Hyperparasitization of Vesicular-Arbuscular Mycorrhizal Fungi

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ABSTRACT

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Sporocarps of two vesicular-arbuscular mycorrhizal fungi, *Glomus epigaeus* and *G. fasciculatus,* often contain hyperparasitized spores even when produced in greenhouse pot cultures. Two of these hyperparasites were isolated on agar media and identified as *Anquillospora pseudolongissima* and *Humicola fuscoatra. Glomus* spores parasitized by *A. pseudolongissima* contained swollen sausage-shaped hyphae which are constricted at the septa, while those parasitized by *H. fuscoatra* contained slender hyphae and/or aleurospores. *G. epigaeus* spores, visibly free from parasites, when added to water agar or autoclaved soil containing *A. pseudolongissima* became 51.4 and 69.3% parasitized, respectively, while *H. fuscoatra* parasitized 48.2 and 70.9%, respectively. Similarly, *G. fasciculatus* became 67.1 and 94.6% parasitized by *A. pseudolongissima* and 56.8 and 91.2% parasitized by *H. fuscoatra,* respectively. Control spores of *G. fasciculatus* or *G. epigaeus* placed in either soil or on agar without parasitic fungi were only 1.3–3.0% parasitized. Of nine fungicides tested, only mancozeb reduced growth of both hyperparasites without inhibiting the germination of mycorrhizal fungi. A third chytridlike hyperparasite, identified as a *Phlyctochytrium* sp. was isolated from *G. fasciculatus* sporocarps and propagated in sterile pond water on *Gigaspora margarita* spores or pollen of a *Liquidambar* sp. Parasitism by this fungus was nearly eliminated by addition of ethazole to the culture water.

Additional key word: hyperparasite.

Spores of vesicular-arbuscular mycorrhizal (VAM) fungi often contain hyperparasites (6). These hyperparasites frequently occur in pot cultures of supposedly purified cultures of mycorrhizal fungi (4,11,14) and may severely limit the population of indigenous VAM fungi in the field (13). The presence and activity of hyperparasites in pot cultures of VAM fungi is of immediate concern if large-scale mycorrhizal inoculation of fumigated, infertile, or disturbed soils is to be accomplished successfully. The presence of hyperparasitized VAM inoculum may explain some erratic results which occur in tests with VAM fungi.

Sporocarpic VAM fungi are desirable for commercial production because of the large numbers of easily extractable clusters of spores. However, the proximity of spores in a sporocarp may also predispose these species to increased hyperparasitism.

The purpose of this study was to investigate several hyperparasites of several species of VAM fungi and to further evaluate possible fungicides to control these hyperparasites.

MATERIALS AND METHODS

Isolation and identification of hyperparasitic fungi. Sporocarps of *Glomus epigaeus* Daniels & Trappe, known to be infected with hyperparasites, were harvested from the soil surface of 3- to 4-mo-old pot cultures containing *Asparagus officinalis* L. and were stored in Ringer's solution (NaCl, 6 g; $CaCl_2$, 0.1 g; KCl, 0.1 g in 1 L of distilled water, then adjusted to pH 7.4 with 0.1 N NaOH) for several months. These stored sporocarps were divided manually into sections containing 50–100 spores, placed in 10-mm-diameter petri dishes containing sterile distilled water, and used as a source of hyperparasitic fungi. Similar portions of sporocarps, visibly free of hyperparasites, were harvested from *Sorghum vulgare* Pers. and surface disinfected in 0.5% sodium hypochlorite (10% Clorox) for 3 min followed by three rinses in sterile water. These portions of sporocarps served as "bait," and were added to petri dishes containing water and hyperparasitized sporocarps.

