Journal of Nematology 43(2):101–109. 2011.

Evaluation of Econem™, a Formulated *Pasteuria* sp. Bionematicide, for Management of *Belonolaimus longicaudatus* on Golf Course Turf[1]

William T. Crow,[2] John E. Luc,[2] Robin M. Giblin-Davis[3]

Abstract: In 2010, a turfgrass bionematicide containing *in vitro* produced *Pasteuria* sp. for management of *Belonolaimus longicaudatus* was launched under the tradename Econem™. Greenhouse pot studies and field trials on golf course fairways and tee boxes evaluated Econem at varied rates and application frequencies. Trials on putting greens compared efficacy of three applications of Econem at 98 kg/ha to untreated controls and 1,3-dichloropropene at 53 kg a.i/ha. Further putting green trials evaluated the ability of three applications of Econem at 98 kg/ha to prevent resurgence of population densities of *B. longicaudatus* following treatment with 1,3-dichloropropene at 53 kg a.i./ha. None of the Econem treatments in pot studies were effective at reducing *B. longicaudatus* numbers ($P \leq 0.05$). Econem was associated with reduction in population densities of *B. longicaudatus* ($P \leq 0.1$) on only a single sampling date in one of the eight field trials and did not improve turf health in any of the trials ($P > 0.1$). These results did not indicate that Econem is an effective treatment for management of *B. longicaudatus* on golf course turf.

Key words: Belonolaimus longicaudatus, bermudagrass, biological control, biopesticide, *Cynodon spp.*, nematode management, *Pasteuria sp.*, sting nematode, turfgrass.

Belonolaimus longicaudatus (sting nematode) is very damaging to turfgrasses, and numerous other crops in the sandy coastal plains of the southeastern United States. This migratory ectoparasite primarily feeds on root tips, causing root abbreviation and stunting (Crow and Han, 2005). Affected turfgrasses have an extremely shallow root system that is unable to withdraw water from the soil profile, leading to turfgrass decline and death from drought stress (Trenholm et al., 2005). The recent cancellation of fenamiphos (Nemacur, Bayer CropScience, Triangle Park, NC) has increased the need for new nematode management tactics for use on turf. Among the options being explored are development of safer chemical nematicides, biopesticides, and tolerant turf cultivars. Use of *Pasteuria* sp. that infects *B. longicaudatus* is among the biopesticide options being evaluated.

Belonolaimus longicaudatus is considered native to the sandy regions of the coastal southern states (Perry and Rhoades, 1982). This region is most likely the point of origin of both *B. longicaudatus* and its *Pasteuria* parasites. *Candidatus* Pasteuria usgae (CPu) is a parasite of *B. longicaudatus* (Giblin-Davis et al., 2003) that has been shown to naturally suppress *B. longicaudatus* on turfgrasses (Giblin-Davis, 2000). Suppression *of B. longicaudatus* was transferable through inoculation with CPu infested soil (Giblin-Davis et al., 2000). However, because CPu is an obligate parasite its use as a commercial biopesticide was not considered practical until recently.

Pasteuria Bioscience LLC has developed techniques for *in vitro* production of *Pasteuria* sp. Among the *Pasteuria* spp. being produced in this manner is a *Pasteuria* sp. isolate found infecting *B. longicaudatus*. This isolate is morphologically similar to the description of CPu, (Luc et al., 2010 a; Giblin-Davis et al., 2001) but it has not been confirmed as CPu with molecular sequencing data. Greenhouse trials with the *in vitro* produced *Pasteuria* sp. from *B. longicaudatus* showed that 280,000 endospores/cm^3 of soil was capable of suppressing *B. longicaudatus* in pots (Luc et al., 2010 a, 2010 b, 2011). A comparison of formulations found that a liquid formulation was more effective at suppressing *B. longicaudatus* in pots than was a granular formulation created from the liquid formulation (Luc et al., 2010 b).

In 2010, Pasteuria Bioscience launched Econem™, the first commercial bionematicide with *in vitro* produced *Pasteuria* sp. endospores as the active ingredient. Econem contains *Pasteuria* sp. from *B. longicaudatus* (0.002% a.i.) on a clay carrier. It is designed to be a topical treatment for the inundative management of *B. longicaudatus* on turfgrass. After application, and subsequent irrigation, the endospores are released from the clay and move into the soil profile. The product is labeled for 98 to 488 kg/ha per application. According to the manufacturer, 98 kg/ha and 293 kg/ha of Econem adds the equivalent of 100,000 and 300,000 endospores/cm^3 of soil to the top 5-cm of soil profile, respectively (T. E. Hewlett, Pasteuria Bioscience, pers. comm.). Therefore, a single application of 293 kg/ha or three applications of 98 kg/ha of Econem should add enough endospores to be in excess of the amount shown to induce suppression of *B. longicaudatus* in greenhouse trials (Luc et al., 2010 a, 2010 b, 2011). This assumes that all the endospores applied stay in the top 5-cm of the soil profile.

The current industry standard for management of *B. longicaudatus* on golf and sports turf in the coastal southeastern states is the nematicide 1,3-dichloropropene (1,3-D; Curfew Soil Fumigant, 97.5% a.i., Dow Agrosciences, Indianapolis, IN). While 1,3-D is very effective against *B. longicaudatus* in turf (Crow et al., 2003, 2005), rapid resurgence in population density of *B. longicaiudatus*

Received for publication March 16, 2011.
[1]Funding provided by the Environmental Institute for Golf, Florida Turfgrass Association, Florida Golf Course Superintendent's Association, Pasteuria Bioscience, and Dow AgroSciences.
[2]Associate Professor and Post-Doctoral Research Associate, respectively. Entomology and Nematology Department, University of Florida, Gainesville, FL, 32611.
[3]Professor and Acting Center Co-Director, Ft. Lauderdale Research and Education Center, University of Florida, Davie, FL, 33314.
Email: wtcr@ufl.edu
This paper was edited by Inga Zasada.

following putting green applications has been observed in some locations (Crow et al., 2005). A possible explanation for this resurgence is lack of natural enemies like *Pasteuria* spp. in these locations. It is unknown if application with Econem following a 1,3-D application could slow down or prevent this effect by inoculating the green with the *Pasteuria* parasite of *B. longicaudatus*.

