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

1995 USGA TURFGRASS RESEARCH REPORT

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1995 OBJECTIVES

1) Finish ultrastructural studies with transmission electron microscopy (TEM) and low-temperature scanning electron microscopy (SEM) for a description of the development and morphology of this new species of Pasteuria (S-1) parasitizing the sting nematode, Belonolaimus longicaudatus from Ft. Lauderdale, Florida.

2) Finish studies to elucidate the population dynamics of this new Pasteuria sp. on sting nematode grown on FX-313 St. Augustinegrass in laboratory pot cultures under controlled conditions.

3) Start monthly survey of several hybrid bermudagrass sites at Ft. Lauderdale Research and Education Center and monitor sting nematodes, S-1 Pasteuria bacteria, and temperature at three different soil depths.

1995 USGA TURFGRASS RESEARCH EXECUTIVE SUMMARY

We are describing a new species of bacterium in the genus, *Pasteuria* that we discovered parasitizing the sting nematode, *Belonolaimus longicaudatus* in Florida. We are hopeful that this obligate bacterial parasite of nematodes (*Pasteuria* n. sp. [S-1]) will have some 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 30-50%

In 1995, we completed ultrastructural studies with transmission electron microscopy (TEM) and low-temperature scanning electron microscopy (SEM) that show that *Pasteuria* n. sp. (S-1) is a new species. These studies have also helped to finish elucidating the development and life cycle of this bacterium. We now have excellent photomicrographs illustrating all aspects of the biology of *Pasteuria* n. sp. (S-1). Use of the new technique of low temperature SEM has helped to visualize spore morphology outside and inside the infected nematodes without the usual artifacts associated with TEM. We have now isolated another population of this bacterium from a golf course in Gainesville, FL and we are doing TEM to confirm that it is ultrastructurally similar to *Pasteuria* n. sp. (S-1). This will give us a better idea of the possible distribution of this bacterium within Florida. A population dynamic study (390 days long) was completed on *Pasteuria* n. sp. (S-1) in laboratory pot cultures of the sting nematode on the model turfgrass host (FX-313 St. Augustinegrass) under controlled conditions. There were four treatments; 1) no sting nematodes with no bacteria, 2) sting nematodes (99 ± 10) with no bacteria, 3) sting nematodes (99 ± 10) + 10 sting nematodes encumbered with 8 ± 6 spores of *Pasteuria* n. sp. (S-1), and 4) sting nematodes (99 ± 10) + 25 sting nematodes encumbered with 8 ± 6 spores of *Pasteuria* n. sp. (S-1). 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 increased by the addition of "sick" nematodes suggesting that spore encumbrance is not a good indicator of spore production or nematode health. Root dry weights for the different treatments confirmed that the greatest root loss occurred in the treatments with 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. In 1995, we also 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 at three different soil depths on sting nematodes. Soil temperature was also monitored at these different depths. After 6 months of sampling, locations that started with low levels of spore encumberance had higher numbers of sting nematodes than areas that started with high encumberance levels, suggesting that Pasteuria n. sp. (S-1) might help produce suppressive soil for the sting nematode. These results are encouraging but will require at least one year of survey work.

BACKGROUND

One major problem encountered in southern United States golf courses, athletic fields, and lawns is the destruction of roots by phytoparasitic nematodes. Recent work in the midwestern and western United States has demonstrated the importance of phytoparasitic nematodes to turfgrass culture in these geographical regions as well. The most damaging nematode in the warmseason turfgrass ecosystem in sand soils is the ectoparasitic sting nematode, Belonolaimus longicaudatus. There are many other species of plant-parasitic nematodes that cause below ground root pruning and damage. However, they are not as important as the sting nematode which is recognized as one of the most pathogenic phytonematodes in the state of Florida. Our research has demonstrated 30-50% root weight reductions in controlled inoculation studies with sting nematode on commonly cultivated hybrid bermudagrasses which are used for golf course greens such as 'Tifgreen' and 'Tifdwarf.'

The sting nematode has a wide host range and is a major pest on a variety of grasses, vegetables, and perennial crops. It is a relatively large plant-parasite (ca. 2000 μm long) and goes through its life cycle in about 28 days. The sting nematode does best in soils with >80% sand. It is a documented pest in the sandy soils of the Coastal Plains from Florida north to Virginia and along the Gulf Coast into Texas. It also occurs in Arkansas, Kansas, Oklahoma, Missouri, and Nebraska. It has also been recently introduced into southern California where it is causing problems in golf course greens.

Currently, management of phytoparasitic nematodes for perennial crops such as turfgrass relies largely on postplant application of organophosphate pesticides. Nematicides labelled for use on turfgrass in 1995 are nematostatic at the concentrations achieved in the field and usually require multiple applications for short-lived (< 4 weeks) suppression of phytoparasitic nematode populations. Chronic exposure of nematodes and the soil microflora to sublethal doses of nematicides can encourage microbial decomposition of pesticides.

Releases of members of the Pasteuria penetrans group, obligate nematode endoparasitic bacteria, may provide an alternative or supplement to chemical control. These endospore-forming actinomycetes attach to, and infest the nematode host via the cuticle. The parasitized nematode is incapable of reproduction and eventually becomes filled with developing endospores of the bacterium, which are released into the environment upon host disintegration. Some forms of the bacteria attack juveniles and do not sporulate until the nematode becomes an adult, ie. P. penetrans sensu strictu. Other species, such as P. thornei can attack and complete their life cycle before the host reaches the adult stage. The assets of members of the P. penetrans group as biological control agents of turfgrass nematodes are; 1) their ability to persist for long periods of time (> 1 year), 2) host specificity, 3) compatibility with pesticides, and 4) lack of environmental risk to humans and other non-target organisms.

Spores of the Pasteuria penetrans group are resistant to heat, desiccation, and exposure to nematicides and have been reported adhering to, or infesting, 205 species of nematodes from 51 countries worldwide. Only three species of the P. penetrans group are well characterized, however, and little is known about the ecology of the group in native or managed soil systems.

We have done survey work from 1985-1989 which suggests that isolates of Pasteuria are widely distributed in bermudagrass fairway turf in southern Florida. Five morphometrically distinct isolates of the bacteria were observed on five species of plant-parasitic nematodes. We have done a one year greenhouse study to determine if soil infested with a large-spored isolate of Pasteuria (6.10 µm endospore diameter) was suppressive to populations of the sting nematode on 'Tifgreen II' bermudagrass. Soil containing this isolate was not suppressive to B. longicaudatus in the first six months but caused a significant decrease in sting nematodes after one year with concomitant increases in numbers of Pasteuria sp.-infested sting nematodes.

These results are encouraging because they suggest that the sting nematode isolate of Pasteuria may be valuable in inoculative biological control of the sting nematode in golf course greens, and other turf situations where spore-infested nematodes or small amounts of soil infested with the bacteria could be used for inoculation. The purpose of this USGA-funded project is to describe this new species of Pasteuria and see if it can be successfully manipulated in the managed turfgrass ecosystem.

1995 RESEARCH PROGRESS

Description of Pasteuria n. sp. (S-1) from the sting nematode from Ft. Lauderdale, Florida: We are currently working on 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): Belonolaimus longicaudatus filled with the endospores or with different stages of the vegetative phase of Pasteuria n. sp. (S-1) from the Ft. Lauderdale Research and Education Center were cut and fixed in 2.5% glutaraldehyde + 0.1 M sodium phosphate buffer (pH 7.4) overnight at 4 C, embedded in 3% agarose, and cut into small blocks. The glutaraldehyde was rinsed from the blocks with five rinses of phosphate buffer and the tissue was postfixed in 2% osmium tetroxide in phosphate buffer. The tissue was rinsed, dehydrated in an ethanol-acetone series, and infiltrated with Spurr's epoxy resin. The tissue was then placed in molds in a 60-C vacuum-oven (6.8-kg vacuum) for 18 hours for resin polymerization. Thin-sections (80 nm) were cut with glass knives on a LKB ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with a Hitachi 7000 or a Philips 201 TEM (60 kv). Over the past two 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 observations were performed on a Hitachi S-4100 field emission scanning electron microscope equipped with an Oxford CT-1500 Cryotrans System. Specimen preparation involved hand-picking live male, female, and juveniles of B. longicaudatus from specimens that were removed from soil by centrifugal-flotation. Specimens were placed on a gold-hinged holder mounted on a Denton complementary freeze-etch specimen cap. The specimens were cryofixed by submerging the cap assembly in the Oxford nitrogen slush chamber, evacuating, and withdrawing the cap into a cryo-transfer arm for transfer to the Oxford prechamber. A precooled pick was then used to fracture the samples by lifting and rotating the fracture arm of the complementary cap 180°. The specimens were then either sputter coated with platinum in the prechamber and inserted onto the cryostage of the microscope or etched for 8 min at -90° C, coated in the prechamber, and moved to the cryostage for observation. Accelerating volatages of 10 kV were used to observe or record images onto Polaroid Type 55P/N film.

