EFFECTS OF IRRIGATION, THATCH, AND WETTING AGENT ON MOVEMENT OF *PASTEURIA* SP. ENDOSPORES IN TURF

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ABSTRACT

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Movement of pesticides and biopesticides into the turfgrass soil profile could be hindered by dense turf growth, thatch, organic matter, black layers, and hydrophobic areas. Conversely, high percolation due to rainfall or irrigation, combined with sand-based construction may cause treatments to leach below the rootzone, reducing effectiveness. We evaluated movement of *in vitro*-produced *Pasteuria* sp. endospores into a simulated putting green profile in lysimeters. Ten treatments included five watering levels: 0.6 cm of water with a wetting agent, 0.6 cm of water without a wetting agent, 2.5 cm of water without a wetting agent, 7.6 cm of water without a wetting agent, 15.2 cm of water without a wetting agent, and two thatch levels: with or without thatch. The endospores were applied as a drench at 1,990,000 endospores/cm^2 of soil surface. A bioassay using *Belonolaimus longicaudatus* was conducted to determine relative endospore attachment at four depths following the water treatments. Application of 15.2 cm of irrigation reduced percent endospore attachment by 62% and 39% at soil depths 0 to 2.5 and 2.5 to 10 cm respectively, and increased percent endospore attachment by 95% and 2297% at soil depths 10 to 20 and 20 to 30 cm, respectively compared with 0.6 cm of irrigation. Thatch and wetting agent treatments showed no significant effect on endospore placement. These results indicate that *in vitro*-produced *Pasteuria* sp. endospore movement into the turf profile is not hindered by thatch and that large irrigation events can move endospores below the turfgrass rhizosphere.

Key words: Belonolaimus longicaudatus, biological control, dispersal, irrigation, *Pasteuria*, sting nematode, turfgrass.

RESUMEN

Luc, J. E., W. T. Crow, W. Pang, R. McSorley, and R. M. Giblin-Davis. 2011. Efectos de la irrigación, los residuos vegetales y el agente humectante sobre el movimiento de endosporas de *Pasteuria* sp. en céspedes. Nematropica 41:185-190.

Factores como la alta densidad de plantas, la acumulación de residuos vegetales, la materia orgánica, capas negras y áreas hidrofóbicas pueden reducir el movimiento de pesticidas y biopesticidas en el perfil del suelo. De otro lado, la alta percolación causada por lluvias o irrigación, combinada con alto el contenido de arena puede causar que los tratamientos se escurran por debajo de la zona de las raíces, reduciendo su efectividad. En este trabajo se evaluó el movimiento de endosporas de *Pasteuria* sp. en un perfil simulado en lisímetros. Los diez tratamientos incluyeron cinco niveles de irrigación: 0.6 cm de agua con agente humectante, 0.6 cm de agua sin agente humectante, 2.5 cm de agua sin agente humectante, 7.6 cm de agua sin agente humectante, 15.2 cm de agua sin agente humectante, y dos niveles de residuos vegetales: con y sin acumulación de residuos. Se aplicaron las endosporas a razón de 1,990,000 endosporas/cm^2 de superficie de suelo. Se utilizó un bioensayo con *Belonolaimus longicaudatus* para deteminar la adhesión relativa a cuatro profundidades después de los tratamientos de irrigación. La aplicación de 15.2 cm de agua redujo el porcentaje de adhesión de endosporas en un 62% y 39% a profundidades de 0 a 2.5 y de 2.5 a 10 cm, respectivamente, y aumentó el porcentaje de adhesión de endosporas en un 95% y 2297% a profundidades de 10 a 20 y de 20 a 30 cm, respectivamente, comparados con 0.6 cm de irrigación. La acumulación de residuos vegetales y el uso de agentes humectantes no mostraron efectos significativos en el movimiento de las endosporas. Estos resultados demuestran que el movimiento de endosporas de *Pasteuria* sp. en el perfil del suelo no ve afectado por la acumulación de residuos vegetales y que la alta irrigación puede mover las endosporas por debajo de la rizosfera de los céspedes.

Palabras clave: Belonolaimus longicaudatus, céspedes, control biológico, dispersión, irrigación, *Pasteuria*.

