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ORIGINAL PAPER

Arbuscular mycorrhizal fungi colonization and phosphorus nutrition in organic field pea and lentil

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Abstract Phosphorus (P) can be low in soil under low input organic management; however, beneficial crop plant associations with arbuscular mycorrhizal fungi (AMF) are known to promote crop nutrition and increase phosphorus uptake. Thus, management strategies that promote AMF associations are particularly desirable for low-input cropping systems. The objectives of this study were to determine the impact of seeding rate on AMF colonization and the impact of AMF colonization on P concentration and uptake by organically grown field pea and lentil. Field experiments examined the impact of three seeding rates of field pea and lentil on P uptake and crop yield. Phosphorus accumulation was examined further in a controlled growth chamber experiment, in which field pea was sown at rates corresponding to those used in the field and harvested at 10-day intervals until 50 days after emergence. In the field, the level of AMF colonization of roots remained at 80% for field pea, while colonization of lentil increased with increasing seeding rates from 77% to 88%. The level of AMF colonization of field pea achieved in the growth chamber after 50 days was 80% for the two highest seeding rates and 60% for the low seeding rate. The rate at which AMF colonization occurred did not vary between treatments. Ultimately, AMF colonization level did not affect P accumulation. In contrast to several previous studies, both field and growth chamber experiments revealed that AMF colonization was not reduced at higher seeding rates. These results suggest that organic farmers may increase seeding rates without adversely affecting P nutrition.

Keywords Organic · Seeding rate · Field pea · Lentil · Phosphorus (P) · Arbuscular mycorrhizal fungi (AMF)

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Introduction

Pulse crops are an important part of an organic crop rotation. Organic pulse seed garners high premiums and can be a good source of income for farmers. These crops also fix up to 80% of their nitrogen (N) requirement from atmospheric N via N_2 fixation and can provide a positive N balance for the following crop (Corre-Hellou and Crozat 2005). Nutrient availability in organically managed soils can be low, and growing pulse crops are an important way to maintain soil nutrient levels. Soil nutrient levels in organic production systems are highly dependent on individual management and time under organic production but are generally considered to be lower than in conventionally managed soils (Gosling and Shepherd 2005; Watson et al. 2002). Mäder et al. (2002) found that nutrient inputs into organic systems were 34% to 51% lower than in conventional systems.

Phosphorus is an important macronutrient in the early stages of plant growth for development of reproductive parts, energy storage, and transfer, as a component of many structural compounds and for increased root growth. On many organic arable farms where animal manure is not

applied, P is the limiting nutrient to crop production (Malhi et al. 2002). There is a general consensus that the P concentration in organically managed soils is depleted over time (Entz et al. 2001; Gosling and Shepherd 2005; Malhi et al. 2002; Oehl et al. 2002). Low soil P levels are thought to reduce N_2 fixation by legume crops (Oberson et al. 2007; Ozanne 1980). In a study by Malhi et al. (2002), higher soil N levels in an organic production system than in a reduced-input production system were suggested to occur due to the very low levels of P, limiting crop growth, and uptake. Consequently, methods to increase crop uptake of P are important for organic systems where P is limiting.

An important mechanism many plants use to increase P acquisition is specialized root structures and increased root growth and associations with soil microorganisms, specifically arbuscular mycorrhizal fungi (AMF) (Gahoonia et al. 2005; Richardson 2001). Arbuscular mycorrhizal fungi are ubiquitous in soil (Mosse et al. 1981) and colonize approximately 80% of all plant species, including field pea and lentil (Smith and Read 1997). Arbuscular mycorrhizal fungi effectively colonize and extend the root systems of plants allowing for greater nutrient uptake while plants supply the fungi with C as an energy source (Gupta et al. 2000). In organic production systems where P is generally limiting, the symbioses between crops and AMF are especially important, as AMF contribute to soil-immobile nutrient uptake (Harley and Smith 1983). According to Kahiluoto et al. (2009), AMF contributions to soil quality and functional capacity of soil are particularly favored in low-input systems as compared to conventional cropping systems. With a high rate of AMF colonization in soil where P availability is low, more P can be accessed from the soil for plants through absorption from an extensive hyphal network (Harley and Smith 1983), thereby increasing potential uptake of other nutrients.