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 (Fig. 1). Basically, the peripheral fibers of the endospore protrude around the exposed spherical outer coat of the spore creating a crenate border which gives the endospre the appearance of a fried egg with a scalloped ring around the yolk (Fig. 1). 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 μm 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 (Fig. 2), similar to P. thornei but different from the other two species (Fig. 3). The spore pore diameter, measured from TEM micrographs, is larger than any other described species of Pasteuria. The endospore shape in Pasteuria n. sp. (S-1) is an oblate spheroid that is a ventrally flattened ellipse in longitudinal sections (Fig. 2). The other species possess endospores which are narrowly or broadly elliptic in longitudinal TEM sections (Fig. 3). The outer coat wall thickness at its thickest point is 1/3 the diameter of the outer coat for Pasteuria n. sp. (S-1) compared with 1/4-1/15 for the other described species of Pasteuria (Fig. 2-3).

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 with internal infections of vegetative and sporulating Pasteuria n. sp. (S-1). A mycelial microcolony (see Figure 1 in 1994 report) is formed which eventually breaks up and is distributed throughout the pseudocoelom (fragmentation). 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 typical for the three other described species of Pasteuria. A septum is formed within the sporangium, the sporangium cytoplast condenses to form a forespore, the endospore walls form, the endospore matures, and areas adjacent to the endospore give rise to peripheral "attachment" fibers.

Host range studies with Paseuria n. sp. (S-1): Host range studies were conducted. Endospores were harvested from spore-filled sting nematodes recovered by centrifugal-flotation using 1M sucrose from field plots previously determined to be infested. Endospore/water suspensions of 1,000 endospores in 100 µl per 250 µl microfuge tube were quantified using a hemocytometer. A set number of a test nematode host species (ie. 200 each for Meloidogyne incognita, M. javanica, M. hapla, M. arenaria, or 60 each for different isolates of Belonolaimus, Hoplolamius galeatus, or Pratylenchus penetrans) were used in each attachment run in the microfuge. The suspension and nematodes were centrifuged 2 min at 9,500 g in a Beckman microfuge. Nematodes were pipetted from the microfuge tubes onto counting dishes in individual drops of water and 20 randomly chosen individuals were examined for successful spore attachment. The results were summarized in Table 3 of the 1994 report. Basically, Pasteuria n. sp. (S-1) spores only attach to Belonolaimus longicaudatus. 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 designed 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]. Washed aerial stolons of FX-313 St. Augustinegrass were planted in autoclaved 60-mesh sand in 26 X 52 mm plastic trays kept on a raised bench for rooting. Stolons were 6-8 cm long terminal cuttings with 2-3 nodes. After 28 days, sprigs were transplanted to square tapered pots (80 mm wide at the top, 60 mm wide at the bottom, 75 mm deep). Sprigs had one strong terminal with two to three nodes and four to six basal roots at transplanting. Pots were filled with 250 ml (378 g) of moist, autoclaved Margate fine sand. Treatments were applied five days after transplanting. Those pots receiving nematodes were inoculated with 99 ± 10 B. longicaudatus without spores of Pasteuria as described below and placed in 0.26 mls of water into a small depression near the base of each plant. The nematode inoculum was obtained by centrifugal flotation from a stock culture maintained on FX-313 St. Augustinegrass.

Treatments involved a harvest factor (to be 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 ± 10) with no bacteria, 3) sting nematodes (99 ± 10) + 10 sting nematodes encumbered with 8 ± 6 spores of Pasteuria n. sp. (S-1), and 4) sting nematodes (99 ± 10) + 25 sting nematodes encumbered with 8 ± 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.

Pots were watered twice weekly to bring soil moisture content up to just below saturation. Pots were situated on a laboratory bench under flourescent lights with a 16 hr photoperiod (photosynthetic photon flux: 138 μmole m^{-2} s^{-2}). Soil temperatures were maintained between 22 and 25°C.

At harvest, the soil was washed from the root ball and nematodes were extracted from the entire soil volume by centrifugal flotation. Cohorts of 15-25 nematodes were stained with crystal violet and examined for infestation with the vegetative and/or spore phase of Pasteuria n. sp. (S-1). Following nematode extraction, roots were separated from stolons and leaves, dried at 60°C for 72 hr, and weighed.

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) (Fig. 4) suggesting that spore encumbrance is not a good indicator of spore production or nematode health. Root dry weights for the different treatments confirmed that root loss was greatest in the treatments receiving the most nematodes (Fig. 5). 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). A map of sample locations A-F is presented in Figure 6. 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 levelled. About 30 sites around the station were pre-sampled for sting nematodes and Pasteuria (S-1) n. sp. Six locations were chosen (Fig. 6). Nematode and bacterial sampling and root dry weights were 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 the 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%) (Fig. 7). Sting nematode densities were highest at 0-10 cm and lowest at 20-40 cm. Location B showed similar trends (Fig. 8), although the densities of sting nematode were not as high as in location A. Location C was chosen because it had moderate levels of sting and high encumberance by Pasteuria. By the time we started regular sampling, the sting numbers had plummeted and Pasteuria levels were high, suggesting an epizootic (Fig. 9). Location D had moderate levels of both sting nematode and Pasteuria which appeared stable throughout the sampling (Fig. 10). Locations E and F both started with moderate levels of sting and high levels of Pasteuria and we observed what appears to be suppresion of the sting nematode (Figures 11,12).

In general, after 6 months of sampling (Figures 6-12), locations that started with low levels of spore encumberance had higher numbers of sting nematodes than areas that started with high encumberance levels, suggesting that Pasteuria n. sp. (S-1) might help produce suppressive soil for the sting nematode in the turfgrass ecosystem. These results are encouraging but require at least one full year of survey time.

FUTURE RESEARCH (1996-1997)

In 1996, we propose to continue monthly survey work in 'Tifgreen' bermudagrass areas where Pasteuria n. sp. (S-1) occurs naturally to assess its suppressive effects on sting nematodes. We also plan to begin a field experiment where soil that is heavily infested with Pasteuria n. sp. (S-1) is harvested and the spore levels quantified by bioassay. This soil will then be used to inoculate plots of 'Tifdwarf' bermudagrass that have high sting nematode levels without Pasteuria n. sp. (S-1). The test sites will then be monitored to determine if the bacteria becomes established and if the sting nematodes are suppressed. We also plan on submitting the description of Pasteuria n. sp. (S-1) for publication.

SUMMARY OF PERSONNEL TIME AND EXPENDITURES MADE DURING 1995

Dr. Robin M. Giblin-Davis (Project P.I.)	30%
Mr. Frank Bilz (State line Biologist)	30%
Mrs. Barbara J. Center (USGA-grant paid laboratory assistant)	50%
Dr. Don W. Dickson (Project Co-P.I.)	07%
Mr. Tom Hewlett (State line Biologist)	20%
Mr. Ross Robinson (USGA-grant paid laboratory assistant)	25%
Dr. John L. Cisar (Project Co-P.I.)	05%
Ms. Karen Williams (State line Biologist)	05%

Salaries and wages:	
Laboratory assistant: Barbara J. Center (Ft. Lauderdale R.E.C. 1/2 time, $12.00/hr)	$11,100
Laboratory assistant (University of Florida 1/4 time, $10.00/hr)	$ 5,200
Operating expenses:	
Materials and supplies	$ 500
Overhead:	
16%	$ 3,200
1995 total	$20,000

