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The role of VAM fungi in nitrogen dynamics in maize-bean intercrops

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Abstract

Nitrogen (N) transfer from N-fixing legumes via vesicular-arbuscular mycorrhizal (VAM) fungi to associated non-fixing plants has been demonstrated in greenhouse experiments. To date, this transfer has been shown only where mineral N is applied shortly before harvest, and hence is readily available. We have yet to demonstrate VAM-mediated N transfer where soil-N is limiting, a condition under which most traditional legume-nonlegume intercrops are grown.

In this study, ^{15}N-enriched soil (with 0.28%N) was used to distinguish between the uptake of soil- and atmospherically-derived N in maize grown with beans in the presence or absence of VAM fungi. VAM infection did not result in transfer of fixed N or soil N from bean to maize, despite a VAM-stimulated increase in N fixation in bean. In fact, beans were more competitive for soil N when mycorrhizal. N content in beans increased by 75% with a concomitant 22% decrease in mg N per maize plant. The competitive effect may have resulted from a VAM-mediated shift in carbon allocation in beans (but not maize) from shoots to roots.

Introduction

Multiple cropping systems are common throughout the world, particularly in the tropics. One of the numerous advantages reported by multiple cropping systems, or intercropping, is higher yield per area (or overyielding) when compared to monocultures of the component species where planting densities of the monocultures are those at which yield per unit area is maximized (Francis, 1986; 1989; Ofori and Stern, 1987; Searle et al., 1981; Trenbath, 1974). It is generally thought that legume-nonlegume intercrops are particularly productive combinations under conditions of limiting soil nitrogen (N), where the nonlegumes (non-N-fixing plants) have the most to gain from inputs of legume-fixed N (Eaglesham et al., 1981; Vest, 1971). The actual mechanisms of the presumed N-mediated benefit of legumes to nonlegumes, however, are still not well understood. This lack of understanding may limit our ability to successfully manipulate legume-nonlegume intercrops to fully exploit both the N-fixing capacity of the legumes and N transfer to associated nonfixing crops.

The mechanisms thought to be responsible for N-mediated overyielding can be divided into two general categories. In the first, interspecific competition for soil N is reduced because the legume substitutes atmospherically derived N for soil N (Trenbath, 1976; Vandermeer, 1989; Willey, 1979). Alternatively, direct transfer of fixed N from the legume to the nonlegume could explain the increased yield of intercrops. This transfer may occur via decomposition and mineralization of legume roots and/or nodules (Burity et al., 1989; Haynes, 1980), legume root exudates (Brophy and Heichel, 1989; Burity et al. 1989; Wacquant et al., 1989), or via vesicular arbuscular mycorrhizal (VAM) fungi through interplant hyphal connections (Haystead et al., 1988; van Kessel et al., 1985).

Vesicular arbuscular mycorrhizal (VAM) fungi (family Endogonaceae) are known to play an important role in nutrient uptake in most agricultural and natural plant communities (Francis et al., 1986; Harley and Smith, 1983; Rabatin and Stinner, 1989). While best known for their role in phosphate assimilation, VAM fungi also play an important role in N transfer to and from plants (Raven et al., 1978). VAM fungi are known to assimilate and transport both NH_4^+ ions and some organic-N compounds to their host plants, particularly under conditions of low pH and low N availability (Raven et al., 1978). While some Endogonaceae species have relatively restricted environmental and/or host ranges, several species in Glomus, Acaulospora, and Scutellospora are found in soils of wide-ranging pH and fertility, and with a wide range of potential host species (Sieverding, 1989).

The direct transfer of N via hyphal connections between roots of two closely associated living plants was suggested in a field experiment with ryegrass (*Lolium perenne*) and clover (*Trifolium repens*), in which VAM infection enhanced the transfer of ^{15}N from the clover (applied as $(^{15}NH_4)_2SO_4$) to the ryegrass (Haystead et al., 1988). Direct transfer of N via hyphal connections has been demonstrated in greenhouse experiments as well (Ames et al., 1983; Francis et al., 1986; Van Kessel et al., 1985). Using a split-root experiment, van Kessel et al. (1985) demonstrated the transfer of N from soybean to maize via VAM fungi. In their experiment, two maize plants and two soybean plants were grown in adjacent pots with the soybean roots split between the pots by a PVC elbow. Half of the maize pots were inoculated with VAM fungi (spores, host-plant roots, and hyphae) and the other half remained in sterilized, uninoculated soil. Forty eight hours after application of ^{15}N-labeled fertilizer to the soybean, ^{15}N appeared in the inoculated maize only. Although these studies clearly demonstrate transfer of soil N from legume to nonlegume via VAM connections, no such studies have demonstrated the transfer of atmospherically-derived (fixed) N.

