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LUCERNE IN ARABLE CROPPING SYSTEMS: POTENTIAL OF DIFFERENT VARIETIES ON BIOMASS PRODUCTION AND NITROGEN BALANCE

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ABSTRACT

Lucerne improves yields and quality of subsequent crops by fixing nitrogen and increasing soil organic matter. In the Ukrainian region of Poltava weather conditions have become more difficult for producing legumes over the past decades. While the biomass productivity of modern lucerne varieties is well known, data on the nitrogen fixation capacity are still missing. Therefore biomass and nitrogen fixation capacity of 4 Ukrainian varieties (Lidiya, Poltavchanka, Vira, Zaykevicha) were evaluated in two field trials in two subsequent years. The seasonal average forage yield of lucerne was 10.5-11.4 t ha^{-1} with no significant differences between the varieties, but with a better performance at 1st and 2nd harvest in the second year because of a successful field emergence and water supply. An analysis of the genotype-by-year interaction on two experimental sites indicated that the old varieties Poltavchanka and Zaykevicha were well adapted to the experimental site environment. These varieties gave consistently stable shoot dry matter yields in both vegetation periods. Biological nitrogen fixation (average of 155 kg N ha^{-1}) and N-balance (average of -159 kg N ha^{-1}) did not differ between the four varieties, therefore the benefit for subsequent crops should be the same or similar.

Key words: *Medicago sativa*, nitrogen fixation, N balance.

INTRODUCTION

Biological N fixation through the symbiosis of legumes with nodule bacteria should be the main source of N for non-legumes in crop rotations. Lucerne (*Medicago sativa* L.) has a variety of positive effects in crop rotations (Olmstead and Brummer, 2008). It improves both yield and quality of subsequent crops (De Kruijff et al., 2008; Stanger and Lauer, 2008), reduces diseases, controls weeds within the crop rotation (David et al., 2005; Dunin et al., 2001), increases soil organic matter content, and improves water infiltration (Blair et al., 2006; Marsi and Ryan, 2006). In stockless organic farming systems, lucerne used for green manure is able to fix 159-230 kg N ha^{-1} per year (Loges et al., 1999; Pietsch et al., 2007). Hirth et al. (2001) concluded that two to three subsequent crops (depending on the weather conditions) could rely on lucerne N. Other works show the positive precrop effect of lucerne on wheat (Dalal et al., 2004; David et al., 2005; Latta and Lyons, 2006), which is especially interesting for organic farming. Modern lucerne varieties are characterized by improved crop survival and yield potential. However, yield, root growth, disease resistance and many other traits depend upon the environment. Varieties best adapted to a target environment must therefore be identified through testing within that environment. Suitable parent material is necessary in the development of new varieties. Lamb et al. (2006) compared ten lucerne cultivars released between 1940 and 1995 in the USA, and found that differences in forage yield were environmentally dependent, assuming that the multiple disease resistance

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of lucerne has increased in recently released varieties. At present, information about biomass productivity of modern Ukrainian lucerne varieties is available, but field data regarding nitrogen fixation capacity are still lacking. Information about N fixation capacity is necessary for breeding purpose and for farmers to promote organic farming.

The objective of this study was to compare the performance of four lucerne varieties in field experiments under the agro-climatic conditions of the Ukraine regarding their biomass production, biological nitrogen fixation and N pre-crop effect. Periodic measurements of shoot/root dry matter and N yield as well as biological nitrogen fixation (BNF) were related to inorganic soil N and weather conditions.

MATERIAL AND METHODS

Plots were established at the Poltava Agricultural State Research Station (49°35′ N, 34°33′ E). Two experiments were situated in two experimental sites close to each other at the Poltava Agricultural State Research Station, i.e. “A” and “B”, starting in summer 2003 and 2004, respectively. The climate is continental, characterized by hot and dry summers with low dew formation (April – September) and cold winters with low snow coverage (October – March). The long-term (1961-1990) annual mean temperature was 7.8°C with an average annual precipitation of 569 mm. Weather data for the experimental seasons were recorded by the weather station of the Poltava Agricultural State Research Station. Soils are Spodosols from loess with a silty loam texture (clay content of 25-45%), an organic carbon content of 2.4-3.5% in the A horizon, and a pH ($CaCl_2$) value of 5.8-5.9. The soils were provided with a moderate level of readily available nutrients in the Ap horizon (mean hydrolysed N of 80 and 40 mg kg soil^{-1}, 74 and 139 mg P kg soil^{-1}, 149 mg K kg soil^{-1} in trial A and trial B respectively, with a soil $N_{inorganic}$ content of 180-241 kg ha^{-1} in trial A and 68-85 kg ha^{-1} in trial B measured in the top 90 cm a few days prior to sowing.

