

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

1996 USGA FINAL TURFGRASS RESEARCH REPORT

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1996 OBJECTIVES

1) Continue and finish ultrastructural studies with transmission electron microscopy (TEM) and low-temperature and regular 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) Develop a bioassay to estimate the numbers of spores of this new Pasteuria sp. in bacteria-infested soil.

3) Continue monthly survey of six 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.

4) 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.

1996 USGA TURFGRASS RESEARCH EXECUTIVE SUMMARY

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 30-50%.

In 1996, we completed ultrastructural 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 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 m² plots with 15 cm borders. Precounts of sting nematodes present per 100 cm³ subsamples 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 diam. cup cutter was used to remove a core from the center of each 1 m² plot. Soil (900 cm³) 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.

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 1996 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 μ m 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.

1996 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 and a golf course in Orlando, FL 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 three 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 voltages of 10 kV were used to observe or record images onto Polaroid Type 55P/N film. In 1996, at the University of Florida, we used regular SEM on sting nematodes encumbered with Pasteuria n. sp. that were critical point dried (CPD) to confirm ultrastructural observations using low temperature SEM. Observations were consistent for both methods.

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 endospore 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, 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 endospore shape in Pasteuria n. sp. (S-1) is an oblate spheroid that is a ventrally flattened ellipse in longitudinal sections. The other species possess endospores which are narrowly or broadly elliptic in longitudinal TEM sections. Another species specific character is the outer spore coat thickness which at its thickest point is 1/3 the outer spore diameter for Pasteuria n. sp. (S-1) compared with 1/4-1/15 the outer spore diameter for the other described species of Pasteuria. The TEM ultrastructure of the isolate of Pasteuria n. sp. from a golf course in Orlando, FL is very similar to Pasteuria n. sp. from Ft. Lauderdale, FL. This confirms that the

distribution of this bacterium is widespread within Florida.

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 may break up and become distributed throughout parts of the pseudocoelom (fragmentation). Mycelial filaments are divided by septa and possess double-layered cell walls with associated mesosomes. Endospores are produced endogenously.

In 1996, we sectioned more Pasteuria n. sp.-infested sting nematodes for a better interpretation of the endospore formation sequence (sporogenesis) for Pasteuria n. sp. (S-1) for comparisons with other described species. General sporogenesis appears typical for the other described species of Pasteuria from nematodes. Basically, there is a stem cell connecting a dyad of sporangia. The stem cell breaks down early in sporogenesis. The sporangial axis during early sporogenesis is vertical, but shifts horizontally as the cortex and epicortex appear. The sporangium cytoplasm condenses to form a forespore, the endospore walls form, the endospore matures, and areas adjacent to the endospore give rise to peripheral "attachment" fibers. Careful examination of Pasteuria n. sp. demonstrates that some of the ultrastructural differences between S-1 and other forms may be due to slight differences in sporogenesis. S-1 sporogenesis proceeds as depicted in Figure 2. Sporangial cytoplasm condenses to form a forespore, no clear septum was observed. About the time of condensation, electron lucent bands can be seen which will give rise to the peripheral fibers. As early sporogenesis proceeds, the relatively amorphous endospore becomes more elliptical in shape, being surrounded by a double-layered membrane. The first apparent endospore wall is the cortex, followed by the epicortex. These layers appear as the peripheral fibers develop laterally and the endospore shifts from a dominant vertical axis to a horizontal axis. At first, the epicortical wall appears as a concentric outer layer to the cortex. But as sporogenesis proceeds, this layer appears to shift ventrally, concurrent with the formation of the outer spore coat on the top of the protoplast. The endospore continues to enlarge. The exosporium enclosing the endospore and electron dense cytoplasm detaches inside the sporangial wall. The outer spore coat appears to continue development ventrally as an inner spore coat appears that separates the cortex and epicortex from the outer spore coat. From this point on, the epicortical wall is relegated to a species characteristic subventral band between the inner spore coat and the cortex. As the endospore continues to mature, the electron dense cytoplasm disappears as the outer spore coat thickens dorsally and laterally in a species specific manner and a surface coat is observed on the dorsal and ventral surfaces of the peripheral fibers. The endospore is not infective until the sporangial wall and the exosporium are removed.

Host range studies with Pasteuria 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 μ l per 250 μ l 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, Hoplolaimus 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 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 fluorescent lights with a 16 hr photoperiod (photosynthetic photon flux: $138 \mu\text{mole m}^{-2} \text{s}^{-1}$). 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 in 1995 report) 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 in the 1995 report). 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 3. 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 diameter 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 (Fig. 3). 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 gone from extremely high to much lower levels in the last 6 months and Pasteuria which was detected at low levels (<13%) at the start as continually increased to apparently suppressive levels (Fig. 4). Sting nematode densities were highest at 0-10 cm and lowest at 20-40 cm. The percentage of Pasteuria n. sp. spore-filled sting nematodes also increased in the last 6 months of the survey (Fig. 5). Location B showed similar trends (Figs. 6,7), 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 encumbrance by Pasteuria. By the time we started regular sampling, the sting numbers had plummeted and Pasteuria levels were high, suggesting an epizootic (Figs. 8,9). The soil appears to have remained suppressive to the sting nematode up until the last 5 months when a small resurgence was detected. It appears that Pasteuria n. sp. is acting in a delayed density dependent fashion and slowly suppressing the sting resurgence. Location D had moderate levels of both sting nematode and Pasteuria which oscillated throughout the sampling without a clear pattern of suppression (Figs. 10,11). Locations E and F both started with moderate levels of sting and high levels of Pasteuria and we observed what appears to be density dependent suppression of the sting nematode (Figures 12-15).

In general 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 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.

Spore encumbrance bioassay for Pasteuria n. sp. (S-1) infested soil: 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.

Inoculation of 'Tifdwarf' hybrid bermudagrass green with Pasteuria n. sp. (S-1)-infested soil: 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 seasonal depth study. The spore encumbrance bioassay was used to estimate the numbers of spores in the randomly mixed and dried soil. There were about 5,000 endospores/g of soil. There were two treatments of the soil; 1) control soil which 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 twenty-five 1 m² plots with 15 cm borders. Precounts of sting nematodes present per 100 cm³ subsamples were taken using the sugar flotation method. Twenty plots were ranked according to sting nematode density and the two treatments were randomly assigned within ranks (10 replicates). A 15 cm diam. cup cutter was used to remove a core from the center of each 1 m² plot. Soil (900 cm³) 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 and plot visual ratings were statistically equal for both treatments ($P > 0.7103$). However, there was a significant difference in the proportion of sting nematodes encumbered ($91.0 \pm 3.2\%$ vs. $7.5 \pm 4.0\%$ [$P < 0.0001$]) and filled ($5.0 \pm 1.7\%$ vs. $0.5 \pm 0.5\%$ [$P < 0.0415$]) 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.

FUTURE RESEARCH (1997-1998)

This is the final report for the three year project ending February 1, 1997. Please see the following appendix outlining proposed research for 1997-98, if additional funds are available for continuation.

