Journal of Nematology 32(2):205–209. 2000.

Yield Reduction and Root Damage to Cotton Induced by *Belonolaimus longicaudatus*[1]

W. T. Crow,[2] D. W. Dickson,[3] D. P. Weingartner,[4] R. McSorley,[3] and G. L. Miller[5]

Abstract: Sting nematode (*Belonolaimus longicaudatus*) is recognized as a pathogen of cotton (*Gossypium hirsutum*), but the expected damage from a given population density of this nematode has not been determined. The objective of this study was to quantify the effects of increasing initial population densities (Pi) of *B. longicaudatus* on cotton yield and root mass. In a field plot study, nematicide application and cropping history were used to obtain a wide range of Pi values. Cotton yields were regressed on Pi density of *B. longicaudatus* to quantify yield losses in the field. In controlled environmental chambers, cotton was grown in soil infested with increasing Pi's of *B. longicaudatus*. After 40 days, root systems were collected, scanned on a desktop scanner, and root lengths were measured. Root lengths were regressed on inoculation density of *B. longicaudatus* to quantify reductions in the root systems. In the field, high Pi's (>100 nematodes/130 cm^3 of soil) reduced yields to near zero. In controlled environmental chamber studies, as few as 10 *B. longicaudatus*/130 cm^3 of soil caused a 39% reduction in fine cotton roots, and 60 *B. longicaudatus*/130 cm^3 of soil caused a 70% reduction. These results suggest that *B. longicaudatus* can cause significant damage to cotton at low population densities, whereas at higher densities crop failure can result.

Key words: Belonolaimus longicaudatus, cotton, crop loss, damage function, damage threshold, *Gossypium hirsutum,* nematode, plant disease loss, root scanning, sting nematode.

Belonolaimus longicaudatus Rau (sting nematode) is a destructive pathogen of numerous crops (Perry and Rhoades, 1982; Smart and Nguyen, 1991). While it can be devastating to crops where it is found, geographic distribution of *B. longicaudatus* is limited primarily to the Coastal Plains of the southeastern United States. Soil texture greatly influences the distribution of *B. longicaudatus,* which is found predominantly in soils composed of >80% sand and <10% organic matter (Robbins and Barker, 1974).

Belonolaimus longicaudatus was first identified as a pathogen of cotton (*Gossypium hirsutum* L.) by Graham and Holdeman (1953), who reported severe yield reductions and root damage in field and greenhouse tests. They described symptoms on cotton roots as ''shrunken lesions along the root axis or at the root tip.'' Sting nematode was later reported to cause stubby root symptoms on 'DPL 50' cotton (Crow et al., 1997). Sting nematode also increased the severity of Fusarium wilt of cotton in greenhouse tests (Holdeman and Graham, 1954; Minton and Minton, 1966; Yang et al., 1976).

The existence of different physiological races of *B. longicaudatus* has been suggested because the host range of populations from different locations has been shown to differ substantially (Abu-Gharbieh and Perry, 1970; Robbins and Barker, 1973). The host status of cotton for *B. longicaudatus* has varied in experiments conducted by different researchers. 'Coker 100WR' cotton was a good host for *B. longicaudatus* from South Carolina (Holdeman and Graham, 1953), but 'Stoneville 7A' cotton was a poor host for populations from North Carolina and Georgia (Robbins and Barker, 1973).

Although *B. longicaudatus* has long been identified as a severe pathogen of cotton, there has been little work devoted to this host-pathogen combination, perhaps because of the lack of cotton production on sandy soils conducive to sting nematode. Recent surveys of cotton fields in South Carolina and Georgia found the incidence of infestation with *B. longicaudatus* to be <1% and

Received for publication 7 May 1999.

[1] A portion of the PhD dissertation by the first author. Florida Agricultural Experiment Station Journal Series no. R-06892.

[2] Former Graduate Research Assistant, Entomology and Nematology Department, University of Florida, Gainesville FL 32611. Present address: Texas A&M University Agricultural Research and Extension Center-Dallas, 17360 Coit Road, Dallas, TX 75252.

[3] Professors, Entomology and Nematology Department, University of Florida, Gainesville FL 32611.

[4] Center Director, University of Florida Agricultural Research and Education Center, Hastings, FL 32145.

[5] Assistant Professor, Environmental Horticulture Department, University of Florida, Gainesville, FL 32611.

E-mail: w-crow@tamu.edu

This paper was edited by E. C. Bernard.

