

## Mycorrhiza-Mediated Nutrient Distribution Between Associated Soybean and Corn Plants Evaluated by the Diagnosis and Recommendation Integrated System (DRIS)

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### Abstract

The objective of the study was to determine if plants whose vesicular-arbuscular mycorrhizal (VAM) mycelium had access to the root zone of an adjacent plant developed different growth, nutritional, and symbiotic characteristics from VAM plants grown under identical conditions, but separated by a barrier in the soil. Soybean (*Glycine max* L. Merr. cv. Clark) plants associated with corn (*Zea mays* L.) by VAM hyphae had greater nodule activity ( $C_2H_2$  reduction) than plants of the nonassociated comparison treatment. In associated corn plants, cob dry mass and VAM colonization were significantly smaller than in nonassociated plants. Concentrations of N in associated soybeans, and P in nonassociated ones, were very significantly greater than in their respective nonassociated or associated counterparts. However, nutrient status evaluated by indices of the Diagnosis and Recommendation Integrated System (DRIS) showed differences in the levels of N, P, and K deficiencies or sufficiencies. Nutrient balance was better in the associated than in the nonassociated plants.

Transport of products of photosynthesis was investigated by exposing the corn plants to  $^{13}CO_2$  and later evaluating the distribution of the C among plants and

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soil. All of these data suggest that nutrient distribution is modified in plant associations that include VAM hyphae. Implications of this phenomenon for agro-ecosystem management are discussed.

Keywords: corn, mycorrhiza, nitrogen fixation, nutrient transfer, plant nutrition, root nodule, soybean

## 1. Introduction

The concept of resource distribution between plants by the hyphae of vesicular-arbuscular mycorrhizal (VAM) fungi which connect the root systems of such associated plants (Reed et al., 1985) opens up important implications for the understanding of plant communities (Fitter, 1985) and for potential agricultural applications in intercrop situations (Bethlenfalvay et al., 1991). Including the entire soil volume that the mycorrhiza (fungus-root) contacts in the VAM-fungus-mediated nutrient exchange system between plants is a logical extension of the concept, since it treats the plant-soil system as a continuum (Bethlenfalvay and Newton, 1990). Thus, VAM-mediated nutrient distribution may be visualized as occurring not only between associated plants but between their respective spheres of nutritional influence (nutrisphere, Bethlenfalvay et al., 1991) as well, since the VAM fungus is an integral component of both its host plant and its host soil.

If host-plant and endophyte growth are influenced by the plant's association with another plant through VAM mycelium, then it is possible that an increase in a parameter of one partner as a result of VAM-mediated nutrient transfer may be accompanied by a complementary, though not necessarily equal, decrease in the other partner. This assumption rests on the premise that source strength and sink demand can be different in the associated plants.

One aspect of such nutrient transfer among associated plants may be evaluated by comparing the nutrient status of two VAM plants, each of whose VAM mycelium has access to the root zone of the other, with that of similar VAM plants separated by a solid barrier. Each companion pair consisted of a soybean and a dwarf corn plant.

The Diagnosis and Recommendation Integrated System (DRIS, Walworth and Sumner, 1987) is useful in performing such nutrient evaluations, since it not only diagnoses deficiencies, but also determines the order of limitation among nutrients, and the relative balance of overall nutrient status (Jones, 1986). Since DRIS is based on nutrient concentration ratios, its use minimizes morphogenic or genotypic effects on the accuracy of deficiency diagnoses (Hallmark et al., 1987), and has been recently adapted to evaluate VAM effects on plant nutrition (Bethlenfalvay et al., 1990).

The allocation of photosynthetically fixed carbon among the members of a mycorrhizal association can be assessed with the aid of isotopically labeled  $\text{CO}_2$ . In this experiment, it was supplied to the corn plant of each companion pair, and subsequently the amounts of the label in plants and soils were determined.

## 2. Materials and Methods

### *Experimental unit, design, and statistics*

Companion plants were grown in containers (Camel et al., 1991) which separated the root compartments of two plants with either a soil bridge or a solid barrier (Table 1). The soil bridge between associated plants was 6 cm wide and was delimited by screens (44  $\mu\text{m}$  openings) on both sides. The screens permitted the passage of VAM hyphae, but not roots. The root-free zone

Table 1. Host-plant and endophyte parameters in plants associated through a common VAM mycelium or separated by a barrier (nonassociated). The growth-container design is indicated by heavy lines around the respective treatment data. Separation of soil volumes by screens permeable to VAM hyphae, but not to roots, is shown by broken lines. Numbers are the means and SE of 5 replications. P-values represent the significance of differences between respective data from the associated vs nonassociated treatments (*t*-test).