Four Ukrainian lucerne varieties were compared. Zaykevicha was selected in 1931 and is described as being well adapted to adverse environmental conditions. Poltavchanka has been used as a national standard since 1987, has moderate frost and drought tolerance and is still highly accepted by farmers. Vira was released in 1999 and is recommended for steppe regions in the Ukraine. Lidiya, released in 2000, exhibits good frost and drought tolerance. The four lucerne varieties and the non-nodulating reference crop *Bromus inermis* were grown in four replications in a completely randomised design with plots measuring 6 x 8 m. Seeding rate of lucerne and the reference crop was 30 kg ha^{-1}. The soil cultivation before sowing was ploughing at 20 to 22 cm depth. The lucerne in trial A was sown in August and harvested in October, May and June of the following year. Trial B was sown in July and harvested in October, May and June of the following year. Plant disease rates were estimated visually after winter. Shoots were controlled for brown and yellow spottiness, rust and *Ascochyta* sp. Roots were checked for rot in spring before the second harvest. No significant differences in disease infestations were observed between experimental years. Both experiments (trial A and trial B) were ploughed in spring 2006 after mulching the lucerne in both fields. No N, P and K fertilizer was applied. The crops were grown without irrigation in both vegetation periods.

Lucerne plants were harvested three times per experimental period, according to their developmental stage (at start of flowering). For each plot, above-ground biomass was collected from four 1 m² sampling areas. Shoots were harvested by hand so as to determine above-ground biomass. In order to investigate root biomass, soil samples (0-30 cm) were collected three times per experimental period in both trials, and were gathered and washed by hand. Shoot and root dry matter yield were determined by drying an aliquot at 65°C until its weight remained constant. Dry samples were ground prior to N determination by Kjeldahl digestion. The biological nitrogen fixation of lucerne was estimated using the total nitrogen difference method (Danso, 1995). This method determines the difference between the total N uptake of the legume and the total N uptake of the non-nodulating reference crop, i.e. *Bromus inermis*. In accordance with this method, biological nitrogen fixation capacity was calculated from the plant and soil analyses as follows:

$$\text{Nitrogen fixation [kg ha}^{-1}\text{]} = (\text{above-ground } N_{Leg} + \text{below-ground } N_{Leg}) \text{ [kg ha}^{-1}\text{]} - (\text{above-ground } N_{Ref} + \text{below-ground } N_{Ref}) \text{ [kg ha}^{-1}\text{]} + (N_{in} \text{ in soil}_{Leg} - N_{in} \text{ in soil}_{Ref}) \text{ [kg ha}^{-1}\text{]} \quad (1)$$

(Above-ground $N_{Leg/Ref}$ + below-ground $N_{Leg/Ref}$) is defined by adding the nitrogen concentration in above-ground biomass at 1st, 2nd and 3rd harvest and the nitrogen concentration in below-ground biomass (0-30 cm) at 2nd harvest (biomass and N root concentration was highest at 2nd harvest).

(N_{in} in soil$_{Leg}$ – N_{in} in soil$_{Ref}$) is defined as the difference between inorganic soil nitrogen under lucerne and reference crop at 2nd harvest.

The percentage of legume N derived from the atmosphere (N_{dfa}) was calculated as follows:

$$N_{dfa} \text{ [\%]} = \text{Biological nitrogen fixation [kg ha}^{-1}\text{]} / (\text{above-ground } N_{Leg} \text{ at 1st, 2nd and 3rd harvest} + \text{below-ground } N_{Leg} \text{ at 2nd harvest) [kg ha}^{-1}\text{]} \quad (2)$$

To estimate the N benefit of lucerne for subsequent crops in the rotation, a simplified N balance calculation was used:

$$\text{N-balance [kg ha}^{-1}\text{]} = \text{N input [kg ha}^{-1}\text{]} - \text{N output [kg ha}^{-1}\text{]} \quad (3)$$

N input is the biological nitrogen fixation and N output is the above-ground N_{Leg} at 1st, 2nd and 3rd harvest, removed as forage.

