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Effect of Mycorrhizal Colonization and Light Limitation on Growth and Reproduction of Lima Bean (Phaseolus lunatus L.)

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Effect of mycorrhizal colonization and light limitation on growth and reproduction of lima bean (*Phaseolus lunatus* L.)

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Summary

Plants can respond with sink stimulation of photosynthesis when colonized with fungal or bacterial root symbionts, compensating costs of carbohydrate allocation to the microbes. However, constraints may arise under light limitation when plants cannot extensively increase photosynthesis. We hypothesize that under such conditions the costs for maintaining the symbiosis outweigh the benefits, ultimately turning the mutualist microbes into parasites, resulting in reduced plant growth and reproduction.

Using lima bean (*Phaseolus lunatus*) as an experimental plant, we applied two levels of light (full light, 75% shading) and microbial inoculation (sterile soil, mycorrhizal fungi) and quantified both vegetative and generative plant traits.

As expected, shaded plants produced less vegetative biomass and seeds than non-shaded plants. However, individual seeds were significantly heavier in shaded plants and required less time for germination. While under both light conditions mycorrhizal plants showed a significantly reduced belowground biomass, mycorrhizal fungi neither enhanced overall plant performance in terms of total biomass and seed production nor resulted in measurable costs in either light condition. Our study suggests that mycorrhizal colonization neither provided benefits to lima bean plants grown under full light, nor created costs when photosynthesis was limited.

Introduction

Mutualistic interactions between plants and microbes are an important component in the determination of plant diversity and ecosystem productivity. One of the most important groups of plant-associated microbial mutualists are mycorrhizal fungi (SMITH and READ, 1997). The symbiosis between plants and mycorrhizal fungi is extremely widespread and ancient in the plant kingdom. Root colonization with mycorrhizal fungi occurs in >80% of all plant species (SMITH and READ, 1997) and has been observed in fossils dating back 400 million years ago (REMY and TAYLOR, 1994). Mycorrhizal fungi colonize the host plant's roots, form extensive networks and participate in the acquisition of phosphorus (P) in the case of arbuscular mycorrhizal fungi (AMF) and nitrogen in ectomycorrhizal fungi (EMF) (SMITH and READ, 1997). Due to their critical impact on plant growth and species composition these microbial symbionts are considered keystone species in terrestrial ecosystems (LODGE et al., 1996).

Root colonization with mycorrhizal fungi generally has positive effects on plant growth (CHALK et al., 2006) and mycorrhizal inoculation is frequently applied to increase crop plant productivity in agricultural systems (LI et al., 2000, 2004; ORTAS et al., 2003; ORTAS, 2010). Positive effects of mycorrhiza on plants include increases in height (HAYMAN, 1986; HOEKSEMA et al., 2010; SAFAPOUR et al., 2011), biomass (VEJSADOVA et al., 1993; MATHUR and VYAS, 2000; RAMANA et al., 2010), shoot:root ratio (GAVITO et al., 2000; VERESOGLOU et al., 2012), production of flowers (DODD et al., 1983; CAREY et al., 1992), and yield in crop plants such as *Phaseolus vulgaris*, *Glycine max*, and *Triticum aestivum* (VEJSADOVA et al., 1993; BETHLENFALVAY et al., 1997; ABDEL-FATTAH, 1997; LI et al., 2005; RAMANA et al., 2010; SAFAPOUR et al., 2011). There is an extensive body of literature on the effects of mycorrhizal fungi in a broad range of plant families including legumes (BAREA and AZCON-AGUILAR, 1983; YANG et al., 1994; OLSEN et al., 1999a; 1999b; LIU et al., 2003; SCHEUBLIN and RIDGWAY, 2004; ORTAS, 2008; MULETA, 2010) but a detailed understanding of costs and benefits arising from the mycorrhizal symbiosis under different abiotic conditions is often lacking. Legumes are both important components in many terrestrial ecosystems and crop plants of world economic importance. Due to their association with another group of microbial symbionts – nitrogen-fixing rhizobia – legumes critically determine the productivity and species composition of ecosystems (SPRENT and SPRENT, 1990) and according to their key function in global nitrogen cycles legumes are considered ecosystem engineers (CARNEY and MATSON, 2005; VAN DER HEIJDEN et al., 2008). In addition to their high impact on natural ecosystems, legumes are of high relevance in agro-ecosystems. Legumes critically enhance the sustainability of agroforestry systems (MULETA, 2010) or pastures (HAYSTEAD et al., 1988) and some legume crop plants are of world economic importance (*Glycine max*, *Pisum sativum*, *Phaseolus vulgaris*, *Phaseolus lunatus*).

