RELATIONSHIPS BETWEEN HOST AND ENDOPHYTE DEVELOPMENT IN MYCORRHIZAL SOYBEANS

BY G. J. BETHLENFALVAY, M. S. BROWN, AND R. S. PACOVSKY

Western Regional Research Center, U.S. Department of Agriculture, Science and Education Administration, Berkeley, California 94710, U.S.A.

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SUMMARY

Symbiotic associations of soybeans and the vesicular–arbuscular mycorrhizal fungus *Glomus fasciculatus* were grown to maturity in a sand–perlite rooting medium watered with a nutrient solution containing growth-limiting amounts of soluble phosphorus. Development of fungal mycelia external and internal to the host plant's root system was measured by determining chitin in isolated fungal mycelium, in the rooting medium, and in the mycorrhizae. The biomasses of the extra- and intraradical mycelia were calculated from the values of chitin obtained spectrophotometrically. The amount of total fungal biomass relative to that of the host plant varied throughout the lifespan of the association and reached a maximum of 2·3 % 10 weeks after planting. The amount of intraradical mycelium increased throughout the host plant's life span. Extraradical fungal structures attained a maximum weight at the onset of logarithmic growth of the soybean pods and decreased thereafter. Cessation of the rapid growth phase of *G. fasciculatus* lagged behind that of the host plant's vegetative structures, but appeared to be related to pod development. The fungus to root dry wt ratio was 12·3 % at senescence. The ratio of extra- to intraradical mycelium decreased throughout the association's lifespan. Since the extraradical hyphae are the organs responsible for enhanced nutrient uptake this ratio is proposed as an index of the endophyte's usefulness to the host. High values for this usefulness index coincided with significant growth enhancement of the host plant. Source–sink relationships in the host appear to be a determining factor in the growth of the fungal endophyte.

INTRODUCTION

Infection of plant roots by vesicular–arbuscular mycorrhizal (VAM) fungi is associated with enhanced mineral uptake and growth when the supply of relatively immobile nutrient ions is limiting (Saunders and Tinker, 1973). When P is provided in soluble form, growth benefit to the host plant is reduced (Murdock, Jackobs and Gerdemann, 1967; Daft and Nicholson, 1969; Powell and Daniel, 1978), and an excess of readily available P may result in parasitic growth of VAM fungi to the detriment of the host plant (Hayman, 1980; Tinker, 1975; Smith, 1980). The amount of fungal material within the host root may be determined by a number of methods (Becker and Gerdemann, 1977; Hepper, 1977; Weijmann and Meuzelaar, 1979; Giovanetti and Mosse, 1980), and it is customarily used as a measure of the degree of infection. However, the intraradical component of the infecting fungus may include a proportionately large number of storage vesicles (Gerdemann, 1968) which do not contribute to host plant nutrition. Therefore it may not be directly related to host plant response to the endophyte. On the other hand, the extraradical component, with the exception of spores, is essential to growth enhancement, since it is responsible for increased mineral uptake

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(Pearson and Tinker, 1975; Rhodes and Gerdemann, 1978). The direction of the growth response may therefore vary with the proportion of extra- to intraradical fungal components. Thus, a negative growth response by the host, or parasitism of the fungus, might result from extensive intraradical fungal growth in the absence of sufficient extraradical mycelium.

The useful contribution of VAM fungi to the symbiotic, plant–fungus association lies in the ability of fungal hyphae to permeate a soil volume far more densely than roots can alone (Hattingh, Gray and Gerdemann, 1973; Rhodes and Gerdemann, 1975). These fungi take up and translocate to the roots relatively immobile nutrients, which would otherwise not be available to the host plant (Sanders and Tinker, 1973). It is therefore axiomatic that the extent and development of the extraradical mycelium, the ion-uptake organ in direct contact with the soil, should be of crucial importance to the association (Sanders *et al.*, 1977). Yet, we are aware of no study which considers the extraradical along with intraradical VAM mycelium as the measure of useful fungal contributions to host nutrition. The objective of this study was to measure the entire mycelium of a VAM fungus, to relate the development of its extra- and intraradical components to that of the host plant, and to determine if a functional relationship between these components could serve as a satisfactory index of endophyte usefulness to the host.