If VAM fungi are involved in the direct transfer of fixed N from legume to nonlegume, legumes with greater N-fixation capability or ability to develop effective infection by VAM fungi should be selected. In this case, the role of VAM fungi is two-fold: a) interplant N transfer, and b) the stimulation of N fixation in the legume by increasing phosphate uptake (Barea, 1989; Smith and Daft, 1977). If however, common mycorrhizal connections between legumes and nonlegumes function to increase the transfer of soil N, then whichever plant develops more effective VAM infection may become more competitive if soil N is limiting.

P. vulgaris is characterized as having a relatively high mycorrhizal dependency (Howeler et al., 1987) and forms associations with several Endogonaceae species (Sieverding, 1989). Likewise, maize responds positively to inoculation with a number of different Endogonaceae species in both greenhouse (Gerdemann, 1965; Howeler et al., 1987; Mosse, 1977; Simpson and Daft, 1990) and field experiments (Jackson, et al., 1972; Khan, 1972; 1975; Mosse and Hayman, 1980).

The purpose of the present study was to determine the role of a VAM fungi in N transfer between maize and beans. A split-root experimental design and soil uniformly labelled with ^{15}N were used to evaluate the effect of VAM formation on total N accumulation in maize and bean, N fixation in bean, and to look for evidence of transfer of fixed N from bean to maize. When the legume and nonlegume are grown together in ^{15}N-enriched soil, inputs to the rhizosphere of fixed N will dilute the ^{15}N in the rhizosphere. Likewise, if bean-fixed N is transferred to the maize, the proportion of ^{15}N in the maize will decrease relative to maize with access to soil N only.

Materials and methods

Experimental design

A modification of the split-root design of van Kessel et al. (1985) was used. One maize plant (*Zea mays* L. var. Los Diamantes 8043) and one bean plant (*Phaseolus vulgaris* L. var. Huetar ICTA-81-64) were grown in adjacent one-L pots and the bean roots were split between the pots by passing them through a PVC elbow (Fig. 1). There were 12 pairs of pots, six inoculated with VAM fungi and six not.

Pots were filled with pasteurized ^{15}N-labeled soil (0.28%N) collected from a field plot labeled with ammonium sulfate (5% atom excess ^{15}N) ten months prior to the initiation of this experiment. The soil at the field site is a loam soil of the Baudrit soil series (a thapto-ustertic dystrandept). The site is moderately acidic (pH 5.2 (KCl), 5.8 (H_2O)), with low to medium percent organic matter (3–8%) and extremely low availability of phosphorus (P) (8 ppm). A laboratory analysis of P fixation indicated a very high rate of fixation (88%). All other routinely measured macro- and micro-nutrients (Ca, Mg, K, Fe, Cu, Mn) were above the critical levels for the production of maize and beans with the possible exception of Zn at only 1 ppm.

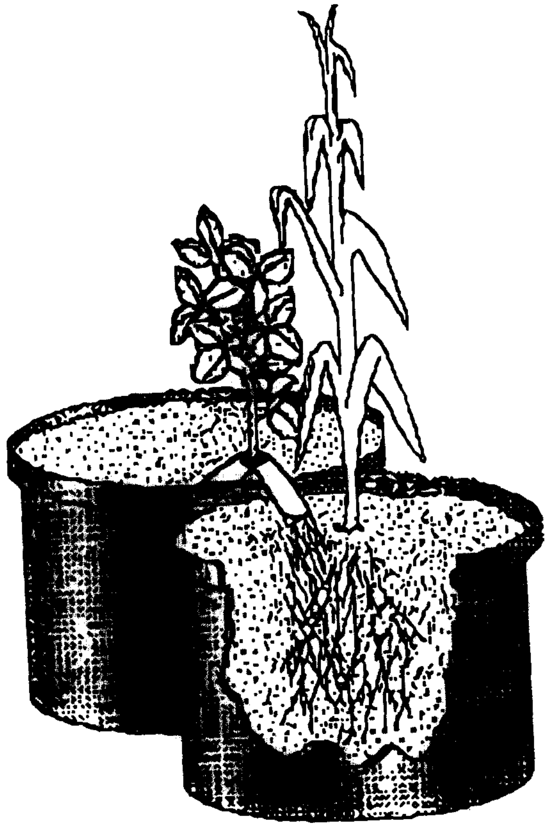

Fig. 1. A split-root system where the roots of the beans are trained to grow into separate pots.

