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In-field lucerne root morphology traits over time in relation to forage yield, plant density, and root disease under two cutting managements

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ABSTRACT

Changes in lucerne (*Medicago sativa* L.) root morphology concurrent with forage growth have been little studied in multi-year field experiments with lucerne stands though they could provide explanations for differences in performance among tested treatments. Our objectives were: (i) to compare lucerne root trait development under three- and four-cut managements over a 4-year period, and (ii) to investigate relationships among root traits, and between root traits and lucerne varieties, cutting frequencies, root sampling periods, lucerne dry matter yield and evidence of root disease incidence and plant density, using multivariate methods. Treatments were a factorial combination of 15 lucerne cultivars with managements of three and four cuts per year. Each spring and autumn, plants were evaluated for root morphology traits and scored for root disease. Root morphology traits were strongly modified by sampling period and plant density, which together explained over 40% of total variability. The development over time was positively associated with an increase in tap-root diameter (TD) and root mass accumulation, whereas root density determined changes in root branching traits. The four-cut management reduced TD and root branching in association with lower forage yield. Variety effect was significant but contributed only 2.8% of total variability. Root branching showed a positive correlation with forage yield through a large increase of TD for branch-rooted plants, but more intensive root branching was associated with a significantly higher disease score than in tap-rooted plants. For optimization of evaluation of root traits, we proposed a root potential index (RPI) integrating TD and plant density. This RPI showed a closer relation to yield than TD, plant density or root biomass alone, in the arable cultivation layer. Our results reveal that monitoring of root morphology is an effective tool to characterize the production potential of lucerne as a sown field crop, and may explain yield differences among the various experimental treatments. Understanding these relationships between root morphology and lucerne stand performance can help in breeding selection as well as effective lucerne stand evaluation.

1. Introduction

The high forage production potential of lucerne (*Medicago sativa* L.) has been attributed in part to its efficient use of water and nutrients as a result of its root architecture, particularly deep tap root system, although its root development is also sensitive to conditions in the rooting zone (Frame, 2005). Root traits also play an important role in the soil carbon balance of agroecosystems (Thivierge et al., 2016). Investigations of the root systems of field-grown plants that have extensive rooting systems, such as lucerne, is very labor intensive and time consuming (Lamb et al., 2000a). This may explain why this type of research is seldom conducted, compared with studies of the above-ground parts of plant development.

Lucerne root morphology has been widely investigated. Almost a century ago, Garver (1922) suggested that root growth was influenced by soil, climate, cultural treatment, and injury. Increasing forage yield is a major goal of most lucerne breeding programs, and root research has focused primarily on supporting this breeding objective. Consequently, the critical role of root morphology in forage crop persistence and productivity has been recognized in many studies (McIntosh and Miller, 1980; Johnson et al., 1996, 1998; Lamb et al., 1999, 2000b). Positive correlations between plant forage yield and the size of the root system have been found (Saindon et al., 1991; Chloupek et al., 1999), suggesting that a selection for larger root systems may increase forage

Abbreviations: DMY, dry matter yield; IRB, Intensity of root-branching; LDM, Lateral root dry matter; LRD, Lateral root diameter; LRN, Lateral root number; LRP, Lateral root position; PD, Plant density; PRDS, Plant root disease score; RB, Percentage of branch-rooted plants; RDM, Total root dry matter; RFM, Plant root fresh matter; RPI, Root potential index; RPIcor, Root potential index corrected for SRDS; SRDS, Stand root disease score; TD, Tap-root diameter; TDM, Tap-root dry matter

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yield. A tendency for higher yield associated with branch-rooted plants producing lateral roots has been reported, usually with medium correlation coefficients (McIntosh and Miller, 1980). Some root traits are heritable (Saindon et al., 1991; Johnson et al., 1996; Lamb et al., 1999) which has supported the idea about the benefits of phenotypic selection on more fibrous or lateral roots for improving forage yield (Lamb et al., 2000b; Vaughan et al., 2002). A suitable methodology for investigation of lucerne root morphology was proposed by Lamb et al. (2000a) for field plots using plants with uniform spacing.

Another group of studies has focused on lucerne root traits in relation to various environmental or anthropogenic factors. Root traits have been investigated in relation to soil salinity (Vaughan et al., 2002), dynamics of nodulation (Chmelíková et al., 2015), drought stress (Annicchiarico, 2007), soil compaction and tractor wheeling (Głąb, 2008), soil tillage (Vasileva and Pachev, 2015), or soil nutrient content and fertilization (Russelle and Lamb, 2011). Cutting management (timing and frequency) is an important factor affecting lucerne stand development (Frame, 2005); however, there is a lack of studies about its effect on root morphology traits.