Although in most cases clearly beneficial for the plants, the associations with mycorrhizal fungi also incur costs as the microbial symbionts may consume up to 16% of photosynthetically-fixed carbon, which otherwise could be allocated to growth and reproductive functions (KASCHUK et al., 2009). However, recent research demonstrated that plants can compensate for this cost through sink stimulation of photosynthesis, which is thought to be an adaptation to take advantage of the nutrient supply provided without compromising the total amount of photosynthates available for plant growth and development (MORTIMER et al., 2008). In natural ecosystems, light availability is often a variable resource due to competition among plant species and, depending on cultivation method, also shows strong variation in agricultural systems (CHIRKO et al., 1996). While sink stimulation of photosynthesis is generally an efficient strategy to compensate for costs of carbohydrate allocation to microbial root symbionts, the question arises as of how plants respond to mycorrhizal inoculation under light-limited conditions when photosynthesis cannot be increased easily.

The interacting effects of mycorrhizal colonization and light limitation on plant vegetative growth have been studied previously (SMITH and READ, 1997), however, the impact of mycorrhiza on plant reproduction and the actual crop yield have received much less attention. One study found maize plants exposed to lower irradiance had a smaller percent mycorrhizal infection and smaller shoot weight, which was equated to yield (DAFT and EL-GIAHMI, 1978). Another study showed that mycorrhizal infection did not affect *Pisum sativum* biomass; however, increased light resulted in enhanced growth once the plant reached the flowering stage (REINHARD et al., 1994). Unfortunately, these studies did not elucidate mycorrhizal fungi's effects on seed production. Analyzing the effects of mycorrhizal

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colonization on actual seed set is of high importance due to two reasons: First, it is a much more precise fitness measure than plant biomass as mycorrhizal symbionts might lead to resource allocation and reduce plant biomass while increasing seed set. Second, for many agricultural plants, seed yield is of larger interest than vegetative parameters (SMITH and SMITH, 2011; RONSHEIM, 2012). In the present study we used lima bean (Fabaceae: *Phaseolus lunatus*) as experimental plant. Lima bean represents an emerging model plant commonly used in studies on indirect and direct plant defense against herbivores (BALLHORN et al., 2008; 2009; 2010), and also bacterial (YI et al., 2009) and fungal pathogens (BALLHORN et al., 2010). Furthermore, this plant is one of the most economically important *Phaseolus* species cultivated for food (FOFANA et al., 1999; ALVES et al., 2008; BONIFÁCIO et al., 2012). To better understand the concerted effects of mycorrhizal colonization and light availability, we exposed mycorrhizal and mycorrhiza-free lima bean plants to two levels of light. We hypothesized that mycorrhizal fungi provide benefits for their host plant under full light regimes, but that reduced light availability might increase the costs of the symbiosis and shift a beneficial interaction to a detrimental one. To our knowledge, this study is the first to analyze the interactive effects of mycorrhizal fungi and light availability in lima beans in order to uncover potential costs of these microbes when photosynthesis is limited.

Materials and methods

Plants

Lima bean plants (*Phaseolus lunatus* cv. Henderson) were grown from seeds (American Meadows Inc., Williston, VT). Plants were cultivated in a greenhouse with light regime of 13:11 light:day. Light in the greenhouse was provided by a combination (1:1) of HQI-BT 400 W (Osram) and RNP-T/LR 400 W (Radium) lamps. Temperature was 27:19 °C (i.e. temperature of 27 °C in the light period and 19 °C in the dark period) and we maintained an air humidity of 70-80%. Plants were grown in containers (one plant per pot) with 12 cm in diameter in Sunshine Mix #1, LC1 (SunGro Horticulture®, Bellevue, WA) 175 g per pot and were watered daily.