Inorganic N was determined from soil samples in three layers (0-30, 30-60 and 60-90 cm), collected randomly from plant-free or harvest areas during plant sampling in both trials. Inorganic N was extracted from soil using a 1 M $CaCl_2$ solution.

Nitrate in the extracts was reduced to nitrite by hydrazine in the presence of copper sulphate. This was then photometrically determined as an azo dye complex (Ukraine State Standard 26488-85 – GOST: Determination of nitrates by the CINAO method). Ammonium-N was assessed according to Searle (1984) using an UV-VIS spectrophotometer. Data were analysed statistically using a linear mixed-effects model with variety as fixed effect and year and variety × year as random effects. Model-selection was based on corrected AIC values (Akaike's Information Criterion). The significance of the fixed effect was tested by *F* test, while the significance of random terms was determined by the LRT (Likelihood Ratio Test) as described by Galwey (2006). Traits with significant variety × year interaction were subjected to a GGE biplot analysis (Yan and Kang, 2003) in order to fully explore the genotype-by-year interaction pattern. Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC) and GenStat 16th Ed. (VSN International Ltd., Hemel Hempstead, UK) software.

RESULTS AND DISCUSSION

In general, the temperature trend from July-trial A to June-trial B correlated with the long-term average (Table 1). The annual precipitation was higher than the long-term mean value (569 mm) in both vegetation periods (trial A: July – June: +205 mm; trial B: July – June: +85 mm) during each of both experiments. In trial B, the monthly precipitation in August was unusually high (150 mm), giving the lucerne seedlings optimal conditions to grow and develop a dense vegetation cover.

Significant differences in inorganic N content were detected between the experimental years in spring, at 1st, 2nd and 3rd harvest (Table 2). The factor variety had no influence on inorganic N.

Table 1. Long term (1960-1991) temperature and rainfall in Poltava, and deviations from long term weather trends in the growing seasons of trial A and B

Month	Temperature (°C)			Rainfall (mm)		
	1960-1991	Trial A	Trial B	1960-1991	Trial A	Trial B
July	20	0.1	-0.2	71	151.4	56.9
August	19	0.8	1.2	46	-3.4	104.0
September	14	0	0.6	44	-28.2	-16.1
October	8	-0.2	0.6	42	90.4	-15.1
November	2	0.7	0.4	49	-23.1	-13.1
December	-2	1.0	1.4	51	-12.1	-24.8
January	-6	3.1	5.8	43	28.7	-10.1
February	-4	0.7	-1.3	37	-1.1	24.1
March	0	3.8	-0.6	35	-0.1	-12.7
April	9	-0.5	1.6	40	-20.7	-26.4
May	15	-1.6	2.4	51	38.3	-25.2
June	19	-1.9	-1.5	60	-15.1	43.7

Table 2. Effects of genotype and year upon mean $N_{inorganic}$ (kg·ha^{-1}) at different times

Specification	$N_{inorganic}$			
	H1	H2	H3	SPR
Variety	0.234	0.199	0.301	0.221
Year	0.034	< 0.001	< 0.001	0.002
Variety × Year	-	-	-	-
Lidiya	45.8	85.2	65.7	58.1
Poltavchanka	46.1	90.9	75.4	59.9
Vira	48.8	79.3	66.0	62.1
Zaykevicha	42.0	79.9	69.4	66.7
s.e.d.	3.2	5.9	5.6	4.2
d.f.	27	27	27	27
l.s.d.	6.6	12.2	11.5	8.7
Trial A	39.8	132.1	92.5	41.4
Trial B	51.5	35.6	45.7	82.0
s.e.d.	2.2	4.2	3.9	2.9

Legend: H1: 1st harvest, H2: 2nd harvest, H3: 3rd harvest; SPR: spring; d.f.: degrees of freedom; s.e.d.: standard error of difference; l.s.d.: least significant difference; significant effects ($p<0.05$).

A significant influence of the year and vegetation period upon shoot DM yield at 1st and 2nd harvest was observed (Table 3), where lucerne in trial B performed better than in trial A. The rainfall distribution might be more meaningful than the rainfall amount for the development and growth of plants (Condon et al., 2004; Keating and McCown, 2001).

