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Influence of phosphorus supply and light intensity on mycorrhizal response in *Pisum-Rhizobium-Glomus* symbiosis

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Abstract. The influence of mycorrhizal colonization with *Glomus mosseae* on parameters of N_2 fixation and plant growth was studied in pot experiments with pea plants (*Pisum sativum* L.) infected with *Rhizobium leguminosarum* and supplied with varied levels of phosphorus (P) and nitrogen (N). Reduced light intensities were used to evaluate the dependence of the microsymbionts on assimilate supply. In plants grown with low P supply, mycorrhization increased the concentration of P in shoots, and thus N_2 fixation. Reduced light intensity significantly depressed mycorrhizal colonization and nodule growth in low-P plants. When P supply did not limit plant growth and N_2 fixation, however, the percentage of mycorrhizal colonization was reduced due to the higher P status, and the microsymbionts were not impaired by low light intensities. To maximize carbohydrate supply, another experiment was carried out at high light intensity of 900 μmol m^{-2} s^{-1} and with non-limiting P supply. Nitrogen fertilization, given as starter N, enhanced plant growth, but delayed nodule formation. Towards flowering, nodulation rapidly increased, but less so in *Glomus* inoculated plants. After 28 days mycorrhizal plants were lower in shoot dry weight, nodule dry weight and nitrogenase activity. The results suggest that under many, but not all, environmental conditions the host plant is able to restrict mycorrhizal colonization and, thus, to prevent impairment of *Rhizobium* symbiosis.
Key words. Arbuscular mycorrhiza; light intensity; N_2-fixation; phosphorus nutrition; *Pisum sativum, Rhizobium leguminosarum.*

Both nodule growth and N_2-fixation in legumes are characterized by a high specific demand for phosphorus[22]. This influence of phosphorus on symbiotic N_2-fixation may be indirect, by stimulation of host plant growth[16,28] or direct, by more specific effects on nodule initiation, growth and functions[15].

Plant uptake of mineral nutrients of low mobility in the soil, such as phosphorus (P), may be limited by root length. Arbuscular mycorrhiza (AM) formation is therefore of major importance in plant species which form a coarse, small root system, especially under conditions of low P availability. The sparsely-developed root system of grain legumes compared with cereals, as for example pea versus barley[18], emphasizes the importance of AM for P uptake by legumes. Mycorrhizal hyphae have access to a greater volume of soil than can be exploited by the plant roots themselves, and can absorb and translocate fairly large amounts of P to the host plant[19,21].

Energy costs of both *Rhizobium* symbiosis and AM formation are high. In soybean, the respiratory cost of N_2-fixation has been calculated to be about 8.4 mol carbon (C) per mol N_2 (ref. 29) with approximately 20% of photosynthates being respired by the nodules[9]. The C allocated to the fungal microsymbiont represents between 5 and 20% of net photosynthates, and is thus in the same range as the C use for the *Rhizobium* symbiosis[11,25,32]. Nodulation and nodule activity are finely tuned to the N demand of the host plant, and are therefore strongly depressed when levels of available N in the substrate are high. In contrast, roots of pea crops, for example, are extensively colonized by AM even in soils which have been treated with large amounts of fertilizer[18]. If the fungi do not contribute to the nutrient acquisition of the plant, AM colonization may depress growth, as has been shown for example in ryegrass[6], pea[27], sunflower[20], soybean[5] and citrus[25]. Since the formation and function of symbiotic associations between plants and microorganisms depend on the supply of energy by the host plant, the development of macro- and microsymbionts may be indirectly influenced by photosynthetic activity and nutritional conditions. It is not clear whether, and to what extent, the host plant is able to optimize the amounts of fungal structures by reducing carbohydrate supply.

In the present work, interactions between symbionts in the *Pisum-Rhizobium-Glomus* symbiosis were studied, primarily under conditions of non-limiting P supply. The extent, activity and time-course of the development of the associated organisms were quantified using a solid substrate with varied levels of P and N supply. To corroborate the relationships between symbiosis and carbon supply, the photosynthetic activity of the plant was varied by using different light intensities.

Materials and methods

Three experiments were performed to study the effect of different rates of N and P supply and of light intensity.

