

## The Effects of a Mycoparasite on the Mycorrhizal Fungus, *Glomus deserticola*

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### ABSTRACT

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In vitro experiments have shown *Anguillospora pseudolongissima* to be a mycoparasite of vesicular-arbuscular mycorrhizal fungus spores. The mycoparasite was added to a low-phosphorus sandy loam containing  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$  serial dilutions of inoculum of the mycorrhizal fungus, *Glomus deserticola*. Onion seeds were planted in the soil-inoculum mixture after a 2-wk incubation period. *A. pseudolongissima* significantly reduced root colonization by *G. deserticola* and growth response of onions after 80 days in treatments where the mycorrhizal inoculum was diluted to  $10^{-3}$  of the

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original inoculum. No significant differences were seen between mycoparasite and nonmycoparasite treatments containing mycorrhizal fungus inoculum diluted  $10^{-1}$  and  $10^{-2}$  times. Most-probable-number calculations showed that *A. pseudolongissima* reduced the initial effective propagule density of the mycorrhizal fungus over 50%. Under conditions of low inoculum density of the mycorrhizal fungus and low phosphorus availability, a mycoparasite can indirectly reduce plant dry weight by adversely affecting the fungal mycorrhizal symbiont.

Mycoparasites of vesicular-arbuscular mycorrhizal (VAM) fungus spores have been frequently observed (2,5-7,9,13,14,16,18-22). Most of these reports have been descriptive only, and in many cases the suspected mycoparasite was not identified. Effects of mycoparasites on populations of VAM fungi and host plant growth have been assumed or ignored. However, during a population dynamics study of two VAM fungi in field plots, Ross and Ruttencutter (13) observed *Phlyctochytrium* sp. and a fungus resembling *Pythium* attacking the spores of *Glomus macrocarpum* var. *geosporum* (Nicol. and Gerd.) Gerdemann and Trappe. The presence of these mycoparasites was correlated with a decline in chlamydospore production of the VAM fungus in soybean [*Glycine max* (L.) Merr.] pot cultures containing mycoparasite-infested field soil. They postulated that mycoparasites might play a role in limiting populations of VAM fungi. Daniels and Menge (5) isolated *Anguillospora pseudolongissima* Ranzoni and *Humicola fuscoatra* Traaen from parasitized spores of *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe and *G. epigeum* Daniels & Trappe in greenhouse pot culture. When the spores of these two VAM fungi were added to water agar or sterile sand containing inoculum of the two mycoparasites, a high percentage of the VAM

spores became infected. It was implied that these mycoparasites could significantly reduce soilborne populations of mycorrhizal fungi. However, it has not been shown conclusively that mycoparasites can reduce populations of VAM fungus to the point that colonization of host plants is reduced and growth of host plants is inhibited.

The purpose of the experiments reported here was to determine if mycoparasites could reduce the effective propagule density of a VAM fungus, root colonization by the VAM fungus, and growth of mycorrhizal plants. We attempted to determine if a mycoparasite of a VAM fungus spore could indirectly reduce plant dry weight by adversely affecting the fungal mycorrhizal symbiont.

### MATERIALS AND METHODS

**VAM fungus inoculum.** Mycorrhizal fungus inoculum was prepared from 1.5-yr-old pot cultures of *Glomus deserticola* Trappe, Bloss, and Menge [= *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. & Trappe] on Troyer citrange [*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osbeck]. The inoculum, consisting of soil, root pieces, and spores, was manually chopped and air-dried for 1 wk prior to incubation with the mycoparasite. The purpose for air-drying was to reduce the inoculum density of VAM fungus hyphae, leaving chlamydospores as the main source of inoculum.