INTRODUCTION

Management of plant-parasitic nematodes in turf has become increasingly difficult over the last decade due to the limited number of effective post-plant nematicides. Sting nematode, *Belonolaimus longicaudatus*, is considered the most destructive nematode on turf in Florida (Crow *et al*., 2003; Crow, 2005). Biological control of *B. longicaudatus* may offer another management tool to turfgrass managers in addition to chemical and cultural control methods. '*Candidatus* Pasteuria usgae' has been recognized as a naturally occurring biological agent that can suppress B. longicaudatus in turf (Giblin-Davis, 2000; Giblin-Davis *et al*., 2003) and use of *in vitro*-produced *Pasteuria* sp. endospores as a bionematicide for sting nematode is being explored. The movement of *in vitro*-produced *Pasteuria* sp. endospores in the soil profile may greatly affect its efficacy and success as a biopesticide on turf. In previous experiments, *in vivo*-produced *Pasteuria* spp. endospores have been inoculated into pots, micro-plots, and field plots using various sources of inoculum laden with endospores: ground root material, soil, second stage juvenile nematodes encumbered with endospores, or endospores in water suspension (Stirling and Wachtel, 1980; Dube and Smart, 1987; Chen *et al*., 1996; Weibelzahl-Fulton *et al*., 1996; Giblin-Davis, 2000; Kariuki and Dickson, 2007). However, methods in some of these experiments required disturbance of the soil profile to incorporate the endospore for testing. Unlike seasonal crops that are cultivated regularly allowing for incorporation of endospores throughout the soil profile, established turfgrass is relatively undisturbed. Movement of topically applied endospores into the turfgrass soil profile might be hindered by dense turf growth, thatch, or localized hydrophobic soil conditions (Murray and Juska, 1977; Tan, 1998). Thatch is an intermingled layer of living and dead plant tissues, and decaying organic matter between the turf canopy and soil surface. Excessive thatch layers (≥ 2.5cm-depth) can affect the movement of air, water, and pesticides (Dunn and Diesburg, 2004). Pesticides applied to control soil pests can be adsorbed and held in the thatch. Likewise, localized hydrophobic soil conditions can be created when fulvic acid coats soil particles and then dries. The dry fulvic acid then repels water hindering pesticide movement (Tan, 1998). Wetting agents can be used to decrease hydrophobic soil conditions and aid pesticide penetration. Golf course greens are constructed predominately of sand to have high percolation rates, and as a result have high leaching potential. Once in the mineral soil, increased percolation due to rainfall or irrigation following treatment may affect the distribution of endospores or cause endospores to be moved below the turf root zone (upper 10 cm of soil), reducing effectiveness (Cetintas and Dickson, 2005; Dabiré *et al*., 2005). The objective of this research was to determine the effect of thatch and wetting agent on movement of *in vitro*-produced *Pasteuria* sp. endospores into the soil profile and to determine if *in vitro*-produced *Pasteuria* sp. endospores were subject to leaching from large irrigation or rainfall events.

MATERIALS AND METHODS

A greenhouse experiment was conducted and replicated at the Turfgrass Envirotron at the University of Florida from August 2007 to August 2008. The ten treatments evaluated were five watering levels: (i) 0.6 cm of water with a wetting agent (Lesco Wet®) (John Deere Landscapes, Troy, MI) at 2.54 ml/m^2, (ii) 0.6 cm of water without a wetting agent, (iii) 2.5 cm of water without a wetting agent, (iv) 7.5 cm of water without a wetting agent, and (v) 15.2 cm of water without a wetting agent. Each watering level was examined with thatch (2.5-cm depth) or without thatch.

Fifty lysimeters (5.08-cm-diam., 45.5-cm-deep, 927-cm^3-volume) were used to simulate a golf course putting green soil profile. In the bottom of the lysimeters was placed 15 cm of gravel (2-mm-diam.) covered with an additional 30 cm of nematode-free U.S. Golf Association specification root-zone sand (Anonymous, 1993). Plugs of recently established 'Tifdwarf' bermudagrass (*Cynodon dactylon* Var. *dactylon* × *C. transvaalensis*) were harvested from pots using a soil probe (5.08-cm-diam.) and planted onto 25 lysimeters to simulate turf with minimal to no thatch. Conversely, plugs of 'Tifdwarf' bermudagrass from a putting green with an extensive thatch layer were harvested and soil removed to simulate turf with a heavy thatch layer. Prior to the turf being harvested, nematode and '*Candidatus* Pasteuria usgae' bioassay tests were performed, and no *B. longicaudatus* or '*Candidatus* Pasteuria usgae' endospores were detected. Following planting, approximately 0.25 cm of sand was added to the surface of each lysimeter as topdressing. During grow-in, the turf was watered 3 times/day with 10 ml of water for 3 weeks.