In spite of well-documented positive relationships between plant root traits and plant productivity, these results cannot always be applied effectively in field situations. Large genotype × environment interactions that alter root branching expression seem to exist (Pederson et al., 1984). Moreover, root traits are continually changing over time (Suzuki et al., 1991) and there is also a strong and direct impact of lucerne stand density on root traits (Hakl et al., 2011). In particular, the density effect could not prevail under the uniform plant spacing that characterizes experiments in controlled conditions, in pot or in a field under uniform plant spatial arrangement, this density effect will not commonly be apparent. In contrast, in common stands of lucerne growing in the field, plant density varies naturally and it decreases over time, as documented in many field studies (Ventroni et al., 2010). Moreover, the difference between root traits at the plant level and at the stand level must be carefully distinguished in field environments. Plant traits are measured for individual plants whereas stand traits are calculated as the average value of plants per unit area. Forage yield is generally expressed per unit area and, therefore, discrepancy between the effects of plant *vs.* stand traits can emerge.

The roots of lucerne can therefore be regarded as a key part of the plant in terms of their importance for productivity of the plant and the stand. It is difficult, however, to measure these root traits. Furthermore, their contribution to the stand performance may be concealed by the effects of other natural factors such as time development. For successful application of knowledge about how lucerne root morphology may support agronomical stand traits, it is necessary to understand the relationships among root morphology traits, applied field management, and natural changes associated with changes over time and in plant density. Despite previous extensive research, there is a lack of multiyear studies investigating the development of lucerne root traits in field environments in order to clarify these relationships at the stand level. Therefore, a field study with lucerne sown in a common stand density was conducted over a 4-year period with the following aims (i) to compare the effect of root sampling periods and of three- and four-cut managements on lucerne root trait development at the plant and stand level; (ii) to evaluate advantages and disadvantages of root branching in the multi-year study; and (iii) to investigate relationships between root traits and lucerne varieties, cutting management, and root sampling periods. The study also focused on investigation of relationships between root morphology and important agronomic traits such as yield and root disease resistance. Clarification of these relationships could be valuable for optimization of lucerne root morphology evaluation in the field environment.

2. Materials and methods

2.1. Field experiment

The experiment was established in April 2006 on a clayey-loam Haplic Luvisol (clay 22.4%) at the experimental field station Červený Újezd (50°04′ N, 14°10′ E; elevation 410 m a.s.l.). The long term (50 year) mean annual temperature of the site is 7.7 °C and annual rainfall is 493 mm. Fifteen lucerne varieties (the French variety Europe, 13 Czech lucerne varieties, and one candidate variety) were established and managed under two cutting frequencies (three or four cuts per growing season). Cutting dates were determined by the flowering stage and bud stage (Kalu and Fick, 1983) for three and four cuts, respectively. A split-plot factorial design with four replicates was used, with cutting management as the main factor (main plots) and lucerne varieties as the subfactor (subplots). Consequently, there were 30 subplots of 7.2 × 2.5 m in each block. Plots were not fertilized.

The plots were established on 28th April by row sowing (with 0.125 m between rows), and the seeding rate was 700 germinated seeds per m^2 for all lucerne varieties (typically equivalent to about 15 kg seed per hectare). In the sowing year there were two harvests only, and in subsequent years the plots were cut three or four times per year depending on the cutting management treatment. Cutting dates and annual weather data are presented in Table 1. Fresh matter yield was assessed by harvesting and weighing from 10 m^2 in the centre of each subplot using a mower MF-70 with working width 1.4 m. A subsample from each plot was oven dried at 60 °C to enable determination of dry

Table 1
Annual mean temperature, cumulated precipitation, harvest dates, annual dry matter yield and root sampling dates and surface of sampling area for the four years of the study.

Year	2006		2007		2008		2009	
Annual temperature mean (°C)	8.8 (+1.11)[a]		9.7 (+1.94)[a]		9.2 (+1.5)[a]		8.8 (+1.07)[a]	
Annual cumulated precipitation (mm)	467 (−26.5)[a]		515 (+21.7)[a]		518 (+25.1)[a]		554 (+61.3)[a]	
Annual dry matter yield ($t\ ha^{-1}$)	2.46		14.95		15.45		17.05	
Harvest management	3 cut	4 cut	3 cut	4 cut	3 cut	4 cut	3 cut	4 cut
Cut 1	19 July		22 May	11 May	2 June	26 May	21 May	11 May
Cut 2	18 October		4 July	19 June	14 July	30 June	16 July	1 July
Cut 3			5 September	14 August	17 September	19 August	9 September	25 August
Cut 4				11 October		13 October		12 October
Root sampling period								
Spring Dates			11 April		21 April		8 April	
Sampling area (cm)			25 × 25		25 × 40		25 × 40	
Plant density			137	128	91	99	83	84
Autumn Dates	28 October		29 October		3 November		5 November	
Sampling area (cm)	25 × 25		25 × 30		25 × 40		25 × 50	25 × 60
Plant density	196	195	129	164	93	96	78	75

[a] Difference relative to long-term mean (1960–2010).