Influence of *Glomus fasciculatus* on *Thielaviopsis basicola* Root Rot of Citrus

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ABSTRACT

DAVIS, R. M. 1980. Influence of *Glomus fasciculatus* on *Thielaviopsis basicola* root rot of citrus. Plant Disease 64:839-840.

Sweet orange seedlings were inoculated with *Glomus fasciculatus* alone, *Thielaviopsis basicola* alone, both fungi, or neither fungus. Seedlings infected with the vesicular-arbuscular mycorrhizal fungus, *G. fasciculatus*, were larger than nonmycorrhizal seedlings. Growth of both mycorrhizal and nonmycorrhizal seedlings was reduced by *T. basicola*, but mycorrhizal seedlings were larger than nonmycorrhizal seedlings. However, the relative reduction of growth by *T. basicola* was about 37% for both mycorrhizal and nonmycorrhizal seedlings. *G. fasciculatus* apparently had little influence on the root disease caused by *T. basicola*.

Additional key words: endomycorrhizae, soil fungi

Vesicular-arbuscular mycorrhizal fungi variously influence a number of diseases. Mycorrhizal soybean (10) and avocado (4) were more susceptible to *Phytophthora megasperma* and *P. cinnamomi*, respectively, than were nonmycorrhizal plants. In some disease interactions, eg. *P. palmivora* on papaya (9), mycorrhizal fungi had no effect. In several cases mycorrhizal plants were more resistant to disease than plants without mycorrhizae. Tobacco and alfalfa infected with the mycorrhizal fungus *Glomus mosseae* were more resistant to *Thielaviopsis basicola* (Berk. and Br.) Ferr. than were nonmycorrhizal plants (2). Furthermore, formation of chlamydospores by *T. basicola* was impaired on mycorrhizal tobacco roots (1).

Thielaviopsis basicola causes a root disease on many crop plants, including citrus. The pathogenic nature of *T. basicola* on citrus has been reported (12), but the work was done in fumigated soil that lacked mycorrhizal fungi. In light of the discovery of the increased resistance of mycorrhizal tobacco and alfalfa to *T. basicola*, this study was initiated to determine if root infection by a mycorrhizal fungus could influence the susceptibility of citrus to *T. basicola*.

MATERIALS AND METHODS

Citrus sweet orange (*Citrus sinensis* (L.) Osbeck 'Pineapple') seeds were sown in two flats of a twice autoclaved sandy soil. The soil, which contained 1.6 μg of P per gram of soil, was amended with finely ground superphosphate [Ca $(H_2PO_4)_2 \cdot H_2O$] at 12 μg of P per gram of soil. One flat received a layer of inoculum of *Glomus fasciculatus* (Thaxter) Gerd. and Trappe (isolate 92) placed 6 cm beneath the seeds. Inoculum consisted of soil, roots, and spores from a pot containing sudan grass (*Sorghum vulgare* Pers.) infected with *G. fasciculatus*. The other flat received a water filtrate from the inoculum of *G. fasciculatus* to include microorganisms associated with the mycorrhizal inoculum. The filtrate was passed through a 20-μm nylon sieve to remove spores of *G. fasciculatus*.

Seedlings were gently removed from the flats after 11 wk of growth and roots were examined for mycorrhizal infection. Randomly selected root samples were stained with 0.05% trypan blue in lactophenol (8), placed on a grid of 1-mm^2 divisions, and examined for arbuscles, vesicles, spores, and hyphae of *G. fasciculatus* in 100 or more 1-mm^2 sections of root tissue (4). Sixty-two percent of the roots of the seedlings inoculated with *G. fasciculatus* were mycorrhizal.

Ten seedlings with or without mycorrhizae were transplanted into 10-cm pots (one seedling per pot) of the sandy soil infested or not infested with *T. basicola*. Inoculum of *T. basicola* consisted of spores and mycelia produced by the method of Tsao and Van Gundy (12). Fifty milliliters of autoclaved V-8 broth in each of 20 250-ml prescription bottles was inoculated with a 5-mm agar plug of *T. basicola* taken from a 7-day-old culture grown on V-8 agar. After the bottles were incubated vertically for 24 hr at 25 C, each bottle was shaken and incubated horizontally for an additional 10 days. The cultures were then pooled, macerated in a blender, rinsed in sterile distilled water, and mixed into the soil. The soil was divided into 20 pots for a final concentration of inoculum of about 50 ml per 10-cm pot.

Seedlings were grown in a glasshouse at temperatures ranging from 16 to 34 C and watered daily with a 14% Hoaglands solution (6) lacking phosphorus. Seedlings were lifted from the soil after 12 wk, and heights and weights were recorded. Samples of roots were stained as previously described to estimate percentages of roots infected with *G. fasciculatus*. Phosphorus content of leaves was determined by the molybdate-$SnCl_2$ method (7).

RESULTS AND DISCUSSION

Growth of seedlings was increased by *G. fasciculatus* and generally decreased by *T. basicola* (Table 1, Fig. 1). Total weights of both mycorrhizal and nonmycorrhizal seedlings were reduced in soil infested with *T. basicola*. In soil with *T. basicola*, however, heights and weights of mycorrhizal seedlings were significantly greater ($P = 0.05$) than those of nonmycorrhizal seedlings. *T. basicola* caused little obvious necrosis to roots; extensive rot of roots was not observed in either mycorrhizal or nonmycorrhizal seedlings. Cessation in the production of feeder roots rather than significant decay of root systems was apparently the cause for the reduction of weights of seedlings inoculated with *T. basicola*. This phenomenon was reported earlier in a study on the pathogenesis of *T. basicola*