MATERIALS AND METHODS

Soybean [*Glycine max* (L.) Merr. cv. Kent] plants were inoculated with the VAM fungus *Glomus fasciculatus* (Thaxt. *sensu* Gerd.) Gerd. and Trappe (Gerdemann and Trappe, 1974) or left uninoculated as controls. Plants were grown in a greenhouse at Berkeley, California, U.S.A., July to September 1980, under conditions as described previously (Bethlenfalvay, Pacovsky and Brown, 1981). Nutrient solution 50 μM in KH_2PO_4 was the only source of P available to the association in the sand–perlite rooting medium. Five replicates of VAM and control plants were harvested at 2-week intervals. Rooting media from VAM and control plants were collected and dried for 2 days at 90 °C. Mycorrhizae (VAM plants) and roots (control plants) were thoroughly washed over a sieve (43 μm mesh size). Washings were returned to the rooting media. Mycorrhizae, roots, shoots, plant reproductive structures and extraradical mycelium (collected from additional plants grown under the same conditions) were dried for 1 day at 80 °C. The chitin (poly-β-1,4-*N*-acetylglucosamine) content of samples of rooting medium, of the extraradical mycelium, of the mycorrhizae and of the roots were determined as described previously (Bethlenfalvay *et al.*, 1981). For the calculation of chitin in the rooting medium, a standard curve was constructed by adding purified chitin to nutrient-solution-washed rooting medium. The dry rooting media of VAM and control plants were thoroughly stirred to achieve a uniform distribution of hyphal fragments and spores. Both were considered to be part of the extraradical mycelium and were not separated. Duplicate, 40 cm^3 samples taken from the stirred medium were mixed with 30 ml of concentrated KOH and processed for chitin analysis. To account for any chitin-positive contamination due to sources other than *G. fasciculatus*, the average absorbance from five replications of control-plant rooting medium was subtracted from the absorbance value obtained from each replicate of VAM plant rooting medium.

Extra- and intraradical fungal biomass per plant was calculated from the chitin content of measured amounts of extraradical mycelium, from the chitin content

of the total rooting medium (1250 cm^3), or from the total dry wts of the mycorrhizae, respectively. Total plant P content was determined colorimetrically by the molybdenum blue method after digestion with perchloric acid (Allen, 1940). Means and standard errors were calculated from five replicates.

Results and Discussion

We measured the development of the extra- and intraradical mycelium of *G. fasciculatus* during soybean ontogeny. The plants which were stressed by a suboptimal (Bethlenfalvay and Yoder, 1981) P regime started flowering without an inductive photoperiod during the sixth week after planting. The shoots senesced, and ripe seed was produced by week 14 (Fig. 1). The biomass of all plant vegetative structures was maximum at 10 weeks after planting, which coincided with early logarithmic growth by the reproductive structures. Dry wts of shoots (Table 1) and roots (Table 2) decreased slightly after week 10, presumably as a result of nutrient export from vegetative organs to the developing pods (Sinclair and de Wit, 1976). Decline in dry wt of host–plant roots (mycorrhizae less intraradical mycelium) during the last 4 weeks of the experiment was 13 % while that of the extraradical mycelium was 29 % (Table 2).

Table 1. *Dry wt and total phosphorus (P) in soybean plant parts inoculated (VAM) or not inoculated (control) with* Glomus fasciculatus†

Time after planting (weeks)	Treatment	Shoot‡		Pod§	
		Dry wt (g)	P (mg)	Dry wt (g)	P (mg)
4	VAM	1·26 ± 0·12*	1·26 ± 0·10*	—	—
	Control	1·01 ± 0·10	1·05 ± 0·06	—	—
6	VAM	2·64 ± 0·43*	2·30 ± 0·06	—	—
	Control	2·17 ± 0·20	2·13 ± 0·17	—	—
8	VAM	4·20 ± 0·50	4·21 ± 0·86	0·17 ± 0·09	0·53 ± 0·28
	Control	4·01 ± 0·40	4·89 ± 0·85	0·24 ± 0·09	0·75 ± 0·34
10	VAM	5·50 ± 0·61	4·32 ± 0·88	2·69 ± 0·40*	5·62 ± 0·72*
	Control	5·89 ± 0·52	4·86 ± 0·52	3·39 ± 0·22	7·16 ± 0·78
12	VAM	5·02 ± 0·61	3·31 ± 0·28	6·87 ± 0·57	14·00 ± 0·98*
	Control	5·53 ± 0·88	3·19 ± 0·25	6·71 ± 1·33	12·79 ± 0·68
14	VAM	—	—	5·17 ± 0·47	15·93 ± 0·77*
	Control	—	—	5·21 ± 0·55	14·74 ± 0·51

* Indicates significant differences ($P = 0·05$) between corresponding VAM and control figures.
† Numbers are means and standard deviations of five replications.
‡ Shoots were senesced at week 14 and were not weighed, as most leaves had abscised.
§ Numbers at week 14 are weight and P content of the mature seeds only.