Treatment	Plant		P-value	
	soybean	corn	soybean	corn
<i>Associated plants</i>				
Shoot dry mass (g)	7.5 ± 0.7	7.3 ± 0.8	0.979	0.435
Root dry mass (g)	3.7 ± 0.2	1.6 ± 0.2	0.922	0.976
Cob dry mass (g)	—	3.8 ± 0.5	—	0.088
VAM colonization (%)	40 ± 5	36 ± 4	0.215	0.088
Nodule activity ( $\text{C}_2\text{H}_2$ ) ( $\mu\text{mole h}^{-1}\text{plant}^{-1}$ )	2.9 ± 0.3	—	0.041	—
<i>Nonassociated plants</i>				
Shoot dry mass (g)	7.5 ± 0.7	8.3 ± 0.8		
Root dry mass (g)	3.7 ± 0.3	1.6 ± 0.3		
cob dry mass (g)	—	5.1 ± 0.4		
VAM colonization (%)	33 ± 2	46 ± 3		
Nodule activity $\text{C}_2\text{H}_2$ ) ( $\mu\text{mole h}^{-1}\text{plant}^{-1}$ )	2.1 ± 0.1	—		

was interposed between the associated plants to minimize nutrient transfer by exudation, diffusion, and root anastomosis, processes that are unavoidable if roots are allowed to intermingle (Haystead et al., 1988; van Kessel et al., 1985). Between the roots of the two plants not associated by VAM-fungal mycelia were two similar 6 cm-wide root-free zones separated from each other by an impermeable barrier. The additional zone served to equalize root-free soil volumes in close proximity to the roots of associated and nonassociated plants, in view of the importance of rooting space to plant development (Stevenson, 1967). The root compartments of all plants had a volume of 4.4 L; the screened bridge or supplementary soil zones, 1.5 L.

The two treatments (associated or nonassociated plants), each with five replications, were arranged in the growth chamber in a random design. The differences between the plants of the two treatments were evaluated by student's *t*-test. Actual significance values of differences are presented (Nelson, 1989).

### *Biological materials*

The soil for each plant was mixed with an inoculum of the VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, WRRRC Isolate 1 (Franson and Bethlenfalvay, 1989), consisting of 50 ml of soil containing approximately 700 sporocarps and 200 colonized root fragments. All compartments also received an inoculum wash, free of VAM-fungal propagules, to equalize the non-VAM microbiota (Ames et al., 1987). Soybean (*Glycine max* L. Merr. cv. Clark) seeds were surface-sterilized, germinated, and selected for uniformity. The seeds were planted in one end compartment of each container, and were inoculated with 10 ml of a suspension containing  $10^8$  cells  $\text{ml}^{-1}$  of *Bradyrhizobium japonicum*. Seeds of dwarf corn (*Zea mays* L. cv. MM926), germinated in the same way as the soybeans, were planted in the opposite end of each container.

### *Growth conditions*

The plants were grown in a walk-in type growth chamber at day/night regimes of 16/8 hr, 27/21° C, and 40/60% RH. A bank of 1500 mA cool white fluorescent and 40 W incandescent lamps provided illumination of approximately  $750 \mu\text{mol m}^{-2}\text{s}^{-1}$  at the shoot tips. The soil utilized was a sandy loam purchased from a local landscaping supply dealer. It had pH of 7.7, bulk density of  $1.5 \text{ g cm}^{-3}$ , organic matter content of 0.2% and available ( $\text{NH}_4\text{HCO}_3$ -DTPA extractable) nutrient concentrations ( $\text{mg kg}^{-1}$ ) of: N, 4.8; P, 5.7; and K, 51. The watering schedule was the same for all compartments of the containers, and solution amounts were proportional to the volumes of the compartments.

For 14 d after planting, all plants were irrigated only with water. After that time, nutrients were added, approximating 1/4-strength Hoagland's solution, but with N and P concentrations that were changed at intervals. The solution for soybean was 2 mM in N ( $\text{NH}_4\text{NO}_3$ ) only during the third week, to encourage nodulation. The solution for the corn plants was 8 mM in N for the remainder of the experiment. Both solutions were 0.2 mM in P ( $\text{KH}_2\text{PO}_4$ ) through the fifth week of growth, after which P was deleted. Other macronutrient concentrations used after the second week were (mM)  $\text{CaCl}_2$ , 1.5;  $\text{K}_2\text{SO}_4$ , 1.0; and  $\text{MgSO}_4$ , 0.25.