Similarly, portions of sporocarps of *Glomus fasciculatus* (Thaxter) Gerd. & Trappe (E_3 as described by Gilmore, [5]) were wet-sieved and decanted (4) from pot cultures known to contain a hyperparasite. Surface sterilized sporocarps from pot cultures of *G. fasciculatus* which appeared to be unparasitized were used as bait, as described above. After 4 days, all "bait" sporocarp sections were examined microscopically and those showing signs of infection were transferred to cornmeal dextrose yeast agar (CMDY) (8) plates or to petri dishes containing sterile pond water plus *Liquidambar* pollen or *Gigaspora margarita* Becker & Hall spores which were surface sterilized with 0.5% sodium hypochlorite as previously described. *Gigaspora margarita* spores were used as bait for chytridlike hyperparasites because of their susceptibility to a chytrid described by Schenck and Nicolson (14).

In addition, hyperparasitized portions of sporocarps of *G. epigaeus* and *G. fasciculatus* were rinsed in sterile water and plated on CMDY agar. Fungi which developed on "bait" sporocarps were compared with those growing directly from hyperparasitized portions of sporocarps on CMDY. Successive, hyphal tip transfers of these fungi on CMDY yielded pure cultures of two potential filamentous hyperparasites. A third chytridlike organism was isolated on pollen and *G. margarita* spores in water cultures.

Chlamydospore inoculation with hyperparasites. To test the parasitic potential of the two filamentous fungi isolated from parasitized sporocarps, both hyperparasites were grown on PDA agar and five agar disks (8 mm in diameter) containing mycelium of each hyperparasitic fungus were transferred to petri dishes containing water agar. A young sporocarp of *G. fasciculatus* or *G. epigaeus,* not visibly infected with hyperparasites, was placed on both sides of each agar disk. As controls, 10 sporocarps of each VAM fungus, not visibly parasitized, were plated alone on water agar. Thus, there were six treatments, each replicated three times. After 3 wk, sporocarps were recovered by sieving and decanting, stained in 0.5% cotton blue in lactophenol (12), and examined microscopically to determine the number of visibly parasitized spores.

A similar experiment was conducted in autoclaved soil. The two filamentous fungi were grown on PDA and four agar plugs (8 mm in diameter) of each, one fungus per vial, were buried in vials containing 10-g samples of moist blow sand (20% MC) that had been autoclaved twice on successive days. After 2 days, four young surface-sterilized sporocarps of *G. fasciculatus* or *G. epigaeus* not visibly infected with hyperparasites were added to the soil infested with each hyperparasite. Vials containing soil not infested with the hyperparasites, but which did contain sporocarps of *G. fasciculatus* or *G. epigaeus* which were not visibly parasitized, served as controls. Thus, there were six treatments, each replicated three

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times. After 3 wk, sporocarps were removed, stained, and examined as in the previous experiment.

Pathogenesis of the chytridlike organism was tested by placing surface-sterilized spores of *Gigaspora margarita, Glomus epigaeus, Glomus constrictus,* or *Glomus fasciculatus* 0-1 (a nonsporocarpic isolate) and 92 (a sporocarpic isolate) into petri dishes containing sterile pond water. Spores of *G. margarita* infected with the chytridlike hyperparasite were added to each petri dish as inoculum. Each treatment was replicated twice.

It was hypothesized that the melanin content of VAM spores might determine their susceptibility to the chytridlike hyperparasite. Therefore, spores were exposed to compounds known to interfere with melanin structure of spore walls (7). *G. epigaeus* spores were soaked in 1 N KOH for 1 hr, 1 N HCl for 1 hr, 3% H_2O_2 for 30 or 60 min, or 0.5% sodium hypochlorite for 3, 5, or 10 min. Control spores were soaked in distilled H_2O for 60 min. Spores were then rinsed in sterile distilled water and transferred to petri dishes containing sterile distilled water. Three spores of *G. margarita* previously parasitized by the chytridlike hyperparasite were transferred to each dish. After 5 days, the number of parasitized spores was determined microscopically.

