Journal of Nematology 45(3):223–227. 2013.

Effects of a Commercial Formulation of *Paecilomyces lilacinus* Strain 251 on Overseeded Bermudagrass Infested with *Belonolaimus longicaudatus*

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Abstract: Belonolaimus longicaudatus is an important parasite of both warm-season bermudagrass and winter overseed grasses used on golf courses in the southeastern United States. Field trials were conducted to study the effects of a commercial formulation of *Paecilomyces lilacinus* strain 251 applied to overseed grasses during the winter and early spring on population density of *B. longicaudatus* and bermudagrass health in late spring after bermudagrass broke dormancy. These studies found that *P. lilacinus* reduced numbers of *B. longicaudatus* in most cases, but not below damaging levels. Multiple applications of 1×10^{10} spores/m^2 were generally more effective than 2×10^{10} spores/m^2 in reducing nematode numbers and improving turf roots. These results indicate that application of this formulation of *P. lilacinus* strain 251 to overseeded turf in the spring may be a useful integrated pest management tool for *B. longicaudatus* on bermudagrass, but is not sufficient as a stand-alone nematode management tactic.

Key words: Belonolaimus longicaudatus, bermudagrass, biological control, *Paecilomyces lilacinus,* sting nematode, turfgrass.

Belonolaimus longicaudatus (sting nematode) is among the most virulent nematodes on many crops in the southeastern United States (Crow and Han, 2005). On golf course turfgrasses *B. longicaudatus* causes severe root reductions that leads to turf decline from drought stress. Increased irrigation frequency and reduced nitrogen interception resulting from the root reductions caused by *B. longicaudatus* can lead to increased potential for groundwater contamination (Trenholm et al., 2005; Luc et al., 2006, 2007). The loss of many of the effective nematicides used on turf in the past has increased the need for environmentally friendly, yet effective treatment options including biopesticides (Crow, 2005).

Paecilomyces lilacinus, a facultative parasite of nematode eggs, has been used to manage *Meloidogyne* spp., and several other plant-parasitic nematode species (Kiewnick and Sikora, 2006; Mendoza et al., 2007; Timper, 2011). A strain of *P. lilacinus,* strain 251, is now the active ingredient in several commercial bionematicides used in a number of countries. In the United States, two *P. lilacinus* strain 251 products have been marketed, MeloCon WG for nematode management on horticultural crops and NemOut WP for agronomic crops. In the southern United States, NemOut has been evaluated in the field against *M. incognita* on corn (Lawrence et al., 2008a, 2008b; Lawrence et al., 2009), *M. arenaria* on peanut (Rich and Barber, 2007; Moore et al., 2008a; Rich and Johnson, 2008a; Castillo et al., 2009a; Rich and Johnson, 2009), and *Rotylenchulus reniformis* on cotton (Rich and Johnson, 2008b; Moore et al., 2008b; Castillo et al., 2009b, 2013) and sweet potato (Adams et al., 2013; Lawrence et al., 2010) with mixed results. MeloCon was evaluated on bermudagrass turf against *M. marylandi*, but was not effective (Starr et al., 2007). It is unknown how effective this fungus would be against an ectoparasitic nematode with dispersed eggs such as *B. longicaudatus* on turfgrasses.

The most common grass species used on golf courses in the southeastern United States is bermudagrass (*Cynodon dactylon* and *Cynodon* hybrids). Bermudagrass is very susceptible to damage from *B. longicaudatus*. This is a warm-season perennial grass that uses a C4 photosynthetic pathway. In much of the region, bermudagrass goes dormant in the winter in response to cold temperatures and reduced day length. Many golf courses overseed during the winter with a cool-season C3 grass such as rough bluegrass (*Poa trivialis*) on greens or perennial ryegrass (*Lolium perenne*) on fairways to provide color and to prevent excess turf wear. With high temperatures and humidity in late spring, the overseed grass dies out concurrently with the bermudagrass breaking dormancy; in the golf course industry this process is termed "transition." Because soil temperatures in Florida rarely drop below the range of activity for *B. longicaudatus* (Mc Groary et al., 2009), population densities of *B. longicaudatus* increase during the winter and spring on the overseed grass roots. When the bermudagrass breaks dormancy in late spring, *B. longicaudatus* numbers are double on overseeded compared with nonoverseeded bermudagrass (Crow et al., 2005b). Earlier experiments applying *P. lilacinus* strain 251 as typical for a conventional nematicide, i.e., following transition when temperatures are high, were not successful in affecting *B. longicaudatus* or improving turf health (W. T. Crow, unpublished data). However, since the reproduction rate of *B. longicaudatus* on golf course turf in Florida is greatest during the spring (Mc Groary et al., 2009), this might be a better season to apply *P. lilacinus* as a biopesticide. The objective of this research was to determine if application of a commercial formulation of *P. lilacinus* strain 251, during the winter and spring prior to or during transition, is effective in reducing damage from *B. longicaudatus* on bermudagrass.