Experimental setup

In a full factorial split plot design with two whole plots we applied four treatments, 15 replicates each (60 plants total) including two levels of light (full light, 75% shading) and two levels of mycorrhizal colonization (with and without mycorrhizal fungi). Light availability was measured at noon on a sunny day (+ additional lighting) and at table height was an average of 525 µmol photon s^{-1} m^{-2} in full light and an average of 138 µmol photon s^{-1} m^{-2} (LI-250 light meter; LI-COR Biosciences, Lincoln, Nebraska, USA) under the shade tent (205 cm × 113 cm × 113 cm). Experimental plants were inoculated with commercial mycorrhizal inoculum [powder inoculant, Bio Organics™ , La Pine, Oregon (*Glomus aggregatum*, *G. etunicatum*, *G. mosseae*, *G. clarum*, *G. deserticola*, *G. monosporus*, *Gigaspora margarita*, *Paraglomus brasilianum*, *Rhizophagus irregularis*), 10 cc (8 g) per plant] when they had developed completely unfolded primary leaves. Plants were not inoculated with any other microbes (such as rhizobia) to avoid uncontrolled cross-effects between fungal and bacterial root symbionts.

Plant biomass and reproduction

Over the experimental period of 14 weeks we measured plant height, leaf number, and initial number of seed pods. At the end of the experiment we evaluated the above and belowground biomass, the final number of seed pods, and determined the shoot:root ratio. To obtain belowground biomass, plant root systems were carefully washed until all potting soil was removed. Above and belowground parts of plants were dried in an oven (IncuMax™ CV250 Convection Oven, Amerex Instruments, Inc., Lafayette, CA) at 70 °C for 5 days until constancy of weight. Seeds produced per plant were counted and weighed. To assess viability of seeds they were germinated by placing them between four 24 cm × 45 cm wet papers towels in the dark at 25 °C. Number of days to germination was recorded for each seed.

Microscopic analysis of mycorrhizal colonization

Mycorrhizal colonization was evaluated by taking 1 g of fresh root samples, from each plant, from 4 separate locations of the washed roots. Root segments were placed into histocassettes (VWR, West Chester, PA). All root samples were cleared with 10% KOH, acidified in 2% HCl, stained with 0.05% trypan blue solution, and preserved in lactoglycerol (PHILLIPS and HAYMAN, 1970). Roots were cut into 1 cm sections and at least 40 cm of roots from each plant were placed on a single microscope slide with lactoglycerol. Microscopic observations were conducted using an AmScope FM320 Trinocular Microscope in both 100x and 400x magnification. Roots were examined for mycorrhizal structures that intersected the microscope eyepiece crosshair at 100 random points using the Magnified Intersections Method (MCGONIGLE et al., 1990). The presence or absence of mycorrhizal structures at 100 intersects was used to calculate percent root length colonization by mycorrhizal fungi per plant.

Data analysis

The effects of 'Mycorrhizal inoculation' and 'Light availability' on plant traits were assessed with 2-way ANOVA split-plot analysis with 'Light Availability' as a blocking factor, using the GLM procedure in SAS. Square root transformation was performed on several plant growth parameters (number of leaves, buds, flowers, and seed pods) to control for normality in tests. 'Mycorrhizal inoculation' and 'Light availability' were set as fixed effects in all tests. Number of inflorescences per plant was set as a covariate in the 2-way ANOVA for number of seed pods at a defined time point (6 weeks after cultivation). All tests were performed in SAS version 9.2.

Results

Light availability

The colonization of experimental plants with mycorrhizal fungi was not affected by light availability (Tab. 1). Light availability significantly affected all considered vegetative plant traits including plant height (F=144.59, P<0.001), number of leaves (L: F=38.93, P<0.001), total biomass (F=103.48, P<0.001), above- (F=70.10,

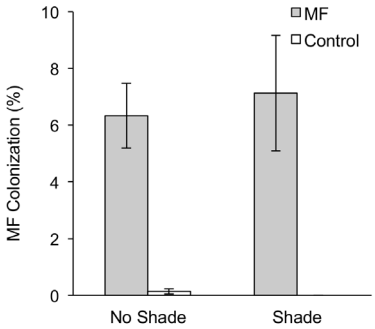

Fig. 1: Percent mycorrhizal colonization. MF = Mycorrhizal Fungi; Values shown are means + SE; n = 15 plants per treatment

Tab. 1: Summary of ANOVA results of the effects of mycorrhizal fungi and light availability on vegetative plant traits.

