DEVELOPMENT OF EXTERNAL HYPHAE BY DIFFERENT ISOLATES OF MYCORRHIZAL *GLOMUS* SPP. IN RELATION TO ROOT COLONIZATION AND GROWTH OF TROYER CITRANGE

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SUMMARY

The hypothesis was tested that the amount of external hyphae of a vesicular–arbuscular mycorrhizal (VAM) fungus extending from roots out into soil is not always proportional to the extent of colonization of the root cortex. Growth enhancement and amount of external hyphae were compared for eight isolates of five *Glomus* spp. that differed in their geographic origin and capacity to enhance growth of Troyer citrange, but were similar in their capacity to extensively colonize Troyer citrange roots. In general, isolates from California increased growth in a P-deficient (9·8 mg kg^{-1}) California soil more than did non-native isolates from Florida soils. The difference between the capacity of California and Florida isolates to enhance growth was not a function of the degree to which they colonized the roots since all had colonized over 95 % of the root length by the time of harvest. Differences in growth enhancement did appear, however, to be a function of the amount of external hyphae that had developed as estimated by the weight of soil they had bound into aggregates. This study suggests that isolates of VA mycorrhizal fungi may differ in their capacity to develop an external hyphal system independent of their capacity to colonize the root cortex, and that we cannot assume that high levels of colonization will necessarily mean the fungus has also developed the mycelium in the soil necessary to transport nutrients responsible for plant growth enhancement.

INTRODUCTION

Citrus, which has a magnolioid root system with poorly developed root hairs (Baylis, 1970), is considered to be highly dependent on vesicular–arbuscular mycorrhizae (VAM) for uptake of phosphorus (P) in soils low in available P (Menge, Johnson and Platt, 1978; Nemec, 1978). For citrus the magnitude of growth response produced by VAM is referred to as mycorrhizal dependency which is determined in part by the efficiency of the fungus. Mycorrhizal dependency may vary with cultivar, soil factors and mycorrhizal fungus (Menge *et al.*, 1978, 1982; Nemec, 1978). In a test of six citrus cultivars, Nemec (1978) found that differences in efficiency of three *Glomus* species varied with both rootstock and phosphorus fertilization. Studies by Mosse (1972a, b) on onion and *Paspalum notatum* demonstrated that potential for growth enhancement by VAM fungi may also vary with soil type. She recognized that the mycorrhizal system is a link between root and soil and judged that the maximum benefit of mycorrhizal fungi,

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with their wide host range, may be determined more by the development of the fungus in soil than by the fungus–host compatibility.

In examining the role of mycorrhizal hyphae in absorption of phosphate, Sanders and Tinker (1971) concluded that while hyphae act to increase the absorptive surface area, the distribution of hyphae beyond the zone of nutrient depletion is crucial if mycorrhizae are to be effective in nutrient uptake. Thus, the development of the soil hyphal network appears to be an important factor determining the capacity of VAM to enhance growth, but the determination of hyphal length and distribution in soil is difficult (Tinker, 1975). Sanders *et al.* (1977) sieved hyphae out of soil and found that the amount of external hyphae was proportional to the length of infected onion root and to growth enhancement for the four VAM fungi examined. However, Mosse (1972b) showed that growth response and percentage infection in onion roots were not always correlated for some of the mycorrhizal fungi she examined, which suggests that the relationship between root colonization and development of external hyphae may vary depending on the fungal endophyte.

The relationships between the percentage of root length colonized by VAM fungi, the amount and distribution of their external hyphae, and host growth response needs clarification. We, and others, have frequently observed and measured extensive root colonization by VAM fungi without concomitant growth enhancement. Accordingly, we tested the hypothesis that the extent of external mycelium is not always proportional to the extent of root colonization and that the amount of external mycelium in a soil may be a more meaningful explanation for why a VAM fungus isolate did or did not enhance growth of plants grown in that soil than the more common explanation of strain differences based on root colonization capacity. In this study we compared root colonization, growth enhancement, and production of external mycelium resulting from inoculation of Troyer citrange with eight different isolates of *Glomus* spp., isolated from citrus from either Florida or California, which were known (J. A. Menge, unpublished data) to enhance growth of Troyer citrange in California soils to different extents, even though their capacity to colonize roots was the same.