**Mycoparasite culture.** *Anguillospora pseudolongissima* Ranzoni was isolated from parasitized spores of *G. deserticola* extracted from pot cultures of Sudan grass [*Sorghum sudanense* (Piper) Stapf]. The fungus was grown on potato-dextrose agar

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(PDA) and transferred to fresh media every 2 mo. The mycoparasite substrate for the greenhouse experiment was prepared by transferring plugs of the fungus grown on PDA to a sterilized mixture of sand and cornmeal (19:1, w/w in 0.95-L (1-quart) mason jars). The sand and cornmeal medium was autoclaved at 125 C for 2 hr on two consecutive days. After 2 mo, the substrate was manually chopped and mixed with the VAM fungus inoculum.

**Experimental design.** The experiment consisted of four treatments including: a control with no VAM fungus and no mycoparasite in twice-autoclaved Delhi sandy loam, VAM fungus only and no mycoparasite, VAM fungus plus mycoparasite, and mycoparasite only (1 part mycoparasite inoculum:9 parts sterile Delhi sandy loam, w/w). The soil had a  $\text{NaHCO}_3$ -extractable P (10) level of 2–3 ppm. In the treatments with the VAM fungus, the inoculum was air-dried and serially diluted with sterile Delhi sandy loam to  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$  (w/w). In the treatment with mycoparasite the concentration of VAM fungus inoculum in the dilutions was slightly higher, to compensate for the subsequent dilution by the mycoparasite substrate. Mycoparasite substrate was mixed with diluted VAM fungus inoculum (1 part mycoparasite substrate:9 parts diluted VAM fungus inoculum, w/w). Thus, the final concentration of VAM fungus inoculum in each dilution was the same in treatments with and without the mycoparasite.

Soil, inoculum, and substrate were combined and mixed for 3 min in a twin-shell blender. The mixture was placed (70 g of soil per tube) in 100 16 × 3-cm-diameter Cone-tainers (Ray Leach "Conetainer" Nursery, Canby, OR). Soil in the tubes was wetted and incubated in the greenhouse for 2 wk prior to planting with onions (*Allium cepa* L. 'Southport Yellow Globe'). Five onion seeds were planted in each tube. One week after germination, the seedlings were thinned to three per tube. The plants were watered daily with 14% Hoagland's solution without P (8). The treatments were arranged in a completely randomized design on the greenhouse bench. Thirty, 40, 50, 60, 70, 80, 100, and 110 days later, plants were harvested from ten replicate tubes in each treatment. The roots were fixed and stained according to the method of Phillips and Hayman (11). The roots were spread across a plastic petri dish top (95-mm diameter) etched with a 1-mm grid. One hundred root sections that crossed over the 1-mm lines in the grid were rated as colonized or uncolonized, based on the presence of arbuscles, vesicles, and internal or external hyphae. The average percent root colonization of each replicate was calculated by using the following equation: number of roots with VAM fungus divided by the number of total roots times 100. The tops of the plants were oven-dried at 60 C for 3 days to determine dry weight. To determine if the mycoparasite substrate altered the level of available P, plants from each harvest between 70 and 110 days in the control and mycoparasite-only treatments were pooled and ground up. Two 250-mg samples from each treatment per harvest were digested in concentrated nitric-perchloric acid (2:1) for 12 hr at 120 C. Phosphate content of the tissue was quantified colorimetrically by using a phosphomolybdate complex (4).

Average shoot dry weight and percent colonization were plotted against time, with linear regressions of the data. Analysis of covariance was performed on the regression lines, with a test of variation among the adjusted means and a pairwise comparison of the adjusted means. Duncan's multiple range test was used to determine the statistical significance of differences between individual treatments at each sampling time. The experiment with *A. pseudolongissima* was repeated with similar results.

**Effect of *A. pseudolongissima* on the effective propagule density of the VAM fungus.** The number of effective propagules of the VAM fungus per gram of soil was determined by using the most-probable-number method (1) to analyze the data for incidence of colonization from 80, 100, and 110 days. The calculated effective propagule density value for VAM fungi is not an absolute value; it is dependent on the time of sampling, the host, and environmental conditions (12,23). Because the effective propagule density values calculated for each treatment were similar at each harvest date, the values were averaged. The most-probable-number method,

sometimes called the extinction dilution method, was used to estimate the population density from a series of 10-fold dilutions of the inoculum. In this experiment, ten plants were harvested from each dilution. The roots were cleared and stained to detect the presence or absence of mycorrhizal fungus colonization, which was an indication of effective propagules.