Effects		Height		Total Leaves		Aboveground Biomass		Belowground Biomass		Total Biomass		Shoot:Root	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Mycorrhiza (M)	1	2.78	0.107	1.43	0.242	1.20	0.283	9.44	0.005*	0.10	0.750	23.49	<0.001*
Light (L)	1	144.59	<0.001*	38.93	<0.001*	70.10	<0.001*	290.16	<0.001*	103.48	<0.001*	4.99	0.034*
M × L	1	0.40	0.534	0.67	0.419	0.61	0.387	3.13	0.088	0.10	0.757	1.17	0.288

* *P*-values are significant (*P*<0.05)

P<0.001) and belowground biomass (F=290.16, P<0.001), as well as shoot:root ratio (F=4.99, P=0.034; Tab. 1). Plants growing under full light were significantly taller, had more leaves, more total biomass, more above- and belowground biomass than plants cultivated under shaded conditions (Fig. 2).

Light availability also significantly affected all generative plant traits analyzed, such as the initial number of pods per plant (L: F=127.88, P<0.001), the final number of pods (L: F=34.28, P<0.001), the number of pods dropped before maturation (F=129.94, P<0.001) as well as the number of seeds (L: F=202.15, P<0.001; Tab. 2). Plants growing under full sun had more initial pods, more final pods, and more seeds, but also dropped more pods than shaded plants (Fig. 3).

Seed quality parameters were significantly affected by light conditions including total seed weight (F=43.56, P<0.001), average seed weight (F=10.72, P=0.002), and days to germination (F=54.13, P<0.001), but not the percentage of seeds that germinated (Tab. 3). Total and average seed weight were higher for shaded plants than for full light controls and seeds from plants under shaded conditions germinated significantly quicker than seeds from plants growing under full light conditions (Fig. 4).

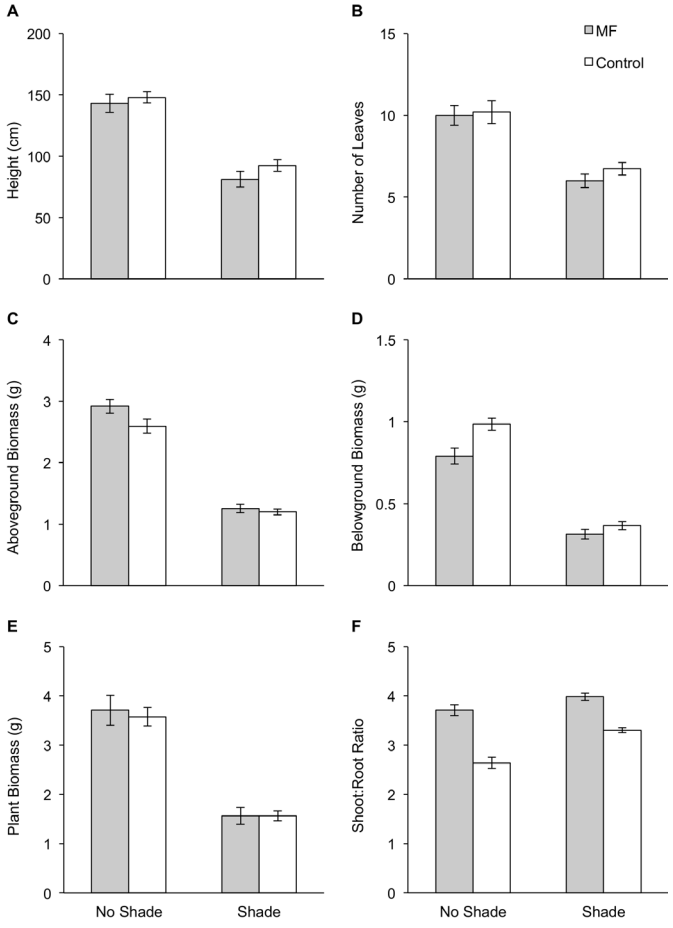

Fig. 2: Effects of mycorrhizal fungi and light availability on vegetative plant and biomass traits. Plant height (a) and number of leaves per plant (b) were determined at the end of the cultivation period. Total plant aboveground biomass (c), belowground biomass (d), total biomass (e) and plant shoot:root ratio (f) were determined on dry weight basis. MF = Mycorrhizal Fungi; Values shown are means + SE; n = 15 plants per treatment