Received for publication October 8, 2012.
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This paper was edited by Abigail Walter.

Materials and Methods

In 2006 and 2007, a series of experiments evaluated the efficacy of *P. lilacinus* strain 251 against *B. longicaudatus* on golf course turf. Research sites selected had population densities of *B. longicaudatus* in excess of "high risk" thresholds used by the Florida Cooperative Extension Service of 25/100 cm^3 of soil (Crow, 2011). The *P. lilacinus* formulation used was a wetable granule product containing 1×10^{10} spores/g (MeloCon WG; PROPHYTA Biologischer Pflanzenschutz, Malchow/Poel, Germany). The *P. lilacinus* formulation was mixed in water and sprayed onto the research plots with a CO_2-powered backpack sprayer (Weed Systems, Hawthorne, FL). After treatment, all the plots were irrigated with 1.25 cm of water to move the spores into the turf root zone.

Nematode samples consisted of nine 1.9-cm-diam. × 10-cm-deep cores from each plot. The top layer of the cores consisting of leaves, stolons, and rhizomes, along with associated organic thatch layer was discarded and the remaining soil fraction was thoroughly mixed. Nematodes were extracted from a 100-cm^3 subsample by centrifugal-flotation (Jenkins, 1964). The plant-parasitic nematodes extracted were then identified and counted. Each research site contained 7 to 12 different genera of plant-parasitic nematodes. However, because the plots used in the trials were selected based on population density of *B. longicaudatus*, only data on this nematode are presented herein.

"Turf percent green cover" is the percentage of the plot surface covered by green turf and is a measurement of turf health. To determine turf percent green cover, a digital photo is taken of the center 1.0 m^2 of each plot. The percentage of the pixels in each photo that are "green" is determined using a macro developed by faculty at the University of Arkansas (Karcher and Richardson, 2005) for use with SigmaScan Pro 5 software (SPSS Inc., Chicago, IL). Percentage of the total pixels in the image that are green is the measure of turf percent green cover. To evaluate treatment effects on root health, root lengths were measured from two 3.8-cm-diam. × 15.25-cm-deep (174 cm^3 volume) cores taken from each plot and combined. Roots were extracted and root lengths determined using WinRHIZO (Regent Instruments, Quebec, Canada) software according to the method of Pang et al. (2011).

Putting green experiment: In 2006, two trials were conducted on putting greens 6 and 8 at Winter Springs Golf Club in Winter Springs, FL. Both of these greens were United States Golf Association specification greens (USGA, 1993) planted with 'Tifdwarf' bermudagrass, and overseeded with rough bluegrass. Plots were 3.34 m^2 with 1.5 m untreated borders between adjacent plots. Treatments were as follows: (i) untreated control, (ii) *P. lilacinus* at 1×10^{10} spores/m^2 (1 g formulation/m^2) applied three times at 6-wk intervals, and (iii) *P. lilacinus* applied at 2×10^{10} spores/m^2 (2 g formulation/m^2) applied three times at 6-wk intervals. The experiments used a randomized-block design with four replications of the two *P. lilacinus* treatments and eight replications of the untreated control. The initial *P. lilacinus* applications were made on 21 February. Prior to each application all plots were sprayed with a soil wetting agent (Affinity; Becker Underwood, Ames, IA) applied at 25 liters/ha, to facilitate spore movement into the soil. Pretreatment nematode samples were collected 1 February and posttreatment nematode samples were collected 6 April and 16 May. Turf percent green cover was measured 7 March, 18 April, and 30 May. Root samples were collected 30 May.

Nematode data were subjected to analysis of covariance with the initial observation as the covariant using SAS statistical software (SAS Institute, Cary, NC). Each *P. lilacinus* treatment was compared with the untreated control and the *P*-value generated was used to determine differences. For percent green cover and root data, each *P. lilacinus* treatment was compared with the untreated control using orthogonal contrasts and the *P*-value generated ($P \leq 0.10$) was used to determine differences.

Originally this experiment was scheduled to run through June, after transition was complete and the greens were completely bermudagrass. However, the golf course was unexpectedly sold, and access to the facility was denied after mid-May while the turf was still transitioning and about 50% rough bluegrass and 50% bermudagrass.