MATERIALS AND METHODS

Seed of Troyer citrange [*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osbeck] was germinated in autoclaved sand and seedlings watered daily with 14% Hoagland's solution minus P (Hoagland and Arnon, 1939). After 2 months, seedlings of uniform size were transplanted into 500 cm^3 clay pots containing autoclaved sandy loam soil (Menge *et al.*, 1978) with a pH of 7·3 and 9·8 mg kg^{-1} available phosphorus as determined by Olsen analysis (Chapman and Pratt, 1961). Ten grams of pot culture inoculum of each of eight isolates of five species of *Glomus* (Table 1) were placed 2 cm below the roots of each of 10 transplanted seedlings. The soil inoculum contained a mixture of 50 to 200 chlamydospores g^{-1} soil depending on the isolate (Table 1), as well as mycorrhizal roots of sudangrass (*Sorghum vulgare* Pers.). Ten uninoculated seedlings received an inoculum water extract from a 10 g composite sample containing 1·25 g of inoculum of each isolate. The extract was prepared by leaching the inoculum on a 38 μm sieve to exclude mycorrhizal propagules. Seedlings were grown in the glasshouse under a maximum light intensity of 1000 μmol M^{-2} s^{-1} at 400 to 700 nm and 29/22 °C day–night temperatures, and were watered as needed with 14% Hoagland's solution minus P.

The technique of Sutton and Sheppard (1976) was modified to estimate soil attachment to roots by mycorrhizal fungus hyphae. Four months after inoculation, plants were harvested by carefully removing the root–soil mass from the pots without disturbing the soil core. Plant tops were removed and their fresh v.t determined. The soil mass was air-dried in the dark at 25 to 27 °C for 48 h, then the roots were vigorously shaken to remove air-dry soil that was not firmly attached. The tightly adhering soil that remained was washed from the roots by plunging the root system into a beaker of water several times. The roots were blotted dry and their fresh wt determined. The soil washed from the roots was allowed to settle out in the beaker, the water was decanted and the soil residue dried and weighed. The amount of soil adhering to roots was expressed as the amount of dry soil attached in milligrams per gram fresh wt of root.

Mycorrhiza formation was assessed for 10 plants per treatment by sampling a cross-section of the root mass, and clearing and staining the sample in KOH and trypan blue-lactophenol (Phillips and Hayman, 1970). The stained root segments were randomly distributed under a grid of 1-mm^2 divisions and examined for the presence or absence of mycorrhizal arbuscules, vesicles, hyphae, and spores in 100 1 mm^2 sections of root tissue.

Results

Four months after inoculation of Troyer citrange, root colonization by *Glomus* was at least 95% for all the isolates examined (Table 2). As a result of VAM formation, growth was significantly ($P = 0{\cdot}01$) increased compared to the uninoculated control plants which were markedly stunted and chlorotic. In general, growth enhancement was significantly greater for *Glomus* isolates from California (92, 0–1, 329, 66; see Table 1) than for Florida isolates (600, 619, 624) even though most of the comparisons were for the same species (*G. fasciculatus* 92 and 0–1 *v.* 624, *G. macrocarpus* 329 *v.* 619). Isolate 474 of *G. fasciculatus* was an exception in that it was the poorest in terms of growth enchancement of all the fungi examined. Excluding isolate 474, *G. fasciculatus* isolates were the most efficient among both California and Florida fungi and isolates of *G. macrocarpus* the least efficient.

Table 1. *Identification and origin of* Glomus *isolates*

Fungus species	Isolate identification no.	Origin	Host	Inoculum spore count* (spores g^{-1} soil)
Glomus constrictus Trappe	66	California	Citrus	100
G. etunicatus Becker & Gerd.	600	Florida	Citrus	100
G. fasciculatus (Thaxter) Gerd. & Trappe	92	California	Citrus	100
G. fasciculatus†	0–1	California	Citrus	200
G. fasciculatus	474	California	Citrus	50
G. fasciculatus	624	Florida	Citrus	200
G. macrocarpus (Tul. & Tul.) Gerd. & Trappe	329	California	Citrus	50
G. macrocarpus	619	Florida	Citrus	200

* Soil inoculum of each isolate was obtained from pot cultures of sudangrass (*Sorghum vulgare Pers.*).
† Isolate no. 0-1 to be described as *G. deserticola*.