SUMMARY OF PERSONNEL TIME AND EXPENDITURES MADE DURING 1996

Dr. Robin M. Giblin-Davis (Project P.I.).....30%
Ms. Donna Williams (State line Microscopist).....15%
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. 3/8 time, \$13.50/hr).....\$11,100

Laboratory assistant (University of Florida 1/4 time,
\$10.00/hr).....\$ 5,200

Operating expenses:

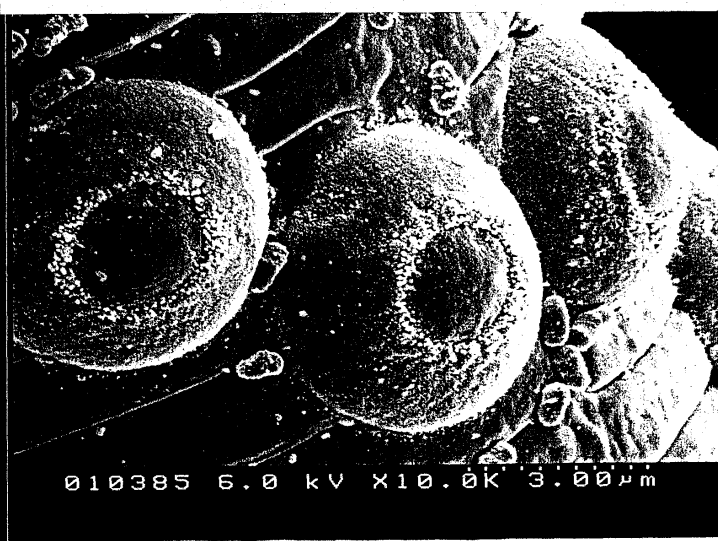
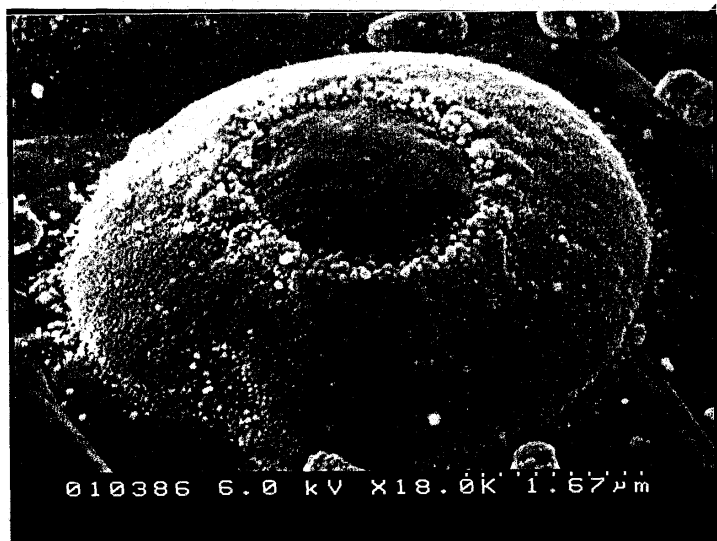
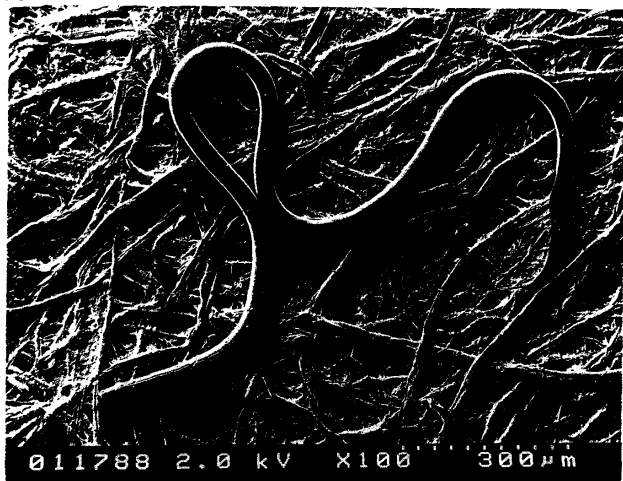
Materials and supplies.....\$ 500

Overhead:

16%.....\$ 3,200

1996 total.....\$20,000

FIGURE 1



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FIGURE 2
SPOROGENESIS OF S-1 *PASTEURIA*

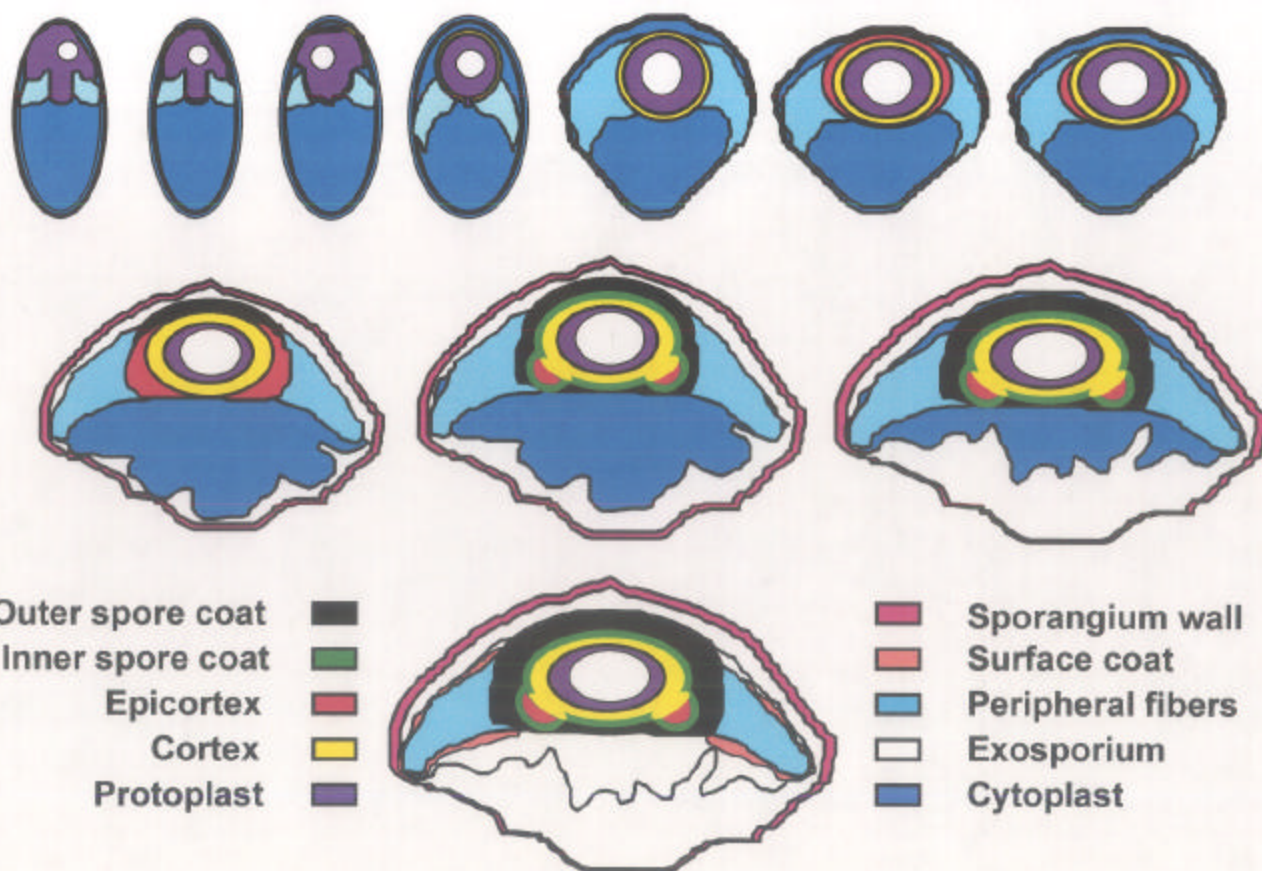


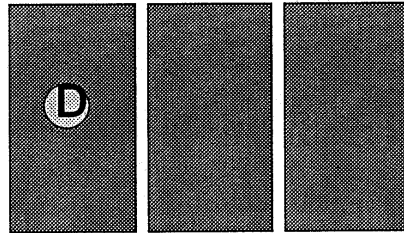
Figure 3

**Plot Plan for
Pasteuria (S-1)
Seasonal Depth
Survey**



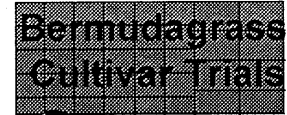
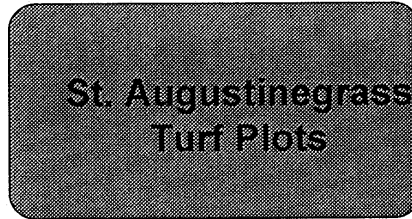
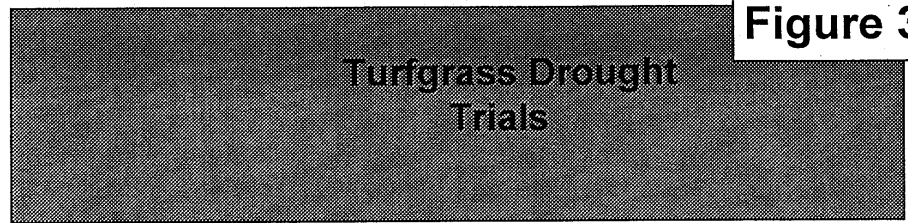
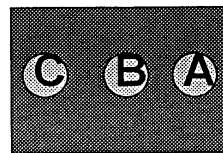
00436

