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Biological control in lucerne crops can negatively affect the development of root morphology, forage yield and quality

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Abstract: Root diseases of lucerne (*Medicago sativa* L.) play a significant role in reducing the persistence and productivity of stands; however, the potential of using biological control in lucerne crops has not yet been investigated. Our objectives were to determine the effect of biological crop protection on (i) lucerne plant density and root traits development and (ii) lucerne forage yield and nutritive value in a two-year field experiment. The lucerne stand was managed under three treatments of disease control: an untreated control and spraying with either Albit (hydrolysate of microorganisms containing microelements and poly-beta-hydroxy butyric acid) or Polyversum (mycoparasitic *Pythium oligandrum*) under five-cut utilization. Application of Albit resulted in a negative yield response, associated with a reduction of root branching, and it also reduced crude protein and increased crude fibre and water-soluble carbohydrates in the second cut of the first year of the experiment. Polyversum increased the percentage of infected plants relative to the control in the last year of the experiment. The study highlights that biological control of lucerne under field conditions may not always be beneficial because of the complex interactions between plant, biological preparation, and environment.

Keywords: Fabaceae; alfalfa; root disease; antifungal agent; fungicide

Root diseases are generally considered to be a serious factor limiting lucerne (*Medicago sativa* L.) persistence and yield through reduction of plant density and productivity (Gray and Koch 2004). No effective approved chemical fungicides are available for elimination of these diseases. Therefore crop protection is based on appropriate management practices such as improving the soil pH or ensuring adequate plant nutrition, as well sowing disease-resistant cultivars (Huang 2003), for which disease resistance has been shown to significantly improve forage yield in environments with severe root disease incidence (Lamb et al. 2006).

Biological control of fungal diseases has been investigated for more than 80 years, but commercial products have become available only in recent years (Suprapta 2012), and about 14 genera of fungi or bacteria are registered in Europe (Gerbore et al. 2014). For lucerne, some positive effects were reported after *Streptomyces* (Xiao et al. 2002) or *Bacillus cereus* application (Handelsman et al. 1990). Benhamou et al. (2012) summarized findings that *Pythium oligandrum*, a non-pathogenic soil-inhabiting mycoparasitic oomycete, can increase crop protection against fungal disease. The utilization of *P. oligandrum* has been focused on laboratory and greenhouse experiments, especially for annual crops (Kowalska and Zbytek 2015). For perennial forage crops, the positive effect of *P. oligandrum* on red clover yield has been reported by Pisarčik et al. (2020).

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In addition to biological control agents, there is also a group of plant stimulants that have potential for reducing disease. Morsy et al. (2011) tested the application of salicylic acid, K_2HPO_4, and neem (*Azadirachta indica*) oil on lucerne and reported a significant reduction of rust, downy mildew, and root rot infestation. The stimulator Albit, based on hydrolysate of microorganisms, microelements and poly-beta-hydroxy butyric acid has been tested in Russia, where its application reduced lucerne disease infestation and increased fresh matter yield by about 15–20% in comparison with the control, as a result of increased stand height and stem number (Kharchenko et al. 2008).

Although biological treatments represent an interesting means of reducing or eliminating lucerne root disease, there is a lack of field-based studies covering the Central Europe region. Therefore, the aim of this study was to investigate the effect of application of Albit and Polyversum on (i) lucerne plant density and root traits development and (ii) lucerne forage yield and nutritive value in a two-year field experiment. These comprehensive evaluations could be valuable for better understanding the simultaneous impact of application of biological treatments on lucerne root development, occurrence of root diseases, and stand performance.

MATERIAL AND METHODS

The field experiment was established in April 2016 in Drválovice in the Czech Republic (elevation 460 m a.s.l.). The long-term (30-year) mean annual temperature is 7.4°C, and cumulative annual rainfall is 545 mm. The soil type is loamy-sandy Cambisol (IUSS Working Group WRB 2015). The lucerne sowing rate was 700 viable seeds of cv. Pálava per m^2 with a row distance of 125 mm.