The objectives of our research were: i) to determine if three applications of 98 kg/ha or a single application of 293 kg/ha of Econem is sufficient to suppress *B. longicaudatus* and improve turf health in the field, ii) to compare Econem to 1,3-D for management of *B. longicaudatus* on putting greens, iii) to determine if Econem is effective is reducing resurgence of *B. longicaudatus* population densities following a 1,3-D application, and iv) to determine if much higher rates or reapplication strategies with Econem suppressed *B. longicaudatus* or led to recycling of *Pasteuria* sp. in greenhouse pot studies on turfgrass hosts.

Materials and Methods

A number of field and greenhouse experiments evaluating Econem were conducted in 2010 and 2011 at the University of Florida. Econem used was obtained from commercial sources. Each years commercial supply of Econem is formulated from a single batch of *in vitro* produced *Pasteuria* endospores. Therefore, while different experiments used different bags of Econem, all of the experiments in each year used Econem originating from a common batch.

All field experiments were conducted on sites that had population densities of *B. longicaudatus* in excess of the "high risk" threshold (25/100 cm^3 of soil) for bermudagrass used by the Florida Cooperative Extension Service (Crow, 2011). Econem applications were made with a walk-behind drop spreader (Gandy, Owatonna, MN). After each application all plots, including the controls, were irrigated with 0.64-cm of water. 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. Turf percent green cover is a measurement of the plot surface covered by green turf. A digital photo was taken of the center m^2 of each plot. The percentage of the pixels in each photo that were "green" was determined using a macro developed by faculty at the University of Arkansas (Karcher and Richardson, 2005) for use with SigmaScan Pro5 software (SPSS Inc., Chicago, IL). Percentage of the total pixels in the image that were green was the measure of turf percent green cover. 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., (2011a).

Fairway Experiment: Two trials were conducted on golf course fairways in 2010 to evaluate Econem as a bionematicide for use on turf for management of *B. longicaudatus*. One site was located at the Babe Zaharius Golf Club in Tampa, FL, the other was at the SummerGlen Golf Club in Ocala, FL. Both sites were planted to 'Tifway' bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) that was overseeded during the winter with perennial ryegrass (*Lolium perenne*). Winter overseeding is used in the region to provide turf cover during the winter when bermudagrass is dormant. During the late spring perennial ryegrass dies under Florida conditions, roughly concurrent with bermudagrass breaking dormancy. In addition to *B. longicaudatus* the Babe Zaharius site was infested with below-threshold population densities of *Helicotylenchus* sp., *Hemicriconemoides annulatus*, *Hemicycliophora* sp., *Meloidogyne* sp., *Mesocriconema ornatum*, *Peltamigratus christiei*, *Trichodorus obtusus*, and *Tylenchorhynchus* sp., and the SummerGlen site with *Helicotylenchus* sp., *H. annulatus*, *Meloidogyne* sp., *M. ornatum*, and *Nanidorus minor*.

Plots were established using a randomized block design, with blocking based on the initial population density of *B. longicaudatus*. There were five replications of each treatment. Plots were 21 m^2 with 1.5 m untreated borders between plots. A 2.3 m^2 subplot in the center of each plot was used for all data collection. Treatments were: i) untreated control, ii) Econem applied at 98 kg/ha at each of three applications at four wk intervals, and iii) Econem applied once at 293 kg/ha. Initial treatment applications were made on 15 April at Babe Zaharius and 5 May at SummerGlen.

Nematode and root samples were collected 1 wk prior to the initial treatment applications, 4 wk after each treatment application, and 8 wk after the final treatment application. Turf percent green cover was measured approximately every 2 wk.

To evaluate treatment effects on *B. longicaudatus*, nematode data were subjected to analysis of variance and treatment means separated according to Fisher's protected least significant difference (LSD) test ($P \leq 0.1$) using SAS software (Cary, NC). For turf percent green cover and root length data, orthogonal contrasts between the Econem treatments and the untreated controls at each measurement date ($P \leq 0.1$) were used to determine if either Econem treatment was different from the untreated.

Tee Box Experiment: To evaluate the efficacy of Econem for suppression of *B. longicaudatus* on turf, two field trials were conducted on golf course tee boxes in 2011. One tee box was located at Miromar Lakes Golf and Beach Club in Estero, FL, the other was at the University of Florida Plant Science Research Unit in Citra, FL. The Miromar Lakes site was planted to Tifway bermudagrass,

the Plant Science site was planted to 'Celebration' bermudagrass. In addition to *B. longicaudatus*, the Miromar Lakes site was infested with below-threshold population densities of *Helicotylenchus* sp., *H. annulatus*, *Hoplolaimus galeatus*, *Meloidogyne* sp., *M. ornatum*, and *N. minor*, and the Plant Science site with *Helicotylenchus* sp., *H. annulatus*, *Hemicycliophora* sp., *H. galeatus*, *P. christiei*, *Pratylenchus hippeastri*, *Meloidogyne* sp., *M. ornatum*, and *N. minor*. The experimental design was completely randomized block with 5 replications. Blocks were based on initial population densities of *B. longicaudatus*. Plots were 1.5 m^2 with 0.6 m untreated borders between plots.