Fairway experiment: In 2007 and 2008, two trials were conducted on overseeded golf course fairways. One of these trials was conducted on a fairway planted with 'Common' bermudagrass (*C. dactylon*) at the Palatka Golf Club in Palatka, FL, and the other on a fairway planted with 'Tifway' bermudagrass (*C. dactylon* × *C. transvaalensis*) at Southern Woods Golf Club in Homosassa, FL. Both sites had been overseeded during the winter with perennial ryegrass.

Plots were 3.34 m^2 with 1.5 m untreated borders between adjacent plots. Treatments were as follows: (i) untreated control, (ii) *P. lilacinus* applied at 1×10^{10} spores/m^2 (1-g formulation/m^2) at each application,

Table 1. Effect of treatment with 0 g/m^2, 1g/m^2, and 2 g/m^2 of MeloCon WG on population density of *Belonolaimus longicaudatus*/100 cm^3 of soil in two trials on putting greens in Winter Springs, FL.

	Green 6			Green 8		
Treatment	1 February	6 April	16 May	1 February	6 April	16 May
Untreated	66 ± 17[a]	92 ±22	15 ± 5	31 ± 7	55 ± 16	56 ± 27
1 g/m^2	88 ±48	58 ± 23*	16 ± 8	21 ± 10	21 ± 8	6 ± 4
2 g/m^2	63 ± 25	52 ± 27	9 ± 3	21 ± 13	17 ± 5*	10 ± 4

[a] Mean and standard error of mean.
* Different from untreated according to analysis of covariance, $P \leq 0.10$.

TABLE 2. Effect of treatment with 0 g/m^2, 1g/m^2, and 2 g/m^2 of MeloCon WG on population density of *Belonolaimus longicaudatus*/100 cm^3 of soil on a fairway in Palatka, FL.

Treatment	26 October	1 December	11 January	23 February	6 April	11 May	8 June
Untreated	28 ± 6[a]	52 ± 7	47 ± 7	84 ± 24	146 ± 32	38 ± 6	164 ± 41
1g/m^2	27 ± 6	74 ± 25	33 ± 9	99 ± 38	149 ± 37	75 ± 30	84 ± 30*
2g/m^2	25 ± 4	71 ± 16	39 ± 10	110 ± 27	206 ± 34	60 ± 18	116 ± 27

[a] Mean and standard error of mean.
* Different from untreated according to analysis of covariance, $P \leq 0.10$.

and (iii) *P. lilacinus* applied at 2×10^{10} spores/m^2 (2-g formulation/m^2) at each application. The experiments used a randomized-block design with five replications. Treatment applications were made every 6 wk starting 17 November at Palatka and 20 November at Homosassa. Both trials ended in mid June. The day preceding each treatment application the plot areas were treated with a wetting agent to aid with uniform movement of *P. lilacinus* spores into the soil. The commercial wetting agent Affinity was applied at 25 liters/ha and a proprietary experimental wetting agent (BU 1136-912; Becker Underwood, Ames, IA) was applied at 16 liters/ha at the Palatka and Homosassa sites, respectively. Initial nematode samples were collected 2 wk prior to the initial treatment applications and 4 wk after each treatment application. Turf percent green cover was measured at 6-wk intervals beginning concurrently with the initial treatment application. Root samples were collected twice, once to measure the ryegrass roots in mid-February and once to measure the bermudagrass roots in mid June.

Nematode data were subjected to analysis of covariance with the initial observation as the covariant. Each *P. lilacinus* treatment was compared with the untreated control and the P-value generated was used to determine differences. For turf percent green cover and root data, each *P. lilacinus* treatment was compared with the untreated control using the orthogonal contrasts and the P-value generated was used to determine differences ($P \leq 0.1$).

RESULTS

In the putting green experiment, population density of *B. longicaudatus* was reduced after the initial application of *P. lilacinus* at 1 g/m^2 on green 6, and 2 g/m^2 on green 8 (Table 1). No differences in percent green cover or root length among treatments were observed from the putting green experiment ($P > 0.10$, data not shown).

In the fairway experiment, population density of *B. longicaudatus* was reduced by *P. lilacinus* at the lower rate compared with the untreated by the end of transition in both trials (Tables 2,3). At the Homosassa site this same treatment reduced population densities of *B. longicaudatus* on four of six postapplication sampling dates (Table 3). Root length of bermudagrass was improved by 1 g/m^2 of *P. lilacinus* on both sites, and 2 g/m^2 of improved bermudagrass root length at one site (Table 4). Percent green cover of bermudagrass was not improved by either *P. lilacinus* treatment at either site ($P > 0.10$, data not shown).