**Detection and isolation of *A. pseudolongissima*.** To determine if the mycoparasite could survive greenhouse conditions, a selective medium was developed to detect and quantify *A. pseudolongissima* in the soil. The medium was a modification of a peptone glucose rose bengal agar (17). Penicillin G and chloramphenicol were added at 200 mg/L each as a substitute for streptomycin. Pentachloronitrobenzene (PCNB) was added at 375 mg/L (500 mg of Terraclor, 75% WP, per liter) as a selective agent to eliminate other fungal contaminants. *A. pseudolongissima* was tolerant to PCNB at 750 mg/L, although its growth rate was inhibited. Soil samples were taken from all the treatments at 0, 1, and 3 mo after mixing the inoculum. A 10-g sample (adjusted for dry weight) was diluted 10-fold with 0.15% water agar. A 0.25-ml aliquot was spread across the surface of the selective medium in 100 × 15-mm plastic petri dishes, and the resulting colonies were counted after 10 days. Ten replicate plates were used for each sample.

## RESULTS

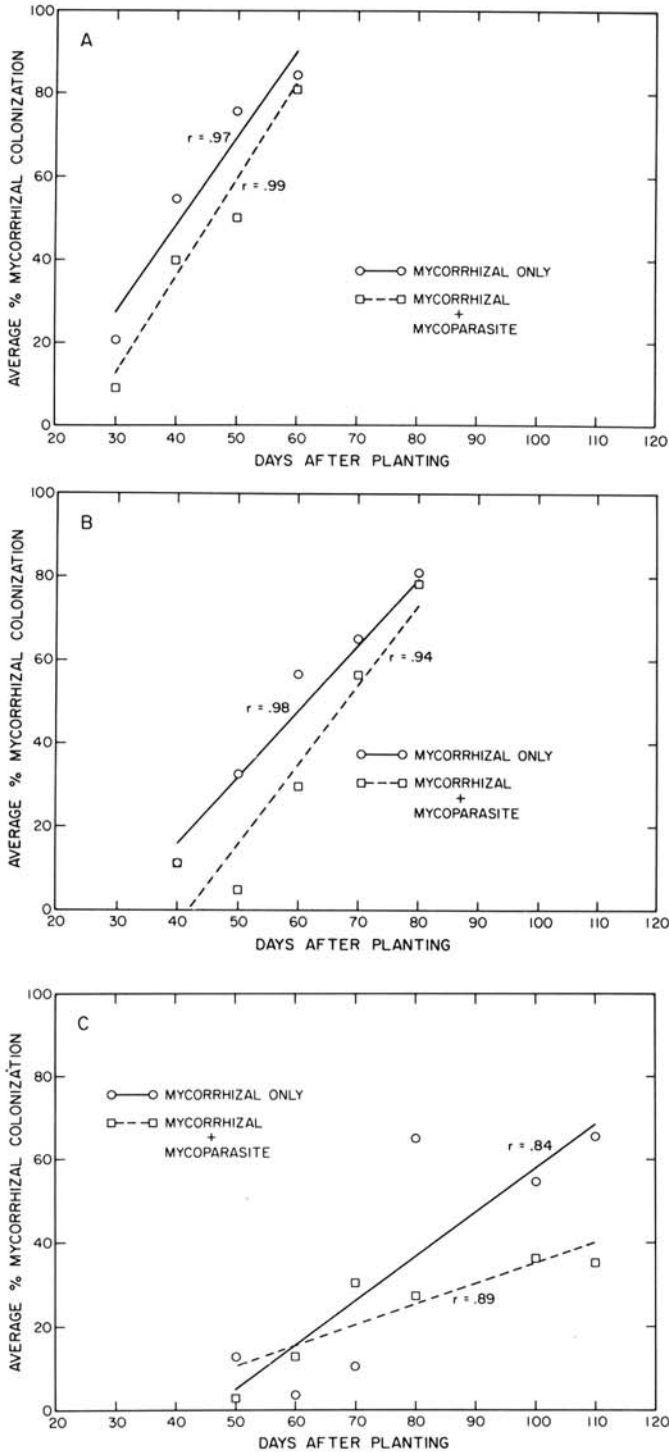
**Effects of diluting mycorrhizal fungus inoculum on root colonization and growth response of onion.** Onions grown in the  $10^{-1}$  dilution were colonized rapidly (>80%) by the VAM fungus within 60 days (Fig. 1A). In the  $10^{-2}$  dilution, colonization above 20% was not detected until 50 days after inoculation (Fig. 1B). The rate of colonization in this dilution was less than the rate in the  $10^{-1}$  dilution. Although the logarithmic phase of colonization was delayed approximately 10 days in the  $10^{-2}$  dilution, the maximum level of colonization in both dilutions was the same. Colonization was delayed even further in the  $10^{-3}$  dilution, and the maximum level was significantly less (Duncan's multiple range test,  $P = 0.05$ ) than in the most concentrated dilution (Fig. 1C).