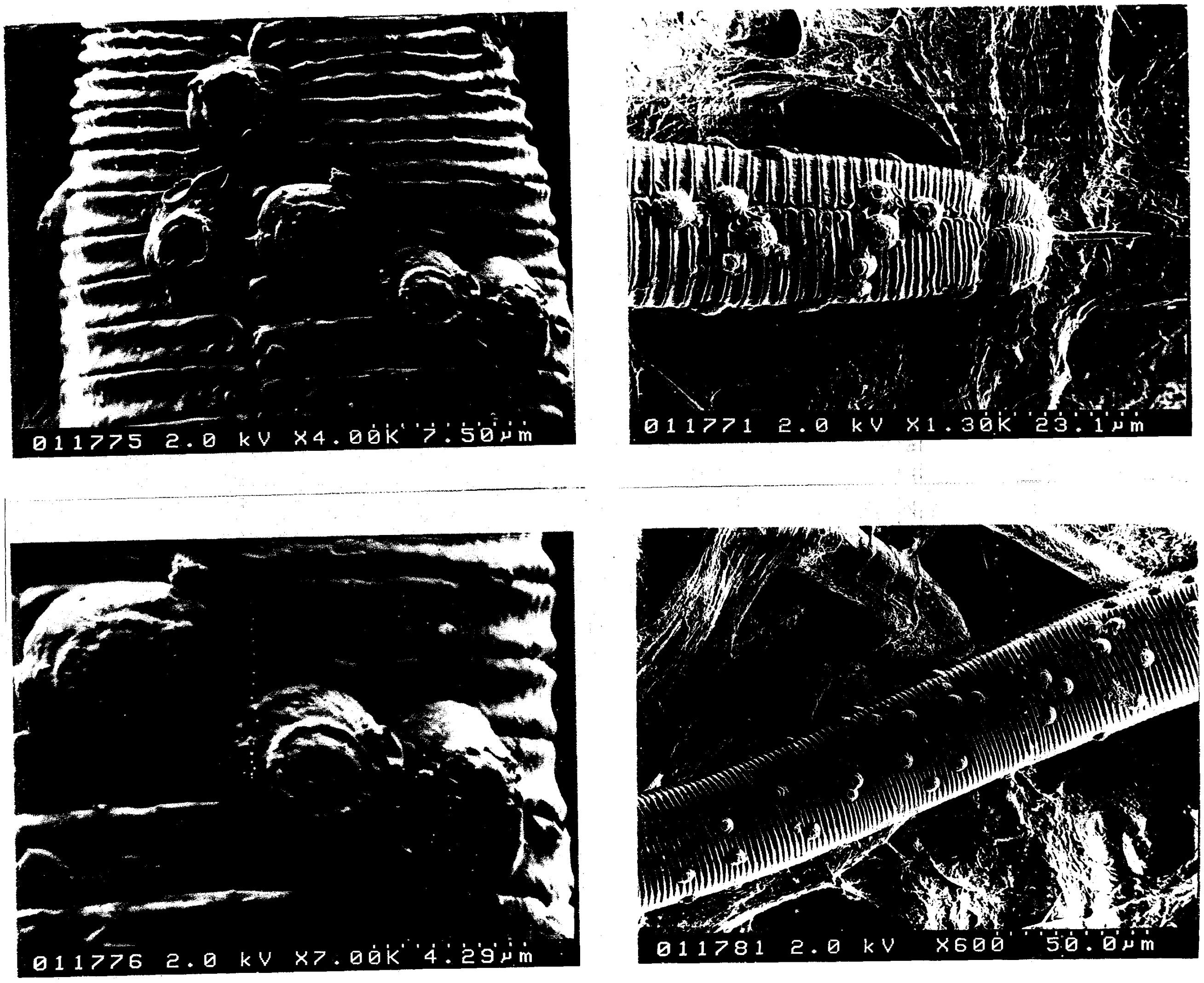

011775 2.0 kV X4.00K 7.50µm
011771 2.0 kV X1.30K 23.1µm
011776 2.0 kV X7.00K 4.29µm
011781 2.0 kV X600 50.0µm

Figure 1.

Figure 2.

Pasteuria sp. (S-1)

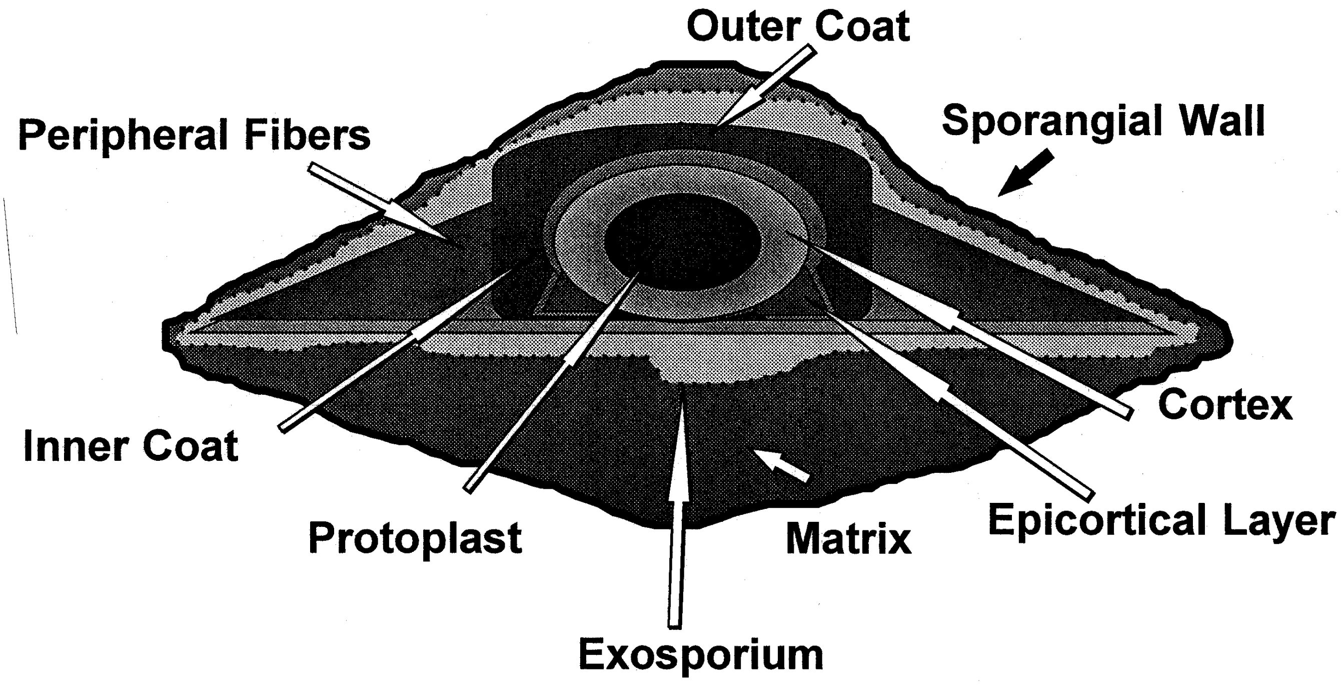
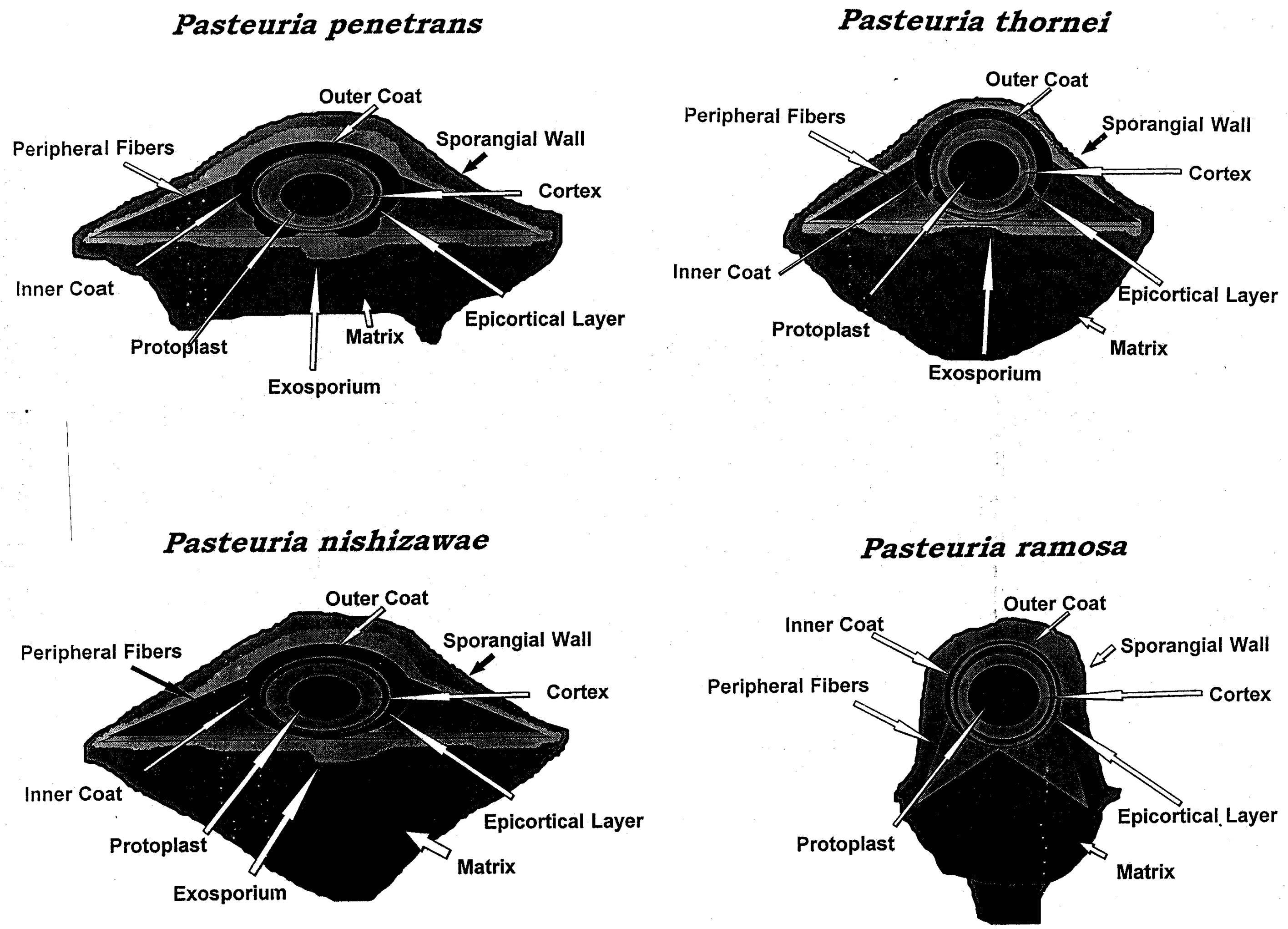