To determine whether the observed differences in *Glomus* isolate efficiency were related to development of external hyphae, the amount of soil attached to roots inoculated with different isolates was compared. Examination of mycorrhizal and non-mycorrhizal roots confirmed that hyphae of the mycorrhizal fungus were responsible for soil attachment to roots [Fig. 1(a) and (b)]. There was little or no

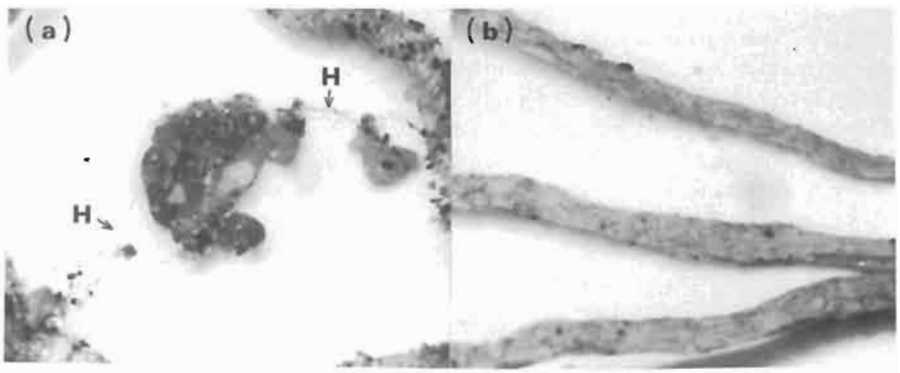

Fig. 1. Development of soil hyphae outside of mycorrhizal and non-mycorrhizal roots of Troyer citrange. (a) Attachment of soil particles to mycorrhizal roots by hyphae (H) of *Glomus fasciculatus* 0–1. × 12. (b) Absence of attached soil particles on non-mycorrhizal roots. × 12.

Table 2. *Vesicular-arbuscular mycorrhiza (VAM) formation, plant growth, and amount of soil attached to roots of Troyer citrange inoculated with isolates of* Glomus *from California (C) and Florida (F)*

Treatment	VAM formation (%)	Plant fresh wt (g)			Dry wt attached soil per fresh root (mg g^{-1})
		Total	Shoot	Root	
Uninoculated	0a*	2·48a*	0·99a*	1·49a*	42a*
Glomus etunicatus 600 (F)	95b	7·56b	4·68b	2·88b	62a
G. macrocarpus 619 (F)	98b	6·79b	4·11b	2·68b	75a
G. fasciculatus 624 (F)	98b	8·76bc	5·30bc	3·45b	68a
G. fasciculatus 474 (C)	95b	6·30b	3·67b	2.60b	53a
G. fasciculatus 92 (C)	99b	15·34e	10·00e	5·34e	130b
G. fasciculatus 0–1 (C)	98b	15·73e	10·93e	4·79de	152b
G. macrocarpus 329 (C)	98b	10·35cd	6·76cd	3.59bc	134b
G. constrictus 66 (C)	96b	11·79d	7·71d	4·08cd	146b

* Values are the mean of 10 replications. Column means followed by the same letter are not significantly different at the $P = 0.01$ level according to Duncan's multiple range test.

soil attached to roots of non-mycorrhizal roots (Mosse, 1959) inoculated with a combined soil extract of mycorrhizal inocula. Our observations support those of Sutton and Sheppard (1976), who found that *Glomus* hyphae were primarily responsible for binding of sand to bean roots growing in sand-dune soil.

Soil attachment to roots by external hyphae of VAM fungus was significantly greater for California *Glomus* isolates (except isolate 474) than Florida isolates, even though all isolates colonized over 95% of the root length (Table 2). Further, California isolates, except isolate 474, also enhanced growth of Troyer citrange

more than did Florida isolates. Thus, growth enhancement appeared to be directly related to the amount of external mycelium as estimated by weighing soil attached to the roots. Florida isolates and California isolate 474 also increased plant growth (though to a lesser extent than most California isolates), compared to non-inoculated controls, but attachment of soil to roots they had colonized was only slightly greater than for non-inoculated control plant roots. We presume that there were some external hyphae coming from those inoculated roots that were responsible for P uptake and the growth enhancement, but that the amount was not detectable by the soil attachment method used. However, even though the method was not absolutely quantitative, it did allow for relative comparisons between isolates.

Discussion

In a sandy loam soil from California, *Glomus* isolates from California increased growth of Troyer citrange more than did Florida isolates. The difference in capacity to enhance growth between California and Florida fungi was not a function of host origin since all isolates were originally from citrus, but was apparently associated with the edaphic origin of the isolates. When Nemec (1978) compared Florida isolates of *G. etunicatus* and *G. fasciculatus* on six different citrus root stocks, the combined top growth of all rootstocks was increased 21-fold by *G. etunicatus* but only sixfold by *G. fasciculatus* in a Florida sandy soil. When we compared the same species from Florida on Troyer citrange in a California soil, *G. fasciculatus* was more efficient than *G. etunicatus*. Taken together, these results support Mosse's (1972a, b) conclusion that the efficiency of isolates may vary with the soil type in which they are compared.