AM fungal colonization

Microscopical analyses revealed successful mycorrhizal colonization in the inoculated group, whereas the control group showed little to no mycorrhizal fungi (Fig. 1). AM fungi influenced far fewer traits than light availability. Specifically, mycorrhizal fungi significantly affected belowground biomass (*F*=9.44, *P*=0.005) and the shoot:root ratio of plants (*F*=23.49, *P*<0.001). Plants inoculated with mycorrhizal fungi had significantly lower belowground biomass than controls and accordingly a higher shoot:root ratio (Fig. 2). All other vegetative plant traits that we analyzed were not significantly changed in response to mycorrhizal colonization including plant height, number of leaves, aboveground biomass and total biomass (Tab. 1).

Reproductive plant traits, which were significantly affected by AM fungal colonization, were the number of initial pods (*F*=7.38, *P*=0.011) and the number of dropped pods (*F*=12.14, *P*<0.001). Plants with mycorrhizal fungi had less initial pods and dropped less pods than plants grown without the symbiotic partner (Fig. 3). However, the total number of final pods and total seed numbers did not show significant variation between mycorrhizal and non-colonized control plants (Tab. 2).

Inoculation with mycorrhizal fungi significantly affected the average seed weight (*F*=11.80, *P*=0.002) as well as the days to germination (*F*=5.27, *P*<0.022). Inoculated plants had significantly higher average seed weight per plant than controls, but seeds of these plants required more time to germinate (Fig. 4). In contrast, the total seed weight and the percent of seeds that germinated were not altered by mycorrhizal fungi (Tab. 3).

Interacting effects of 'Light availability' and 'Mycorrhizal fungi'

The interaction of 'Light availability' and 'Mycorrhizal fungi' (M×L) did not significantly affect any of the vegetative plant parameters assessed (Tab. 1). Of the reproductive plant traits analyzed, the number of initial pods (*F*=5.37, *P*=0.028) and the number of dropped pods (*F*=10.28, *P*=0.003) were significantly affected by the interaction of 'Light availability' and 'Mycorrhizal fungi', while the number of final pods and the number of seeds per pod were not affected

Tab. 2: Summary of ANOVA results of the effects of mycorrhizal fungi and light availability on reproductive plant traits.

Effects		Total Initial Pods		Total Final Pods		Total Dropped Pods		Total Seeds	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Mycorrhiza (M)	1	7.38	0.011*	0.05	0.821	12.14	0.002*	1.14	0.294
Light (L)	1	127.88	<0.001*	34.28	<0.001*	129.94	<0.001*	202.15	<0.001*
M × L	1	5.37	0.028*	1.54	0.225	10.28	0.003*	1.14	0.294

* *P*-values are significant ($P<0.05$)

