Glomus fasciculatum, a Weak Pathogen of Heterodera glycines[1]

L. J. Francl and V. H. Dropkin[2]

Abstract: The occurrence of chlamydospores of *Glomus fasciculatum* (Gf) within cysts of the soybean cyst nematode, *Heterodera glycines,* and the effects of vesicular-arbuscular mycorrhizae on nematode population dynamics and soybean (*Glycine max*) plant growth were investigated. Chlamydospores occupied 1–24% of cysts recovered from field soil samples. Hyphae of Missouri isolate Gf1 penetrated the female nematode cuticle shortly after she ruptured the root epidermis. Convoluted hyphae filled infected eggs, and sporogenesis occurred within infected eggs. *G. microcarpum, G. mosseae,* and two isolates of Gf were inoculated with *H. glycines* on plants of 'Essex' soybeans. Each of the two Gf isolates infected about 1% of the nematode eggs in experimental pot cultures. The Gf1 isolate decreased the number of first-generation adult females 26%, compared with the nonmycorrhizal control. The total numbers of first-generation plus second-generation adult females were similar for both Gf isolates and 29–41% greater than the nonmycorrhizal control. Soybean plants with Gf and *H. glycines* produced more biomass than did nonmycorrhizal plants with nematodes, but only Gf1 delayed leaf senescence.

Key words: soybean cyst nematode, vesicular-arbuscular mycorrhiza, *Glycine max,* biocontrol.

Root-parasitic nematodes and mycorrhizal fungi both obtain their nutrition from higher plants, but most often they have opposite effects on plant growth. When they coexist in the same root system, an intermediate plant growth response usually results (10). The relationship between nematode and fungus may be stimulatory, depressive, or neutral to one or both organisms. Deleterious effects to nematodes are specific in particular interactions (13). Nematode attraction, penetration, juvenile development, or reproduction may be affected when nematode populations are depressed in the presence of mycorrhizae (8). Indeed, few nematodes penetrate and juvenile development is retarded in several interactions (2,14,15). The mechanisms governing these effects are unknown, but changes in root exudates could alter nematode attraction, structural modifications such as thick-walled vesicles could deter penetration, and shifts in physiology might retard nematode development and reproduction.

Nematodes in the family Heteroderidae are sedentary endoparasites, and many are important to agriculture. The female body wall forms a cyst in most species of this family. There have been reports (5,20) of *Heterodera avenae* Woll. cysts containing chlamydospores of vesicular-arbuscular mycorrhizal (VAM) fungi, but the question of whether a VAM fungus is capable of infecting sedentary nematodes is still open (19). An alternative explanation for the presence of chlamydospores within cysts is that the fungus sporulates preferentially in soil voids and that empty or partially empty cysts serve as suitable sporulation sites. VAM fungal chlamydospores have been reported to occupy seeds and dead insects in soil (12,16).

Chlamydospores of the VAM fungus, *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. and Trappe (Gf), were found within cysts in a greenhouse population of the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe. SCN egg production was lower than expected in this population. Our objective was to determine if Gf was infecting SCN and, if so, whether this fungus was a potentially useful biocontrol agent. Plant response to the paired combinations of *H. glycines* with *Glomus microcarpum* Tul. and Tul., *G. mosseae* (Nic. and Gerd.) Gerd. and Trappe, and two isolates of *G. fasciculatum* were also investigated.

MATERIALS AND METHODS

Survey: SCN cysts from four Missouri locations and the greenhouse SCN population were examined for *Glomus* spp. chlamydospores. Cysts were eluted from soil

and all cysts were dissected to ensure a complete inventory of cysts containing chlamydospores.

Mycorrhizal associations: Formation of a mycorrhizal association of a greenhouse isolate of *G. fasciculatum* (Gf1) originally from Marthasville, Missouri, with soybean, *Glycine max* (L.) Merr. 'Williams', was determined. Soybean seeds were surface sterilized for 5 minutes in 1% sodium hypochlorite, rinsed, and germinated on water agar. Fine-textured river sand, previously treated with methyl bromide, was placed in sodium hypochlorite disinfected polyvinyl chloride (PVC) pipes (2.5 cm d × 15 cm long). An aqueous suspension of 50 spores was removed from dissected cysts and pipetted into a depression in the sand. One 3-day-old soybean seedling was inserted into each depression. Sand in control pipes was not inoculated with spores. Root systems were evaluated for VAM 30 days later (11).