The greater efficiency of most California isolates was associated with the presence of more external hyphae as measured by hyphal attachment of soil to roots. Sanders *et al.* (1977) also found that the amount of external mycelium was correlated with growth enhancement of onion by the four VAM fungi studied. However, they reported that in each case the quantity of external mycelium was correlated with the level of internal root colonization, which in their time course study was related to the rate of colonization. One of their isolates produced no growth increase and had no external mycelium, but was also slow to colonize the roots. Thus, their study suggested that maximum development of external mycelium may only occur after roots are colonized to a maximum extent. Our study offers a different perspective in that all our isolates had colonized roots to a maximum extent by the time of the harvest. Yet, external hyphae did not develop for some isolates, suggesting that the external mycelium stage is controlled by factors different from or at least independent of those controlling colonization. Thus, our results show that internal root colonization may occur to a maximum extent without the development of external mycelium presumably because of restrictive factors in the soil.

Lambert, Cole and Baker (1980) suggested that indigenous mycorrhizal fungi may be better adapted to soil factors than otherwise more efficient introduced fungi because these non-native fungi are unable to adapt to edaphic conditions. The greater efficiency of California versus non-native Florida isolates in a California soil supports their conclusion. The exception is *G. fasciculatus* 474 which was isolated from a high phosphorus soil (J. Menge, unpublished data). Normally, high levels of available phosphorus in soils result in an increase in root content of P which is not only inhibitory to mycorrhiza formation but also reduces the

development of external hyphae in soil and minimizes their role in P uptake (Sanders, 1975). Isolate 474 may be adapted to the high P soil by limiting hyphal growth in soil, while maintaining growth within the root.

A number of other soil factors may influence the efficiency of mycorrhizal fungi. Menge *et al.* (1982) found that the mycorrhizal dependency of Troyer citrange on *G. fasciculatus* isolate 0–1 in 26 citrus soils from California was inversely correlated with soil P, Zn, Mn, Cu, percentage organic matter and cation exchange capacity, and was positively correlated with soil pH. In general, arid soils are alkaline in pH, as was the California soil we used (pH 7·3), whereas soils from humid regions, such as Florida, have a pH below 7 (Brady, 1974). Of the isolates we tested, *G. fasciculatus* 0–1 was the most efficient and had the greatest amount of external hyphal development in the California soil. It is possible that the Florida fungi isolated from acidic soils were less efficient as a result of the inhibition of hyphal development by higher soil pH.

The data presented here strongly support the idea presented earlier by other workers (Sanders *et al.*, 1977) that host plant growth enhancement from VAM fungus inoculation, due largely to increased P uptake, is proportional to the extent of external hyphal development. We recognize that weighing the soil bound by that mycelium (Sutton and Sheppard, 1976) is not as precise as if one could harvest and weigh all the mycelium. The latter method, too, would surely have considerable error, and thus would only show relative differences. These methods also fail to take into account the spatial distribution of the hyphae which may have profound impact on whether or not P is acquired beyond the root depletion zone. Further, these methods do not deal with the problem of relative differences in isolate efficiency of uptake and translocation once the hyphae are in place. It seems possible that some isolates may be more efficient than others such that the hyphal inflow of P could vary even though the amount of external mycelium was the same. That variation could result from genetic and physiological differences between strains, or to the relative age of the external hyphae (Mosse, 1959). While a multiple harvest experiment would have yielded data on progressive development of colonization and perhaps external hyphal development as well, as in the study by Sanders *et al.* (1977), it would not have yielded information on relative uptake and translocation efficiency. A later harvest could, however, have told us whether or not external hyphae would ever have been developed by the inefficient Florida isolates if given more time. Since we had maximum colonization of the roots by the time of our harvest, we can assume that uptake of P was not directly related to that phase of the process. An earlier harvest, when colonization might have still been incomplete, as in the study by Sanders *et al.* (1977), would not have shown us that external hyphal development is not always correlated with the extent of root colonization. By waiting until colonization was complete, we could eliminate the variable of different rates of colonization.

The practical aspect of this study is that one cannot always quantify root colonization and predict how much the resultant mycorrhizae may enhance growth because those morphological features do not totally reflect P uptake potential. Specifically, the lack of growth enhancement could reflect the lack of external hyphae. The soil aggregation method is a reasonably simple way to monitor external hyphae, and could be used to give clues as to what soil variables may prevent or enhance their formation.

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