Following establishment the turf was watered daily with 25 ml of water. Turf was fertilized every 2 wk with Peters® 24-4-16 (N-P_2O5-K_2O) fertilizer (United Industries Corp., St. Louis, MO). Nutrient inputs were 49 kg/ha N, 3.6 kg/ha P, 27.1 kg/ha K/month, along with trace amounts of essential micronutrients (0.8 kg/ha Mg, 9.6 kg/ha S, 6.9 g/ha B, 40.8 g/ha Cu, 102.1 g/ha Fe, 40.8 g/ha Mn, 1.8 g/ha Mo, and 40.8 g/ha Zn). Turf was trimmed to 3-cm-height once/wk. The turf was allowed to establish a root system for 7 mo before applying endospores.

In vitro endospores were produced from an isolate of *Pasteuria* sp. that was collected and cultured from *B. longicaudatus* on turf from Sebring, Florida. The endospores were cultured *in vitro* by Pasteuria Bioscience LLC (Alachua, FL) and kept refrigerated at 4°C for 3 d to allow time to quantify endospores/ml and obtain morphometric measurements. *In vitro* endospore measurements indicated that mean core diam was

consistent with previously published measurements for '*Candidatus* Pasteuria usgae'; however mean sporangium diam of in vitro endospores was variable (Giblin-Davis *et al*., 2001). *In vitro*-produced *Pasteuria* sp. endospores were applied topically at 1,990,000 endospores/cm^2 of soil. Endospores were applied to all lysimeters as a drench using 0.6 cm (12 ml) of water, spore media, and wetting agent if assigned. Following treatment, the lysimeters remained undisturbed for 2 d, and then water treatments were applied once.

Destructive sampling occurred one week after the water treatments were applied. The entire soil profile (5.08-cm-diam., 30-cm-deep) was removed from each lysimeter. These soil profiles were cut into sections to determine endospore presence at four soil depths (0 to 2.5 cm, 2.5 to 10 cm, 10 to 20 cm, and 20 to 30 cm-deep) providing four depth samples per soil profile. Each sample was placed in a paper bag and allowed to air dry.

After drying, each sample was gently mixed, and a sub sample (100 g) was placed onto a coffee filter within a sterile polyethylene container (6.5-cm-wide, 6.5-cm-length, 10-cm-deep). Twenty-five ml of water were added to each sample to rehydrate the soil allowing for increased nematode movement (Brown and Smart, 1984). Two hundred mixed life stages of *B. longicaudatus* in 5 ml of water were added and the containers left uncovered at room temperature. Three days later, the nematodes were extracted from the soil using the centrifugal-flotation method (Jenkins, 1964). Subsequently, 20 nematodes were randomly selected from each sample population and number of endospores attached per nematode was determined by observation with an inverted microscope at ×400 magnification (Chen and Dickson, 1997).

Data for thatch and wetting agent were subjected to analysis of variance (ANOVA) and orthogonal contrast with SAS software (SAS Institute, Cary, NC). Microsoft Excel (Microsoft, Redmond, WA) was used for regression analysis. Data for percent endospore attachment were transformed using natural log (y) before statistical analysis. Transformed numbers are presented in the figures and untransformed data are presented in the table.

RESULTS

During trial one, increasing the amount of irrigation reduced ($P \leq 0.001$) percent endospore attachment in soil depths 0 to 2.5 and 2.5 to 10 cm and increased ($P \leq 0.001$) percent endospore attachment at soil depths 10 to 20 and 20 to 30 cm, compared with 0.6 cm of irrigation. Application of 2.5, 7.6, and 15.2 cm of irrigation reduced percent endospore attachment by 16%, 47%, and 73%, respectively, compared with 0.6 cm of irrigation at soil depth 0 to 2.5 cm (Figs. 1A). Similarly, irrigation treatments reduced percent endospore attachment by 8%, 26%, and 46%, respectively, compared with 0.6 cm of irrigation at a soil depth of 2.5 to 10 cm (Figs. 1B). However, at soil depth 10 to 20 cm, irrigation treatments increased percent endospore attachment by 12%, 51%, and 136%, respectively, compared with 0.6 cm of irrigation (Figs. 1C). At the deepest soil depth (20 to 30 cm), irrigation treatments increased percent endospore attachment by 54%, 359%, and 2512%, respectively, compared with 0.6 cm of irrigation (Figs. 1D).

