Nutrition of Mycorrhizal Soybean Evaluated by the Diagnosis and Recommendation Integrated System (DRIS)

Gabor J. Bethlenfalvay,* Raymond L. Franson, and Milford S. Brown

ABSTRACT

Colonization of roots by vesicular-arbuscular mycorrhizal (VAM) fungi affects host-plant growth and nutrition, but standard methods to evaluate VAM effects are lacking. The Diagnosis and Recommendation Integrated System (DRIS) may serve this purpose, and an analysis of mycorrhiza-mediated nutrient-uptake effects on soybean [*Glycine max* L.) Merr. cv. Hobbit] plants by DRIS is presented. Plants were grown either colonized by one of three geographical isolates of the VAM fungus *Glomus mosseae* (Nicol & Gerd) Gerd and Trappe or as nonVAM controls in a nutrient-deficient soil. Plant development and nutritional status differed among the treatments. Analysis by DRIS confirmed that N, P, and K were limiting, and established different rankings in the degree of deficiency for each nutrient. Nitrogen was least limiting in all four treatments and K or P were most limiting in each of two treatments. The analysis permitted a ranking of the *G. mosseae* isolates as to their effects on the uptake of each nutrient. Evaluation of VAM-mediated nutrient-uptake effects by DRIS and by nutrient-dilution and concentration analyses could become useful in the selection of specific VAM fungal isolates for their specific crop responses.

UTILIZATION of VAM fungi with maximum effectiveness in large-scale agricultural applications will necessitate an evaluation of fungal effects on crop plants. Therefore, all criteria which promise to have an impact on such an evaluation should be explored. The best-known VAM effect on plants is nutritional. When mediated by VAM fungi, nutrient enhancement is a more complex phenomenon than that obtained with chemical fertilizer applications. The fungi may inhibit as well as enhance nutrient uptake from soil (Ames and Bethlenfalvay, 1987; Stribley, 1987) or from biological N_2 fixation (Bayne and Bethlenfalvay, 1987). Because of such differences, there is general agreement on the need to screen isolates for specific applications (Sylvia and Burks, 1988). One experimental plan for the selection of VAM inocula was proposed by Abbott and Robson (1985). In includes criteria for the collection and culture of the fungi under different edaphic conditions, and for evaluation of physiological and ecological responses.

The DRIS has served as a useful tool in diagnosing and predicting nutrient disorders in plants (Walworth and Sumner, 1987) since its introduction by Beaufils (1973). Analysis by DRIS is based on nutrient balance ratios. Its use minimizes morphogenic and genotypic effects on the accuracy of deficiency diagnoses, and predicts which nutrient is most limiting to yield. Treating dry matter (DM) as a mineral nutrient in DRIS calculations permits determining whether any of the tissue nutrients are limiting (Hallmark et al., 1987). Thus, plant nutritional responses to VAM fungi in a given soil may be evaluated for each nutrient affected. Comparisons between such evaluations can provide a ranking for limitation by each nutrient as well as an order of preference for fungal inocula most suited to counteract that limitation.

The purpose of this report was to test the applicability of DRIS in evaluating soybean nutritional responses to VAM fungi, and to compare DRIS with another system of evaluation based on nutrient concentrations (Jarrell and Beverly, 1981).

MATERIALS AND METHODS

Three isolates of *Glomus mosseae* were grown on Hobbit soybean inoculated with *Bradyrhizobium japonicum*, Nitragin strain 61A118 (Nitragin Co., Milwaukee, WI) as a bacterial suspension in yeast-mannitol broth (10^9 cells mL^{-1}, 10 mL pot^{-1}). The fungal isolates were collected from California and Nevada (isolate numbers WRRC-1 and WRRC-2) or obtained from the International Culture Collection for VAM Fungi, University of Florida, Gainesville (isolate INVAM-156). They are referred to as CAL, NEV and FLO and were described elsewhere (Bethlenfalvay et al., 1989). Soybean plants not colonized by a VAM fungus were used as (nonVAM) controls. Propagule densities in the VAM-fungal inocula were determined by a method (Franson and Bethlenfalvay, 1989) which relies on a direct count of discreet infection units formed in roots during a 14-d growth period. Inocula of equal potential, in amounts calculated to produce 10^6 infection units kg^{-1} of root fresh mass, were applied.

