PASTEURIA SP. FOR BIOLOGICAL CONTROL OF THE STING NEMATODE, *BELONOLAIMUS LONGICAUDATUS*, IN TURFGRASS

1997 USGA TURFGRASS ANNUAL AND FINAL REPORT

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1997 EXECUTIVE SUMMARY

A new species of *Pasteuria* (S-1) was discovered that parasitizes the sting nematode, *Belonolaimus longicaudatus*. Host range studies with several species of soil inhabiting nematodes have demonstrated that this obligate endoparasitic bacterium only attaches to members of the genus of *Belonolaimus*. Ultrastructural and morphometric studies of mature and developing endospores with transmission and scanning electron microscopy (TEM and SEM) have shown that this *Pasteuria* is unique relative to the other described species of *Pasteuria* but that different geographical isolates are morphologically and morphometrically constant. A two-year survey was conducted with 6 different sites of hybrid bermudagrass (Fairways) at the Ft. Lauderdale Research and Education Center where *Pasteuria* (S-1) occurs naturally at different levels to monitor its suppressive effects at three different soil depths on sting nematodes. Density dependent regulation of sting nematodes appears to be occurring in areas with *Pasteuria* (S-1). Survey locations that started with low levels of spore encumbrance showed a building trend in encumbrance levels and a corresponding decline in the numbers of sting nematodes. Locations with high spore encumbrance levels cycled and appeared to suppress sting nematode population resurgences, suggesting that *Pasteuria* (S-1) might help produce suppressive soil for the sting nematode in the turfgrass ecosystem. An eighteen month study was completed that compared the effects of inoculation with 900 mls of *Pasteuria* (S-1) spore-infested soil (ca. 5,000 endospores/ml) versus 900 mls of autoclaved soil in 1 m^2 'Tifdwarf' bermudagrass (Greens) plots. This study showed that a relatively small amount of *Pasteuria*-infested soil can be introduced into a USGA green with high numbers of sting nematodes and bring about density dependent suppression within about 12 months.

1993-1997 RESEARCH SUMMARY

Description of *Pasteuria* n. sp. (S-1) from the sting nematode from Ft. Lauderdale, Florida: I am currently writing the description of *Pasteuria* n. sp. (S-1) from the sting nematode in southern Florida based upon ultrastructure, morphometrics, development, and host range studies.

Transmission electron microscopy (TEM): Ultrastructural studies of the new species of *Pasteuria* from the sting nematode are now complete. Over the past 3 1/2 years, we have continued to examine spore-filled and spore-encumbered nematodes to get publication quality photomicrographs of all aspects of the biology and development of *Pasteuria* n. sp. (S-1). In addition, with the help of Dr. Bill Wergin at the USDA in Beltsville, MD, we examined the external morphology of *Pasteuria* n. sp. (S-1) with low temperature scanning electron microscopy (SEM).

SEM work demonstrates that the external morphology of attached spores of *Pasteuria* n. sp. (S-1) is significantly different than any of the described species. Basically, the peripheral fibers of the endospore protrude around the exposed spherical outer coat of the spore creating a crenate border which gives the endospore the appearance of a fried egg with a scalloped ring around the yolk. All of the other spores described from nematodes appear like a "fried egg" without a scalloped border. The sporangium and endospore diameters of *Pasteuria* n. sp. (S-1) were on the average at least 1.0 and 0.5 m wider than these respective measurements for the other described species of *Pasteuria* or other host isolates of *Pasteuria* from southern Florida fairways (1994 report). In TEM, the epicortical wall of *Pasteuria* n. sp. (S-1) surrounds the cortex in a sublateral band and the basal cortical wall thins to expose the inner endospore, similar to *P. thornei* but different from the other two species. The spore pore diameter, measured from TEM micrographs, is larger than any other described species of *Pasteuria*. The outer coat wall thickness at its thickest point is 1/6 the diameter of the central body for *Pasteuria* n. sp. (S-1) compared with 1/8-1/30 for the other described species of *Pasteuria*.

