil N concentrations for faba bean and other legumes restrict symbiotic N_2 fixation (Eaglesham, 1989; Gan et al., 2003; Hardarson, Atkins, 2003; Kage, 1995; Salvagiotti et al., 2008; Wojcieska et al., 1994). The fertilization of legumes with nitrogen is still being investigated (Prusiński, Kotecki, 2006; Zeidan, 2003). Kocoń et al., (1995) and Wojcieska, Kocoń (1997) reported that symbiotic N_2 fixation in the reproductive phase of faba bean development is reduced naturally as a result of decreased nitrogenase activity. This seems to be a result of inadequate N levels to cover the need of the faba bean during pod and seed development. Nitrogen is an essential element for the plant to fulfil its genetic potential yield.

Research into finding an effective method of feeding N to faba bean plants during this essential development period should be focused on gaining knowledge of physiological processes. This includes symbiotic N_2 fixation, intensity of growth and development, intensity of gas exchange, accumulation of N and dry matter (DM) including analyses of the faba bean seed yield. Currently, there is limited knowledge on foliar N feeding of faba bean, and particularly on its influence on physiological processes. Research of Wojcieska and Kocoń (1997) and by this author using marked ^{15}N; identified that faba bean uses N from foliar feeding more efficiently than from soil top-dressing (Kocoń, 2003).

The aim of this study was to evaluate the effectiveness of foliar and soil top-dressed urea on faba bean with respect to symbiotic N_2 fixation, intensity of net photosynthesis, N accumulation and seed yield of faba bean.

MATERIAL AND METHODS

Studies with the faba bean (*Vicia faba* L.) cultivar Nadwiślański were conducted as part of a pot trial over two years in an experimental greenhouse in IUNG-Puławy. A completely randomized design was used in a factorial experiment, the first factor was basal N fertilizer, and the second factor was top-dressing with urea.

Mitscherlich pots were filled with 7.8 kg of quartz sand rinsed with demineralised water. Liming was made by mixing sand with 5 g of $CaCO_3$ pot^{-1}, and adding the following mineral nutrients per pot: 1250 mg P (in $NaH_2PO_4 \cdot H_2O$), 1800 mg K (in K_2SO_4), 390 mg Mg (in $MgSO_4 \cdot 7H_2O$), 50 mg Fe ($C_6H_5O_7) \cdot 3H_2O$, 10 mg H_3BO_3,

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Table 1. Urea top dressing scheme.

Basic fertilizer (N mg pot^{-1})	Treatment	Urea top dressing (mg N pot^{-1}) Plant development stage (according to the BBA scale – Ostrowska, Kucińska, 2000)		
		57	62	64
10, 300, 900	Control (C)	0	0	0
	Soil top dressing (S)	150	+150 (300)	+150 (450)
	Foliar application to leaves (L)	150$^{\#}$	+150$^{\#}$ (300)	+150$^{\#}$ (450)

after addition of 25% extra sprayed N

10 mg $MnSO_4 \cdot 4H_2O$, 1 mg $CuSO_4 \cdot 5H_2O$, 1 mg $ZnSO_4 \cdot 7H_2O$, 0.5 mg $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ and 0.5 mg $CoCl_2$. K, P, and Mg were applied in two doses: half before sowing and half at the 4–5 leaf phase. Microelements were applied before sowing.

Basic nitrogen fertilizer application to the faba bean was in 3 doses: 10 (starter dose), 300, and 900 mg N pot^{-1}. Nitrogen was added to the soil before sowing in NH_4NO_3.

During flowering, some plants were used for urea foliar feeding whilst others for soil top-dressing. As a result of this treatment plants received an additionally 450 mg N pot^{-1} (Table 1), as three equal doses of 150 mg – plants were sprayed by small portions of urea at 7.00–8.00 a.m. for five consecutive days. No surfactant was used while spraying. Prior to this the author (Alexander, 1986; Kocoń, 2003; Skiba et al., 1995) had demonstrated that with spraying, about 25% of the N applied is lost due to unmanageable targeting of the spray solution around the plant. Thus in foliar feeding, the dose of N was increased by 25% with respect to the recommended dose applied to the soil. The soil surface in-between foliar fed plants was shielded with absorbent during treatment to stop the N solution from coming in contact with the soil. Control plants were given basic N fertilizer (before sowing) and were sprayed with water (no top dressing with urea).

