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POTENTIAL FOR BIOLOGICAL CONTROL OF PHYTOPARASITIC NEMATODES IN BERMUDAGRASS TURF WITH ISOLATES OF THE *PASTEURIA PENETRANS* GROUP[1]

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Abstract. **Survey work done from 1985-1989 suggests that members of the *Pasteuria penetrans* group of endoparasitic bacteria are widely distributed in bermudagrass turf in southern Florida. Five morphometrically distinct isolates of the bacteria were observed on five species of plant-parasitic nematodes. These endospore-forming actinomycetes are obli-**

grasses or other plant species. Some of these nematodes can be managed with frequent applications of postplant nematicides (organophosphates) which are currently labelled for turfgrass. Because most nematicides are highly toxic and must remain for a time in the root zone for effective management of nematodes there is always the potential for ground water contamination by the parent compound or a more toxic and soluble degradation product. Also, frequent use of some nematicides may artificially select for rhizosphere microflora which can quickly degrade such materials. Recently, biological control and cultural management tactics have received attention because they can be used alone or in combination with pesticides or other agents for nematode management with less threat to the environment.

Different species or pathotypes of obligate endoparasi-

High densities of *B. longicaudatus* were established on 'Tifgreen II' bermudagrass as follows: Nematode-free bermudagrass sod was established from aerial sprigs and transplanted over autoclaved sand in polypropylene beakers (14.8 cm d x 14 cm high). Each beaker was inoculated with 125-625 *B. longicaudatus* from greenhouse cultures on green beans (*Phaseolus vulgaris* L.). Beakers were suspended in a water bath (1.0 x 2.0 x 0.3 M) in which soil temperature was controlled at 27 ± 2 C with a model CFF-500 Remcor liquid circulator (Remcor Products Co., Franklin Park, IL 60139). The bermudagrass was maintained with weekly or more frequent watering to field capacity and mowed to a 1.3 cm height. After nine months the nematode populations appeared stabilized and mean densities of 485 ± 177 *B. longicaudatus* per 100 cm^3 were recorded from 16 beakers on 20 July 1989, the date of inoculation with *Pasteuria*-infested soil.

Soil infested with the *B. longicaudatus* isolate of *Pasteuria, B. longicaudatus,* and *Tylenchorhynchus annulatus* was collected from Plantation Golf Club (PGC), Broward Co., Florida at site 1 (Fairway 6) on 14 July 1989. Soil was either autoclaved at 120°C at 103 Kpa for 1 hour for sterilization (control) or heat-treated for 48 hours at 47°C to kill the nematodes but leave the *Pasteuria* isolates viable (treated). For introduction of control or treated soil, the soil column was removed from the 16 beakers with *B. longicaudatus*-bermudagrass cultures and cut in half vertically. Half of the column was returned to the beaker and the empty volume was filled with 1 kg of moist strelized fine sand from the Ft. Lauderdale Research and Education Center and topped off with either 500 g autoclaved control soil from PGC site 1 (eight beakers) or 500 g heat-treated soil from PGC site 1 (eight beakers). The beakers were randomly placed in the temperature controlled water bath in the greenhouse. At 6 months and 12 months post-inoculation, three soil cores were reomved from the disturbed half of the beaker in which the bermudagrass had become established, and extracted to determine *B. longicaudatus* densities and proportions of nematodes encumbered or filled with the large-spored *Pasteuria* isolate from *B. longicaudatus* from PGC site 1 (sporangium diameter = 6.10 μ; endospore width = 2.93 μ [3]). Each 100-cm^3 subsample was extracted for nematodes using the sugar-flotation technique (6). Nematodes in the sample were heat killed, fixed in 2.5% formalin-glycerol (12), and stained with crystal violet for visualization of spores. All quantifications of spore-filled or encumbered nematodes were done with subsamples that were examined at least at 400X with a compound microscope.