Diseased SCN eggs filled with aseptate hyphae occurred together with Gf1 chlamydospores inside cysts from a greenhouse nematode population. Eggs were placed on acidified potato dextrose and water agar media to attempt to isolate nonmycorrhizal fungi. Also, infected eggs were used as mycorrhizal inoculum on soybean plants. Eggs were separated from Gf1 chlamydospores by micropipet, and four seedlings received 85 eggs each via the system described earlier for chlamydospore inoculation. Four noninoculated seedlings were planted as a control. In a second test, cysts were collected from an SCN stock culture containing Gf1 and grouped according to age by sorting into white and brown colors. Eggs were collected from cysts that did not contain chlamydospores by crushing the cysts on a 150-μm-pore sieve and centrifuging in a sucrose density gradient (1). Sand in PVC pipes was not inoculated or inoculated with 2,500 eggs or chopped mycorrhizal roots. Treatments were replicated four times. Whole crushed cysts were used as inoculum instead of an egg suspension in a third trial.

Coinoculation experiments: Greenhouse time-course experiments were conducted to determine the sequence of events in the Gf1–SCN association. Dexter fine sand (Typic Ultic Hapludalf) fumigated with

TABLE 1. Occurrence of *Glomus* spp. chlamydospores inside cysts collected from four Missouri locations and from a greenhouse population derived from the Marthasville location.

Location	Total cysts observed	Cysts containing chlamydospores
Center	35	7
Marthasville	167	39
Portageville	479	3
Steele	25	6
Greenhouse	23	13

methyl bromide was used as the plant growth medium for these experiments and in experiments described in the next section. Chlamydospores for inoculum were obtained by wet sieving Gf1 cultured on bahiagrass, *Paspalum notatum* Flugge (4). Eggs were collected from nonmycorrhizal cultures of SCN, and motile juveniles were separated from eggs by Baermann funnel. Juveniles were surface sterilized for 20 minutes in an aqueous solution containing 50 g chlorhexidine diacetate per liter to further reduce the possibility of adding egg pathogens. Inoculation rates were 700–1,000 Gf1 chlamydospores and 500–1,500 SCN juveniles per plant. PVC pipes and 3-day-old seedlings were used as before. Single or replicate plants were harvested 12–75 days after coinoculation.

Fungus preinoculation: Based on the low rates of egg infection in coinoculation experiments, we attempted to maximize the effect of VAM fungi on the SCN population by inoculating Essex soybean seedlings with VAM spores 14 days before adding nematodes and by using high rates of chlamydospore inoculum to minimize the distance between spores and SCN females. Chlamydospores were obtained from bahiagrass cultures by wet sieving and were uniformly mixed with sand in PVC pipes. Essex soybean seedlings and nematode juveniles were prepared as described for coinoculation experiments, except that a second group of plants was placed in larger PVC pipes (4 cm d × 30 cm long) to permit longer plant growth. The experiment was begun on 9 March 1983 with five replications per treatment in both pipe sizes. The control group had autoclaved chopped mycorrhizal root pieces mixed with soil. The

FIG. 1. Chlamydospores of *Glomus fasciculatum* developing within eggs from a *Heterodera glycines* cyst collected from a pot culture. Distance between bars = 10 μm.

Glomus species tested were *G. mosseae* (source: University of Missouri), *G. microcarpum* (source: Abbott Laboratories), *G. fasciculatum* (Gf1) (source: Marthasville, Missouri), and *G. fasciculatum* (Gf2) (source: Abbott Laboratories); inoculation rates were 2,100, 2,400, 20,000, and 7,000 chlamydospores per plant, respectively. Soybeans were inoculated with 4,000 SCN juveniles 2 weeks after planting. Half of the juveniles were delivered through a 7-cm-long tube inserted 5 cm deep into the sand at planting, and half were applied to the sand surface and washed in with water. Plants received weekly applications of 15 ml Hoagland's solution (7) lacking phosphorus.

Plants were harvested 27 and 45 days after inoculation with nematodes. Roots were sprayed with water to remove adult SCN females for counting. First-generation and second-generation females were distinguishable by color and size at 45 days. Second-generation females still attached to roots after spraying were counted in situ. Roots and tops were oven dried at 50 C for 4 days for dry weight determination. Plant height, number of trifoliate leaves, and number of senescent trifoliates were also measured.

A second experiment was begun on 25 May 1983. Gf1 was inoculated at 5,000 and 20,000 chlamydospores per plant to determine if the difference between Gf1 and Gf2 in the first experiment resulted from a dose response to Gf1. Gf2 was added at 5,000 chlamydospores per plant, similar to the 7,000 chlamydospore dosage used in the first experiment. SCN juveniles were added at 2,300 per plant. The treatments were replicated seven times for the 27-day harvests and six times for the 45-day harvests. All other experimental parameters were the same as in the first experiment. After an exploratory statistical analysis, data from repeated treatments of the two experiments (Gf1, Gf2 and controls) were combined to maximize analytical power.

