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Arbuscular mycorrhizal colonization and growth of wild and cultivated lettuce in response to nitrogen and phosphorus

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Abstract

Lettuce (*Lactuca sativa*) is a crop with high water and nutrient demand. Its nearest wild relative, *Lactuca serriola*, is tolerant of low moisture and nutrient-poor conditions. Due to selection in different types of environments, the two taxa may differ in response to mycorrhizal colonization. Pot experiments were used to determine if mycorrhizal colonization, growth, root allocation, and P and N tissue concentrations differed between the two *Lactuca* taxa when inoculated with *Glomus intraradices*. In Experiment 1, two levels of P [0 (P0) or 0.25 (P1) mmol P kg⁻¹] were added to a sand/soil medium with and without *G. intraradices* at low N supply (0.89 mmol N kg⁻¹) for 4 weeks. In Experiment 2, two levels of N [1.7 (N1) or 5.0 (N2) mmol N kg⁻¹] were supplied to the same soils containing 0.25 (P1) mmol P kg⁻¹, and plants were grown for 6 weeks. In Experiment 1, the two taxa had similar mycorrhizal colonization, and shoot and root dry weight. In Experiment 2, cultivated lettuce had slightly higher colonization and growth than wild lettuce. Mycorrhizal plants at N1 were small with high root allocation and colonization. Colonization decreased and growth increased at N2. In these experiments, no major difference in mycorrhizal response occurred between *L. sativa* and *L. serriola*. Use of wild lettuce for breeding improved cultivars of lettuce, will at least maintain any ability, to benefit from mycorrhizas, especially in farming systems with low P inputs. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: *Glomus intraradices* Schenck and Smith; *Lactuca sativa* L.; *Lactuca serriola* L.; Mycorrhizal colonization; Root allocation

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1. Introduction

Mycorrhizal colonization increases phosphorus (P) uptake of many plants and has been shown to increase nitrogen (N) uptake in some species as well (Smith and Read, 1997). For agricultural species, considerable effort has been directed towards comparing mycorrhizal responsiveness among old and modern crop cultivars and their wild relatives, but the results are not consistent from species to species. In wheat, growth of modern cultivars tended to benefit less from mycorrhizal inoculation than older cultivars or crop ancestors without the DD genome (Hetrick and Wilson, 1992). Similarly, improved soybean cultivars showed a lower growth response to colonization than older cultivars (Khalil et al., 1994). No consistent trend has been observed among corn cultivars (Toth et al., 1990; Khalil et al., 1994). Cultivated tomatoes and oats, however, are more responsive to mycorrhizal colonization than closely related wild species (Koide et al., 1988; Bryla and Koide, 1990). Comparison of responses to mycorrhizal colonization between cultivated species and their wild relatives may be particularly useful where the latter are being used in breeding programs, so that mycorrhizal colonization and responsiveness can be deliberately included with potential benefit for low-input agriculture.

Lactuca serriola L., wild lettuce, is an annual species native to disturbed habitats in the summer-dry Mediterranean Basin (Ryder and Whitaker, 1976). It is a close relative of cultivated lettuce, *Lactuca sativa* L., and is probably its wild ancestor (Kesseli et al., 1991). *L. serriola* is being used in a marker-assisted breeding program to increase the rooting depth of cultivated lettuce, a shallow-rooted crop with high N and water demand (Jackson, 1995; Johnson et al., 2000). Cultivated lettuce is known to be responsive to mycorrhizal colonization which can reach 80% of root length (Azcón et al., 1996; Azcón-Aguilar, 1998) and contribute to increased P and N uptake (Azcón et al., 1992, 1996; Hepper, 1983).

Our objective was to compare mycorrhizal and non-mycorrhizal *L. sativa* and *L. serriola* for differences in growth, root and shoot partitioning of dry matter, N and P tissue concentrations, and mycorrhizal colonization. We used low levels of N and P supply because mycorrhizal colonization and responsiveness were expected to be highest under low nutrient regimes (Azcón et al., 1992, 1996; Hepper, 1983). Characters in *L. serriola* of value for breeding programs might include rapid colonization leading to high mycorrhizal responsiveness. Such cultivars would be most advantageous in cropping systems with low inputs of inorganic P fertilizer, reducing potential offsite impacts of excessive fertilizer application. This approach would be particularly effective where application of fungicides is also low, because these decrease mycorrhizal colonization (Smith and Read, 1997; Miller and Jackson, 1998).

2. Materials and methods

2.1. Experimental design

Two pot experiments were conducted in a growth chamber to compare shoot and root growth between the two *Lactuca* taxa, with and without mycorrhizal colonization by *Glomus intraradices* Schenck and Smith. Crisphead lettuce, *L. sativa* L. ‘Salinas’ was

compared with wild lettuce, *L. serriola* L. (UC92G489) originating from the collection of R.W. Michelmore (University of California at Davis). Both populations consisted of individuals resulting from several generations of self-pollination, and had been used in previous studies (Jackson, 1995; Gallardo et al., 1996). Briefly, Experiment 1 examined the effect of two P levels on the growth of the two lettuces at low N supply. Experiment 2 was conducted at low P supply, with two levels of N supply.

We chose 4–6 weeks as a suitable duration for the experiments because previous work had shown that total biomass of the two taxa was similar at that time (Jackson, 1995). Similar total biomass provides an appropriate basis for realistic comparisons of root and shoot growth and nutrient uptake, avoiding potentially confounding factors (very familiar to all workers on mycorrhizal responses) related to differences in plant size.