The density of lucerne plants in trial B (92 plants m^{-2}) exceeded values of trial A (60 plants m^{-2}). This can be explained by a better field emergence and water supply in trial B, following higher rates of precipitation after sowing in August/September. The lucerne varieties showed similar shoot DM yields at all harvests, the influence of the variety was not significant (Table 3). Only the influence of the interaction effect (year × var) on the total shoot DM yield (p=0.026) and at the 3rd harvest (p=0.020) was significant (Table 3). The lucerne shoot DM yields were in accordance with the results of Moghaddam et al. (2013), who compared lucerne varieties in a similar environment and measured shoot yields of 9.6-14 t ha^{-1} and Ardakani et al. (2009), who observed 8.2-8.8 t ha^{-1} on the same experimental site.

The partitioning of the genotype and genotype-by-year interaction (Figure 1) clearly shows that the two study years were significantly different and rated as two macro-environments. The highest-yielding genotype in trial A, during which weather conditions were unfavourable in spring, was Poltavchanka, while in trial B it was Lidiya and Zaykevicha. Vira was inferior in both years. The significant interaction was caused by differences in rank and scale. Poltavchanka showed a stable performance (11.7 and 11.3 t ha^{-1}) in both years and was, therefore, ranked first in trial A, but only fourth in trial B. Lidiya, the best performing genotype in trial B (12.5 t ha^{-1}) was ranked only third in trial A (9.9 t ha^{-1}). Zaykevicha and Vira showed similar scale effects as Lidiya (2 t ha^{-1}) across the two years. However, Zaykevicha (10.3 and 12.3 t ha^{-1}) performed better than Vira (9.6 and 11.5 t ha^{-1}) in the same time period.

Table 3. Effects of genotype and year upon mean shoot DM yield and root DM yield (kg·ha^{-1}) at different times

Specification	Shoot-DM				Root-DM
	H1	H2	H3	Total	H2
Variety	0.900	0.194	0.707	0.928	0.875
Year	0.041	0.007	-	-	-
Variety × Year	-	-	0.026	0.020	-
Lidiya	1117.5	4199.0	5841.1	11158	1938.5
Poltavchanka	1044.6	4192.7	6238.0	11475	2013.5
Vira	1089.5	3888.5	5568.1	10546	2032.0
Zaykevicha	1025.1	4433.3	5862.4	11321	1966.7
s.e.d.	135.2	243.4	554.8	1513	126.2
d.f.	27	27	4	4	28
l.s.d.	277.4	499.5	1540	4200	258.4
Trial A	835.9	3525.7	-	-	-
Trial B	1302.3	4831.0	-	-	-
s.e.d.	93.72	170.7	-	-	-

Legend: see Table 2; Total: H1+H2+H3.

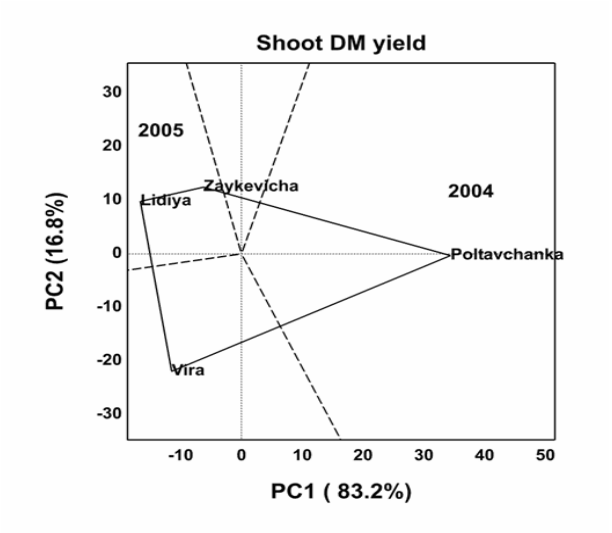

Figure 1. GGE biplot (symmetrical scaling) of lucerne variety performance with respect to total shoot dry matter yield (The vertex cultivar in each sector is the best performing cultivar in the respective year.)

The root DM yields of all varieties were similar, and no influence of variety and year on the root yields was found (Table 3). Nor were varietal differences in shoot and root N yield detected (Table 4). The influence of the year was significant for shoot N yields at 1st, 2nd harvest, total harvest, and for root N yield at 2nd harvest (Table 4). The above-ground/below-ground N yield is a calculated value of dry matter yield multiplied by N concentration. The above-ground N concentration at 1st and 2nd harvest was higher in trial B (3.5%) than in trial A (2.8%). Therefore, also the above-ground N at 1st and 2nd harvest performed better in trial B compared to trial A. Additionally, we observed an influence of the year upon total above-ground N, as well as upon below-ground N at 2nd harvest. Generally, there can be large differences in the performance of varieties across years and harvests, and within a regional area.