† deceased in May 1994

Pisum sativum L. cv. Belman plants were grown in PVC-tubes of 35 × 4.5 cm (volume 445 mL). Subsoil of a Luvisol (loess), low in extractable P (3.8 mg P kg^{-1}, extracted with 0.5 M $NaHCO_3$ [23]) and organic matter (0.2% C_{org}), and with pH ($CaCl_2$) 7.3, was mixed with washed quartz sand and vermiculite (40% soil:55% sand:5% vermiculite, w/w) to obtain a well-aerated substrate. Before γ-sterilization, mineral nutrients were supplied as follows (mg kg^{-1} substrate): 400 K, 180 Mg, 8 Cu, 8 Fe, 7 Zn, given as K_2SO_4, $MgSO_4$, $CuSO_4$, Sequestren (Fe-ethylenediamine-di-o-hydroxyphenylacetic acid) and $ZnSO_4$. In experiment 1, plants were fertilized with (mg kg^{-1}) 50 P and 16 N; in experiment 2, with 100 P and 16 N, and in experiment 3 with 100 P and 16 or 100 N, given as $Ca(H_2PO_4)_2$ and NH_4NO_3, respectively.

Rhizobium leguminosarum viciae (DSM 30132) was cultivated in a liquid medium after Holsten et al.[14] with modifications as follows: as Fe source instead of $FeCl_3$ 8.7 mg L^{-1} Fe-ethylenediamintetraacetic acid was used, and as carbon source instead of mannitol 1.5 g L^{-1} each of arabinose and galactose. The pH was adjusted to 6.8. For inoculation 10 mL of a suspension containing approximately 10^8 mL^{-1} bacteria was added to each tube. Plants were inoculated with the mycorrhizal fungus *Glomus mosseae* isolated from a Luvisol near Göttingen in northern Germany, or left uninoculated. AM infected maize roots with adhering soil were used as inoculum and per tube 5 g of fresh or, for uninoculated plants, autoclaved roots were mixed with the substrate. All treatments received 9 mL of a soil filtrate of AM infected maize roots.

All experiments were conducted in growth chambers with day/night temperatures of 20/15 °C and a photoperiod of 16 h. In experiment 1, irradiation was 680 µmol m^{-2} s^{-1} in the high and 160 µmol m^{-2} s^{-1} in the low light intensity (shaded) treatment. In experiment 2, irradiations were 500 µmol m^{-2} s^{-1} and 250 µmol m^{-2} s^{-1}. In experiment 3, plants were grown with an irradiation of 900 µmol m^{-2} s^{-1}. In this experiment, after planting the seedlings were allowed to adapt gradually to the very high irradiation. The light intensity was increased from 400 to 900 µmol m^{-2} s^{-1} in steps of 100 µmol m^{-2} s^{-1} every second day by reducing the distance between plants (canopy) and the light source. The weight of PVC tubes with plants and substrates was checked three times a week and, if necessary, distilled water was added up to a substrate water content of 17.3%.

Plants were harvested after 30 days of growth (end of vegetative growth stage) in experiment 1, and harvested sequentially after 18, 30 and 37 days in experiment 2 and after 7, 11, 14, 18, 21 and 28 days in experiment 3. Nitrogenase activity was estimated using the acetylene reduction technique according to Hansen et al.[10] Immediately after separating them from the shoots, the root systems were carefully shaken to remove excessive soil and subsequently incubated for 10 min in an atmosphere of 6% acetylene. Ethylene production was measured after 5 and 10 min with a gas chromatograph (model 5890, Hewlett Packard, Bad Homburg, Germany). Endogenous ethylene production by the roots or soil microorganisms was found to be insignificant.

After the assay, nodules were collected, counted and dried for 24 h at 70 °C and subsequently weighed. Washed roots were cut into 1 cm pieces and thoroughly mixed. In a subsample of 25–50%, based on fresh weight, total root length was determined using the line intersect method[35]. After clearing with 10% KOH and straining with 0.1% trypan blue in lactic acid (90%) (modified after Philips et al.[26]), percentage AM colonization was determined.

Shoots and roots were dried at 70 °C for 48 h and subsequently weighed. After drying, ground samples were ashed at 550 °C for 8 h and the ash dissolved in 7.4 M nitric acid. Phosphorus was determined colorimetrically according to Gericke and Kurmies.[8] Total nitrogen analyses were carried out by an automatic N analyzer (Macro-N, Foss Heraeus, Hanau, Germany).

Soluble carbohydrates were extracted with water (60 °C, 1 h) and enzymatically determined after digestion to oligosaccharides (Test-Combination Saccharose, D-Glucose/D-Fructose, Boehringer, Mannheim, Germany).

Data were analyzed separately for the different light regimes by analysis of variance ($p = 0.05$), and means of mycorrhizal and non-mycorrhizal treatments were separated in experiments 1 and 2 by the Scheffé test[33] and in experiment 3 by the Student-Newman-Keuls test[33].

Results

In plants grown under conditions of low P supply, mycorrhizal colonization markedly increased P concentrations in the shoots, regardless of the light intensity (table 1). Mycorrhization promoted plant growth, although increase in dry matter production was not significant. This was true for both light intensities. Compared with high light intensity, low light intensity reduced root dry weight. The relative decrease in shoot dry weight was smaller, which led to a decline in root-to-shoot dry weight ratio. Under the growth conditions given, low light intensity did not influence shoot and root growth responses to AM.

