Global J. Environ. Sci. Manage., 1(4): 333-339, Autumn 2015
DOI: 10.7508/gjesm.2015.04.008

Nitrogen use efficiency and life cycle of root nodules in Alfalfa after different mineral fertilization and soil cultivation

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Received 29 May 2015; revised 20 June 2015; accepted 4 August 2015; available online 1 September 2015

ABSTRACT: Nitrogen use efficiency and life cycle of root nodules in alfalfa after different mineral fertilization and soil cultivation practices were studied. Field trial was conducted in the Institute of Forage Crops, Pleven, Bulgaria on leached chernozem subsoil type and no irrigation. The next treatments were tested: i) for fertilization as follows: N0P0K0 (control); N60P100K80 (an accepted technology); N23P100K35 (nitrogen was applied 1/2 in first year of growing and 1/2 in third year); N23P100K35 (nitrogen was supplied pre-sowing); N35P80K50, and Amophose - 250 kg/ha, calculated at fertilizing rates N27P120K0; ii) for soil cultivation as follows: soil loosing 10-12 cm, plough at depth 12-15 cm, 22-24 cm (an accepted technology), 18-22 cm and 30-35 cm. It was found that soil cultivation and mineral fertilization had effect on nitrogen use efficiency and life cycle of root nodules in Alfalfa. Nitrogen use efficiency was found to be highest at N23P100K35 and plough at the depth of 22-24 cm. Life cycle of root nodules was the longest at N35P80K50 and plough at the depth of 18-22 cm. The better root mass to nodule number ratio was found at N23P100K35 and plough at the depth of 22-24 cm.

Keywords: *Alfalfa; Life cycle of root nodules; Mineral fertilization; Nitrogen use efficiency; Soil cultivation.*

INTRODUCTION

Application of synthetic nitrogen fertilizers in agriculture is essential for crop productivity (Tilman *et al.*, 2002, Mueller *et al.*, 2012, Sinclair and Rufty, 2012). They are a part of "green revolution" after which although not enough, but the plant protein have to be found increasing (Lassaletta *et al.*, 2014a). However, at present most of the half of nitrogen used for fertilization is lost to the environment. For most of Europe, the losses are above 50 kg N ha/yr. This conclusion was done after extensive survey on nitrogen use efficiency (applied to the crops as synthetic fertilizers, manure, symbiotic nitrogen fixation and atmospheric deposition). This study was done on the base of FAO data and 124 counties were involved (Power and Alessi, 1971; Tilman *et al.*, 2002; Herridge *et al.*, 2008; Bouwman *et al.*, 2009). Results showed that nitrogen use efficiency was generally higher for agricultural systems with higher proportion of N inputs derived from symbiotic nitrogen fixation. Conversely, nitrogen use efficiency was generally lower for a higher proportion of synthetic fertilizers in total fertilization scheme. The higher nitrogen use efficiency associated to nitrogen fixation is likely explained by a higher efficiency in the incorporation by legumes of their self-supplied nitrogen (Herridge *et al.*, 2008).

Although signiûcant improvement in nitrogen use efficiency has occurred in many countries, an increase of nitrogen fertilization would result in a disproportionately low increase of crop production with further environmental changes (Lassaletta *et al.*, 2014b). Using nitrogen inputs with low efficiency leads to environmental problems such as pollution of groundwater by nitrates, ammonia emissions and

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Note. This manuscript was submitted on May 29, 2015; approved on August 1, 2015; published online September 1, 2015. Discussion period open until December 1, 2015; discussion can be performed online on the Website "Show article" section for this article. This paper is part of the Global Journal of Environmental Science and Management (GJESM).

greenhouse gases. Therefore, the efficiency of resource use is crucial and its improvement is the key challenge for sustainable agriculture. Profitable, but at the same time environmentally and socially sustainable, agro-ecosystems are needed. Proper schemes of fertilization and soil cultivation practices are among the factors that are important for nitrogen and phosphorus use efficiency improvement. An increase of nitrogen fixing potential as a source of nitrogen can also contribute to increasing nitrogen use efficiency at the local and global scale (Herrero *et al.,* 2010, Lassaletta *et al.,* 2014a, Bonaudo *et al.,* 2014, Soussana and Lemaire, 2014).

