

THE GLYCINE-GLOMUS-RHIZOBIUM SYMBIOSIS
III. ENDOPHYTE EFFECTS ON
LEAF CARBON, NITROGEN, AND PHOSPHORUS NUTRITION

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Milford S. Brown and Gabor J. Bethlenfalvay

United States Department of Agriculture
Agricultural Research Service
Western Regional Research Center
Albany, California 94710

ABSTRACT

Soybean plants [Glycine max (L.) Merr.] were grown in pots and inoculated with Rhizobium japonicum and/or Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe, either at planting or 20 days later. Nitrogen was supplied in the nutrient solution to plants without nitrogen-fixing bacteria, and P was added to those without the mycorrhizal fungus. At harvest, 50 days after planting, all plants had leaves of similar dry mass. Each root symbiont grew best in the absence of the other. Growth of Glomus reflected the duration of its growing time and the presence and duration of competition from Rhizobium. Nodule weight in the tripartite

associations, on the other hand, was inhibited only by the earlier introduction of Glomus.

Dipartite associations and the plants inoculated with both root symbionts at planting had the highest concentration of leaf N, and the lowest was in those inoculated with both organisms at d 20. Leaf P was highest in plants inoculated only with Rhizobium, and lowest in those tripartite associations involving any inoculation at day 20. The low values were presumably a result of the short duration of endophyte-mediated P uptake before the plants were harvested.

Although there was almost no difference in leaf sugar concentrations, starch concentrations reflected the duration of Glomus growth, and were greatest in those plants that had supported it for the least time. Uninoculated plants contained the least starch, but produced a greater fresh mass of leaf tissue than any of the tripartite symbionts.

INTRODUCTION

The role of vesicular-arbuscular mycorrhizal (VAM) fungi in the uptake of phosphorus by roots is well documented 5, 9, 20. By providing a much greater area of contact with the soil, they make it possible for the roots to extract sufficient P (as well as certain other mineral elements) from a soil in which that element is not sufficiently available for normal plant growth. Rhizobium, another root symbiont, serves as a biological fixer of N₂ and helps to satisfy the host's N requirement in the absence of sufficient

nitrogen in the soil. In return, the host plant supplies the microsymbionts with carbon compounds that provide energy and serve as metabolic intermediates for their growth.

Uptake of P by VAM fungi helps to satisfy the high P requirement for N₂ fixation by Rhizobium⁷ when P is limiting, and most reports on mycorrhiza-Rhizobium interactions have in the past focussed on this effect². Kucey and Paul¹¹ reported greater nitrogen fixation in VAM faba beans (Vicia faba L.) than in those without the fungus, and Daft and El-Giahmi⁸ observed similar behavior in alfalfa (Medicago sativa L.). The VAM fungus has a large N requirement because of the amino sugar component of its cell walls.

The photosynthetic system of the host plant supports all 3 members of this tripartite symbiosis. Daft and El-Giahmi⁸ noted a greater reduction of dry matter production in VAM plants than in controls when daylength was shortened, reducing the ability of the plant to supply the needs of its microsymbiont through photosynthesis. Thus, the inputs and requirements of the symbiotic partners are links in a closed chain of interdependence in the source-sink relationships of the association³. We examined the leaf composition of plants in the absence or presence of one or both microsymbionts, but plants lacking one or both of the root symbionts were supplied with equivalent amounts of N and/or P in their nutrient solutions to eliminate the effects of nutrient deficiencies. The objectives of the study were (1) to determine the

effects of VAM fungi and Rhizobium as the primary sources of P and N on leaf nutrition, and (2) to evaluate the effects of the microsymbionts, which are sinks for C, on leaf carbohydrate composition.

MATERIALS AND METHODS

Sterilized and germinated seeds of soybean [Glycine max (L.) Merr. cv. 'Hobbit'] were planted in pots containing 1 L of an autoclaved clay loam soil severely deficient in N and P. Inocula of the VAM fungus Glomus mosseae (Nicol. & Gerd.) Gerd. & Trappe and/or Rhizobium japonicum strain 61A118 were added at planting or 20 d later to all except the uninoculated control plants, as described previously⁴. All plants were grown in a growth chamber with $800 \mu\text{Em}^{-2}\text{s}^{-1}$ of photosynthetically active radiation on a 16/8 h day/night schedule. Day/night temperature and relative humidity were 27/21°C and 60/90%.

Glomus and Rhizobium were introduced singly only at planting (NG₁, R₁P). Four combined treatments included the addition of both symbionts at planting (R₁G₁) or at day 20 (R₂₀G₂₀), as well as the initial introduction of either symbiont at day 1 followed by the other at day 20 (R₁G₂₀, R₂₀G₁). Symbiotic plants without Rhizobium received N (2 mM as NH₄NO₃) in their nutrient solution, and those without Glomus received P (0.2 mM as KH₂PO₄). Remaining nutrients were supplied in amounts equivalent to one-quarter strength Hoagland's solution⁴. A control group of plants without

either microsymbiont received both N and P from the nutrient solution (NP). Plants were watered only with these solutions, and for those inoculated at day 20, N and P supplies were discontinued at that time. All treatments were replicated 6 times.