In mid April, in both years, 10 faba bean seeds were sown into each pot, inoculated with active *Rhizobium leguminosarum viceae* biotype, (inoculum AS from the collection of the Department of Agricultural Microbiology – IUNG). After germination, plants were thinned to 5 healthy plants pot^{-1}. Soil moistness was kept at a level of 60% of field capacity, and was applied as demineralised H_2O. The reaction of so prepared undersoil in pots measured as pH_{KCl} was about 7.

During the whole period of growth the following measurements were taken at plant development stages (in BBA scale Ostrowska and Kucińska, 2000) described in the Tables 2-4:

a) Measurements of nitrogenase activity using the acetylene reduction method (Hardy et al., 1968; 1973) a gas chromatograph PYE - UNICAM 204 was used. This was done by measuring the entire root system with nodules from a single pot (5 plants). Directly after removal from the soil they were rinsed in water at 18–20°C. Plants were then incubated with 10% C_2H_2, at 25°C for 1 hour. The results (average of three parallel estimates) were in µmol of ethylene pot^{-1}. During harvests, observations were made on root nodules to measure nitrogenase activity. Nodules were then separated from roots to determine their dry weight.

b) Measurements of net photosynthesis and leaves transpiration at the individual plant levels of the main stem (every third leaf, starting with the leaf third from the bottom). This was recorded by measuring infrared absorption, using a CO_2 LCA-4 analyser, working in tandem with a leaf PLC camera. The results are given as arithmetic means for the entire foliage from five replicates. Each repetition on average was measured 40 times. Water use efficiency (WUE) was calculated from the relationship between net photosynthesis to transpiration.

c) Nitrogen content was evaluated from mature plants, with two averaged parallel readings using a spectrophotometer. Nitrogen accumulation was estimated based on the percentage N contents in individual plant parts in their DM. The relative methodological error of determination of N fluctuated between 5 to 8%.

Faba bean plants were harvested at full maturity. Plants were divided into component parts and dried to determine DM yield. The results are the arithmetical mean of 5 replicates after two years research.

Results were statistically analysed using analysis of variance. The least significant difference at P = 0.05 was calculated using Tukey's test.

RESULTS AND DISCUSSION

Pre-sowing soil fertilization with N of faba beans from ammonium nitrate always led to a significant reduction in root nodule mass and limited nitrogenase activity (Table 2). This enzyme is responsible for symbiotic N_2 fixation. The restricting influence of N decreases as soil N is used; therefore, with consecutive sampling, the root nodule mass rose and reached a maximum during pod development. The increase in root nodule mass was not in line with the increase in nitrogenase activity. Nitrogenase activity only increased until flowering and then fell during pod development. The problem of decreased nitrogenase activity in faba bean, and a reduction in symbiotic N_2 fixation during pod development of pods, was indicated by Hardarson and

Table 2. The effect of a basal application of ammonium nitrate and top dressing with urea on the dry mass of faba bean nodules and their nitrogenase activity.

Basal fertilizer [N mg pot^{-1}]	Urea top dressing	Dry root nodule mass [g pot^{-1}]			Nitrogenase activity [µmol C_2H_4 pot^{-1} h^{-1}]		
		Plant development stage#					
		62	64	72	62	64	72
10	C	0.77	0.87	1.18	82.4	139.0	89.8
	S	0.59	0.62	0.82	59.5	109.2	80.8
	L	0.63	0.71	1.02	63.5	118.1	92.6
300	C	0.69	0.70	0.97	51.5	85.6	64.6
	S	0.56	0.58	0.88	39.2	79.5	68.8
	L	0.58	0.60	0.92	42.7	86.3	78.8
900	C	0.34	0.61	1.05	16.6	69.3	63.5
	S	0.14	0.34	0.82	9.6	51.6	60.4
	L	0.22	0.48	1.08	11.3	60.9	69.1
Mean	C	0.60	0.72	1.06	50.2	98.0	72.6
	S	0.43	0.51	0.84	36.1	81.1	70.0
	L	0.48	0.59	1.01	39.2	88.4	80.2
LSD (0.05) for:							
basic fertilization (I)		0.108	0.074	ns	5.74	19.18	7.92
top dressing (II)		0.084	0.058	0.087	4.45	14.69	2.97
interaction							
I x II			0.011			8.84	
II x I			0.128			9.96	

