Aphelenchus avenae, a Potential Biological Control Agent for Root Rot Fungi

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ABSTRACT

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Various numbers of *Aphelenchus avenae,* a mycophagous nematode, were added to pots of soil artificially infested with virulent isolates of *Rhizoctonia solani* or *Fusarium solani.* Control of these root rot fungi was determined by bioassay with alfalfa seedlings transplanted to the pots after a 3-wk fungus–nematode interaction period. Concentrations of 250,000 to 1 million nematodes per 15.2-cm (6-in.) standard clay pot of fungus-infested soil consistently produced healthy, dark green plants. Plants that survived in pots treated only with a fungus were stunted and chlorotic and had necrotic roots.

The ideal approach to controlling root rot fungi has been to use resistant cultivars. Because many different pathogenic fungus species usually inhabit the rhizosphere of crop plants, resistance to the more important species is desirable. Cultivars with specific resistance to individual soil pathogens have been developed, but none has the desired multiple resistance. Consequently, soil fungitoxicants have come into wide use; however, few control a wide spectrum of pathogens. Repeated applications of the same or several different chemicals are frequently necessary for control, which increases both the cost of control and the risk of phytotoxicity and environmental pollution.

Control by biological means has the potential to become the best approach to pathogen control, because growers may have better choices of cultivars if effective biological control agents can be established in the field. Biological control approaches include 1) incorporating into the soil certain organic materials that promote an increase in the existing populations of organisms antagonistic to pathogens and 2) adding cultures of highly active antagonists to soil to increase populations of existing antagonists.

Several investigators have found that the nematode *Aphelenchus avenae* has a very wide fungal host range, including both plant-pathogenic and saprophytic species, under laboratory and greenhouse conditions (2,4,5,11–16,18,19). Although *A. avenae* is a mycophagous nematode, its ability to reproduce on tobacco callus tissue and on certain other plant tissues under controlled laboratory conditions is limited (1,3,6,10). However, it has failed to attack many plant species under greenhouse conditions (2,4,5,12–16,18). Two early investigators (7,17) found *A. avenae* in root tissues but thought that the nematode was probably feeding on an invading fungal pathogen.

Studies in our laboratory have shown that *A. avenae* is not pathogenic to alfalfa (S. Al-Saloom, *unpublished*). This biological control agent might maintain itself on resident saprophytic and pathogenic soil fungi, thus reducing root rots and seedling damping-off; moreover, its use would be nonpolluting and nonphytotoxic. We tested the effectiveness of *A. avenae* as a biological control agent for two root rot fungi. A preliminary report of our results has been published (4).

MATERIALS AND METHODS

Test organisms. *A. avenae* was originally obtained from agricultural soil. Cultures were maintained and increased on sterilized oat grain cultures of *Rhizoctonia solani* Kuehn at 25 C. A local modification of the Christie-Perry technique (8) was used to separate *A. avenae* from the fungus cultures.

Highly virulent isolates of *R. solani* and *Fusarium solani* (Mart.) Appel & Wr., obtained from infected alfalfa roots, were used as test root pathogens. Both pathogens were cultured on Bacto Proteose-Peptone No. 2 (Difco)-dextrose agar, and stock cultures were preserved on this medium at 24 C under a sterile mineral oil seal.

Greenhouse bioassay. A steam-sterilized greenhouse potting medium (sandy clay

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Table 1. Percentage survival of Cody alfalfa plants in soil infected with either *Rhizoctonia solani* or *Fusarium solani* and treated with a mycophagous nematode, *Aphelenchus avenae*[w]

Treatment	Fungal species	
	F. solani	*R. solani*
Sterilized soil (SS)	93 a[x]	93 a
SS + sterilized oats (SO) (25 g per pot)[y]	94 a	93 a
SS + *A. avenae*:		
250,000 per pot	93 a	86 a
500,000 per pot	87 a	92 a
1 million per pot	92 a	90 a
SS + SO (25 g per pot) + *A. avenae*:		
250,000 per pot	93 a	92 a
500,000 per pot	86 a	92 a
1 million per pot	97 a	92 a
SS + fungus oat culture (25 g per pot)	42 b[z]	4 b[z]
SS + fungus oat culture (25 g per pot) + *A. avenae*:		
250,000 per pot	88 a	69 c
500,000 per pot	87 a	74 c
1 million per pot	92 a	64 c

[w] Seedlings were planted after a 3-wk fungus–nematode interaction period. Plant survival data were recorded 1 mo after planting. All tests were run in a greenhouse at 26–33 C. Each treatment consisted of five pots (10 seedlings per pot) arranged in an incomplete block design. The data are the means from 12 tests with *F. solani* and 11 tests with *R. solani*.