The degree of mycorrhizal colonization, however, was reduced from 44.2% at high light intensity to 28.5% at low light intensity (table 2). The colonized root length, which may be better correlated with the fungal mass than percentage infection, was twice as high under high light than under low light conditions. Uptake of P was clearly enhanced in AM plants compared with non-infected plants under both light intensities.

As expected, N_2-fixation responded positively to the higher internal P-status of mycorrhizal plants, which

Table 1. Shoot and root dry weight, shoot P and N concentrations as influenced by mycorrhiza treatment (*Glomus mosseae*) in 30-day-old *Pisum sativum* plants inoculated with *Rhizobium leguminosarum*

Treatment		Dry weight (mg plant^{-1})		Shoot concentration (mg g^{-1} dry matter)	
Irradiation (μmol m^{-2} s^{-1})	AM Inoculation	Shoot	Root	P	N
680	–	1.03[a] (0.13)	0.31[a] (0.07)	1.56[a] (0.20)	26.60[a] (3.39)
	+	1.19[a] (0.07)	0.37[a] (0.09)	3.03[b] (0.20)	35.13[b] (3.08)
160	–	0.81[a] (0.26)	0.17[a] (0.03)	2.08[a] (0.12)	35.37[a] (3.06)
	+	1.02[a] (0.15)	0.21[a] (0.08)	3.23[b] (0.19)	36.23[a] (1.82)

Plants were grown under different light intensities and at low P supply (Expt. 1). Values represent means; standard deviations are given in brackets. Means followed by different letters are significantly different ($p < 0.05$, $n = 4$). Data were separately analyzed after the plants had been grown under high or low light intensities.

Table 2. Mycorrhizal (AM) colonization, nodule number (No.) and dry weight (nodule dry wt.) per plant, acetylene reduction activity (ARA, μmol C_2H_4 h^{-1} plant^{-1}), specific nitrogenase activity (μmol C_2H_4 h^{-1} g^{-1} nodule dry wt.) and relative increase of shoot N and P contents (non-mycorrhizal plants = 100) as influenced by mycorrhiza treatment (*Glomus mosseae*) in 30-day-old *Pisum sativum* plants inoculated with *Rhizobium leguminosarum*

Treatment		AM colonization (%)	Nodules plant^{-1}		ARA (μmol h^{-1})		Relative increase of content in shoot	
Irradiation (μmol m^{-2} s^{-1})	AM Inoculation		No.	Dry wt. (mg)	(plant^{-1})	(specific)	N	P
680	–	0	89[a] (25)	22.2[a] (4.8)	0.92[a] (0.29)	42.6[a] (10.8)	100	100
	+	44.9 (3.7)	117[a] (59)	47.3[b] (13.5)	3.21[b] (1.96)	70.2[a] (41.4)	145	213
160	–	0	55[a] (35)	11.3[a] (3.1)	1.59[a] (0.33)	135.6[a] (11.4)	100	100
	+	28.5 (5.2)	77[a] (8)	25.0[b] (4.2)	2.66[b] (0.59)	106.2[a] (11.4)	131	209

Plants were grown under different light intensities and at low P supply (Expt. 1). Values represent means; standard deviations are given in brackets. Means followed by different letters are significantly different ($p < 0.05$, $n = 4$). Data were separately analyzed after the plants had been grown under high or low light intensities.

was reflected by an increase in nodule number per plant and, more distinctly, by an increase in nodule dry weight and total acetylene reduction activity (ARA) (table 2). Consequently, N concentrations in the shoots, and N uptake, were higher in AM plants under high light intensity, while they remained unaffected under low light intensity (table 1, table 2).

Although nodule dry weight and AM-colonized root length were markedly reduced by shading, growth promotion was similar in both light intensities, as shown by data of relative enhancement of P and N uptake, indicating an adaptation of the specific nitrogenase activity to these growth conditions (table 2).

Storage of starch in plant shoots was highest in non-mycorrhizal plants at high light intensity, while it was reduced by either AM or shading (table 3). In contrast, sugar concentrations in shoots were not influenced by the treatments, except that there were much lower sucrose concentrations in the low light plants.

No starch was detectable in roots, and sugar concentrations were much lower in roots than in shoots. Mycorrhization decreased the sucrose concentrations under high light intensity, but caused no further reduction under low light. Reducing sugars in the roots were not affected by the presence of mycorrhiza. Concentrations of reducing sugars in nodules were comparable to those in roots, and similar in all treatments. However, sucrose concentrations in nodules were markedly higher than in roots. Mycorrhization enhanced sucrose concentrations in nodules under both light treatments, indicating a greater supply by the host plant (table 3).