Plants were grown in a growth chamber under a day/night regime of 16/8 h, 27/21 °C, 60/90% relative humidity and photosynthetically active radiation of 800 $\mu mol\ m^{-2}\ s^{-1}$. The soil used was a heavy silt loam (Balcom Series Typic Xerorthent) mixed with fine sand (2:1, v/v, soil/sand). After sterilization by autoclaving, the soil mixture (pH 8) had plant-available P and K concentrations (extracted with NH_4HCO_3–diethyltriamine pentaacetic acid) of 1.7 and 131 mg kg^{-1}, respectively, and total N of 0.47 g kg^{-1}. The VAM inoculum was evenly mixed into the soil, and a wash of the combined inocula, sieved free of VAM propagules (43-μm screen), was applied to all treatments to minimize differences in the microbiota of the treatments. The plants were grown in 1.5-L pots, watered with deionized water twice per week, harvested at 50 d and evaluated as part of a larger experiment (Bethlenfalvay et al., 1989).

The nutrient status of plants was assessed using DRIS by comparing ratios of element concentrations with the same ratios (reference norms) from high-yielding (in excess of 2700 kg ha^{-1}) soybean crops. Reference norms were presented by Hallmark et al. (1987) and were derived from 1535 trifoliate leaf samples at the early reproductive stage. From these comparisons a DRIS index was computed for each nutrient, including DM. The indices were ranked, with the most negative index indicating the most limiting nutrient. The indices for N, P, K and DM were calculated according to Hallmark et al. (1987) as

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

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

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

USDA-ARS, Western Regional Research Center, Albany, CA 94710. Contribution from the Plant-Soil Symbiosis Group, Plant Development-Productivity Unit, WRRC. Received 9 Feb. 1989. *Corresponding author.

Published in Agron. J. 82:302-304 (1990).

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

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

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

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

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

RESULTS AND DISCUSSION

A comparison of the nutrient concentrations of the present experiment (Table 1) with critical values used in the literature (42.6, 2.6 and 17.1 mg/g for N, P, and K, respectively; Small and Ohlrogge, 1973) revealed severe nutrient deficiencies in our soil mix. This poor growth medium provided a good test vehicle for the analysis of changes in leaf nutrient content (Table 1) and total plant dry mass (Table 2) in response to colonization by *G. mosseae* isolates. Differences between colonization by the isolates (CAL, 43.5%; FLO, 38.2%; NEV, 41.2%) were not significant ($P > 0.05$, Duncan's multiple range test).

When nutrients such as N, P, and K are applied as fertilizers, changes in the concentration of a given element are difficult to predict. Concentration of a nutrient depends on the influence of the other nutrients available in the soil and on the effects of the applied nutrient on plant growth (Munson and Nelson, 1973). However, when nutrient input is mediated by VAM fungi, the mechanisms responsible for observed plant responses are not limited to a single element (Jarrell and Beverly, 1981).

In this study, the CAL isolate increased uptake and concentration of K and P, while the FLO and NEV isolates increased P but decreased K relative to the control (Table 1). In the case of legumes depending on N_2 fixation for their N input, effects of other nutrients on nodule activity may influence N stress. In the plants colonized by the CAL isolate, a 20% increase in leaf P concentration was accompanied by a significant 14% increase in N concentration over that of the nonVAM controls. Conversely, the change in N was not significant (9%, $P > 0.05$) in the plants colonized by the other two isolates, apparently due to growth-limiting K concentrations in host tissues.