[x] Statistically significant differences were determined with Student's *t* test of least-square means in an analysis of variance.

[y] Standard 15.2-cm (6-in.) clay pots.

[z] Most surviving plants were stunted and chlorotic and exhibited necrotic root systems.

loam, builder's sand, and peat moss [1:2:1]) was moistened and infested with oat cultures of *R. solani* or *F. solani* at 25 g per 15.2-cm (6-in.) standard clay pot. This rate was found to ensure a high level of disease development by both fungi while allowing a few plants to survive, thus simulating a severe disease situation.

The inoculum was mixed thoroughly into the potting medium with a Patterson-Kelley laboratory V-blender equipped with an auxiliary mixing bar. Some pots were drenched with suspensions of *A. avenae*, previously extracted from 14- to 21-day-old *R. solani* cultures, at 250,000, 500,000, or 1 million per pot. An incomplete randomized block design with five replications was used, with treatments as follows: 1) sterile soil, 2) sterile soil plus sterile oats, 3) sterile soil plus *A. avenae*, 4) sterile soil plus sterile oats plus *A. avenae*, 5) sterile soil plus fungal inoculum, and 6) sterile soil plus fungal inoculum plus *A. avenae*.

All pots were placed on benches in a greenhouse (ambient temperature 26–33 C) and watered immediately. Thereafter, pots were watered as required until the end of the experiment.

After a 3-wk fungus–nematode interaction period, 10 seedlings of alfalfa (*Medicago sativa* L. 'Cody') 7–10 days old, obtained by germinating seeds on moist filter paper in petri dishes, were planted in a uniformly spaced arrangement in each pot. One month later, the number and condition of surviving plants in each pot were ascertained. Data were obtained from 11 tests with *R. solani* and 12 tests with *F. solani*. Survival percentages were tabulated and analyzed by analysis of variance; Student's *t* test was used to compare pairs of treatment means.

RESULTS

The F-test from the analysis of variance for both fungal species indicated that the treatments were not homogeneous ($P < 0.05$) with respect to survival percentage (Table 1).

Plant survival in pots inoculated with *F. solani* and treated with *A. avenae* did not differ significantly from that in check pots (sterile soil only, sterile soil plus sterile oats, sterile soil plus *A. avenae*, and sterile soil plus sterile oats plus *A. avenae*) (Table 1). Survival ranged from 88% with 250,000 nematodes per pot of *F. solani*-inoculated soil to 92% with 1 million nematodes per pot. These results indicate that *F. solani* was unable to cause high levels of disease at any of the *A. avenae* population levels. When the data obtained at the three nematode levels in *F. solani*-inoculated soil are compared with the data from soil containing only *F. solani*, at least a doubling in plant survival is evident at each nematode population.

R. solani is much more virulent than *F. solani*, as is apparent from comparisons similar to those made above. Regardless of which check treatment (no fungus inoculum) is compared with *R. solani* plus any of three levels of *A. avenae*, the nematodes consistently provided survival rates between 64 and 74%, or more than 17 times the survival rate in the *R. solani* only treatment.

DISCUSSION

More work is needed to determine the potential of *A. avenae* as a biological control for root rots under field conditions. *A. avenae* should be tested against other root rot pathogens, such as species of *Phytophthora, Helminthosporium,* and *Pythium*.

To measure field effectiveness under field conditions, mass-culture techniques and delivery systems need to be tested and evaluated. For mass culture, the technique of rearing *A. avenae* in a liquid nutrient medium (20) should be investigated. Because *A. avenae* can survive desiccation (9), it might be preserved, stored, marketed, and dispensed in the dried state in a protective medium. Delivery systems applicable to dispensing fertilizers and granular pesticide formulations into field soils should be tested and evaluated for dispensing dried *A. avenae* formulations. The probable high initial costs of using *A. avenae* could limit its use for root rot control to high-value crops at first.

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