Outer Coat
Peripheral Fibers
Sporangial Wall
Cortex
Inner Coat
Epicortical Layer
Protoplast
Matrix
Exosporium

Figure 3.



Pasteuria penetrans

Outer Coat
Peripheral Fibers
Sporangial Wall
Cortex
Inner Coat
Epicortical Layer
Protoplast
Matrix
Exosporium

Pasteuria thornei

Outer Coat
Peripheral Fibers
Sporangial Wall
Cortex
Inner Coat
Epicortical Layer
Protoplast
Matrix
Exosporium

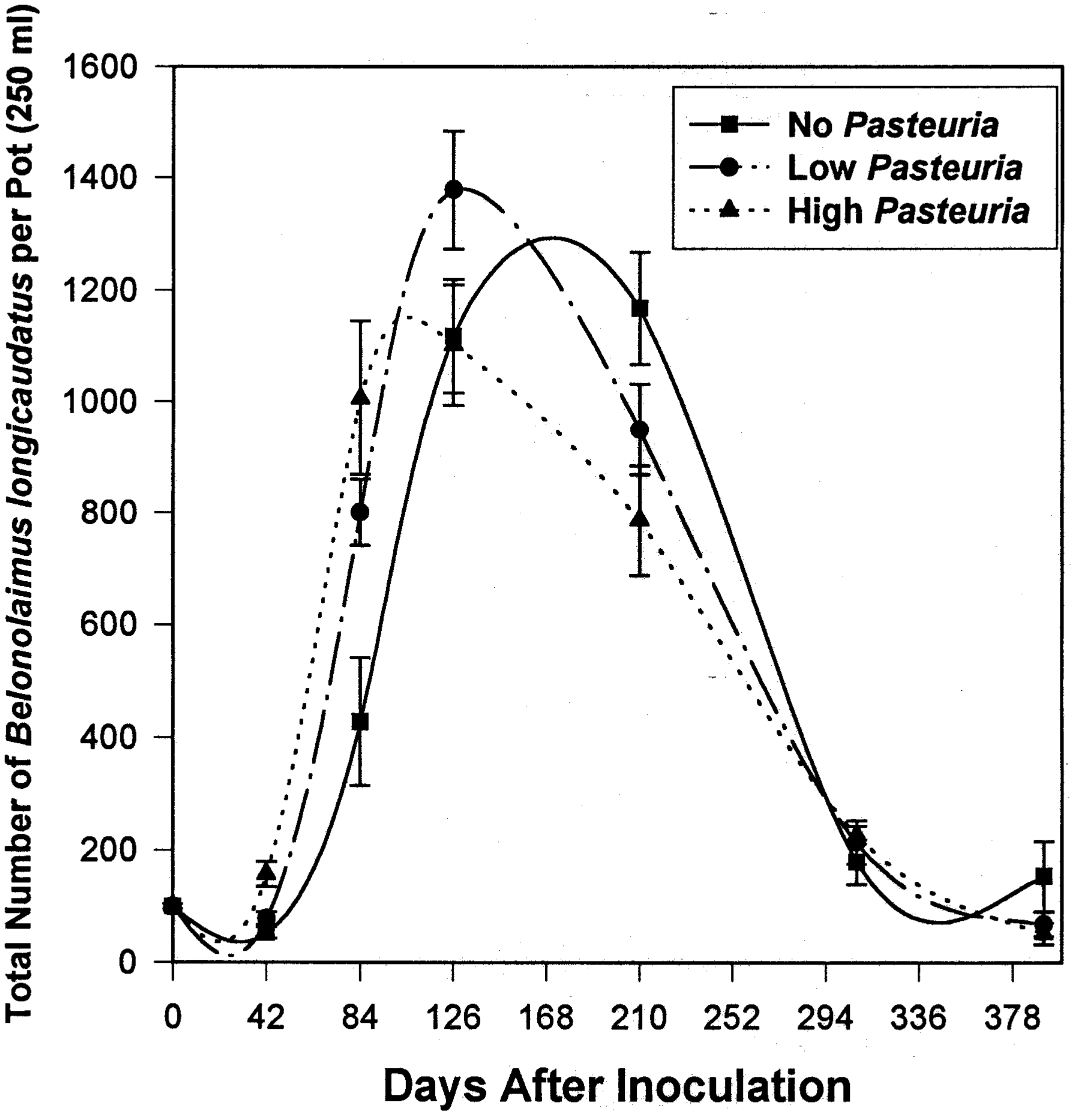
Pasteuria nishizawae

Outer Coat
Peripheral Fibers
Sporangial Wall
Cortex
Inner Coat
Epicortical Layer
Protoplast
Matrix
Exosporium