RESULTS

Survey: Chlamydospores were found inside cysts collected from all four Missouri locations (Table 1). When many chlamydospores were within a cyst, the cyst wall had noticeable protrusions. Also, the round spore shape contrasted with the oval shape of eggs when viewed through translucent cyst walls. The predominant species observed was *G. fasciculatum*, although *G. microcarpum* also may have been present be-

TABLE 2. Effect of two isolates of *Glomus fasciculatum* on *Heterodera glycines* female numbers and egg production.

| Treatment | Numbers of females | | | Eggs at 45 days | |
	First generation	Second generation	Total*	Total	Per cyst*
Gf1	479 b	882 a	1,435 a	42,650 b	83 a
Gf2	644 a	732 a	1,306 a	54,820 a	97 a
Control	621 a	397 b	1,015 b	53,740 a	92 a
P (a = b)	< 0.001	< 0.001	< 0.001	< 0.05	n.s.

Data from two experiments are combined and all values represent least squared means from a linear model. Analysis of variance and mean separations are based on square root transformations of female counts and on rank transformations of total eggs and eggs per cyst.

* First-generation females were counted at 27 and 45 days after inoculation on different plants; therefore, total females and eggs per cyst are not calculable from first-generation plus second-generation females in these summary data.

cause some of the chlamydospores were smaller than the size reported for *G. fasciculatum* (17).

The condition of the cyst contents was quite variable. Some cysts contained only egg shells and chlamydospores; others had chlamydospores, healthy eggs, and juveniles; still others had chlamydospores and eggs infected with fungi. Sporogenesis occurred within infected eggs (Fig. 1).

Mycorrhizal associations: Chlamydospores dissected from cysts formed mycorrhizal associations with soybean. In two trials, all 14 inoculated root systems showed characteristic vesicles and arbuscules but nine noninoculated root systems were not colonized.

Neither VAM fungi nor nonmycorrhizal fungi could be isolated directly from fungus-infected eggs placed on agar media. Roots were not colonized with VAM when fungus-infected eggs were used as inoculum, although roots inoculated with chlamydospores became mycorrhizal.

Coinoculation experiments: Most SCN females ruptured the root epidermis 12 days after inoculation with juveniles. At 15 days, hyphae from a sporocarp were observed penetrating the cuticle of a young female. After about 20 days, cysts enmeshed by Gf1 hyphae usually contained infected eggs. Infected eggs were filled with convoluted, aseptate hyphae. Eggs laid in the egg sac matrix and eggs retained within the female both became infected. Usually, infective hyphae originated from sporocarps in close proximity to females, but hyphae growing along the surface of the root also were infective. White, immature chlamydospores were seen inside cysts 56 days after inoculation. The incidence of egg infection was

quite low, regardless of time after coinoculation. No other fungal pathogens of cysts or eggs were observed.

Fungus preinoculation: Roots infected with Gf1 in the first experiment had fewer ($P = 0.05$) first-generation SCN females attached than did roots of nonmycorrhizal plants. Other VAM fungi had no such effect; roots infected with *G. microcarpum* had more females than did nonmycorrhizal plants ($P = 0.05$). All mycorrhizal treatments averaged more second-generation females than did nonmycorrhizal controls. Aliquots of 2,500–4,400 eggs from crushed cysts were examined for infected eggs at 45 days. Infected eggs were estimated at 1.1% for Gf1, 0.7% for Gf2, 0.2% for *G. microcarpum,* and 0.0% for *G. mosseae* and the nonmycorrhizal controls. Oily eggs (19) occurred in all treatments at a similar low level.

Gf1 inoculum at dosages of 5,000 and 20,000 chlamydospores per plant in the second experiment did not differ ($P = 0.05$) in their effects on numbers of females and plant growth. The effect of Gf2 on SCN and plant growth also was consistent over two trials. These results therefore were combined over two experiments (Table 2). Gf1 applied at 20,000 chlamydospores per plant suppressed the first-generation female population by 23% and 26% relative to Gf2 and the control, respectively. Second-generation and total female populations for both Gf1 and Gf2 were significantly higher than for the controls. Total females produced on mycorrhizal plants were 29% (Gf2) and 41% (Gf1) higher than on nonmycorrhizal plants.

The combined analysis of eggs per plant and eggs per cyst at 45 days required non-

TABLE 3.   Effects of two *Glomus fasciculatum* isolates on soybean growth parameters 27 and 45 days after inoculating *Heterodera glycines*.

