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Below-ground interactions between a seedling soybean and pre-established soybean plant with and without mycorrhizal fungi. 1. Plant biomass, root growth, and mycorrhizal colonization**

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Abstract

Nurse–seedling relationships mediated below-ground are not well-known. Seedlings of a non-nodulating isoline of soybean were planted alone or with a 19 day old pre-established nodulating soybean as partner, with the vesicular–arbuscular mycorrhizal (VAM) fungi *Glomus mosseae* or control inoculum, and with one of four levels of below-ground barrier in the middle of the pot (no barrier, a 20 μm screen, a 1 μm screen, or a solid barrier). This study asked how a stressed soybean seedling interacts below-ground with an older non-stressed soybean with and without VAM fungi, in terms of plant biomass, root growth and VAM colonization. Plants were grown for 53 days in a greenhouse with the seedlings on the north side of each pot, shaded by above-ground barriers in the middle of each pot. Non-nodulating soybeans grew best when growing alone with no below-ground barrier in the pot, least with a nodulated pre-established soybean as partner and intermediate when the pot was split in half by a solid barrier. Screen barrier treatments produced intermediate biomass values between the values for no barrier and solid barrier treatments. Biomass results were consistent with a model of resource competition with the larger established nodulated soybean being the superior competitor. Percent VAM colonization was highest in the seedlings when the plants were grown together. In spite of this result, VAM fungi did not change the competitive relations between the two plants.

1. Introduction

Much of the work on interactions between plants has been done on even-aged plants (Eissenstat and Newman, 1990). In natural and in many intercrop systems, plants at varying stages of growth interact. Beneficial non-competitive interactions between plants are of particular interest in sustainable agriculture systems where high yield of both crops is the goal. A well known example of non-competitive interactions between plants is the nurse–seedling relationship that has been demonstrated in the Sonoran desert, where seedlings of *Cereus*, *Agave*, and *Ferocactus* survive only if they are growing under an appropriate nurse plant which provides shade to ameliorate the microclimate around the seedling

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(Nobel, 1989). Legumes are commonly used in inter-cropping systems to improve the nitrogen status of non-fixing crops. Nodulated soybeans can release a significant amount of symbiotically-fixed N into the rhizosphere (Brophy and Heichel, 1989). They may therefore serve as a nurse plant to a nitrogen deficient seedling.

Vesicular-arbuscular mycorrhizal (VAM) fungi are important to plant growth and nutrient uptake (Harley and Smith, 1983). It has been suggested that they may also be important to interactions between plants (Eissenstat and Newman, 1990), as they are in ectomycorrhizal fungi which have been shown to transfer photosynthetic products from a photosynthesizing plant to a non-photosynthesizing plant (Bjorkman, 1960; Reid and Woods, 1969).

Because below-ground mediated nurse–seeding relationships are not well known (Gliessman, 1986), the authors chose to maximize the stress to a seedling by growing a shaded non-nodulating strain of soybean under a low N and P regime, to maximize the likelihood of establishing a source–sink relationship between the nurse and the seedling. The stressed non-nodulating shaded soybean seedling (seedling) was grown alone or with a 19 day old nodulating soybean (partner) under mycorrhizal and non-mycorrhizal conditions with varying degrees of below-ground barrier to interaction between the plants. The investigation was to determine how an older non-stressed soybean interacts with a stressed soybean seedling with and without VAM fungi. If the relationship was competitive, the larger, healthier soybean would be expected to be the superior competitor. If the relationship between the plants was commensal, then the healthier soybean would be expected to transfer nutrients and/or photosynthetic products to the stressed soybean allowing it to benefit by the presence of the partner.