The lucerne stand was managed under three treatments of biological disease control (untreated control, spraying with Albit or Polyversum) with a five-cut schedule at the early bud stage in post-seeding years. The preparation of Albit represents a 0.62% concentration of poly-beta-hydroxy butyric acid supplemented with mineral nutrients (OOONPF Albit, Pushino, Russia), and Polyversum contains 1 000 000 active oospores of *Pythium oligandrum* M1 per gram (Biopreparáty spol. s.r.o., Úherce, Czech Republic). Applied rates and timing of application are summarized in Table 1. Polyversum was activated 3 h before application. Rates of preparations were applied in 300 L of water per hectare, always in humid weather without direct sunshine. The experiment was arranged in completely randomized blocks in four replicated plots of each of the 3 treatments, which resulted in 12 plots each with a harvested area of 10 m^2.

Forage and root sampling and evaluation. All dried forage samples from 10 cuts (5 in each year) were milled to pass through a 1-mm screen and were scanned with a Foss NIR System 6500 (Hilleroed, Denmark) equipped with a spinning sample module, in reflectance range 1100–2500 nm, bandwidth 2 nm, measured in small ring cups. The estimated qualitative traits (g/kg DM) were crude protein (CP), crude fibre (CF), water-soluble carbohydrates (WSC), and organic matter digestibility (OMD, %).

In each autumn, plant root systems were dug to a depth of about 20–25 cm in each plot. Plant density (PD, plant/m^2) was calculated from the number of plants per sample and the size of the root sampling area. For each plant, the tap-root diameter below the crown (TD, mm) and lateral root number per plant tap-root (LRN, when larger than 1 mm) were

Table 1. Annual temperature and precipitation, description of treatments, application dates of preparations, root sampling area, and sampling dates, and forage harvest dates, annual temperature, and precipitation for the two years of the study

			2017	2018
Annual temperature mean (°C)			8.4 (+1.0)*	9.6 (+2.2)*
Annual cumulated precipitation (mm)			539 (–6)*	401 (–144)*
		dose	application, sampling and harvest dates	
Treatment	untreated control	–	–	–
	Albit	40 mL/ha	23 May	15 May
	Polyversum	100 g/ha	23 May	15 May
Root sampling dates and sampling area			14 Nov; 50 × 12.5 cm	1 Nov; 50 × 12. 5 cm
Forage harvest dates			11 May, 16 Jun, 19 Jul, 24 Aug, and 11 Oct	4 May, 1 Jun, 19 Jul, 29 Aug, and 9 Oct

*difference to long-term mean (1981–2010) evaluated. Fine root mass (FRM, less than 1 mm) occurrence was scored subjectively from 1 to 5 with scores of 1, 3, and 5 indicating none, moderate, and many, respectively. The percentage of branch-rooted plants (RB) was calculated for each sample. The root potential index (RPI) integrating TD and plant density was assessed according to Hakl et al. (2017). Plant root disease score (PRDS) was scored subjectively and based on discoloration on a cross-cut of the tap-root with ratings from 0 (= healthy plant) to 7 (= dead plant) following Hakl et al. (2017). The ratio of infected plants (IP) was assessed as a proportion of plants with visible root discoloration, and PRDS values were averaged for infected plants.

Statistical analysis. The effect of biological control on root traits was analyzed by one-way ANOVA within each year where PD was used as a covariate for some analyses. Influence on annual forage yield was evaluated by two-way ANOVA. Forage yield and nutritive value in each cut were analyzed by three-way ANOVA with interaction. Significant differences between means were reported using the Tukey's *HSD* (honestly significant difference) test at α = 0.05. Principal component analysis (PCA) was used for interpreting the relationships among forage yield and quality, root morphology and root disease occurrence. The PCA was used for calculating a component weight for the investigated variables (Meloun and Militký 2011). All these analyses were carried out using the Statistica program (StatSoft 2012).

RESULTS

Application of Albit significantly reduced FRM and tended to the lowest values of TD ($P = 0.053$) and RB ($P = 0.063$) among all treatments (Table 2) in 2017. In 2018, the Polyversum treatment showed lower FRM and higher IP in comparison with the untreated control. Differences between years were detected in LRN and FRM, with higher values observed in 2018.

Forage yield was significantly reduced under treatment with Albit application compared with both the other treatments (Table 3). The most important negative impact was observed in 2017 when Albit caused stem dying-off and discoloration (yellowing) after application. The stand was regenerating until the end of the growing season. The Polyversum treatment did not differ from the untreated control. Interaction of cut × year showed significant differences among all cuts in 2017, whereas in 2018 only the first cut was different from others (data not shown).