Onions grown in the  $10^{-1}$  and  $10^{-2}$  dilutions showed similar increases in shoot dry weight over time (Fig. 2A and B). After 50 days, the mycorrhizal plants were significantly larger than the nonmycorrhizal controls. By the end of the experiment, shoot dry weights of the mycorrhizal plants were three to four times greater.

**Effect of *A. pseudolongissima* on VAM fungus colonization and growth response of onions.** No significant differences in root colonization between the mycoparasite and nonmycoparasite treatments were detected in the  $10^{-1}$  VAM fungus inoculum dilution (Fig. 1A). In the  $10^{-2}$  dilution (Fig. 1B), VAM fungus colonization was slightly delayed in the mycoparasite treatment, but these differences were not significant ( $P = 0.05$ , Duncan's multiple range test and analysis of covariance to test variation among adjusted means). By 80 days, the colonization curve was similar for both treatments. In the  $10^{-3}$  dilution, significantly less colonization occurred on onion roots with the mycoparasite and the VAM fungus at 80 days and after (Fig. 1C). *A. pseudolongissima* had similar effects on shoot dry weights (Fig. 2A to C). No significant differences were seen in the  $10^{-1}$  and  $10^{-2}$  dilutions. In the  $10^{-3}$  dilution (Fig. 2C), onions in the mycoparasite-VAM fungus treatment had significantly less shoot dry weights than onions in the VAM fungus-only treatment after 80 days. No significant differences in shoot dry weight were seen between the mycoparasite-only and control treatments. Phosphorus analyses of plant tissues from these two treatments showed no significant differences ( $P = 0.05$ , Duncan's multiple range test,  $n = 8$ ). Tissue P concentrations in these treatments ranged from 690 to 876 ppm dry weight, which were in the deficiency range. This suggests that the mycoparasite inoculum did not significantly affect the level of available P in the soil mixtures.