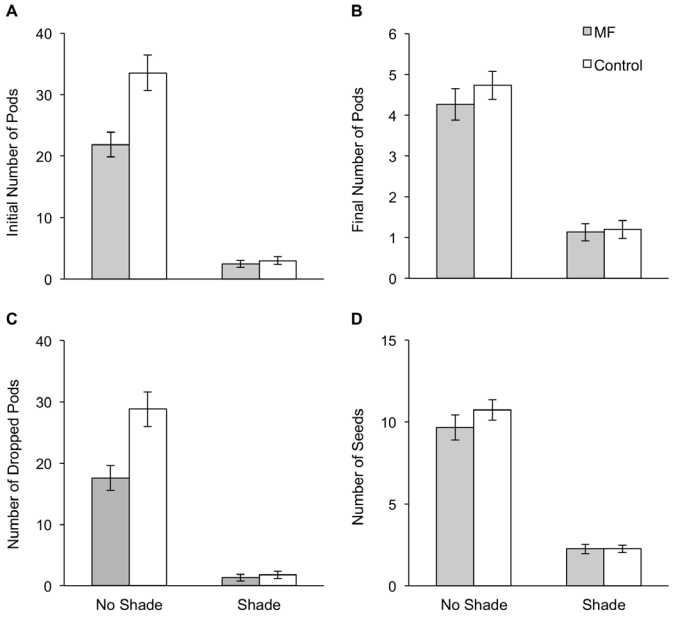

Fig. 3: Effects of mycorrhizal fungi and light availability on seed production. Number of initial pods per plant (a) was determined 6 weeks after planting. Final number of pods (b), dropped pods (c), and total seed production per plant (d) was determined at the end of the cultivation period. MF = Mycorrhizal Fungi; Values shown are means + SE; n = 15 plants per treatment

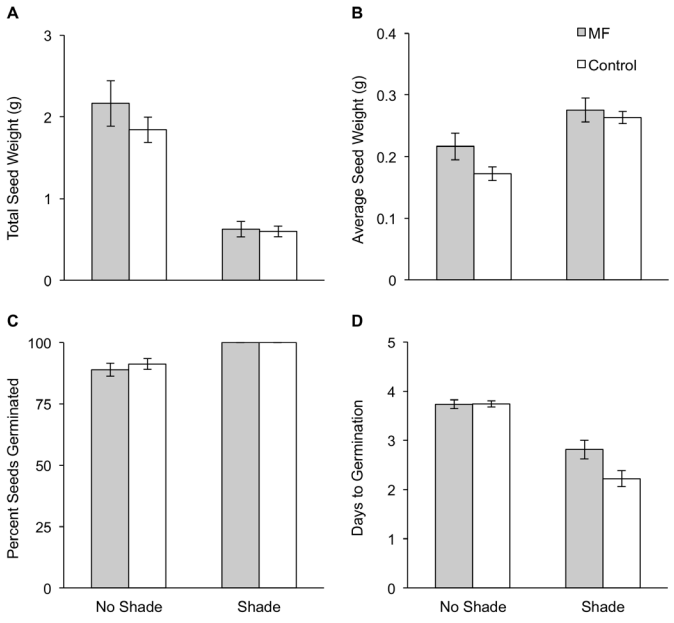

Fig. 4: Effects of mycorrhizal fungi and light availability on weight and viability of seeds. Total weight of seeds per plant (a) and average seed weight (b) were determined on dry weight basis. Percent seeds germinated per plant (c) and days to seed germination per plant (d) were determined over an 8-day germination period. MF = Mycorrhizal Fungi; Values shown are means + SE; n = 15 plants per treatment for (a); for (b), (c), and (d), n = 27 seeds per treatment for shaded plants, n = 129 seeds for inoculated, no shade plants, and n = 147 seeds for control, no shade plants

(Tab. 2). The only seed trait to be significantly affected by the interacting term of both variables was the days to germination ($F=4.93$, $P=0.027$), while all other seed traits were not significantly altered (Tab. 3).

Discussion

Even though the benefits of root-associated microbes are well studied in many cases, it remains widely elusive as to now the outcome of this mutualism is affected by external abiotic conditions. In our study we quantitatively analyzed the effects of mycorrhizal fungi and light availability on growth and reproduction of lima bean. We hypothesized enhancing effects of growth and reproduction by mycorrhizal fungi under full light whereas we expected reduced plant development in mycorrhizal plants under shaded conditions due to constraints for plants to support both growth and the mycor-

Tab. 3: Summary of ANOVA results of the effects of mycorrhizal fungi and light availability on seed traits and viability.

Effects		Total Seed Weight		Average Seed Weight		Percent Seed Germination		Days to Germination	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Mycorrhiza (M)	1	1.93	0.178	11.80	0.001*	0.52	0.470	5.27	0.022*
Light (L)	1	543.56	<0.001*	10.72	0.002*	3.20	0.081	54.13	<0.001*
M × L	1	0.42	0.526	0.46	0.498	0.52	0.470	4.93	0.027*