Maximum development of *G. fasciculatus* lagged behind that of the host's vegetative structures and was not attained until week 12 (Fig. 1). The inflection point in the growth curve describing total endophyte development was at approximately 9 weeks after planting. The decline in the endophyte's rate of growth from this time on coincided with the accelerated rate of pod growth (Fig. 1). Similar sink effects by newly-developing plant reproductive structures on another symbiotic association (root nodules) have been noted (Bethlenfalvay and Phillips, 1977a, b). Development of the reproductive phase in soybeans has been

Table 2. *Dry wt of soybean roots inoculated (VAM) or not inoculated with* Glomus fasciculatus†

Time after planting (weeks)	Treatment	Dry wt		
			Fungus (mg)	
		Root‡ (g)	Extraradical	Intraradical
4	VAM	0·7±0·1	14·1±1·2	1·9±0·9
	Control	0·6±0·1		
6	VAM	1·1±0·2	48·1±4·4	18·1±2·3
	Control	1·4±0·2		
8	VAM	2·3±0·3	65·8±6·5	45·9±4·2
	Control	2·0±0·3		
10	VAM	2·3±0·2	108·3±5·2	129·6±8·4
	Control	2·4±0·1		
12	VAM	2·1±0·2	96·5±7·0	152·8±13·6
	Control	2·2±0·4		
14	VAM	2·0±0·1	76·8±3·7	174·6±6·9
	Control	2·0±0·1		

† Numbers are means and standard deviations of five replications.
‡ VAM–plant root weights do not include the fungal component.

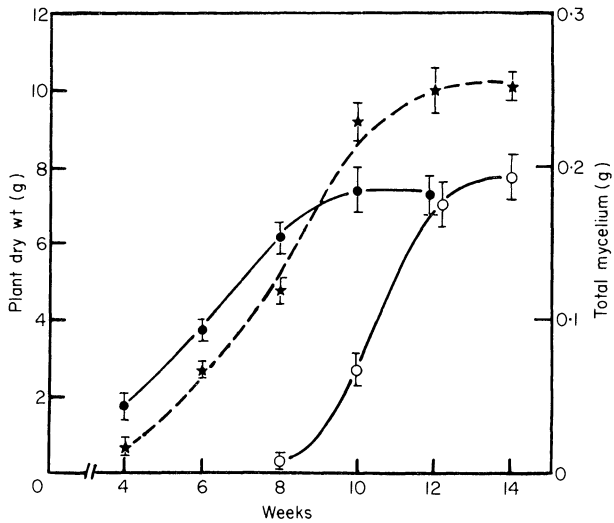

Fig. 1. Development of soybean plant reproductive and vegetative structures, and of the extra- and intraradical mycelium of *Glomus fasciculatus*. Shoots at week 14 yielded no meaningful measurement as most leaves had abscised. ★, Mycelium; ●, plant vegetative structures; ○, plant reproductive structures.

described as a self-destructive phenomenon (Sinclair and de Wit, 1976). Cessation of total fungal growth (Fig. 1), and declines in the extraradical mycelium (Fig. 2 and Table 2) and in the ratio of fungus to host plant biomass (Fig. 3) indicated that the endophyte, like the host's vegetative organs, was subject to destructive sink demands by the developing seeds.

Weights of all fungal structures were calculated from the chitin content of

extraradical mycelium collected at weeks 9 and 12. Chitin content of the extraradical mycelium was 88·5 μg chitin mg^{-1} fungal dry wt. This was equivalent to 32·9 μg glucosamine mg^{-1} fungal dry wt (Bethlenfalvay *et al.*, 1981), in general agreement with Hepper (1977). Development of the extraradical mycelium in terms of biomass was significantly ($P < 0{\cdot}05$) greater than that of the intraradical mycelium up to 8 weeks after planting (Fig. 2 and Table 2). The decline in the extraradical mycelium, and continued increase in the intraradical mycelium after week 10 (Table 2), may be due to resorption of external hyphae and continued production of vesicles in the root cortex at the expense of other fungal organs. A quantitative analysis for different fungal structures in terms of chitin content was not feasible (Whipps and Lewis, 1980), as it would have required physical separation of these structures. However, a proliferation of vesicles with increasing age of the association was observed qualitatively in stained root sections. Objections to the use of extraradical mycelium to calculate total fungal biomass, which is based on the assumption that the chitin content of all fungal structures is invariant with morphology, age and growth conditions, were summarized by Hepper (1977).