In order to achieve an adequate P supply irrespective of mycorrhizal colonization, in experiment 2 plants were grown with 100 mg kg^{-1} phosphorus. In this experiment, mycorrhization did not influence shoot or root growth (fig. 1) or plant P concentration (table 4). Light intensity did not influence plant growth either, until 30 days after planting. However, at flowering time, 37 days

Table 3. Concentration of starch in shoots and of reducing (red.) sugars and sucrose in shoots, roots and nodules as influenced by mycorrhiza treatment (*Glomus mosseae*) in 30-day-old *Pisum sativum* plants inoculated with *Rhizobium leguminosarum*

Treatment		Shoot			Root		Nodule	
Irradiation ($\mu mol\ m^{-2}\ s^{-1}$)	AM Inoculation	Starch ($mg\ g^{-1}$ dry matter)	Red. sugars	Sucrose	Red. sugars ($mg\ g^{-1}$ dry matter)	Sucrose	Red. sugars ($mg\ g^{-1}$ dry matter)	Sucrose
680	–	129.3[a] (17.6)	38.1[a] (2.7)	56.0[a] (20.9)	8.7	3.9	4.3	13.3
	+	69.6[b] (13.5)	41.5[a] (1.4)	50.5[a] (8.3)	7.7	0.3	4.3	24.9
160	–	59.1[a] (10.4)	41.4[a] (9.2)	34.8[a] (8.0)	5.1	0.5	6.0	5.7
	+	45.3[a] (8.2)	63.1[a] (4.4)	24.9[a] (2.3)	4.1	1.5	11.9	25.0

Plants were grown under different light intensities and at low P supply (Expt. 1). Shoot values represent means; standard deviations are given in brackets. Means followed by different letters are significantly different ($p < 0.05$, $n = 4$). Data were separately analyzed after the plants had been grown under high or low light intensities. Data of root and nodule carbohydrate concentrations are obtained from samples pooled within treatments.

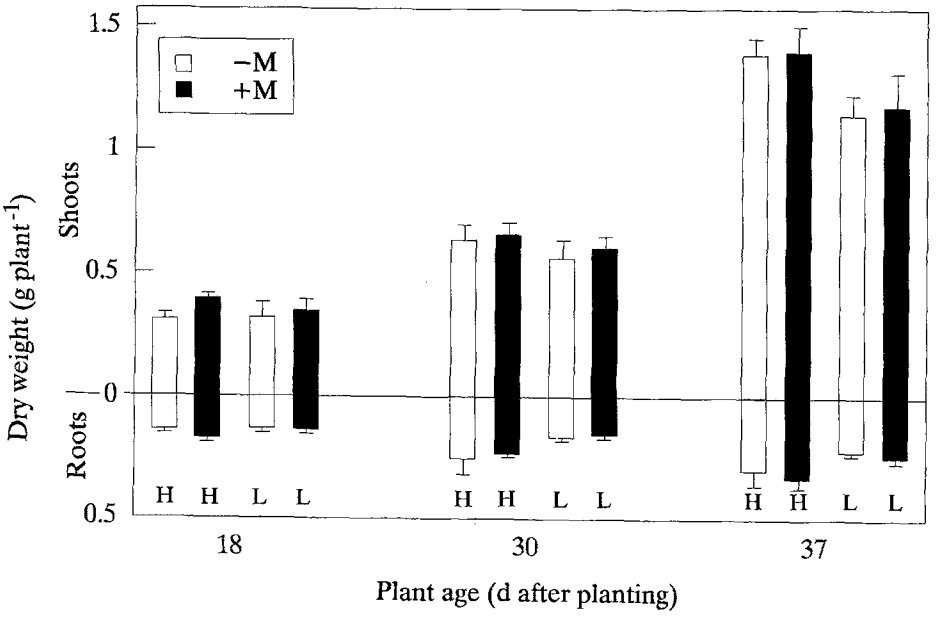

Figure 1. Shoot and root dry weight at three harvest dates in non-mycorrhizal (open segments) and mycorrhizal (filled segments) *Pisum sativum* plants inoculated with *Rhizobium leguminosarum*. Plants were grown under high (H) ($500\ \mu mol\ m^{-2}\ s^{-1}$) or low (L) light intensity ($250\ \mu mol\ m^{-2}\ s^{-1}$) and under non-limiting P supply (Expt. 2). Crossbars indicate standard deviations ($n = 4$).

after planting, shoot and root dry weight was slightly enhanced by high light intensity (fig. 1).

The degree of mycorrhizal colonization was markedly lower in the high-P plants as compared with the low-P plants in experiment 1. Mycorrhizal colonization reached a plateau of about 10% in 30-day-old plants and was hardly affected by light intensity. Only at the first harvest date, 18 days after planting, did mycorrhizal colonization tend to be decreased by low light intensity (table 4).

