

PHOTOSYNTHETIC WATER- AND NUTRIENT-USE EFFICIENCY
IN A MYCORRHIZAL LEGUME.

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Introduction. Two aspects of plant science find a good fit in mycorrhizal research: the need to utilize symbiotic plant associations in more efficient, sustainable modern agriculture, and the desire for a better understanding of a living system for its own sake. The tripartite legume association includes cooperative but also competitive interactions by organisms from three biological kingdoms. It is therefore a particularly challenging and complex exercise in the search for basic principles which, when fully understood, may be utilized as management tools in agricultural productivity.

Early impetus in the use of vesicular-arbuscular mycorrhizal (VAM) fungi as legume associates stemmed from their dramatic effect on nodule activity (1). The synergistic growth response to the combined effects of the VAM-fungus and Rhizobium endophytes which is sometimes observed in the host plant has led to a view of the microsymbionts as biological substitutes for fertilizers (3). While this view certainly holds promise in terms of agricultural productivity, its merits must now be reexamined in terms of the underlying biological mechanisms. The promise had been based largely on comparisons of VAM and non-VAM plants grown under nutrient limitation. Such comparisons have little significance in applied or basic studies, since a non-VAM legume is unlikely to occur or survive for long in the field, while the stunted non-VAM control plant does not serve as a meaningful comparison under experimental conditions. Thus, the "big plant-little plant" syndrome, which generated so much of the excitement during the early part of the era of enlightenment (13) of mycorrhizal work, is bound to change in perspective as emphasis shifts from descriptive, natural-history oriented projects to goal-oriented, physiological experimentation. This shift is already in progress and is likely to accelerate in the coming years, founded on the necessary and growing data base of ecological and agro-ecological observations. Of the many possible and available areas of interest to future work we will single out here previews of coming attractions in gas exchange phenomena in the soybean leaf, as influenced by endophyte-modified C,N,P and water uptake.

The non-VAM comparison plant. The VAM growth effect is often thought of as a P effect. This is not surprising in view of the VAM-fungal contribution to host P nutrition (4), the scarcity of available P in most soils (10) and the evolutionary importance of phosphates in biochemical transformations (16). But is the supply of P the only, or even the major VAM-fungal effect on the host, as the little plant-big plant comparison would indicate? And is the higher P concentration in VAM-plant tissues generally observed in comparison to those of non-VAM plants of the same size (15) a necessary expression of the symbiotic condition per se, or an artifact of differences in ontogenetic development, of the timing, kind, and quantity of P supplements for the non-VAM comparison plant, or of the efficacy of the fungus as a source of P and sink for C? An answer could be learned from the "construction" of nutrient-supplemented non-VAM plants equivalent in all respects to VAM plants. Unfortunately, such equivalence may

not be possible to achieve (and equally difficult, in practice, to duplicate from time to time), since different aspects of the host's form and function react differently to VAM colonization or fertilization (12). This makes the interpretation of VAM effects problematic. If P levels in VAM-plant tissues are higher than in non-VAM counterparts of the same size and age, we call the effect nutritional; if P levels are lower, other, little known mechanisms need to be invoked. In what follows, let us demonstrate both possibilities.

Water uptake. Three groups of VAM and non-VAM soybean plants, well watered for 21 d, were either exposed to cycles of severe or moderate drought or to no stress for a subsequent period of 28 d. The cycles were repeated by rewatering at soil water potentials of -1.0, -0.3 or -0.05 MPa. Plants were grown in a sand-soil mix severely deficient in nutrients and supplemented by one-quarter strength Hoagland's solution, less P. Non-VAM plants received P (KH_2PO_4) timed to coincide with 60% VAM root colonization at 21 d, in an amount producing growth equivalent to that of VAM plants in the treatments under moderate stress. All plants were taken to permanent wilting at harvest, at which time soil moisture content was determined (Bethlenfalvai et al. unpublished data).

In the absence of drought stress, dry weights of non-VAM plants were significantly greater than those of VAM plants, while VAM plants produced significant growth enhancement under severe stress, even though P levels in non-VAM plants were markedly higher in all three stress treatments (Table 1). These results indicated VAM effects independent of P nutrition, and were related to increases in the extraradical mycelium with increasing stress. Importantly, all VAM plants had lower soil moisture contents at permanent wilting, suggesting that VAM roots may have access to soil water which is not available to the plant alone.

Table I. Host, endophyte and soil parameters of VAM plants relative to non-VAM plants under drought stress.

Parameter ^a	Stress (MPa)		
	-1.0	-0.3	-0.05
	-----% change ^b -----		
Growth response	17.5	2.5	-15.9
P concentration	-36.9	-40.0	-45.0
Soil water content	-10.3	-6.0	-8.1
	-----m/g soil-----		
VAM hyphae in soil	10.4	8.8	7.0

^aGrowth response refers to total plant dry weights; P concentration to roots and leaves; soil water content is at permanent wilting (-1.5 MPa).

^bPercent change reflects difference between VAM and non-VAM parameters referred to the non-VAM control; minus signs indicate larger non-VAM parameter. All differences between VAM and non-VAM parameter means were significant at least at the 5% level.

