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Defoliation effects on mycorrhizal colonization, nitrogen fixation and photosynthesis in the *Glycine-Glomus-Rhizobium* symbiosis

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Soybean [*Glycine max* (L.) Merr. cv. Wells] plants grown in a greenhouse were inoculated with *Rhizobium japonicum* strain 61A118 and the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* (Thaxt. *sensu* Gerd.) Gerd. & Trappe. Plants were defoliated (26, 48 and 66%) throughout the growth period and evaluated for VAM colonization, N_2 fixation and photosynthesis at harvest (six weeks). Photosynthate stress as a result of defoliation affected nodulation and nodule activity most severely. Colonization of the roots by the VAM fungus was little affected in comparison, and the intensity of colonization increased with increasing stress. The CO_2-exchange rate decreased less with defoliation than did leaf mass, and photosynthetic efficiency increased with the severity of defoliation. The increase in photosynthetic efficiency was significantly correlated with increases in leaf P ($r = 0.91$) and N ($r = 0.97$) concentrations. The results suggest that the VAM fungus should not be regarded as a simple P source and C sink in the tripartite legume association. Threeway source/sink relationships (VAM-P, Rhizobium-N, and host leaf-C) are discussed.

Additional key words – CO_2-exchange rate, hydrogen evolution, nitrogen content, phosphorus content, root nodule, soybean.

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Introduction

Observations that the mycorrhizal symbiosis may be parasitic as well as mutualistic indicate that the allocation of limiting resources is of consequence in intersymbiont activity and growth response (Bethlenfalvay et al. 1983, Buwalda and Goh 1982, Munns and Mosse 1980). When a tripartite legume association is grown in soil severely limiting in P and N, growth enhancement due to increased uptake of P by vesicular-arbuscular mycorrhizal (VAM) fungi and to fixation of atmospheric N in the root nodules is observed (Asai 1944). Both microsymbionts rely on the host plant for carbohydrates (Warembourg and Morrall 1978), the allocation of which to the endophytes has been determined in a few associations (Paul and Kucey 1981, Snellgrove et al. 1982, Warembourg et al. 1982). Experiments showing that carbohydrate limitation imposed on the legume symbiosis affects the development of the endophytes (Bethlenfalvay and Pacovsky 1983, Bethlenfalvay et al. 1978) pose the question of competition between the endophytes for reduced C (Bethlenfalvay et al. 1982).

The purpose of the present study was to investigate the effects of carbohydrate stress on the development and activity of the VAM fungal and rhizobial microsymbionts, as well as the ability of the host plant to react to stress by altering the rate of net CO_2 assimilation.

Abbreviations – CER, CO_2-exchange rate; PE, photosynthetic efficiency; PPFD, photosynthetic photon flux density; VAM, vesicular-arbuscular mycorrhizal.

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Materials and methods

Growth conditions

Soybean [*Glycine max* (L) Merr. cv Wells] plants were grown in 1.5 l white plastic pots in a greenhouse at Albany, CA during October and November 1983. Temperature and relative humidity varied from day to day within the day/night ranges 28/21°C and 60/67%, respectively. On sunny days photosynthetic photon flux density (PPFD) averaged 1000 μmol $m^{-2}s^{-1}$; the majority of the days during the experimental period were sunny. Day length was extended to 16 h by General Electric Multi-Vapor 1000-W metal halide lamps mounted vertically in parabolic reflectors and arranged to provide uniform supplementary PPFD of 500 μmol $m^{-2}s^{-1}$ at soil level. The growth medium consisted of a silty clay loam soil (Balcom series, from Yolo County, CA, USA) mixed 2:1 with sand and covered by a 2.5 cm layer of perlite. Plant-available ($NaHCO_3$-extractable) and total P of the soil were 3.3 and 750 μg (g soil)$^{-1}$, respectively. Soil pH was 8.0. Plants were watered with a nutrient solution (pH 7.0) consisteing of 1.5 m*M* $CaCl_2$, 0.5 m*M* K_2SO_4, 0.25 m*M* $MgSO_4$. Micronutrients (without Mn) were according to Johnson et al. (1957) at one-quarter strength.