Control soil and *Pasteuria*-infested soil were compared for their effects on *B. longicaudatus* densities per 100 cm^3 of soil at T = 0, T = 6, and T = 12 months post-inoculation with a SAS general linear models procedure (8).

Results and Discussion

A survey of the occurrence of morphometrically different isolates of the *Pasteuria penetrans* group on phytoparasitic nematodes in bermudagrass fairways in southern Florida was conducted between 1985-89 (3). Significantly different-sized spore isolates of the *Pasteuria penetrans* group were associated with different species of nematodes. Sporangium and endospore diameter (in microns) means and standard deviations were 7.26 ± 0.36 and 3.54 ± 0.24 (n = 80) and 3.87 ± 0.33 and 1.95 ± 0.22 (n = 92) for two co-occurring isolates on *Hoplolaimus galeatus*, 6.10 ± 0.39 and 2.93 ± 0.24 (n = 412) for *Belonolaimus longicaudatus*, and 4.55 ± 0.33 and 2.53 ± 0.17 (n = 41) for *Tylenchorhynchus annulatus*, 3.87 ± 0.39 and 1.97 ± 0.22 (n = 220) for *Helicotylenchus microlobus*, and 3.42 ± 0.27 and 1.58 ± 0.16 (n = 182) for *Meloidogyne* spp., respectively (3). The *Pasteuria* isolate from *H. microlobus* and the small-spored isolate from *H. galeatus* were not morphometrically different from each other (3). All of the above isolates were observed to sporulate within their respective hosts (3). Most of the isolates of the *Pasteuria penetrans* group appeared to parasitize and sporulate in juveniles or adults of their respective nematode hosts. However, only second stage juveniles of *Meloidogyne* spp. were recovered filled with endospores. This is interesting because it has been reported that *P. penetrans* completes its life cycle only in adult root-knot nematodes (13). Apparently, this isolate of the *P. penetrans* group was able to complete its life cycle in second stage juveniles of *Meloidogyne* spp. Davies *et al.* (2) have reported a similar *Pasteuria* isolate that completes its life cycle in second-stage juveniles of another sedentary endoparasite, the cereal-cyst nematode, *Heterodera avenae.*

Sporangium and endospore diameter differences were relatively consistent for host specific isolates of the *P. penetrans* group at different sites and at different sampling times (3). Host specific morphometric differences in the *P. penetrans* group have been previously summarized (10,11). In fact, the host specific morphometrics for most of the isolates that were observed by Giblin-Davis *et al.* (3) were similar to previously reported values for members of the *P. penetrans* group from their respective nematodes. Experiments can now be conducted to determine if these are different host-specific strains or species of *Pasteuria. Criconemella ornata, Hemicriconemoides annulatus,* and *Hemicycliophora* sp. were present in large numbers at many of the sites surveyed but no isolates of the Pasteuria penetrans group were observed on them (3).

All four locations surveyed (one each in Collier Co. and Palm Beach Co. and two in Broward Co., Florida) had sample sites where one or more isolates of the bacteria were present, suggesting that the *Pasteuria penetrans* group is widespread in its distribution in southern Florida and persists in the intensively managed golf course environment (3). PGC site 1 in Broward Co., Florida showed the most diversity of any of the sites surveyed with three morphometrically different *P. penetrans* group isolates parasitizing three different phytoparasitic nematodes; *B. longicaudatus, T. annulatus,* and *Meloidogyne* spp. (3).

Do any of these isolates of the *Pasteuria penetrans* group suppress their nematode hosts to an acceptable level in bermudagrass? The results from the greenhouse study in this paper suggest that the *Pasteuria* isolate from *B. longicaudatus* does affect sting nematode densities.

