Pasteuria sp. for Biological Control of the Sting Nematode, *(Belonolaimus longicaudatus)*, in Turfgrass

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Goals:

- *Examine bacteria ultrastructure with transmission electron microscopy and begin describing a new species of Pasteuria that was discovered parasitizing the sting nematode, Belonolaimus longicaudatus.*
- *Perform host range studies on this new Pasteuria sp.*
- *Begin studies to elucidate the population dynamics of this new Pasteuria sp. on sting nematode grown on St. Augustinegrass in laboratory pot cultures under controlled conditions.*

We are describing a new species of bacterium in the genus *Pasteuria,* discovered parasitizing the sting nematode, *Belonolaimus longicaudatus* in Florida. This obligate bacterial parasite of nematodes (*Pasteuria* n. sp. [S-1]) may have potential for inoculative biological control in golf course greens against the sting nematode; a destructive ectoparasite that can reduce the root dry weight of turfgrasses and other crops in sandy soils by as much as 30 to 50%.

In 1996, we completed ultrastructua1 studies with transmission electron microscopy (TEM) and low-temperature and regular scanning electron microscopy (SEM) that show that *Pasteuria* n. sp. (S-1) is a new species. We have elucidated the development and life cycle of this bacterium with excellent photomicrographs over the past 3 years.

In 1995, we began a monthly survey of 6 different sites of TIFDWARF and TIFGREEN hybrid bermudagrass (fairway conditions) at the Ft. Lauderdale Research and Education Center where *Pasteuria* n. sp. occurs naturally at different levels to monitor its suppressive effects on sting nematode populations at three different soil depths. Soil temperature was also monitored at these different depths.

After 18 months of sampling, we have documented what appear to be epizootics of the sting nematode caused by the *Pasteuria* n. sp. Locations that started with low levels of spore encumbrance have shown increases in the numbers of nematodes encumbered with spores and a decrease in the total sting

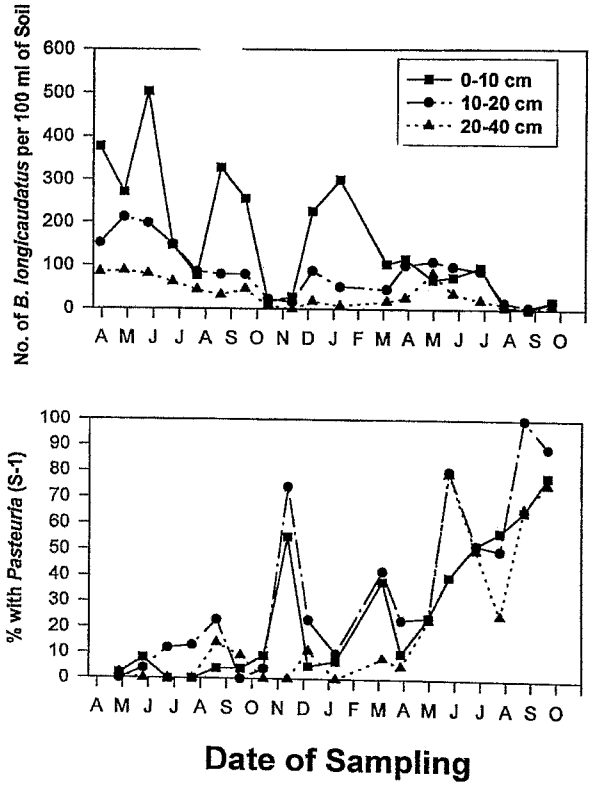

Figure 12. One of six field locations where sting nematodes and *Pasteuria* (s-1) n. sp. bacterial sampling and root dry weights were recorded. In the location depicted above, sting nematode populations were reduced as the *Pasteuria* (s-1) n. sp. populations increased.

nematode numbers. Areas that started with high encumbrance levels, suggesting that *Pasteuria* n. sp. was established, have continued to be suppressive in what appears to be a density dependent manner.

In 1996, a *Pasteuria* n. sp. spore encumbrance bioassay was developed using spores extracted from spore-filled cadavers and inoculated into 1 gm of soil in tubes at different doses (0, 10, 100, 500, 1000, 5000, 10,000, and 100,000 spores). Ten sting nematodes were then inoculated into the soil and incubated at 25 C for 21 days, and extracted and stained and counted for spore-encumbrance levels. These data are being used for a model to estimate spore-densities from unknown soils.

In 1994-1995, our 390-day lab study demonstrated that inoculative release of *Pasteuria* n. sp. encumbered sting nematodes was unacceptable for establishment and population suppression of healthy sting nematodes. Therefore, a field study was undertaken in 1996 to determine whether inoculation of *Pasteuria* n. sp.-infested soil from one of the survey areas which appeared to be suppressive had any promise. Soil was collected and pooled from a heavily *Pasteuria* n. sp.-infested area near to location E from the field epizootic study.

The spore encumbrance bioassay was used to estimate the numbers of spores in the randomly mixed and dried soil. There were two treatments of the soil: 1) control soil that was autoclaved for 2 hours, killing all nematodes and *Pasteuria* n. sp.; and 2) soil heated to 47 C for 48 hours to kill the sting nematodes but not the *Pasteuria* n. sp. A plot of TIFDWARF bermudagrass was divided up into a grid of 1 m^2 plots with 15 cm borders. Pre-counts of sting nematodes present per 100 cm^3 subsample were taken using the sugar flotation method.

Plots were ranked according to sting nematode density and treatments were randomly assigned within ranks. A 15 cm diameter cup cutter was used to remove a core from the center of each 1 m^2 plot. Soil (900 cm^3) was removed and replaced with an equal volume of the assigned treated soil. The core was then replaced and leveled. Six months after inoculation, sting nematode densities were statistically equal for both treatments. However, there was a significant difference in the proportion of sting nematodes encumbered (90% vs. 7%) and filled (5% vs. 1 %) for the heat-treated vs.

autoclaved soil treatments, respectively) with *Pasteuria* n. sp. endospores.

These data suggest that *Pasteuria* n. sp. was present but undetected before the experiment was started and that the inoculation of soil was successful at establishing the *Pasteuria* n. sp. in the turfgrass ecosystem. We are continuing to monitor the spread (increase of the radius of the *Pasteuria* n. sp. infestation) and whether sting nematode densities are suppressed by the bacterial disease over time in a golf course green.