Results of trial two were similar to those of trial one. Application of 2.5, 7.6, and 15.2 cm of irrigation reduced percent endospore attachment by 12%, 37%, and 62%, respectively compared with 0.6 cm of irrigation at soil depth 0 to 2.5 cm (Figs. 2A). Likewise, irrigation treatments reduced percent endospore attachment by 6%, 21%, and 39%, respectively, compared with 0.6 cm of irrigation at soil depth 2.5 to 10 cm (Figs. 2B). Conversely, at a soil depth of 10 to 20 cm, irrigation treatments increased percent endospore attachment by 9%, 38%, and 95%, respectively compared with 0.6 cm of irrigation (Figs. 2C). At the deepest soil depth (20 to 30 cm), irrigation treatments increased percent endospore attachment by 52%, 358%, and 2297%, respectively, compared with 0.6 cm of irrigation (Figs. 2D). During both trials, thatch and wetting agent treatments showed no effect ($P \leq 0.05$) on movement of endospores into the soil profile (Table 1).

DISCUSSION

Movement by water is the basic method of bacteria dispersal throughout the soil profile and is a major reason for *Pasteuria* spp. endospore losses from the cultivated soil horizon (Gammack *et al*., 1992). One application of 0.6 cm of irrigation was sufficient to place *in vitro*-produced *Pasteuria* sp. endospores within the turfgrass rhizosphere (0 to 10 cm soil depths), with some endospores moving into the 10 to 20 cm depth. A slight reduction in irrigation to 0.5 cm might prevent the movement of endospores below the root zone, concentrating the endospores where most nematodes are actively feeding and causing damage. Previous research has shown that repeated applications of water could leach *Pasteuria* spp. endospores, reducing endospore levels and effectiveness (Zyman and Sorber, 1988; Cetintas and Dickson, 2005; Dabiré *et al*., 2005). In our studies, a single application of water simulating a large rainfall event was sufficient to move many of the *in vitro*-produced *Pasteuria* sp. endospores below the turf rhizosphere. Timing of application may need to be scheduled at times when rainfall is minimal. In Florida, this might be best accomplished during early fall and spring. Reapplication of *in vitro*-produced *Pasteuria* sp. endospores following rainfall events might be required to maintain desired nematode reductions.

Thatch did not hinder the movement of endospores into the soil profile and is unlikely to impact its utility as a bionematicide. Wetting agents are used to improve the movement of water and other liquids into the soil