In organic production systems, where herbicide use is prohibited, producers can alter seeding rates to provide a competitive advantage to the crop (Stockdale et al. 2001; Nazarko et al. 2003). Increased crop density has been shown to reduce weed densities (Townley-Smith and Wright 1994), which is a desirable outcome; however, increased crop density may simultaneously affect beneficial associations with AMF. A number of studies revealed decreased AMF colonization with increasing plant density (Abbott and Robson 1984; Jakobsen and Nielsen 1983; Koide and Dickie 2002; Schroeder and Janos 2005). Warner and Mosse (1983), however, found that increasing the density of clover increased colonization when AMF inoculum originated from a single location. While much research has been devoted to the study of plant density and AMF colonization rates, little research has been conducted examining whether colonization levels change with seeding rates within organic production systems.

Given the importance of the AMF association, particularly in organic production systems, there is a need to examine the impact of seeding rate on this association. The objectives of this study were: (1) to determine the impact of seeding rate on AMF colonization and (2) to determine if AMF colonization rate and level affects P concentration and uptake by field pea and lentil in an organic system.

Materials and methods

Field study

Experimental design and plot management

Sites were established on preexisting organic farms in 2005 and 2006. The site locations were south of Vonda and west of Delisle, SK in 2005 and Vanscoy and Vonda, SK in 2006. Sites were organically managed for approximately 3 years at Vanscoy, 8 years at Delisle, and 20 years at Vonda. Site descriptions are given in Table 1. In each location, organic lentil ("CDC Sovereign") and field pea ("CDC Mozart") were seeded in a randomized complete block design with four replicate blocks that included three target densities of 15, 94, and 375 plants per square meter and 10, 62, and 250 plants per square meter , respectively. The single plot size for each treatment was 2×6 m. Crops were seeded at a 23-cm row spacing at a depth of 2.5 cm using a cone seeder with an offset disk drill. The number of seeds planted was increased based on the percentage germination determined from a germination test to achieve target plant densities. When seeding, Nodulator® granular *Rhizobium* inoculant (Becker Underwood, Saskatoon, SK) was placed with the seed at the recommended rate for lentil.

Preseeding tillage was performed by the landowner at all sites to manage early-emerging weeds. In-crop harrowing using an Einbock tined weeder was performed in the same direction as the crop row with two passes for all seeding rates, with the tines set to 45° and a travel speed of 10 km h^{-1}, when the crop was judged to be sufficiently resilient (approximately 1 month after seeding). Crop densities were determined by counting the number of crop plants in two randomly selected 0.25 m^2 quadrats in each plot approximately 1 week after the in-crop harrowing was performed. A hand harvest of four 1-m long rows (equivalent to 0.81 m^2) was taken from each plot at harvest to measure phosphorus in seed and straw.

Soil analysis

Soil samples for general site characteristics were taken to a depth of 15 cm at five random locations within each trial area just prior to seeding. Samples were combined and

Table 1 Locations and soil characteristics in the upper 15 cm of the soil profile of trial sites for four site–years in central Saskatchewan in 2005 and 2006

Site	2005		2006		Growth
	Vonda	Delisle	Vonda	Vanscoy	Chamber[a]
Location	52°18′25″ N	51°49′31″ N	52°17′50″ N	51°57′24″ N	52°19′24″ N
	106°06′03″ W	107°19′01″ W	106°06′05″ W	106°56′44″ W	106°01′35″ W
Soil association	Oxbow	Elstow	Oxbow	Asquith	Oxbow
Soil zone	Black	Dark Brown	Black	Dark Brown	Black
Soil texture	Clay loam	Loam	Clay loam	Clay loam	Sandy loam
pH	8.4	7.2	8.2	6.3	8.3
EC (mS cm^{-1})	0.2	0.3	0.2	0.2	0.2
Nitrate (kg ha^{-1})	3.1	4.6	2.4	1.8	2.6
Phosphorus (kg ha^{-1})	2.2	4.4	1.1	>9.9[b]	4.4

[a] Characteristics of soil collected from the top 15 cm of the soil profile mixed with silicate sand (1:1) (*v*/*v*) used in the growth chamber

[b] High value for Vanscoy soil test P may have been due to fall manure application

analyzed for pH, electrical conductivity, inorganic N (2.0 M KCl extract) (Maynard and Kalra 1993), and phosphorus (modified Kelowna extract) (Qian et al. 1994) (Table 1).