DISCUSSION

These results indicate that applications of *P. lilacinus* strain 251 during the winter and spring to overseeded golf course turf can suppress *B. longicaudatus* and aid bermudagrass during turf transition. These applications were much more effective than those in unpublished trials conducted by the author, where the applications were made to turf infested by *B. longicaudatus* during the summer months, and those of Starr et al. (2007) who applied *P. lilacinus* to turf infested by *M. marylandi* in July and March. These results are similar to those reported by Crow (2012) who found that application of another turfgrass bionematicide, *Bacillus firmis* strain I1582, were much more effective when applied in February or March than later in the year.

Crow et al. (2003, 2005a) reported that the current industry standard nematicide used on golf courses in

TABLE 3. Effect of treatment with 0 g/m^2, 1g/m^2, and 2 g/m^2 of MeloCon WG on population density of *Belonolaimus longicaudatus*/100 cm^3 of soil on a fairway in Homosassa, FL.

Treatment	30 October	4 December	16 January	26 February	9 April	14 May	11 June
Untreated	134 ± 27[a]	321 ± 88	630 ± 140	347 ± 58	273 ± 28	243 ± 83	361 ± 66
1g/m^2	135 ± 31	329 ± 91	456 ± 82*	203 ± 24*	179 ± 37**	169 ± 60	218 ± 38*
2g/m^2	137 ± 31	287 ± 45	512 ± 106	368 ± 92	234 ± 42	303 ± 70	315 ± 70

[a] Mean and standard error of mean.
*,** Different from untreated using orthogonal contrast, $P \leq 0.10$, 0.05, respectively.

TABLE 4. Effects of treatments with 0 g/m^2, 1g/m^2, and 2 g/m^2 of MeloCon WG on cm of root/350 cm^3 of soil of perennial ryegrass and bermudagrass on fairways at Palatka, FL, and Homosassa, FL.

	Palatka		Homosassa	
Treatment	Ryegrass 23 February	Bermudagrass 8 June	Ryegrass 26 February	Bermudagrass 11 June
Untreated	409 ± 51[a]	21 ± 8	84 ± 61	73 ± 21
1g/m^2	420 ± 66	124 ± 21***	86 ± 29	173 ± 50*
2g/m^2	293 ± 59	85 ± 22**	36 ± 31	59 ± 32

[a] Mean and standard error of mean.
*,**,*** Different from untreated using orthogonal contrast, $P \leq 0.10$, 0.05, 0.01, respectively.

Florida, 1,3-dichloropropene (1,3-D), reduced population densities of *B. longicaudatus* between 85% and 93% on fairways and between 72% and 97% on putting greens. Their studies showed that 1,3-D reduced population density of *B. longicaudatus* below the high risk threshold (25/100 cm^3 of soil) and improved turf density and root length in the majority of cases. In the current trials, *P. lilacinus* suppressed *B. longicaudatus* as much as 49% on fairways, but population densities remained ≥ 84/100 cm^3 of soil. While numbers of *B. longicaudatus* were reduced, they remained in the high-risk category and the turf was not visually improved. Therefore, the results from the commercial formulation of *P. lilacinus* evaluated were not as directly effective as those expected from the standard nematicide.

1,3-D may be applied to golf course turf only once per year. Crow et al. (2005a) found that population density of *B. longicaudatus* can resurge rapidly following a 1,3-D application, so often a single 1,3-D application is not sufficient for annual management of *B. longicaudatus*. Also, because of regulation and environmental concerns, many golf courses are unable or unwilling to use 1,3-D. Therefore, there is demand for integrated pest management (IPM) strategies for golf courses in Florida that supplement, reduce reliance on, or replace 1,3-D. *Paecilomyces lilacinus* strain 251 may be an effective candidate for inclusion into these IPM programs.

It is unknown why the lower rate of *P. lilacinus* was more effective than the higher rate on the fairways. It is possible that higher rates stimulate activity of organisms suppressive to *P. lilacinus* in the soil. Castillo et al. (2013) found that 0.2% v/v of *P. lilacinus* strain 251 was less suppressive to *R. reniformis* than either 0.1% or 0.3% in nonautoclaved soil, but not in autoclaved soil. Further studies are needed to explore the causes of this phenomenon.

Development of IPM programs using multiple chemical on biological nematicides for management of *B. longicaudatus* on golf course turf is underway (Crow and Kenworthy, 2013). Future studies developing integrated pest management for *B. longicaudatus* on golf course turf should include *P. lilacinus* as a component of the program.

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