Conventional plant tissue analysis reveals only one deficiency at a time, while other nutrients in short supply may accumulate due to reduced growth caused by the primary deficiency (Ulrich and Hills, 1973). Therefore, such analysis is of limited utility in analyzing effects of VAM colonization. Jarrell and Beverly (1981) clarified the interpretation of shifts in nutrient concentration by pointing out the need to evaluate these concentrations in relation to plant responses in biomass and elemental content. A decrease in concentration is interpreted as dilution when both nutrient content and dry matter simultaneously increase, but as antagonism when nutrient content or nutrient content and dry matter decrease.

In the present case, for instance, the decrease in K concentration of the plants colonized by the FLO isolate relative to the controls (Table 1) could be erroneously interpreted as dilution in view of significantly greater plant biomass (Table 2), if all factors were not considered simultaneously (Table 3). Consideration of

Table 1. Leaf dry mass and nutrient content, concentration and concentration ratios. Nodulated soybean plants were grown with one of three isolates (CAL, FLO, NEV) of *Glomus mosseae* or without (nonVAM control).

Leaf parameter	*G. mosseae* isolate†			NonVAM control	SE	Norm‡	
	CAL	FLO	NEV			Mean	CV
Content (mg)							
K	7.5a	2.3c	2.2c	3.1b	0.102		
N	24.5a	16.3b	16.8b	14.3b	0.053		
P	0.88a	0.58b	0.65b	0.49c	0.012		
Dry mass (g)	1.10a	0.72b	0.79b	0.73b	0.048		
Concentration (mg g^{-1})§							
K/DM	6.8a	3.2c	2.8c	4.2b	0.094	1.96	19.9
N/DM	22.3a	22.6a	21.3ab	19.6b	0.044	4.80	11.9
P/DM	0.80a	0.80a	0.82a	0.67b	0.011	0.331	21.4
Nutrient concentration ratio							
N/P	27.87a	28.26a	26.15a	29.49a	2.887	14.92	16.9
N/K	3.31c	7.07a	7.67a	4.65b	0.460	2.56	19.6
P/K	0.119a	0.257c	0.293c	0.158b	0.103	0.174	24.9

† Numbers are the means of six replications, and when followed by the same letter are not significantly different ($P < 0.05$, Duncan's multiple range test). SE is the standard error of the treatment means.
‡ Means and coefficients of variation (CV, %) of the norms were adpated from Hallmark et al. (1987).
§ Nutrient concentrations of the norms are expressed as percentage of dry matter (DM), rather than in SI units.

Table 2. Total plant dry mass, DRIS indices and the order of nutrient requirement including dry matter (DM) in VAM and nonVAM soybean. Notation and statistics as in Table 1.

Treatment†	DRIS index				Plant dry mass, g	Order of nutrient requirement
	K	N	P	DM		
NonVAM	−78	−5	−81	164	2.9c	P > K > N > DM
CAL	−30	−10	−72	113	5.1a	P > K > N > DM
FLO	−122	17	−61	166	3.5b	K > P > N > DM
NEV	−146	15	−52	183	3.3bc	K > P > N > DM
SE‡					0.26	

† CAL, FLO, and NEV refer to isolates of the VAM fungus *G. mosseae*.
‡ SE = standard error of treatment means.

Table 3. Changes in leaf nutrient content, dry mass, and nutrient concentration in VAM and nonVAM soybean. Treatment effects due to colonization by the *G. mosseae* isolates (increase = +; decrease = −; no change = 0) relative to the nonVAM controls are interpreted according to Jarrel and Beverly (1981).