#### *Uptake of labeled carbon*

Exposure of the corn plants to  $^{13}\text{CO}_2$  was accomplished in a box approximately 1.2 m long  $\times$  0.3 m wide  $\times$  0.8 m high). The box had 5 gasketed openings in each of 2 opposite sides, through which one end compartment of a plant container could be inserted. Thus, ten plants could be exposed to  $^{13}\text{CO}_2$  at one time. With the plants in place, the internal volume of the box was 270 L. The air within the box was circulated by four small fans to distribute the  $^{13}\text{CO}_2$  uniformly. The plants were illuminated by four 1.2 m-long VHO cool white fluorescent tubes suspended above the clear acrylic plastic top of the box. To monitor the internal atmosphere, the sensor assembly of a LI-COR 6200 Portable Photosynthesis System was mounted over a hole in the  $^{13}\text{CO}_2$ -exposure box. This provided air temperature (27° C) and relative humidity (70 to 76%) measurements, and a relative reading of the changes in  $\text{CO}_2$  concentration. Since the instrument is calibrated with atmospheres containing the normal distribution of C isotopes, its reading is lower than the actual level of  $^{13}\text{CO}_2$  (Svejcar et al., 1990). Six-45 ml portions of 99 atom %  $^{13}\text{CO}_2$  were added to the exposure box over a 1 hr period. Because the isotope ratio changed constantly as the plants absorbed both the original  $\text{CO}_2$  (predominantly  $^{12}\text{C}$ ) and the added  $^{13}\text{CO}_2$  (not necessarily in the same proportions), the calculation of Svejcar et al. (1990) based on measurements in the dark, i.e. in the absence of photosynthesis, was not applicable to determination of the composition of the atmosphere during photosynthesis. An additional 0.5 hr was allowed for uptake after the last portion was added, after which the plants were returned to the growth chamber. One day after  $^{13}\text{CO}_2$  exposure, leaf samples were taken from the soybean plants to determine if C had been exported from the corn to the soybean plants, and the plants were removed from the soil for measurement and analysis.

### *Measurements and analysis*

At harvest, 60 d after planting, weights of roots and shoots were determined after drying for 2 d at 70° C. Leaf tissue nutrient analyses were performed by the Research Extension Analytical Laboratory, Ohio State University, Wooster, OH 44691. Percent VAM colonization of roots was determined by the grid intercept method after staining with trypan blue in lactic acid and glycerol (Kormanik and McGraw, 1982). Nodule activity ( $C_2H_2$  reduction) was measured as reported previously (Bethlenfalvay et al., 1982). Levels of  $^{13}C$  in plant tissues and soil were determined by Isotope Services, Inc., Los Alamos, NM 87544. From the  $\delta^{13}C$  values (relative to the international PDB standard) reported by the analytical laboratory, fractional abundances (F) of  $^{13}C$  ( $^{13}C$ -isotope fraction in plant and soil samples) were calculated according to Svejcar et al. (1990). The extent to which F values in the soybean compartments exceeded those present prior to exposure of the corn plants and compartments to  $^{13}C$  was interpreted as a measure of C transport between compartments.

### *DRIS evaluation*

Changes in the order of nutrient deficiencies and nutrient balance of leaves were evaluated by the DRIS. This system utilizes a comparison of ratios of element concentrations in experimental plants with the same ratios (reference norms) compiled from an extensive sampling of field-grown crops. Reference norms for soybean were taken from Hallmark et al. (1987), and for corn, from Elwali et al. (1985). From these comparisons, a DRIS index was computed for each nutrient. The indices were ranked, with the most negative index indicating the most limiting nutrient. The sum of the absolute values of the indices is a measure of nutrient balance: the lower the sum, the better the balance (Jones et al., 1986). The indices for N, P and K were calculated according to Hallmark et al. (1987) as:

$$N \text{ index} = [f(N/P) + f(N/K)]/X$$

$$P \text{ index} = [-f(N/P) + f(P/K)]/X$$

$$K \text{ index} = [-f(N/K) - f(P/K)]/X$$

with X equal to the number of functions in the numerator. The functions of the nutrient ratios were derived from the formulas (Walworth and Sumner, 1987):

$$f(N/P) = [N/P]/(n/p) - 1)(1000/cv), \text{ when } N/P > n/p$$

$$f(N/P) = [1 - (n/p)/(N/P)](1000/cv), \text{ when } N/P < n/p,$$

where capital letters refer to the nutrient concentrations of the sample tissues, lower case letters to the concentrations of the reference norms, and cv to the coefficients of variation of the respective concentration-ratio norms.

