The development of lucerne root morphology traits under high initial stand density within a seven year period

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ABSTRACT

The root system of plants is generally regarded as a factor, which is in relation to important agronomic and ecological characteristics. The aim of this study was to investigate the effect of high initial stand density on the reduction in development of lucerne root morphology traits and how long-term this effect would be. In spring 2003, a field experiment with six lucerne entries in randomized blocks was established. Broadcast sowing was used and the seeding rate was 5000 germinated seeds per m^2. In 2003–2009, the plants were sampled in each plot in autumn; the average depth of sampling was 0.2 m. The stand density reached an average value of 860 plants per m^2 in the autumn of the seeding year and this strongly reduced the root weight, tap-root diameter, position and number of lateral roots. The subsequent decrease of stand density to 57 plants/m^2 in 2009 was not linear but it was extremely quick from the 1st to 2nd year and, by contrast, it was extremely slow in the last three years. It indicates that older plants with larger tap-root diameter probably have a higher persistency. All evaluated root traits were developed slowly, nevertheless, they reached common values during a seven year period. The intensity of the relation of stand density to root weight or tap-root diameter increased over time whilst it decreased to the ratio of root-branched plants. Results suggest that an assessment of density in samples should be recommended for the varieties evaluation in case of irregularly-spaced plants because the differences in root morphology among varieties could be caused by the differences in density among the varieties. It is possible to conclude that lucerne stands under higher initial density provided a strongly reduced speed of root development with an impact on important agronomic traits connected with root morphology.

Keywords: alfalfa; *Medicago*; long-term experiment; tap-root; lateral root

The root system of plants is generally regarded as a very important factor, which is in relation to its considerable agronomical and ecological characteristics. In respect of agricultural crops, this research is concerned mainly with important annual crops such as wheat, maize or soybean etc. (e.g. Gregory 2006, Svoboda and Haberle 2006). Studying field-grown root systems of lucerne (*Medicago sativa* L.) is labour intensive and time consuming (Lamb et al. 2000a) and this could be the reason why this type of research is conducted relatively seldom. Lucerne plants generally have a single deep taproot, with variation in the number and size of lateral roots (Johnson et al. 1998). The most frequently observed parameters are the tap-root diameter and lateral root number, alternatively also the lateral root diameter and position and fibrous root mass. The depth of root sampling usually focused on the arable layer and varied from 22 cm (Lamb et al. 2000a) to 25 cm (Johnson et al. 1998). The often evaluated root system length (Gregory 2006) has no sense in case of lucerne due to the unique very deep root system which reaches to a depth of several metres (Frame et al. 1997). This unique root system is a reason that lucerne is regarded as the best crop for indicating sub-soil archaeological features during the dry summer (Hejcman and Smrž 2010).

The traits associated with the persistence and productivity in perennial lucerne are believed to be influenced by root morphology (Johnson et al. 1998). Several researchers reported a correlation between the lucerne root morphology and yield (e.g. McIntosh and Miller 1980) and Lamb et al.

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(2000b) confirmed that the selection for fibrous and lateral roots within the lucerne germplasm increased herbage yield. Johnson et al. (1998) described the relation of root morphology traits to fall dormancy and geographic origin among the plant introductions. The tap-root and lateral root diameter were positively correlated and lateral root number and position, and fibrous root mass were negatively correlated with fall dormancy. Hakl et al. (2007b) noted that also the amount of root reserve nutrients before overwintering is in relation to the amount of lucerne root biomass. In the case of red clover (*Trifolium pratense* L.), Hejduk and Knot (2010) described considerable differences in the persistence among varieties, which are associated with different adaptability and disease resistance. These described differences among the varieties could be also related to different root morphology but this was not investigated in their study.

Lucerne root morphology traits are heritable (Lamb et al. 1999) but many environment or management factors can influence them. It has long been appreciated that the growth of root systems in soils is affected by a wide range of soil properties but, in turn, the properties of soils are modified by roots (Gregory 2006). Except for the soil properties, root growth of lucerne is largely controlled by the supply of reserve substances from shoots (Luo et al. 1995). Šantrůček and Svobodová (1988) concluded that the ratio of root-branched plants was also influenced by the term of the stand establishment, stand density, and soil compaction. The effect of soil compaction on root growth within the same soil type was confirmed by Hakl et al. (2007b) with lucerne and, similarly, significant relations between the bulk density and length of lateral roots or branching density was described by Konopka et al. (2009) with maize roots. According to Luo et al. (1995), cutting of lucerne produced an initial decrease in fine root mass followed by a recovery towards the end of the harvest cycle. Frequent cutting decreased the overall growth and vigour (Garver 1922). The role of lucerne varieties in relation to root morphology was investigated very rarely and the focus was on the lucerne plant introduction in relation to fall dormancy and geographic origin (Johnson et al. 1998). This research suggests that the variation observed in root morphological traits among all the evaluated lucerne entries indicated that selection for specific root modifications could be effective.