Plant analysis

Biomass samples taken at harvest were air-dried in a covered facility in cloth bags for 1 week then moved indoors and dried further at approximately 30°C. Samples were weighed prior to threshing and then threshed mechanically. Seed weight was determined from the threshed, cleaned seed. Seed and straw samples were ground for P analysis. All samples were assessed for P content by acid digestion (Thomas et al. 1967). Phosphorus levels in the digested samples were determined colorimetrically using a Technicon AutoAnalyzer II (Technicon Industrial Systems, Tarrytown, NJ).

Root sampling and analysis

Five representative bulk root samples from each treatment were collected by excavating the soil from around the crop rows to a depth of 15 cm at the bud stage (Germida and Walley 1996). Samples were stored at 4°C until soil was washed from the roots by wrapping plastic mesh around the sample and gently massaging underwater until only roots and debris were left within the mesh enclosure. The roots were separated from the debris by immersing in water and removing the roots using tweezers. Roots were sliced into 1-cm sections and stored at 4°C in a 50% ethanol solution until further analysis was performed (Koske and Gemma 1989). Percent colonization of roots by AMF was determined using the ink and vinegar staining technique described by Vierheilig et al. (1998). Briefly, root samples were placed into cassettes and cleared by immersion in a boiling 10% KOH solution for 12 min. Samples were then rinsed in tap water and placed in a boiling solution of 5% black India ink and vinegar for 3 min to stain the fungal structures. Samples were rinsed again with tap water and placed into a destaining solution of tap water and a few drops of vinegar for 7 days at room temperature (Vierheilig et al. 1998). Percent colonization was assessed by the gridline intersect method (Giovannetti and Mosse 1980). Root samples with a fresh weight of approximately 1 g were placed in a Petri dish with gridlines 0.5 cm apart. Roots were laid out so as to not overlap and were assessed with a dissecting microscope at ×50 magnification for any AMF structures, including hyphae and vesicles. One hundred observations were recorded for each sample, and samples from each of the four blocks were sampled for the low, middle, and high seeding rates at each site.

Growth chamber experiment

Experimental design

The soil used for this experiment was collected from the Ap horizon (0–15 cm) of an organically managed field near Vonda that had not had peas grown on it for the previous 3 years to reduce potential incidence of disease. The soil was air-dried for 2 weeks then mixed with silicate sand at a ratio of 1:1 (*v*/*v*). A bulk soil sample was collected from the field, and the soil characteristics were assessed by ALS Laboratory Group as previously described (Table 1).

Three densities of organic field pea ("CDC Mozart") corresponding to low (10 plants per square meter), medium (62 plants per square meter), and high (250 plants per square meter) seeding rate targets used in the field were

planted in pots with a surface area of 615 cm^2 and 20-cm depth. Four replicates and five sampling times were used for a total of 60 pots. The low seeding rate treatment contained one plant per pot, the medium seeding rate contained four plants per pot, and the high seeding rate contained 16 plants per pot. Two seeds were planted for the low rate, six seeds for the midrate, and 20 seeds for the high rate, and then plants were thinned at emergence to achieve the target plant densities. Seeds were pregerminated in the dark on moist paper towel for 72 h and then planted to a depth of 2 cm (Manitoba Agriculture 2004). Seeds were inoculated with *Rhizobium leguminosarum* (strain P108) from Philom Bios (Saskatoon, SK). The inoculant was diluted in a phosphate-buffered saline solution to a concentration of 4×10^8 cfu mL^{-1}. One milliliter of solution was pipetted onto each seed before covering with soil.