0.3%, respectively (Baird et al., 1996; Martin et al., 1994). A survey of nematodes in cotton fields in Florida found no sting nematodes in any sample fields (Kinloch and Sprenkel, 1994). If cotton production expands into soils with a high sand content, sting nematode may become a significant problem (Starr, 1998). The objective of this study was to quantify reductions in yield and root systems in response to increasing population densities of *B. longicaudatus.*

Materials and Methods

Yield reductions in the field: Yield reductions caused by *B. longicaudatus* in the field were quantified in a 2-year trial carried out at the Yelvington Farm University of Florida Agricultural Research and Education Center at Hastings, Florida, in 1997 and 1998. The site selected was naturally infested with *B. longicaudatus, Meloidogyne incognita* race 1, *Paratrichodorus minor, Pratylenchus brachyurus, P. zeae, Tylenchorhynchus* sp., *Mesocriconema* sp., *Dolichodorus heterocephalus,* and *Hemicycliophora* sp. Soil at the research site was an Ellzey fine sand (sandy, silicaceous, hyperthermic Arenic Ochraqualf) consisting of 95% sand, 2% silt, 3% clay; <1% organic matter; pH 6.5 to 7.0.

To study effects of sting nematode on cotton over a range of population densities, initial population densities (Pi) of *B. longicaudatus* in 25 field plots were adjusted as follows. Twenty plots were planted to cotton following 8 months of clean fallow. Ten of these plots were fumigated 3 weeks before planting with 1,3-dichloropropene (1,3-D) at a broadcast rate of 56 liters/ha (570 ml/100-m row) applied with a single chisel per row. Fumigation resulted in low population densities at planting (Pi) of *B. longicaudatus.* The remaining 10 plots were untreated and had moderate Pi densities. To obtain high population densities (>100 nematodes/130 cm^3 of soil), cotton was planted into an additional five plots where potato (a host for *B. longicaudatus*) had been grown the preceding winter. Only 1 week of fallow occurred between the potato and cotton crops in these plots, and no nematicides were used on either crop.

The experiment was carried out on ridged rows with 102-cm spacing between rows, and the plot area was watered with seepage irrigation (Campbell et al., 1978; Rogers et al., 1975). Field plots were 4 rows wide and measured 9 m long. Yield and nematode data were collected from the center two rows in each plot.

Nematode population densities in all plots were assayed 3 weeks following fumigation of plots receiving 1,3-D. Twelve cores (2.5-cm-diam.) were taken 20 cm deep from the center rows of each plot to form a composite sample. Nematodes were extracted from a 130-cm^3 subsample with modifications of the centrifugal-flotation technique (Jenkins, 1964). Soil was not passed through a 2-mm-pore sieve during the washing process to remove large debris because *B. longicaudatus* is long enough to become enmeshed by the sieve (McSorley and Frederick, 1991). Additionally, the concentration of the sucrose solution was doubled by adding 0.908 kg of sugar to 1 liter of water. Numbers of all genera of plant-parasitic nematodes extracted were counted with the aid of an inverted light microscope at ×32.

'DPL 5415' cotton seeds were planted immediately following the collection of the Pi soil samples. Seedlings were thinned to 15 cm between plants following emergence. Planting dates were 22 May 1997 and 30 June 1998, and harvest dates were 17 October 1997 and 9 December 1998. Cotton was harvested with a single-row mechanical harvester, and seed cotton weights were recorded. Following harvest, cotton yield was regressed on Pi densities of all plant-parasitic nematodes present by stepwise multiple-regression analysis (Ott, 1993). This analysis provided for identification of plant-parasitic nematodes with strongest relationships to yield reductions (McSorley and Waddill, 1982). The multiple-regression analysis was performed with SAS software (SAS Institute, Cary, NC). Cotton yield also was regressed on Pi density of *B. longicaudatus* by simple linear regression with Excel software (Microsoft, Redmond, WA).

Controlled environmental chamber study: To quantify effects of increasing population

densities of *B. longicaudatus* on cotton roots, tests were conducted under controlled environmental conditions. These studies were carried out in two trials, with the second trial having two additional Pi treatments.

A population of *B. longicaudatus* was collected from the Hastings field site and reared on bermudagrass (*Cynodon dactylon*) in pots filled with pasteurized soil in the greenhouse. Nematodes were extracted from greenhouse soil with the use of a modified Baermann method (McSorley and Frederick, 1991). The inoculum consisted of mixed life stages of *B. longicaudatus* suspended in water.