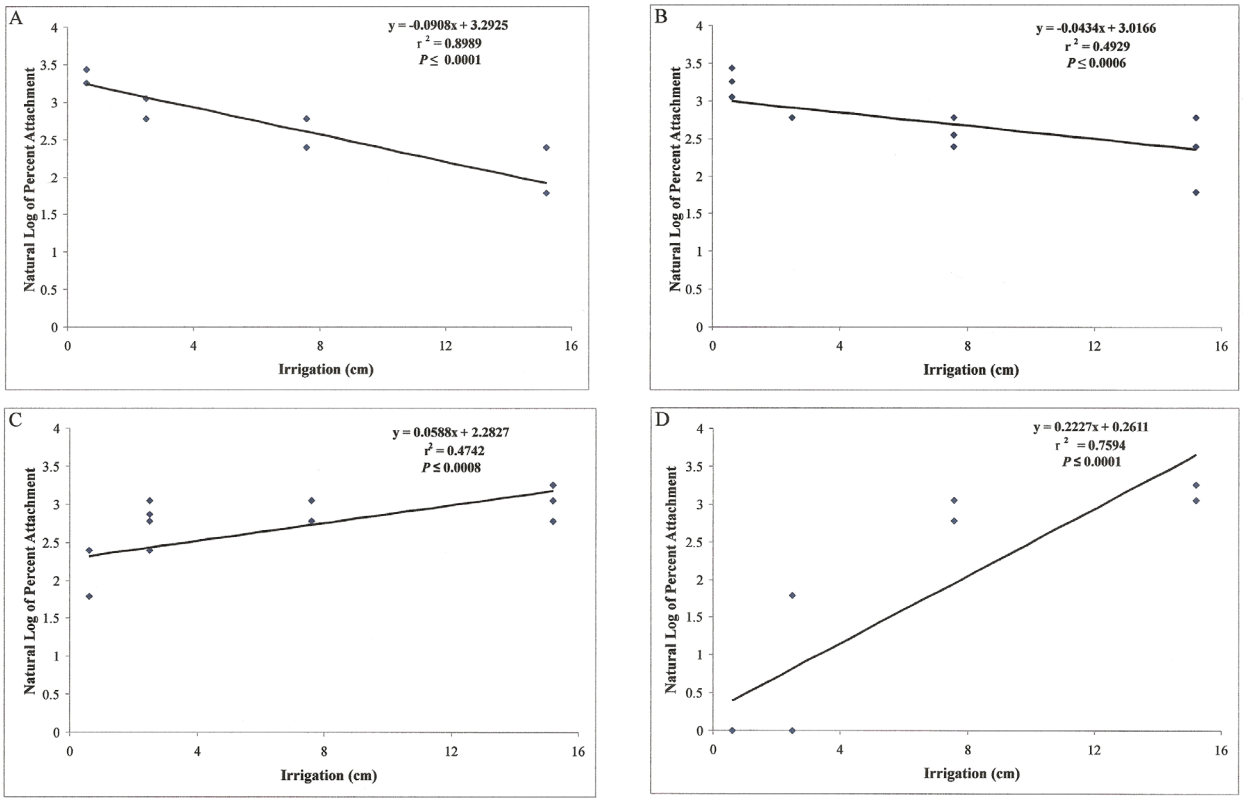

Fig. 1. Effect of increasing irrigation on depth placement of *in vitro*-produced *Pasteuria* sp. endospores in soil columns as determined by bioassay with *Belonolaimus longicaudatus* during trial 1. Soil depth ranges evaluated were: A) 0.0 to 2.5cm, B) 2.5 to 10.0 cm, C) 10.0 to 20.0 cm and D) 20.0 to 30.0 cm. Data are means and standard deviations of five replications.