2. Materials and methods

Seedlings of a non-nodulating isoline of soybean (*Glycine max* (L.) Merr.) cultivar 'Clark' were planted alone or with a 19 day old pre-established nodulating isoline of 'Clark' soybean as partner, with the VAM fungus *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe or non-VAM control inoculum, and with one of four levels of below-ground barrier in the middle of the pot (no barrier, a 20 μm screen, a 1 μm screen, or a solid barrier). The design was evaluated as two separate factorials, one for the seedling and the second for the partner plant. For the seedling, the factors were: (1) inoculation (with or without the VAM fungus); (2) barrier (four levels); (3) partner (presence or absence of the partner plant), giving 16 treatments with five replications for a total of 80 seedlings. Because the partner was always grown with a seedling, there were only eight treatments and 40 partner plants.

The below-ground barriers were chosen to control the degree of below-ground interaction between plants as well as the space available to each plant. A 20 μm screen allows VAM hyphae, and the soil solution but not roots, to cross. A 1 μm screen allows the passage of soil solution and bacteria, but was supposed to stop mycorrhizal hyphae. A solid barrier cuts the below-ground space available to the plant in half. This arrangement assumes that resource competition is the main below-ground interaction, a seedling grown alone in the largest space should achieve the highest biomass, and that biomass decreases with the presence of a partner and with decreases in below-ground space. If there is a significant co-operative interaction between the seedling and the partner, the biomass of the seedling will decrease less in the presence of the partner than it does when the space available to the seedling is reduced.

To obtain partner plants, 60 pre-germinated seeds were chosen for uniformity and planted in 14 cm high tapering tubes filled with microwave-pasteurized coarse sand. Tubes contained a 5 g layer of *G. mosseae* or control inoculum and 5 ml of yeast extract media broth containing *Bradyrhizobium japonicum* (strain USDA 110) at 4.9×10^{8} cells ml^{-1}. *G. mosseae* inoculum comprised soil from a pot culture of *Sorghum* containing spores and colonized root fragments. Control inoculum was prepared by microwave pasteurization of *G. mosseae* inoculum. Plants were grown in a growth chamber at 28°C under a day/night regime of 13/11 h. Plants were watered from a drip as needed with a nutrient solution containing (μg ml^{-1}): N, 253 (as NH_4NO_3); Ca, 4; K, 47; Mg, 12; S, 32; P, 6.2; Fe, 0.30 (as sodium ferric ethylenediamine di-(o-hydroxyphenylacetate)); B, 0.07; Zn, 0.06; Cu, 0.01; Mo, 0.02. The pre-establishment period was 19 days. Each tube received a total of 234 ml of nutrient solution. Twenty VAM and 20 non-VAM control plants were selected for uniformity (by size) and weighed.

Pre-established partners were transplanted at 19 days into containers made of clear acrylic tubing 25.5 cm high and 12.5 cm in diameter, with a volume of 3.1 l. The tubes were cut lengthwise, to allow insertion of barriers. Three 3 mm diameter holes 1 cm from the bottom of the containers were drilled on each side to allow drainage. Grey above-ground barriers (15 cm×10 cm) were attached above the middle of each pot to prevent shading differences between treatments. After 13 days these barriers were replaced with white shade barriers (30 cm×24 cm). Containers were assembled with duct tape and wrapped in aluminum foil to prevent light penetration to the roots. Containers were filled to a volume of 3 l with a microwave pasteurized (Ferriss, 1984) mixture of coarse beach sand and fine river bottom sand (2:1, v:v). A cylindrical hole (3 cm in diameter, 6 cm deep) was bored into the soil mix in the centre of each side of each pot and 17.4 g of *G. mosseae* inoculum or control inoculum was added. A spore free wash was added to all pots to reintroduce non-VAM microflora to control pots (Ames et al., 1987). An inoculum of *Bradyrhizobium japonicum* (strain USDA 110, 5 ml, 9.4×10^8 cells ml^{-1}) was added to each side of each container so that each pot received the same amount of *B. japonicum* inoculum whether it contained a partner plant or not. Seedlings were germinated as per the partners, selected for uniformity and planted on one side of each container. The 19-day-old partners were then planted in half of the pots on the opposite side from the seedlings.