C – control (no top dressing); S – top dressing on soil; L – top dressing on leaves

\# development stages (Ostrowska, Kucińska, 2000): 62 – flowering of first flower bunches; 64 – flowering on three flower bunches; 72 – first visible pods

ns – non significant

Table 3. The effect of a basal application of ammonium nitrate and top dressing with urea on net photosynthesis rate, transpiration rate and WUE of leaves of flowering field bean plants (stage 64 – flowers on three flower bunches).

Basic fertilizer [mg N pot^{-1}]	Urea top dressing	Net rate of photosynthesis [µmol CO_2 m^{-2} s^{-1}]	Transpiration rate [mmol H_2O m^{-2} s^{-1}]	WUE [µmol CO_2 per mmol H_2O]
10	C	10.69	5.75	1.86
	S	11.44	7.25	1.58
	L	12.95	7.75	1.67
300	C	10.72	6.34	1.69
	S	10.98	7.28	1.51
	L	12.23	7.51	1.63
900	C	10.03	7.29	1.37
	S	10.33	7.27	1.42
	L	11.27	7.24	1.56
LSD (0.05) for:				
basic fertilization (I)		ns	0.214	
top dressing (II)		1.140	0.317	
interaction				
I x II			0.448	
II x I			0.371	

For explanation see Table 1, 2.

Atkins (2003), Wojcieska and Kocoń (1997) and in different legumes by Gan et al., (2003) and Ruszkowska et al., (1991). The reduction in nitrogenase activity, which reduces fixation N_2, can negatively affect the yield of faba bean seed, because during the period of high plant demand for N (flowering and pod development), a consequence of insufficient N for the developing pods can be a reduced yield.

Top-dressing of faba beans with urea at the start of flowering negatively influenced root nodule mass and nitrogenase activity. Generally, continual feeding of plants with urea increased nodule mass and nitrogenase activity (Table 2), often exceeding the activity of this enzyme in test pots – not fed N. Foliar feeding of faba bean with urea, during this period, was a more favourable way of supplementing N, compared with side-dressing of N, because side-dressing restricted an important process of symbiotic N fixation. There was a more beneficial influence of foliar feeding of faba bean with urea on symbiotic N_2 fixation, as reported earlier (Kocoń et al., 1995), with work on peas Wojcieska et al., (1994), with soybean Gan et al., (2003), lucerne Ruszkowska et al., (1991). It was associated with a lower soil N concentration in the soil environment of the plants.

The influence of pre-sowing fertilization of soil with ammonium nitrate on the intensity of photosynthesis of faba bean leaves was minimal (Table 3). However, transpiration intensity increased with increased N fertilizer. Plants were more inefficient in managing water during photosynthesis. However, feeding faba beans with urea, (particularly their foliage), always increased the intensity of the net photosynthesis in their leaves. Consequently, it often also increased transpiration intensity; thus, it gave similar indications of the photosynthetic effectiveness of water use – similar to the value of WUE, independent of method of urea feeding (Table 3).

Table 4. The effect of basic application of ammonium nitrate and top dressing of urea on nitrogen accumulation and yield of dry mass in ripened plants (5 plants).

Basal fertilizer [mg N pot^{-1}]	Urea top dressing	Nitrogen accumulation [mg N pot^{-1}]			Dry matter yield [g pot^{-1}]		
		seed	other organs	whole plant	seed	other organs	whole plant
10	C	2431	999	3430	59.1	91.6	150.7
	S	2663	1058	3721	60.2	96.3	156.5
	L	2820	987	3807	66.3	88.2	154.5
300	C	2887	968	3855	61.3	92.1	153.4
	S	2937	929	3866	62.4	87.3	149.7
	L	3002	1025	4027	65.6	87.9	153.5
900	C	2379	1037	3416	51.8	88.2	140.0
	S	2561	994	3555	53.3	86.9	140.2
	L	2960	1032	3992	65.0	89.2	154.2
LSD (α=0.05) for							
basic fertilization (I)					3.64	ns	8.95
top dressing (II)					3.47	ns	7.02
interaction:							
I x II					4.17		
II x I					4.62		

For explanation see Table 1, 2.