On 10 May 1988, 2 kg of $(NH_4)_2SO_4$ 5 atom % ^{15}N excess (ICON Services Inc., Summit, NJ) was dissolved in 12 liters of water and spread equally in three passes over a 13 m × 17.5 m plot, using a CO_2 pressure-regulated sprayer (R and D Sprayers Inc., Opelousas, LA.). Immediately following the ammonium sulfate application, 48 kg of sucrose (giving a C:N ratio of 50:1) was similarly dissolved in water and applied to the plot. The soil was thoroughly rototilled every 10 days for one month at which time another 48 kg of sucrose was applied to the plot, as before, and rototilled twice more at two-week intervals.

Potted soil was fertilized with potassium (K_2O) and phosphorus (P_2O_5) at per plant rates equivalent to $60\,\mathrm{kg\,P\,ha^{-1}}$ and $40\,\mathrm{kg\,K\,ha^{-1}}$: 0.3 g K and 0.55 g P for beans and 1.25 g K and 2.0 g P for maize. Two days later 73 g of air-dried VAM fungi inoculum was added to six maize pots and 73 g of air-dried pasteurized inoculum added to another six maize pots. Inoculum was prepared by finely chopping roots of dried maize plants previously grown in one-L pots in the greenhouse and inoculated with *Glomus etunicatum* (voucher deposited with INVAM, W. Virginia University). The chopped roots were mixed with 876 g of the soil in which the host maize plants were grown and this mix used as inoculum.

Bean seeds were surface-sterilized (by washing for 1 minute in 95% ethanol followed by 1 minute in 2.5% chlorox solution) and planted in vermiculite on 21 February, 1989. On 1 March, 1989 and tap root of germinated beans was cut and the lateral roots fed into the PVC elbow (22 mm i.d.) and planted into the two adjacent pots. At this time bean roots were inoculated with an aqueous suspension of *Rhizobium leguminosarum* biovar. *phaseoli* (prepared in the Laboratory of Soil Microbiology, Center for Agronomic Research, University of Costa Rica, San José). At the same time, maize seeds were surface-sterilized (using the above procedure) and planted. All bean plants developed effective (pink) nodules.

Plant harvest and processing

Forty-nine days after the maize was planted, all plants were harvested and shoots and roots separated and fresh weight recorded. All maize and bean fresh roots were evaluated for mycorrhizal infection using trypan blue in lactophenol. Presence or absence of infection was verified using the rapid assessment method of Phillips and Hayman (1970).

Maize and bean shoots and bean grain were dried to constant weight at 60°C and ground to <1 mm in a Wiley Mill. Ground samples were thoroughly mixed and subsamples analyzed for total N using a Kjeldahl procedure modified with a salicylic acid predigestion to include all NO_x (Bremner, 1965). All N was converted to NH_3 and distilled into a boric acid indicator solution for total N determination (procedure of the International Fertilizer Development Center, IFDC, Muscle Shoals, Alabama). Samples were acidified and evaporated, and the remaining NH_4 salts shipped to the IFDC for ^{15}N determination. Total N analyses and ^{15}N sample preparation were conducted in the Soil Microbiology Laboratory of the Center for Agronomic Research, University of Costa Rica.

Percent P in maize shoots was determined at the Soils Testing Laboratory, Michigan State University. Bean samples remaining after N analyses were insufficient for P analysis.

Statistical analyses

The effects of inoculation with VAM on maize and bean response variables were analyzed using independent t-tests when assumptions of homogeneity of variances and normality were not violated. Two variables: bean dry shoot biomass and bean root in maize pots were transformed using natural logarithms. Atom % excess ^{15}N in bean grain was not correctable by transformation and treatment means were analyzed using a Kruskal-Wallis nonparametric analysis (K-W). Results were considered significant at the 5% level.

All plants inoculated with VAM fungi were infected with the possible exception of one bean plant which was subsequently eliminated from analyses. One uninoculated bean plant appeared to a have a low level of mycorrhizal infection (2.1%) and was similarly eliminated from analyses.