Biological materials

Seeds were germinated for 3 days at 30°C. Seedlings were selected for uniformity and the surrounding soil was inoculated at planting with 7.8×10^8 cells of *Rhizobium japonicum* strain 61A118 and with the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* (Thaxt. sensu Gerd.) Gerd. and Trappe. The fungal inoculum consisted of 15 g soil containing approximately 25 spores and 375 root fragments partially (80%) infected by *G. fasciculatum*. Plants were subjected to one of four levels of defoliation: (1) no defoliation (control), (2) one leaflet of each trifoliate leaf excised, (3) two leaflets of each trifoliate excised, (4) one of the two unifoliate leaves and one leaflet of every second trifoliate left unexcised. Plants were harvested at six weeks after planting.

Assays

Nodule activity as measured by C_2H_4 accumulation (Minchin et al. 1983) and H_2 evolution (Skøt 1983) was determined as described previously (Bethlenfalvay et al. 1982). Ethylene analyses were made with a Varian Model 1400 gas chromatograph equipped with a flame ionization detector, using a 0.32 × 183 cm stainless steel column filled with 80–100 mesh Porapak N. Hydrogen evolution was measured with a Hewlett-Packard Model 5880 gas chromatograph equipped with a thermal conductivity detector, using a 0.32 × 183 cm column filled with 60–80 mesh molecular sieve 5A. Helium served as the carrier gas for ethylene, and N_2 for H_2, both at a rate of 30 ml min^{-1}. Oven temperatures were 85°C for ethylene and 50°C for H_2.

Intraradical VAM-fungal biomass was determined by the chitin assay of Bethlenfalvay et al. (1981). Percentage colonization of host-plant root length was estimated by visual observation of stained root segments using the gridline intercept method (Giovanetti and Mosse 1980).

The rate of photosynthesis was measured with a flow-through gas exchange apparatus similar in principle to the system described by Jarvis and Čatský (1971). The entire shoot was placed in an acrylic plastic chamber and illuminated with a 1000 w metal halide lamp (G. E. Multi-Vapor) that provided 700 μmol m^{-2} s^{-1} at the top of the plant. Heat from the lamp was absorbed by a 7.5 cm deep layer of water in an acrylic tank beneath the lamp to maintain the chamber air temperature at 30.8 ± 0.5°C, and the leaf temperature at 30.0 ± 0.5°C. Air containing 345–360 μl CO_2 l^{-1} was supplied to the chamber at a flow rate of 5 l min^{-1}. Relative humidity, measured with an E. G. & G. Inc. Model 911 Digital Humidity Analyzer, was 50 ± 4%. The CO_2 concentrations of air entering and leaving the chamber were measured with an infrared gas analyzer (Beckman Model 865), and the carbon dioxide exhange rate (CER) was calculated from the difference between these measurements. Photosynthetic efficiency (PE) was calculated as CER/leaf area. Plant N and P content were determined by standard analytical methods.

Results and discussion

Defoliation of soybean plants resulted in varied responses by the plant and endophytes (Tabs 1 and 2). Root and nodule dry weights, intraradical VAM-fungal biomass, CER/plant, and nodule activity declined with increasingly severe defoliation. The concentrations of N and P in both leaves and roots, the intensity of VAM colonization, as well as PE increased.

The evaluation of these findings is facilitated by comparing changes in plant and endophyte parameters due to defoliation with the undefoliated controls. The relative slopes of lines representing defoliation and its various consequences indicate the severity of carbohydrate stress on growth and functioning of the host plant and endophytes (Figs 1 and 2). Also, by relating these changes at each level of defoliation to the change in leaf area (a measure of photosynthate stress) the effects of the severity of defoliation are emphasized.

Similar reductions in leaf area and dry weight (Fig. 2) showed that defoliation did not change leaf density in the unexcised leaves. The CER response to defoliation was smaller than the percentage change in leaf area and declined uniformly over the range of defoliation (Fig. 1). Changes in the weight of roots from defoliated plants relative to the controls were smaller than changes in leaf weights (Fig. 2). This indicates that a proportionately larger share of the plant's resources was allocated to the roots at a high level of defoliation. Nodule

Tab. 1. Leaf parameters of nodulated mycorrhizal soybean plants grown in a soil deficient in N and P. Plants were subjected to different levels of defoliation. Numbers are means of 8 replications and are not significantly different ($P > 0.05$) by Duncan's multiple range test when followed by the same letter.