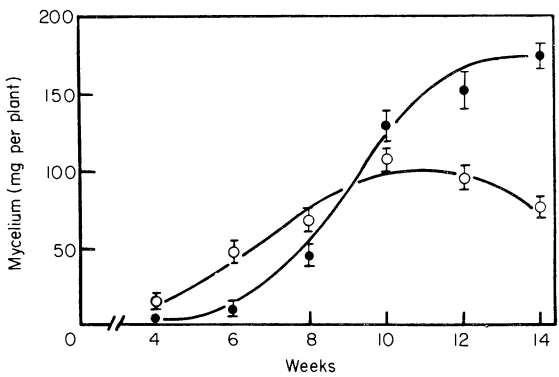

Fig. 2. Growth of the extra- (○) and intraradical (●) mycelia of *Glomus fasciculatus* during soybean plant ontogeny.

If benefits derived by the host from its endophyte depend on an extensive development of the extraradical fungal structures, the ratio of extraradical to intraradical mycelium (Fig. 3) may serve as an index of the endophyte's usefulness to the association. The high ratios at weeks 4 and 6 corresponded to significantly ($P < 0{\cdot}05$) higher shoot dry wt in VAM than in control plants (Table 1). At later stages VAM and control plant dry wts were not significantly different. This was reflected by low values of this index. Pod dry wt at week 10 was significantly higher in the controls than in VAM plants (Table 1), when fungal biomass was maximum (2·3 %) with respect to host plant dry wt (Fig. 3). This may have reflected sink demand on the host by the endophyte (Tinker, 1975). The proportion of fungal material in the mycorrhizae varied with plant age (Fig. 3) and was within the range found by Hepper (1977) for intraradical and by Sanders *et al.* (1977) for extraradical mycelia in associations made up of symbionts of species different from those used in this study. The significantly ($P < 0{\cdot}05$) higher P content in the pods (week 12) and seeds (week 14) of VAM plants as compared to the controls (Table 1) suggests a reallocation of P to the plant's reproductive structures from the entire association, including the endophyte.

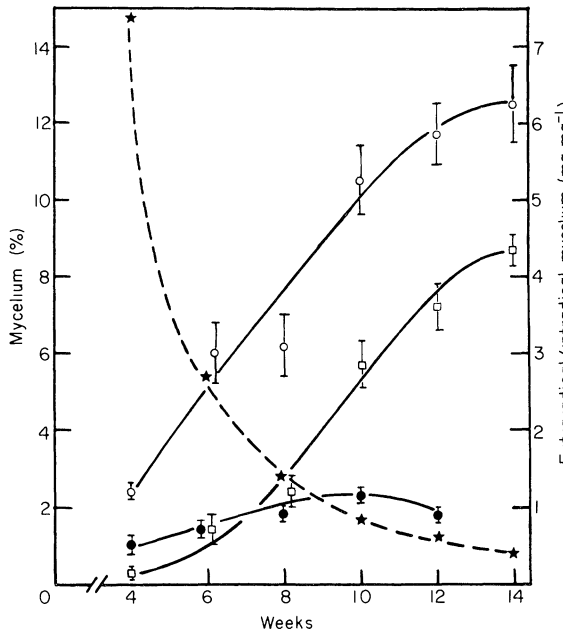

Fig. 3. Relationship of host and endophyte biomass in the soybean–*Glomus fasciculatus* association. Values represent fungal dry wt as a percentage of total association dry wt, or of the dry wt of the roots. The usefulness index, E/I (★), was computed as the ratio of the biomasses of extraradical to intraradical mycelium. ○, Total mycelium/root; □, intraradical mycelium/root; ●, total mycelium/plant.

CONCLUSIONS

These results indicate that structures of *G. fasciculatus* external or internal to soybean roots develop at different rates and are affected differently by host plant development. Total production of fungal biomass may be dependent on source–sink relationships in the host. Seed development has a similar inhibitory effect on the endophyte and on the vegetative structures of the host. Under the conditions of this experiment the intraradical mycelium did not accurately reflect host growth enhancement. Measurement of the extraradical mycelium, a relatively simple process in an inert rooting medium, may be difficult in many soils where high levels of interfering substances could mask the measurement of VAM fungal chitin. Use of the extraradical mycelium in evaluating VAM fungal effects by the chitin assay may therefore be of limited value in field applications, especially as it involves availability of non-VAM controls. However, an assessment of the extraradical mycelium is essential to a basic understanding of the endophyte's contribution to the association (Sanders *et al.*, 1977). Such an assessment is relatively easy in artificial rooting media, whose use is justified, since it simplifies little-understood biotic (Hubbell and Gaskins, 1980; Ross, 1980) and chemical (Bowen and Bevege, 1976) interactions in the rhizosphere.

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