Water-use efficiency and N_2 fixation. The relative importance of water uptake vs. P nutrition mediated by VAM fungus in determining the host growth response to VAM colonization is controversial (2,11). As pointed out above, stress, independently of P nutrition, may influence the growth response. This situation is particularly complex in the legume association, where water and P availability affect the activity of the N source as well (4). Thus, an entire chain of cause-effect relationships may be envisioned in which the activities of the C,N, and P sources (chloroplast, root nodule, VAM fungus) of

the tripartite symbiosis are involved, and which is to some extent controlled by moisture conditions. The availability of P_i is a function of soil water content (10). In the plant, P_i plays a regulatory role in photosynthesis (14) and is required in high concentrations for nodule activity (4). These processes are in turn interdependent: an increase in the products of one enhances the output of the other, thus forming an autocatalytic cycle (5). Some effects of the VAM fungus on these processes, which appear to be independent of the relative P concentrations in host- or non-VAM comparison-plant tissues, is illustrated below.

In a drought cycle experiment (6) similar to the one described above, unstressed non-VAM plants received soluble P to produce plants equal in dry weight to non-P supplemented VAM plants. In the absence of stress, these non-VAM plants fared better than their VAM comparisons in root P content (1.2 vs. 1.0** mg/g), nodule dry weight (0.32 vs. 0.23** mg) and nodule activity (9.6 vs. 6.0* $\mu\text{mol C}_2\text{H}_2/\text{h} \cdot \text{plant}$). Under stress, however, non-VAM plants fared worse in root P content (0.8 vs. 1.2* mg/g), and nodule weight (0.05 vs. 0.14* mg) and activity (1.7 vs. 9.4** $\mu\text{mol C}_2\text{H}_2/\text{h} \cdot \text{plant}$). Leaf conductance was significantly ($P < 0.05$) greater in the VAM than in non-VAM plants during the first 3 days of the final drought cycle, with values converging with increasing stress (Table 2).

Table 2. Leaf conductance in VAM and non-VAM soybean plants during a 7-day drought cycle.

Treatment	Time (d)						
	1	2	3	4	5	6	7
	-----s/cm-----						
VAM	0.91*	0.71*	0.50*	0.38	0.20	0.09	0.07
non-VAM	0.67	0.45	0.38	0.31	0.15	0.08	0.05

While the enhancement of nodule and leaf gas exchange may be ascribed here to the higher P concentrations of VAM plants under stress, another drought stress experiment yielded contrary results. Utilizing the same host variety and endophyte strain under similar growth conditions, the ratios of net CO_2 uptake vs. transpiration (water-use efficiency) were determined as a function of soil water content (Figure 1). Values for VAM plants were significantly higher than for non-VAM plants although leaf P concentrations of the former were significantly lower (1.1 vs. 2.0*** mg/g).

Nutrient-use efficiency. These apparent inconsistencies raise the question of VAM effects independent of those of mineral nutrition. Harley's concept of opposing effects (P gain vs. C loss, 8) on the host due to mycorrhizal colonization has been confirmed and expanded by Paul and coworkers (9) in a sequence of nutrient-balance experiments showing enhanced photosynthetic rates in symbiotic plants in response to increased sink demand. The roles of the three symbionts of the legume association as interdependent and interacting sources and sinks is well established (4). The question is therefore not whether our current, nutritional concept of the VAM effect is correct, but rather if it is not too restrictive. Our present data suggest a modification to the concept (7). We used four sets of plants: nodulated + VAM, VAM, nodulated, or non-symbiotic, grown with N and/or P supplements in the absence of one or both microsymbionts so as to produce the same dry weights ($P > 0.05$) in all four sets. The elimination of size and developmental differences

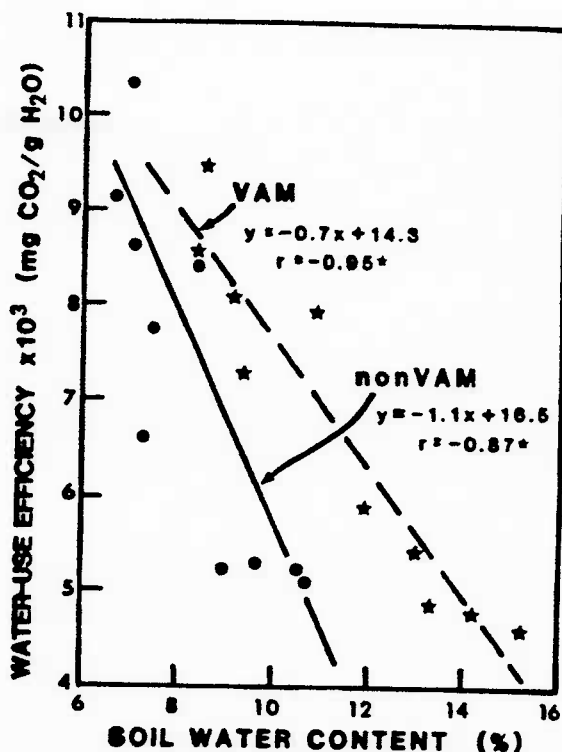


Figure 1. Water-use efficiency as a function of soil water content in VAM and non-VAM soybeans.

between treatments revealed that symbiotic plants had higher rates of photosynthesis than non-symbiotic plants (as expected) but at significantly lower N and/or P concentrations (Table 3).