The number of nodules was affected by light intensity (table 4) and did not increase between 30 and 37 days after planting (data not shown), indicating that the infection phase had been terminated at the end of the vegetative growth stage. However, nodule dry weight still increased towards flowering, and was enhanced by high light intensity. Mycorrhizal colonization impaired nodulation under low light conditions at the first harvest date (table 4), when nodules and mycorrhizal structures had not become fully established, but not at later harvest dates (data not shown).

In experiment 3, plants were supplied with the same level of P as in experiment 2, but nitrogen supply was varied (16 or $100\ mg\ N\ kg^{-1}$). High light intensity ($900\ \mu mol\ m^{-2}\ s^{-1}$) was used to provide optimal plant growth conditions (fig. 2).

Mycorrhizal colonization was lower (5 to 10%) in this experiment, and was not influenced by the N treatment.

Table 4. Mycorrhizal (AM) colonization, nodule number (No.) and nodule dry weight per plant and concentrations of P and N in shoots as influenced by mycorrhiza treatment (*Glomus mosseae*) in 18-day-old *Pisum sativum* plants inoculated with *Rhizobium leguminosarum*

Treatment		AM Colonization (%)	Nodules plant^{-1}		Shoot concentrations (mg g^{-1} dry matter)	
Irradiation (μmol m^{-2} s^{-1})	AM Inoculation		No.	Dry wt. (mg)	P	N
500	−	0	152^a (54)	18.7^a (5.6)	4.70^a (0.20)	28.48^a (0.67)
	+	8.6 (1.9)	132^a (31)	18.9^a (4.5)	4.50^a (0.50)	21.18^b (1.57)
250	−	0	151^a (48)	22.1^a (8.4)	4.67^a (0.48)	29.27^a (1.89)
	+	6.4 (2.7)	92^a (16)	12.3^b (2.1)	4.35^a (0.25)	22.58^b (0.69)

Plants were grown under different light intensities and at non-limiting P supply (Expt. 2). Values represent means; standard deviations are given in brackets. Means followed by different letters are significantly different ($p < 0.05$, $n = 4$). Data were separately analyzed after the plants had been grown under different light intensities.

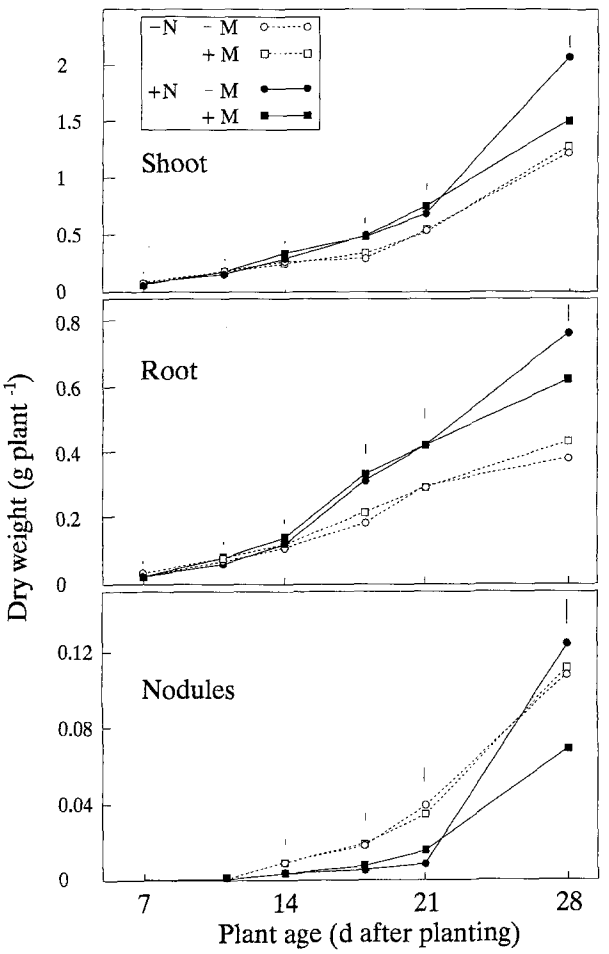

Figure 2. Shoot (above), root (middle) and nodules dry weight (below) at six harvest dates in non-mycorrhizal (−M) and mycorrhizal (+M) *Pisum sativum* plants inoculated with *Rhizobium leguminosarum*. Plants were grown without (−N) or with starter N (+N) under high light intensity (900 μmol m^{-2} s^{-1}) and under non-limiting P supply (Expt. 3). Vertical bars indicate standard error of means ($df = 16$).