Pots were placed in a growth chamber and covered with a transparent polypropylene sheet after seeding to conserve soil moisture. The sheet was removed once all plants had emerged and were thinned 6 days after the seeds were planted. After thinning the plants to the target density, plastic beads were placed on the soil surface to reduce evaporation. Pots were watered to 65% of field capacity by weight every day. The growth chamber provided a 16 h photoperiod and day/night temperatures of 21/18°C. Light intensity in the growth chamber varied by up to 50% between locations, from 262 to 525 μmol m^2 s^{-1}. The pots were rerandomized every 5 days. Weed seedlings that emerged were removed daily.

Biomass sampling and analysis

Harvests occurred at 10-day intervals, beginning 13 days after planting or 10 days after emergence (AE). Four replicates of each seeding rate were selected at random for each harvest. The aboveground plant tissue was removed, placed in a paper bag, and dried for 72 h at 60°C. Plant biomass was weighed and ground, and P content in both seed and straw samples was determined (Thomas et al. 1967). Phosphorus levels in the digested samples were determined colorimetrically using a Technicon AutoAnalyzer II (Technicon Industrial Systems, Tarrytown, NJ). Soil was washed from the roots with pressurized water. Roots were collected, stained, and assessed as previously described.

Statistical analysis

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute 2004) with block and site–year as random effects for the field study. Sites and years were combined for each crop, as the site–year×treatment interaction was not significant at $P\leq 0.05$. Percentage data (i.e., AMF colonization) were arcsine-transformed prior to analysis. Nutrient data were transformed using natural log or square root transformations as required to meet the assumption of homogeneity of variance. Back-transformed data are presented. Treatment effects were considered significant at $P\leq 0.05$.

A logistic growth equation was fitted to the means for P concentration and uptake data using the PROC NLIN procedure of SAS (SAS Institute 2004). The logistic function has been used to describe theoretical P uptake by mycorrhizal plants (Janos 2007) and actual P uptake by crops, such as winter wheat (Barraclough 1989). A four-parameter logistic equation was used in this case, as it provided the best fit to the data using Sigmaplot 10.0 (Systat software, Inc.):

$$y = y_0 + a/\left[1 + (x/x_0)^b\right] \quad (1)$$

where y_0=the lower asymptote of P uptake, a=the upper asymptote of P uptake, x_0=days after emergence where half of P uptake occurs, and b=the slope factor that describes how quickly P is increasing in the plant.

Results

AMF colonization

In the growth chamber experiment, a visual assessment of the roots at all harvests indicated good nodulation of all treatments. There were no noticeable differences in degree of nodulation between treatments; however, nodules on roots in the highest density treatment were slightly smaller than in the other two treatments. The assessment of roots from the growth chamber experiment for AMF colonization indicated a steady increase in percent colonization between the first and third harvests (Fig. 1). After the third harvest 30 days AE, the lowest plant density reached a plateau at approximately 60% root colonization whereas the higher two plant densities continued to experience increased AMF colonization. At the final harvest 50 days AE, both the medium and high seeding rate treatments showed significantly higher root colonization rates than the low seeding rate treatment. The medium and high density growth chamber treatments reached an average root colonization rate of over 80% at 50 days AE (Fig. 1).

The field experiment establishment rates resulted in actual plant densities ranging between 61% and 83% of the target density as seeding rate increased. Actual mean densities were 7, 46, and 175 plants per square meter for field pea and 12, 77, and 229 plants per square meter for lentil. Field samples supported the findings of the growth chamber experiment in that relatively high levels of AMF root colonization were achieved (Fig. 2). Roots from the