Soil from the field site was autoclaved at 250 °C and 131 kPa for 45 minutes, then placed into 15-cm-diam. black plastic pots. Approximately 750 cm^3 of soil was added per pot. Nematode inoculum was pipeted into five 2-cm-deep holes in the soil per pot at 0, 20, and 60 nematodes/130 cm^3 of soil in the first trial and 0, 10, 20, 40, and 60 nematodes/130 cm^3 of soil in the second trial. Following addition of nematodes, three 'DPL 5415' cotton seeds were planted 1 cm deep into each pot and soil was wetted to 12% soil moisture (w/w). The pots were then placed in controlled environmental chambers that were maintained at 28.5 °C. Soil moisture in each pot was kept between 5% and 12% (w/w). Plants were given 16 hours of light each day. Following emergence, seedlings were thinned to one per pot and grown for 40 days. The plants were removed, and the soil was washed from the roots.

Each root sample was immersed in 150 ml of water in plastic cups that had three drops of 1% methylene blue added. The stained roots were then placed into a glass-bottom tray and scanned with an HP ScanJet 2cx desktop scanner (Hewlett Packard, Boise, ID) to create a bitmap image of the roots (Kaspar and Ewing, 1997; Pan and Bolton, 1991). Bitmap images were imported into the GSRoot (Louisiana State University, Baton Rouge, LA) software program to measure root lengths and surface areas from the scanned images. In this program, root-diameter ranges of interest are entered, and individual measurements for roots of each diameter range are given. Diameter ranges measured were: <0.25 mm, 0.26 to 0.5 mm, 0.51 to 1.0 mm, and >1.0 mm. The root-length measurements for each range were regressed on Pi of *B. longicaudatus* with Excel software (Microsoft, Redmond, WA).

Results

Yield losses in the field: Sting nematode had the greatest degree of association ($R^2 \geq 0.72$) with cotton yield losses in the field, and no other nematodes were consistently associated with yield reductions during both years (Table 1). Separate linear regressions of cotton yield on Pi density of *B. longicaudatus* from the 2 years were tested for heterogeneity of the slopes. Because the slopes for the 2 years were not different from each other ($P = 0.68$), data from the 2 years were combined into a single data set for further regression analysis. The association between

Table 1. Results of stepwise multiple-regression analysis of cotton yield on initial population densities of all plant-parasitic nematodes present at the Yelvington Farm near Hastings, Florida, in 1997 and 1998.

	1997		1998	
Nematode	R^2	Prob > F	R^2	Prob > F
Belonolaimus longicaudatus	0.76	0.0001	0.72	0.0001
Meloidogyne incognita	0.02	0.14	NS	NS
Paratrichodorus minor	NS[a]	NS	0.04	0.07
Pratylenchus spp.	NS	NS	NS	NS
Tylenchorhynchus sp.	0.04	0.05	NS	NS
Mesocriconema sp.	NS	NS	NS	NS
Dolichodorus heterocephalus	NS	NS	NS	NS
Hemicycliophora sp.	NS	NS	NS	NS

[a] NS = Regression was not significant at $P \leq 0.15$.

Pi density of *B. longicaudatus* (x) and cotton yield (Y) for both years was described by the linear equation $Y = -18.36x + 2{,}848$ with an $r^2 = 0.78$ ($P < 0.0001$) (Fig. 1). Poor cotton stands were observed with Pi densities >60; surviving plants in these plots tended to be stunted and many lodged.

Controlled environmental chamber study: The relationships between Pi of *B. longicaudatus* and root length were described by negative exponential equations for the different root-diameter ranges (Fig. 2). The data used in the exponential equations for root-length measurements were transformed (ln x) in order to linearize the slopes so that heterogeneity of slopes could be statistically tested. Although the second trial had two additional Pi's, the slopes of the regression lines from the two trials were not different for roots with diameter <0.25 mm ($P = 0.12$). Therefore, the data from the two trials were combined into a single data set (Fig. 2A). The slopes of the two trials were heterogenous, with P values of 0.05 and 0.07 for roots with diameters of 0.25 to 0.5 and 0.51 ≤1.0 mm, respectively. Therefore, regression lines for the two trials were shown separately for these diameter ranges (Fig. 2B, C). Root lengths of all diameter ranges < 1.0-mm-diam. were reduced ($P < 0.001$) in response to increasing Pi's of *B. longicaudatus*. Pi of *B. longicaudatus* had no effect ($P \geq 0.05$) on roots with diameters >1 mm.