Table 3. Photosynthetic N⁻ and P-use efficiency in VAM and/or nodulated (Nod) soybeans.

Parameter ¹	Treatment ²			
	VAM + Nod	VAM + N	Nod + P	N + P
Photosynthesis	18.4a	17.8ab	16.2bc	15.3c
N concentration	29.2a	32.9b	30.7a	40.6c
P concentration	1.3a	1.4a	2.0b	1.9b

¹Photosynthesis in $\mu\text{mole CO}_2/\text{s} \cdot \text{m}^2$, concentrations in mg/g leaf dry weight.

²Numbers not followed by the same letter are significantly different at the 5% level.

Concentrations of P in the leaf (Fig. 2) which are relevant here may be summarized in five steps: 1. Overall influx of P_i into the leaf is modified by the VAM fungus, 2. The vacuole serves as a sink for excess P_i in the cytosol, 3. Uptake of P_i by the chloroplast and output of fixed C as triose-phosphate (TP) are controlled stoichiometrically by the phosphate translocator, 4. Recycling of organic P within the chloroplast to starch and P_i keeps P_i available to photophosphorylation, and 5. Recycling of organic P in the cytosol as a result of sucrose export due to sink demand makes P_i available for re-entry into the chloroplast. Thus, VAM fungi are crucial in the regulation of CO_2 fixation by P_i (14) in step 1, and share a role with *Rhizobium* in step 5 through their effects on sink formation and activity throughout the symbiotic association. The other three steps provide some degree of stability to P_i concentrations in

stroma and cytosol (10,14) especially under conditions of P deficiency. In the present case, leaf P_i concentrations in VAM plants were subcritical (6), while in non-VAM plants they were low to sufficient. The same conditions held also for the relationship between nodulation and N concentrations. One may assume that under critical P deficiency the phosphate translocator determines the rate of CO_2 fixation, since the efflux of triose phosphate (TP) from the chloroplast is limited by an equal influx of P_i . Nonetheless, our critically P-deficient VAM plants fixed more CO_2 than their P-sufficient non-VAM counterparts. The problem may be resolved, if CO_2 is assumed to be the primary limiting factor. Better leaf conductance (through microsymbiont-mediated changes in water status, organic osmotica, ion fluxes and phytohormone levels) may provide the leaf additional access to CO_2 .

and N_2 fixation and the improvement of water-use and nutrient-use efficiency through a modification of plant water status and leaf gas exchange.

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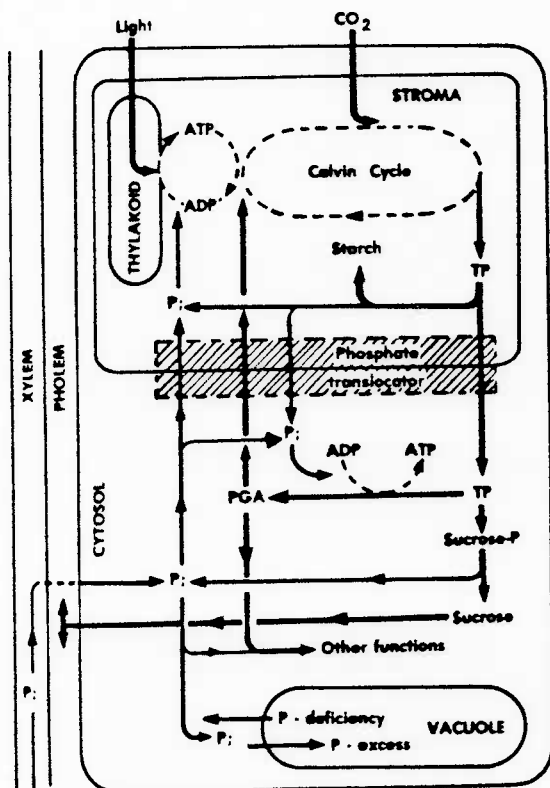


Fig. 2. Phosphorus fluxes in leaf cells.

Conclusions: The concept of cost-benefit relationships in plant symbioses recognizes the influence of changing conditions, such as nutrient or water availability, on the balance of supply and demand between the symbionts. Such changes may bring about temporary shifts in the symbiotic condition, causing it to alternate between the mutualistic and parasitic modes. The expression of these modes may be subtle and difficult to document due to their transience. The prevailing mode will be determined by environmental stimulation or inhibition of the contributions or requirements of each symbiont. The carbon-nitrogen-phosphorus supply/demand relationship in the tripartite legume association is a fundamental expression of the three symbionts' activities as sources or sinks for each other's products. We suggest that source-sink relationships may not be the only means by which the symbiotic partners interact. Examples are the stimulation of CO_2