Application of Albit reduced forage CP in contrast to Polyversum, with a simultaneous increase in WSC content in comparison with the untreated control. Differences between years were observed for CP and WSC, whereas cuts were significantly different in all

Table 2. Effect of biological control and year on lucerne plant density (PD); tap root diameter (TD); root potential index (RPI); percentage of branch-rooted plants (RB); lateral root number (LRN); fine root mass (FRM); percentage of infected plants (IP) and plant root disease score (PRDS)

Year	Treatment	PD	TD	RPI	RB	LRN	FRM	IP	PRDS	*n*
2017	untreated control	158	8.00	89.7	78.7	2.32	2.91^b	66.7	1.95	60
	Albit	210	6.77	81.6	57.8	2.71	2.12^a	66.1	2.05	62
	Polyversum	179	7.92	94.7	70.5	2.38	2.40^{ab}	70.6	1.73	68
	P	0.674	0.053	0.913	0.063	0.613	< 0.001	0.839	0.483	
	density (covariate)	–	0.449	–	0.042	0.028	0.908	–	–	
2018	untreated control	165	7.97	88.1	77.1	3.37	3.87^b	48.9^a	2.09	47
	Albit	172	7.90	87.0	72.4	3.08	3.60^{ab}	57.1^{ab}	1.75	49
	Polyversum	161	7.75	86.3	74.5	2.56	3.06^a	76.1^b	1.83	46
	P	0.990	0.934	0.995	0.867	0.229	0.007	0.023	0.424	
	density (covariate)	–	< 0.001	–	0.024	< 0.001	0.018	–	–	
2017		182	7.48	88.6	68.1	2.36	2.45	67.9	1.90	190
2018		166	7.99	87.1	75.8	3.11	3.53	60.6	1.87	142
P		0.649	0.127	0.910	0.121	0.003	< 0.001	0.168	0.866	

P – probability of one-way ANOVA, different letters indicate statistical differences between treatments for Tukey's *HSD* (honestly significant difference), α = 0.05

Table 3. Effect of year, biological control and cut order on lucerne dry matter forage yield (DMY, t/ha); crude protein (CP, g/kg); crude fibre (CF, g/kg); water-soluble carbohydrates (WSC, g/kg) and organic matter digestibility (OMD, %)

		Annual DMY	Cut DMY	CP	CF	WSC	OMD
Year	2017	12.19	2.44	210	213	40.9	74.5
	2018	7.64	1.53	214	216	39.0	74.6
	P	< 0.001	< 0.001	0.032	0.130	0.003	0.673
Treatment	untreated control	10.58^{b}	2.12^{b}	213^{ab}	215	38.6^{a}	74.5
	Albit	8.83^{a}	1.77^{a}	208^{a}	217	41.1^{b}	74.5
	Polyversum	10.33^{b}	2.07^{b}	215^{b}	211	40.2^{ab}	74.6
	P	0.040	0.003	0.011	0.110	0.004	0.960
Cut	1		3.82^{d}	209^{a}	217^{a}	47.5^{c}	74.8^{b}
	2		2.20^{c}	212^{a}	221^{a}	40.5^{b}	75.4^{bc}
	3		1.67^{b}	208^{a}	223^{ab}	35.9^{a}	73.1^{a}
	4		1.40^{b}	194^{b}	230^{b}	36.3^{a}	73.2^{a}
	5		0.84^{a}	238^{c}	180^{c}	39.7^{b}	76.2^{c}
Cut			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Year × treatment		0.932	0.896	0.309	0.162	0.917	0.210
Cut × year			< 0.001	0.056	< 0.001	< 0.001	< 0.001
Cut × treatment			0.913	0.256	0.415	< 0.001	0.153
Cut × treatment × year			0.497	0.048	0.026	0.009	0.474

P – probability; two or three-way ANOVA with interactions, different letters indicate statistical differences between treatments or cuts for Tukey's *HSD* (honestly significant difference), α = 0.05

traits of forage quality. Interaction with treatments was significant only with cut for WSC, where the Albit treatment had higher WSC content than the control in the second cut. Analyses of triple interactions showed that differences in forage quality (CP, CF, WSC) between treatments were detected, especially in the first year from the second to the third cut (data not shown) where the Albit treatment had forage with lower CP and higher WSC and CF.