Chemical control of hyperparasites. To identify specific fungicides or nematicides which inhibited radial growth of the filamentous hyperparasitic fungi, the hyperparasites were plated on cornmeal agar alone or on cornmeal agar containing the following concentrations of fungicides: 10, 20, or 40 μg/g DBCP (1,2-dibromo-3-chloropropane); 12.5, 25, or 50 μg/g ethazole (5-ethoxy-3-trichloromethyl-1,2,4-thiazole); 50, 100, or 200 μg/g PCNB (pentachloronitrobenzene); 2.5, 5, or 10 μg/g captan [*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide]; 50, 100, or 200 μg/g ridomil [*N*-2,6-(dimethylphenyl)-*N*-(methoxyacetyl)-alanine methyl ester]; 0,8, 1.6, or 3.2 μg/g mancozeb (manganese ethylenebisdithiocarbamate plus zinc ion); 0.375, 0.75, or 1.5 μg/g chloroneb (1,4-dichloro-2, 5-dimethoxybenzene); 1.5, 3.0, or 6.0 μg/g thiram (tetramethylthiuram disulfide); and 50, 100, or 200 μg/g dichloran (2,6-dichloro-4-nitroaniline). Each of these 20 treatments was replicated five times. Radial growth of each colony was measured after 11 days.

Those fungicides from the previous experiment which inhibited radial growth of the filamentous hyperparasitic fungi on agar were then tested for control of hyperparasitism in sterile sand. Autoclaved moist (20% MC) blow sand was amended with DBCP, ethazole, chloroneb, PCNB, or mancozeb at the concentrations used previously. Samples of each amended sand were transferred to 35-mm-diameter petri dishes (20 g of sand per dish). Each treatment was replicated twice. Five g of autoclaved blow sand, previously infested with the hyperparasites, was added to each petri plate. After 1 wk, 15 mm^2 pieces of 38 μm Nytex nylon mesh were placed on the soil surface of each petri dish. Three small surface sterilized *G. epigaeus* sporocarp sections were placed on each piece of nylon. After 10 days, portions of each sporocarp section were removed, stained with cotton blue in lactophenol, and examined microscopically. Spores which appeared vacuolated (containing 1 large vacuole rather than numerous oil globules) or necrotic were considered dead.

The tolerance of VAM fungal spores to various fungicide concentrations was tested by germinating *G. epigaeus* spores in nonsterile soil containing fungicides (*G. epigaeus* spores germinate readily in nonsterile soil [3]). Spores of *G. epigaeus* were separated from visibly parasite-free sporocarps, by agitating them for 30–60 sec in a blender. These spores were surface sterilized in 10% Clorox, added (20 spores per gram of sand) to 30-g samples of nonsterile, moist blow sand (20% MC) into which fungicides had been incorporated at the rates described earlier. Only fungicides previously determined to inhibit growth of both hyperparasitic fungi in water agar were used. Each treatment was replicated three times. After 2 wk, spores were sieved, decanted from the petri plates, and examined microscopically to assess percent germination.

Chemical control of the chytridlike organism was tested by placing approximately 100 *G. margarita* spores in sterile water containing 0, 25, 50, 100, 200 μg/g ethazole. The treatments were replicated twice. After 5 days, the number of infected spores was assessed microscopically.

RESULTS

Isolation and identification of hyperparasitic fungi. Colonies identified as *Humicola fuscoatra* Traaen grew from hyperparasitized *G. epigaeus* spores on CMDY. The same fungus was apparent on bait sporocarps of *G. epigaeus* and *G. fasciculatus* only when they came into contact with hyperparasitized *G. epigaeus* spores in water. After 4 days aleurospores of *H. fuscoatra* were abundant in agar near the host, but infection was not yet apparent.