2.2. Pot experiments

The soil medium was a mixture of soil, sand, and soil inoculum containing clover roots that were either colonized with *G. intraradices*, or were free of mycorrhizal colonization (see below). Sterilized Mallala soil with low bicarbonate-extractable P (0.52 mmol kg⁻¹ dry soil; Colwell, 1963) and KCl-extractable N (0.12 mmol kg⁻¹ dry soil; Keeney and Nelson, 1982) was mixed with sterilized medium-grain sand (1:9, w/w). Pot culture inoculum (*G. intraradices* or non-mycorrhizal, see below) was incorporated in the sterile mix (1:9, w/w) to produce the soil medium for the experiments. Phosphorus solutions containing NaH₂PO₄ and modified full-strength Long Ashton solution without P (Smith and Smith, 1981) were mixed thoroughly into the dry soil medium before adding water to bring the soil to 10% gravimetric moisture at planting. Nitrogen was supplied as equimolar amounts of ammonium and nitrate using NH₄NO₃.

The pot culture inoculum was prepared as follows: Sterilized pots were filled with a mixture of sterilized Mallala soil and sterilized sand (1:9, w/w). For one set of pots, colonized root material and spores of *G. intraradices* were incorporated into the mix and the pots were watered to 10% moisture. Other pots contained the same soil without the *Glomus* inoculum. Five sterilized and pre-germinated seeds of *Trifolium subterraneum* L. 'Mt Barker' were sown and the pots were placed in transparent Sunbags[®] (44 cm × 20.5 cm) with a 24 mm diameter disc with a 0.02 μm filter that permits gas exchange (Sigma, St. Louis, MO). The Sunbags[®] were sealed to reduce evaporation and aid in maintaining stable soil moisture with less frequent watering. The Sunbags[®] were used to avoid leaching of nutrients, intermittent water stress, and inadvertent introduction of unwanted mycorrhizal spores and disease propagules. For the first 2 weeks, only de-ionized (DI) water was added to the pots. Thereafter, 10 ml of modified Long Ashton solution (-P) was added weekly, in addition to periodic watering to replenish soil to 10% moisture. Colonization in all the pot cultures was evaluated in a 1 cm diameter core, removed after 4 months of growth. Pots were left to air-dry, then plant tops were removed and a single composite inoculum of each type (+ or -*G. intraradices*) was prepared by thorough mixing. Inoculum was stored in sterile bags up to 3 months before use.

Lettuce seeds were sterilized in 30% NaOCl solution for 10 min and rinsed thoroughly with DI water before sowing in sterilized pots containing the soil media at 10% moisture. Pots contained 3 kg dry soil medium in Experiment 1, and 1 kg in Experiment 2. The pots

were placed in sealed Sunbags[®] in a growth chamber. Light was provided by R-Multi-Vapor metal halide lamps (G.E. Lighting, Nela Park, Cleveland, OH) with a photoperiod of 14 h and irradiance of $0.45 \text{ mmol photons m}^{-2} \text{ s}^{-1}$. Day temperature was $20 \text{ }^\circ\text{C}$ and night temperature was $10 \text{ }^\circ\text{C}$. After 1 week, germinating seedlings were thinned to one per pot. Pots were weighed twice weekly, and water was added as needed to bring soil to 10% moisture. Nutrient additions are described below. Plants were grown for 4 weeks in Experiment 1 and 6 weeks in Experiment 2. Short experiments were conducted to emphasize growth differences during the initial phases of mycorrhizal colonization.

In Experiment 1, there were two lettuce taxa (*L. sativa* and *L. serriola*), two mycorrhizal treatments (+ and -*G. intraradices*), and two P levels (addition of either 0 or $0.25 \text{ mmol P kg}^{-1}$ soil medium) designated P0 and P1. Pots were arranged in a randomized block design. There were four blocks and one replicate per treatment per block. Pots contained 3 kg dry soil medium in Experiment 1. Before planting, 60 ml of the modified Long Ashton solution (-P) was added, then 35 ml per week was added in twice-weekly additions. N availability was low; a total of $0.89 \text{ mmol N kg}^{-1}$ soil medium was added during the course of the experiment.

In Experiment 2, there were two lettuce taxa, and two mycorrhizal treatments as in Experiment 1, but one P level (P1, i.e., $0.25 \text{ mmol P kg}^{-1}$ soil medium) and two N levels. The low N (N1) and higher N (N2) treatments received 1.7 and $5.0 \text{ mmol N kg}^{-1}$ soil medium, respectively, during the 6-week experiment. Pots contained 1 kg dry soil medium in Experiment 2. Before planting, 20 ml of modified Long Ashton solution (-P) was added, then twice-weekly additions of 25 ml were given. There were four blocks and one replicate per treatment per block.

Plants were harvested by cutting the shoot at the soil surface. In Experiment 2, shoot fresh weight was recorded immediately. Shoots were oven-dried ($65 \text{ }^\circ\text{C}$) to a constant weight. Shoot material was ground with a mortar and pestle. Samples were digested with nitric acid and analyzed by inductively coupled plasma atomic emission spectrometry (ICPAES) for micro- and macro-nutrients, including P. Total N was analyzed by combustion using a Carlo Erba analyzer. In Experiment 1, nutrient analysis was conducted after combining the plants for each treatment from all four blocks due to the small size of the individual plants.

Roots were washed free of soil, rinsed, blotted and weighed. The root system was cut into 1 cm pieces, and two subsamples were weighed immediately. One subsample was cleared and stained for determination of mycorrhizal colonization and root length, and the other was weighed after oven-drying so that total root dry weight could be calculated.