In contrast to annual crops, lucerne belongs to perennial legumes. Its root morphology development over time was studied by Upchurch and Lovvorn (1951) or Suzuki (1991) and they all reported that the tap-root diameter and lateral roots number increased with age in lucerne. This could be explained by the changes in stand density during the stand development across the years. As noted by Suzuki (1991), morphological and physiological changes associated with aging of the crown and roots are so slow that such a change may be recognized only by continuous observations over many years.

Stand density is also one of the most important factors associated with the root morphology traits. As noted by Nelson et al. (1998), the percentage of live seeds that established a plant one month after seeding in the field was independent of the seeding rate. This indicated that the seed-soil contact and diseases or insects, and not plant competition, were the primary factors affecting emergence and early survival. Stands thinned rapidly, especially those at high densities, as plants increased in size during late spring and summer, indicating that the plant-to-plant competition was the major factor. According to Hall et al. (2004), the higher plant densities experienced eight times higher plant deaths in the first year after planting compared with the lower plant densities. Increasing alfalfa seeding rates above the recommended levels (approximately 1000 seeds per m^2) provided no measurable long-term benefit.

Investigation by Lamb et al. (2000a) revealed that all lucerne root traits were affected by plant spacing. Solid seeded plants needed more time to show a maximum expression of root traits and scored lower tap-root diameters than spaced plants. Decreasing stand density generally increased the taproot size and increased the branching of lucerne roots (Garver 1922, Upchurch and Lovvorn 1951). The significant effect of stand density on the lucerne root morphology was also described by Hakl et al. (2007a). Decrease of stand density and the following lucerne plant development over the years is also associated with the changes in the forage quality (Doležal and Skládanka 2008, Hakl et al. 2010) or weed infestation (Hakl et al. 2006).

In this regard, the density factor and its influence on root morphology seems to be interesting because it could be influenced by the seeding rate. Previous research was conducted only under common densities or with spaced plants but never under high initial stand density and it suffered from a lack of long-term experiments. In our opinion, high initial stand density considerably influences the root morphology development in the following years. In the present study, the results of an experiment are reported which investigated the changes in the lucerne root morphology traits under extremely high initial stand density within a seven year period. The aim of this paper was therefore to answer the following questions: can a high initial stand density reduce the root morphology traits development in comparison with common stand and how long-term will this effect be? There is also a question of the influence of used variety and if the effect of density will be stable across the year in relation to the actual value of stand density. These results could be useful for understanding of the development of lucerne root system under specific arrangements with a possibility of influencing the root morphology under field conditions.

MATERIALS AND METHODS

In April 2003, an experiment with five lucerne candivars (ŽE XLI, XLII, XLV, IL, L) and the control variety Jarka was established at the experimental field station of the Czech University of Life Sciences Prague in Suchdol (286 m above sea level, 50°08'N, 14°24'E). The soil is a deep loamy degraded chernozem with permeable subsoil. The detailed subscription of site soil characteristic was presented by Černý et al. (2010). The long-term annual temperature is 9.3°C and precipitation 510 mm.

The plot experiment was established in randomized blocks with four replicates for each lucerne entry. The size of each plot was 3 × 2 m. Broadcast sowing was used and the seeding rate was 5000 germinated seeds per m^2. Three weeks after seeding, all plots were treated by bentazone at the rate of 1200 g per hectare. Except for the sowing year with two harvests, plots were clipped four times per year mostly during the bloom stage. Total cover of weeds was estimated in the first cut.

During the 2003–2009 period the plants were sampled in each plot in autumn; the average depth of sampling was 0.25 m. The size of the sampling area was increased during the seven year period due to a decrease in the stand density (Table 1). The number of plants was determined in the samples and density calculated per m^2. The tap-root diameter under the crown (TD, in mm), position of lateral roots closest to the crown (LRP, in mm), and lateral roots number per plant (LRN, pieces) were measured for each individual plant and the percentage ratio of root branching plants (RBP) was calculated. After the measurements, the root samples were washed, oven-dried at 60°C, the root weight (RW) was assessed and calculated per m^2. The effect of time and lucerne entry was statistically evaluated by a two-way ANOVA or ANCOVA. The relations between the evaluated morphological traits were assessed by a correlation and partial correlation analysis. All statistical procedures were performed using the Statistica 9.0 (StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