Pasteuria ramosa

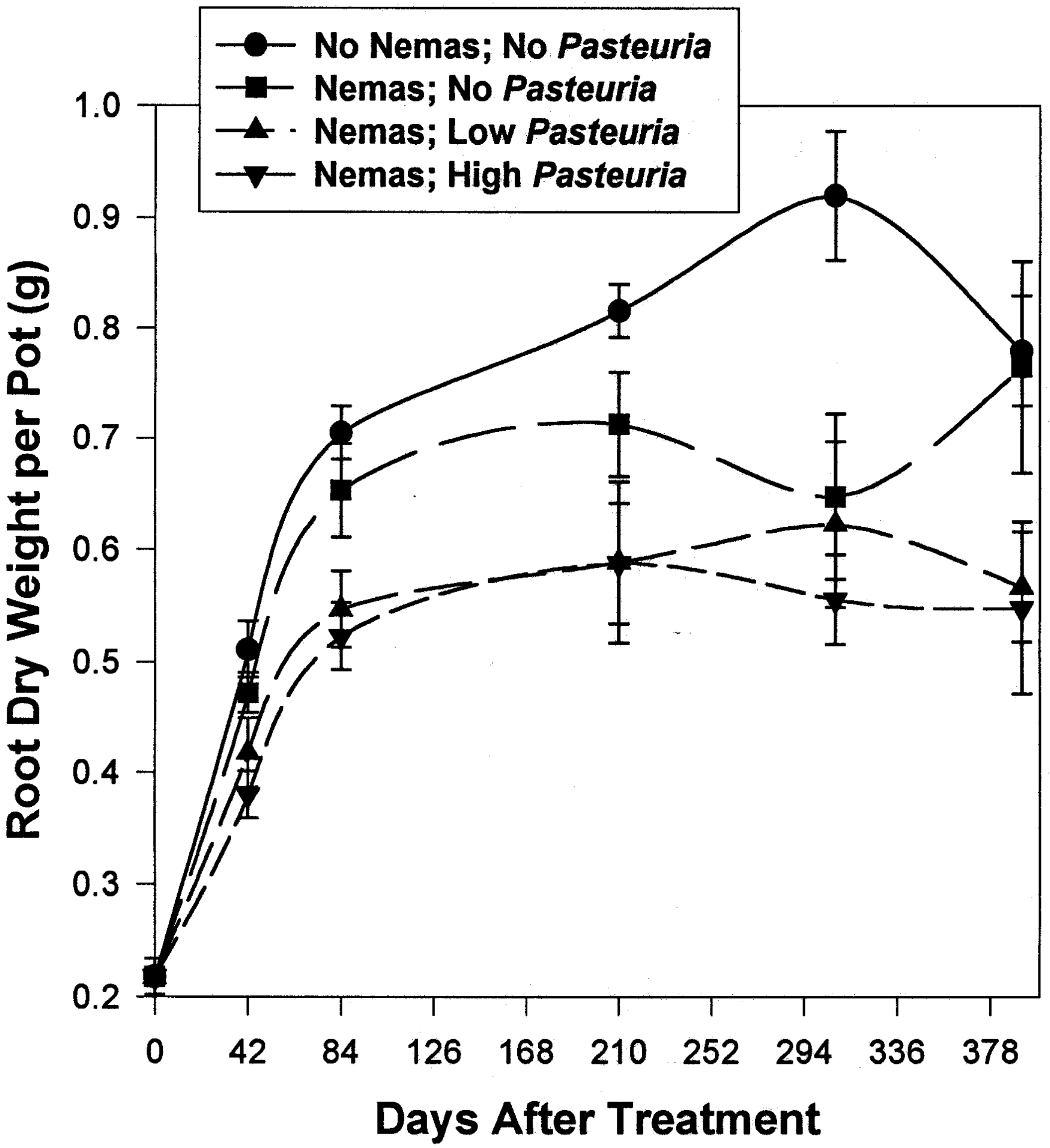
Outer Coat
Inner Coat
Sporangial Wall
Peripheral Fibers
Cortex
Epicortical Layer
Protoplast
Matrix

Figure 4.



Total Number of *Belonolaimus longicaudatus* per Pot (250 ml)
1600
1400
1200
1000
800
600
400
200
0
0
42
84
126
168
210
252
294
336
378
Days After Inoculation
No *Pasteuria*
Low *Pasteuria*
High *Pasteuria*

Figure 5.



No Nemas; No *Pasteuria*
Nemas; No *Pasteuria*
Nemas; Low *Pasteuria*
Nemas; High *Pasteuria*
Root Dry Weight per Pot (g)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
42
84
126
168
210
252
294
336
378
Days After Treatment

Figure 6. Plot Plan for *Pasteuria* (S-1) Seasonal Survey

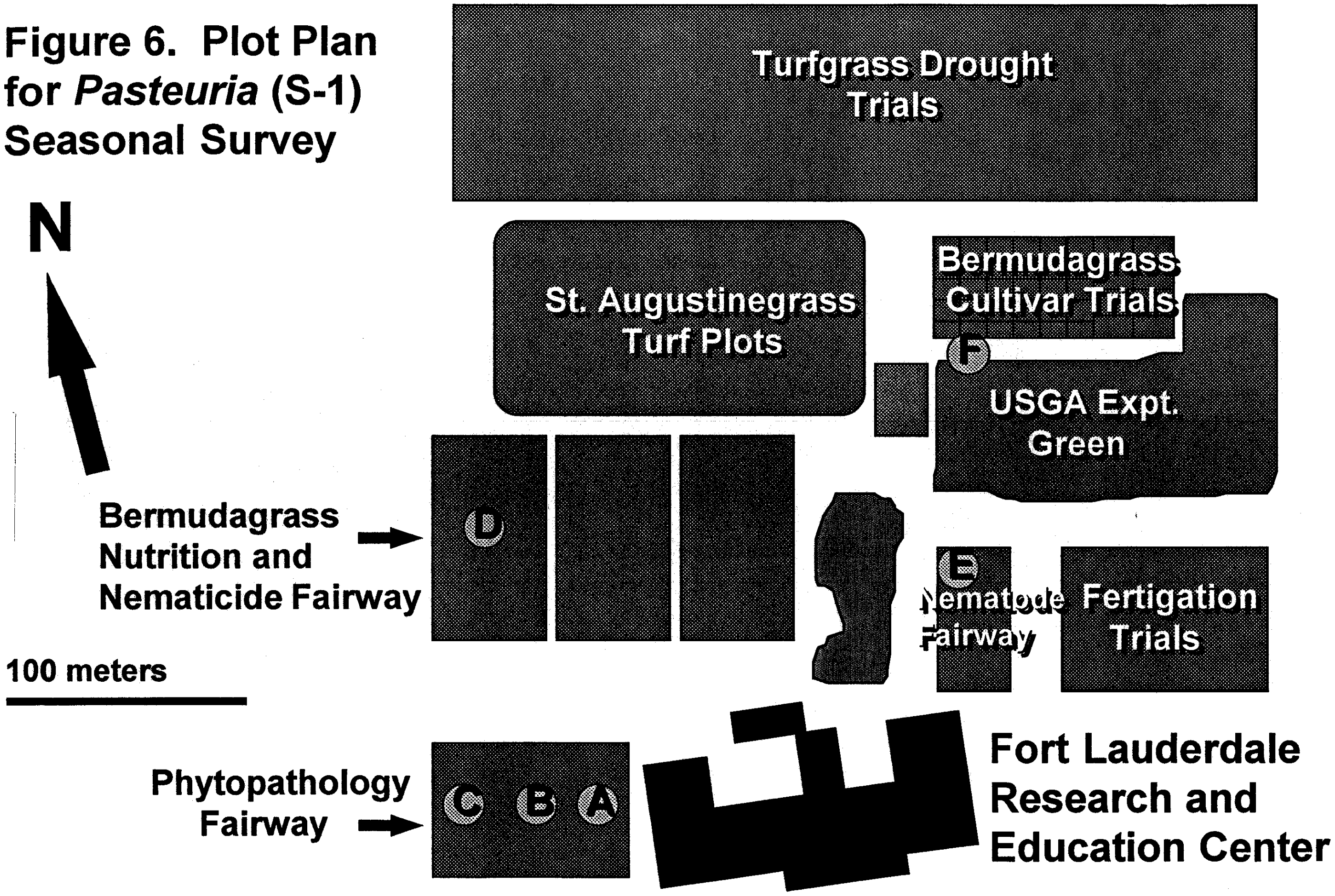

Turfgrass Drought Trials
N
St. Augustinegrass Turf Plots
Bermudagrass Cultivar Trials
F
USGA Expt. Green
Bermudagrass Nutrition and Nematicide Fairway
D
E
Nematode Fairway
Fertigation Trials
100 meters
Phytopathology Fairway
C
B
A
Fort Lauderdale Research and Education Center

Figure 7.