Leaf parameters	Defoliation			
	No	Light	Moderate	Severe
Leaf area (dm^2)	3.0a	2.3b	1.6c	1.0d
Leaf dry weight (g)	1.8a	1.3b	1.0c	0.6d
CO_2 exchange rate ($\mu mol\ CO_2\ plant^{-1}h^{-1}$)	741	584	448	320
Photosynthetic efficiency ($\mu mol\ CO_2 dm^{-2}h^{-1}$)	245	252ab	290bc	315c
Phosphorus content (%)	0.11a	0.13ab	0.14b	0.21c
Nitrogen content (%)	2.69a	3.18b	3.58b	4.13c

weight, on the other hand, declined much more than root weight at moderate levels of defoliation, but further reduction was not as severe at the greatest defoliation level. In contrast to the weight changes, nodule activity (H_2 evolution) remained relatively high at the lowest defoliation level, and only declined with greater amounts of defoliation (Fig. 1). These observations indicate a different pattern of response by the nodule to photosynthate stress in comparison to other plant parameters. At low levels of stress, nodule activity was maintained with little change at the expense of nodule development, and only at a high level of stress were both development and activity affected equally. Fungal biomass was affected less than leaf area, and its response to defoliation was essentially uniform over the range of defoliation studied (Fig. 2).

In the tripartite legume association, the responses of the symbionts to stress must be considered in the context of three-way source-sink relationships (source or sink strength = size × activity, Wareing and Patrick 1975). Defoliation not only reduces the C-source capacity of the host, but at the same time also reduces its sink size for N and P. The strengths of the N and P sources (nodules and the VAM fungus) did not decrease to the same extent as the main N and P sink (foliage). This was reflected in increased plant N and P and nodule P concentrations. Increasing leaf N and P concentrations with more severe levels of defoliation were significantly correlated with increases in PE (N, r = 0.97; P, r = 0.91). Due to this interaction between the rate of C assimilation and leaf N and P status (Herold and Walker 1979, Raper et al. 1977), PE may be regarded as a measure of both C-source activity and N- and P-sink activity.

Defoliation thus affected leaf sink strength directly by decreasing its size and indirectly by increasing its activity. Likewise, the C-sink size of the fungal symbiont decreased but its activity (as measured by the increase in colonization intensity) increased. As this decrease in size was relatively small (Tab. 2), sink strength of the fungal symbiont decreased least among the symbiotic partners. As a P source, the fungal symbiont increased leaf-P content by almost 100% in the severely defoliated plants relative to the controls (Tab. 1). As the extraradical VAM-fungal mycelium could not be measured by the chitin assay (Pacovsky and Bethlenfalvay 1982) in this soil, fungal effects on the association must be inferred from the intraradical mycelium. The comparable increase in leaf-N content was only 53%. This difference was reflected in the larger decrease in N-source strength (nodule weight and activity) relative to P-source strength. Nitrogen fixation, which has a high P requirement, was not limited by P availability. This was shown by the 43% increase in nodule P content in the severely defoliated plants (Tab. 2). Although photosynthesis is

Tab. 2. Root parameters of nodulated, vesicular-arbuscular mycorrhizal (VAM) soybean plants grown in a soil deficient in N and P. Plants were subjected to different levels of defoliation. Values are means of 8 replications, and are not significantly different by Duncan's multiple range test when followed by the same letter.

Root parameter	Defoliation			
	No	Light	Moderate	Severe
Root dry weight (g)	1.7a±0.1	1.3b ±0.1	1.2b ±0.1	0.8c ±0.1
Nodule dry weight (mg)	59.8a±3.9	37.5ab±3.1	28.4bc±4.3	21.9c±3.3
C_2H_4 accumulation ($\mu mol\ plant^{-1}\ h^{-1}$)	0.65a±0.11	0.53ab±0.14	0.43b±0.10	0.27c±0.05
H_2 evolution ($\mu mol\ plant^{-1}\ h^{-1}$)	0.56a±0.13	0.53a ±0.19	0.41a±0.08	0.19b±0.03
VAM fungal biomass (mg $plant^{-1}$)	92.2a±8.4	78.5a ±3.8	74.9ab±6.5	62.9b±3.5
VAM fungal biomass (% of VAM root)	5.5a±0.4	6.1ab±0.3	6.1ab±0.9	7.0b±0.3
Root P content (%)	0.10a	0.11ab	0.11ab	0.12b
Root N content (%)	1.64a	1.60a	1.77b	1.89c
Nodule P content (%)	0.31a	0.35ab	0.33ab	0.44b