At harvest, 50 d after planting, leaf blades were removed, measured with a LI-COR leaf area meter, weighed (fresh wt.), frozen in liquid nitrogen, lyophilized, weighed (dry wt.), and ground for analysis. Phosphorus and N contents were determined^{15, 17} from wet digests²², soluble hexose by the anthrone method^{1, 12}, and starch by enzymatic hydrolysis and glucose measurement²¹. Nodules were removed from the roots of Rhizobium-inoculated plants, dried, and weighed. Percent VAM-fungal colonization was determined by the grid-intercept method¹³.

RESULTS AND DISCUSSION

Symbiotic Relationships

The highest level of VAM-fungal colonization was found in the plants without Rhizobium (NG₁), and the greatest nodule mass was found in the absence of Glomus (R₁P) (Table 1). In the tripartite symbioses, the extent of host-root colonization by either endophyte depended on the timing of the second microsymbiont's introduction. Early introduction of Glomus produced a higher level of root colonization than inoculation at day 20, and within each of these groups, early inoculation with Rhizobium was more inhibitory than late inoculation. However, nodule formation was reduced

TABLE 1
Leaf Mass and Composition, and Extent of Root Colonization by Microsymbionts.

Treatment ^a	Leaf				Root				
	Fresh mass g	Dry mass g	Area dm ²	N	P	Sugar	Starch	Nodule mass g	VAM fungus % L ^b
R ₁ G ₁	13.0 bcd ^c	3.95 ab	4.4 c	0.75 a	0.096 cd	3.5 ab	24 b	0.46 b	50 c
R ₁ G ₂₀	11.4 d	3.48 b	5.1 bc	0.52 bc	0.088 de	3.4 b	28 a	0.45 b	40 d
R ₂₀ G ₁	12.8 cd	3.67 ab	5.7 ab	0.47 c	0.090 de	3.7 a	24 b	0.31 c	63 b
R ₂₀ G ₂₀	13.5 bc	4.03 a	6.1 a	0.36 d	0.087 e	3.4 b	30 a	0.46 b	50 c
R ₁ P	14.7 ab	4.14 a	5.2 bc	0.70 a	0.126 a	3.5 ab	22 b	0.60 a	
NG ₁	14.1 bc	3.68 ab	5.0 bc	0.79 a	0.110 b	3.5 ab	18 c		89 a
NP	15.9 a	4.21 a	5.3 b	0.59 b	0.100 bc	3.5 ab	17 c		

^a R = Rhizobium, G = Glomus; N, P = nitrogen, phosphorus from nutrient solution.

^b % L = percent of root length colonized by VAM fungus.

^c Numbers followed by the same letter are not significantly different at $p = 0.05$.

only in the case of early Glomus inoculation followed by late introduction of Rhizobium ($R_{20}G_1$). Unlike mycorrhizal development, however, the extent of nodulation did not depend upon inoculation time if Glomus was added at d 20.

The dry masses of the leaves from all treatments were similar, an indication that nutrient inputs from solution and root symbionts were equivalent. Inoculation with both symbionts at day 20 produced plants with the highest leaf area; and inoculation with both at planting, the smallest area. Inoculation with only one symbiont, or with both separately, produced plants of intermediate leaf area.

Leaf Nitrogen and Phosphorus

The highest level of leaf N was found in plants inoculated with one or both symbionts at planting only, while inoculation with both root symbionts at day 20 produced the lowest N. Of those with only one root symbiont, inoculation with Rhizobium at planting produced plants with a higher level of leaf P than those inoculated with Glomus at that time. In general, both leaf area and starch concentrations were inversely related to N and P concentrations. Plants supplied with N and P from the nutrient solution had leaves of intermediate N and P concentration. Early development of nodules allowed the leaves to accumulate a high level of N unless there was an additional requirement for N by late Glomus development (R_1G_{20}). The greater N content of VAM plants reported by Nielsen¹⁶ was not confirmed in this experiment.

Late addition of both symbionts produced the lowest level of leaf N. Late addition of a second symbiont to a previously inoculated plant also resulted in low leaf N.

Low leaf N in plants inoculated late with Rhizobium may in part be a consequence of the reduced N supply between the time of inoculation, at which the nutrient solution was changed to one without N, and the time of full nodule activity. In addition, the extent of late nodulation was affected adversely by prior development of the fungus. However, this did not occur after simultaneous late introduction of both symbionts. This suggests an interference with the process of nodulation by established fungal structures or some product of their metabolic processes, followed by distribution of the products of nitrogen fixation according to needs within the entire tripartite symbiosis. Of plants supplied with N in the nutrient solution, those inoculated with Glomus at planting accumulated more N in their leaves than did those plants without the root symbionts. The relatively high P content and fresh mass of the NG₁ plants, with low starch and moderate sugar levels, suggest a balanced supply of mineral nutrients for proper growth.