**Bermudagrass
Nutrition and
Nematicide Fairway** →

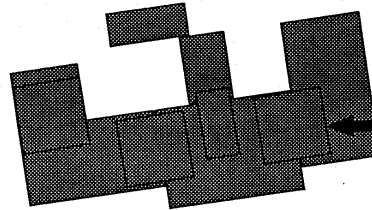
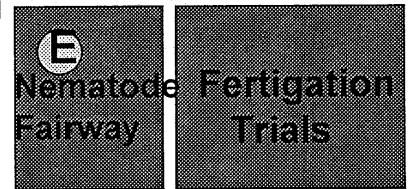
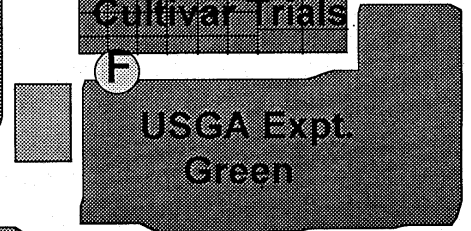


100 meters

**Phytopathology
Fairway** →



F



**Fort Lauderdale
Research and
Education Cent**

FIGURE 4

Location A (1995-96)

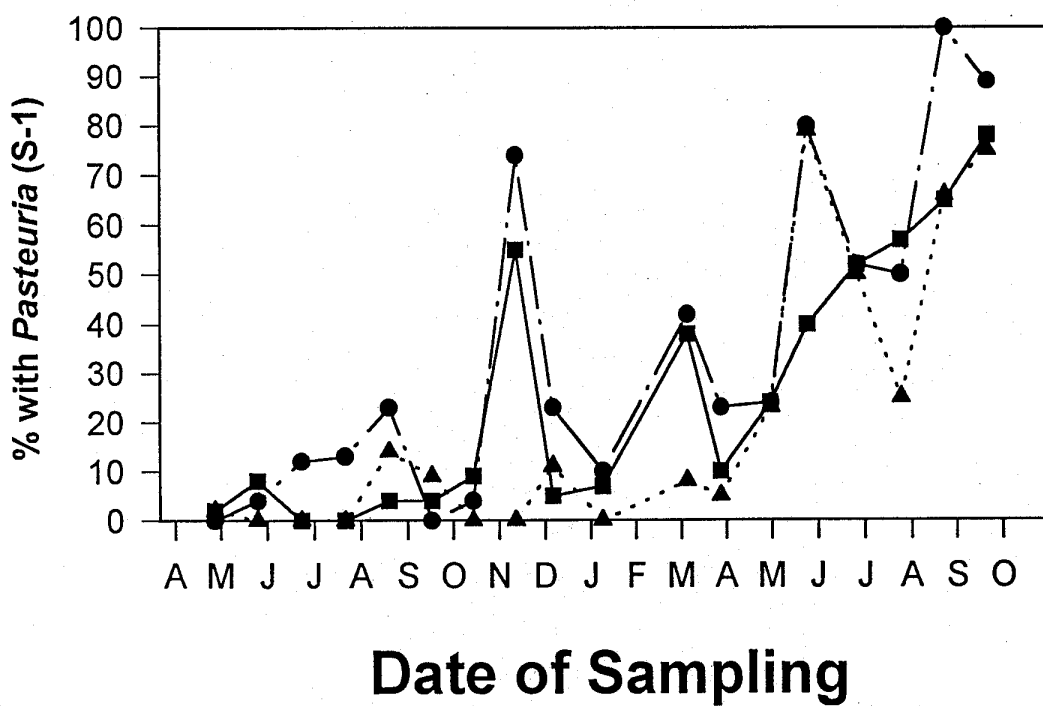
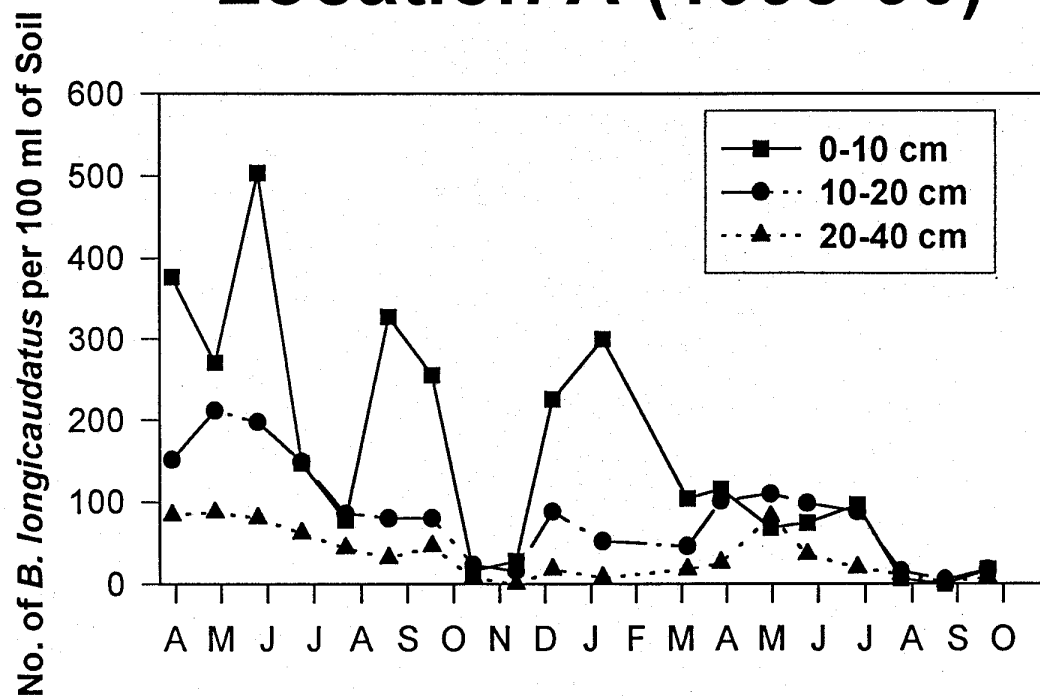