Throughout the seven years of the experiment, the lucerne stand survived without any substantial damage by pests or root disease. The total cover of all weed species in the stand did not exceed 15%. The dominance of couch grass (*Elytrigia repens* L.) was decreasing and this species was not observed during the last three years, whilst the dandelion (*Taraxacum* sp.) dominance was increased and

Table 1. The means and standard error (SE) of stand density (D) and dry matter of root weight (RW)

Year	Sampling area (cm)	D (plants/m^2)		RW (g/m^2)		*n*
		mean	SE	mean	SE	
2003	25 × 25	860^a	66	17.7^a	1.7	12
2004	30 × 30	272^b	23	120.3^b	9.8	24
2005	30 × 30	144^c	11	179.8^b	14.3	24
2006	30 × 30	81^{cd}	6	161.4^b	12.0	24
2007	35 × 35	96^{cd}	6	374.4^c	19.2	24
2008	35 × 35	66^d	5	304.8^d	22.9	24
2009	35 × 35	57^d	5	344.0^{cd}	17.9	24

Different letters document statistical differences between years for Tukey HSD, α = 0.05

Table 2. The means and standard error (SE) of tap-root diameter (TD) and percentage ratio of root-branching plants (RBP) in all evaluated plants

Year	TD (mm)		RBP (%)		n
	mean	SE	mean	SE	
2003	1.41[a]	0.13	0.0	–	301
2004	5.06[b]	0.10	4.4[a]	0.8	543
2005	6.22[c]	0.14	4.9[a]	1.2	287
2006	8.82[d]	0.20	13.2[a]	1.8	177
2007	12.01[e]	0.29	33.6[b]	3.0	256
2008	12.71[ef]	0.37	46.3[c]	3.8	175
2009	13.11[f]	0.35	66.2[d]	3.9	151

Different letters document statistical differences between years for Tukey HSD, α = 0.05

it was a dominant weed species in the final years of the experiment.

Changes over the years. The means and statistical differences of the stand density and root weights are shown in Table 1. The average stand density decreased from 860 to 57 plants/m^2 in 2003 and 2009, respectively. It is obvious that the decrease in the stand density during the observed period was not linear but it was extremely quick from the 1st to 2nd year and, by contrast, extremely slow in the last three years. A similar trend in the density changes was presented by Suzuki (1991) for the seeding year. In respect of the seeding rate, 850 seeds per m^2 were used in the ten following years. Six hundred or more plants emerged each seeding year but the density decreased to 300 or less by late autumn of the seeding year. A comparable decrease in the density in the seeding year is generally presupposed. According to Nelson et al. (1998), this decrease is caused firstly by factors such as disease or seed soil contact and only later by plant to plant competition. As noted by Hall et al. (2004), the higher plant densities generally experienced higher plant deaths during the first year after planting compared with the lower plant densities. By contrast, a considerably higher density was obtained when the seeding rate of 5000 germinated seeds per m^2 was used. Probably, under optimal conditions with a higher seeding rate, the stand density could hold on to a higher level over the seeding year. With regard to the root weight in the arable layer, this value increased until the 5th year with a subsequent slow decrease in the final years.

The changes in root morphology within seven years are presented in Tables 2 and 3. The TD and LRN values increased over all seven years in agreement with the results of Upchurch and Lovvorn (1951) or Suzuki (1991) but TD reached a lower value than what was presented by Suzuki (1991) or Hakl (2006) for a stand of comparable age. In regard to a non linear decrease of the stand den-

Table 3. The means and standard error (SE) of lateral root position (LRP) and lateral root number (LRN) at root branched plants

Year	LRP (mm)		LRN (pcs)		n
	mean	SE	mean	SE	
2003	0.0	–	0.0	–	0
2004	3.10	0.46	1.68	0.21	22
2005	20.14	1.71	1.64	0.17	14
2006	34.60	6.37	1.06	0.06	23
2007	39.78	4.12	2.69	0.22	86
2008	39.48	3.41	2.83	0.31	82
2009	32.93	2.99	2.75	0.18	100

sity, it indicated that the remaining plants with a larger TD have a higher persistency. The intensity of branching is correlated with the TD value (Hakl et al. 2007a); therefore LRN was increasing concurrently with the increase of TD under a decline in the stand density. The LRP value increased until the 5th year with subsequent stagnancy which was in relation to the root weight. The effect of time on LRN ($P = 0.004$) and LRP ($P < 0.000$) was significant but numbers of root branching plants that were too low were observed in some years so a post hoc test was not performed between the years.