Results

Bean and maize biomass response

Mycorrhizae inoculation caused a shift in the distribution of biomass and N between maize and bean in favor of the latter (Tables 1 and 2). Bean shoot and grain biomasses significantly increased with inoculation by VAM fungi. There was a concomitant 31% decrease in maize shoot biomass although this was only marginally significant (Table 1). Bean root growth showed the largest response to inoculation by VAM fungi. Bean fresh root biomass in maize pots inoculated by VAM fungi was nearly eight times higher than that of bean roots in uninoculated maize pots. Bean root biomass in the pot without maize increased nearly two and a half times in the presence of VAM fungi, but this increase was only

Table 1. Mean per plant dry-shoot, dry-grain and fresh-root biomass response to VAM fungi in a maize-bean intercrop. (**A**) Bean response (n = 5). (**B**) Maize response (n = 6)

	With VAM	No VAM	t-stat.	*p* value	% change
A. Bean					
Dry shoot (g)[a]	0.96 ± 0.17	0.57 ± 0.04	5.82	0.000	+ 68
Dry grain (g)	1.62 ± 0.28	0.89 ± 0.16	5.11	0.001	+ 82
Root: bean pot (g)	1.13 ± 0.75	0.45 ± 0.28	1.92	0.097	+ 151
Root: maize pot (g)[a]	0.31 ± 0.16	0.04 ± 0.01	6.48	0.000	+ 675
Fresh shoot : root	4.97 ± 4.51	14.59 ± 8.69	2.20	0.059	− 66
B. Maize					
Dry shoot (g)	4.13 ± 1.55	5.98 ± 1.71	1.96	0.079	− 31
Fresh root (g)	24.55 ± 5.31	27.90 ± 11.37	0.65	NS	
Fresh shoot : root	1.32 ± 0.37	1.41 ± 0.41	0.42	NS	

[a] *t*-test performed on data transformed by natural logarithms.

Table 2. Mean per plant nitrogen and phosphorus in response to VAM: **A)** maize + bean, **B)** bean (n = 5), **C)** maize (n = 6). All N measures for bean are of bean grain, all N measures for maize are for shoots

	With VAM	No VAM	t-stat.	*p* value	% change
A. Bean grain + maize shoots					
Total N (mg) in 2 pots:					
a bean + maize pair	116.19 ± 9.95	108.15 ± 11.88	1.16	NS	
B. Bean grain					
% N	3.84 ± 0.13	4.01 ± 0.19	1.69	NS	
Total N (mg)	62.01 ± 9.50	35.35 ± 5.25	5.49	0.001	+ 75
^{15}N (mg)	240.50 ± 34.76	140.20 ± 21.20	5.51	0.001	+ 72
a.% e.^{15}N[a]	0.022 ± 0.005	0.030 ± 0.001	3.72	0.009	− 27
C. Maize shoots					
% N	1.46 ± 0.28	1.27 ± 0.19	1.37	NS	
Total N (mg)	57.05 ± 12.48	73.37 ± 10.60	0.06	0.031	− 22
^{15}N (mg)	222.59 ± 49.86	285.90 ± 40.92	2.44	0.037	− 22
a.% e.^{15}N	0.024 ± 0.003	0.023 ± 0.001	2.40	NS	
% P	0.21 ± 0.03	0.22 ± 0.02	0.70	NS	
Total P (mg)	8.33 ± 2.63	12.84 ± 3.52	2.51	0.031	− 31

[a] The *p* value is given for a Kruskal–Wallis non-parametric comparison. Percent ^{15}N is shown as atom % excess (a.% e.) ^{15}N (the percent enrichment over natural abundance of 0.3663%.

marginally significant. Bean responses to VAM formation resulted in a decrease in fresh shoot/root ratio from 14.59 (with VAM) to 4.91 (without VAM). Maize fresh root biomass did not differ between treatments, nor did the shoot/root ratio.

The formation of VAM decreased the competitive effect of maize on bean growth, particularly root growth. For uninoculated bean plants, root biomass in the bean-only pot was 11 times higher than in the maize pot. In the presence of VAM, bean root biomass in the bean only pots was four times that of roots growing in the maize pots.

N and P response

Mycorrhizae had no significant effect on total N accumulation per maize-bean pair (Table 2A). Similarly, N concentration (%N) did not differ between treatments in either beans (Table 2B) or maize (Table 2C). However, presence of VAM did influence the distribution of N between maize and bean pots. Total N (mg) and ^{15}N (μg) were 75% and 72% higher in bean with VAM than in the uninoculated treatment. These increases were accompanied by 22% decreases in both total N (mg) and ^{15}N (μg) in maize, thus explaining the lack of significance in overall N accumulation.