A brief description of the life cycle of *Pasteuria* n. sp. (S-1) based upon LM and TEM follows; after attachment of a mature endospore to the cuticle of the host, penetration ensues via a germ tube through the cuticle into the pseudocoelom of the sting nematode. All stages from J2 through adults were observed with attached endospores on the cuticle and J3 through adults were observed with internal infections of vegetative and sporulating *Pasteuria* n. sp. (S-1). Counts of spore-filled cadavers showed that juveniles (J3-J4; N = 5), males (N = 5), and females (N = 5) contained averages of 4,700, 1,483, and 3,633 spores/nematode, respectively. A mycelial microcolony is formed which increases in size in the pseudocoelom. Mycelial filaments are divided by septa and possess double-layered cell walls. Endospores are produced endogenously and the formation sequence (sporogenesis) for *Pasteuria* n. sp. (S-1) is similar to the three other described species of *Pasteuria* and species of *Bacillus*. We have observed stage II; the formation of the forespore septum, stage III; engulfment of the forespore and appearance of peripheral fibers, stage IV; formation of the cortex, appearance of the sporangial wall, enlargement of the peripheral fibers, and dorso-ventral flattening of the

spore, stage V; formation of the spore coats; epicortex appears first and surrounds most of the cortex, thinning near the pore. As stage V progresses the epicortex appears to migrate equatorially. Stage VI; formation of the exosporium, the exosporium appears as the spore coats are still forming. The outer spore coat appears dorsally as a cap and descends around the cortex as an inner spore coat is formed. Concordant with the development of the outer and inner spore coats the epicortical region continues to recede into a sublateral band and the cytoplasm bounded by the exosporium is reduced. Stage VII; spore maturation, outer spore coat continues to thicken dorasIly and laterally as the cytoplasm in between the spore and the exosporium disappears, a microfibrillar layer appears on the peripheral fibers.

Host range studies with *Pasteuria* n. sp. (S-1): Host range studies have been completed. Basically, *Pasteuria* n. sp. (S-1) spores only attach to *Belonolaimus longicaudatus* isolates and *B. enthychilus*. This is consistent with field work that we have done in southern Florida fairways. We only see the *Pasteuria* n. sp. (S-1) attaching and completing its life cycle in sting nematodes, even when there are many other species of nematodes in the same sample (i.e. *Hoplolaimus galeatus, Tylenchorhynchus annulatus, Meloidogyne spp., Helicotylenchus microlobus, Hemicriconemoides annulatus, Criconemella ornata, Trichodorus proximus*, and several freeliving nematode species).

Laboratory time-course study of sting nematode with or without *Pasteuria* n. sp. (S-1): We completed a laboratory pot assay to study the population dynamics of the sting nematode and compare the ability of *Pasteuria* to suppress the establishment of *B. longicaudatus* on FX-313 St. Augustinegrass [*Stenotaphrum secundatum*]. Treatments involved a harvest factor (harvested 42, 84, 126, 210, 308, and 392 days after inoculation) and a *Pasteuria* encumbrance factor. There were four treatments; 1) no sting nematodes with no bacteria, 2) sting nematodes (99 $\pm$ 10) with no bacteria, 3) sting nematodes (99 $\pm$ 10) + 10 sting nematodes encumbered with 8 $\pm$ 6 spores of *Pasteuria* n. sp. (S-1), and 4) sting nematodes (99 $\pm$ 10) + 25 sting nematodes encumbered with 8 $\pm$ 6 spores of *Pasteuria* n. sp. (S-1). Spore encumbered *B. longicaudatus* were harvested from a Ft. Lauderdale, FL, field site with *Pasteuria* n. sp. (S-1). The resulting 20 combinations were arranged in a randomized complete block design with 9 replications for time periods 42, 84, and 126 days and 6 replications for the 210, 308, and 392 day time periods.