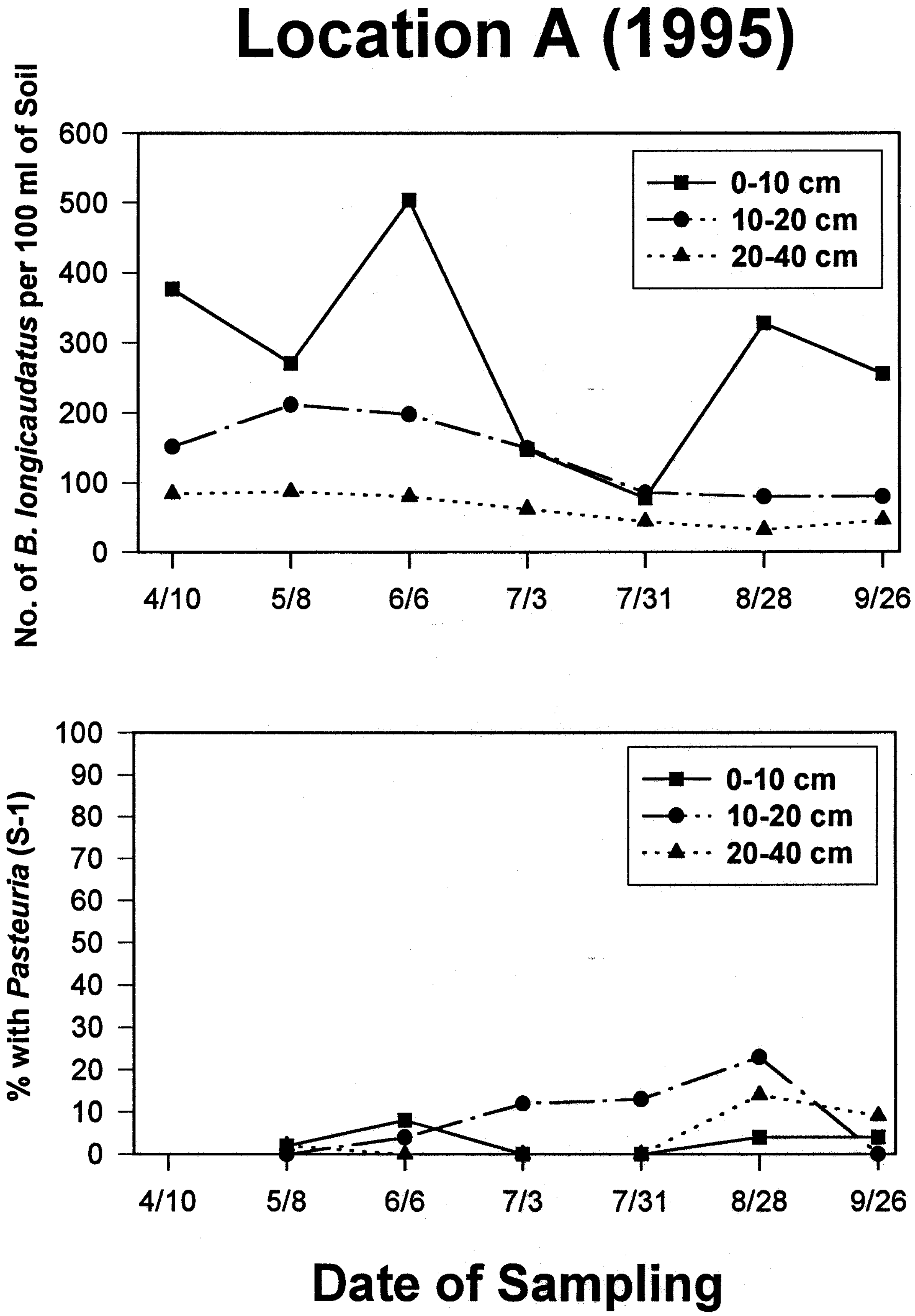

Location A (1995)
No. of *B. longicaudatus* per 100 ml of Soil
600
500
400
300
200
100
0
0-10 cm
10-20 cm
20-40 cm
4/10
5/8
6/6
7/3
7/31
8/28
9/26
% with *Pasteuria* (S-1)
100
90
80
70
60
50
40
30
20
10
0
0-10 cm
10-20 cm
20-40 cm
4/10
5/8
6/6
7/3
7/31
8/28
9/26
Date of Sampling

Figure 8.

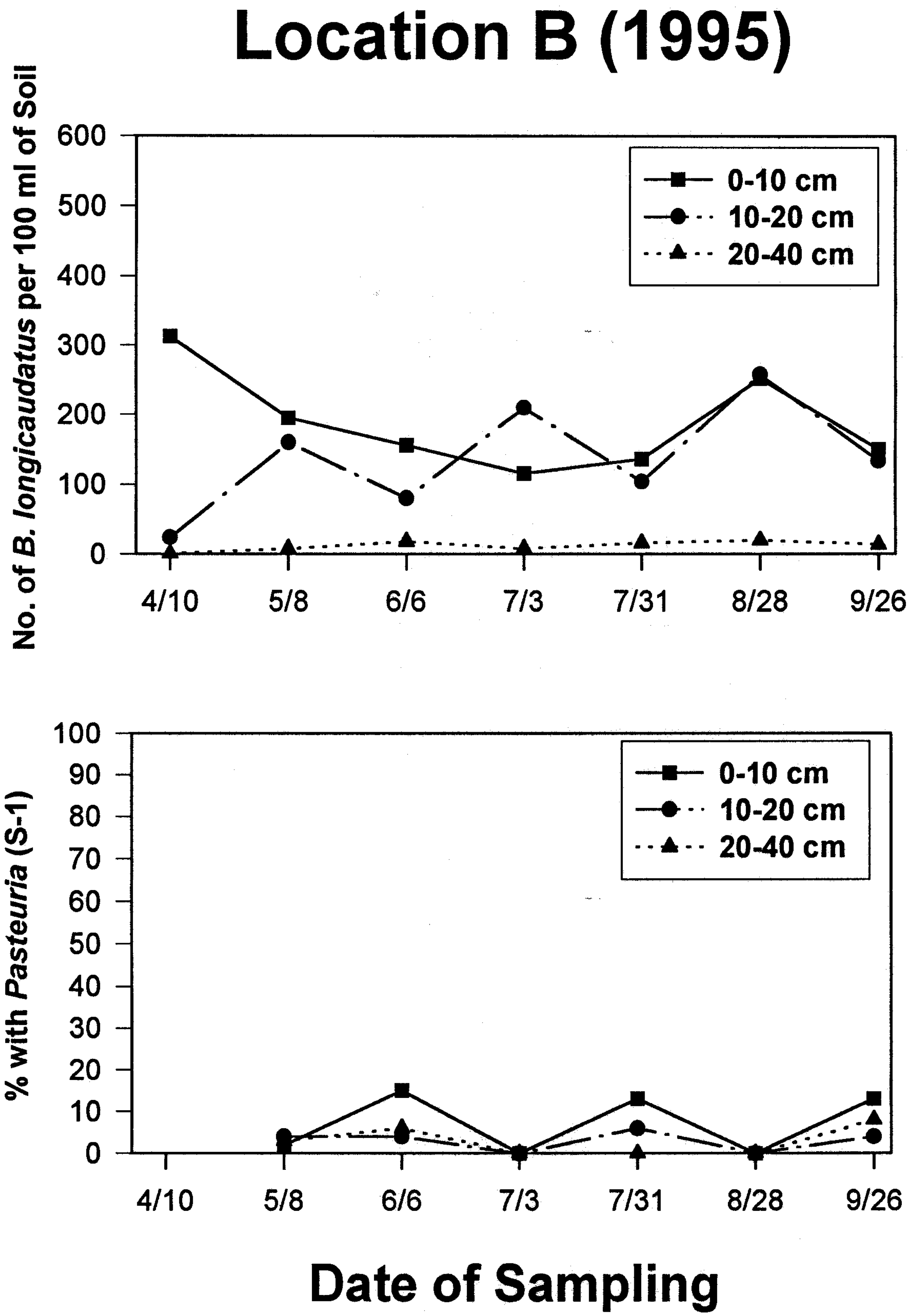

Location B (1995)
No. of B. longicaudatus per 100 ml of Soil
600
500
400
300
200
100
0
0-10 cm
10-20 cm
20-40 cm
4/10
5/8
6/6
7/3
7/31
8/28
9/26
% with Pasteuria (S-1)
100
90
80
70
60
50
40
30
20
10
0
0-10 cm
10-20 cm
20-40 cm
4/10
5/8
6/6
7/3
7/31
8/28
9/26
Date of Sampling

Figure 9.