FIGURE 5

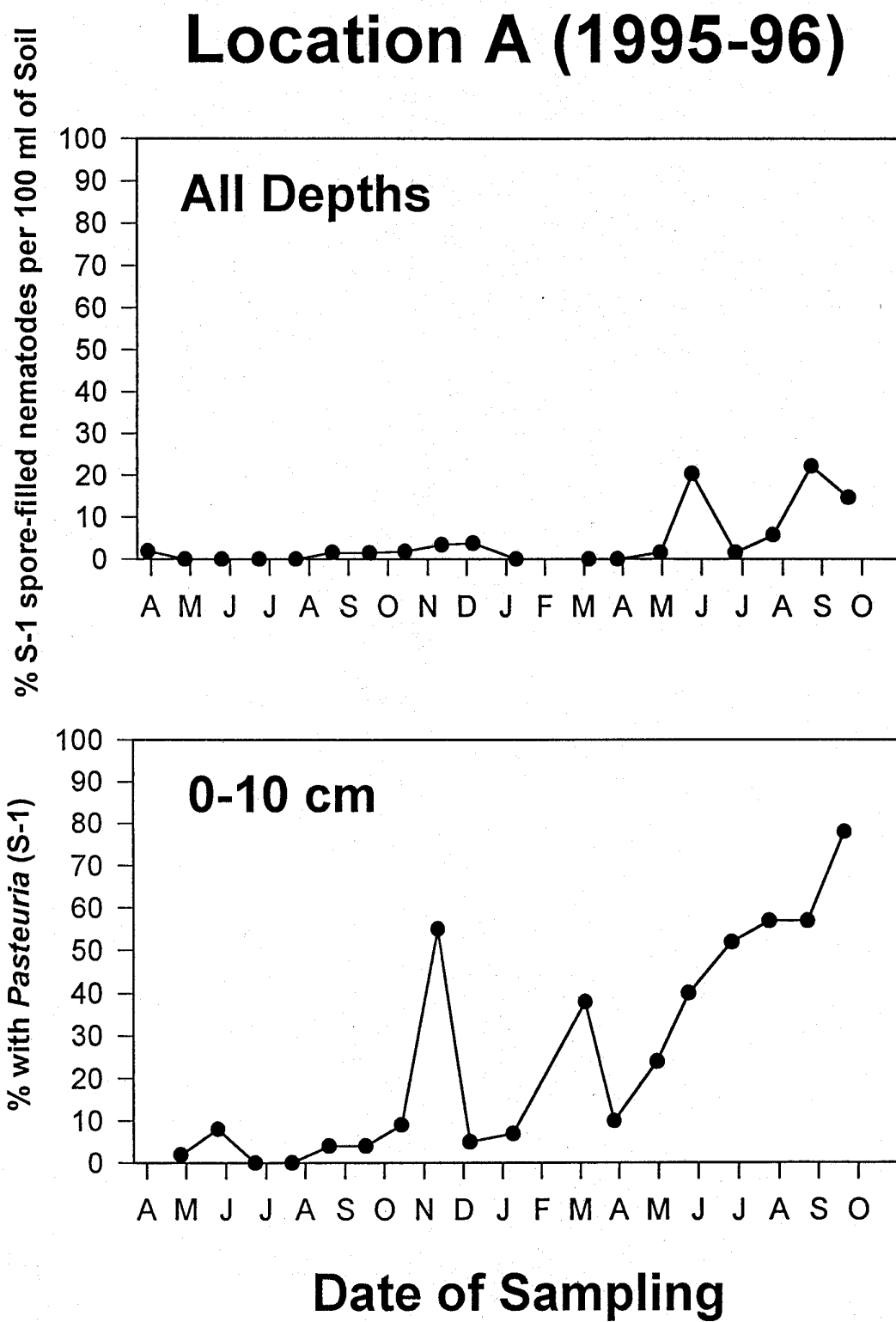
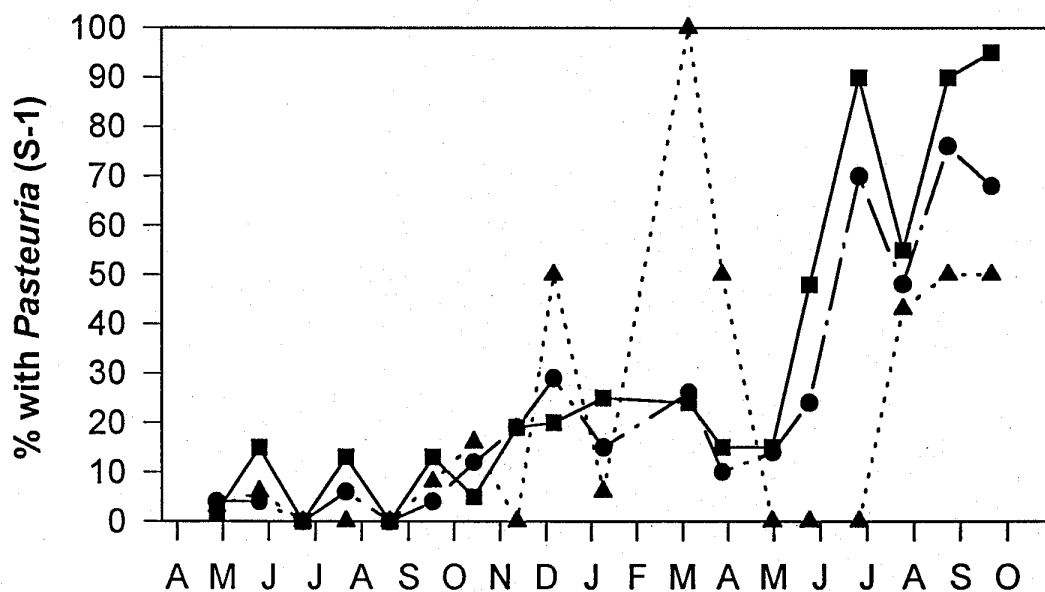
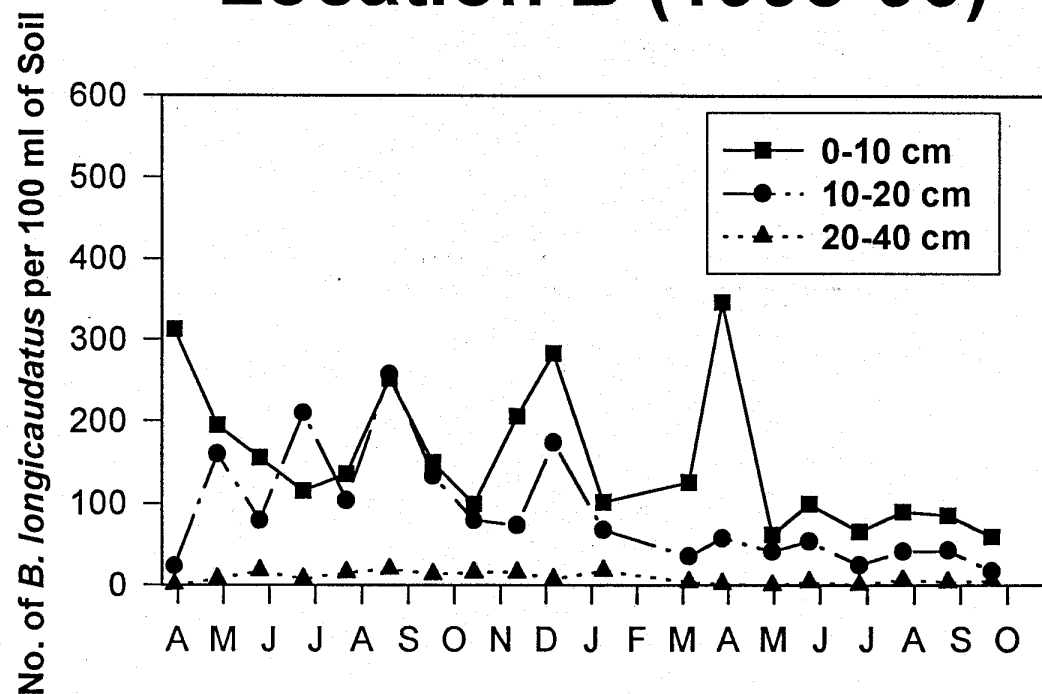


FIGURE 6

Location B (1995-96)