G. mosseae isolate	Nutrient content	Dry mass	Nutrient concentration	Effect
Potassium				
CAL	+	+	+	Synergism
FLO	−	0	−	Antagonism
NEV	−	0	−	Antagonism
Nitrogen				
CAL	+	+	+	Synergism
FLO	0	0	+	Concentration
NEV	0	0	0	No effect
Phosphorus				
CAL	+	+	+	Synergism
FLO	+	0	+	Synergism
NEV	+	0	+	Synergism

Table 4. Treatment comparisons of nutrient limitation in VAM and nonVAM soybean. Nutrients become more limiting from left to right. Notation as in Table 1.

Nutrient	Order of limitation†
Potassium	CAl < Control < FLO < NEV
Nitrogen	FLO < NEV < Control < CAL
Phosphorus	NEV < FLO < CAL < Control
Dry matter	CAL < Control < FLO < NEV

† Control, nonVAM plants; CAL, FLO, and NEV are isolates of *G. mosseae.*

all variables (nutrient content, dry mass, and concentration) indicated a synergistic effect of nutrient content and dry mass on nutrient concentration in the plants colonized by the CAL isolate for all nutrients, but only for P in the other two VAM treatments. Thus, the role of P in enhancing plant growth appears to be the same for all three isolates, but is modified by the differences in K uptake.

Computation of the DRIS indices (Table 2) revealed the relative importance of P and K in VAM-mediated growth responses in soybean. Phosphorus was more limiting in plants colonized by the CAL isolate and in the nonVAM controls than K, while K was more limiting in the plants colonized by the FLO and NEV isolates. Nitrogen was least limiting in all treatments. However, since the N-indices were lower than the DM-indices, N was also diagnosed to be deficient (sensu Hallmark et al., 1987). Plants colonized by the CAL isolate showed the best nutrient balance (the lower the sum of DRIS-index values irrespective of sign, the more the nutrients are balanced, Jones et al., 1986), and this was clearly related to their significantly greater biomass (Table 2). Of course, the same conclusion can be reached by simply comparing the nutrient concentrations of Table 1 with critical values found in the literature. But such direct comparisons would be biased by differences in cultivars, plant age and leaf position, and would not provide relative rankings in terms of nutrient requirements (Sumner, 1977).

With special regard to VAM treatments, the DRIS indices also permit a ranking of the organisms themselves as to their effects on plant nutrient status (improvement or aggravation of deficiencies or alleviation of toxicity). Such a ranking (Table 4) is expected to apply only to specific host-endophyte combinations in the test soil. Rankings also need to be verified in field applications, where competition by the introduced fungus with the native VAM mycoflora (Hepper et al., 1988) and other environmental effects (Smith and Roncadori, 1986) are likely to be factors. Nutrient imbalances created in the soil by overfertilization have been addressed as to their effects on plants (Kádár and Pusztai, 1983), but their differential impact on individual members of the VAM mycoflora are still unknown.

In order to utilize VAM fungi as biological fertilizers (Azcón et al., 1979) in sustainable agriculture (Mosse, 1987), a complex screening and selection phase of different isolates will be needed to proceed with the utilization of these organisms on more than a hit-or-miss basis. The DRIS (Beaufils, 1973) and the dilution-concentration (Jarrel and Beverly, 1981) analyses are two complementary methods which will be useful in the screening of VAM fungi for beneficial nutritional host-plant responses.

REFERENCES

Abbott, L.K., and A.D. Robson. 1985. Selection of 'efficient' mycorrhizal fungi. p. 89–90. *In* R. Melina (ed.) Proc. 6th North American Conf. on Mycorrhizae, Bend, OR. June 1984, Forest Res. Lab., College of Forestry, Oregon State Univ., Corvallis, OR.

Ames, R.N., and G.J. Bethlenfalvay. 1987. Mycorrhizal fungi and the integration of plant and soil nutrient dynamics. J. Plant Nutr. 10:1313–1321.

Azcón-G. de Aguilar, C., R. Azcón, and J.M. Barea. 1979. Endomycorrhizal fungi and *Rhizobium* as biological fertilizers for *Medicago sativa* in normal cultivation. Nature 279:325–327.