Component weights of PC1, PC2, and PC3 explained together 93% of the variability (Figure 1). The PC1 axis characterizes the relationships between density, root morphology (RB, TD), and forage quality (OMD, CP, and WSC), whereas PC2 and PC3 demonstrate negative relation of yield to root branching (LRN, FRM) or forage quality (WSC). The analysis separated the biological treatment Albit (right side of Figure 1) and Polyversum (left upper side of PC3) from the untreated control.

DISCUSSION

Effect of biological control on forage yield and root disease score in association with root morphology. Application of Albit caused an average reduction of forage yield of about 16.5% with lower values in the first (–11.8%) than in the second year (–23.7%) of the experiment. This is in contrast to the results of Kharchenko et al. (2008), where Albit was associated with increased fresh yield of lucerne of about 15–20%. The negative effect on forage yield obtained in our study could be associated primarily with reduced intensity of root branching and TD in lucerne under this treatment in 2017, corresponding with PCA analysis. These results are in line with a positive correlation between lucerne yield and root branching published by Hakl et al. (2017). Polyversum reduced FRM in the last year of the experiment without any negative correlations with TD, RB or forage yield. In summary, application of Albit may cause a negative lucerne yield response through damage to stem regrowth with subsequent reduction of root development and branching. The negative yield effect was more pronounced in the dry year of 2018. Our study suggests that an inappropriate date of foliar application about crop management and environment can probably cause a negative lucerne yield response, which is in contrast to the positive effect reported by Kharchenko et al. (2008).

A positive effect of *P. oligandrum* in the reduction of disease infestation has been reported in many studies, especially under greenhouse or laboratory conditions (e.g. Daraignes et al. 2018). In last year of our field experiment, application of Polyversum caused even significant