Date of Sampling

FIGURE 7

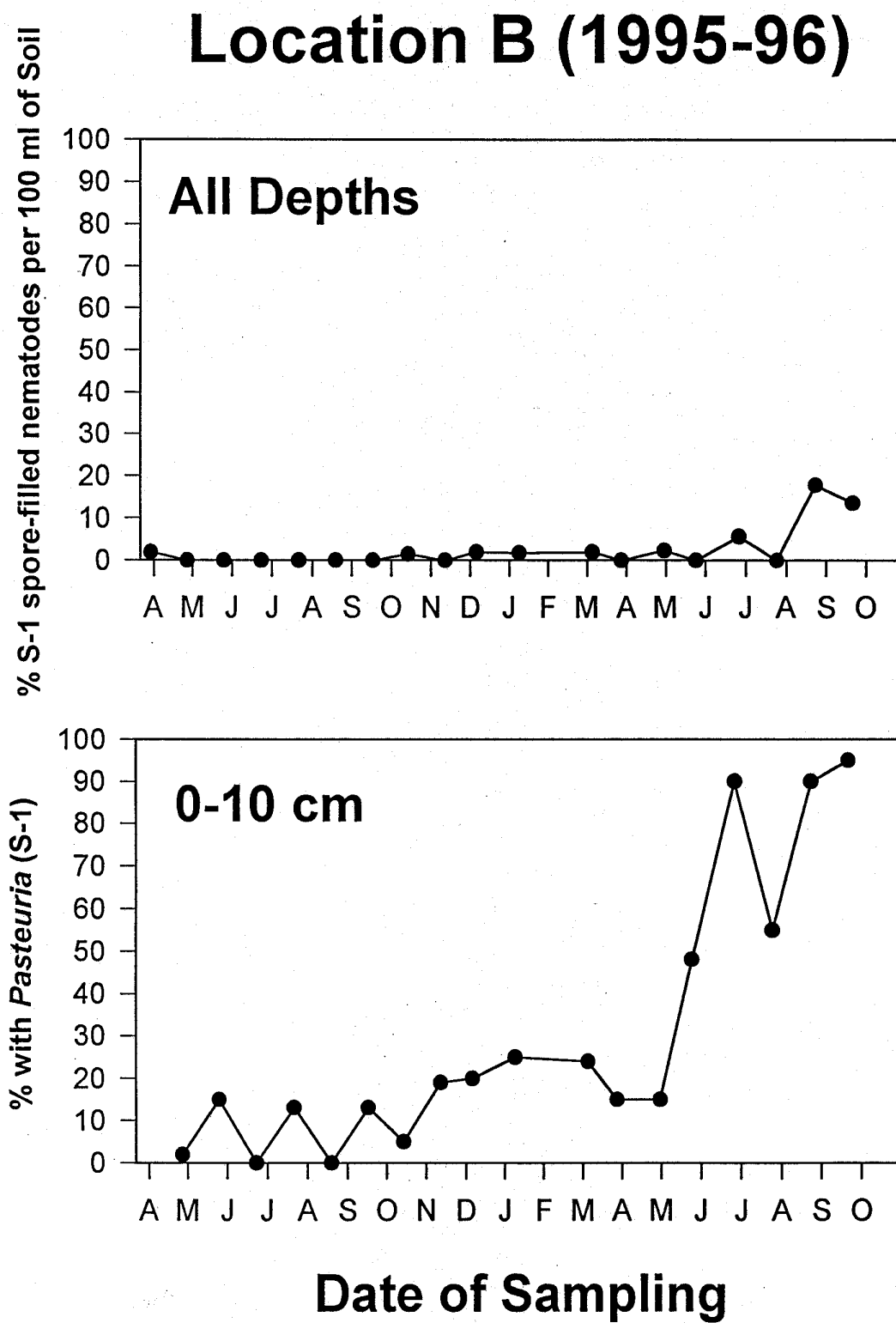


FIGURE 8

Location C (1995-96)