The highest leaf P concentration occurred not in those plants whose uptake was assisted by the root symbiont (Glomus), but in those nodulated plants that received P in their nutrient solution. Leaf P concentration was low in plants of the four tripartite symbioses, presumably a consequence of the requirement

for P in metabolic components involved in energy transfer reactions in the establishment and functioning of the two root symbionts.

The pattern of P accumulation in leaves was similar to that of N. Early introduction of Glomus was as effective as supplying P directly from the nutrient solution, and late development of either or both symbionts acted as the sink (Rhizobium), or the lack of a source (Glomus), that lowered leaf P concentration. As with N, the presence of the noncontributing symbiont (Rhizobium in this case) increased the uptake of P beyond that of the nonsymbiotic controls. Again, the pattern of development of the symbiont under various combinations of inoculation time and symbiosis was not always reflected in the distribution of the product of its functioning; P needed for the development of either symbiont was supplied at the expense of leaf P.

Leaf Carbohydrates

Carbohydrate concentrations were indicative of the demands of the root symbionts for reduced C. The timing of Rhizobium introduction had no effect on the concentrations of leaf sugar and starch. Although its introduction at planting contributed to a higher level of N in the leaves, the initial energy requirement for nodule development may have been similar to the later energy utilization by nitrogenase. Thus there was little difference in the overall effect of time of symbiont introduction on carbohydrate storage in the leaves, although previous work¹⁹ did not find

an increase in nitrogen fixation in response to greater CO₂ uptake by soybeans.

Plants inoculated late with Glomus, on the other hand, contained the highest concentration of starch. Phosphorus levels in the environment of the chloroplasts influence chloroplast activity¹⁰, with low levels of P favoring starch accumulation in the chloroplast rather than the export of triose phosphates. Plants supplied with N from the nutrient solution (NP and NG₁) had the lowest levels of starch in their leaves, although their total masses were among the largest. Their high levels of N and P apparently stimulated the export of C from the chloroplasts and its incorporation into structural materials. This confirms the findings of Same, et al.¹⁸ that additional P reduces the concentration of soluble carbohydrate, but increases the dry mass of the plant. With adequate P, an essential requisite of all energy transfer reactions in the plant, the products of photosynthesis can be used immediately for growth.

Leaf N-P-C Interactions

A salient feature of the data was the discrepancy between the similarly large leaf fresh weights of the non-symbiotic controls and the dipartite associations, and the dissimilarity of their P and N concentrations. The higher leaf N concentrations in the NG relative to the NP plants, and the higher P concentration in the leaf of the RP relative to the NP plants indicates higher demand for these nutrients by the symbiotic plant. Furthermore,

in the tripartite symbioses, it may mean that the demands of Glomus for N and of Rhizobium for P are first communicated to the plant, and then to the other endophyte.

Conversely, the high N concentration of the R₁P relative to the NP treatment indicates that uptake mediated by an endophyte utilizes a more efficient mechanism than is available to the nonsymbiotic plant. The mineral and carbohydrate composition of the leaves shown in Table 1 is the status of the plants at the age of 50 days, but this represents the summation of nutrient uptake and export during the entire life of the plant. During the latter part of this experiment, major changes in mineral nutrient and photosynthate demands occurred in those plants inoculated with either or both of the root symbionts at day 20, thus complicating comparisons with the results of earlier work at this or other laboratories. Comparisons between plants whose nutrition is mediated by beneficial endophytes and those whose nutrients are taken directly from the soil are problematic. In each case, the energy costs and benefits must be considered (e.g. nitrogenase vs. nitrate reductase, or supporting the growth of VAM fungus vs. obtaining P from the soil solution) and balanced against the availability of soil nutrients in sufficient concentration to support plant growth. Further, the problem of endophyte inhibition by its substrate (VAM fungi by P, diazotrophs by N) makes the use of nutrient-sufficient controls an elaborate process.

In this experiment, the late addition of the root symbionts to some of the plants resulted in unusually low levels of N and P in the leaves. In order to maintain comparability of dry mass with the symbiotic plants, the control plants received N and P at a lower level than would normally be considered adequate. Failure to do so would have resulted in plants of different size, an additional variable the effects of which could not be isolated in this experiment. Nonetheless, present data show negative interactions between developing endophytes that cannot be explained by previous nutrient treatments.

In future studies, determination of the nutrient and carbon status of the plants at the time of the late introduction of the root symbionts (d. 20) could provide additional information about these interactions. In addition, measurements of carbon uptake rates at various stages of plant growth might provide an insight to the way in which the plant responds to the presence of the root symbionts at various stages of its development.

DISCLAIMER

Reference to a company and/or product is for purposes of information only, and does not imply approval or recommendation of the product to the exclusion of others that may also be suitable.

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