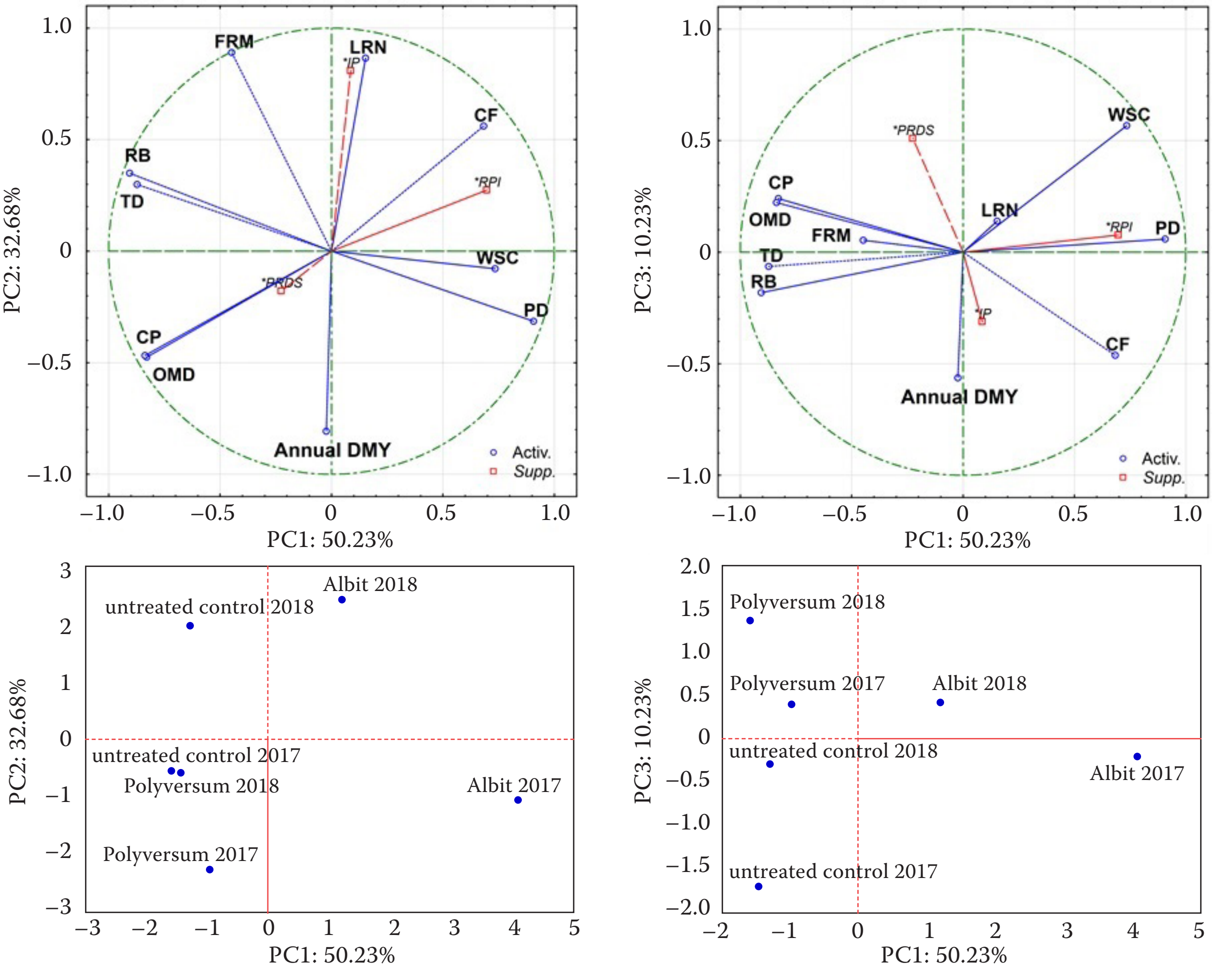

Figure 1. Relationships between forage and root traits under biological control of lucerne over the two years analyzed by principal component analysis. Annual DMY – dry matter forage yield; CP – crude protein; CF – crude fiber; WSC – water soluble carbohydrates; OMD – organic matter digestibility; PD – plant density; TD – tap root diameter; RPI – root potential index; RB – percentage of branch-rooted plants; LRN – lateral root number; FRM – fine root mass; IP – percentage of infected plants; PRDS – plant root disease score

increase of IP up to 76%, in contrast to 49% at untreated control. However, any negative yield effect was not observed due to low PRDS from 1.7 to 2.1. These results correspond with the few studies reporting negative effect of *P. oligandrum* application. Boček et al. (2013) reported a negative effect on disease incidence in strawberries in one year out of two with significantly reduced yield.

A negative effect of Polyversum application in lucerne seems to have been promoted under conditions of drought stress in the second year of the experiment. The growth period from April to September 2018 was warm (+3.5°C above normal) and dry (–75 mm below normal) with a marked reduction in forage yield from 12.2 to 7.6 t/ha. This is in line with the negative effect of drought on lucerne productivity through reduced stem length and density (Saeed and El-Nadi 1997). Our study also demonstrated that this stress also resulted in cessation of common development of root morphology traits. Tap-root diameter did not differ between years; this was in contrast to regular annual increase about 3 mm per year reported by Hakl et al. (2017).

Influence of biological control on forage quality. Application of Albit led to generally lower forage quality in terms of lower CP and higher CF content in the harvested forage of some cuts. On the other hand, there was a higher WSC content. Lopachev et al. (2013) reported that high doses of Albit reduced the content of CP in barley, similar to the CP decrease in lucerne after Albit application. The impact on plant CP content could be associated with fact that the poly-beta-hydroxy butyric acid contained in Albit is also produced by Rhizobia as an energy source (Kıvanc and Dombaycı 2016). According to Ratcliff et al. (2008), there can be a conflict of interest between rhizobia and legumes due to a metabolic tradeoff between N_2 fixation and poly-beta-hydroxy butyric acid accumulation. It seems that application of preparations containing poly-beta-hydroxy butyric acid in lucerne stands could influence these relationships with negative impacts on CP content. Results of PCA also suggest possible correlations among root morphology traits and forage quality, but more definite conclusions would need to be supported by monitoring of stand structure parameters.

In summary, the different methods of disease control should be tested under field conditions because results show that negative effects on forage yield and quality can be observed. There are questions about the optimum timing of the application during the cutting schedule and also the impact of drought and temperature on the efficacy of the applied preparations. This study does not diminish the potential importance of biological control methods for forage legume crops, but it does highlight that applications under field conditions may not always give beneficial outcomes, in contrast to results of laboratory experiments, because of complicated interactions between plant, organism/preparation, and environment.

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