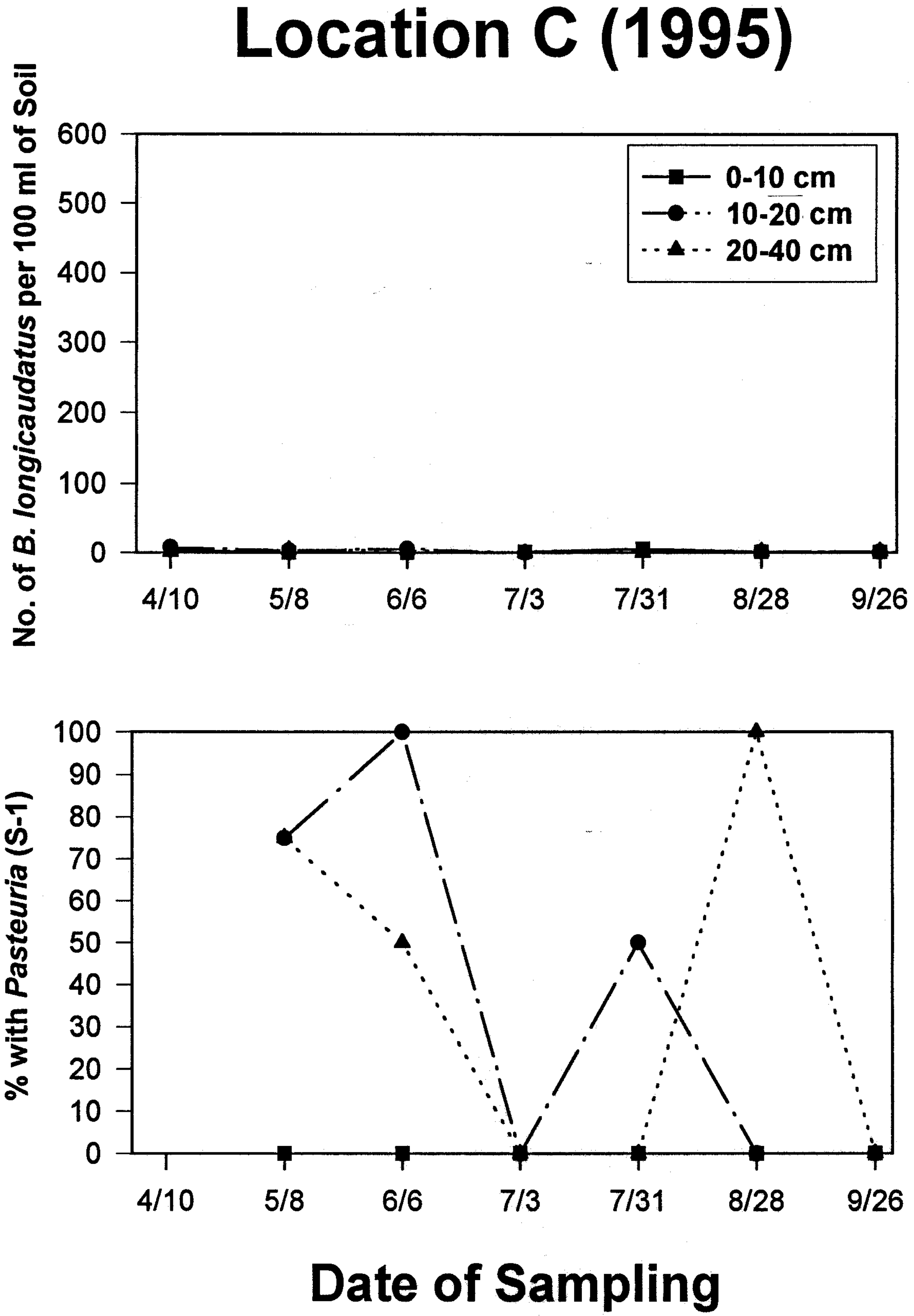

Location C (1995)
No. of *B. longicaudatus* per 100 ml of Soil
600
500
400
300
200
100
0
0-10 cm
10-20 cm
20-40 cm
4/10
5/8
6/6
7/3
7/31
8/28
9/26
% with *Pasteuria* (S-1)
100
90
80
70
60
50
40
30
20
10
0
4/10
5/8
6/6
7/3
7/31
8/28
9/26
Date of Sampling

Figure 10.

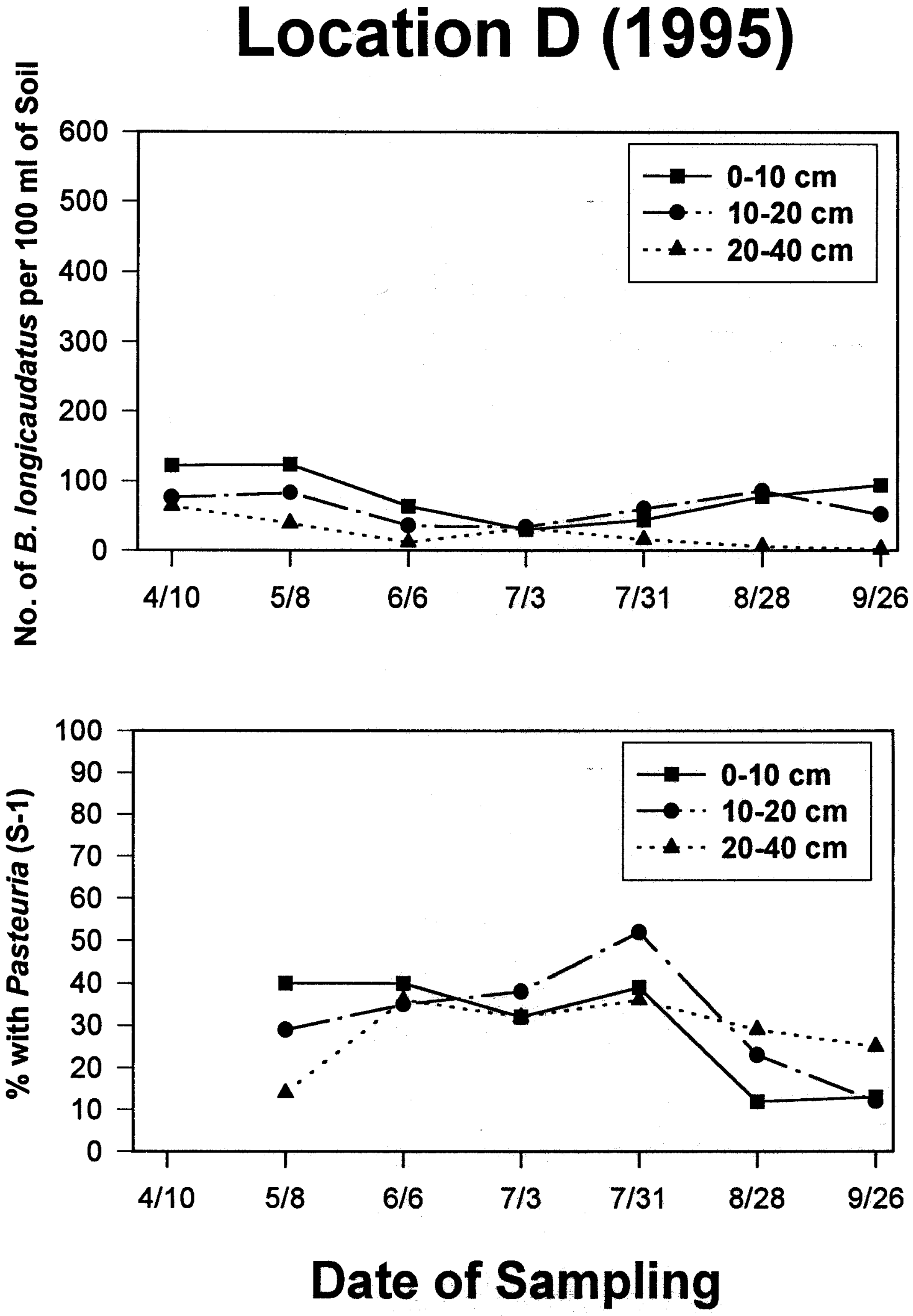

Location D (1995)
No. of B. longicaudatus per 100 ml of Soil
600
500
400
300
200
100
0
0-10 cm
10-20 cm
20-40 cm
4/10
5/8
6/6
7/3
7/31
8/28
9/26
% with Pasteuria (S-1)
100
90
80
70
60
50
40
30
20
10
0
0-10 cm
10-20 cm
20-40 cm
4/10
5/8
6/6
7/3
7/31
8/28
9/26
Date of Sampling

Figure 11.