Treatment	Top dry weight (mg)	Root dry weight (mg)	Total biomass* (mg)	Shoot–root* ratio	Number of trifoliates	Number of se- nescent trifoliates
27 days						
Gf1	532 a	259 a	798 a	2.1 a		
Gf2	484 a	265 a	752 a	1.8 b		
Control	396 b	245 a	589 b	1.4 c		
P	< 0.05	n.s.	< 0.01	< 0.05		
45 days						
Gf1	873 a	716 a	1602 a	1.3 a	6.5 a	0.7 a
Gf2	747 b	703 a	1473 a	1.1 a	6.5 a	2.1 b
Control	518 c	553 b	1084 b	1.0 a	6.0 b	2.2 b
P	< 0.05	< 0.01	< 0.01	n.s.	< 0.05	< 0.01

Dry weight values are geometric least squared means from a logarithmic transformation; other values are untransformed least squared means.

* Total biomass and shoot–root ratios are not calculable from these summary data because of the display of geometric least squared means.

parametric methods because the variance among treatments was not homogeneous after transformation. Data are presented as least squared means with an analysis of variance based on ranking the variates (Table 2). Plants treated with Gf1 had fewer ($P = 0.05$) eggs than did nonmycorrhizal plants or plants treated with Gf2. Numbers of eggs per first-generation cyst were not significantly different. The experiment was ended before eggs were produced by second-generation females.

Plant growth responses were combined over two experiments (Table 3). Higher values for most weight measurements, in the shoot–root ratio at 27 days and in leaf measurements at 45 days, occurred in VAM versus nonmycorrhizal plants. The Gf1 and Gf2 treatments gave similar results, except Gf1-treated plants yielded greater shoot dry weights and less leaf senescence after 45 days. Plant heights at 27 or at 45 days were not significantly different among the treatments ($P = 0.05$).

DISCUSSION

The survey of four Missouri locations, published reports from England (5,19,20), and personal communication with nematologists in the United States confirm that VAM chlamydospores within cysts of Heterodidae have been observed in widely separated geographical areas. The observed variability in the condition of the cyst contents apparently reflects the age of the specimen as well as the status of the cyst when the encounter with the fungus took place. Higher percent occupation of SCN cysts in some soil samples probably results from factors that might include differences in fungal strain, soil environment, and perhaps presence of empty cysts.

Evidence for infection of SCN eggs by Gf is conclusive, but predisposing factors were not studied. Involvement of other microorganisms was not rigorously eliminated, although precautions were taken and no unexpected organisms were observed. One approach to resolving the question of predisposition would be to aseptically culture Gf, SCN, and soybeans together (6,9). *G. microcarpum* also may infect SCN eggs. *G. microcarpum*, *G. fasciculatum*, and *G. macrocarpum* are considered to form a taxonomic continuum (18) and might be expected to behave similarly toward cyst nematodes.

The level of Gf-infected eggs occurring under experimental conditions was low, and VAM infection of roots via inoculation with Gf1-infected eggs was not accomplished. Nematode infection by *Glomus* seemed to depend on the vigor of fungal growth, either when the fungus was mobilizing stored substrate in chlamydospores or when the plant was providing substrate. The nutritional aspect of the relationship requires further exploration.

In experiments where plants were preinoculated with VAM fungi, suppression of populations of first-generation SCN females occurred only with Gf1. Suppression of females appears to be distinct from egg infection by Gf1 because Gf2 also in-

fected SCN eggs without depressing the number of first-generation females and because SCN infection by Gf1 seemed too low to account for the population suppression. Number of eggs per cyst did not differ among the treatments, suggesting that reproduction was not affected.

Although numbers of first-generation females were suppressed by Gf1, suppression was not carried over into the second generation. Application of both Gf1 and Gf2 resulted in more females compared to the numbers of females produced on nonmycorrhizal plants. The greater biomass of mycorrhizal plants at 27 days evidently supported development of greater numbers of nematodes than in nonmycorrhizal plants.

The beneficial effect of VAM on soybean growth in the presence of SCN is obvious, but responses of plants whose roots were colonized by Gf1 and Gf2 differed significantly in several parameters. Fewer first-generation SCN females on Gf1-infected roots than on Gf2-infected plants presumably stressed the plants less and promoted better shoot growth and leaf maintenance. Differences in plant growth response to various species and isolates of VAM fungi have been reported (3).

We have shown that one, and possibly two, species of *Glomus* can infect SCN eggs. Further, one isolate of *Glomus* suppressed SCN female populations initially but was not capable of limiting the population in the second generation. Therefore, biocontrol of SCN by these fungi is unlikely. Since there was considerable variation in response of the SCN–soybean system to *Glomus* isolates and species, it is probable that yet uncharacterized VAM isolates are even more detrimental to SCN. A large screening operation would be required to find a beneficial economic response.

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