The experiment was conducted in a greenhouse, equipped with automatic temperature controls, during February, March and April at Albany, Ca. Day/night temperatures were 24–32°C/10–24°C. Day length was 12–14 h. Seedlings were on the north side of the pots, so that they were shaded by the above-ground barriers whether a partner was present or not. Plants were watered once a day, or twice a day on very sunny days. Beginning at 14 days after planting, plants were fertilized once a week at the rate of 100 ml per side of the growth containers. The nutrient solution was Johnson's solution (Johnson et al., 1957) modified to allow growth of non-nodulating, non-VAM soybean plants without inhibiting VAM colonization in either soybean isoline or nodulation in the nodulating isoline. Element concentrations in the nutrient solution in μg ml^{-1} as NH_4, NO_3. Growth containers were randomized weekly. As partner plants began to fruit, any pods were removed to delay senescence. After 53 days of growth, one replicate of each treatment was harvested per day on 5 consecutive days.

At harvest, shoots were cut at the base, leaves were removed and leaf areas were measured with a LiCor Li 3000 leaf area meter (Li-Cor, Lincoln, NE). Leaves and stems were then bagged separately, dried at 70°C and weighed. Containers were cut open and immersed in water to facilitate removal of soil from roots. For the treatments with two plants per pot and no barrier, intermingled roots were washed free of soil, placed in clear water and carefully separated by hand. Any nodules present were removed, counted, dried (70°C) and weighed. Roots were then re-wetted, blotted and measured for fresh weight. A 0.4 g random sample was then removed for staining with trypan blue for determination of VAM colonization and root length (Marsh, 1971; Kormanik and McGraw, 1982).

Seedlings were analyzed by a three-way ANOVA (inoculation, barrier, partner). Partner plants were analyzed by a two-way ANOVA (inoculation, barrier). Comparisons of interest between treatment means were tested with linear contrasts (Statistical Analysis Systems Institute Inc., 1987).

3. Results

For both seedling and partner plants the 20 μm screen and the 1 μm screen treatments did not differ for any response variable. Microscopic examination of the 1 μm screens at the end of the experiment showed that hyphae (possibly of *G. mosseae*) could pass across the 1 μm opening. This data for the two screen barrier treatments were therefore combined.

Partner plants had higher N levels than seedlings. DRIS analysis showed partner plants to be limited for P but relatively balanced for N. The DRIS indices for seedlings showed both P and N to be limiting. The nutrient analyses will be presented in a subsequent paper.

3.1. Partner plants

Presence of a barrier did not influence partner plants for any response variable. The VAM partner plants had larger values than non-VAM plants only for: root dry mass, nodule dry mass, and root/shoot ratio (Table 1). Roots of VAM plants had 47% colonization by the fungus. Partners growing alone in half of the space responded in the same way as they did to growing with the seedling in twice the space. In the presence of the seedling, the partner was not able to take advantage of the increased space.

Table 1
Effect of colonization by the vesicular–arbuscular mucorrhizal (VAM) fungus *Glomus mosseae* on plant parameters. Plants were pre-established (19 days) nodulating soybeans associated with a non-nodulating strain of soybean seedlings

Plant parameter	Treatment	
	VAM	non-VAM
Root dry mass (g)	1.89 A	1.53 B
Shoot dry mass (g)	4.15 A	4.19 A
Total dry mass (g)	6.04 A	5.72 A
Leaf area (m^2)	0.027 A	0.026 A
Nodule dry mass (g)	0.33 A	0.030 B
Root/shoot ratio	0.45 A	0.37 B
VAM root colonization (%)	47.4	trace
Root length (m)	128 A	132 A
Specific root length ($m\ g^{-1}$)	8.0 A	8.6 A

Means represents 20 plants. Means with the same letter in a row are not significantly different at the 0.05 level by ANOVA.