Treatments were untreated control and a single application of 293 kg/ha of Econem. Treatments were made on 8 March at the Miromar Lakes site and 15 March at the Plant Science site. Nematode samples were collected 2 wk before the Econem applications, and 2 and 10 wk after the Econem applications. Turf percent green cover was evaluated every 2 wk. Initial root length samples were collected the day of treatment and 4, 8, and 18 wk after treatment.

To evaluate treatment effects on *B. longicaudatus*, nematode data were subjected to analysis of variance and treatment means separated according to Fisher's protected LSD test ($P \leq 0.1$). For turf percent green cover and root length data, analysis of covariance was conducted with the initial measurement used as the covariate. The Econem treatment was compared to the untreated control and the P-value generated ($P \leq 0.1$, 0.05, 0.01) for the comparison was used to determine differences.

Putting Green Experiment: In 2010, two trials were conducted to compare the effects of Econem to the nematicide, 1,3-D on golf course putting greens. One trial was conducted at Venetian Bay Golf Club in New Smyrna Beach, FL, the other at the Avila Club in Tampa, FL. Both sites were putting greens planted with 'Tifdwarf' bermudagrass. In addition to *B. longicaudatus* the Venetian Bay site also was infested with below-threshold population densities of *Dolichodorus* sp., *Hemicycliophora* sp., *Meloidogyne* sp., *M. ornatum*, and *N. minor*, and the Avila Club site with *Helicotylenchus* sp., *H. annulatus*, *Meloidogyne* sp., *M. ornatum*, and *N. minor*. The experimental design was completely randomized block with four replications. Blocks were based on spatial location on the green and not nematode population densities. Plots were 26 m^2 with 1 m untreated borders between plots. In the center of each plot a 3.34 m^2 subplot was established for collection of all data.

Treatments were: an untreated control, Econem applied at 98 kg/ha at each of three applications at 4 wk intervals, and 1,3-D applied at 53 kg a.i./ha once by slit injection as described by Crow et al. (2005). 1, 3-D was applied by a commercial applicator (Southern Soils Turf Management, Lake Mary, FL). The initial Econem applications were made on 5 April at Venetian Bay and 16 March at Avila Club. The 1,3-D applications were made on the same day as the second Econem applications.

Nematode samples were collected 2 wk before the initial Econem applications, 2 wk after the 1,3-D/second Econem applications, and 4 wk after the final Econem applications. Turf percent green cover was measured every two wk. Root samples were collected 2 wk after the final Econem application. To evaluate treatment effects on *B. longicaudatus*, nematode data were subjected to analysis of variance and treatment means separated according to Fisher's LSD test ($P \leq 0.1$). For turf percent green cover and root length data, orthogonal contrasts between each experimental treatment and the untreated controls at each measurement date ($P \leq 0.1$) were used to determine if experimental treatment was different from the untreated.

Population Resurgence Experiment: Two field trials were conducted in 2010 to determine if applications of Econem could slow down resurgence in *B. longicaudatus* population density following treatment with 1,3-D. One trial was conducted at the MetroWest Golf Club in Orlando, FL, the other was at the Continental Country Club in Wildwood, FL. In addition to *B. longicaudatus* the MetroWest site was infested with below-threshold population densities of *Helicotylenchus* sp., *H. galeatus*, *Meloidogyne* sp., and *M. ornatum*, and the Continental site with *Helicotylenchus* sp., *Meloidogyne* sp., *M. ornatum*, and *T. obtusus*. Both sites had a history of resurgence of *B. longicaudatus* within a couple of months following application of 1,3-D.

Both golf courses had commercial applications of 1,3-D at 53 kg a.i./ha made to all their putting greens for management of *B. longicaudatus* 2 wk prior to the initiation of the trials; therefore, the entire experimental area was treated with 1, 3-D. The experimental design was randomized complete block with five replications. Blocks were based on spatial location on the green and not nematode population densities. Plots were 13.4 m^2 with 1 m untreated borders between plots. In the center of each pot a 3.34 m^2 subplot was established for collection of all data. The two treatments were untreated control and Econem applied at 98 kg/ha at each of three applications at 4 wk intervals.

Nematode population density, turf percent green cover, and root length data were obtained as described for the previous experiments. Nematode samples were collected the day of the initial Econem applications, and 4 and 8 wk after the final Econem application. Turf percent green cover was evaluated every 2 wk. Root samples were collected 4 wk after the final Econem application.

Nematode and turf percent green cover and data was subjected to analysis of covariance with the initial measurement used as the covariate. The Econem treatment was compared to the untreated control and the P-value generated ($P \leq 0.1$, 0.05, 0.01) for the comparison was

used to determine differences. For root length data, orthogonal contrasts between the Econem treatment and the untreated controls ($P \leq 0.1$) were used to determine differences.

Greenhouse Pot Experiments: Two separate greenhouse experiments were conducted in 2010-2011 to determine if different rates or reapplication strategies with Econem suppressed *B. longicaudatus* or led to recycling of *Pasteuria* sp. in model bentgrass or St. Augustinegrass hosts. Both experiments were conducted at greenhouses at the UF-IFAS Fort Lauderdale Research and Education Center (FLREC). Soil used in these experiments was a Margate fine sand with 3.8% OM.