No contribution of mycorrhiza to P acquisition occurred, as concentrations of P in shoots and roots were similar in mycorrhizal and non-mycorrhizal plants (table 5). At low N supply, mycorrhizal colonization had no effect on plant growth or N_2 fixation (table 5). During the first two weeks after planting, plant growth was similar at both N supply rates, but nodulation was delayed in high-N plants. At the first harvest date (7 days after planting), nodules were absent, but early AM infection stages were visible as appressoria and hyphal coils. Later on, the AM infection developed in distinct infection units of approximately 0.5 cm length. At 11 days after planting, small nodules were observed in low-N plants. Nitrogenase activity was detected in low-N plants 11 days after planting and in high-N plants 18 days after planting. Shoot and root growth rates were higher in high-N plants, but nodule numbers were markedly lower (fig. 2). After three weeks, with declining shoot N concentrations (data not shown), nodulation was intense, leading to an increase both in number and in dry weight of nodules in high-N plants. Within one week, between 21 and 28 days after planting, approximately 200 new nodules per plant were formed in both mycorrhizal and non-mycorrhizal plants. While nodule dry weight of non-mycorrhizal plants was steeply increased in this period, it remained markedly lower in mycorrhizal plants. As the nodule number was similar in mycorrhizal and non-mycorrhizal plants, lower nodule dry weight in mycorrhizal plants was due to reduced growth of individual nodules, accompanied by lower absolute and specific nitrogenase activity in mycorrhizal plants (table 5).

At the final harvest, shoot and root growth and dinitrogen fixation were significantly decreased by mycorrhizal treatment at high N supply (fig. 2, table 5). Shoot and root N concentrations were similar in mycorrhizal and non-mycorrhizal plants (data not shown). However, due to reduced dry matter production in mycorrhizal plants, N contents per plant were considerably lower than in non-mycorrhizal plants.

Sucrose concentrations in the roots (data not presented) were low, and were not affected by N treatment or mycorrhizal colonization.

Table 5. Acetylene reduction activity (μmol C_2H_4 h^{-1} $plant^{-1}$), specific activity (μmol C_2H_4 h^{-1} g^{-1} nodule dry wt.), nodule number per plant, concentrations of P in shoots and roots, and plant N content, as influenced by mycorrhiza treatment (*Glomus mosseae*) in 28-day-old *Pisum sativum* plants inoculated with *Rhizobium leguminosarum*

Treatment		ARA (μmol h^{-1})		Nodules ($plant^{-1}$)	P-concentration (mg g^{-1} dry matter)		N-content (mg $plant^{-1}$)
N-fertilization	AM Inoculation	($plant^{-1}$)	(specific)		Shoot	Root	
− N	−	5.44[a]	51.3[a]	463[a]	3.35[a]	5.92[a]	40.7[a]
	+	6.54[a]	57.9[a]	453[a]	3.48[a]	5.98[a]	48.5[a]
+ N	−	5.57[a]	45.2[a]	307[b]	3.52[a]	4.33[b]	69.5[b]
	+	2.36[b]	31.6[b]	272[b]	3.44[a]	4.44[b]	54.4[a]

Plants were grown without or with starter N and at non-limiting P supply. Light intensity was 900 μmol m^{-2} s^{-1} (Expt. 3). Means followed by different letters are significantly different ($p < 0.05$, $n = 5$).

Discussion

Enhancement of plant growth by AM infection is a well known response under conditions of limited P supply[3,13]. Phosphorus was a growth-limiting factor in experiment 1, where low P concentrations in the tissues of non-mycorrhizal plants limited N_2-fixation. The contribution of mycorrhiza to P acquisition enhanced shoot and root P concentrations and thus nodule growth and function. The beneficial effect on plant dry matter production resulted from both improved P and improved N nutritional status.

Plants grown with a higher P supply in experiment 2 showed no growth response to mycorrhization. Enhanced P fertilization resulted in higher P concentrations and a reduced degree of mycorrhizal colonization compared with plants in experiment 1. As a rule, with increasing P availability mycorrhizal colonization decreases[2,34]. Reasons suggested for this decrease are restricted carbon supply to the fungus, indicated by lower carbohydrate concentrations in root extracts[37] or changes in the anatomy of cortical cells limiting the number of entry points[1]. In experiment 3, with high P supply, expression of appressoria occurred early, but extension of distinct infection units was limited. This may be explained by restriction in the formation of secondary appressoria.

In plants grown under conditions of low P supply, mycorrhizal colonization was reduced at low light intensity. Plants high in P were hardly influenced by low light intensity, but infected root length was much lower.