Hyperparasitized *G. fasciculatus* spores characteristically contained swollen sausage-like hyphae which were constricted at the septa (Fig. 1). These infected spores when plated on CMDY initially produced thin threadlike mycelium, 2–4 μm in diameter. After 4 days, however, swollen sausage-like hyphae, 6–8 μm in diameter were evident in the media as well (Fig. 2). This fungus failed to produce spores in culture even after several months. However, spores readily were formed after cultures were flooded with water, allowing identification of this fungus as *Anguillospora pseudolongissima* Ranzoni. Swollen sausage-like structure initials appeared to be formed in "bait" *G. epigaeus* and *G. fasciculatus* spores which came in contact with infected *G. fasciculatus* sporocarps in water. *H. fuscoatra* was isolated at least once from infected *G. fasciculatus* plated on CMDY. Similarly, *A. pseudolongissima* was isolated on agar from infected *G. epigaeus,* but less frequently than from *G. fasciculatus.*

The chytridlike organism was originally isolated not only from hyperparasitized *G. fasciculatus*, but subsequently from naturally infected *G. epigaeus* and *G. margarita* spores as well.

Numerous sporangia of the chytrid were formed on *G. margarita* spores (Fig. 3) or pollen of a *Liquidambar* sp. The size of the sporangia varied depending on the host they infected. For instance, sporangia formed on *G. margarita* spores averaged 47–56 μm in diameter, while sporangia formed on *G. fasciculatus* spores averaged 10–14 μm in diameter. Sporangia contained one to three exit papillae.

Following transfer to fresh sterile water, the sporangia released posteriorly uniflagellate zoospores with a prominent central starch granule. The zoospores were elongated (6–8 × 2.5–4 μm) when first released. Within minutes the zoospores became globose (2.4–3.4 μm in diameter) with 14–17 μm long whiplash-type flagella. Resting spores were not evident. A thin threadlike, unbranching rhizoidal system, however, was observed in the host spores. This chytridlike organism was identified as a *Phlyctochytrium* sp. by Donald J. S. Barr (*personal communication*).

Chlamydospore inoculation with hyperparasites. From the previous experiments, it was not entirely clear whether *A.*