* *P*-values are significant ($P<0.05$)

rhizal partner when photosynthesis is limited. Studies in the past have shown decreases in plant growth in lower light conditions, mainly seen as decreased plant biomass (DAFT and EL-GIAHMI, 1978; BETHLENFALVAY and PACOVSKY, 1983; TESTER et al., 1986; PEARSON et al., 1991). Thus, under such conditions the costs for maintaining the mutualism with mycorrhizal fungi may outweigh the benefits, which ultimately turns the mutualistic microbes into parasites that exploit resources and reduce host fitness (BETHLENFALVAY and PACOVSKY, 1983; REINHARD et al., 1993). Contrary to our expectations, under full light, mycorrhizal fungi did not significantly enhance plant performance. Inoculated plants did not produce more biomass than the respective controls (Fig. 2), and mycorrhizal plants actually had significantly less pods than controls (Fig. 3). However, in our study the significantly negative effects of mycorrhizal fungi disappeared on the level of actual number and weight of seeds, which showed no significant differences between the treatments (Figs. 3 and 4). These overall neutral effects of mycorrhizal fungi on plant reproductive structures observed in our study are in line with a recent meta-analysis looking at various legume species including *Cicer arietinum*, *Lens culinaris*, *Phaseolus vulgaris*, *Pisum sativum*, and *Vicia faba*. In these plants, mycorrhizal fungi were found to have no effect on crop yield (KASCHUK et al., 2010). In our study the only factor affecting biomass and seed production was light availability. Under reduced light, plants produced less biomass and less seeds compared to plants growing under full light conditions.

There are several possible explanations for the limited impact of mycorrhizal fungi on plant growth and reproduction as observed in the present study. One possibility is that mycorrhizal fungi may play a less important role for grain legumes than for other plant species in general (KASCHUK et al., 2010) or that lima bean in particular is not adapted to form a symbiosis with mycorrhizal fungi. Even though lima bean represents an important crop plant, to the best of our knowledge there is no information available on the colonization of lima bean plants with mycorrhizal fungi under laboratory or field conditions. On the other hand, studies on a closely related plant species (snap bean, *Phaseolus vulgaris*) showed positive effects of mycorrhizal fungi on growth and nutrient uptake (HACISALIHOGLU et al., 2005; CIFTCI et al., 2010). Another possibility that could explain the limited impact of mycorrhizal fungi on lima bean observed in our study is that the commercial inoculum we used may not have contained fungal strains that provide a benefit to lima bean plants. Although we could show mycorrhizal colonization of the plants roots and formation of haustoria microscopically, this does not necessarily mean the fungi efficiently provided nutrients for their host plant. Furthermore, the rate of mycorrhizal colonization was relatively low compared to other plant species. However, this might be due to sampling roots towards the end of the plants' life cycle, which was required, as we needed to collect data on seed production of the same individual plants. At earlier stages of plant development, the rate of mycorrhizal colonization likely might have been higher as colonization rates decrease with plant age, as has been reported for *Hordeum vulgare*, *Secale cereale* and *Triticum aestivum* (DODD and JEFFRIES, 1986; BOSWELL et al., 1998; LI et al., 2005). Furthermore, together with our microscopic proof of successful colonization, the observation that mycorrhizal colonization of lima bean resulted in a significantly increased shoot:root ratio compared to the respective controls supports a good matching quality of plants and fungi rather than limited compatibility. A relatively lower root biomass in mycorrhizal plants compared to uncolonized conspecifics is a common phenomenon and indicates an efficient transport of minerals and water via mycorrhizal fungi (KOTHARI et al., 1990). Thus, the increased shoot:root ratio in mycorrhizal lima bean plants we observed here suggests an actual interaction between both partners.