### 3. Results

Our results indicated support for the assumption that complementary transfer of resources can occur in plants associated through VAM mycelium. In soybeans, nodule activity was significantly higher ( $P = 0.041$ ) in the associated than the nonassociated plants (Table 1) and was reflected in significantly ( $P = 0.002$ ) higher leaf N concentrations (Table 2). In the associated corn plants, on the other hand, VAM colonization and cob dry mass were lower ( $P = 0.088$ ) in the associated than in the nonassociated corn plants (Table 1). Thus, although all plants of both treatments had the same ( $P > 0.4$ ) shoot and root dry mass, the high energy requirement of  $N_2$  fixation may have been supplemented in soybean by the diversion of reduced carbon from the cobs of the associated corn plants.

The 27% ( $P = 0.134$ ) increase in K concentration in associated vs nonassociated corn leaves was not reflected by a significant ( $P = 0.694$ ) decline in associated soybean (Table 2).

Labeled C assimilated by the corn plants was not detectable in any of the soybean leaves one day after exposure. However, the fractional abundance (F) of  $^{13}C$  in the soil surrounding the roots of nonassociated corn ( $8.50 \times 10^{-3}$ ) was significantly ( $P = 0.015$ ) higher than that in the soil on the soy side of the barrier ( $8.37 \times 10^{-3}$ ). In contrast, F values of both associated plant soils (soybean,  $8.69 \times 10^{-3}$ ; corn,  $8.62 \times 10^{-3}$ ) were statistically the same ( $P = 0.468$ ),

Table 2. Leaf nutrient concentrations of plants associated by VAM mycelium or separated by a barrier (nonassociated). Numbers are the means and SE of 5 replications. P-values denote the significance of differences (*t*-test) between respective treatment data.

Treatment	Nutrients (mg g <sup>-1</sup> )					
	N		P		K	
	Soybean	Corn	Soybean	Corn	Soybean	Corn
Associated	29.0±0.7	24.2±0.8	2.7±0.1	4.1±0.4	13.3±0.6	18.3±1.8
Nonassociated	24.1±0.6	24.2±0.8	3.5±0.2	3.1±0.3	13.7±0.8	14.3±1.5
P-value	0.002	0.974	0.009	0.080	0.694	0.134

indicating a distribution of the labeled C to the soil of the associated plant, whereas such transport did not occur in the nonassociated system. Lower values in the nonassociated vs associated corn plant soils (soybean,  $P = 0.0001$ ; corn,  $P = 0.005$ ) may be ascribed to decreased flux of shoot C down to and out of associated corn roots in the absence of sink demands by the soybean and its nitrogen-fixing symbiont.

In terms of apparent nutrient fluxes between associated plants, as evaluated by comparisons with the nonassociated controls, only a movement of P from soy to corn was evident: comparisons of P concentrations in the associated plants indicates a gain by corn plants (32.2%,  $P = 0.080$ ) at the expense of soybeans (-22.9%,  $P = 0.009$ ). A difference of N content comparable to the significantly higher N concentration of associated vs nonassociated soybean did not appear in the corn plants. The 27% ( $P = 0.134$ ) increase in K concentration in associated vs nonassociated corn leaves was not reflected by a significant decline ( $P = 0.694$ ) in associated soybean plants (Table 2).

The evaluation of nutrient relationships by DRIS analysis showed no difference in the order of nutrient deficiencies in the plants of the two soybean treatments (Table 3), but the magnitudes of the indices indicated differences

Table 3. Relative nutrient deficiencies and balances in leaves of plants associated by VAM mycelium or separated by a barrier (nonassociated), evaluated by the Diagnosis and Recommendation Integrated System (DRIS). More negative DRIS indices reflect greater deficiency. The sum of the absolute values of the indices is an expression of nutrient balance; the smaller the number, the more balanced the nutrients.