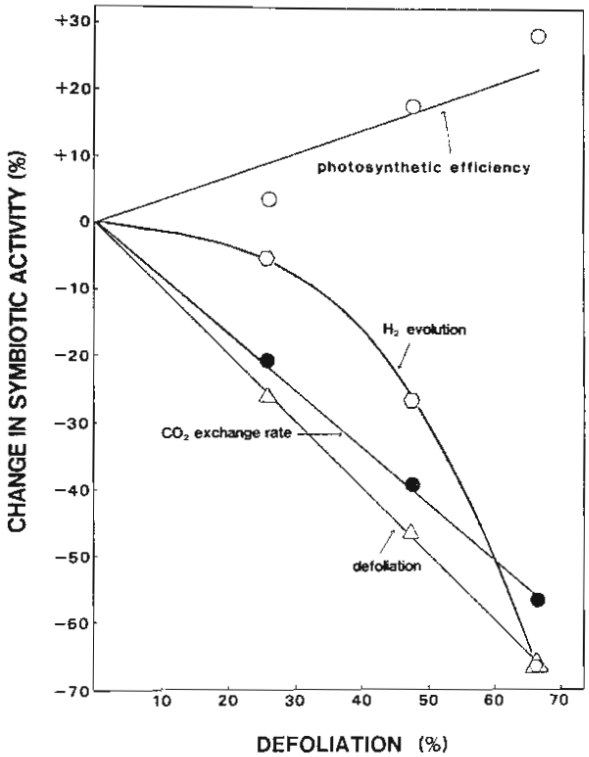

Fig. 1. Changes in symbiotic activity (photosynthesis, H_2 evolution) as a function of defoliation. Values represent changes relative to those of the undefoliated controls. Absolute values of controls: see tables.

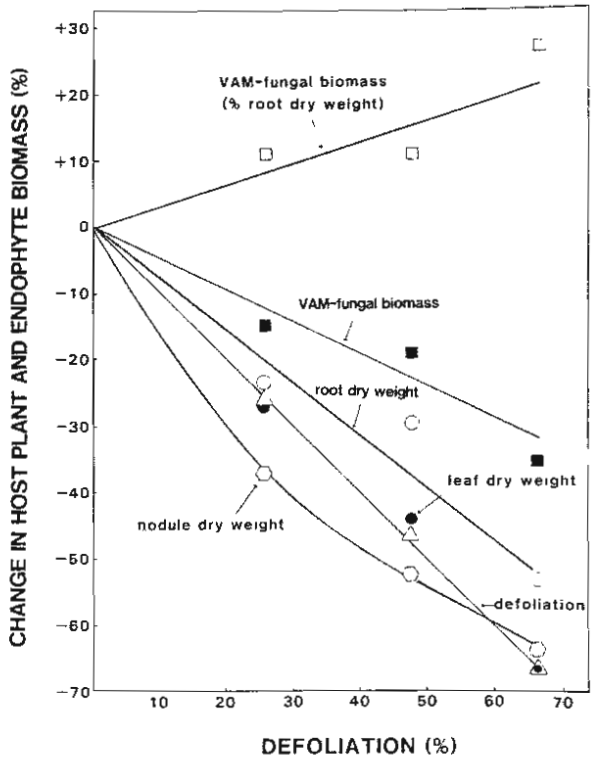

Fig. 2. Changes in host-plant and endophyte biomass as a function of defoliation. Values represent changes relative to those of the undefoliated controls. Absolute values of controls: see tables.

regarded as having excess source potential (Herold 1980, Paul and Kucey 1981), the levels of stress imposed by this experiment (similar in severity to some agricultural practices, such as grazing or mowing) exceeded the capability of the C source to respond to all demands. Nodules were the least effective competitors as shown by their low activity and poor development.

The increase in the intensity of VAM-fungal colonization in spite of decreased C availability from the leaves and P demand by the entire association is unexplained. Apparently, the VAM fungus-host plant relationship cannot be explained satisfactorily by treating the fungus as a simple P-source and C sink, thus supporting a previous observation (Bethlenfalvay and Pacovsky 1983). It is conceivable that fungal proliferation is encouraged as a survival mechanism under carbohydrate stress. Under field conditions, both increases (M. F. Allen, personal communication) and decreases (Bethlenfalvay and Dakessian 1984) in VAM fungal colonization have been noted in plants under severe grazing stress.

The results show that carbohydrate stress has differential effects on VAM colonization and nodulation in soybeans. The fungal endophyte responded uniformly to increasing defoliation, and may have competed increasingly for carbohydrates with the root nodules and other host tissues as photosynthetic products became more limiting, so that the severe inhibition of nodulation and nodule activity may be partially attributed to the presence of the VAM fungus.

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