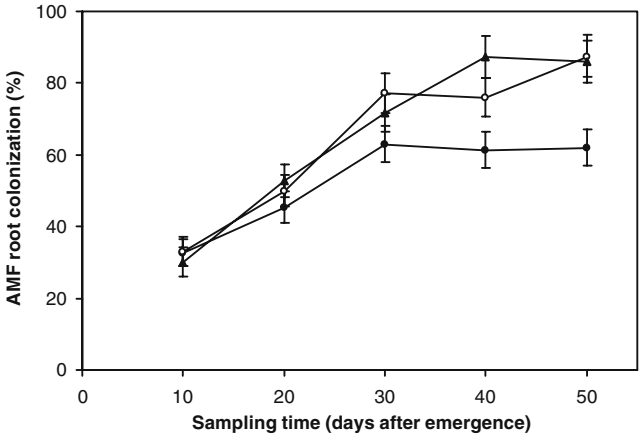

Fig. 1 Percent of field pea root colonized by AMF at five harvest dates in the growth chamber. Plant densities correspond to low (10 plants per square meter; *filled circle*), medium (62 plants per square meter; *white circle*), and high (250 plants per square meter; *filled triangle*) target densities used in field trials

low, medium, and high seeding rates in the field were assessed for both crops. Field peas showed considerable variability within treatments, but no significant differences were detected between the seeding rates (Fig. 2). Conversely, lentil root colonization levels showed significant differences between the three seeding rates (15, 94, and 375 viable seeds per square meter) and less variability within treatments (Fig. 3). The root colonization levels of the three lentil seeding rates were 77%, 83%, and 88% for the low, middle, and high seeding rates, respectively. All plant densities for both crops exhibited high AMF colonization levels.

Phosphorus concentration and uptake

Phosphorus concentration in field pea in the growth chamber experiment did not show any clear trend between plant densities. At the final harvest 50 days AE, the medium and high plant density treatments had P concentrations of 2.5 mg g^{-1}, while the low plant density treatment showed a P concentration of 3.4 mg g^{-1}. Phosphorus uptake per plant was similar for all three treatments for the first three harvests then significantly different between all seeding rates at the fourth and fifth harvests (40 and 50 days AE; Fig. 4). The final values for P uptake per plant 50 days AE were 93.4, 50.2, and 24.4 mg P per plant for the low, medium, and high seeding rates, respectively. While the lowest plant density showed the highest P uptake per plant, the opposite occurred when assessing each pot. Total P uptake per pot increased with increasing plant density (Fig. 4). At the final harvest, the highest plant density removed 385 mg P per pot, the medium density removed 200 mg P per pot, and the lowest density removed 105 mg P per pot in the aerial biomass. Phosphorus uptake per plant decreased with increasing plant density (Fig. 5).

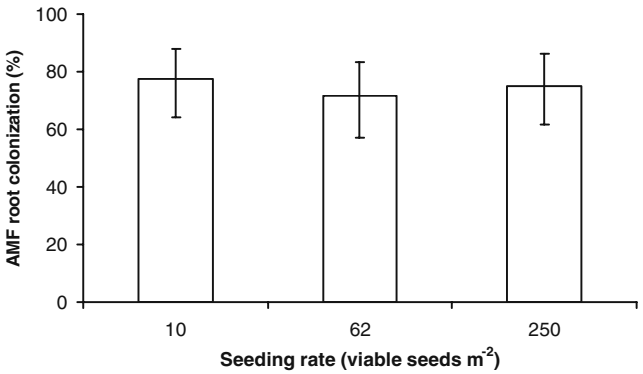

Fig. 2 Impact of seeding rate on root colonization by AMF in organic field-grown field pea. Data combined for sites in central Saskatchewan in 2005 and 2006. *Bars* indicate standard error of the means. Differences were not significant at $P \leq 0.05$

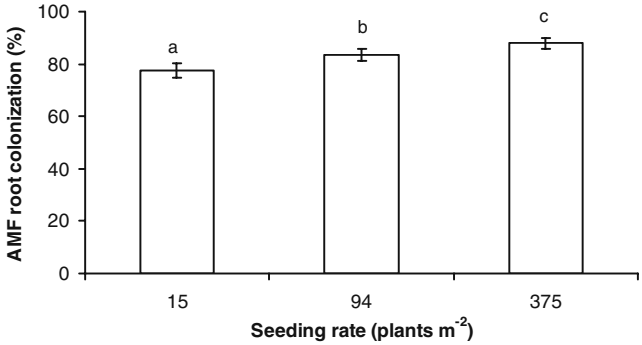

Fig. 3 Impact of seeding rate on root colonization by AMF in organic field-grown lentil. Data combined for sites in central Saskatchewan in 2005 and 2006. *Bars* indicate standard error of the means. *Letters* denote significant differences at $P \leq 0.05$