The assumption was that inoculated sick nematodes would not add to the population growth of the healthy sting nematodes but would die and release bacteria that would negatively affect the healthy population. Our results demonstrate that this was not the case. Population dynamics of the healthy sting nematodes were significantly increased by the addition of "sick" nematodes (high *Pasteuria* treatment was highest at 84 days, the low *Pasteuria* treatment was highest at 126 days, and the no *Pasteuria* treatment did not peak until 168 days) suggesting that spore encumbrance is not a good indicator of spore production potential. Root dry weights for the different treatments confirmed that root loss was greatest in the treatments receiving the most nematodes. Although spore encumbered sting nematodes were recovered throughout most of the 390 day study the levels were never greater than 1% from treatments receiving spores which suggests that inoculative release of "sick" nematodes is unacceptable

for establishment and population suppression work.

Seasonal depth survey of hybrid bermudagrass sites with different levels of sting nematode and *Pasteuria* n. sp. (S-1):

In 1995, we began a monthly survey of 6 different sites of hybrid bermudagrass (fairway conditions) at the Ft. Lauderdale Research and Education Center where *Pasteuria* n. sp. (S-1) occurs naturally at different levels to monitor its suppressive effects on sting nematodes at three different soil depths (0-10 cm, 10-20 cm, and 20-40 cm). This study was completed this year (April 7, 1997). Weekly maximum and minimum soil temperatures were recorded at 5, 15, 25, and 40 cm using Fisher Digital internal/external thermometers with external sensors. The recording units were housed in a white-vented wooden box that stood 1.7 m off the ground. The external sensors were routed from the box through tygon tubing inside pvc pipe down to a wooden sensor holder. The board (2 cm thick) had 5 cm diam holes cut and staggered from the vertical axis at each depth. A 1 cm diam copper pipe was threaded through a drilled hole that came from the side of the board through the center of the hole. The copper tube was sealed with silicon sealant. The board was positioned in the soil and anchored with a stake. The entire wooden sensor board was surrounded by a grounded extruded aluminum cage. All soil was replaced and leveled. About 30 sites around the station were pre-sampled for sting nematodes and *Pasteuria* (S-1) n. sp. Six locations were chosen. Nematode and bacterial sampling and root dry weights have been done as described above. The general trend for all locations was that >98% of all roots recovered were from the top 10 cm. Maximum and minimum soil temperatures were highest and lowest, respectively for 5 cm and the level of fluctuation flattened out at 40 cm. Location A was chosen because it had high sting levels with no *Pasteuria*. Over the course of the survey the densities of sting nematode have held high and *Pasteuria* has been detected at low levels (<13%). Sting nematode densities were highest at 0-10 cm and lowest at 20-40 cm. Location B showed similar trends, although the densities of sting nematode have not been as high as in location A. Location C was chosen because it had moderate levels of sting and high encumbrance by *Pasteuria*. By the time we started regular sampling, the sting numbers had plummeted and *Pasteuria* levels were high, suggesting an epizootic. Location D had moderate levels of both sting nematode and *Pasteuria* which appeared stable throughout the sampling. Locations E and F both started with moderate levels of sting and high levels of *Pasteuria* and we have observed what appears to be suppression of the sting nematode.

In general, after 24 months of sampling, we have observed what appears to be density dependent regulation of sting nematodes in areas with *Pasteuria* n. sp. (S-1). Locations that started with low levels of spore encumbrance showed a building trend in encumbrance levels and a corresponding decline in the numbers of sting nematodes. Locations with high spore encumbrance levels cycled and appeared to suppress sting nematode population resurgences, suggesting that *Pasteuria* n. sp. (S-1) might help produce suppressive soil for the sting nematode in the turfgrass ecosystem. These results are encouraging and we are planning to analyze them using a Lotka-Volterra predation model.

Effects of *Pasteuria* (S-1) Infested Soil on Sting Nematode in a Bermudagrass Green: Soil that was infested with S-1 *Pasteuria* was collected from a bermudagrass fairway area on 29 February 1996. Soil was mixed uniformly, cleaned of roots and rock with a #10 screen, sampled for sting nematode and spore counts, and divided in half. One half of the soil was autoclaved for 90 min. and dried in a drying oven at 45-46 C for 48 hours to kill all *Pasteuria*. The other half of the soil was only dried in the drying oven at 45-46 C for 48 hours to kill nematodes but leave the *Pasteuria* alive. An area of 'Tifdwarf' bermudagrass (certified from Tifton, Georgia stock) (obtained from Rapid-Turf) that had been established in 20 X 25 ft plots since 20 November 1992 was pre-sampled for nematodes and discovered to have high counts (200 sting/ 100 cc) without *Pasteuria* (S-1). This area was marked in a grid of 1 m^2 plots with 15 cm borders and pre-sampled and stratified for sting nematode counts. Plots with equal sting counts were paired and inoculated with 900 g of soil (autoclave treatment versus dried soil). There are 10 replications for each treatment. Inoculations were done on 28 March 1996 by removing a 15 cm core from the center of each plot and removing 900 g of soil which was replaced with the treated soil.

