Evaluation of *Arthrobotrys amerospora* as a Biocontrol Agent for *Heterodera glycines* on Soybean

T. L. NIBLACK, Graduate Research Assistant, and R. S. HUSSEY, Professor, Department of Plant Pathology, University of Georgia, Athens 30602

ABSTRACT

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A nematode-trapping fungus, *Arthrobotrys amerospora*, combined in three commercial preparations with *Rhizobium japonicum* inoculum was evaluated for control of *Heterodera glycines* on soybean (*Glycine max* 'Bragg') in the field and greenhouse. In four successive greenhouse studies, soybeans were inoculated at planting with *H. glycines* eggs (0, 10,000, or 20,000 per 15-cm-diameter pot) and treated with one of the *A. amerospora* preparations or aldicarb at recommended rates or were left untreated. Two field studies of the same nematode-control treatments plus ethylene dibromide (EDB) were conducted in soil naturally infested with *H. glycines*. In the greenhouse, only aldicarb significantly reduced nematode populations compared with the untreated controls, whereas in the field, only EDB provided significant nematode control. Yield was not affected by any of the treatments in either the greenhouse or the field. *A. amerospora* is not considered a promising biocontrol agent for *H. glycines* on soybean.

Nematode-trapping fungi generally have not shown promise as biocontrol agents in production agriculture (3,9,11). However, Cayrol (2) recently reported significant control of *Meloidogyne incognita* (Kofoid & White) Chitwood on field-grown tomato with a commercial preparation of *Arthrobotrys irregularis* (Matr.) Mekht. in the absence of added organic matter. *A. amerospora* Schenck, Kendr., & Pramer, like *A. irregularis*, is a fast-growing, efficient but nonspecific nematode trapper in vitro that captures its prey by means of adhesive hyphal loops or networks. Recently, *A. amerospora* was made available in commercial preparations (Kalo Agricultural Chemicals, Inc., Columbus, OH) in combination with *Rhizobium japonicum* Kirch. and intended for in-furrow application with soybeans at planting. These products had potential for control of *Heterodera glycines* Ichinohe on soybean according to preliminary studies (R. Gonzales, Agrigenetics Corp., Boulder, CO, *personal communication*). *H. glycines* is one of the primary soybean pathogens in Georgia for which there is no effective, economical means of chemical control. Therefore, we determined whether commercial preparations of *A. amerospora* would provide yield-enhancing control of *H. glycines* on a susceptible soybean cultivar.

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MATERIALS AND METHODS

Glycine max (L.) Merr. 'Bragg', susceptible to *H. glycines*, was used in all field and greenhouse tests. A commercial preparation of *R. japonicum* was added to the check pots and to those receiving the nematicide. Soil samples were processed for nematode extraction by elutriation and centrifugal flotation (6). The extraction efficiency of this procedure for second-stage juveniles was 35%, and data presented are not adjusted for extraction efficiency. Hussey and Boerma's method (5) was used for egg extraction. Nematodes were counted with a dissecting microscope at ×40. Nematode counts were transformed to $\log_{10}(X + 1)$ values to stabilize variance; nematode data reported are antilogs. All data were subjected to analysis of variance appropriate to the experimental design. Single degree-of-freedom comparisons were used to investigate significant main effects.

In vitro assays. Assays for the presence of nematode-trapping fungi in field and greenhouse soil samples were made on five replicates per 500-cm^3 soil sample by a semiquantitative method (8) on 1:3 cornmeal/water agar. Two of the products were granular formulation (Dormal and Legume-Aid), and the third was a frozen liquid concentrate (Rhizobia+). Five replicates of 0.1-g samples of Legume-Aid and Dormal were assayed twice on the same medium by sprinkling the sample on the agar surface in petri dishes. Fungi were isolated from 0.5-ml samples from serial dilutions of Rhizobia+ ($1:10^1$–$1:10^6$).

Preplant soil samples from field plots were taken in 1984 for population estimates of bacteria, actinomycetes, and fungi by the dilution plate method (7). Bacteria were isolated on soil extract agar, actinomycetes on Benedict agar, and fungi on peptone-dextrose-rose bengal agar containing 2 μg/ml of aureomycin (7). The microorganisms were screened for inhibition of radial colony growth of *A. amerospora* on Czapek's medium with two replicates per test organism.