Experiment 1 was conducted to determine if Econem in a single or multiple applications at 488, 4,883, or 48,830 kg/ha (or 5×10^5, 5×10^6, and 5×10^7 endospores/cm^3 of soil to the top 5-cm of soil profile, respectively) suppressed *B. longicaudatus* in pots of cultured *Pasteuria*-free nematodes on a St. Augustinegrass (*Stenotaphrum secundatum*) host. Single aerial cuttings of 'FX-313' St. Augustinegrass was sprigged with into bulb pots (17 cm effective diam. × 8 cm depth) filled to within 2 cm of the top with autoclaved soil on 1 June 2010. Biweekly fertilization was started on 4 June 2010 with each pot receiving 10 ml of solution containing 18-18-21 + micros fertilizer (Miracle-Gro, Scotts, Marysville, OH) at 4.9 cm^3/liter. All pots receiving *B. longicaudatus* were inoculated with *ca* 488 *B. longicaudatus*/pot in 130 ml of soil on 11 August 2010. The soil was from *B. longicaudatus* stock cultures on FX-313 St. Augustinegrass (Giblin-Davis et al., 1992) to avoid handling damage to the nematodes during inoculation. Soil for the noninoculated control was treated with boiling water to kill the nematodes and allowed to cool before inoculation. Pots were completely randomized on a bench in a greenhouse receiving overhead irrigation every other day. The *B. longicaudatus* populations were allowed 48 days to establish before they were sampled to estimate the pre-treatment population density by pulling two random cores with a #8 cork borer (1.5 cm diam. × 11.5 cm depth) to produce a measured volume of 25 ml which was extracted using sugar flotation and counted as described above. There were eight treatments with four replicates in a completely randomized design. The treatments were applied to the *Pasteuria*-free *B. longicaudatus* pots on 8 October 2010 and included; 1) a control with no Econem, 2) a single application of Econem at 488 kg/ha, 3) a single application of Econem at 4,883 kg/ha, 4) a single application at 48,830 kg/ha, 5) a single application at 488 kg/ha followed by four additional monthly applications at same rate, 6) a single application at 4,883 kg/ha followed by four additional monthly applications at same rate, 7) a single application at 48,830 kg/ha followed by two additional monthly applications at same rate, and 8) a "no nematode" and "no Econem" control to be used only in turf quality assessments. Originally, treatment 7 was going to receive four additional applications as for treatments 5 and 6 instead of just two. However, the pots were too full to add additional Econem after the third application.

Nematode population dynamics (nematode soil counts) were determined with subsampling as done for pretreatment counts 6 wk, 12 wk, and 26 wk posttreatment using sugar flotation extraction. Turf quality in each pot was scored separately for color and coverage using a 0-10 scale to evaluate overall plant performance prior to taking nematode samples. Roots from the final nematode harvest at 26 wk were collected, cleaned of adhering sand and dried for 48 hr before being weighed. Foliar plant performance was examined weekly for phytotoxicity. Nematode count data (square root transformed), averaged color and coverage scores, and root dry weights were analyzed using PROC ANOVA followed with a Waller-Duncan k-ratio test for means separation ($P \leq 0.05$). In addition, a subsample of at least 10 *B. longicaudatus* harvested from each pot at each harvest date were stained with 1% crystal violet and examined under a compound microscope to look for endospore encumbrance and/or spore-filled cadavers, indicative of attachment of and/or recycling of *Pasteuria* sp. in the pots.

Experiment 2 was conducted to determine if Econem in a single application at 98, 196, 293, or 392 kg/ha (or 1×10^5, 2×10^5, 3×10^5 and 4×10^5 endospores/cm^3 of soil to the top 5-cm of soil profile, respectively) suppressed *B. longicaudatus* in bentgrass-seeded pots of cultured *Pasteuria*-free nematodes. Soil from *B. longicaudatus* stock cultures grown on potted FX-313 St. Augustinegrass (Giblin-Davis et al., 1992) was collected and thoroughly mixed and sub-sampled to estimate the population density of nematodes in the soil before filling 25 square tapered pots (80 mm wide at top, 60 mm wide at the bottom, and 75 mm deep) with the drainage holes covered with an 85-mesh synthetic fabric. The total amount of soil added to each pot was about 300 g wet weight (ca 250 g dry weight) with *ca* 220 *B. longicaudatus*/100 cm^3 of soil (=*ca* 660 nematodes per pot). There were four treatments of Econem applied at 98, 196, 293, or 392 kg/ha for a pot surface area of 62.41 cm^2 and an untreated control with five replicates. All pots were then seeded with *ca* 0.08 g each of 'Penncross' creeping bentgrass (*Agrostis palustris*) (98 kg/ha) and lightly covered with a small amount of autoclaved soil. Pots were completely randomized on a mist bench in a greenhouse. The experiment was conducted for 35 days and adjusted daily for misting time and duration to prevent water flow through the plot or lethal wilting (ranging for 10-20 seconds mist for every 15-30 min). Nematode numbers were determined with destructive sampling and sugar flotation extraction of each pot on 16 June, 2011. Nematode count data was analyzed using PROC ANOVA followed with a Waller-Duncan k-ratio test for means separation ($P \leq 0.05$).

Results

Fairway Experiment: In both trials, population densities of *B. longicaudatus* in plots treated with either Econem regime were not different ($P > 0.1$) from the untreated at any sampling date (Table 1). Similarly, in both trials neither Econem regime improved turf percent green cover or root length at any observation date (data not shown).

Tee Box Experiment: At the Miromar Lakes site there were no differences in population density of *B. longicaudatus* between treatments at any sampling date (Table 2). However, plots treated with Econem exhibited less ($P \leq 0.1$) root length than the untreated plots on the third root sampling date, root lengths were 352 cm and 216 cm for the untreated and Econem treated, respectively. At the Plant Science site, Econem treated plots had a lower ($P \leq 0.1$) population density of *B. longicaudatus* than the untreated control plots on the third sampling date only (Table 2). The Econem treated had greater ($P \leq 0.1$) root length than the untreated control on the second sampling date only, root lengths were 394 cm and 710 cm for the untreated and Econem treated, respectively. There were no differences in turf percent green cover between treatments on any observation date in either trial (Data not shown).