3.2. Seedlings

Seedlings grown alone were larger than seedlings grown with a partner (Fig. 1(a), $P<0.01$). For seedlings grown alone, seedlings without a solid barrier (no barrier or screen barrier) were larger than seedlings with a solid barrier (Fig. 1(a), $P<0.01$). Conversely, for seedlings grown with a partner, seedlings without a solid barrier (no barrier or screen barrier) were smaller than seedlings with a solid barrier ($P<0.01$).

Seedlings grown alone with a solid barrier were larger than plants with a solid barrier and a partner ($P<0.01$). Because both above-ground and below-ground barriers were present, this difference suggests there was shading by partner plants during the first 13 days before placement of the larger above-ground barriers and is evident in seedling plant dry mass where seedlings were smaller when a partner was present (Fig. 1(a)).

The VAM seedlings had higher root/shoot ratios than non-VAM seedling (Fig. 2(a), $P<0.01$). Seedlings grown alone had a higher root/shoot ratio than seedlings with a partner (Fig. 1 (b), $P<0.01$).

Seedlings grown alone had longer roots (147 m) than seedlings grown with a partner (97 m, $P<0.001$). Non-VAM seedlings had a longer root length than VAM seedlings (Fig. 2(b), $P = 0.01$). Non-VAM seedlings had a higher specific root length (SRL) than VAM seedlings (Fig. 2(c), $P<0.001$). Seedlings with no barrier had smaller SRL than plants with a screen or solid barrier (Fig. 1(c), $P = 0.04$). Higher SRL in non-VAM plants is commonly observed (Marschner et al., 1986; Price et al., 1989), and shows that VAM plants have shorter thicker roots than non-VAM plants, with VAM hyphae providing the majority of the surface area for nutrient absorption in VAM plants. Seedlings also increased their SRL when space was decreased by a barrier whether a partner was present or not.

Seedlings showed a growth response to the presence of VAM ($P<0.01$) for leaf area and the

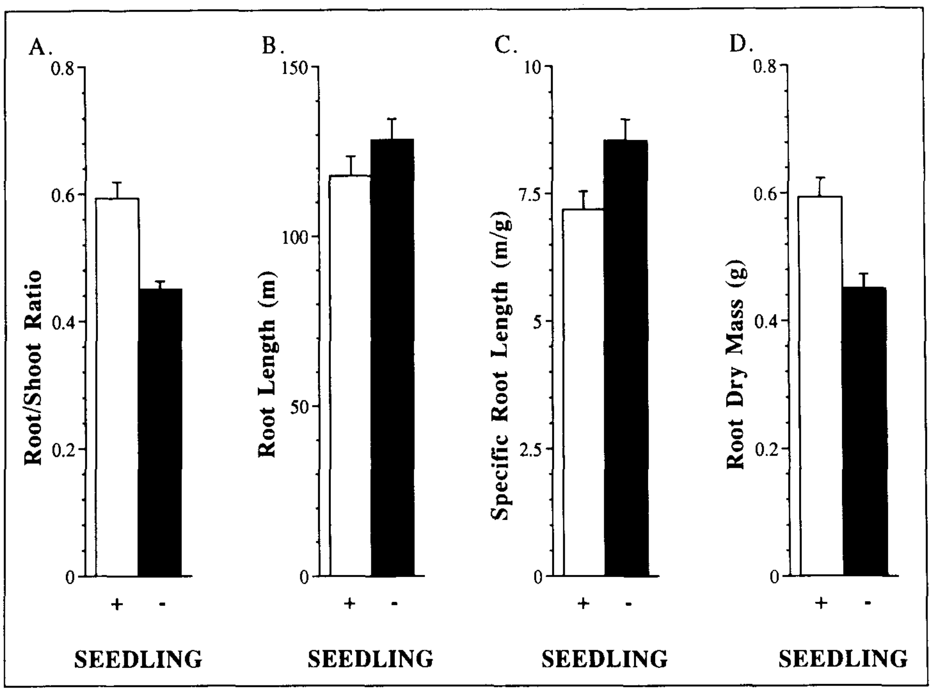

Fig. 1. Effect of vesicular–arbuscular mycorrhizal (VAM) fungi on root growth of non-nodulating soybean seedlings. (A) Root/shoot ratio, (B) length, (C) specific root length and (D) root dry mass vs. inoculation for seedlings. Inoculation: + Glomus mosseae, – control. Bars represent standard error of mean. $N = 40$.