A. amerospora isolated from the Rhizobia+ formulation was tested for trapping activity on 1:3 cornmeal/water agar. After 48 hr, either 20 *H. glycines* juveniles, 20 *Rhabditis* sp. (microbivorus nematodes), or a combination of 10 of each were added to each of five plates. Trapped nematodes were counted after 24 and 48 hr.

Field experiments. Field tests were conducted in 1983 and 1984 at the University of Georgia Plant Sciences Farm near Athens on Appling coarse sandy loam (69% sand, 12% silt, 19% clay; 5.8 pH) containing less than 2% organic matter and naturally infested with *H. glycines*. Trifluralin (0.56 kg a.i./ha) was applied before planting each year for weed control. In 1983, the experiment was conducted in a randomized complete block design with four blocks of five treatments: Legume-Aid, Dormal, Rhizobia+, aldicarb (Temik 15G), and a check. In 1984, the experiment was completely randomized with five replicates of six treatments; the additional treatment was ethylene dibromide (EDB). Aldicarb was applied in-furrow at the rate of 2.24 kg a.i./ha, and EDB was applied 20 cm deep 12.5 cm on each side of the row at the rate of 36 kg a.i./ha. Dormal and Legume-Aid were applied in-furrow at rates of 2.2 and 5.6 kg/ha, respectively. Rhizobia+ was applied in-furrow at the rate of 146 ml/ha. All treatments were applied at planting on 14 June 1983 and 4 June 1984. Treated plots in both tests consisted of two adjacent 6.1-m rows spaced 96.5 cm apart. Alleys between blocks of adjacent plots were 4.6 m wide, and border rows were planted to Bragg soybean. Plots were irrigated with 2.5 cm of water when needed to avoid severe drought stress and maintained until harvest with recommended cultural practices.

Soil samples for nematode and fungus assays were collected before planting and 6 and 12 wk after planting in both tests. In 1984, an additional sample was taken 2 wk after planting. Plot samples consisted

of 10 soil cores 2.5 cm in diameter by 20–30 cm that were mixed and subsampled for assays as described.

Greenhouse experiments. In 1983, two tests were conducted, both with factorial treatment designs arranged in six randomized complete blocks. The first was a 5 × 3 factorial conducted without supplemental lighting in May and June. The treatments were recommended rates (given previously) of Legume-Aid, Dormal, Rhizobia+, aldicarb 15G, and check, each with 0, 10,000, or 20,000 *H. glycines* eggs per pot. The second test excluded the Rhizobia+ treatment and was conducted in August and September with supplemental lighting from 400W Multi-Vapor phosphor-coated GE lamps. In 1984, two additional tests were conducted in June and July and in July and August, each in six randomized complete blocks with each of the treatments containing *A. amerospora* applied at 1× and 2× the recommended rates. Nematode inoculum levels were unchanged from 1983.

In each of the four tests, *A. amerospora* and aldicarb treatments were applied in a sterilized sand carrier and check treatments received an equivalent volume of sterilized sand alone. Experimental units were single plants thinned from five seeds planted per 15-cm-diameter pot containing 1,600 cm^3 of sterilized soil.

Nematode eggs used for inoculum were extracted from greenhouse cultures maintained on Bragg soybean. Treated plants receiving no eggs were given an equivalent volume of eggfree filtrate from the egg suspension. Pots were fertilized at weekly intervals in 1983 and at biweekly intervals in 1984 with a soluble trace-element mix and 20-20-20 (N = 20%, P = 8.7%, K = 16.6%). The studies were terminated 65 days after planting, and the following data were collected: plant height, dry shoot weight, and nematode juveniles and eggs per 100 cm^3 of soil.

RESULTS

In vitro assays. In 1983, fungi isolated from samples of Dormal and Legume-Aid that parasitized *H. glycines* juveniles in vitro were in the genera *Arthrobotrys, Dactylaria*, and *Verticillium*. Only *Arthrobotrys* was isolated from the 1984 materials. The 1983 preparation of Rhizobia+ likewise contained a *Verticillium* species, but only *Arthrobotrys* was found in the 1984 preparation. The *Arthrobotrys* species was confirmed to be *A. amerospora* by morphological comparisons with the fungus description (12) and with the type culture (American Type Culture Collection 34468). The concentrations of *A. amerospora* in the Rhizobia+ and Legume-Aid formulations were equivalent to 10^4–10^5 colony-forming units (cfu) per meter of row applied at recommended rates and to no more than 10^3 cfu/m in the Dormal formulation.