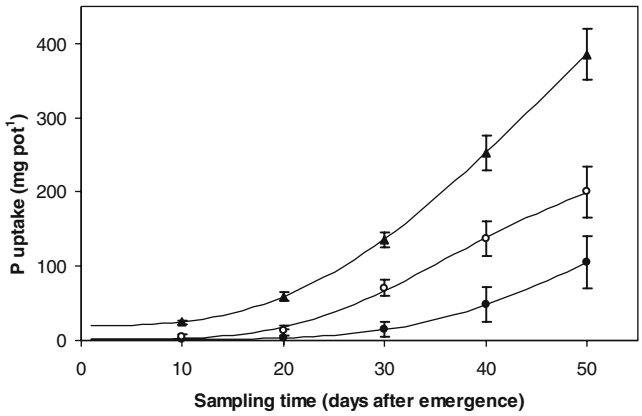

Fig. 4 Phosphorus uptake per pot by field pea grown in organically managed soil in a growth chamber at five sampling times. A four-parameter logistic equation was fitted to the data for each plant density. Plant densities are one plant per pot (*filled circle*), four plants per pot (*white circle*), and 16 plants per pot (*filled triangle*). The data point for the first harvest of the lowest plant density was the result of combining all four reps to accumulate enough plant material for a single analysis

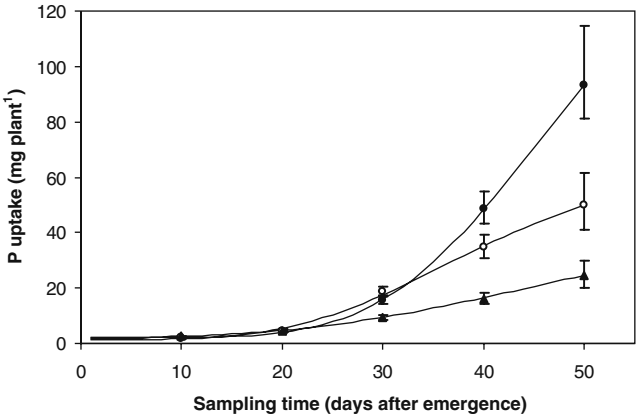

Fig. 5 Phosphorus uptake per plant by field pea grown in organically managed soil in a growth chamber at five sampling times. A four parameter logistic equation was fitted to the data for each plant density. Plant densities are one plant per pot (*filled circle*), four plants per pot (*white circle*), and 16 plants per pot (*filled triangle*). The data point for the first harvest of the lowest plant density was the result of combining all four reps to accumulate enough plant material for a single analysis

Phosphorus removal per pot was significantly higher in the high density treatment for the first, second, and fifth harvest dates. At the third and fourth harvests, all treatments were significantly different. The logistic curves fitted to the data were similar to those for P uptake per plant (Fig. 5) except that the curves representing the lowest and highest plant densities were reversed. The upper asymptote and slope values for the one plant per pot treatment were higher than the 16 plant per pot treatment when comparing P uptake per plant (Table 2). The lower asymptote values (y_0) were similar between treatments. When comparing treatments in terms of P uptake per pot, again, the slope value was higher for the one plant per pot treatment than the 16 plants per pot treatment (Table 2). In this case, however, the upper asymptote value was much higher for the 16 plants per pot treatment than any other. Another finding for the P uptake per pot measurements was that the starting value for P uptake (y_0) was higher for the 16 plants per pot treatment than any other. This is not surprising as there were many more plants taking up P at the highest density than at the medium and low plant densities.