There are conflicting reports on the influence of light intensity on mycorrhizal colonization. Colonization remained unaffected in experiments with onions and *G. mosseae*[31,34]. In contrast, the data of our first experiment (table 2), showing reduced AM-colonization under low light intensity, are supported by previous findings in alfalfa and *G. mosseae*[7], soybean and *G. fasciculatum*[4] and in clover and *G. mosseae*[36]. Despite a similar percentage of colonization under low light intensity, arbuscule development may be delayed in onion and *G. mosseae*[24], while lower arbuscule numbers were found in onion and *G. fasciculatum*[12]. In conclusion, the response to reduced light intensities can be confounded by several other factors, i.e. plant species, AM isolate and environmental conditions. This may be responsible for the divergence between our results and some previous findings.

In dual-infected plants, rhizobia and AM consume carbohydrates for growth and maintenance, but the host plants are able to a certain degree to control the energy consumption of their microsymbionts by restricting both AM formation and nodulation (experiment 1, 2 and low N plants in experiment 3). Sink competition for carbohydrates between host and symbionts, however, might have impaired nodule and shoot growth in mycorrhizal plants fertilized with starter-N. With high P supply and starter-N, combined with high light intensity, growth rates were enhanced but nodulation delayed.

After the starter-N had been utilized up by the plants, N concentration decreased, intensive nodulation began and the nodule number increased steeply. At this stage, the simultaneous occurrence of high growth rates of both nodules and host plants impaired parameters of N_2-fixation (ARA, table 5) and subsequently plant growth (fig. 2). A similar effect has been described previously[27] for a growth depression at the flowering stage in the *Pisum-Rhizobium-Glomus* symbiosis, when with a non-limiting P supply the formation of pods intensified competition for carbohydrates between aerial and below-ground parts of the plants.

The absence of starch and the low sucrose concentrations in the roots suggests that the symbionts rely on a continuous photosynthate supply via the phloem. In mycorrhizal pea plants, rapid nodule development constitutes a considerable sink activity, which may exceed the energy supply provided by the shoot, and thus impair root growth and consequently shoot growth as well.

The present data suggest that the interactions observed result from sink competition more than from direct effects on the development of microsymbionts. When both organisms are inoculated at the same time, nodulation and AM colonization proceed simultaneously, indi-

cating that the endophytes do not compete for infection sites or depress each other. Even when nodule formation was delayed (experiment 3), the subsequently produced nodule number was not influenced by previous AM colonization.

These results indicate that under many, but not all, environmental conditions the host is able effectively to restrict the access of AM to carbohydrates and thus to prevent severe impairment of *Rhizobium* symbiosis.