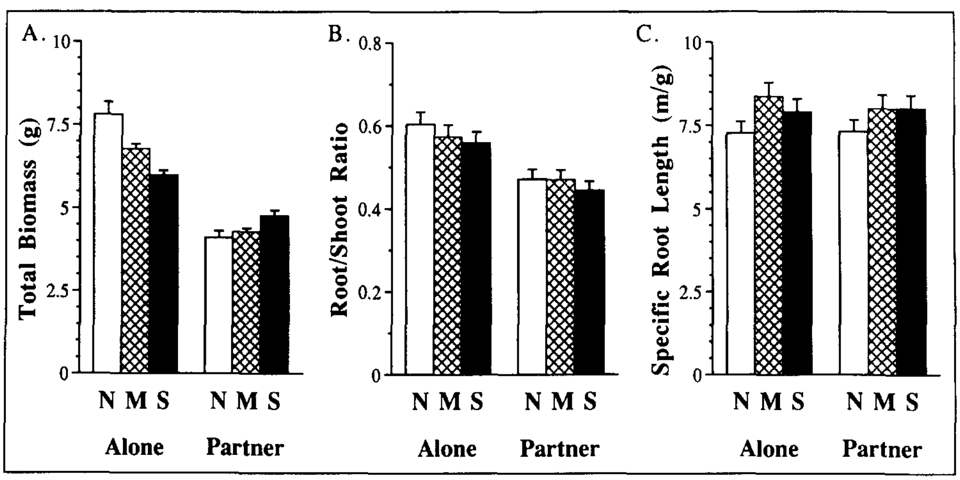

Fig. 2. Effect of below-ground barrier and presence or absence of a pre-established partner plant on growth of non-nodulating soybean seedlings. (A) Total dry mass, (B) root/shoot ratio and (C) specific root length vs. barrier by partner for seedlings. Barrier: N, none; M, screen (20 and 1 micron screens averaged); S, solid. Partner: ALONE, no partner; PARTNER, pre-established nodulating soybean as partner. Error bars are standard error of mean. $N = 10(N), 20(M), 10(S)$.

biomass response variables: root, shoot, and total dry weight (Fig. 2(d)). Seedlings grown with no barrier had a higher percentage colonization by VAM fungi than seedlings grown with a barrier.

To test whether or not the VAM fungus af-

Table 2
Measurement of the effect of vesicular-arbuscular mycorrhizal (VAM) fungi on interactions between a seedling and a pre-established partner soybean plant: biomass ratio comparison of seedlings grown with a partner to seedlings grown without a partner for VAM and non-VAM treatments

Variable	Inoculation	Ratio	LL	UL
Root dry mass	VAM	0.44	0.33	0.56
	non-VAM	0.46	0.29	0.65
Shoot dry mass	VAM	0.55	0.49	0.61
	non-VAM	0.59	0.53	0.66
Total dry mass	VAM	0.51	0.44	0.57
	non-VAM	0.55	0.47	0.63

Inoculation: VAM, *Glomus mosseae*; non-VAM, control inoculum.
LL, lower limit of 95% confidence interval; UL, upper limit of 95% confidence interval.
Ratios are means of five replicates of each treatment.