Bayne, H.G., and G.J. Bethlenfalvay. 1987. The *Glycine-Glomus-Rhizobium* symbiosis. IV. Interactions between nitrogen-fixing and mycorrhizal endophytes. Plant Cell Environ. 10:607–612.

Beaufils, E.R. 1973. Diagnosis and Recommendation Integrated System (DRIS). A general scheme for experimentation and calibration based on principles developed from research in plant nutrition. Soil Sci. Bull. Univ. Natal 1:1–132.

Bethlenfalvay, G.J., R.L. Franson, M.S. Brown, and K.L. Mihara. 1989. The *Glycine-Glomus-Bradyrhizobium* symbiosis. IX. Nutritional, morphological and physiological responses of nodulated soybean to geographic isolates of the mycorrhizal fungus *Glomus mosseae.* Physiol. Plant. 76:226–232.

Franson, R.L., and G.J. Bethlenfalvay. 1989. The infection unit method of VA mycorrhizal propagule determination. Soil Sci. Soc. Am. J. 53:754–756.

Hallmark, W.B., J.L. Walworth, M.E. Sumner, C.J. deMooy, J. Pesek, and K.P. Shao. 1987. Separating limiting from nonlimiting nutrients. J. Plant Nutr. 10:1381–1390.

Hepper, C.M., C. Azcón-Aguilar, S. Rosendahl, and R. Sen. 1988. Competition between three species of *Glomus* used as inocula for leek (*Allium porrum* L.). New Phytol. 110:207–215.

Jarrell, W.M., and R.B. Beverly. 1981. The dilution effect in plant nutrition studies. Adv. Agron. 34:197–224.

Jones, M.B., D.M. Center, C.E. Vaughn, and L.B. Fremont. 1986. Using DRIS to assay nutrients in subclover. Calif. Agric. Sept.–Oct.:19–21.

Kádár, I., and A. Pusztai. 1983. Effect of NPK overfertilization on nutrient turnover in soil and plant. III. Proportions of nutrient elements in six-leave maize. Növénytermelés 32:49–59.

Mosse, B. 1986. Mycorrhiza in sustainable agriculture. Biol. Agric. Hortic. 3:191–209.

Munson, R.D., and W.L. Nelson. 1973. Principles and practices in plant analysis. p. 223–248. *In* L.M. Walsh and J.D. Beaton (ed.) Soil testing and plant analysis. SSSA, Madison, WI.

Small, H.G., and A.J. Ohlrogge. 1973. Plant analysis as an aid in fertilizing soybeans and peanuts. p. 315–328. *In* L.M. Walsh and J.D. Beaton (ed.) Soil testing and plant analysis. SSSA, Madison, WI.

Smith, G.S., and R.W. Roncadori. 1986. Responses of three vesicular-arbuscular mycorrhizal fungi at four soil temperatures and their effects on cotton growth. J. Nematol. 18:208–216.

Stribley, D.P. 1987. Mineral nutrition. p. 59–70. *In* G.R. Safir (ed.) Ecophysiology of VA mycorrhizal plants. CRC Press, Boca Raton, FL.

Sumner, M.E. 1977. Preliminary N, P, and K foliar diagnostic norms for soybeans. Agron. J. 69:226–230.

Sylvia, D.M., and J.N. Burks. 1988. Selection of a vesicular-arbuscular mycorrhizal fungus for practical inoculation of *Uniola paniculata.* Mycologia 80:565–568.

Ulrich, A., and F.J. Hills. 1973. Plant analysis as an aid in fertilizing sugar crops: Part I. Sugar beets. p. 271–288. *In* L.M. Walsh and J.D. Beaton (ed.) Soil testing and plant analysis. SSSA, Madison, WI.

Walworth, J.L., and M.E. Sumner. 1987. The diagnosis and recommendation integrated system (DRIS). Adv. Soil Sci. 6:149–188.