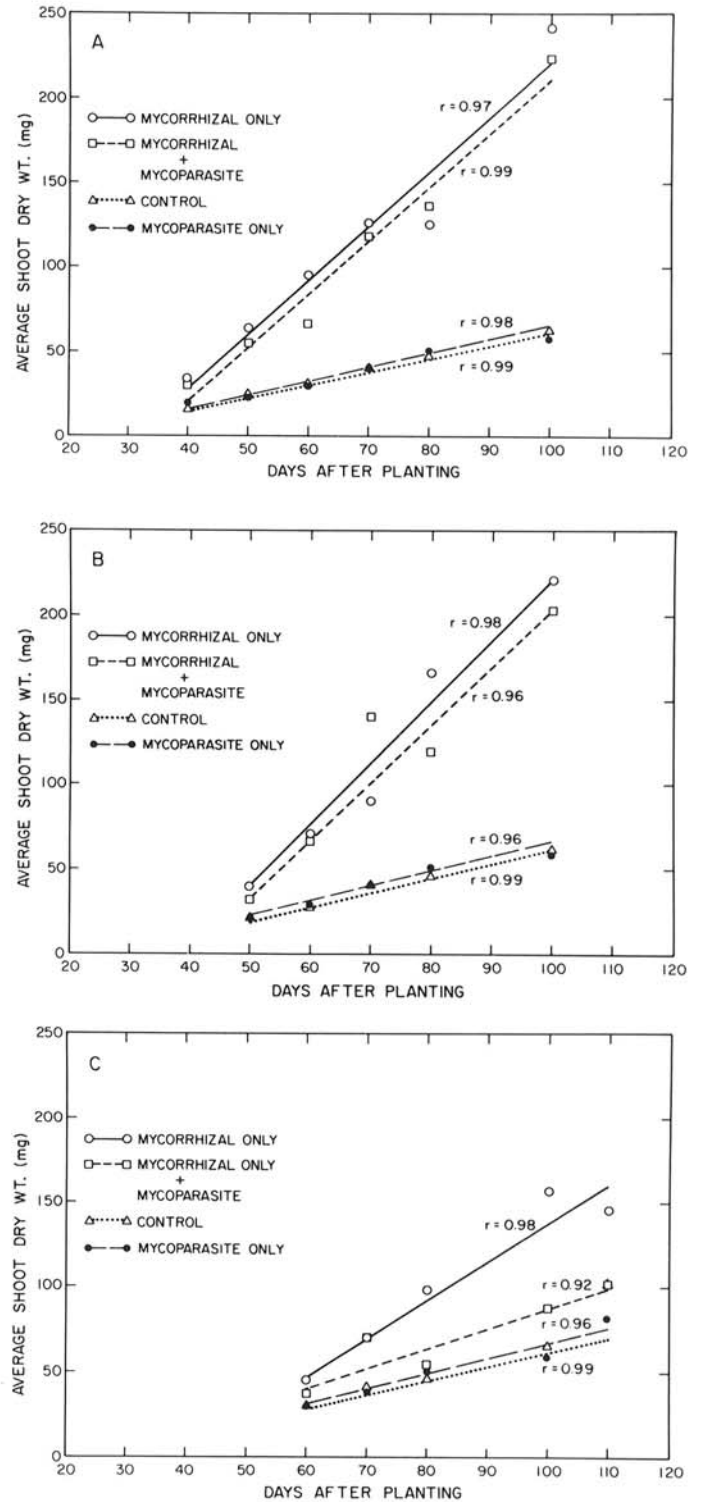
**Effect of *A. pseudolongissima* on the effective propagule density of the VAM fungus.** The effective propagule density of the VAM fungus in the nonmycoparasite treatments at the time of planting was  $26.4 \pm 5.6$  effective propagules/g of undiluted inoculum, and in the mycoparasite treatments it was  $10.3 \pm 2.7$  effective propagules

per gram of undiluted inoculum. Thus, *A. pseudolongissima* reduced the initial effective propagule density of the VAM fungus by 61% during the 2 wk prior to planting. Most-probable-number methods have a low order of statistical precision (1), but the results were similar at 80, 100, and 110 days, increasing the level of confidence. In the repeated experiment, it was calculated that the mycoparasite reduced the initial effective inoculum density of the VAM fungus 48%, from  $2.80 \pm 1.84$  effective propagules per gram to  $1.45 \pm 0.45$  effective propagules per gram of undiluted inoculum.



**Fig. 1.** Effects of the mycoparasite *Anguillospora pseudolongissima* on root colonization of *Allium cepa* by the vesicular-arbuscular mycorrhizal (VAM) fungus, *Glomus deserticola*. Lines represent linear regression of means from the logarithmic phase of colonization. Each data point is the average of 10 replicates. **A**, VAM fungus inoculum dilution =  $10^{-1}$ ; **B**, VAM fungus inoculum dilution =  $10^{-2}$ ; and **C**, VAM fungus inoculum dilution =  $10^{-3}$ .