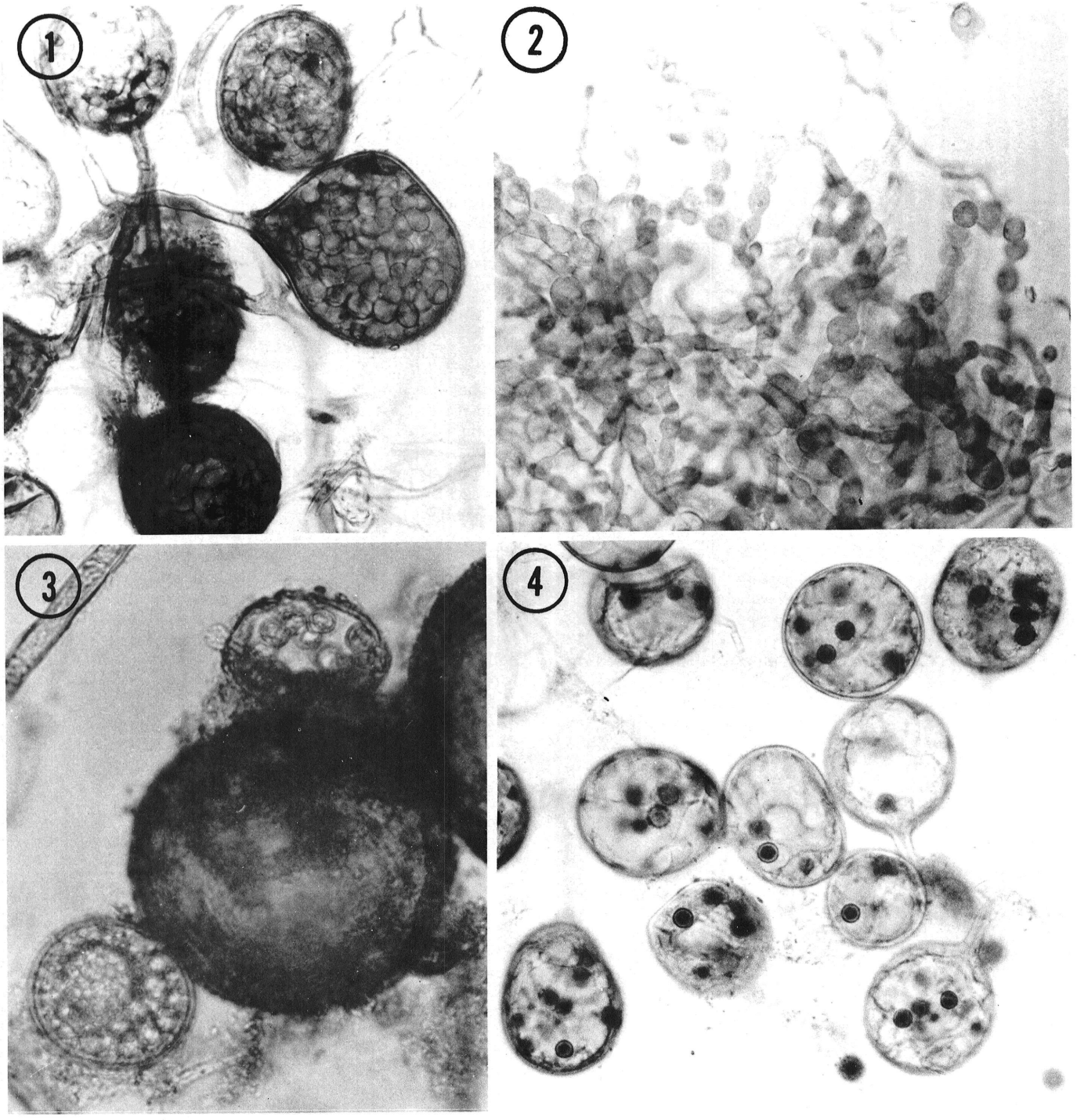
TABLE 1. Percent visibly parasitized spores of *Glomus epigaeus* and *Glomus fasciculatus* after incubation for 3 wk on agar or in autoclaved blow sand inoculated with *Anguillospora pseudolongissima* or *Humicola fuscoatra*

Host/parasite combination	Parasitism[a] (%)	
	On water agar[b]	In autoclaved soil[b]
G. epigaeus/*A. pseudolongissima*	51 C	69 B
G. epigaeus/*H. fuscoatra*	48 C	71 B
G. fasciculatus/*A. pseudolongissima*	67 B	95 A
G. fasciculatus/*H. fuscoatra*	57 C	91 A
G. epigaeus	3 D	1 D
G. fasciculatus	3 D	2 D

[a] After 3 wk on water agar, some spores in each sporocarp appeared to have collapsed. Whether this resulted from parasitism or drying out of spores was difficult to determine so these spores were not counted. Therefore, the number of spores parasitized on water agar may have been greater than indicated here. Spores containing large, swollen, sausage-like hyphae of *A. pseudolongissima* or aleurospores and hyphae of *H. fuscoatra* were considered parasitized.

[b] Values in both columns not followed by identical letters are significantly different, $P = 0.05$.