Putting Green Experiment: At the Venetian Bay site, population densities of *B. longicaudatus* in all treatments dropped from $\geq 55/100$ cm^3 of soil two wk before treatment to $\leq 5/100$ cm^3 of soil 2 wk after treatment, and remained low for the rest of the study. This is below the "moderate risk" threshold of $10/100$ cm^3 of soil for *B. longicaudatus* on bermudagrass used by the Florida Cooperative Extension Service (Crow, 2011). There were no differences ($P > 0.1$) in population density of *B. longicaudatus* (Table 3) or in root lengths (Figure 1) among treatments at the Venetian Bay site, although there were increases ($P \leq 0.1$) in turf percent green cover from 1,3-D compared to the untreated on two of the observation dates (Figure 2). At the Avila Club site population densities of *B. longicaudatus* were reduced ($P \leq 0.1$) by 1,3-D compared to the untreated control two wk after application (Table 3), but the population densities rebounded by the final sample date. Similarly, 1,3-D increased ($P \leq 0.1$) turf root lengths, and improved ($P \leq 0.1$) turf percent green cover on one observation date compared to the untreated control (Figures 1, 2). Econem was not different from the untreated control for any parameter evaluated in either trial ($P > 0.1$).

Population Resurgence Experiment: Population densities of *B. longicaudatus* increased in both trials over the course of the experiment (Table 4). However, population density of *B. longicaudatus* in Econem treated plots was not different from the untreated at any sampling date in either trial. Similarly, there were no differences in root length or percent cover between treatments in either trial (data not shown).

Greenhouse Pot Experiments: In experiment 1, there were no differences ($P > 0.05$) in *B. longicaudatus*/100 cm^3

Table 1. Effects of a single application of Econem at 293 kg/ha, and three applications of Econem at 98 kg/ha at 4 wk intervals on population densities of *Belonolaimus longicaudatus* on golf course fairways at two sites.

Rate of Econem applied	Weeks after initial treatment application				
	-1 (Pi)	4	8	12	16
Babe Zaharius Site					
0 kg/ha (Untreated)	64	52	19	45	8
98 kg/ha (3 applications)	64	75	29	47	23
293 kg/ha (1 application)	65	41	19	20	13
SummerGlen Site					
0 kg/ha (Untreated)	151	11	71	47	47
98 kg/ha (3 applications)	151	26	105	45	45
293 kg/ha (1 application)	151	8	87	34	34

Data represent *B. longicaudatus* population density means/100 cm^3 of soil from five replications.

No differences among treatments were found at any sampling date in either trial ($P > 0.1$).

Table 2. Effects of a single application of Econem at 293 kg/ha on population densities of *Belonolaimus longicaudatus* on golf course tees at two sites.

Rate of Econem applied	Weeks after treatment application		
	-2 (Pi)	2	10
Miromar Lakes Site			
0 kg/ha (Untreated)	70 a	93 a	78 a
98 kg/ha (3 applications)	71 a	89 a	70 a
Plant Science Site			
0 kg/ha (Untreated)	78 a	84 a	143 a
98 kg/ha (3 applications)	82 a	63 a	84 b

Data represent *B. longicaudatus* population density means/100 cm^3 of soil from five replications.

Date followed by common letters within a trial are not different according to Fisher's protected least significant difference ($P > 0.1$).

Table 3. Effects of three applications of Econem at 98 kg/ha at 4 wk intervals and Curfew Soil Fumigant at 53 kg a.i/ha on population densities of *Belonolaimus. longicaudatus* on golf course putting greens at two sites.

Treatment	Weeks after initial treatment application		
	-2 (Pi)	6	10
Venetian Bay Site			
Untreated	55 a	5 a	4 a
Econem	55 a	5 a	2 a
Curfew	57 a	2 a	12 a
Avila Club Site			
Untreated	235 a	429 a	81 a
Econem	227 a	384 a	72 a
Curfew	226 a	74 b	111 a

Data represent *B. longicaudatus* population density means/100 cm^3 of soil from four replications.

Date followed by common letters with a trial are not different according to Fisher's protected least significant difference ($P \leq 0.1$).

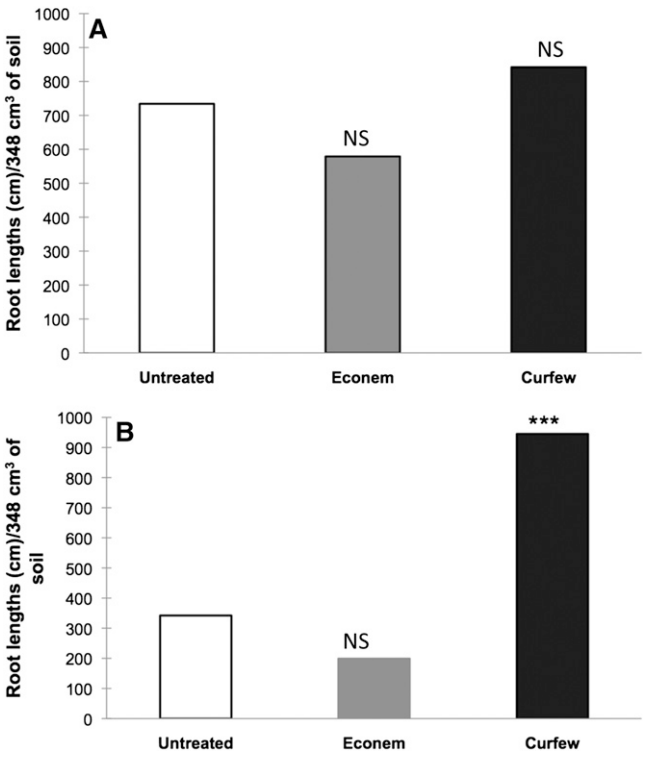

FIGURE 1. Effects of three applications of Econem at 98 kg/ha at 4 wk intervals and 1,3-D at 53 kg a.i.//ha on root length/348 cm^3 of soil from 'Tifdwarf' bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) on golf course putting greens at the A) Venetian Bay Golf Club, and B) Avila Club. NS = Not different from the untreated according to orthogonal contrast ($P > 0.1$). ***Different from untreated according orthogonal contrast ($P \leq 0.01$).