The subsample of fresh roots was cleared in 10% KOH at room temperature, rinsed with water, neutralized with 1N HCl, and stained in 0.06% Trypan blue in lactoglycerol for 1 h, rinsed in DI water, and stored in lactic acid:glycerol (1:1, v/v). This is a modification of the method of Phillips and Hayman (1970). Root length per sample was determined with a grid intersect method. Mycorrhizal colonization was determined by the magnified intersects method (McGonigle et al., 1990). Presence of hyphae, arbuscules or vesicles was recorded for 150 root intersections per plant. Total mycorrhizal colonization was calculated as the percentage of intersections with any occurrence of mycorrhizal structures.

Differences between treatments were determined with analysis of variance (ANOVA) using the GLM procedures of SAS (SAS Institute, 1991) and the Duncan's mean separation

test. Statistical comparisons were considered significant at $P \leq 0.05$. In the three-way ANOVA, error terms include the three-way interactions, because these were rarely found to be significant.

3. Results

3.1. Experiment 1: plant response to two levels of soil P

There was no significant difference between lettuce species in the total fraction of the root length colonized, nor in the development of different structures. Arbuscules were frequent in most root segments, but vesicles were relatively rare (Table 1). Arbuscules were significantly higher at P0, but there was a significant species by P level interaction that indicated greater relative reduction in arbuscules in *L. serriola* at P1.

L. serriola and *L. sativa* had similar shoot and root dry weights at the time of harvest and showed similar growth responses to soil P and mycorrhizal treatments (Fig. 1, Table 2). The treatment that caused the highest biomass production for both species was the +*Glomus* treatment with no added P (P0). Non-mycorrhizal plants showed a positive response to increased P supply, but for mycorrhizal plants, dry weight decreased at P1. This is shown by the significant *Glomus* effect and P level by *Glomus* interaction in the three-way ANOVA. Allocation of dry weight to roots was similar in wild and cultivated lettuce, and there were no significant differences between mycorrhizal or P treatments (ANOVA,

Table 1
Colonization by various mycorrhizal structures in Experiment 1^a

	<i>L. sativa</i> (cultivated lettuce)				<i>L. serriola</i> (wild lettuce)			
	P0		P1		P0		P1	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
<i>Colonization %</i>								
Total	79.8	2.5	60.6	4.8	90.8	2.3	59.6	11.0
Internal hyphae only	6.5	0.6	9.6	0.5	6.5	1.7	8.3	3.0
Arbuscules	73.3	2.4	51.0	4.5	84.3	3.2	51.3	13.0
Vesicles	4.5	1.9	0	0	3.5	0.8	0.3	0.2
	Species	P level	Species × P level					
<i>ANOVA significance levels</i>								
Total	ns	***	ns					
Internal hyphae only	ns	ns	ns					
Arbuscules	ns	***	**					
Vesicles	ns	ns	ns					

^a P0 indicates no P addition, P1 indicates addition of 0.25 mmol P kg⁻¹ soil medium. Species and soil phosphorus (P) level comparisons.

** $P \leq 0.01$.

*** $P \leq 0.001$.

ns $P > 0.05$.

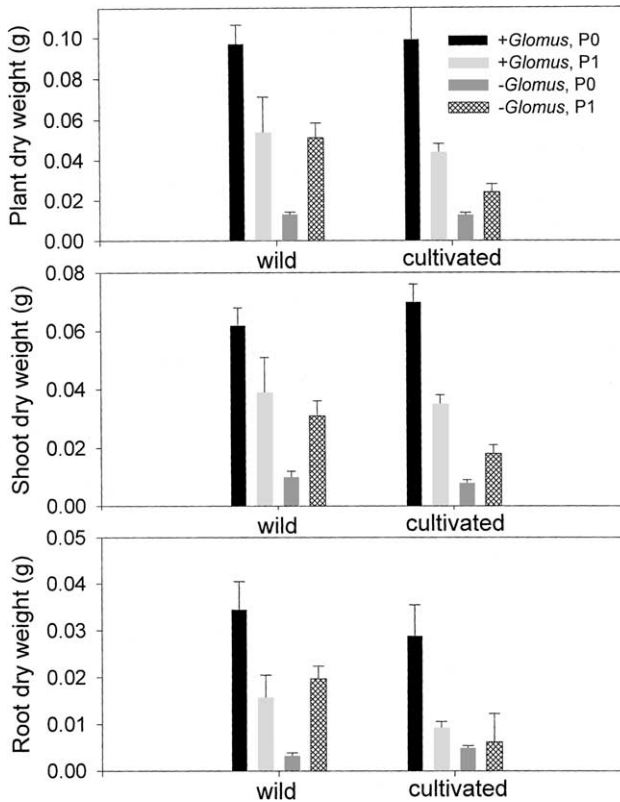


Fig. 1. Plant, shoot and root dry weight for wild (*L. serriola*) and cultivated (*L. sativa*) lettuce in Experiment 1 with and without *G. intraradices*. See Table 2 for ANOVA results. P0 indicates no P addition. P1 indicates addition of 0.25 mmol P kg⁻¹ soil medium.

$P > 0.05$, data not shown). The mean value for all plants was 30.4% dry weight allocation to roots.

The P concentration at P0 in shoots of mycorrhizal and non-mycorrhizal plants was 2.8 and 1.0 g P kg⁻¹ dry weight, respectively, based on data for samples pooled in all treatments. This increased to 4.5 and 4.3 g P kg⁻¹ dry weight, respectively, at P1. The N concentration was 41 and 58 g N kg⁻¹ dry weight in mycorrhizal and non-mycorrhizal plants at P0, respectively, and 47 g N kg⁻¹ dry weight in all plants at P1.