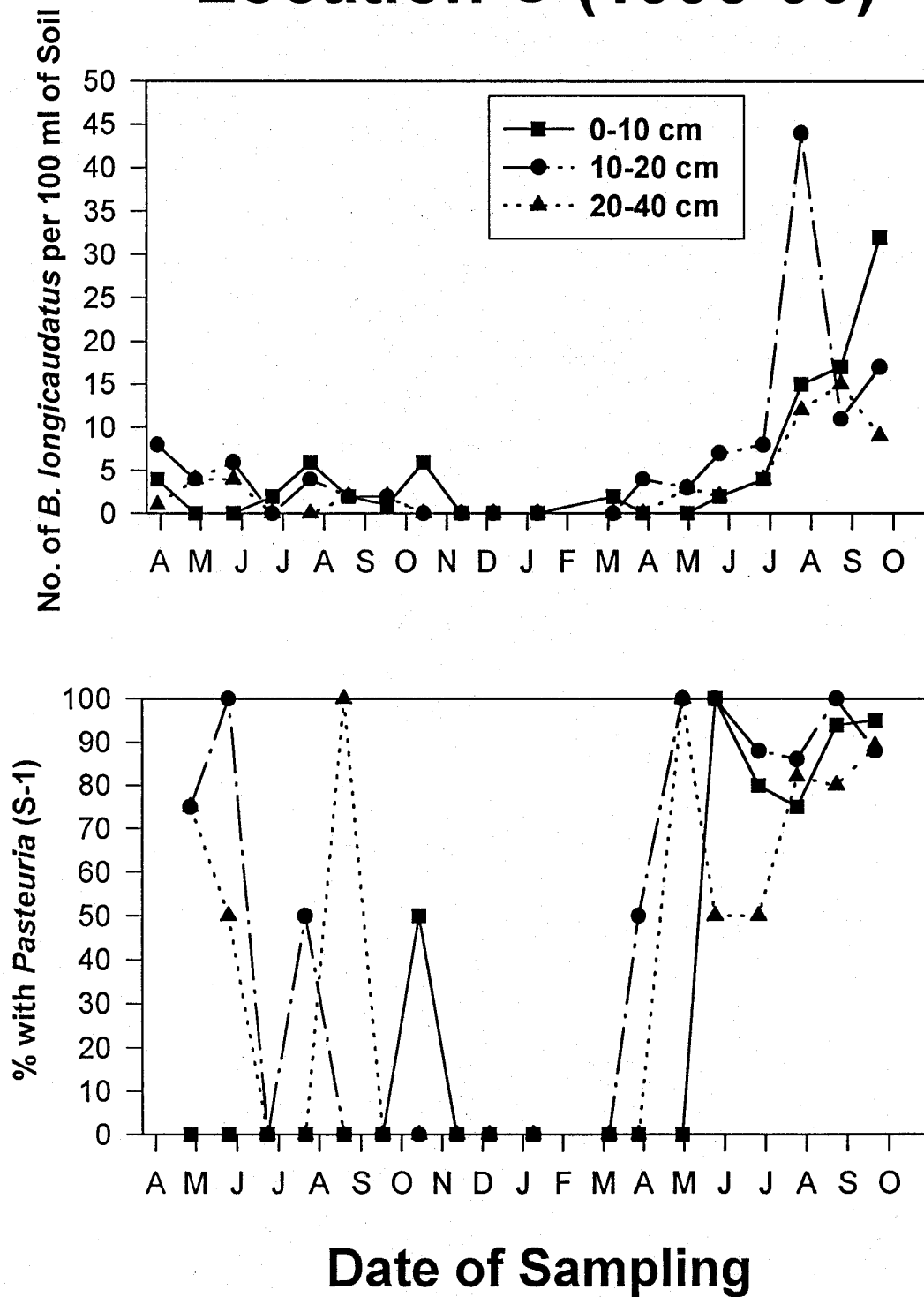


FIGURE 9

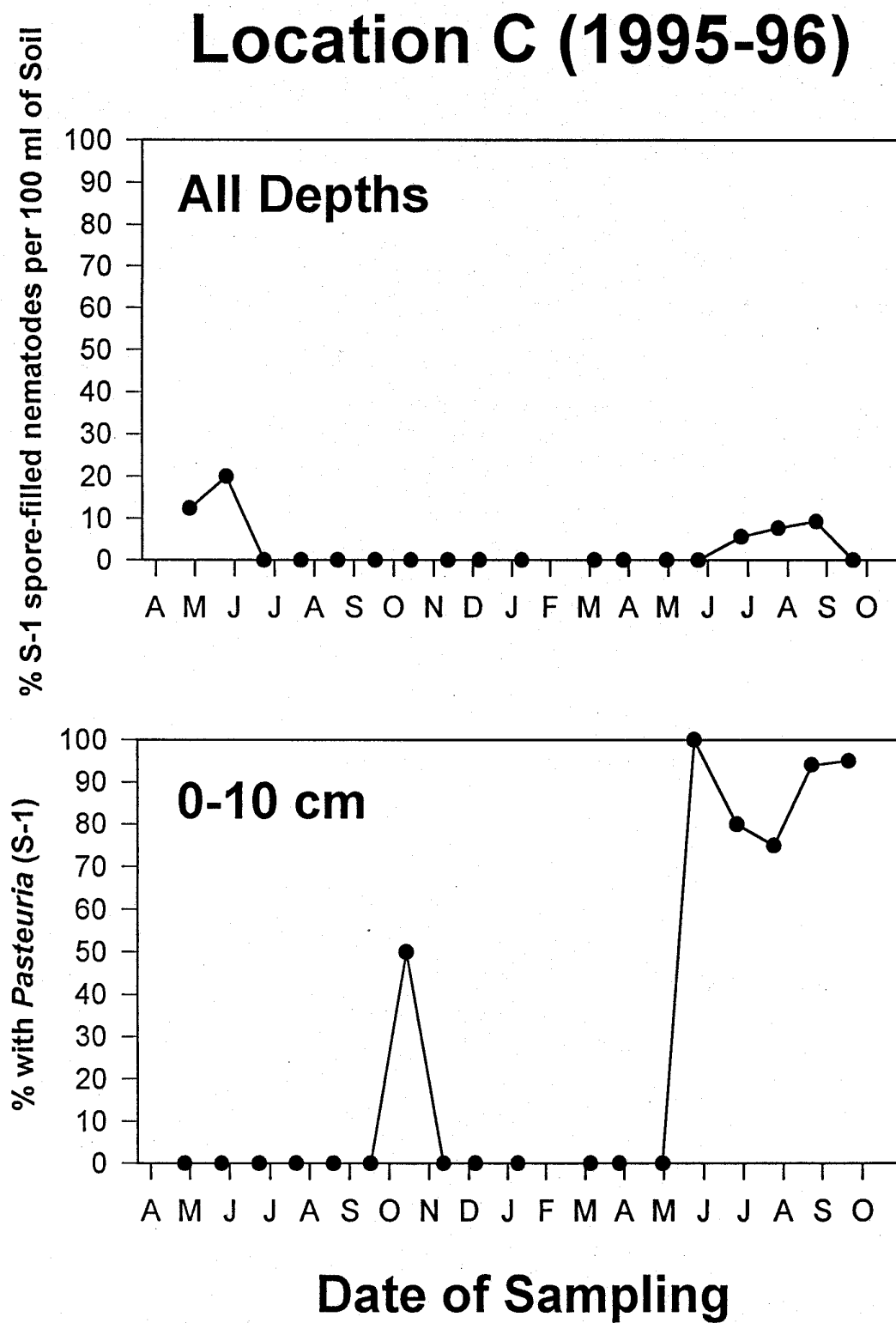
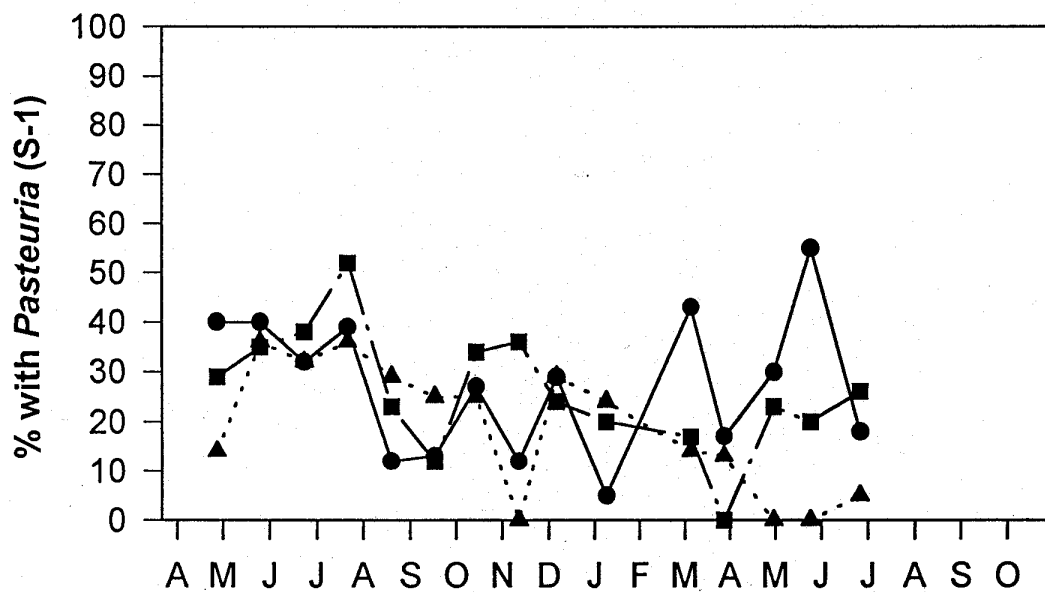
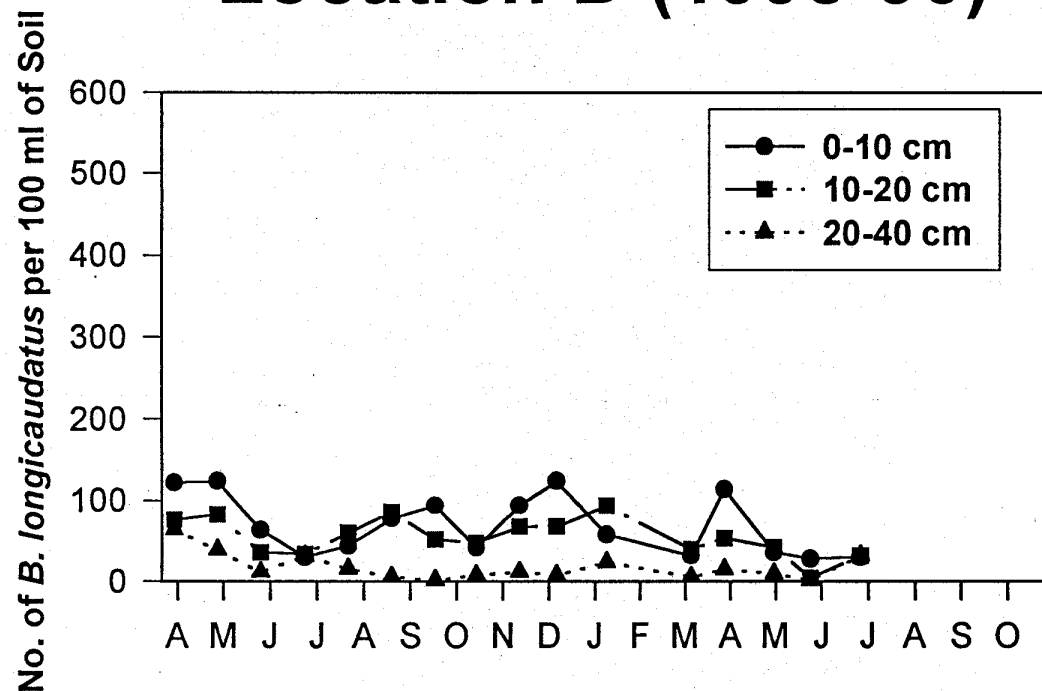


FIGURE 10

Location D (1995-96)