of soil among treatments. No typical looking attached CPu endospores as described by Giblin-Davis et al. (2001), or any spore-filled cadavers were observed. Econem at the highest rate (48,830 kg/ha with single or multiple applications) consistently produced turf coverage and color comparable with the noninoculated/nontreated control pots. These treatments were significantly improved over the other Econem treatments and the inoculated control without Econem at 12, and 26 weeks post-treatment. At 6 weeks post-treatment, Econem at the highest rate (48,830 kg/ha with single or multiple applications) produced better turf coverage and color performance than all other treatments ($P > 0.05$). Root dry weights for the noninoculated/nontreated control were significantly greater than any of the other treatments with or without Econem, which were extremely variable but statistically similar. No phytotoxicity was observed during the experiment.

In experiment 2, there were no differences ($P > 0.05$) between *B. longicaudatus* counts at 35 days post-treatment among treatments. The mean (± SD) counts of *B. longicaudatus* per pot were 294 (98), 286 (138), 446 (280), 292 (157), 349 (52) in the Econem treatments at 0 (control), 98, 196, 293, or 392 kg/ha, respectively.

DISCUSSION

The Econem formulation of *Pasteuria* sp. was not effective in suppressing populations of *B. longicuadatus* in any of the experiments. This is different from the results achieved in earlier experiments with *in vivo* produced endospores of CPu in pots and the field (Giblin-Davis, 2000) and *in vitro* produced *Pasteuria* sp. endospores in pots (Luc et al., 2010 a, 2010 b, 2011). Possible explanations for these differences include loss of virulence during the *in vitro* culture process or during formulation, or environmental factors occurring in the field.

During earlier experiments, *in vitro* produced *Pasteuria* sp. applied at 280,000 endospores/cm^3 of soil suppressed population densities of *B. longicaudatus* on creeping bentgrass (*Agrostis palustris*) in pots (Luc et al., 2010 a, 2010 b, 2011), but not in the current studies using the Econem formulation. Even though the *Pasteuria* sp. in Econem is derived from the same isolate as the one used by Luc et al. (2010 a, 2010 b, 2011), the *in vitro* process used to produce the endospores and the formulation were different between these studies. While the process used for *in vitro* production of *Pasteuria* sp. is proprietary, the endospores used by Luc et al. (2010 a, 2010 b, 2011) were produced using laboratory-scale fermenters and the formulation was simply endospores in liquid growth media, whereas the endospores contained in Econem are produced in large-scale commercial fermenters that are then formulated into a commercial granular product. It is possible that during the scale-up in production or during the formulation process the endospores lost virulence. The granular formulation evaluated by Luc et al. (2010 b) was made by pipetting the liquid growth media containing endospores onto clay granules and letting it dry, versus the concentrated, formulated endospores on clay granules contained in Econem. The lab formulated product was not as effective as the straight liquid formulation but it did have some efficacy (Luc et al., 2010b), whereas the commercially formulated Econem product used in the current studies was not effective.

From the greenhouse experiment 2, examination of *B. longicaudatus* harvested from each pot at each harvest date with a compound microscope was conducted. While endospores observed attaching to the cuticle and occasionally filling cadavers with the earlier liquid formulation of *in vitro*-produced *Pasteuria* sp. (Luc et al., 2010 a, 2010 b; 2011) appeared similar to those reported in natural populations of CPu, , nothing that could unambiguously be assigned to CPu was observed attaching to or filling *B. longicaudatus* in the pot studies conducted with Econem herein. Although visual improvement in turf was noted in greenhouse experiment 1, no effects on *B. longicaudatus* or roots were observed. This suggests that the very high rates of clay added in