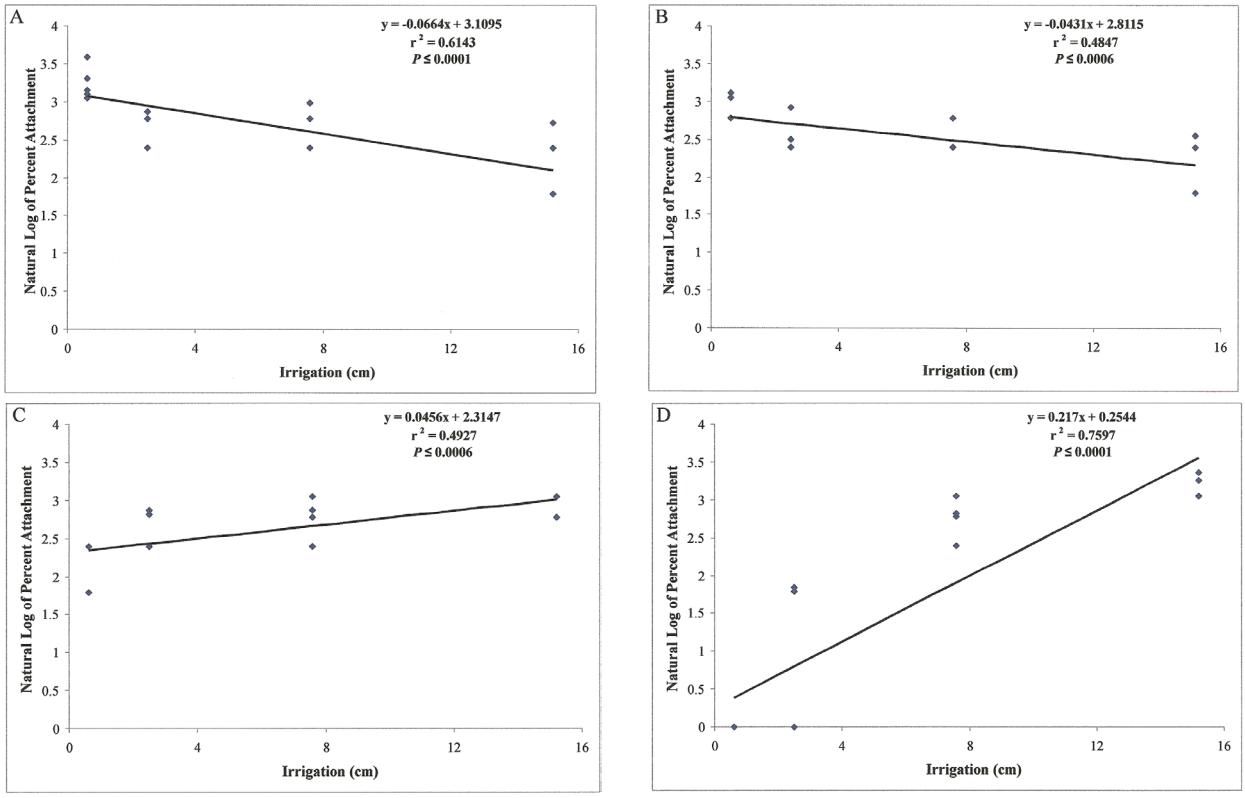

Fig. 2. Effect of increasing irrigation on depth placement of *in vitro*-produced *Pasteuria* sp. endospores in soil columns as determined by bioassay with *Belonolaimus longicaudatus* during trial 2. Soil depth ranges evaluated were: A) 0.0 to 2.5cm, B) 2.5 to 10.0 cm, C) 10.0 to 20.0 cm and D) 20.0 to 30.0 cm. Data are means and standard deviations of five replications.

Table 1. Effect of thatch or wetting agent on movement of topically applied *in vitro*-produced *Pasteuria* sp. in greenhouse lysimeters planted with 'Tifdwarf' bermudagrass at four soil depths determined by bioassay of endospore attachment to *Belonolaimus longicaudatus*. Thatch data pooled across irrigation levels because thatch and irrigation level interaction was not significant ($P \leq 0.05$).

	Percent of *Belonolaimus longicaudatus* with endospores attached					
	(Trial 1)					
Soil Depth (cm)	Thatch[v]	No Thatch	*P*	Wetting Agent[w]	No Wetting Agent	*P*
0 to 2.5	11.18 ± 10.22[x]	11.40 ± 12.54	NS[y]	25.78 ± 2.91[Z]	25.94 ± 3.08	NS
2.5 to 10.0	20.62 ± 5.58	18.11 ± 8.28	NS	22.21 ± 3.48	22.36 ± 4.63	NS
10.0 to 20.0	17.19 ± 7.67	16.50 ± 8.06	NS	1.50 ± 3.34	1.50 ± 3.37	NS
20.0 to 30.0	9.97 ± 9.33	11.87 ± 8.43	NS	1.03 ± 2.16	0.00 ± 0.00	NS
All depths	14.74 ± 8.97	14.47 ± 9.41	NS	12.63 ± 11.94	12.45 ± 12.33	NS
	(Trial 2)					
0 to 2.5	20.28 ± 8.79	12.79 ± 12.79	NS	28.05 ± 8.39	24.09 ± 4.64	NS
2.5 to 10.0	15.55 ± 4.96	17.28 ± 17.28	NS	18.08 ± 4.16	19.41 ± 2.96	NS
10.0 to 20.0	9.19 ± 8.16	13.71 ± 13.71	NS	1.00 ± 2.11	1.50 ± 3.37	NS
20.0 to 30.0	11.06 ± 10.53	10.23 ± 10.23	NS	0.00 ± 0.00	1.00 ± 2.11	NS
All depths	14.02 ± 9.29	12.79 ± 7.93	NS	11.78 ± 12.83	11.50 ± 11.01	NS

[v]Thatch layer was 2.54 cm in depth.
[w]Wetting agent was Lesco Wet ® at 2.54 ml/m2.
[x]Means and standard deviation for 25 replications.
[y]NS = No statistical difference P ≤ 0.05.
[z]Means and standard deviation for five replications.

profile, especially when the soil is hydrophobic. In this experiment, turf was watered daily, preventing the soil from becoming dry or hydrophobic. However in the field, wetting agents might help to provide a more even distribution of endospores by creating conditions more favorable to uniform water movement. Because the wetting agent did not increase leaching of endospores it should not hinder spore movement in turf profile.

In conclusion, greenhouse studies indicate that irrigation has a great impact on the movement of *in vitro*-produced *Pasteuria* sp. endospores in turf and thereby can affect its utility as a bionematicide. Movement of topically applied endospores into the turfgrass rhizosphere is likely achieved by routine irrigation practices. Conversely, too much irrigation or rainfall might leach the endospores out of the turf rhizosphere and thereby reduce efficacy. Clearly, water management is critical for placement and retention of *in vitro*-produced *Pasteuria* sp. endospores and other biopesticides in the typically sandy soils of turf in Florida.

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