Soil dilution plates yielded an average of either 101,000 fungal, 2.76 × 10^6 bacterial, or 1.02 × 10^6 actinomycete colony-forming units per gram of soil. *A. amerospora* was inhibited on Czapek's medium by two fungi, three bacteria, and one actinomycete; in all cases, *A. amerospora* was weakly inhibited in paired cultures as indicated by narrow (<1 cm) zones of inhibition.

In the test of trapping activity, 100% of the rhabditid nematodes were captured in both single and combined treatments after 48 hr of exposure to *A. amerospora* compared with 20–80% of the *H. glycines* juveniles. There were no differences in fungal sporulation with either nematode as a food source.

Greenhouse experiments. Combined analysis for treatment combinations that were constant over the 2 yr, for relative rather than absolute values of response variables, showed that responses did not differ among experiments (Table 1). Shoot dry weight was affected by both treatment and nematode inoculum level, whereas shoot height variation was due to nematode inoculum level only. There were no treatment × *H. glycines* inoculum level interactions for any response variable. Because partitioning the *H. glycines* inoculum level main effect quantitatively (Table 1) did not elucidate the nature of its effect and because there were too few levels for reliable regression analysis, the *H. glycines* inoculum level effect (2 df) was partitioned qualitatively for plant responses in further analysis: the average effect of the two egg-containing treatments was compared with the 0 level, and the 10,000 and 20,000 levels were compared. The 0 level was not included in analyses of nematode responses.

In the 1984 experiments, treatment rates had no effect on either plant or nematode responses and did not differ among the treatments containing *A. amerospora* (Table 2). As in the 1983 and combined analyses, the effects of the 10,000- and 20,000-egg *H. glycines* inoculum levels on both plant responses were not different, whereas the average

Table 1. Effects of four nematode-control treatments on soil populations of *Heterodera glycines* and growth and yield of Bragg soybean in four greenhouse experiments[a]

		Response variable[b]			
Source of variation	df	Shoot height	Shoot dry weight	Eggs	Juveniles
Experiment	3	NS[c]	NS	NS	NS
Treatment[d]	4	NS	*	**	**
Aldicarb vs. D-L-R	1	NS	*	**	**
Check vs. D-L-R	1	NS	*	NS	NS
Check vs. aldicarb	1	NS	**	**	*
Dormal vs. L-R	1	NS	NS	NS	NS
Nematode inoculum level	2	**	**	**[e]	**[e]
Linear	1	**	**	...	...
Quadratic (deviations from linear)	1	**	**	...	...

[a] Main effect sums of squares are partitioned into sets of orthogonal contrasts.
[b] Analyses are based on values relative to the check.
[c] NS = nonsignificant, * = significant at $P = 0.05$, and ** = significant at $P = 0.01$.
[d] Treatments: Check, aldicarb, D = Dormal, L = Legume-Aid, and R = Rhizobia+.
[e] The nematode inoculum level main effect had only one degree of freedom for analysis of variance in egg and juvenile counts.

Table 2. Effects of aldicarb 15G and commercial preparations of *Arthrobotrys amerospora* at two treatment levels on growth of Bragg soybean and soil populations of *Heterodera glycines* in greenhouse tests[a]

Treatment	Rate	Nematode eggs/pot (×1,000)	Shoot height (cm)	Shoot dry weight (g)	Eggs/ 100 cm^3 soil	Juveniles/ 100 cm^3 soil
Check	...	0	50.2	13.5	...	...
	...	10	42.2	6.7	5,695	98
	...	20	44.0	7.3	6,452	55
D-L-R[b]	1×	0	48.0	11.3	...	...
	1×	10	39.6	5.9	12,777	272
	1×	20	39.7	5.8	7,188	141
	2×	0	47.1	10.9	...	...
	2×	10	40.4	6.5	13,790	587
	2×	20	40.1	6.1	11,212	231
Aldicarb	...	0	49.6	5.6	...	...
	...	10	48.3	4.9	75	15
	...	20	48.0	7.4	4	8

[a] Data are means from two tests conducted in 1984.
[b] Treatment effects of D (Dormal), L (Legume-Aid), and R (Rhizobia+) were not significantly different at $P < 0.05$. Data given are means of the three treatments containing *A. amerospora*.

effect of these levels versus the 0 level was significant. The *H. glycines* inoculum level main effect was significant for juveniles but not for egg counts. In both 1984 experiments (Table 2), egg counts were intermediate to those in the two 1983 experiments (Table 3) for corresponding treatments, whereas juvenile counts corresponded to those observed in the second 1983 experiment.