3.2. Experiment 2: plant response to two levels of soil N

Colonization levels were generally similar to the previous experiment (40–90%), but colonization by hyphae, arbuscules and vesicles was significantly lower in *L. serriola* than *L. sativa* (Table 3). Higher N (N2) significantly decreased mycorrhizal colonization at this P level (P1, i.e., 0.25 mmol P kg⁻¹ soil medium). Vesicles were more greatly reduced by N2 in *L. serriola* than *L. sativa*, as shown by the significant species by N level interaction.

Table 2

Significance levels of species comparisons (*L. sativa* vs. *L. serriola*), mycorrhizal (+/–*Glomus*), phosphorus (P), and nitrogen (N) treatments for the different plant characteristics shown in Figs. 1 and 2 for Experiments 1 and 2

Experiment	Characteristic	Species	P level	<i>Glomus</i>	P level × <i>Glomus</i>	Species ×P level	Species × <i>Glomus</i>
Fig. 1	1	Plant dry weight (g)	ns	ns	***	***	ns
		Shoot dry weight (g)	ns	ns	***	***	ns
		Root dry weight (g)	ns	ns	***	***	ns
		Species	N level	<i>Glomus</i>	N level × <i>Glomus</i>	Species ×N level	Species × <i>Glomus</i>
Fig. 2	2	Plant dry weight (g)	*	***	***	*	ns
		Shoot dry weight (g)	**	***	***	**	ns
		Root dry weight (g)	ns	ns	***	ns	ns
		Root allocation (%)	***	***	***	***	ns
		Fresh:dry weight ratio	***	***	***	*	***

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

ns $P > 0.05$.

L. serriola was significantly smaller than *L. sativa*, and produced approximately 20% less plant dry weight (Fig. 2, Table 2). Plant dry weight for either plant taxon whether colonized or not, tended to be higher with higher N supply. Mycorrhizal colonization significantly affected plant dry weight; growth of mycorrhizal plants was lower at N1,

Table 3

Colonization by various mycorrhizal structures in Experiment 2^a

	<i>L. sativa</i> (cultivated lettuce)				<i>L. serriola</i> (wild lettuce)			
	N1		N2		N1		N2	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
<i>Colonization %</i>								
Total	85.9	4.7	54.4	5.5	58.9	6.5	42.2	6.2
Internal hyphae only	11.9	1.5	6.2	1.6	18.1	3.5	11.2	1.7
Arbuscules	74.0	5.9	48.2	4.9	40.8	3.6	31.0	6.1
Vesicles	17.0	2.3	0.8	0.5	4.6	1.2	0.8	0.6
	Species	N level	Species	N level				

ANOVA significance levels

Total * *** ns

Internal hyphae only *** *** ns

Arbuscules ** *** ns

Vesicles ** *** **

^a N1 and N2 indicate addition of 1.7 (N1) or 5.0 (N2) mmol N kg⁻¹ soil medium. Species and soil nitrogen (N) level comparisons.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

ns $P > 0.05$.

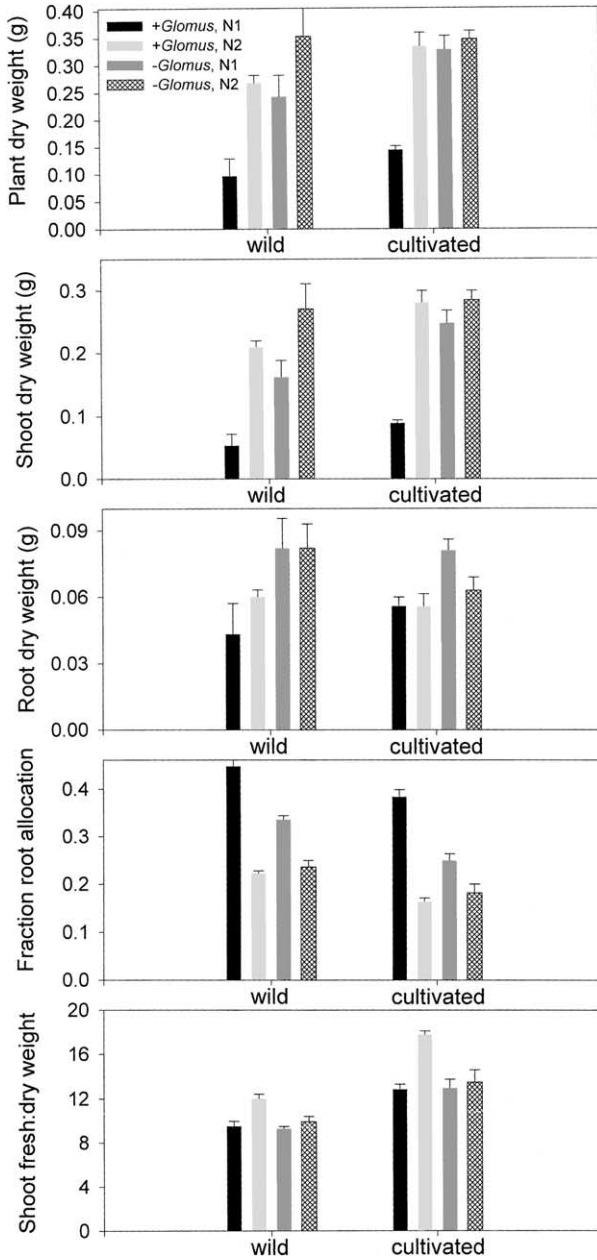


Fig. 2. Plant, shoot and root dry weight, root allocation and shoot fresh:dry weight ratio in Experiment 2 for wild (*L. serriola*) and cultivated (*L. sativa*) lettuce with and without *G. intraradices*. See Table 2 for ANOVA results. N1 and N2 indicate addition of 1.7 (N1) or 5.0 (N2) mmol N kg⁻¹ soil medium.

resulting in a significant N level by *Glomus* interaction. Thus, depending on the N supply, colonization by *Glomus* caused plant dry weight to either decrease (N1) or be similar to (N2) non-mycorrhizal plants. Shoot dry weight showed much the same pattern as plant dry weight.