Phosphorus is the second most vital plant nutrient apart from nitrogen, but for legumes, it presumes primary significance which plays an important role in root proliferation (Tairo and Ndakidemi, 2013). Richardson and Berea (2009) reported that growth, yield and yield components in legumes improved significantly by adequate supply of phosphorus. One of the most important forage legume species is Alfalfa (*Medicago sativa* L.). It belongs to order Family *Fabaceae*, genus *Medicago*. The accepted name is *Medicago sativa* L. It is a valuable protein source for animals with high nutritive value and rich in minerals (Barnes *et al.,* 1995; Kertikova, 2008; Keskin *et al.,* 2009). As a legume Alfalfa is nitrogen fixing crop and the potential for nitrogen fixation through symbiosis is about 450 kg/ha/year (Heichel and Henjum, 1991; Starchenkov and Kot's, 1992).

The issue of the additional introduction of nitrogen to Alfalfa and how the nitrogen use efficiency will be, is debatable in the literature (Oliveira *et al.,* 2004; Werner and Newton, 2005). Many authors support the thesis for application of nitrogen fertilization (Cihacek, 1994; Petkova, 1994; Sharma and Sharma, 1995; Butorac *et al.,* 1988; Raun *et al.,* 1999; Trepachev, 1999; Delgado *et al.,* 2001; Pachev, 2001; Tufenkci *et al.,* 2006; Vasileva *et al.,* 2006; 2011; Vasileva and Ilieva, 2011). The need of nitrogen fertilization was confirmed by Raun *et al*. (1999). They found an additional applying of nitrogen fertilizer (once in the spring) enhanced dry mass productivity in the cuts, harvested later, which is in line with declined then nitrogen fixation capacity. In the same time high doses of mineral nitrogen inhibit the biological fixation process (Streeter, 1985a,b; Kot's *et al.,* 1990, 1996; Streeter, 1993; Kot's, 2001; Vasileva, 2004). The aim of this work was to study the nitrogen use efficiency and life cycle of root nodules after different mineral fertilization and soil cultivation practices in Alfalfa.

This study has been performed in the Institute of Forage Crops, Pleven, Bulgaria in 2003-2006.

MATERIALS AND METHODS

The experiment was carried out in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria (2003-2006) on leached chernozem soil subtype without irrigation. Alfalfa variety Obnova 10 was sown. Long plot method was used and plots size was 10 m^2. The treatments being 4-times replicated were: i) for fertilization as follows: N0P0K0 (control); N60P100K80 (by accepted technology); N23P100K35 (nitrogen was applied 1/2 in first year of growing, 1/2 in third year, and P and K supply); N23P100K35 (nitrogen was supplied pre-sowing, K supply, P by 1/3 in the first, second and third year of growing); N35P80K50, and Amophose - 250 kg/ha, calculated at fertilizing rates N27P120K0; ii) for soil cultivation as follows: soil loosing 10-12 cm, plough at depth 12-15 cm, 22-24 cm (an accepted technology), 18-22 cm and 30-35 cm. Most important agrochemical characteristics of the soil (determined by Page *et al.,* 1982), were as follows: N, 31.5/1000 g soil; P (P_2O_5), 5.19 mg/100 g soil; K (K_2O), 25.4 mg/100 g soil; pH (H_2O), 6.95; humus, 1.77%.