In the field experiment, there were no significant differences between treatments in P concentration for seed or straw in field pea or lentil (Table 3). There were differences, however, in P uptake. As seeding rates increased from 15 to 375 plants per square meter, seed P increased from 0.6 to 3.8 kg ha^{-1} and straw P increased from 0.05 to 1.7 kg ha^{-1} for lentil. In field pea, seed P increased from 0.5 to 3.8 kg ha^{-1} and straw P increased from 0.2 to 2.7 kg ha^{-1} as seeding rate increased from 10 to 250 plants per square meter (Table 3). The increases in P uptake were due to increased yield and biomass as seeding rate increased, as P concentrations did not vary significantly between seeding rates.

Discussion

While establishment rates for field pea and lentil grown in the field varied from 61% to 83%, they fell within a normal range for the region. Establishment rates of 60–92% were reported for field pea grown in Saskatchewan and Alberta in a conventional management study (Johnston et al. 2002). Mycorrhizal fungi colonized approximately 80% of crop roots in the growth chamber experiments. This is similar to colonization rates recorded by other researchers. Inoculated field bean roots showed 80% colonization by AMF in a growth chamber experiment (Kucey and Bonetti 1988). Arbuscular mycorrhizae colonized approximately 75% of plant roots 50 days after field pea seedling emergence in a conventional field study (Jakobsen and Nielsen 1983).

In the present study, AMF colonization of roots increased in field-grown lentil and in the growth chamber-grown field pea with increased plant density and remained steady in field-grown field pea. This refutes considerable research that has shown a reduction in AMF colonization as plant density increases (Jakobsen and Nielsen 1983; Koide and Dickie 2002; Schroeder and Janos 2005). Jakobsen and Nielsen (1983) found that as root density increased, AMF colonization levels decreased from 80% to 30% in barley. Schroeder and Janos (2005) found a negative correlation between mycorrhizal colonization and plant density of *Capsicum annuum* and *Zea mays* grown in pots in a growth chamber experiment similar to the present one. Schroeder and Janos (2005) and Abbott and Robson (1984) suggest that the reason for reduced benefits of AMF at high plant densities was a reduction in the cost/benefit ratio in supplying the AMF with carbon in exchange for increased P uptake.

One of the several factors that could have contributed to the higher AMF colonization of crop roots at higher seeding rates for the field-grown lentils and in the growth chamber is the proximity of roots to AMF hyphae. Warner and Mosse (1983) found that hyphae from AMF could colonize roots up to 20 mm away. Another factor in the growth chamber experiment was the low soil P, where the association with AMF may have been more important to acquire more soil P for high plant densities than at the lower plant densities. Galvez et al. (2001) found that the colonization potential was higher in soils where the P concentration was lower. Plants grown in soil high in P generally have lower levels of colonization by AMF (Mosse et al. 1981).

The difference in results on AMF colonization between the field and growth chamber studies was likely due to the

Table 2 Parameter estimates (±S.E.) for Eq. 1 fitted to P uptake data for growth chamber field pea biomass

Pea density	Parameter estimates			
	a	*b*	x_0	y_0
P uptake per plant				
1 plant per pot	172.0 (20.72)[a]	−5.0 (0.36)	48.8 (2.32)	2.0 (0.63)
4 plants per pot	70.9 (17.94)	−3.9 (0.96)	41.0 (5.46)	1.4 (1.73)
16 plants per pot	65.5 (0.51)	−2.8 (0.01)	63.1 (0.29)	2.2 (0.01)
P uptake per pot				
1 plant per pot	272.1 (0.73)	−4.9 (0.01)	55.2 (0.01)	1.6 (0.01)
4 plants per pot	287.7 (71.21)	−3.9 (0.96)	41.0 (5.32)	1.7 (6.91)
16 plants per pot	976.6 (87.85)	−2.9 (0.10)	59.6 (3.15)	19.8 (1.56)

[a] ±Standard error in parentheses

constraints of the pot experiment in terms of the quantity of soil available for roots to explore. The field experiment offered a greater volume of soil for root exploration and nutrient uptake, and field pea and lentil in a field situation may have had greater access to nutrients than those confined to pots in a growth chamber. Despite this, the growth chamber pot experiment provided valuable data regarding AMF colonization at varying plant densities without the compounding factors of climate, varying AMF inoculum, soil variability, and weed growth.