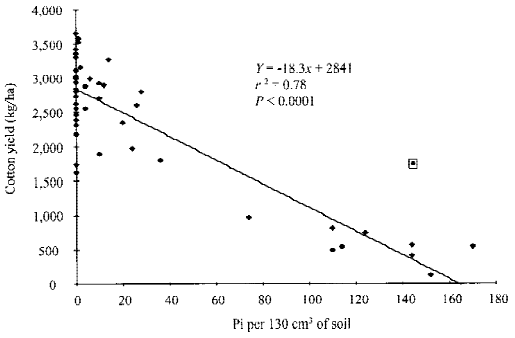

FIG. 1. Effect of increasing *Belonolaimus longicaudatus* densities at planting (Pi) on seed cotton yield in 1997 and 1998. Data from both years were combined for analysis. ⊡ = Outlier plot; i.e., *B. longicaudatus* in this plot had a high incidence of attachment by *Pasteuria* sp., an endospore-forming parasite of nematodes, and was not included in the analysis.

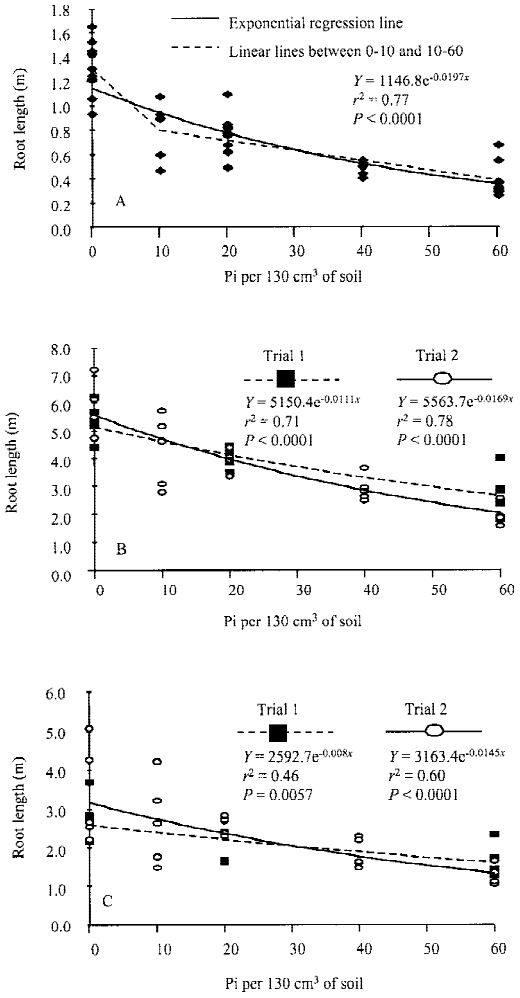

FIG. 2. Effect of increasing *Belonolaimus longicaudatus* densities at planting (Pi) on root length of cotton grown in controlled environmental chambers. A) Roots with diameters <0.25 mm. B) Roots with diameters 0.26 to 0.5 mm. C) Roots with diameters 0.51 to 1.0 mm.

Roots <0.25-mm-diam. growing in soil infested with the highest Pi of *B. longicaudatus* (60/130 cm^3 of soil) had length reductions of 70% in comparison with those grown in uninfested soil. The greatest reduction in root length for roots <0.25-mm-diam. (39%) occurred between Pi's of 0 and 10 nematodes/130 cm^3 of soil. Root-length responses to Pi's between 10 and 60 *B. longicaudatus*/130 cm^3 of soil followed a linear trend (Fig. 2A).

DISCUSSION

Belonolaimus longicaudatus is an aggressive pathogen, causing root damage and yield

losses at relatively low population densities. In the controlled environment studies, Pi's as low as 10 *B. longicaudatus*/130 cm^3 of soil caused nearly a 40% reduction in fine cotton roots. The current extraction efficiency estimate for *B. longicaudatus* with the centrifugal-flotation method is 30% (McSorley and Frederick, 1991). Therefore, it is reasonable to expect that any detectable Pi of *B. longicaudatus* in the field may cause damage to cotton.

Because *B. longicaudatus* is damaging at low Pi's and is capable of reducing yield to near zero at high Pi's, the damage function was linear in shape. Linear damage functions also have been used for *Belonolaimus* spp. on other crops (Crow et al., 2000; McSorley and Dickson, 1989; Todd, 1989). Damage functions such as those derived in this study will be useful in establishing economic thresholds dependent upon local conditions and costs (Ferris, 1978).

Literature Cited

Abu-Gharbieh, W. I., and V. G. Perry. 1970. Host differences among Florida populations of *Belonolaimus longicaudatus* Rau. Journal of Nematology 2:209–216.

Baird, R. E., R. F. Davis, P. J. Alt, B. G. Mulinix, and G. B. Padgett. 1996. Frequency and geographical distribution of plant-parasitic nematodes on cotton in Georgia. Supplement to the Journal of Nematology 28:661–667.