One cut in the first experimental year and two cuts in the next three years were harvested. Nitrogen use efficiency (NUE) (kg/kg) was calculated using formulae of Bowen and Zapata (1991) and Yousefi and Mohammadi (2011), equation no. 1. Phosphorus use efficiency (PUE) (kg/kg) was calculated using formulae of Bowen and Zapata (1991), equation No. 2:

$$NUE = \text{dry mass yield (kg/ha)}/\text{nitrogen applied (kg/ha)} \quad (1)$$

$$PUE = \text{dry mass yield (kg/ha)}/\text{phosphorus applied (kg/ha)} \quad (2)$$

Soils from soil profile were taken (20/30/40 cm). Root mass was washed with tap water and measured (Beck *et al.,* 1993): duration of the life cycle of root nodules – recorded on the basis of internal color (days) (Milev, 2014), roots to number of root nodules ratio was calculated (g roots/number root nodules) - dry root mass, dried at 60^{o}C was divided to number of root nodules. Experimental data were averaged for the period of study and statistically processed using SPSS software program (2012).

RESULTS AND DISCUSSION

Agro meteorological conditions for the period of study were unfavaourable (Table 1). Prolonged drought period occurred in the first year. Early spring drought as well as unevenly distributed rainfall occurred in the second experimental year. The third year was determined as favorable (with more and evenly distributed rainfall). Scarce rainfall and drought marked the last year.Nitrogen use efficiency is an agronomical parameter representing the ratio between yield obtained and nitrogen applied. Results showed greater nitrogen use efficiency for low doses of nitrogen fertilization, where nitrogen intake was better (Table 2). Hartwig and Soussana (2001) found that Alfalfa used the soil or fertilizer nitrogen during the initial development of the plants, since nitrogen assimilation needs lower levels of CO_2 and energy as compared to nitrogen fixation process, therefore requires applying nitrogen. Vasileva and Pachev (2015) suggest that the primary development of Alfalfa plants need nitrogen to avoid the retention of root development. Justes *et al*. (2001) considered that Alfalfa plants without nitrogen fertilization had a significantly lower root dry mass than plants with nitrogen fertilization.

There were small differences in nitrogen use efficiency at N23P100K35, although nitrogen was applied in different ways (1/2 in first year of growing, 1/2 in third year or pre-sowing). Relatively bigger differences were found for the shallowest and deepest soil plowing. For different soil cultivation depths the nitrogen use efficiency for N23P100K35 could be arranged in decreasing order as follows: plough at the depth of 22-24 cm, soil loosing at the depth of 12-15 cm, plough at the depths 12-15, 30-35 and 18-22 cm. The yield responses to N addition could be limited by imbalances with other nutrients such as phosphorus (van der Velde *et al.*, 2014).

Phosphorus is important for aboveground and root mass growing of Alfalfa. It plays a key role for the nodule parameters such as number, size and activity (Armstrong, 1999). Vasileva and Pachev (2009) and Jing-Wei Fan (2015) found both higher nodulation ability and dry mass productivity in Alfalfa plants which were better supplied with phosphorus. Data in the Table 3 showed that phosphorus use efficiency

Table 1: Agro meteorological conditions for the period of study

Months	First year		Second year		Third year		Fourth	
	t	rainfall	t	rainfall	t	rainfall	t	rainfall
	ºC	l/m2	ºC	l/m2	ºC	l/m2	ºC	l/m2
I	-0.1	53.4	-2.2	26.1	2.4	55.0	-2.9	8.8
II	-3.4	27.6	3.2	23.9	-1.7	46.3	0.6	121.0
III	4.7	10.2	7.4	41.6	5.1	75.8	6.8	176.0
IV	10.9	83.6	13.1	6.9	12.4	57.4	13.2	30.9
V	20.5	74.5	16.1	87.5	17.5	101.0	17.8	33.2
VI	23.9	12.8	20.3	70.3	19.8	95.9	21.0	47.3
VII	23.7	49.7	23.5	38.5	22.6	115.0	23.0	78.4
VIII	25.5	1.4	22.6	82.4	21.4	156.0	23.2	63.4
IX	17.7	67.6	18.4	42.2	17.4	225.0	18.7	48.5
X	10.7	107.0	14.0	14.6	17.4	25.5	17.4	25.5
XI	7.4	24.8	7.9	21.6	5.1	24.0	5.1	24.0
XII	1.2	28.2	2.6	43.3	2.7	41.7	3.3	32.5

Table 2: Nitrogen use efficiency of Alfalfa after different fertilizing rates and soil cultivation