Date of Sampling

FIGURE 11

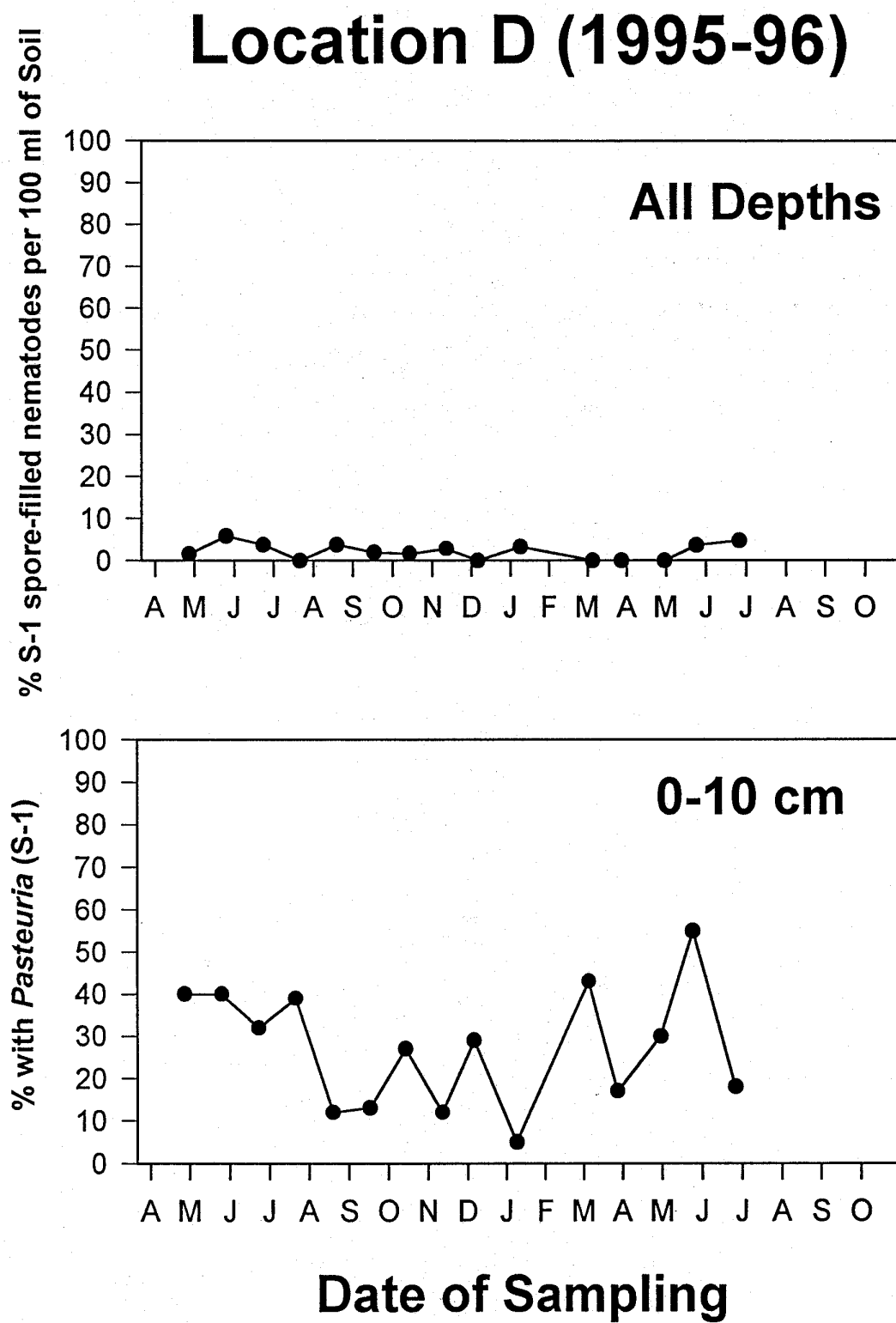
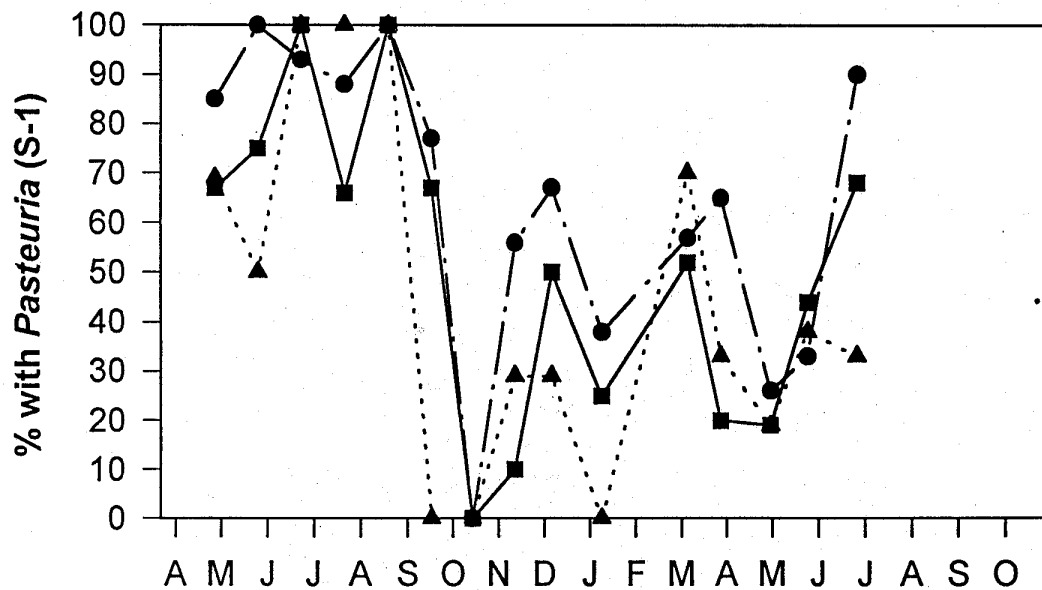
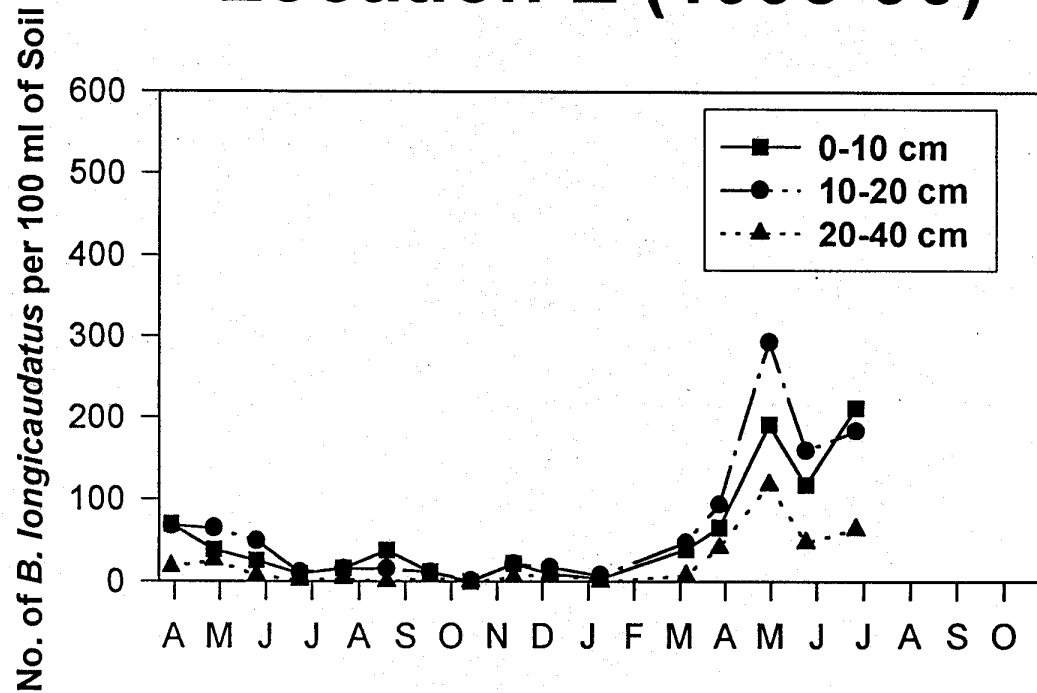