In 1983, there was a large disparity in the final numbers of juveniles and eggs extracted in the first and second experiments, although differences were not detected among the three treatments containing *A. amerospora* in either experiment (Table 3). Juvenile and egg counts in aldicarb-treated pots consistently differed from those in check and *A. amerospora* treatments over *H. glycines* inoculum levels; the *H. glycines* inoculum level main effect was significant for all but egg counts in the second experiment. Treatments containing *A. amerospora* differed from the check for juveniles in the second experiment and for eggs in the first experiment; these differences disappeared when values relative to the checks were analyzed (Table 1).

Nematode-destroying fungi extracted from pots at harvest included *A. amerospora, A. conoides, Catenaria auxiliaris*, and a *Harposporium* sp. *A. amerospora* was recovered from all pots treated with the fungus.

Field experiments. The initial *H. glycines* populations in the field plot 1 mo before planting in 1983 and 1984 were estimated at 14 and 13 juveniles per 100 cm^3 and 12,520 and 13,890 eggs per 500 cm^3 of soil, respectively. Nematode-destroying fungi isolated at planting in 1983 and 1984 included members of the genera *Arthrobotrys, Catenaria, Dactylaria, Harposporium*, and *Monacrosporium*. Six and 12 wk after planting, only *Dactylaria, Harposporium*, and *Verticillium* were isolated from the field plots. In 1984, *A. amerospora* was recovered 2 and 8 wk but not 12 wk after planting.

Treatments had no effect on yield and very little effect on *H. glycines* populations (Tables 4 and 5). *H. glycines* juveniles extracted from field soil were observed infrequently to have fungal ring structures attached. Juvenile counts were lowest in the Legume-Aid- and aldicarb-treated plots at 12 wk in 1983 (Table 4), but egg counts did not differ from those in the check plots. In 1984 (Table 5), only the EDB treatment at 12 wk reduced juvenile and egg counts below those of untreated plots. Counts of the numbers of free-living, fungivorous, plant-parasitic (other than *H. glycines*), and predaceous nematodes did not differ among treatments in either 1983 or 1984.

Table 3. Effects of aldicarb 15G and commercial preparations of *Arthrobotrys amerospora* on soil populations of *Heterodera glycines* race 3 in 1983 greenhouse tests

Treatment	Nematode eggs/pot (×1,000)	Juveniles/100 cm^3 soil		Eggs/100 cm^3 soil	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2
Check	0	...	...	...	...
	10	27	208	749	30,500
	20	175	356	4,672	27,140
D-L-R[a]	0	...	...	...	...
	10	49	156	1,604	27,230
	20	168	266	5,387	29,990
Aldicarb	0	...	...	...	...
	10	5	8	40	140
	20	16	9	221	622

[a] Treatment effects of D (Dormal), L (Legume-Aid), and R (Rhizobia+) were not significantly different at $P<0.05$. Data given are means of the three treatments containing *A. amerospora*. Rhizobia+ was excluded from the second experiment.

Table 4. Effects of aldicarb 15G and three commercial preparations of *Arthrobotrys amerospora* on the yield of Bragg soybean and soil populations of *Heterodera glycines* in a 1983 field trial

Treatment	Yield (kg/ha)	*H. glycines*			
		8 wk		12 wk	
		Juveniles/100 cm^3 soil	Eggs (×1,000)/500 cm^3 soil	Juveniles/100 cm^3 soil	Eggs (×1,000)/500 cm^3 soil
Check	1,143.3 a[z]	138 a	11.4 a	130 a	16.2 a
Legume-Aid	934.8 a	130 a	7.2 a	63 b	22.9 a
Rhizobia+	1,217.2 a	115 a	8.0 a	120 a	15.7 a
Dormal	1,345.0 a	135 a	7.9 a	198 a	14.8 a
Aldicarb 15G	1,176.9 a	98 a	11.0 a	103 b	26.3 a

[z] Means followed by the same letter are not significantly different ($P<0.05$) according to the Waller-Duncan k-ratio t test.