Treatments	Soil loosing	Plough			
	12-15 cm	12-15 cm	22-24 cm	18-22 cm	30-35 cm
	kg/kg				
N60P100K80	67.33	72.17	78.17	61.83	70.67
N23P100K35*	210.00	196.09	210.87	182.61	203.91
N23P100K35**	208.26	209.13	208.26	188.26	194.35
N35P80K50	126.00	117.43	127.43	120.00	110.00
Amophose	161.11	155.56	167.41	155.93	156.30
SE (P=0.05)	26.8	25.3	25.2	23.3	25.4
max	210.00	209.13	210.87	188.26	203.91
min	67.33	72.17	78.17	61.83	70.67

* nitrogen was applied 1/2 in first year of growing, 1/2 in third year, and P and K supply

** nitrogen was supplied pre-sowing, K supply, P by 1/3 in the first, second and third year of growing

Table 3: Phosphorus use efficiency of Alfalfa after different fertilizing rates and soil cultivation

Treatments	Soil loosing	Plough			
	12-15 cm	12-15 cm	22-24 cm	18-22 cm	30-35 cm
	kg/kg				
N60P100K80	40.40	43.30	46.90	37.10	42.40
N23P100K35*	48.30	45.10	48.50	42.00	46.90
N23P100K35**	47.90	48.10	47.90	43.30	44.70
N35P80K50	55.13	51.38	55.75	52.50	48.13
Amophose	36.25	35.00	37.67	35.08	35.17
SE (P=0.05)	3.30	2.75	2.88	3.03	2.29
max	55.13	51.38	55.75	52.50	48.13
min	36.25	35.00	37.67	35.08	35.17

* nitrogen was applied 1/2 in first year of growing, 1/2 in third year, and P and K supply

** nitrogen was supplied pre-sowing, K supply, P by 1/3 in the first, second and third year of growing

Table 4: Duration of the life cycle of root nodules of Alfalfa after different fertilizing rates and soil cultivation

Treatments	Soil loosing	Plough			
	12-15 cm	12-15 cm	22-24 cm	18-22 cm	30-35 cm
	days				
N0P0K0	34.1	32.2	34.2	38.4	36.0
N60P100K80	39.1	37.0	35.4	39.0	39.2
N23P100K35*	35.4	38.6	39.5	38.8	37.3
N23P100K35**	36.3	33.7	38.6	40.2	39.8
N35P80K50	35.0	34.6	35.8	41.6	38.3
Amophose	34.5	37.4	35.6	41.4	39.6
SE (P=0.05)	0.74	1.00	0.84	0.56	0.60
max	39.1	38.6	39.5	41.6	39.8
min	34.1	32.2	34.2	38.4	36.0

* nitrogen was applied 1/2 in first year of growing, 1/2 in third year, and P and K supply

** nitrogen was supplied pre-sowing, K supply, P by 1/3 in the first, second and third year of growing

Table 5: Root mass to nodule number ratio in Alfalfa after different fertilizing rates and soil cultivation

Treatments	Soil loosing	Plough			
	12-15 cm	12-15 cm	22-24 cm	18-22 cm	30-35 cm
	g roots/number root nodules				
N0P0K0	2.6214 (4.58/1.8)	0.7608 (4.56/6.0)	0.5462 (4.64/8.5)	0.6756 (5.74/8.5)	0.6758 (5.23/7.8)
N60P100K80	0.9690 (5.08/5.3)	0.6387 (4.95/7.8)	0.5344 (4.81/9.0)	0.6703 (5.69/8.5)	0.4759 (5.23/11.0)
N23P100K35*	2.4857 (4.35/1.8)	0.5046 (4.41/8.8)	0.4288 (5.14/12.0)	0.6732 (5.72/8.5)	0.6746 (5.90/8.8)
N23P100K35**	2.2089 (4.97/2.3)	0.9150 (4.57/5.0)	0.5092 (4.96/9.8)	0.6850 (5.82/8.5)	0.5498 (5.91/10.8)
N35P80K50	2.2422 (5.04/2.3)	0.9895 (4.94/5.0)	0.6144 (5.53/9.0)	0.6897 (6.55/9.5)	0.6568 (6.07/9.3)
Amophose	1.6438 (5.34/3.3)	0.6213 (4.81/7.8)	0.7348 (5.69/7.8)	0.6514 (5.70/8.8)	0.7315 (6.03/8.3)
SE (P=0.05)	0.2523	0.0007	0.0004	0.0005	0.0003
max	2.6214	0.9895	0.7348	0.6897	0.7315
min	0.9690	0.5046	0.4288	0.6514	0.4759