pseudolongissima and *H. fuscoatra* were hyperparasitic on *Glomus* spores or only saprophytic on dead spores and/or the peridial material surrounding spores in sporocarps. However, sporocarps of *G. fasciculatus* and *G. epigaeus,* not visibly parasitized, became >48% parasitized when placed on water agar or in autoclaved soil containing either *A. pseudolongissima* or *H. fuscoatra* (Table 1). The low level (<4.0%) of infection recorded in the controls reflects the number of spores which appeared to be vacuolated. Whether those spores were senescent or injured during handling is unclear. Parasitized sporocarps also contained vacuolated spores, but in greater numbers (>20%), this being the final result of parasitism. A spore parasitized by *H. fuscoatra* contained hyphae and/or aleuorospores (Fig. 4). Determination of parasitism by *A. pseudolongissima* was based on presence of sausage-like hyphae within the host spores. *G. fasciculatus* appeared to be significantly more susceptible to infection by both *A. pseudolongissima* and *H. fuscoatra* in autoclaved sand and to *A. pseudolongissima* on water agar than did *G. epigaeus.* In young spores, hyphae of *H. fuscoatra* appeared to enter the subtending hyphae of *Glomus* spores and then proceed into the spore itself. This mode of entry was less apparent in older *G. fasciculatus* spores once the passage was occluded by thickening of the spore wall at the point of hyphal



Figs. 1–4. Fungal hyperparasitization of vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus fasciculatus* and *Gigaspora margarita*). **1,** Spores of *G. fascuclatus* containing large swollen sausage-like hyphae of *Anguillospora pseudolongissima* (×368). **2,** Axenic water agar culture (growing from mycelium in a disk of potato dextrose agar) of *A. pseudolongissima*; the hyphae resemble those formed in hyperparasitized VAM fungus spores (×368). **3,** *G. margarita* spores with two *Phlyctochytrium* sp. sporangia attached to one of them. Note the zoospores in one of the sporangia (×460). **4,** *G. fasciculatus* spores containing aleurospores and hyphae of *Humicola fuscoatra* (×368).

attachment.

Pathogenesis of the *Phlyctochytrium* sp. was tested on spores of four species of VAM fungi including two isolates of *G. fasciculatus*. An average of 85% of the *G. margarita* spores became parasitized after 5 days, while only 17% of the *G. epigaeus* spores were similarly parasitized. The sporocarpic isolate of *G. fasciculatus* became 36% parasitized, while the nonsporocarpic, darker-spored isolate was 27% parasitized. Spores of *G. constrictus* appeared to be entirely resistant to this hyperparasite. The susceptibility of *G. epigaeus* spores to *Phlyctochytrium* sp. increased following soaking in H_2O_2, sodium hypochlorite, and KOH for any of the time intervals tested (Table 2). Susceptibility of spores soaked in acid was reduced, however.

TABLE 2. Effects of various chemical compounds on the susceptibility of *Glomus epigaeus* spores to hyperparasitism by a *Phylctochytrium* sp.

Chemical	Treatment time (min)	Hyperparasitism (%)[a]
H_2O	60	20.5 C
H_2O_2	30	82.0 AB
	60	85.0 AB
NaClO	3	92.5 A
	5	85.0 AB
	10	86.2 AB
KOH	60	79.3 B
HCl	60	0

[a]Values in each column not followed by the same letter are significantly different, $P = 0.05$.

TABLE 3. Radial growth (cm) of *Anguillospora pseudolongissima* and *Humicola fuscoatra* after 11 days on cornmeal agar plates containing varied concentrations of fungicides

Fungicide	Concentration (μg/g)	Radial growth (cm)[a] *A. pseudolongissima*	*H. fuscoatra*
Chloroneb	0.4	1.0 L	0.3 I
	0.8	0.8 M	0.3 I
	1.6	0.7 MN	0.0 I
Dichloran	50.0	4.1 CDE	3.3 F
	100.0	2.9 G	1.9 G
	200.0	2.6 H	1.1 H
Metalaxyl	50.0	4.4 ABC	4.7 ABC
	100.0	4.5 A	5.1 A
	200.0	4.2 BCD	5.2 A
Thiram	1.5	3.5 F	4.6 BC
	3.0	3.0 GH	4.9 AB
	6.0	1.4 K	4.8 AB
Mancozeb	0.8	1.9 IJ	4.3 CD
	1.6	1.3 K	3.9 DE
	3.2	0.5 N	3.4 F
Captan	2.5	4.4 AB	4.8 AB
	5.0	4.1 CDE	5.1 A
	10.0	4.3 ABC	4.9 AB
DBCP	10.0	4.3 ABC	4.9 AB
	20.0	4.4 AB	4.9 AB
	40.0	4.4 AB	4.8 AB
Ethazole	12.5	4.3 ABC	4.6 BC
	25.0	3.9 E	4.2 CD
	50.0	4.0 DE	3.3 EF
PCNB	50.0	1.8 J	0.8 H
	100.0	1.8 J	0.9 H
	200.0	2.1 I	1.0 H
Control	···	4.28 ABC	4.98 AB