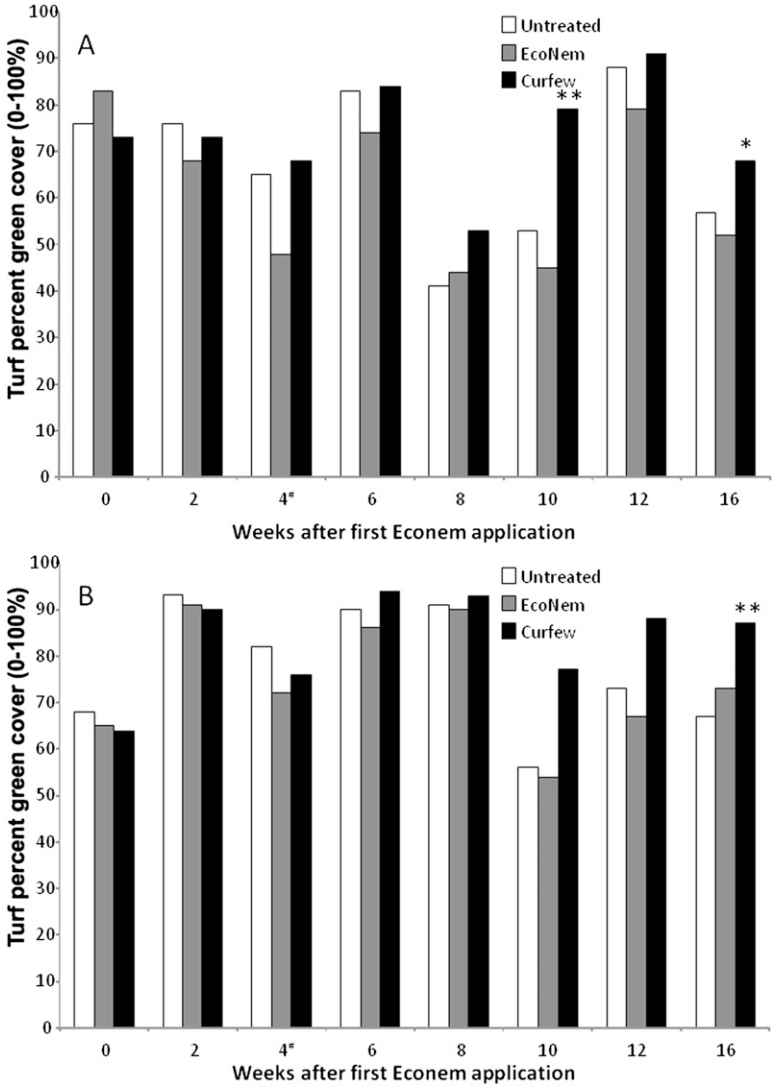

FIGURE 2. Effects of three applications of Econem at 98 kg/ha at 4 wk intervals and Curfew Soil Fumigant at 46.75 l/ha on turf percent green cover (0-100%) of 'Tifdwarf' bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) on golf course putting greens at the A) Venetian Bay Golf Club, and B) Avila Club. # Indicates date of 1,3-D application. *,**Different from untreated at the observation date according to analysis of covariance ($P \leq 0.1, 0.05$, respectively).

these treatments may have improved plant growth without affecting *B. longicaudatus* numbers.

Environmental factors also might have played a part in the lack of efficacy of Econem. While bermudagrass has the genetic potential to establish roots as deep as 2 m or more (Lunt, 1968), under Florida golf course conditions the root depth in putting greens seldom exceeds 10-cm-deep (W. T. Crow, pers. observation). Therefore, the treatment zone for turfgrass nematicides in Florida is considered 5 to 10-cm-deep. Luc et al. (2010 a, 2010 b) found that 280,000 endospores/cm^3 of soil of *in vitro* produced *Pasteuria* sp. endospores were required to suppress population densities of *B. longicaudatus*. While 293 kg/ha of Econem adds enough endospores to reach this target rate, this assumes all the endospores come off the clay and that most do not leach beyond 5-cm of the soil profile. Currently, the release rate of *Pasteuria* sp. endospores from Econem with irrigation is unknown. Also, Luc et al. (2011) found that *Pasteuria* sp. endospores are leachable in sand, with many of the endospores moving beyond the target 5-cm of soil profile with increasing amounts of irrigation. Therefore, it is possible that the amount of *Pasteuria* sp. endospores in the top 5-cm of soil profile at any given time is insufficient to adequately suppress *B. longicaudatus*.

As mentioned previously, both *B. longicaudatus* and its *Pasteuria* parasites may be native to Florida and therefore commonly occur concomitantly in most turf sites in the region. In an attempt to locate turf field sites for experiments having *B. longicaudatus* but no detectible level of preexisting *Pasteuria* attachment, 43 golf course and athletic field sites across the state of Florida were sampled. However, *Pasteuria* sp. attachment to *B.*

TABLE 4. Effects of three applications of Econem at 98 kg/ha at 4 wk intervals on resurgence of population densities of *Belonolaimus. longicaudatus* following an application of Curfew Soil Fumigant at 46.75 l/ha on golf course putting greens at two sites.

Treatment	Weeks after initial Econem application		
	0 (Pi)	6	10
	MetroWest Site		
Untreated	11	78	88
Econem	7	60	86
	Colonial Site		
Untreated	28	38	80
Econem	28	49	93

Data represent *B. longicaudatus* population density means/100 cm^3 of soil from five replications.

No differences between treatments were found at any sampling date in either trial ($P > 0.1$).

longicaudatus was observed in all 43 sites (W. T. Crow, unpubl. data). In Florida, it can be assumed that the vast majority of bermudagrass locations infested with *B. longicaudatus* already have some level of biological suppression of the nematodes from *Pasteuria* spp. occurring. While biological suppression of *B. longicaudatus* is likely occurring in most locations and preventing turf damage from being as severe as it would otherwise, in many cases this natural suppression is insufficient to prevent root damage to a degree acceptable for most turf managers. This lack of acceptable suppression could be due to low CPu endospore production in *B. longicaudatus* and environmental conditions in the turf root zone. *Belonolaimus longicaudatus* causes severe root reductions and moreover, the carrying capacity for *B. longicaudatus* on most bermudagrass cultivars is < 300/150 cm^3 of soil (Pang et al., 2011 b). Individual *B. longicaudatus* cadavers were found to produce 1,483-4,700 CPu endospores/cadaver (Giblin-Davis, 2000) depending on nematode life-stage and sex. Therefore, CPu endospore production is much lower than would be expected for *P. penetrans* on *Meloidogyne* spp. Due to low endospore production and leaching, it is likely a rare occurrence that naturally occurring CPu endospore densities in soil reach the 280,000/cm^3 of soil required to prevent *B. longicaudatus* population increases as reported by Luc et al. (2010 a).