L. serriola allocated a greater percentage of dry weight to roots than did *L. sativa* (Fig. 2, Table 2). Root allocation at N1 increased with mycorrhizal colonization in both taxa. At N1 root allocation was more greatly affected by mycorrhizal colonization than at N2, as indicated by the significant N level by *Glomus* interaction.

The ratio of shoot fresh to dry weight was higher in cultivated lettuce (Fig. 2, Table 2). For both taxa, mycorrhizal plants that were grown at higher N supply (N2) had the highest ratio. This was less pronounced for *L. serriola*. Although both N level and *Glomus*

Table 4

Shoot phosphorus (P) and nitrogen (N) concentration and content for the two lettuce species with and without mycorrhizal inoculation in Experiment 2^a

<i>Glomus</i> treatment	<i>L. sativa</i> (cultivated lettuce)				<i>L. serriola</i> (wild lettuce)			
	N1		N2		N1		N2	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
<i>Shoot P concentration (g kg⁻¹)</i>								
+	5.20	0.23	4.26	0.14	4.12	0.15	3.98	0.07
-	3.88	0.14	3.95	0.03	4.62	0.42	3.44	0.24
<i>Shoot N concentration (g kg⁻¹)</i>								
+	28.80	0.08	58.40	0.51	29.50	1.50	59.40	0.51
-	25.00	1.14	49.00	2.55	31.80	1.50	52.00	3.38
<i>Shoot N/P concentration ratio</i>								
+	5.6	0.3	13.8	0.5	7.5	0.3	14.9	0.2
-	6.4	0.1	12.4	0.7	7.0	0.4	15.2	0.7
<i>Shoot P content (mg per plant)</i>								
+	0.75	0.03	1.41	0.07	0.39	0.12	1.06	0.04
-	1.27	0.08	1.37	0.06	1.06	0.11	1.19	0.16
<i>Shoot N content (mg per plant)</i>								
+	4.19	0.25	19.58	1.57	5.01	0.97	15.92	0.71
-	8.18	0.53	17.01	1.09	7.56	1.04	18.28	2.88
Nutrient	Species	N level	<i>Glomus</i>	Species	Species	N level		
				×N level	× <i>Glomus</i>	× <i>Glomus</i>		
<i>ANOVA significance levels</i>								
P (g kg ⁻¹)	ns	**	*	ns	*	ns		
N (g kg ⁻¹)	*	***	***	ns	ns	**		
N/P ratio	***	***	ns	ns	ns	ns		
P (mg per plant)	***	***	***	ns	ns	***		
N (mg per plant)	ns	***	ns	ns	ns	ns		

^a N1 and N2 indicate addition of 1.7 (N1) or 5.0 (N2) mmol N kg⁻¹ soil medium.

* $P \leq 0.05$.

** $P \leq 0.01$.

*** $P \leq 0.001$.

ns $P > 0.05$.

treatments had significant effects, there was a significant N level by *Glomus* interaction that demonstrates the higher ratio at N2 compared to N1.

No overall differences were found between the two taxa in shoot P concentration, which increased in shoots of +*Glomus* treatments (Table 4). An exception was *L. serriola* at N1, accounting for a significant species by *Glomus* interaction. Shoot P concentration was higher at N1. The N/P ratio in the shoots increased at the higher level of N supply. The ratio was not different between mycorrhizal and non-mycorrhizal plants. *L. serriola* had a higher N/P ratio than *L. sativa*. *L. sativa* plants, which were larger (Fig. 2, Table 2), accumulated more P than *L. serriola* (Table 4).

L. sativa had slightly lower N concentrations than *L. serriola* (Table 4). For both taxa, plants grown with a higher N supply had higher N concentration. In the +*Glomus* treatments, shoot N concentration was significantly higher than in the –*Glomus* treatments. A significant N level by *Glomus* interaction occurred, due to a greater increase in shoot N concentration in the +*Glomus* treatments at higher levels of N supply. *L. serriola* and *L. sativa* plants accumulated similar amounts of N despite the lower dry weight of *L. serriola* (Fig. 2, Table 2).

4. Discussion

No major differences in the growth responses of *L. sativa* ‘Salinas’ and *L. serriola* occurred to either nutrient supply or to mycorrhizal colonization at 4–6 weeks of growth. The timing of the harvests was successful in achieving comparisons of plants of similar total biomass, particularly in Experiment 1. In both taxa, the application of P in Experiment 1 and N in Experiment 2 lowered colonization significantly, but not to the extent that has been observed in some plant species (Sylvia and Neal, 1990; Baon et al., 1992; Bruce et al., 1994; Nadian et al., 1996). Different growth responses were observed for mycorrhizal plants in response to P vs. N. Low P resulted in greater growth of mycorrhizal plants, but low N resulted in lower growth of mycorrhizal plants. Several factors are discussed below to explain the responses to soil P and N availability in mycorrhizal and non-mycorrhizal plants. These include, the strong growth responses to P availability in mycorrhizal lettuce, the capacity for N uptake by mycorrhizal lettuce, plastic changes in root allocation in relation to soil N availability, and effects of the ratio of P and N availability in the soil.