[a]Values in each column not followed by identical letters are significantly different, $P = 0.05$.

Chemical control of hyperparasites. Growth of *A. pseudolongissima* and *H. fuscoatra* was significantly reduced on agar by chloroneb, DBCP, mancozeb, ethazole, and PCNB (Table 3). However, in sterile sand, only high concentrations of DBCP, mancozeb, and PCNB significantly reduced the numbers of both hyperparasites (Table 4).

Germination of *G. epigaeus* was entirely inhibited in all but the mancozeb-treated soil. At the highest mancozeb concentration, germination also was inhibited, but an average of 16 and 57% germination occurred at the medium and low levels of mancozeb, respectively. Germination at the low level of mancozeb (61%) did not differ significantly from germination in nontreated soil.

Parasitism of *G. margarita* by the *Phlyctochytrium* sp. was completely controlled in sterile water containing 100 or 200 ppm ethazole. In contrast, 71% parasitism occurred in water without ethazole. In water containing 25 or 50 ppm ethazole, 44 and 29% hyperparasitism occurred, respectively.

DISCUSSION

Spores of *G. epigaeus* and *G. fasciculatus* were readily parasitized by either *H. fuscoatra* or *A. pseudolongissima*. The pluglike hyphal attachment characteristic of *G. epigaeus* spores may have made entry by hyperparasites less likely in mature spores or even in those approaching maturity. *H. fuscoatra*, thought to be a common soil saprophyte (2), also has been demonstrated to parasitize oospores of *Phytophthora* and *Pythium* spp. (15). Ross and Ruttencutter (13) described a *Phlyctochytrium* sp. hyperparasite of *Glomus macrocarpus* which appears similar to the one described here, although it is not possible to determine positively whether it is the same species. However, the *Phlyctochytrium* sp. described in this paper has also been isolated from hyperparasitized *Glomus* spores by R. H. Estey (*personal communication*). Thus, a *Phlyctochytrium* sp. hyperparasite on *Glomus* spores has been isolated on three occasions from very dissimilar environments. Apparently it is an important and widely distributed hyperparasite.

TABLE 4. Hyperparasitism of *Glomus epigaeus* spores by *Anguillospora psuedolongissima* and *Humicola fuscoatra* in sterile sand soil amended with various fungicides

Fungicide	Conc (μg/g)	*G. epigaeus* spores parasitized (%)[a] *A. pseudolongissima*[b]	*H. fuscoatra*[b]
DBCP	50.0	58.77 AB	22.76 EFG
	100.0	62.52 AB	31.22 DEF
	200.0	16.58 DE	19.51 G
Mancozeb	0.8	52.71 AB	67.05 A
	1.6	56.06 AB	36.32 CDE
	3.2	10.85 E	36.91 CDE
Ethazole	12.5	41.76 ABCD	45.01 BCD
	25.0	36.45 BCDE	37.53 CDE
	50.0	45.88 ABC	20.11 EFG
PCNB	50.0	45.23 ABC	34.00 CDEF
	100.0	41.16 ABCD	16.23 FG
	200.0	25.56 CDE	18.98 EFG
Chloroneb	0.4	60.60 AB	51.49 ABCD
	0.8	46.96 ABC	48.93 ABCD
	1.6	61.17 AB	42.13 BCD
No fungicide + hyperparasite	···	67.31 A	57.19 AB
No fungicide no hyperparasite	···	9.82 E	9.82 E

[a]Spores containing large, swollen, sausage-like hyphae of *A. pseudolongissima* or aleurospores and hyphae of *H. fuscoatra* were considered parasitized.