Unlike the classical biological approach described by Giblin-Davis (2000) with CPu infested soil, use of Econem should be considered an augmentative/inundative biopestide approach. By adding a large amount of the biological control organism to what is already present, the host-pathogen equilibrium is tilted toward host suppression for a short period. The Lotka-Volterra model (Lotka, 1925; Volterra, 1926) predicts that equilibrium would be reestablished over time, but in the short-term, a level of host suppression is achieved. None of these studied were successful in demonstrating efficacy of the Econem formulation of *Pasteuria* sp. at reducing population densities of *B. longicaudatus* or improving turf health in the short-term. This lack of efficacy is most likely due to factors related to the Econem product and/or the turfgrass environment. This does not mean that other *Pasteuria*-based biopesticides or applications of Econem to other cropping systems will not be effective. However, this does stress the importance of thorough field testing of biopesticide products in the target cropping system to determine efficacy.

Acknowledgments. Special thanks to Ms. Tracey Wasylik, Mrs. Barbara J. Center, Dr. Dorota Porazinska and Tom Hewlett for assistance with the pot studies at the FLREC. Also, to Mr. Tom Bean, Dr. Wenjing Pang, Ms. Jay Cee Turner, and Mr.Yun Zhang for their assistance with the field trials.

LITERATURE CITED

Crow, W. T. 2011. Nematode Management on Golf Courses in Florida. ENY-008, Entomology and Nematology Dept., Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, Gainesville, FL.

Crow, W. T., and Han, H. R. 2005. Sting nematode. The Plant Health Instructor: DOI: 10.1094/PHI-I-2005-1208-01

Crow, W. T., Giblin-Davis, R. M., and Lickfeldt, D. W. 2003. Slit Injection of 1,3-dichloropropene for management of *Belonolaimus longicaudatus* on established bermudagrass. Journal of Nematology 35:302–305.

Crow, W. T., Lickfeldt, D. W., and Unruh, J. B. 2005. Management of sting nematode (*Belonolaimus longicaudatus*) on bermudagrass putting greens with 1,3-dichloropropene. International Turfgrass Society Research Journal 10 (2), 734–741.

Giblin-Davis, R. M. 2000. *Pasteuria* sp. for biological control of the sting nematode, *Belonolaimus longicaudatus*, in turfgrass. Pp. 408–426 *in* J.M. Clark, and M.P. Kenna, eds. Fate and Management of Turfgrass Chemicals, American Chemical Society Symposium Series No. 743. New York: Oxford University Press.

Giblin-Davis, R. M., Busey, P., and Center, B. J. 1992. Dynamics of *Belonolaimus longicaudatus* parasitism on a susceptible St. Augustinegrass host. Journal of Nematology 24:432–437.

Giblin-Davis, R. M., Williams, D. S., Bekal, S., Dickson, D. W., Brito, J. A., Becker, J. O., and Preston, J. F. 2003. ' *Candidatus* Pasteuria usgae' sp. nov., an obligate endoparasite of the phytoparasitic nematode *Belonolaimus longicaudatus*. International Journal of Systematic and Evolutionary Microbiology 53:197–200.

Giblin-Davis, R. M., Williams, D. S., Wergin, W. P., Dickson, D. W., Hewlett, T. E., Bekal, S., and Becker, J. O. 2001. Ultrastructure and development of *Pasteuria* sp. (S-1 strain), an obligate endoparasite of *Belonolaimus longicaudatus* (Nemata: Tylenchida). Journal of Nematology 33:227–238.

Jenkins, W. R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Karcher, D. E., and Richardson, M. D. 2005. Batch analysis of digital images to evaluate turfgrass characteristics. Crop Science 45:1536–1539.

Lotka, A. J. 1925. Elements of Physical Biology. Baltimore: Williams and Wilkins.

Luc, J. E., Crow, W. T., McSorley, R., and Giblin-Davis, R. M. 2010 a. Suppression of *Belonolaimus longicaudatus* with in vitro-produced *Pasteuria* sp. endospores. Nematropica 40:217–225.

Luc, J. E., Pang, W., Crow, W. T., and Giblin-Davis, R. M. 2010 b. Effects of formulation and host density on the ability of *in vitro*-produced *Pasteuria* endospores to control its host *Belonolaimus longicaudatus*. Journal of Nematology 42:87–90.

Luc, J. E., Crow, W. T., Pang, W., McSorley, R., and Giblin-Davis, R. M. 2011. Effects of irrigation, thatch, and wetting agent on movement of *Pasteuria* sp. endospores in turf. Nematropica 41:185–190.

Lunt, O. R. 1968. Depth of rooting of bermudagrass. California Turfgrass Culture 18:29.

Pang, W., Crow, W. T., Luc, J. E., McSorley, R., Giblin-Davis, R. M., Kenworthy, K. E., and Kruse, J. K. 2011 a. Comparison of water displacement and WinRHIZO software for plant root parameter assessment. Plant Disease 95:1308–1310.

Pang, W., Luc, J. E., Crow, W. T., Kenworthy, K. E., Giblin-Davis, R. M., McSorley, R., and Kruse, J. K. 2011 b. Bermudagrass cultivar responses to sting nematode. Crop Science 51:1–5.

Perry, V. G., and Rhoades, H. 1982. The genus *Belonolaimus*. Pp. 144–149 *in* R.D. Riggs, ed. Nematology in the Southern Region of the United States. Southern Cooperative Series Bulletin 276. Fayetteville, AR: University of Arkansas Agricultural Publications.

Trenholm, L. E., Lickfeldt, D. W., and Crow, W. T. 2005. Use of 1,3-dichloropropene to reduce irrigation requirements of sting nematode infested bermudagrass. HortScience 40 (5), 1543–1548.

Volterra, V. 1926. Variations and fluctuations of the number of individuals in animal species living together. Pp. 409–448 *in* R. N Chapman, ed. Animal Ecology. New York: McGraw-Hill.