Plant N acquisition may have been enhanced by increased exploration of the soil volume by mycorrhizal hyphae. Uptake of N by mycorrhizal fungi is known to occur in cultivated lettuce (Azcón et al., 1992, 1996). Mycorrhizal fungi may have been more effectively scavenging N from the soil at P0 than at P1 in Experiment 1. Greater responsiveness to and dependence on mycorrhizal fungi is characteristic of mycorrhizal plants grown at lower soil P concentrations and low tissue P concentration (Smith and Read, 1997). In fact, tissue P concentrations at P0 were below adequate ($\leq 4.5 \text{ g P kg}^{-1}$ dry weight; Bergmann, 1992; Piggott, 1986). High mycorrhizal dependence at P0 may have resulted in both higher P and N uptake and less limitation of N for plant growth than at P1. This could explain the greater growth of mycorrhizal plants at P0 compared to all other treatments in Experiment 1. Shoot concentrations of other nutrients, except P (see above) and N (see below), were within adequate ranges for both mycorrhizal and non-mycorrhizal plants in both experiments

(data not shown). This supports the idea that N, rather than other nutrients, may have been a limiting factor in Experiment 1. In Experiment 2, mycorrhizal plants demonstrated a greater growth response to the increase in N supply than non-mycorrhizal plants. The carbon costs of the mycorrhizal symbiosis may have been compensated by greater N uptake at the higher N level, resulting in increased growth.

Root allocation patterns may be explained by plastic responses to N availability, as well as by mycorrhizal effects. The proportion of dry weight allocated to roots typically increases at low soil N availability, and this promotes acquisition of the limiting nutrient (Chapin, 1991). Shoot N concentration at N1 in Experiment 2 indicated N deficiency (i.e., $\leq 40 \text{ g N kg}^{-1}$; Bergmann, 1992; Piggott, 1986). Thus, high root allocation at N1 may have been an inherent plastic response to acquire more of the limited N supply in both mycorrhizal and non-mycorrhizal plants. At N2, shoot N concentrations were higher, suggesting that the soil N supply was adequate. At adequate levels of N supply, roots of wild and cultivated lettuces are typically 10–25% of total plant dry weight (Jackson, 1995; Gallardo et al., 1996), as was observed for plants grown at N2. Root allocation was slightly lower in mycorrhizal plants compared to non-mycorrhizal plants at N2, as is commonly observed (see Smith and Read, 1997). Decreased root allocation at N2 may partially explain the pronounced growth increase in mycorrhizal plants compared to N1. Higher shoot allocation at N2 would have promoted more rapid C gain and dry weight production.

Increasing N supply was associated with significantly reduced mycorrhizal colonization, as has been observed previously with clover, soybean and onion (Chambers et al., 1980; Vejsadová et al., 1990; Sylvia and Neal, 1990). Another study on lettuce presented conflicting results (Hepper, 1983); increasing the availability of soil nitrate and/or the N concentration in root tissue increased mycorrhizal colonization especially at low soil P. The apparent discrepancy between these studies of lettuce may derive from two factors: the ratio of N and P supplied, where an imbalance is more likely to lead to a decline in colonization than an increase in uptake of a single nutrient per se; and the form in which the N is supplied, with ammonium having a much more inhibitory effect than nitrate (Chambers et al., 1980). Ammonium can suppress root colonization in sand and solution culture due to its effect on pH (Thompson, 1986). This was probably not an issue in these experiments because N additions were small, equimolar ammonium and nitrate were applied, and the growing medium contained soil that buffers against changes in pH. Thus, the ratio of N and P availability may be a reason for the discrepancy in N responses between our study and Hepper (1983). Yet these ratios are nearly impossible to evaluate without measurements of soil N pools, especially when N is added as nitrate that can be readily lost by denitrification and leaching.

Mycorrhizal fungi place demands on the plant for organic carbon (C) leading to slower growth rates, reduced mycorrhizal responsiveness at low irradiance, and reduced colonization in some plant species at low irradiance, particularly when P is also increased (Son and Smith, 1988; and see Smith and Read, 1997). Photosynthetic light saturation in wild lettuce occurs at approximately $1.5 \text{ mmol photons m}^{-2} \text{ s}^{-1}$ (Werk and Ehleringer, 1985), which is higher than the light level in our growth chamber. Colonization in our experiments was consistently high, leading to the conclusion that either the plants were not in fact light-stressed at the levels of nutrients supplied or that colonization in *Lactuca* is not very sensitive to the conditions under which the host plants are grown. Low irradiance and

mycorrhizal infection have been shown to increase shoot fresh:dry weight ratio (Evans and Hughes, 1961; Snellgrove et al., 1982; Tester et al., 1986), possibly as a means of maximizing leaf surface area and photosynthesis under conditions of limited C supply to plants. In Experiment 2, mycorrhizal plants at N2 had markedly higher shoot fresh:dry weight ratio and shoot N concentration than at N1, and than non-mycorrhizal plants. C limitation, if any, was not due to either mycorrhizal colonization or to low irradiance per se, but may have occurred in mycorrhizal plants with higher N supply. This suggestion is strengthened by the reduction in fungal vesicles at high N, possibly indicating reduced C storage by the fungi. Higher photosynthetic capacity is highly correlated with N concentration in plant tissue, and Rubisco and chlorophyll content increase when N availability increases (Pons et al., 1994; Lambers et al., 1998). The mycorrhizal plants at N2 apparently experienced less N limitation than any of the other treatments. This could have increased photosynthetic capacity, which consequently could have caused greater perception of light-limitation under the growth chamber light levels.