Belonolaimus longicaudatus densities in the *Pasteuria*-inoculated beakers were significantly lowered ($P < 0.001$) six-fold at 12 months post-inoculation (Fig. 1). There were no significant differences in sting nematode densities between the two treatments at 6 months post-inoculation (Fig. 1) (3). Examination of the spore encumbrance classes and the proportion of endospore-filled sting nematodes revealed a general trend for decreasing proportions of

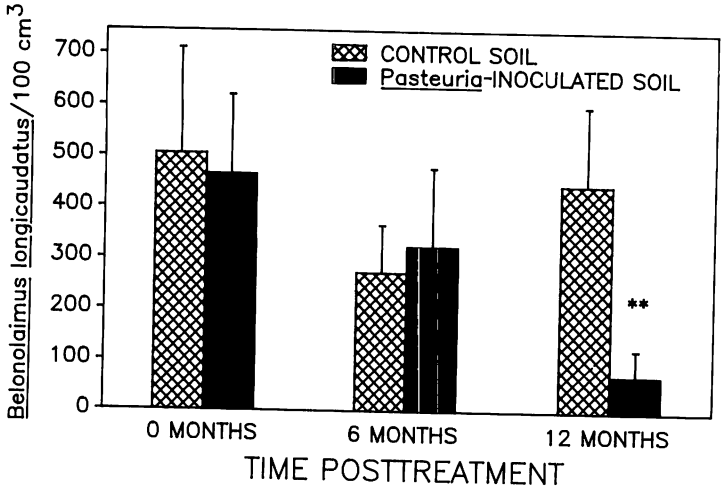

Fig. 1. Effects of inoculation with soil infested with the *Belonolaimus longicaudatus* isolate of *Pasteuria* from PGC site 1, Broward Co., Florida on *B. longicaudatus* densities per 100 cm^3 of soil. ** = a significant difference ($P < 0.001$) between treatments for a particular time posttreatment. Sting nematode densities for T = 0 months were measured from the 500 g of soil used for inoculum.

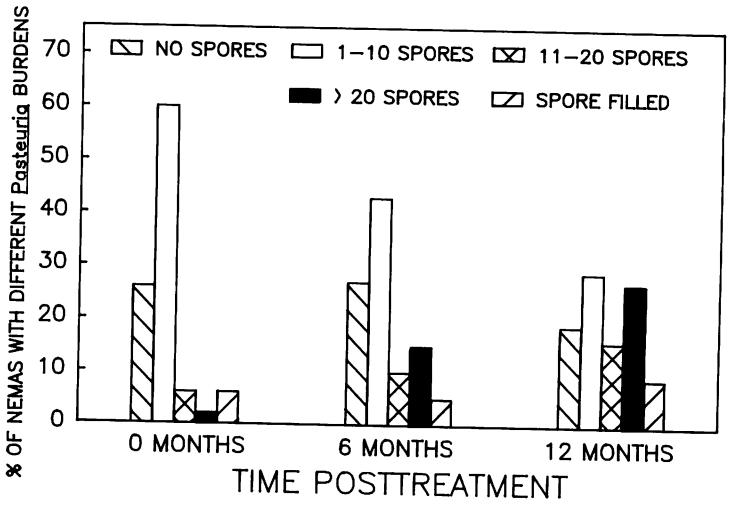

Fig. 2. Proportions of juveniles, males, and females of *Belonolaimus longicaudatus* encumbered with different spore classes or filled with endospores of the *B. longicaudatus* isolate of *Pasteuria* from PGC site 1, Broward Co., Florida. N = 47 nematodes examined for T = 0, n = 256 nematodes examined for T = 6, and n = 135 nematodes examined for T = 12 months posttreatment from beakers with *Pasteuria*-infested soil.

nematodes encumbered with a few spores (Class = 1-10) with concomitant increases in the proportions of nematodes encumbered with large spore burdens (Classes = 11-20 and >20) (Fig. 2). The same trends were noted in juveniles, males, and females.

Obviously with a study like this there is no direct proof of causality. However, the trends in figure 2 provide circumstantial evidence that it was the *Pasteuria* isolate that caused the significant decline in *B. longicaudatus* densities in the greenhouse study.

The *B. longicaudatus* isolate of *Pasteuria* was relatively slow to affect the population densities of sting nematodes

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