A significant decrease in atom % excess ^{15}N (the % enrichment of ^{15}N over natural abundance of 0.3663%) in bean with VAM indicates that in the presence of VAM, beans fixed more N than when they were not infected with VAM fungi. Percent ^{15}N did not differ between inoculated and uninoculated maize.

As with N, total P accumulation in maize inoculated with VAM fungi was 35% less than in uninoculated maize. There was not sufficient plant material for P analysis of bean grain or bean shoots.

Discussion

VAM had a positive and highly significant effect on N fixation, biomass accumulation, and N accumulation in bean. Mycorrhizal maize exhibited a concomitant negative response that was significant for N accumulation and marginally significant for biomass accumulation. There was no evidence of fixed-N transfer from bean to maize. Thus, it appeared that VAM increased the competitive ability of beans to the disadvantage of maize. The increase in total N in mycorrhizal beans (about 27 mg per plant) was larger than the total N decrease in maize (about

16 mg per plant), the difference being due to increases in uptake of soil N and N fixation in mycorrhizal beans. Additional N probably accumulated in bean roots, but all roots were used for VAM analysis and were not available for N analysis. The competitive effect was manifested in terms of P accumulation as well, where mycorrhizal maize had 35% less P than nonmycorrhizal maize. Barea et al. (1989) reported similar results in a greenhouse experiment with alfalfa and ryegrass. The legume responded much more (dry matter accumulation, N and P content) when mycorrhizal than did the grass. VAM also stimulated N fixation in the alfalfa. The authors presented no evidence of VAM-mediated N transfer.

The findings here may be explained as VAM fungal effectiveness being greater in bean than in maize. Although maize response to VAM is generally positive (increased shoot biomass and decreased root : shoot ratio), results in the literature are inconsistent, varying with level of available soil nutrients (Gryndler et al., 1989), cropping history (Johnson et al., 1991), cultivar (Hall, 1978), phenological stage (Khan, 1975), or influence of associated crops (Stejskalová, 1989). Since plant taxa differ dramatically in their response to VAM fungi (Koide, 1991), it is not unexpected that the presence of VAM fungi can dramatically influence the competitive interactions of species, especially those differing in response to VAM fungi. The results of the present experiment are consistent with those of Fitter (1977) in which *Holcus lanatus* showed a much greater competitive advantage over *Lolium perenne* in dry-biomass accumulation, and in P and K uptake with than without VAM inoculation. Similarly, inoculation of two grass species with VAM fungi resulted in an increase in shoot biomass when each were grown in mixed culture with a non-mycorrhizal species, but not when they were grown in pure culture (Allen and Allen, 1984). Thus, differences in response to VAM fungi between crop species or between varieties within species may account, in part, for the inconsistent results of maize-bean intercropping experiments ranging from overyielding to underlying in one or both species.

Alternatively, the apparent contrast between the results of this experiment and those of others demonstrating legume-to-nonlegume N transfer (Ames et al., 1983; Francis et al., 1986; van Kessel et al., 1985), may be due to differences in growing conditions such that N transfer occurs where soil N is not severely limiting as is the case where ^{15}N-labeled fertilizer is added directly to the system to demonstrate N transfer. In all cases where VAM-mediated N transfer from legume to associated nonlegume has been demonstrated, it was when ^{15}N-labeled fertilizer was added to the legume and was subsequently measured in the associated nonlegume, thus demonstrating the transfer of fertilizer N (Barea et al., 1989; Van Kessel et al., 1985). In contrast, evidence for transfer of atmospherically-derived N would include a decrease in $\%^{15}$N in the nonfixing plant relative to ^{15}N-enriched soil N. Despite several studies demonstrating VAM-mediated N transfer from legume to associated nonlegume, none have presented evidence of fixed-N transfer via VAM fungi. Although data presented by Barea et al. (1989) suggest that transfer of fixed N (at one of four P levels), may have occurred, the appropriate analysis was not presented. Since legume to nonlegume N transfer is most often considered important under conditions of low N availability, tests of N transfer should be conducted under such conditions.

Until such tests are performed, claims of VAM-mediated legume to nonlegume N transfer in low-input intercropping systems will remain questionable. Furthermore, if VAM fungi can cause shifts in the competitive outcome of maize-bean interactions in the field, as was demonstrated in the greenhouse, then studies of competitive interactions should consider the relative effectiveness of different VAMF-host associations of the intercropped species. Perhaps it may be possible to select fungi-host combinations such that both species perform relatively well in the intercrop or favor that species which is economically most important.

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