Lower mycorrhizal dependency in wild tomatoes and oats has been related to lower inherent growth rates and low nutrient demand as adaptations to lower nutrient availability in natural ecosystems (Koide et al., 1988; Bryla and Koide, 1990). These same considerations may not be directly applicable to the two lettuce taxa. Under the high light conditions typical of their ruderal and agricultural habitats, *L. serriola* has a higher photosynthetic rate and a higher growth rate than *L. sativa*, although *L. serriola* initially has a slower growth rate that can be attributed to its smaller seed (Jackson, 1995; Gallardo et al., 1996). The similarities in colonization and responsiveness of *L. sativa* 'Salinas' and the likely progenitor of cultivated lettuces, *L. serriola*, suggest that breeding programs have not had major effects on the capacity of this cultivar of *L. sativa* to form mycorrhizas, nor on its ability to respond to colonization.

Use of *L. serriola* in ongoing breeding for deeper root systems with efficient use of applied water and nitrogen will be likely to at least maintain the ability of new cultivars to capture the benefits of mycorrhizas. If there is variation in mycorrhizal colonization among commercial cultivars, crosses with *L. serriola* could enhance this potential. Little information is available on differences in mycorrhizal colonization and responsiveness among lettuce cultivars. In a small study of colonization of four lettuce cultivars sampled at harvest maturity from a field under recent organic production in the Salinas Valley, CA, we found that mean colonization ranged from 14 to 31%, with highest colonization in the romaine lettuce, *L. sativa* 'Paragon' (Table 5). These preliminary results suggest more genetic

Table 5

Mean percent mycorrhizal colonization of four different lettuce types from the same on-farm organic vegetable field^a

Lettuce type and cultivar	% colonization	% arbuscular colonization
Red leaf 'New Red'	14.4 a	7.0 ac
Green leaf 'Waldman'	12.6 a	3.4 a
Romaine 'Paragon'	31.2 b	14.6 b
Crisphead 'Sharpshooter'	23.2 ab	12.8 c

^a Roots in the top 10 cm of soil were sampled. Plants were at harvest maturity. No vesicles were observed. Values followed by the same letter are not statistically different at $P \leq 0.05$ using *t*-tests. $n = 5$ plants per cultivar.

variation in colonization among lettuce cultivars than that might have been predicted from the comparison of *L. serriola* and *L. sativa* ‘Salinas’. Without comparative field data for *L. sativa* ‘Salinas’, we cannot rule out the possibility that soil/crop conditions also contributed to the generally lower values of mycorrhizal colonization in these cultivars. Studies to evaluate colonization and responsiveness in the field with a number of lettuce varieties would be a logical step forward. In any event, it seems likely that enhancement of the value of mycorrhizal symbiosis can be brought about by a combination of cultivar evaluation and breeding, as well as adoption of farming practices conducive to mycorrhizal survival and persistence, such as reduced use of fungicides, other pesticides, and P and N fertilizer as well as increased use of arbuscular mycorrhizal hosts in rotations (Miller and Jackson, 1998).

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References

- Azcón, R., Gomez, M., Tobar, R., 1992. Effects of nitrogen source on growth, nutrition, photosynthetic rate and nitrogen metabolism of mycorrhizal and P-fertilized plants of *Lactuca sativa* L. *New Phytol.* 121, 227–234.
- Azcón, R., Gomez, M., Tobar, R., 1996. Physiological and nutritional responses by *Lactuca sativa* L. to nitrogen sources and mycorrhizal fungi under drought conditions. *Biol. Fert. Soils* 22, 156–161.
- Azcón-Aguilar, R., Handley, L.L., Schrimgeour, C.M., 1998. The $\delta^{15}\text{N}$ of lettuce and barley are affected by AM status and external concentration of N. *New Phytol.* 138, 19–26.
- Baon, J.B., Smith, S.E., Alston, A.M., Wheeler, R.D., 1992. Phosphorus efficiency of three cereals as related to indigenous mycorrhizal infection. *Aust. J. Agric. Res.* 43, 470–491.
- Bergmann, W., 1992. *Nutritional Disorders of Plants*. Jena, Gustav Fisher Verlag, Germany, 741 pp.
- Bruce, A., Smith, S.E., Tester, M., 1994. The development of mycorrhizal infection in cucumber: effects of P supply on root growth, formation of entry points and growth of infection units. *New Phytol.* 127, 507–514.
- Bryla, D.R., Koide, R.T., 1990. Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants. II. Eight wild accessions and two cultivars of *Lycopersicon esculentum* Mill. *Oecologia* 77, 537–543.
- Chambers, C.A., Smith, S.E., Smith, F.A., 1980. Effects of ammonium and nitrate ions on mycorrhizal infection, nodulation and growth of *Trifolium subterraneum*. *New Phytol.* 85, 47–62.
- Chapin, F.S., 1991. Effects of multiple environmental stresses on nutrient availability and use. In: Mooney, H.A., Winner, W.E., Pell, E.J. (Eds.), *Response of Plants to Multiple Stresses*. Academic Press, San Diego, CA, pp. 67–88.
- Colwell, J.D., 1963. The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales. *Aust. J. Exp. Agric. Anim. Husbandry* 3, 190–197.
- Evans, G.C., Hughes, A.P., 1961. Plant growth and the aerial environment. I. Effect of artificial shading on *Impatiens parviflora*. *New Phytol.* 60, 150–180.
- Gallardo, M., Jackson, L.E., Thompson, R.B., 1996. Shoot and root physiological responses to localized zones of soil moisture in cultivated and wild lettuce (*Lactuca* spp.). *Plant Cell Environ.* 19, 1169–1178.
- Hepper, C.M., 1983. The effect of nitrate and phosphate on the vesicular–arbuscular mycorrhizal infection of lettuce. *New Phytol.* 9, 389–399.