In an experiment with the same lucerne candivars and control variety Jarka, Hakl (2006) describes an average TD of 5 mm and RBP around 50% in the seeding year. In this case, the stand density ranged from 140 to 248 plants/m^2. From the 2nd to 4th year of vegetation with the density from 60 to 150 plants/m^2, the average TD and RBP ranged from 9.8 to 15.0 mm and 60 to 70%, respectively. In 2009, the control variety Jarka was seeded in the same location with a regular space of 125 mm between the rows, with the seeding rate of 800 germinated seeds per m^2. In the autumn of the seeding year, the plants reached an average density of 320 plants per m^2, TD 5.6 mm, and RBP 84% (Hakl, unpublished). In the present experiment, no root-branched plants were detected in the seeding year. In the subsequent years, the RBP value increased from 4% in 2004 to 66% in 2009 (Table 2). The development of TD was very slow and the common RBP value around 50% in lucerne stand was not obtained until the 6th year. Based on these results, it seems that the ratio of root branching plants was eliminated by an extremely high stand density and this stand had not reached the usual ratio until the 6th year. This is in agreement with the results presented by Lamb et al. (2000a) who commented that all root traits were affected by plant spacing and solid seeded plants needed more time to show maximum expression of the root traits.

It is possible to assume that these mentioned changes related to a slower development of roots influenced all agronomic traits which are commonly associated with root morphology such as persistency, productivity or root reserve accumulation. Regarding the productivity, with the year 2006 with the highest average yield (sum of four harvests) being considered as 100%, the productivity in 2004 was developed as follows: 38–69–100–92–84–88%. It seems that the productivity basically corresponded with the root weights with regard to the weather conditions in a particular year.

Lucerne entry effect. For TD, the lucerne entries effect was significant ($P = 0.035$) when the significantly higher value was observed in the variety Jarka in comparison with the candivars ŽE XLV, XLI and IL. When the stand density was used as a covariate, this effect was not significant. This indicated that the density in the samples could be the reason for the differences between the entries in this trait. It must be remembered that TD value is significantly correlated with other traits as LRN or LRD (Hakl et al. 2007a). It is possible to conclude that for irregularly spaced plants the density in the samples must be included for the correction of inter-varietal differences in the root morphology. The entries represented only around 1% of variability explained by this model whilst the year and density represented 60 and 40%, respectively. The contribution of the used entries for explained variability of root morphology was accordingly very small in comparison with the age of the plants or stand densities.

For RBP, the effect of lucerne entries was not significant. The direct effect of lucerne entries for

Table 4. Correlation and partial correlation matrix including covariates among evaluated factors

	Age	Density	RW	TD	RBP	Density	Age
Age	1.00					–	1.00
Density	**–0.70**	1.00				1.00	–
RW	**0.66**	**–0.30**	1.00			**0.31**	**0.66**
TD	**0.83**	**–0.68**	**0.58**	1.00		0.09	**0.67**
RBP	**0.80**	**–0.51**	**0.56**	**0.72**	1.00	0.07	**0.72**
Covariate						age	density

$n = 156$; age – in years; density – plants/m^2; RW – root weight (g/m^2); TD – tap-root diameter (mm); RBP – percentage ratio of root-branching plants. Correlations significant at $P < 0.05$ are in bold

Table 5. Correlation between density and selected morphological parameters within evaluated years

	density						
	2003	2004	2005	2006	2007	2008	2009
RW	0.09	**0.45**	**0.56**	0.30	**0.58**	**0.78**	**0.66**
TD	–0.22	**–0.48**	**–0.42**	**–0.66**	**–0.46**	**–0.53**	**–0.58**
RBP	–	–0.34	**–0.39**	–0.16	–0.36	–0.24	–0.05

n = 24, n = 12 at 2003; correlations significant at $P < 0.05$ are in bold

LRP and LRN was not evaluated due to the low numbers of root-branched plants per each entry in some years.

Relations among traits. Correlations among the evaluated traits are shown in Table 4. The age of plants was significantly positively correlated with all evaluated morphological traits whilst the stand density was correlated significantly negatively. This is in accordance with the previous results (Garver 1922, Upchurch and Lovvorn 1951, Hakl et al. 2007a) that stand density showed significantly negative relations to TD and LRN values. After excluding the age of plants affected by partial correlation, the stand density had a significant positive effect on the root weight. Hence, higher stand density reduced all morphological parameters during the time but after excluding the time effect the changes in these relations were observed. After excluding the stand density effect, the age of plants still had a significant positive effect on all morphological parameters.

The effect of the stand density on root morphology was significant but the results of the correlations within the evaluated years presented in Table 5 indicate that the relation of the stand density to the root weights or tap-root diameter increased over time whilst it was decreased in the ratio of the root branched plants. It is possible to conclude that the effect of density on root morphology was not stable across a given year in relation to the actual stand density. It could be important for the evaluation of the stand density effect in some experiments where the intensity of this effect could vary in dependence on the actual value of density.

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