FIGURE 12

Location E (1995-96)



Date of Sampling

FIGURE 13

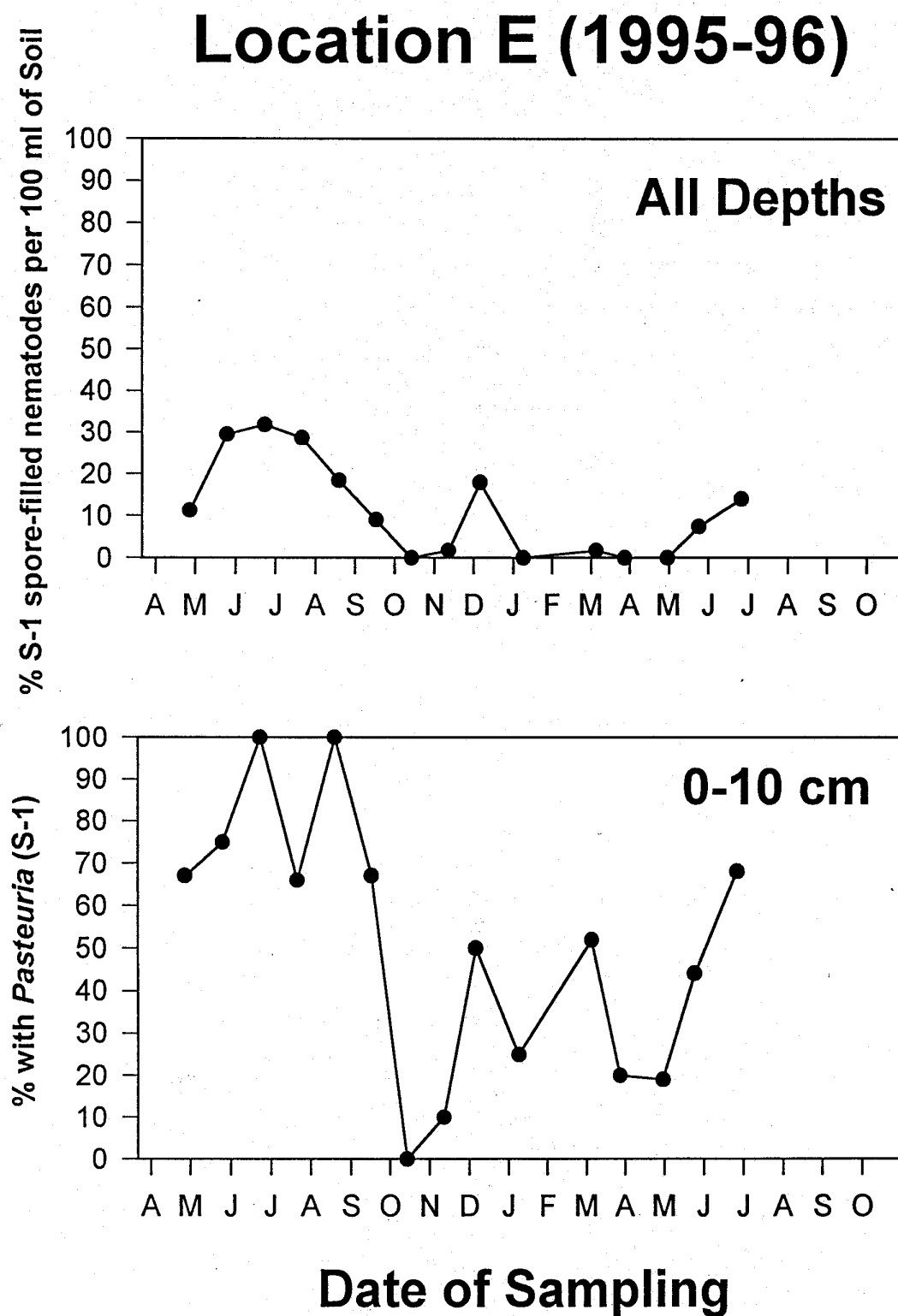


FIGURE 14

Location F (1995-96)

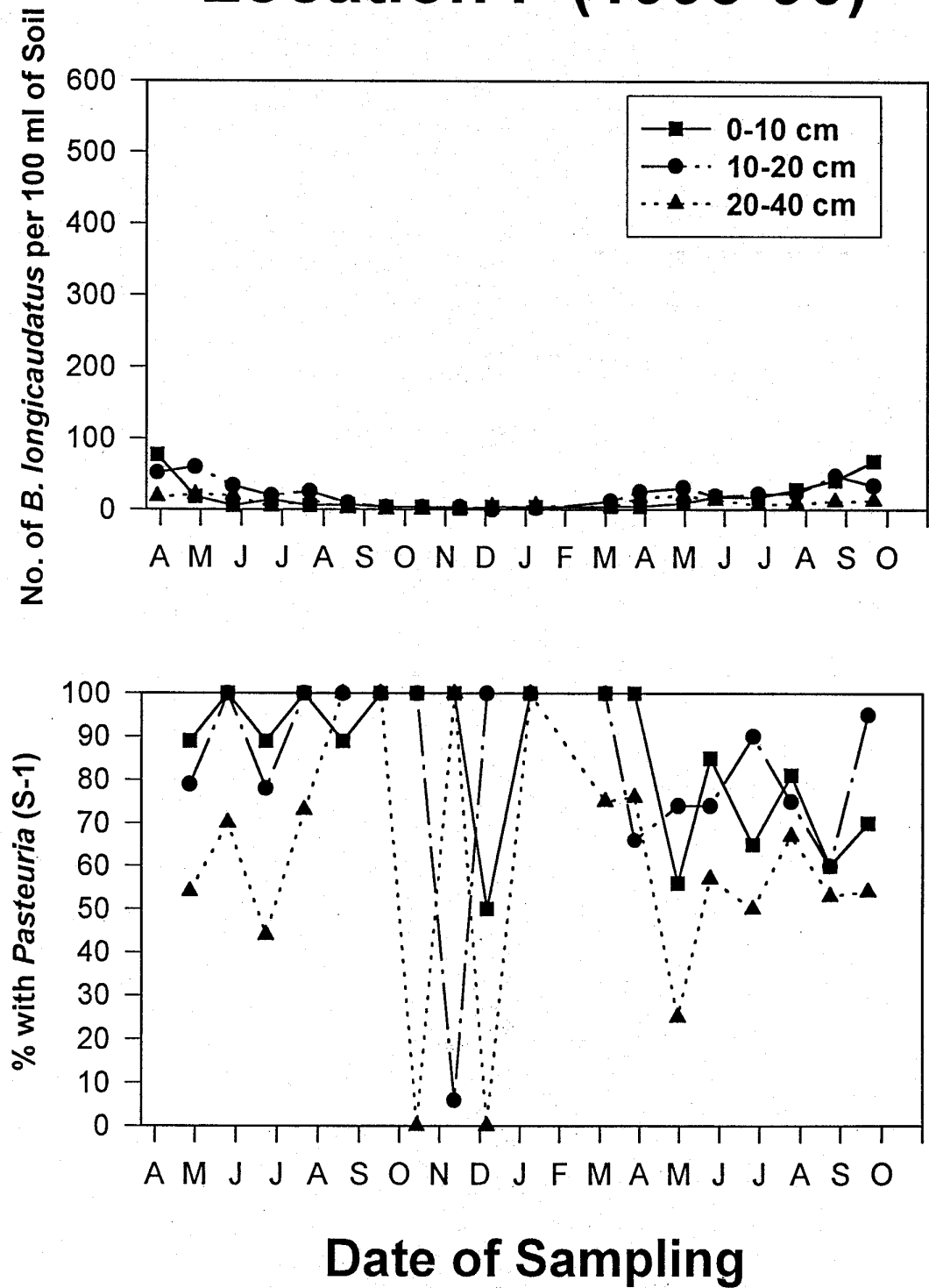


FIGURE 15