- Hetrick, B.A.D., Wilson, G.W.T., 1992. Mycorrhizal dependence of modern wheat cultivars, landraces, and ancestors. *Can. J. Bot.* 70, 2032–2040.
- Jackson, L.E., 1995. Root architecture in cultivated and wild lettuce (*Lactuca* spp.). *Plant Cell Environ.* 18, 885–894.
- Johnson, W.C., Jackson, L.E., Ochoa, O., van Wijk, R., Peleman, J., St. Clair, D.A., Michelmore, R.W., 2000. Lettuce, a shallow-rooted crop, and *Lactuca serriola*, its wild progenitor, differ at QTL determining root architecture and deep soil water exploitation. *Theor. Appl. Genet.* 101, 1066–1073.
- Keeney, D.R., Nelson, D.W., 1982. Nitrogen-inorganic forms. In: Page, A.L., Baker, D.E., Ellis, R., Keeney, D.R., Miller, R.H., Rhoades, J.D. (Eds.), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, No. 9, 2nd Edition. Agronomy Society of America, Madison, WI, USA, pp. 643–698.
- Kesseli, R.V., Ochoa, O., Michelmore, R.W., 1991. Origin of *Lactuca sativa* (lettuce). *Genome* 34, 430–436.
- Khalil, S., Loynachan, T.E., Tabatabai, M.A., 1994. Mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars. *Agron. J.* 8, 949–958.
- Koide, R., Minguang, L., Lewis, J., Irby, C., 1988. Role of mycorrhizal infection in the growth and reproduction of wild vs. cultivated plants. I. Wild vs. cultivated oats. *Oecologia* 77, 537–543.
- Lambers, H., Chapin III, F.S., Pons, T.L., 1998. *Plant Physiological Ecology*. Springer, New York, 540 pp.
- McGonigle, T., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular–arbuscular fungi. *New Phytol.* 115, 495–501.
- Miller, R.L., Jackson, L.E., 1998. Survey of vesicular–arbuscular mycorrhizae in lettuce production in relation to management and soil factors. *J. Agric. Sci. Camb.* 130, 173–182.
- Nadian, H., Smith, S.E., Alston, A.M., Murray, R.S., 1996. The effect of soil compaction on growth and P uptake of *Trifolium subterraneum*: interactions with mycorrhizal colonization. *Plant Soil* 182, 39–49.
- Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.* 55, 158–160.
- Piggott, T.J., 1986. Vegetable crops. In: Reuter, D.J., Robinson, J.B. (Eds.), *Plant Analysis: An Interpretation Manual*. Inkata Press, Melbourne, Australia, pp. 148–187.
- Pons, T.L., Van der Werf, A., Lambers, H., 1994. Photosynthetic nitrogen use efficiency of inherently slow- and fast-growing species: possible explanations for observed differences. In: Roy, J., Garnier, E. (Eds.), *A Whole-plant Perspective of Carbon–Nitrogen Interactions*. SPB Academic Publishing, The Hague, The Netherlands, pp. 61–77.
- Ryder, E.J., Whitaker, T.W., 1976. Lettuce. In: Simmonds, N.W. (Ed.), *Evolution of Crop Plants*. Longman, London, pp. 39–41.
- SAS Institute, 1991. *SAS System for General Linear Models*. SAS Institute, Cary, NC.
- Smith, S.E., Read, D.J., 1997. *Mycorrhizal Symbiosis*, 2nd Edition. Academic Press, San Diego, CA, 605 pp.
- Smith, F.A., Smith, S.E., 1981. Mycorrhizal infection and growth of *Trifolium subterraneum*: use of sterilized soil as a control treatment. *New Phytol.* 88, 299–309.
- Snellgrove, R.C., Splittstoesser, W.E., Stribley, D.P., Tinker, P.B., 1982. The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular–arbuscular mycorrhizas. *New Phytol.* 92, 75–87.
- Son, C.L., Smith, S.E., 1988. Mycorrhizal growth responses: interactions between photon irradiance and phosphorus nutrition. *New Phytol.* 108, 305–314.
- Sylvia, D.M., Neal, L.H., 1990. Nitrogen affects the phosphorus response of VA mycorrhiza. *New Phytol.* 115, 303–310.
- Tester, M., Smith, S.E., Smith, F.A., Walker, N.A., 1986. Effects of photon irradiance on shoot and root growth, on rate of initiation of mycorrhizal infection and on the growth of infection units in *Trifolium subterraneum* L. *New Phytol.* 103, 375–390.
- Thompson, J.B., 1986. Soilless culture of vesicular–arbuscular mycorrhizae of cereals: effects of nutrient concentration and nitrogen source. *Can. J. Bot.* 64, 2282–2294.
- Toth, R., Toth, D., Starke, D., Smith, D.R., 1990. Vesicular–arbuscular mycorrhizal colonization in *Zea mays* affected by breeding for resistance to fungal pathogens. *Can. J. Bot.* 68, 1039–1044.
- Vejsadová, H., Hřelová, H., Prikyl, Z., Vancura, V., 1990. Effect of different phosphorus and nitrogen levels on development of VA mycorrhiza, rhizobial activity and soybean growth. *Agric. Ecosyst. Environ.* 29, 429–434.
- Werk, K.S., Ehleringer, J., 1985. Photosynthetic characteristics of *Lactuca serriola* L. *Plant Cell Environ.* 8, 345–350.