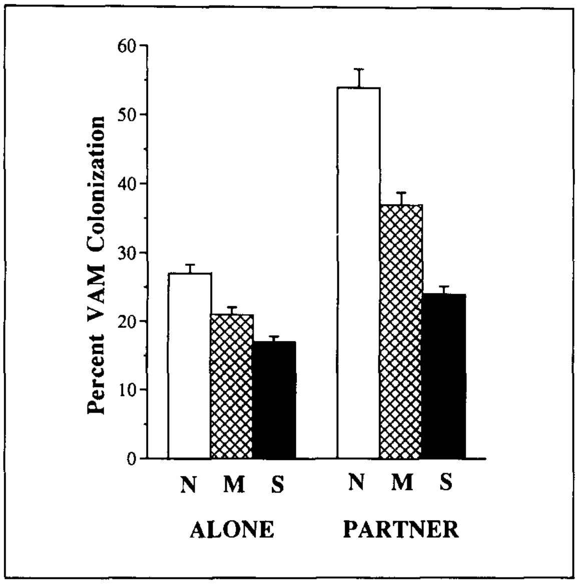

Fig. 3. Effect of below-ground barrier and presence or absence of a pre-established partner plant on percent colonization of a non-nodulating soybean seedling by *Glomus mosseae*. Barrier: N, none; M, screen (20 and 1 micron screens averaged); S, solid. Partner: ALONE, no partner; PARTNER, pre-established nodulating soybean as partner. Error bars represent standard error of mean. $N = 5$(N), 10(M), 5(S).

fected seedling interactions with the partner, the ratio of the biomass of a seedling grown with no barrier and a partner to the biomass of a seedling grown alone with no barrier was calculated for VAM and non-VAM seedlings (Table 2; Fig. 3). Overlapping confidence intervals (95%) for the ratios for root, shoot, and total biomass show that the ratios did not differ significantly as a result of colonization.

4. Discussion

Seedlings grew best when alone and with no barrier in the pot. They grew least in the presence of a partner, and intermediate when the below-ground space was split in half by a solid barrier. Screen barrier treatments gave intermediate values between the values for no barrier and solid barrier. The seedling with the most space had the highest biomass. The seedling grown at the highest density (two plants per pot) had the lowest biomass. These results are consistent with a model of resource competition because the seedling biomass decreased with increasing plant density and increased with increasing below-ground space. As seedling growth was more inhibited by a partner than by smaller rooting volume, the pre-established nodulating soybean did not nurse them. These results show that setting up a stress-induced sink demand (shading and lack of nodules) is not enough to guarantee establishment of a beneficial nurse–seedling relationship.

The competitive relations between the plants were not changed by the VAM fungus (*Glomus*) as shown by the absence of significant differences among the biomass ratios of seedlings grown alone to seedlings grown with a partner for VAM and non-VAM plants. Even though there was a growth response to the presence of the VAM fungus, the seedling–partner interaction was the same in VAM and non-VAM treatments. In this study, the three-way interaction (soybean–*Glomus*–soybean) was qualitatively the same as the soybean–soybean interaction.

In a similar study, Eissenstat and Newman (1990) grew seedlings of *Plantago lanceolata* with and without a pre-established *Plantago* and with and without an unidentified VAM fungus. They also concluded that VAM fungi did not affect the outcome of the interaction between a seedling and an established plant of the same species. However, they did not calculate or test the ratio of a seedling grown alone to a seedling grown with an established *Plantago* for VAM and non-VAM plants. From their data for shoot dry mass, the VAM *Plantago* seedlings grown with an established *Plantago* produced 75% of the biomass that they produced when growing alone, while a non-VAM *Plantago* seedling growing with a nurse only produced 62% of the biomass it achieved when growing alone.

The present results and those of Eissenstat and Newman (1990) agree with the hypothesis of Allen and Allen (1990) which states that mycorrhizal fungi [will] have no effect on plant competition unless the competitive abilities of the plants differ.

In terms of plant biomass the pre-established nodulated soybean did not nurse the non-nodulating soybean and VAM fungi did not change the form of the net interaction between the two plants in spite of a growth response to VAM fungi in both plants.

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