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1 Amijee, F., Colonization of roots by vesicular-arbuscular mycorrhizal fungi in relation to phosphorus and carbon nutrition. Aspects appl. Biol. *22* (1989) 219–225.
2 Amijee, F., Tinker, P. B., and Stribley D. P., The development of endomycorrhizal root systems VII. A detailed study of effects of soil phosphorus on colonization. New Phytol. *111* (1989) 435–446.
3 Barea, J. M., and Azcon-Aguilar, C., Mycorrhizas and their significance in nodulating nitrogen-fixing plants. Adv. Agron. *36* (1983) 1–54.
4 Bethlenfalvay, G. J., and Pacovsky, R. S., Light effects in mycorrhizal soybeans. Pl. Physiol., *73* (1983) 969–972.
5 Bethlenfalvay, G. J., Brown, M. S., and Pacovsky, R. S., Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: The development of the host plant. Phytopathology *72* (1982) 889–893.
6 Buwalda, J. G., and Goh, K. M., Host-fungus competition for carbon as a cause of growth depressions in vesicular-arbuscular mycorrhizal ryegrass. Soil Biol. Biochem. *14* (1982) 103–106.
7 Daft, M. J., and El-Giahmi, A. A., Effect of arbuscular mycorrhiza on plant growth. VIII. Effect of defoliation and light on selected hosts. New Phytol. *80* (1978) 365–372.
8 Gericke, S., and Kurmies, B., Die kolorimetrische Phosphorsäurebestimmung mit Ammonium-Vanadat-Molybdat und ihre Anwendung in der Pflanzenanalyse. Z. Pfl-Ernähr. Düng. Bodenk. *59* (1952) 235–247.
9 Gordon, A. J., Carbon metabolism in the legume nodule, in: Carbon Partitioning Within and Between Organisms, pp. 133–156. Eds C. J. Pollack, J. F. Farrar, and A. J. Gordon. Bios, Oxford, UK 1992.
10 Hansen, A. P., Pate, J. S., and Atkins, C. A., Relationship between acetylene reduction, hydrogen evolution and nitrogen fixation in nodules of *Accacia* spp.: Experimental background to assaying fixation by acetylene reduction under field conditions. J. expl Bot. *38* (1987) 1–12.
11 Harris, D., Pacovsky, R. S., and Paul, E. A., Carbon economy of soybean-*Rhizobium*-*Glomus* associations. New Phytol. *101* (1985) 426–440.
12 Hayman, D. S., The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Can. J. Bot. *61* (1983) 944–963.
13 Hayman, D. S., Plant growth repsonses to vesicular-arbuscular mycorrhiza. VI. Effect of light and temperature. New Phytol. *73* (1974) 71–80.
14 Holsten, R. D., Burns, R. C., Hardy, R. W. F., and Hebert, R. R., Establishment of *Rhizobium* and plant cells *in vitro*. Nature *232* (1971) 173–176.
15 Israel, D. W., Investigation of the role of phosphorus in symbiotic dinitrogen fixation. Pl. Physiol. *84* (1987) 835–840.
16 Jakobsen, I., The role of phosphorus in nitrogen fixation by young pea plants (*Pisum sativum*). Physiologia Pl. *64* (1985) 190–196.
17 Jakobsen, I., Vesicular-aruscular mycorrhiza in field-grown crops. III. Mycorrhizal infection and rates of phosphorus inflow in pea plants. New Phytol. *104* (1986) 573–581.
18 Jakobsen, I., and Nielsen, N. E., Vesicular-arbuscular mycorrhiza in field grown crops. I. Mycorrhizal infection in cereals and peas at various times and soil depths. New Phytol. *93* (1983) 401–413.
19 Jakobsen, I., Abbott, L. K., and Robson, A. D., External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. New Phytol. *120* (1992) 371–380.
20 Koide, R., The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal infection. New Phytol. *99* (1985) 449–462.
21 Li, X.-L., George, E., and Marschner, H., Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Pl. Soil *136* (1991) 41–48.
22 O'Hara, G. W., Boonkered, N., and Dilworth, M. J., Mineral constraints to nitrogen fixation. Pl. Soil *108* (1988) 93–110.
23 Olsen, R., Cole, C. V., Watanabe, F. S., and Dean, L. A., Estimation of available phosphorus in soils by extraction with $NaHCO_3$. Circular no. 939, United States Department of Agriculture Washington DC 1954.
24 Pearson, J. N., Smith, S. E., and Smith, F. A., Effect of photon irradiance on the development and activity of VA mycorrhizal infection in *Allium porrum*. Mycol. Res. *95* (1991) 741–746.
25 Peng, S., Eissenstat, D. M., Graham, J. H., Williams, K., and Hodge, N., Growth responses in mycorrhizal citrus at high-phosphorus supply. Pl. Physiol. *101* (1993) 1063–1071.
26 Philips, J. M., and Hayman, D. S., Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. *55* (1970) 158–161.
27 Reinhard, S., Martin, P., and Marschner, H., Interactions in the tripartite symbiosis of pea (*Pisum sativum* L.), *Glomus* and *Rhizobium* under non-limiting phosphorus supply. J. Pl. Physiol. *141* (1993) 7–11.
28 Robson, A. D., O'Hara, G. W., and Abbott, L. K., Involvement of phosphorus in nitrogen fixation by subterranean clover (*Trifolium subterraneum* L.). Aust. J. Pl. Physiol. *8* (1981) 427–436.
29 Ryle, C. J. A., Arnott, R. A., Powell, C. L., and Gordon, A. J., N_2 fixation and the respiratory costs of nodules, nitrogenase and nodule growth and maintenance in Fiskeby soyabean. J. expl Bot. *35* (1984) 1156–1165.
30 Same, B. I., Robson, A. D., and Abbott, L. K., Phosphorus, soluble carbohydrates and endomycorrhizal infection. Soil Biol. Biochem. *15* (1983) 593–597.
31 Smith, S. E., and Gianinazzi-Pearson, V., Phosphate uptake and arbuscular activity in mycorrhizal *Allium cepa* L.: Effects of photon irradiance and phosphate nutrition. Aust. J. Pl. Physiol. *17* (1990) 177–188.
32 Snellgrove, R. C., Splitstoesser, W. E., Stribley, D. P., and Tinker, P. B., The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytol. *89* (1982) 75–87.
33 Sokal, R. R., and Rohlf, F. J., Biometry. W. H. Freeman and Company, New York 1981.
34 Son, C. L., and Smith, S. E., Mycorrhizal growth responses: Interactions between photon irradiance and phosphorus nutrition. New Phytol. *108* (1988) 305–314.
35 Tennant, D., A test of a modified line intersect method of estimating root length. J. Ecol. *63* (1975) 995–1001.
36 Tester, M., Smith, F. A., and Smith, S. E., Phosphate inflow into *Trifolium subterraneum* L.: Effects of photon irradiance and mycorrhizal infection. Soil Biol. Biochem. *17* (1985) 807–810.
37 Thomson, B. D., Robson, A. D., and Abbott, L. K., Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. New Phytol. *103* (1986) 751–765.