**Detection and isolation of *A. pseudolongissima*.** Dilution plate counts of soil samples from pots with *A. pseudolongissima* at the start of the experiment (before incubation with the VAM inoculum) were  $58,000 \pm 10,760$  colony forming units per gram of soil. After 1.5 mo,  $11,900 \pm 3,977$  colony forming units were detected. After 3.5 mo, the populations declined to  $8,000 \pm 8,620$



**Fig. 2.** Effects of the mycoparasite *Anguillospora pseudolongissima* on shoot dry weight of *Allium cepa* colonized by the vesicular-arbuscular mycorrhizal (VAM) fungus, *Glomus deserticola*. Lines represent linear regression of dry weight means. Each data point represents the average of 10 replicates. **A**, VAM fungus inoculum dilution =  $10^{-1}$ ; **B**, VAM fungus inoculum dilution =  $10^{-2}$ ; and **C**, VAM fungus inoculum dilution =  $10^{-3}$ . Control was without VAM fungus or the mycoparasite.

colony forming units per gram. In the repeat experiment, the population of the mycoparasite showed a similar reduction over time.

## DISCUSSION

The colonization of onion roots by *G. deserticola* can be represented by a sigmoidal curve with a distinct lag phase, logarithmic phase, linear phase, and a maximum plateau. Graphs from the study showed this type of curve, but only data points from linear portions of the curve were plotted, along with a linear regression line of those points. The dilution of the VAM fungus inoculum had three main effects on root colonization. First, colonization was delayed. Second, the maximum percent root colonization was less at the lower effective inoculum density of the VAM fungus because the incidence of infection was reduced. Third, the rate of increase in root colonization was reduced, probably because of a reduction in the rate of formation of primary points of penetration (24). Dilution of VAM fungus inoculum had similar effects on shoot dry weights. These results agree with data for mycorrhizal colonization in other studies (3,15,24).

The effect of the mycoparasite, *A. pseudolongissima*, on the VAM symbiosis was evident only at the lower VAM fungus inoculum concentration ( $10^{-3}$ ) after 80 days. The primary effect of the mycoparasite was to reduce the number of effective propagules of the VAM fungus which resulted in a delay of VAM colonization and reduced incidence of colonization. When the effective propagule density of the VAM fungus is low, the mycoparasite can lower the effective propagule density even further, to the point that a plant can completely escape colonization by the VAM fungus. If plants are grown in low-P soil, then P deficiencies will result from lack of mycorrhizal infection. At higher VAM propagule densities, this reduction of colonization and dry weight would not be seen because sufficient numbers of VAM fungus spores would survive to rapidly initiate colonization of roots.

These results show that mycoparasites of VAM fungi can act indirectly to reduce dry weights of plants. This effect is seen when three conditions are met. First, the effective propagule density of the VAM fungus is low. This condition might be common in many soils where anaerobic conditions, microbial degradation, fumigation, absence of plant host, and fungicides could reduce the effective propagule density of a VAM fungus to the 2.64 propagules per gram level used in this experiment. Second, the plants are grown in soil with a low concentration of available P. These types of agricultural soils are common, especially in the tropics, where highly leached soils have a high phosphorus-fixing capacity. Third, the propagule density of the mycoparasite is high. Little research has been done on this subject. Mycoparasites are present in agricultural soils, but the populations are often very low. In most cases, mycoparasites were isolated from only 1–3% of VAM fungus bait spores incubated in the soils (T. C. Paulitz, unpublished). However, higher populations were detected at two field sites, where mycoparasites were isolated from 16% of the bait VAM fungus spores. Mycoparasites of VAM fungi discovered to date appear to be facultative parasites and probably have a certain degree of saprophytic ability. Therefore, the populations of these mycoparasites may not necessarily be dependent on VAM fungi.

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