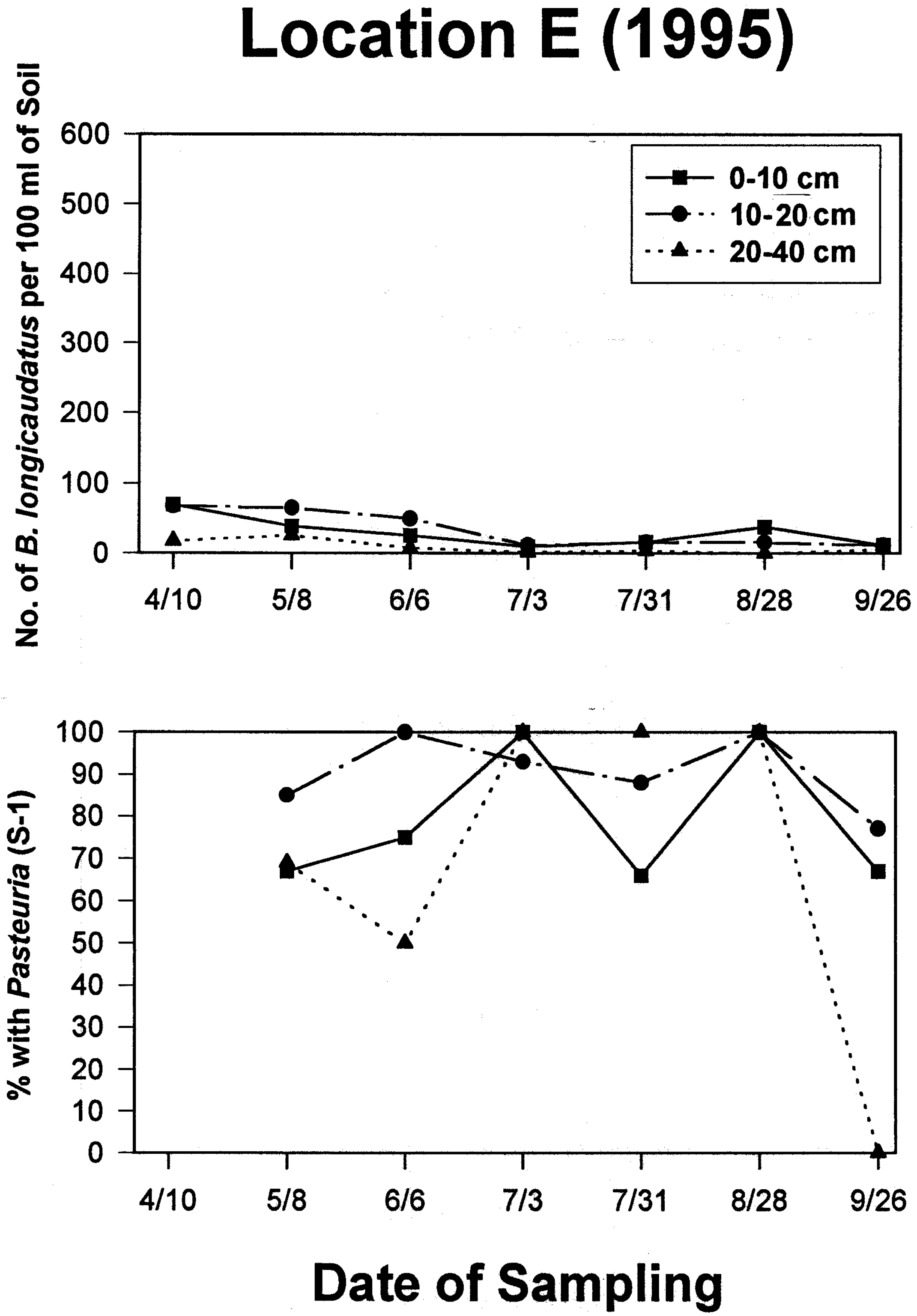
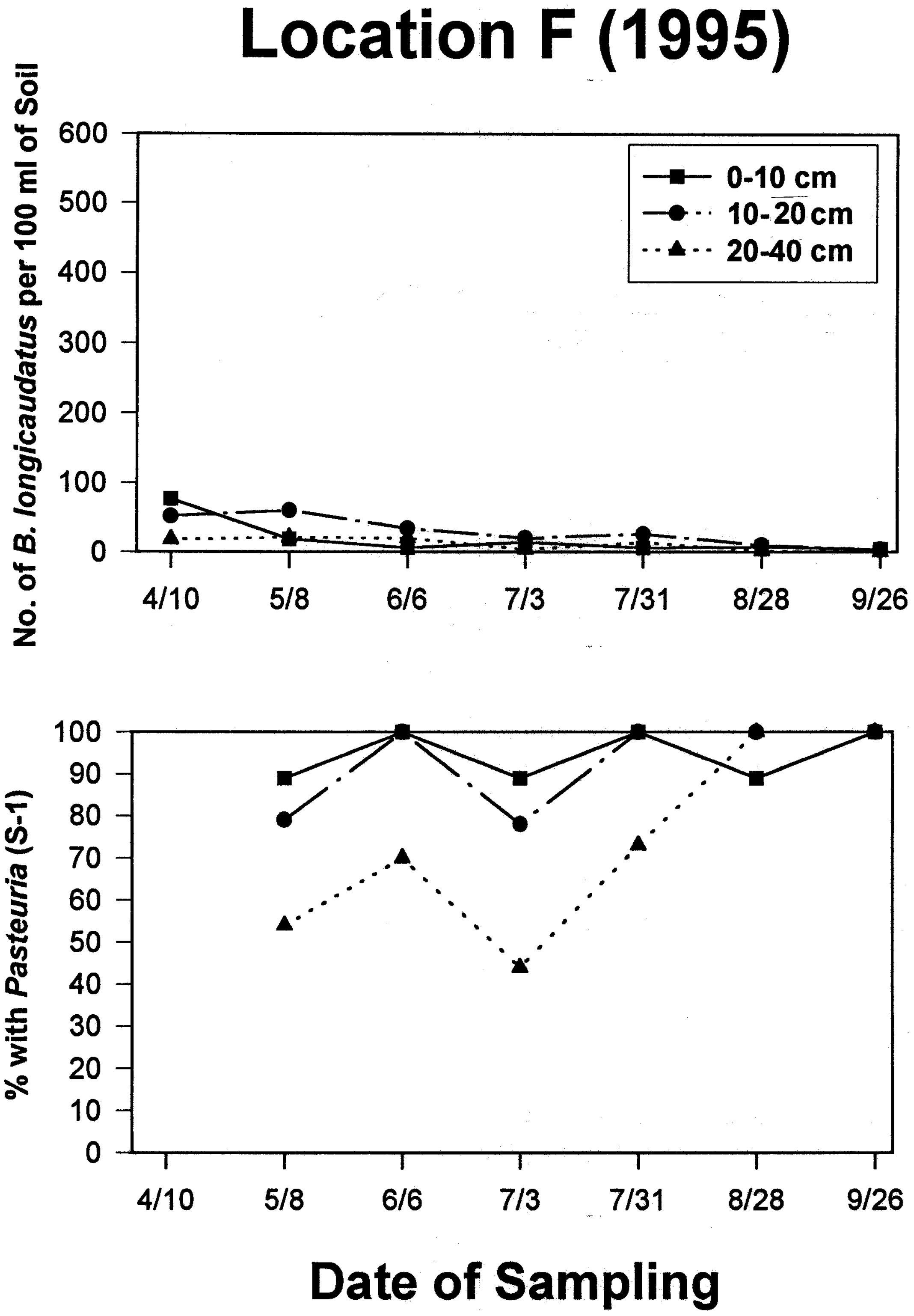

Location E (1995)
No. of B. longicaudatus per 100 ml of Soil
600
500
400
300
200
100
0
0-10 cm
10-20 cm
20-40 cm
4/10
5/8
6/6
7/3
7/31
8/28
9/26
% with Pasteuria (S-1)
100
90
80
70
60
50
40
30
20
10
0
4/10
5/8
6/6
7/3
7/31
8/28
9/26
Date of Sampling

Figure 12.


Location F (1995)
No. of *B. longicaudatus* per 100 ml of Soil
600
500
400
300
200
100
0
0-10 cm
10-20 cm
20-40 cm
4/10
5/8
6/6
7/3
7/31
8/28
9/26
% with *Pasteuria* (S-1)
100
90
80
70
60
50
40
30
20
10
0
4/10
5/8
6/6
7/3
7/31
8/28
9/26
Date of Sampling