[b]Values in both columns not followed by identical letters are significantly different, $P = 0.05$.

Sneh et al (15) reported that oospores, like chlamydospores of *G. epigaeus* and *F. fasciculatus*, appeared more resistant to parasitism by *H. fuscoatra* and *A. pseudolongissima* at maturity, following melanization. VAM spores responded similarly to parasitism by the *Phlyctochytrium* sp. VAM species which produced dark-colored, heavily melanized spores were more resistant to parasitism. In fact, *G. constrictus* spores, which are almost black, were entirely resistant. Conversely, *G. margarita* spores which are white were most susceptible. This trend was evident even within species. The dark-spored isolate of *G. fasciculatus* was more resistant to parasitism than was the light-spored isolate.

The melanin content of spore walls therefore may be related to susceptibility of VAM spores to hyperparasitization. This is demonstrated by the increased susceptibility of *G. epigaeus* spores exposed to KOH or to strong oxidizing agents such as H_2O_2 or sodium hypochlorite. These substances are known to decolorize or in some way deactivate melanin pigments (7). Of particular interest is the increased susceptibility of spores to hyperparasitism following treatment with sodium hypochlorite since this treatment is one well-accepted method of surface sterilization. In situations where hyperparasites are a potential danger (eg, in pot culturing), perhaps alternate surface sterilization methods should be sought.

Methods for the commercial production of VAM fungi have been designed (10). However, hyperparasites of VAM spores could be a serious problem in the commercial production of VAM fungi and no doubt play a major role in the variable results provided by different batches of mycorrhizal inoculum. If the melanization which occurs at spore maturity affords protection from hyperparasitism, then the use of fungicides such as mancozeb and ethazole which retard hyperparasite growth may allow VAM spores to "escape" parasitism. Application of chemicals which reduce hyperparasites of VAM fungi in pot cultures may thus allow a greater number of spores to mature and insure a higher level of VAM spore viability and inoculum dependability. Menge et al (9) demonstrated that application of two pesticides actually increased infection and sporulation of VAM fungi. They speculated that this stimulation resulted from reduced populations of VAM hyperparasites. In view of our results, this interpretation seems probable.

Hyperparasites in pot cultures of VAM fungi may be controlled by direct application of chemicals such as mancozeb which inhibit growth of hyperparasites without entirely inhibiting the VAM fungi. Direct application of fungicides may not be feasible, however, if these chemicals have deleterious effects on the VAM fungi. In this study, ethazole controlled *Phlyctochytrium* hyperparasitism, but also inhibited VAM spore germination. In contrast, Menge et al (9) have shown stimulation of VAM fungi by ethazole when it was added after infection had taken place. Apparently the timing of application of fungicides is important and ethazole, though it inhibited spore germination, may still be useful when directly applied to pot cultures. Alternatively, fungicides could be used to presoak spores, prior to inoculation of pot cultures.

Use of hyperparasites has been suggested as a control for plant pathogenic organisms (1). However, results reported in this paper demonstrate that parasites of pathogenic fungi also may be parasites of VAM fungi. Therefore, care must be exercised when initiating biological control programs based on amendment or increase of hyperparasite populations. Plants such as citrus and sweetgum (*Liquidambar styraciflua* L.) are extremely dependent on mycorrhizal fungi for survival. Reduction of mycorrhizal infection in these plants due to hyperparasitism of VAM fungal spores would, in effect, cause disease in these plants. In this respect, hyperparasites of VAM fungi could be considered to be secondary plant pathogens, even though they are not primary pathogens of higher plants.

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