Campbell, K. L., J. S. Rogers, and D. R. Hensel. 1978. Watertable control for potatoes in Florida. Transactions of the American Society of Agricultural Engineering 21:701–705.

Crow, W. T., D. W. Dickson, and D. P. Weingartner. 1997. Stubby-root symptoms on cotton induced by *Belonolaimus longicaudatus*. Journal of Nematology 29:574 (Abstr.).

Crow, W. T., D. P. Weingartner, R. McSorley, and D. W. Dickson. 2000. Damage function and economic threshold for *Belonolaimus longicaudatus* on potato. Journal of Nematology, in press.

Ferris, H. 1978. Nematode economic thresholds: Derivation, requirements, and theoretical considerations. Journal of Nematology 10:341–350.

Graham, T. W., and Q. L. Holdeman. 1953. The sting nematode *Belonolaimus gracilis* Steiner: A parasite on cotton and other crops in South Carolina. Phytopathology 43:434–439.

Holdeman, Q. L., and T. W. Graham. 1953. The effect of different plant species on the population trends of the sting nematode. Plant Disease Reporter 37:497–500.

Holdeman, Q. L., and T. W. Graham. 1954. Effect of the sting nematode on expression of Fusarium wilt in cotton. Phytopathology 44:683–685.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Kaspar, T. C., and R. P. Ewing. 1997. ROOTEDGE: Software for measuring root length from desktop scanner images. Agronomy Journal 89:932–940.

Kinloch, R. A., and R. K. Sprenkel. 1994. Plant-parasitic nematodes associated with cotton in Florida. Supplement to the Journal of Nematology 26:749–752.

Martin, S. B., J. D. Mueller, J. A. Saunders, and W. I. Jones. 1994. A survey of South Carolina cotton fields for plant-parasitic nematodes. Plant Disease 78:717–719.

McSorley, R., and D. W. Dickson. 1989. Effects and dynamics of a nematode community on maize. Journal of Nematology 21:462–471.

McSorley, R., and J. J. Fredrick. 1991. Extraction efficiency of *Belonolaimus longicaudatus* from sandy soil. Journal of Nematology 23:511–518.

McSorley, R., and V. H. Waddill. 1982. Partitioning yield loss on yellow squash into nematode and insect components. Journal of Nematology 14:110–118.

Minton, N. A., and E. B. Minton. 1966. Effect of root knot and sting nematodes on expression of Fusarium wilt of cotton in three soils. Phytopathology 56:319–322.

Ott, R. L. 1993. An introduction to statistical methods and data analysis. Belmont, CA: Wadsworth Publishing.

Pan, W. L., and R. P. Bolton. 1991. Root quantification by edge discrimination using a desktop scanner. Agronomy Journal 83:1047–1052.

Perry, V. G., and H. L. Rhoades. 1982. The genus *Belonolaimus*. Pp. 144–149 *in* R. D. Riggs, ed. Nematology in the southern region of the United States. Arkansas Agricultural Experiment Station Bulletin 276, University of Arkansas, Fayetteville, AR.

Robbins, R. T., and K. R. Barker. 1973. Comparisons of host-range and reproduction among populations of *Belonolaimus longicaudatus* from North Carolina and Georgia. Plant Disease Reporter 57:750–754.

Robbins, R. T., and K. R. Barker. 1974. The effects of soil type, particle size, temperature, and moisture on reproduction of *Belonolaimus longicaudatus*. Journal of Nematology 6:1–6.

Rogers, J. S., D. R. Hensel, and K. L. Campbell. 1975. Subsurface drainage and irrigation for potatoes. Soil and Crop Science Society of Florida Proceedings 34:16–17.

Smart, G. C., and K. B. Nguyen. 1991. Sting and awl nematodes: *Belonolaimus* spp. and *Dolichodorus* spp. Pp. 627–667 *in* W. R. Nickle, ed. Manual of agricultural nematology. New York: Marcel Dekker.

Starr, J. L. 1998. Cotton. Pp. 359–379 *in* K. R. Barker, G. A. Pederson, and G. L. Windham, eds. Plant and nematode interactions. Madison, WI: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Todd, T. C. 1989. Population dynamics and damage potential of *Belonolaimus* sp. on corn. Supplement to the Journal of Nematology 21:697–702.

Yang, H., N. T. Powell, and K. R. Barker. 1976. Interactions of concomitant species of nematodes and *Fusarium oxysporum* f. sp. *vasinfectum* on cotton. Journal of Nematology 8:74–80.