The test plots have been monitored every 6 months for eighteen months. In the first 6 months, *Pasteuria* encumbrance increased significantly at the point of inoculation (center) and 25 cm from the center of the plot in soil that was not autoclaved (Table 1). Unfortunately, there was contamination of the control plots (autoclaved) (Table 1). There was a very small but detectable level of *Pasteuria* in the plots prior to the start of the experiment (Table 1). *Pasteuria*-infested nematodes may be spreading the bacteria or equipment, water, etc may possibly help spore movement. At one year after treatment, there was a significant decline in the sting nematode counts from *Pasteuria*-treated plots at the center and at 25 cm from inoculation (Table 1). This suggests that the significant increase in the proportion of sting nematodes encumbered with *Pasteuria* observed at 6 months after treatment led to a decline in the populations of the sting nematodes at one year after treatment. These data are consistent with previous greenhouse work and with the 2-year field survey work (see above) where density dependent regulation of sting nematodes appears to be occurring. I am still analyzing the data concerning the number of spores per sting nematode. Preliminary analysis suggests that these numbers are inversely correlated with the sting nematode counts. These data are very encouraging because they suggest that a relatively small amount of *Pasteuria*-infested soil can be inoculated into a USGA site with high numbers of sting nematodes to bring about density dependent control within a fairly short period of time.

Part of the explanation for the precipitous decline in sting nematode numbers may be that spore encumbered nematodes do not move as well as unencumbered nematodes. Sting is a relatively large nematode that requires coarse sand soils (>80% sand) to survive. An increase in clay or silt to > 20% prevents this nematode from prospering. *Pasteuria* S-1 is a large spore (6 m) that could hinder the movement of these nematodes once it becomes attached. It is possible that the surface sugar proteins that are so host specific on these bacteria may be potentially useful for the development of soils that selectively bind to and tie up sting nematodes or can be used as targeting molecules to carry toxins to the nematode for biorational control strategies.

Table 1. Sting nematode counts from plots on a 'Tifdwarf' green before and after treatment with 900 cc of soil infested with about 10,000 spores of *Pasteuria* S-1/cc (*Pasteuria*) or autoclaved soil.

	Treatment	Pre-counts		6 months			12 months			18 months	
			Center	25 cm	50 cm	Center	25 cm	50 cm	Center	25 cm	50 cm
Sting	Autoclaved	296 A	100 A	183 A	139 A	143 A	123 A	109 A	14 A	9 A	8 A
per 100 cc	Pasteuria	294 A	88 A	103 A	129 A	7 B	33 B	87 A	0 B	0 B	0 B
Visual	Autoclaved	5.8 A		3.9 A			6.7 A			5.2 A	
Ratings	Pasteuria	5.8 A		3.5 A			6.6 A			5.3 A	
% Sting	Autoclaved	0.0 A	7.9 B	12.0B	10.1A	55.5A	77.1A	50.5A	57.7A	48.2A	63.3A
with spores	Pasteuria	0.5 A	91.0A	74.5A	28.0A	50.7A	52.2A	55.5A	20.0A	30.0A	20.0B
%filled	Autoclaved	0.0 A	0.5 A	0.5 A	0.0 A	2.5 A	0.5 A	0.5 A	6.2 A	2.2 A	4.6 A
with spores	Pasteuria	0.1 A	5.0 A	4.2 A	0.5 A	0.0 A	1.3 A	0.0 A	0.0 A	0.0 A	0.0 A