Table 4. Effects of genotype and year upon mean shoot N yield and root N yield (kg ha^{-1}) at different times

Specification	Shoot-N				Root-N
	H1	H2	H3	Total	H2
Variety	0.372	0.415	0.714	0.534	0.219
Year	0.019	0.004	-	0.026	0.097
Variety × Year	-	-	-	-	-
Lidiya	39.2	134.6	148.2	322.0	35.0
Poltavchanka	32.7	133.3	150.7	316.7	42.4
Vira	35.3	122.2	137.6	295.1	35.5
Zaykevicha	31.4	140.7	148.0	320.1	36.3
s.e.d.	4.7	11.0	12.2	20.4	3.9
d.f.	27	27	28	27	27
l.s.d.	9.6	22.5	24.9	41.8	7.9
Trial A	23.6	96.9	-	269.9	41.9
Trial B	45.7	168.5	-	357.0	32.6
s.e.d.	3.3	7.7	-	14.2	2.6

Legend: see Table 2 and 3.

The total biological nitrogen fixation ranged from about 129 kg N ha^{-1} (Vira, trial A) to 195 kg N ha^{-1} (Poltavchanka, trial A) and N_{dfa} 40-54%. The estimated nitrogen fixation was low compared to Moghaddam et al. (2013) with 249-385 kg N ha^{-1} or Ardakani et al. (2009) with 243-284 kg N ha^{-1}. Generally, no variety or interaction effect was observed in terms of nitrogen fixation (Table 5). The influence of the year was significant for N_{dfa}. Our results support the observation that high contents of mineral N can limit nitrogen fixation by legumes (Vessey and Waterer, 1992). In trial A, inorganic N started at a lower level in spring (mean value 40 kg N ha^{-1}) compared to trial B (81 kg N ha^{-1}). Due to a significantly lower soil N content, the percentage of nitrogen fixation was higher in trial A (N_{dfa} 55%) than in trial B (N_{dfa} 34%).

Table 5. Effects of genotype and year upon mean N_{dfa} (%), nitrogen fixation and simplified N-balance (kg ha^{-1}) regarding biological nitrogen fixation and N in forage

Specification	N-fix	N_{dfa}	N-balance
Variety	0.828	0.521	0.389
Year	-	0.018	< 0.001
Variety × Year	0.062	-	-
Lidiya	162.1	46.2	-159.9
Poltavchanka	170.0	47.0	-146.8
Vira	129.9	40.6	-165.2
Zaykevicha	156.3	44.2	-163.9
s.e.d.	45.2	4.6	11.7
d.f.	4	27	27
l.s.d.	125.5	9.5	24.0
Trial A	-	55.3	-94.1
Trial B	-	33.7	-223.8
s.e.d.	-	3.2	8.2

Legend: see Table 2: N-fix: total biological nitrogen fixation from H1 to H3;
N_{dfa}: nitrogen derived from atmosphere.

The simplified N-balance of the lucerne varieties was similar for all tested varieties; the variety effect was not significant. The influence of the year on the N balance of the lucerne varieties was significant (Table 5); we found big differences between both vegetation periods (mean value trial A: -94 kg N ha^{-1}; trial B: -224 kg N ha^{-1}). This result is based on the increase of total above-ground DM and N yield (= N output) and the decrease of nitrogen fixation (= N input) in trial B compared to trial A. In the Ukraine, legumes are usually used as forage in animal production. As a consequence, biomass and, hence, fixed N are removed from the field, which reduces the benefits for subsequent crops and the N-balance is negative. Normally, in livestock systems the N balance improves through the application of farmyard manure, wherein a large portion of nitrogen, approx. 60% of harvested N (according to Stein-Bachinger et al., 2004) maximum 40% N-losses during stable, storage and application procedure of farmyard manure), returns to the field. Therefore, in an advanced N balance considering the return of N by farmyard manure, after one year lucerne growing the balance is usually positive or almost consolidated (estimated advanced N balance for our experiments: +67 kg N ha^{-1} for trial A, -9 kg N ha^{-1} for trial B). This very significant contribution must be taken into account when assessing the contribution of biological nitrogen fixation to the N balance of forage legumes.

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