Treatment	Plant					
	Soybean			Corn		
	N	P	K	N	P	K
<i>DRIS Indices</i>						
Associated	-16	+15	+1	-5	+ 4	+ 2
Nonassociated	-46	+44	+2	-9	+22	-14
<i>Order of Nutrient Deficiency</i>						
Associated	N > K > P			N > K > P		
Nonassociated	N > K > P			K > N > P		
<i>Nutrient Balance</i>						
Assoc. vs nonassoc.	Assoc.	>	Nonassoc.	Nonassoc.	>	Assoc.
	(32)		(92)	(45)		(11)

in the degree of nutrient deficiency (N index: -16 vs -46 in associated vs nonassociated plants) or sufficiency (P index: +15 vs +44). In nonassociated corn, however, the order of deficiency for N and K was reversed. Thus, the "nonsignificant" increase ( $P = 0.134$ ) in K concentration in the associated vs nonassociated corn plants (Table 2) was reflected as consequential by the shift in relative deficiencies in the DRIS analysis. Significantly, the nutrient balance comparisons (Table 3) showed that both plants benefitted from being grown in association.

#### 4. Discussion

The influences of source and sink sizes and activities on each other within a plant are well-known (Marschner, 1986), but little-appreciated as they apply to plant associations (Bethlenfalvay et al., 1991). A consideration of parameters which might appear to be properties of each individual plant, but which apparently are also influenced by adjacent plants in the presence of VAM mycelium (Table 1), raises the question of the validity of relating inferences drawn from individual-plant evaluations under artificial conditions to field situations. Since plant responses to stresses or stimuli differ with species, two plants, such as the soybean and corn of this experiment, may pass through stages of source and sink activity out of synchrony with each other. In the context of VAM-mediated nutrient distribution, a definition of the source of nutrients to associated plants becomes useful: our term 'nutrisphere' (Bethlenfalvay et al., 1991) is equivalent in its geometric extent to the 'mycorrhizosphere' (Linderman, 1988) in that it includes the same root-soil volume permeated by the VAM mycelium, but it refers to the entire nutritional content of this volume, including other roots as well as soil, which is available to each associated plant.

The suggested inhibition of cob development in the associated corn plants (Table 1) is an example of applications for the results of VAM-mediated nutrient-distribution experiments. If corn seed yield is of primary interest in an intercrop situation, then it is important to know beforehand whether (1) soybean is too vigorous a sink and another legume should be used, (2) there is a discrepancy between the growth stages of the associated plants which is detrimental and the legume should be planted later, or (3) host-endophyte compatibility favors one plant partner or another, indicating that other VAM fungi or crop cultivars should be used. The stimulation of nodule activity, apparently at the expense of the corn plant (Tables 1 & 2), is another example of unevenly distributed cost-benefit ratios for associated plants. It remains to be seen whether advantages, such as the improvement of nutrient balance in soybean (Table 3), are temporary and growth-stage dependent, or if they

shift during the growth cycle from partner to partner and balance only when considered over the entire growing season.

Within the context of nutrient partitioning in symbiotic plant physiology (Paul, 1981; Smith and Smith, 1990), it is of interest whether apparent C (Francis and Reed, 1984) or other nutrient (Bethlenfalvay et al. 1991; Ritz and Newman, 1986) transfer between plants actually originates from the plants themselves, from the extraradical C compounds of rhizodeposition (Lynch and Whipps, 1990), or from other mineral sources within the nutrisphere. In the agro-ecological context (Bethlenfalvay and Newton, 1991) such a distinction is of little consequence; economic yield is affected in the same way regardless of the immediate source of a nutrient being made available to the strongest sink within the plant association. This is precisely the reason why a knowledge of VAM-mediated nutrient-distribution mechanisms is important in managing intercrop (and weed-crop) associations: the VAM-fungal component of the association should be selected to ensure satisfaction of the primary crop's requirements. For example, if in a cereal-legume association with low P availability, the legume plays a supporting role of biological N input, the system must be so managed that potential (VAM-mediated) loss of P from the nutrisphere of the cereal to that of the legume does not adversely affect the cereal's growth (Bethlenfalvay et al., 1991).

The insight that in plant associations the nutritional and growth status of plants is not only a function of the individual plant's ability to cope with a competitive environment, but also of ever-changing supply-demand relationships with its associates, puts knowledge of VAM host-endophyte compatibility at a premium in systems modeling and management. The effectiveness of a native vs an exotic plant in combination with a VAM-fungus in influencing successful crop growth in associative situations is little known (Hetrick and Wilson, 1990), but, as the present results suggest, is likely to be influenced by interplant nutrient distribution.

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