Phosphorus nutrition for field peas and lentils is particularly important in early growth and development of the plants. The rate of AMF colonization for field pea during the first 30 days AE in the growth chamber experiment was consistent between seeding rates (Fig. 1). This indicates that there is no nutritional advantage or disadvantage to the crop based on seeding rate. Despite high AMF colonization of roots at higher plant densities in the growth chamber experiment, P concentration decreased as plant density increased. The results of this study indicate that availability of AMF for root colonization is not a limiting factor in this particular soil. In this case, a potential reduction in plant-available P may have increased the rate and level of AMF colonization of plant roots at high densities. The steady level of AMF colonization for the low plant density between 30 and 50 days AE may indicate that the plant was likely able to access enough P for growth and did not require further colonization by AMF for that purpose. The medium and high plant densities, however, increased AMF colonization another 20% beyond the highest level of colonization of the low density. Phosphorus deficiencies may have resulted in higher colonization for those treatments.

In the growth chamber experiment when P uptake per pot was converted to P uptake in kilograms per hectare, the amount of P removed by aerial biomass alone would have been 60, 31, and 16 kg P ha^{-1} for the high, middle, and low densities. The lowest plant density of one plant per pot would have had ample access to available soil P based on uptake in aerial biomass and the soil test prior to beginning the experiment, and so P was likely not limiting for that density. The two highest densities would have required more available P than the soil provided according to the soil test at the initiation of the experiment (27 kg ha^{-1}). Phosphorus concentrations per plant at these densities were also lower, and P may have been limiting.

In the field experiment, there was no correlation between AMF colonization and P concentration in either field pea or lentil. Ryan and Angus (2003) found that colonization level by AMF was not correlated to P uptake in field pea or

Table 3 Phosphorus concentration and P uptake in seed and straw for organic field pea and lentil grown in the field at various seeding rates combined for 2005 and 2006

Crop	Seeding rate	Seed P		Straw P	
	Viable seeds m^{-2}	mg kg^{-1}	kg ha^{-1}	mg kg^{-1}	kg ha^{-1}
Field pea	10	2307	0.5 a[a]	652	0.2 a
	62	2209	2.1 b	635	0.9 bc
	250	2218	3.8 c	726	2.7 c
P>*F*		NS[b]	<0.001	NS	<0.001
Lentil	15	2781	0.6 a	430	0.05 a
	94	2738	1.9 b	359	0.6 b
	375	2917	3.8 c	597	1.7 c
P>*F*		NS	<0.001	NS	<0.001

[a] Values within a crop and column with different letters differ at $P<0.05$

[b] *NS* not significant at $P\leq0.05$

wheat even when soil P was low (11 mg kg^{-1}). High densities of organic field pea and lentil may require more AMF colonization to acquire soil-immobile nutrients, especially P, where these nutrient levels are low. This was shown in the field experiment, as P concentrations in aerial biomass were similar despite plant density, while AMF colonization increased with increasing density in most cases.

Phosphorus depletion of the soil is an important concern in organic crop production, especially where organic manure is not available. There is a general consensus in the organic crop production literature that P concentrations in organically managed soils are depleted over time, especially where legume green manure crops are not incorporated into a rotation (e.g., Entz et al. 2001; Malhi et al. 2002; Oehl et al. 2002; Gosling and Shepherd 2005). The results of this study indicate that P may be depleted over time, and more quickly where seeding rates are increased. Further study of how increasing seeding rate affects the sustainability of organic crop production over time is important.

Organic field peas and lentils benefit from AMF colonization of roots by increased P uptake from soils that are generally low in P. The present study has shown that, contrary to several other studies, AMF colonization of crop roots is steady or increases as seeding rate increases. Increasing the seeding rate of these legumes aids in weed control and improves yields (Baird et al. 2009a, b) with no adverse effect on P concentration in the crop. The long-term effects of increased P uptake from higher seeding rates on AMF inoculum and labile soil P, however, are not known and warrant further study.

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