Table 1. Influence of *Thielaviopsis basicola* on growth of mycorrhizal (*Glomus fasciculatus*) and nonmycorrhizal citrus[a]

Fungus	Height (cm)	Dry weight (g) Total	Dry weight (g) Root
None	12.1 z	2.90 y	1.53 yz
T. basicola	10.2 z	1.77 z	0.89 z
G. fasciculatus	29.3 x	7.88 w	3.74 x
T. basicola + *G. fasciculatus*	22.6 y	5.04 x	2.10 y

[a] Values represent mean of 10 seedlings. Means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Influence of *Thielaviopsis basicola* on infection by *Glomus fasciculatus* and on concentrations of phosphorus in leaves of mycorrhizal and nonmycorrhizal citrus[a]

Fungus	Root tissue infected with *G. fasciculatus* (%)	Phosphorus in leaves (%)
None	0 z	0.06 z
T. basicola	0 z	0.04 z
G. fasciculatus	82.6 x	0.17 y
T. basicola + *G. fasciculatus*	64.8 y	0.16 y

[a] Values represent mean of 10 seedlings. Means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

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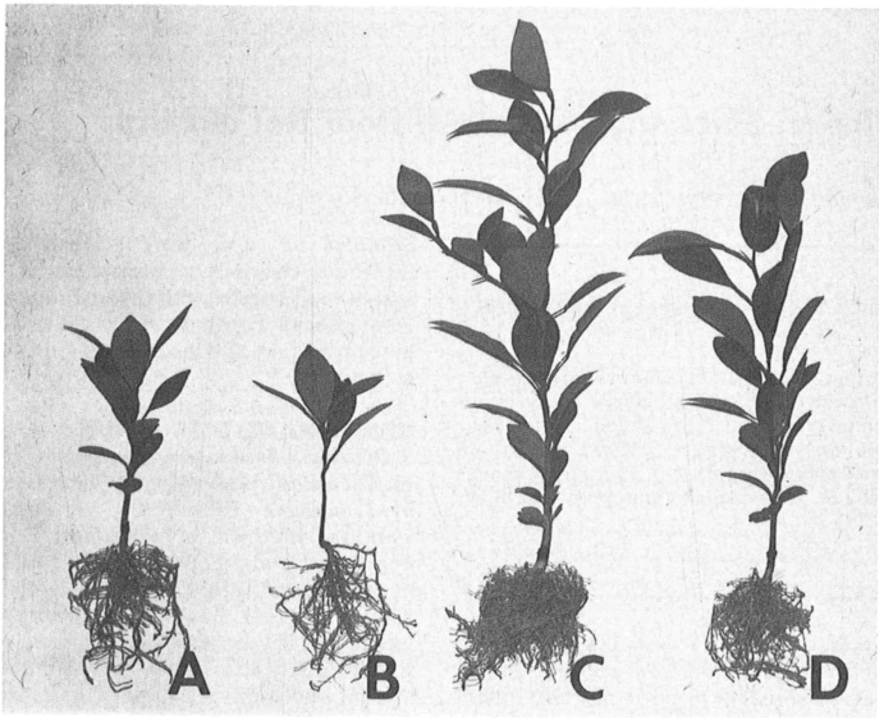

Fig. 1. Growth responses of sweet orange seedlings infected with **(A)** no fungus, **(B)** *Thielaviopsis basicola*, **(C)** *Glomus fasciculatus*, or **(D)** *T. basicola* and *G. fasciculatus*. *T. basicola* caused growth reduction of both mycorrhizal and nonmycorrhizal seedlings.

on citrus (12). Few chlamydospores of *T. basicola* were found on roots of any seedling grown in infested soil.

The percentage of roots infected with *G. fasciculatus* was reduced by *T. basicola*, but the percentage remained high (Table 2). Concentrations of phosphorus in leaves of mycorrhizal seedlings with or without *T. basicola* were greater than in those of nonmycorrhizal seedlings, indicating that mycorrhizae were still functioning in the presence of *T. basicola*. The soil contained less than optimum levels of phosphorus for adequate growth of nonmycorrhizal citrus; hence, growth of seedlings infected with *G. fasciculatus*, which increases phosphorus uptake (5), was superior to that of nonmycorrhizal seedlings.

Although mycorrhizal seedlings in soil infested with *T. basicola* were larger than nonmycorrhizal seedlings, the relative reduction of growth of both mycorrhizal and nonmycorrhizal seedlings by *T. basicola* was similar. The total weights of mycorrhizal seedlings and nonmycorrhizal seedlings were reduced 39 and 36%, respectively. Based on the relative reduction of total weights, no increased resistance or susceptibility was indicated in mycorrhizal citrus. The nutritional superiority associated with the substantial percentage of roots that remained mycorrhizal in soil with *T. basicola* offset the effects of the pathogen in mycorrhizal seedlings compared with nonmycorrhizal seedlings, but no added resistance was apparent in seedlings infected with *G. fasciculatus*.

Baltruschat and Schönbeck (2) found mycorrhizal (*G. mosseae*) tobacco and alfalfa more resistant to *T. basicola* than nonmycorrhizal plants. Although no resistance was found in mycorrhizal (*G. fasciculatus*) citrus to *T. basicola* in this study, the influence of mycorrhizae varies with the disease complex. Different cultivars of citrus responded in a number of ways to various species of mycorrhizal fungi and *P. parasitica* (3) and mycorrhiza-nematode interactions differed with different cultivars of soybean (11). Because mycorrhizal fungi other than *G. fasciculatus* might increase susceptibility or resistance of citrus to *T. basicola*, future experiments will include various species and isolates of mycorrhizal fungi.

ACKNOWLEDGMENT

I thank C. F. Webster for technical assistance.

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