* nitrogen was applied 1/2 in first year of growing, 1/2 in third year, and P and K supply

** nitrogen was supplied pre-sowing, K supply, P by 1/3 in the first, second and third year of growing

In the brackets - (root mass/root nodule number)

was found to be higher for lower doses of phosphorus fertilization and vice versa. Thus, the phosphorus use efficiency for all depths of soil cultivation was the highest at the dose of fertilization N35P80K50. It varied in a relatively narrow limits for the rest experimental doses, where the supply with phosphorus was 100 kg/ha. Phosphorus use efficiency was the highest at the plough at the depth of 22-24 cm and N35P80K50.

Symbiotic fixations of nitrogen take place in the root nodules (Serraj *et al.*, 1999). Pink colored and located on the main root nodules are indication for their effectiveness (Beck *et al.*, 1993; Athar and Johnson, 1996; Athar and Shabbir, 1997; Kostov and van Cleemput, 1997). Life cycle duration of root nodules is important for the normal functioning of these structures and fixing of more nitrogen as well. Milev (2014) found the value of this index in pea was directly proportional to the vegetation rainfall during the year and the duration was between 30 and 52 days. Life cycle duration of root nodules in this study varied between 32.2 and 41.6 days (Table 4). Duration of life cycle of root nodules was the lowest in the control. The longest duration of 39.1 days was found at the shallow soil cultivation and higher nitrogen supply (N60P100K80). Longer life cycle of root nodules was observed in the plants well supplied with phosphorus (for the plough at the depths of 22-24, 18-22 and 30-35 cm). The findings of Asuming-Brempong *et al*. (2013) were similar for cowpea. Having in mind the characteristics included in the calculation of root mass to nodule number ratio, lower values of this ratio indicated better provision of root mass with root nodules (Table 5). The better insurance of root mass with root nodules was found at N23P100K35 (nitrogen was applied 1/2 in first year of growing, 1/2 in third year) and plough at the depth of 22-24 cm. Nitrogen use efficiency and the better root mass to nodule number ratio in Alfalfa was found to be highest at N23P100K35 (nitrogen was applied 1/2 in first year of growing, 1/2 in third year) and plough at the depth of 22-24 cm. Growth rate as well biomass yield of alfalfa have to be studied more detail. High quality of forage after fertilizing with rates N35P80K50 was found by Naydenova and Pachev (2009).

CONCLUSION

Soil cultivation and mineral fertilization had effect on nitrogen use efficiency and life cycle of root nodules in Alfalfa. Nitrogen use efficiency was found to be highest at N23P100K35 (nitrogen was applied 1/2 in first year of growing, 1/2 in third year) and plough at the depth of 22-24 cm; and the phosphorus use efficiency was the highest at the same depth of soil cultivation and N35P80K50. Life cycle of root nodules was the longest at N35P80K50 and plough at the depth of 18-22 cm. The better root mass to nodule number ratio was found at N23P100K35 (nitrogen was applied 1/2 in first year of growing, 1/2 in third year) and plough at the depth of 22-24 cm.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests regarding the publication of this manuscript.

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How to cite this article:
Vasileva, V.; Pachev, I., (2015). Nitrogen use efficiency and life cycle of root nodules in Alfalfa after different mineral fertilization and soil cultivation. Global J. Environ. Sci. Manage., 1 (4): 333-339.

DOI: 10.7508/gjesm.2015.04.008

URL: http://gjesm.net/article_13842_1612.html