Table 5. Effects of aldicarb, EDB, and three commercial preparations of *Arthrobotrys amerospora* on yield of Bragg soybean and soil populations of *Heterodera glycines* in a 1984 field trial

Treatment	Yield (g/plot)	Eggs (×1,000)/500 cm^3 soil			Juveniles/100 cm^3 soil		
		2 wk	8 wk	12 wk	2 wk	8 wk	12 wk
Check	466.8 a[z]	1.7 a	9.8 ab	29.9 a	19 a	26 ab	39 a
Dormal	477.5 a	1.2 a	21.0 a	38.1 a	7 a	33 a	40 a
Legume-Aid	475.6 a	1.5 a	12.1 a	31.3 a	10 a	24 ab	55 a
Rhizobia+	413.5 a	1.4 a	16.7 a	30.5 a	10 a	24 ab	32 a
Aldicarb	442.2 a	1.9 a	12.9 a	27.9 a	18 a	12 ab	40 a
EDB	468.9 a	1.5 a	3.0 ab	5.3 b	7 a	7 b	8 b

[z] Means followed by the same letter are not significantly different ($P<0.05$) according to the Waller-Duncan k-ratio t test.

DISCUSSION

The only consistent differences in plant growth and nematode populations relative to the checks in the greenhouse experiments were due to the aldicarb treatment. Aldicarb apparently was effective in controlling nematodes and thereby increasing plant height compared with the other treatments, including *H. glycines* under greenhouse conditions. Aldicarb treatments did not result in an increase in dry shoot weights, however, and they retarded plant growth during the first 2–3 wk in both the greenhouse and field experiments. Dormal, Legume-Aid, and Rhizobia+ were all ineffective in controlling nematodes and producing plant growth increases in the greenhouse. These results are in agreement with those obtained by Rhoades (10) on corn and okra.

The disparity in nematode counts among greenhouse experiments cannot be accounted for accurately. Differences in light sources, times the tests were conducted, and fertility levels suggest sources of variation. Analysis of counts relative to those in check treatments, however, removed these effects; therefore, the treatments were ranked reliably. The presence of at least three genera of nematode-destroying fungi in the pots suggests that there is a resident population of these fungi in the greenhouse that may or may not compete with any fungi applied in the treatments.

In the field, none of the treatments were effective in controlling *H. glycines* or increasing yield. Early retardation of plant growth observed in aldicarb-treated plots did not affect yield loss at harvest. The failure of EDB to enhance the yield in 1984 suggests that even the low numbers

of *H. glycines* in the soil were above the tolerance limit for Bragg, a highly susceptible cultivar.

Recovery of *A. amerospora* from the commercial materials and from treated field and greenhouse soils verified application of viable fungal inoculum. The apparent absence of *A. amerospora* from treated field plots by 8–12 wk after planting, however, indicates a major problem with the use of nematode-trapping fungi to control phytonematodes (9). These fungi are not obligate predators, and their ability to form traps depends on their establishment in the soil through saprophytic growth. Cayrol (2) found that *A. irregularis* required 20–30 days for establishment in a soil where soil organic matter content was not limiting to provide control of *M. incognita* on tomato. Although we demonstrated the presence in field soil of microorganisms that inhibited *A. amerospora* growth, there is no evidence that they do so in nature, or that such organisms were present in greenhouse soil where results were similar even at twice the recommended treatment rates. The low organic matter content of our soils may have been a main factor limiting establishment of *A. amerospora* for any significant length of time.

Another problem with using nematode-trapping fungi is their nonspecificity (3,9). Only a portion of the nematode fauna in an agricultural habitat consists of plant parasites, and it may be that more actively moving nematodes are more likely to be trapped than the generally sluggish plant parasites, as our in vitro test suggests. It is unlikely that this had any effect on our results, however, because this particular fungus species was not found naturally in the field and did not become established after being introduced. The moderate success of Al-Hazmi et al (1) in controlling *M. incognita* on corn, although in fumigated microplots or sterilized greenhouse soil mix, may have been related to the adaptation of their fungus (*A. conoides*) to soils in their area. However, Eren and Pramer (4) found that their isolate of *A. conoides* was able to act predaceously only in sterilized soil, with live nematodes as the only amendment to support fungal growth.

The overall result of this series of studies was to underscore the difficulties involved in using a nematode-trapping fungus to control a target nematode species under accepted cultural practices. Nonetheless, the data are sufficient to conclude that *A. amerospora* in the commercial preparations was not effective as a biocontrol agent for *H. glycines* on a susceptible soybean cultivar. The formulations were effective in delivering viable fungal inoculum, however, and may prove useful with more promising biocontrol agents.

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