Nitrogen belongs to the group of nutrients, which given at an appropriate dose generally positively influence the process of the gas exchange in plants with regard to the intensity of net photosynthesis. Jla and Gray (2004) reported on the increase of net photosynthesis intensity under the influence of N feeding in faba bean and Borowski and Michałek (2000) in broad bean. Starck (2002) and Kościelniak et al., (1990), consider an increase in intensity of this process can be related with increased RuBisCo enzyme activity as well as a rise in assimilate export from leaves to roots. Moreover, a high level of assimilates in plant roots beneficially influenced symbiotic N_2 fixation (Ayaz et al., 2004; Muraoka et al., 1991; Starck, 2002).

Pre-sowing soil fertilization with ammonium nitrate, as a basic N fertilizer, in doses up to 300 mg N increased the amount of N in seed and in the entire plant (Table 4). However, high doses of N applied at 900 mg N $dose^{-1}$ to a pot had a detrimental effect on N accumulations and biomass yield, particularly of seed. Post flowering fertilization with urea of plants in the reproductive phase always leads to a higher amount of N in plants. Consequently, it also lead to increases in yield, especially of seed, independent of basic N fertilisation. Therefore, the most beneficial treatment was foliar feeding with urea (Table 4).

Behairy et al., (1988), Kocoń et al., (1995), Muraoka et al., (1991), Podsiadło (2001), Wojcieska and Kocoń (1997) reported an increase in seed yield of faba bean in plants fertilized with small doses of N. Higher doses of N were less effective (Behairy et al., 1988;, Hardarson, Atkins, 2003; Hardarson et al., 1991; Kulig, Ziółek, 1997; Muraoka et al., 1991; Zeidan, 2003). Similar relations have been reported in other legumes (Borowski, Michałek, 2000; Prusiński, Kotecki, 2006; Salvagiotti et al., 2008).

The reason for the reduced seed yield at the highest dose of N (900 mg) was a reduction in nodules and nitrogenase activity, which reduced symbiotic bonding of N_2 during initial plant growth. After the mineral N was used up the amount of available symbiotically fixed N_2 was not sufficient for the plants needs. Only top-dressing of faba bean with urea (during flowering and pod development) had an effect on the nutritional needs of plants that had been supplemented with nitrogen and consequently gave higher seed yields. In pots, when fed with urea, particularly foliage fed plants had a higher intensity of net photosynthesis. This confirms that urea in doses, as applied in this experiment, favourably interacted with the process of gas exchange of plant leaves and did not reduce plant photosynthesis (Borowski, Michałek, 2000; Jla, Gray, 2004). This work and earlier work using ^{15}N (Kocoń, 2003), and Gan et al., (2003) with soybean, confirms that foliage feeding of faba bean with N during flowering and podding supplements N deficiencies and is more effective than side dressed N fertilizer.

CONCLUSIONS

1. Top-dressing faba bean with urea, as well as basic fertilization with ammonium nitrate reduces root nodule mass and nitrogenase activity. A decrease in nitrogenase activity as a result of N fertilizer is a temporary character after which growth recovers.
2. The restricting influence of fertilizing with urea on symbiotic N_2 fixation was less with foliar feeding N than with soil applied N.
3. Feeding faba bean with urea during reproductive development increased net photosynthesis of leaves, accumulation of N in plants and seed yield. Foliar feeding with urea was much more effective than a side-dressing.
4. This research confirmed that there is a benefit in applying small doses of nitrogen pre-sowing. The yield of faba bean seed increased, when a dose of 300 mg N pot^{-1} was applied, and decreased at a dose of 900 mg N pot^{-1}. It was shown that there was a benefit of foliar feeding with N in the reproductive phase of faba bean development.

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