In addition to effects of mycorrhizal fungi on above- and belowground biomass of the lima bean plants themselves, we also considered effects of mycorrhizal inoculation on germination and viability of produced seeds, that is, on the next generation of host plants. Compared to plants growing under full light conditions, shaded plants produced overall significantly fewer but heavier seeds. Nevertheless, within each light treatment, mycorrhizal inoculation had no significant effect on the total seed weight produced per plant (Fig. 4a). Mycorrhizal colonization, however, increased the average seed weight with heaviest seeds in shaded plants (Fig. 4b); in plants grown in full light and in shade, this increase was significant. Increases in average seed weight due to mycorrhizal inoculation has been described previously for various plants such as *Triticum aestivum* and *Abutilon theophrasti* (LU and KOIDE, 1994; KARAGIANNIDIS and HADJISAVVA-ZINOVIADI, 1998). While the underlying mechanisms are little understood, changes in resource allocation within the plant are likely as mycorrhizal fungi represent a significant carbon sink (MATHUR and VYAS, 2000; CHALK et al., 2006; KASCHUK et al., 2009). Based on the results of our study we cannot make predictions on whether the observed effects of mycorrhizal colonization on seeds are positive or negative for plants in nature; however, other studies showed that changes in seed traits can have a far reaching impact on plant fitness. Seed weight and size have been identified as plant traits strongly influencing the dispersal, establishment, survival and growth of seedlings (HARPER et al., 1970; HARPER, 1977; WESTOBY, 1998; WEIHER et al., 1999; LEISHMAN et al., 2000; MOLES and WESTOBY, 2004), particularly at early seedling stages (LEISHMAN et al., 2000; COOMES and GRUBB, 2003). FENNER and THOMPSON (2005) showed that large seeds had an increased probability of establishment under detrimental conditions. Generally seedlings developing from large seeds cope better than those of smaller seeded species under competition (PARRISH and BAZZAZ, 1985; REES, 1995), drought, nutrient limitation (LEE and FENNER, 1989; JURADO and WESTOBY, 1992; LEISHMAN and WESTOBY, 1994) and depth of seedling emergence (GULMON and URL, 1992; PETERSON and FACELLI, 1992; VÁZQUEZ-YANES and OROZCO-SEGOVIA, 1992). In line with our study, deep shade has also been identified as a factor selecting against small seed size (GRIME and JEFFREY, 1965; LEISHMAN and WESTOBY, 1994). Thus, development of larger seeds under shaded conditions and the increases in average seed weight in response to mycorrhizal colonization as we observed in our study might represent fitness relevant parameters.

Beyond changes in seed weight, seeds produced by mycorrhizal and non-mycorrhizal plants showed differences regarding germination time (Tab. 3). While seeds from mycorrhizal parent plants took equal time to germinate compared to mycorrhiza-free controls when plants were grown in full light, seeds produced by colonized plants under shaded conditions germinated significantly later than seeds produced by mycorrhiza-free plants (Fig. 4). Whether these changes were due to variation in the thickness of seed coats determining water intake as the first step of the germination process or whether the observed variation is due to different enzymatic activities in seeds derived from the different treatments remains elusive and, again, it is difficult to predict if these changes in germination time increase or decrease plant fitness as this depends on the specific environmental conditions. In general, the benefits to shorter germination time in plants is variable, however in large-seeded plants shorter germination time has been demonstrated to enhance growth and fecundity (VERDÚ and TRAVESET, 2005).

Conclusions

Mycorrhizal fungi play a key role for plant performance and productivity in natural and agricultural ecosystems yet their effects on actual seed production in interdependence with variable abiotic conditions remains elusive in many cases. Thus, as mycorrhizal symbiosis holds

great potential to improve sustainable crop production (PLENCHETTE et al., 2005) there is an urgent need to functionally study this almost ubiquitous interaction and analyze the effects of environmental factors on the symbiosis (ORTAS, 2012). Using lima bean (*Phaseolus lunatus*) as an experimental plant, we hypothesized that for plants under light limitation the costs for maintaining the symbiosis outweigh the benefits of the fungal partner, ultimately turning the beneficial microbes into parasites, resulting in reduced plant growth and reproduction. Contrary to our expectations, we found that mycorrhizal colonization neither provided benefits in terms of increased biomass and total seed number and total seed weight to plants grown under full light, nor created costs under shaded conditions. Mycorrhizal colonization did, however, significantly increase the average seed weight in both light treatments with heaviest seeds in shaded plants and lengthened germination time of seeds produced by shaded plants. Our study shows that the effects of mycorrhizal symbionts go beyond mere effects on plant biomass production as they significantly alter plant reproductive traits. Results of our study suggest that even though mycorrhiza commonly enhance plant growth, prior to costly inoculation of agricultural systems with commercial mycorrhizal strains, preceding experiments are required to test for actual effects of mycorrhizal fungi on the specific crop plant.

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