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Belonolaimus longicaudatus: An Emerging Pathogen of Peanut in Florida

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Abstract: Sting nematode (*Belonolaimus longicaudatus*) is an economically important ectoparasitic nematode that is highly pathogenic on a wide range of agricultural crops in sandy soils of the southeastern United States. Although this species is commonly found in Florida in hardwood forests and as a soilborne pathogen on turfgrasses and numerous agronomic and horticultural crops, it has not been reported infecting peanut. In the summers of 2012 and 2013, sting nematode was found infecting three different peanut cultivars being grown on two separate peanut farms in Levy County, FL. The damage consisted of large irregular patches of stunted, chlorotic plants at both farms. The root systems were severely abbreviated and there were numerous punctate-like isolated lesions observed on pegs and pods of infected plants. Sting nematodes were extracted from soil collected around the roots of diseased peanut over the course of the peanut season at both farm sites. Peanut yield from one of these nematode-infested sites was 64% less than that observed in areas free from sting nematodes. The morphological characters of the nematode populations in these fields were congruous with those of the original and other published descriptions of *B. longicaudatus*. Moreover, the molecular analyses based on the sequences of D2/D3 expansion fragments of 28S rRNA and internal transcribed spacer (ITS) rRNA genes from the nematodes further collaborates the identification of the sting nematode isolates as *B. longicaudatus*. The sequences were deposited in GenBank (accession no. KF963097, KF963098 for ITS, and KF96399, KF963100 for D2-D3). The results of the phylogenetic analysis using the sequences of these isolates from peanut compared with those of other isolates from Florida suggests that the sting nematode from both peanut farms are genetically close to *B. longicaudatus* populations occurring in the state. Peanut plants inoculated with both nematode isolates showed punctate-like isolated lesions on pods and pegs, and an abbreviation of their root systems, whereas those symptoms were not observed on noninoculated peanut plants. To our knowledge, this is the first report of large-scale field damage caused by sting nematode infecting peanut grown under field conditions in Florida.

Key words: Arachis hypogaea, *Belonolaimus longicaudatus*, emerging pathogen, morphology, pathogenicity, peanut, phylogenetics, sting nematode.

Sting nematode, *B. longicaudatus*, is a highly virulent soilborne pathogen that is capable of causing severe damage to peanut in some growing regions. The species is known to have a very wide host range and prefers soils with a high sand content. It causes severe damage on numerous agronomic and horticultural crops and is considered the most destructive soilborne pathogen of turfgrasses in Florida (Crow and Brammer, 2001). Roots of infected plants are generally extremely abbreviated resulting in above-ground plant parts also being severely stunted and chlorotic. The nematode occurs in soils with a minimum of 80% sand and a maximum of 10% clay. Because of this soil preference, sting nematode is mostly found along the eastern seaboard of the United States (Smart and Nguyen, 1991).

The first report of sting nematode (identified as *B. gracilis*) damage on peanut was described in Virginia by Owens in 1951. Rau (1958) later described *B. longicaudatus* and reported it as the most common sting nematode occurring in the southeastern USA. Production losses of peanut by sting nematode, *B. longicaudatus*, have only been reported in North Carolina (Sasser, 1961), Oklahoma (Russell, 1969), and Virginia (Owens, 1951). Sasser (1961) mentioned that the greatest economic losses of peanut occurred where sting nematode appeared in production fields in North Carolina. Although sting nematode is commonly found in sandy soils in Florida, there have been no reports of this nematode infecting peanut. However, an isolate of sting nematode collected from citrus was reported to infect peanut in greenhouse studies (Abu-Gharbieh and Perry, 1970).

During the summer of 2012, numerous large patches of peanut cv. Tifguard were observed to be severely stunted in one large peanut production farm in Levy Co., FL. Root systems showed severe stunting, a typical symptom induced by sting nematode similar to that already reported on other agronomic and vegetable crops (Robbins and Barker, 1973; Smart and Nguyen, 1991). Nematode extraction from soil collected around stunted peanut roots averaged 44 sting nematodes/100 cm^3 of soil. Pods and pegs showed distinctive symptoms of numerous small discrete brown lesions. Again, in 2013, large patches of stunted peanut cv. Georgia-06G were observed in another large production farm in Levy Co. The above- and below-ground symptoms were similar to that observed the previous year on peanut.

The objectives of this project were to (i) elucidate the morphological characteristics of the two sting nematode

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populations infecting peanut in Levy County, FL, (ii) use molecular characteristics to confirm the morphological identification of these sting nematode populations (iii) describe the symptoms they caused on peanut and estimate yield suppression, (iv) describe changes in their population densities over the crop season at both infested sites, and (v) isolate the nematodes and investigate their effect on peanut under greenhouse conditions.

Materials and Methods

Nematode isolates: Two isolates of sting nematodes were collected, the first from the peanut production farm designated as 35 Farms, 2012 and the second from a farm designated as Brown Farm, 2013. The sting nematode infested fields were ca. 200 ha in size. The nematodes were extracted from soil samples by the modified Baermann method (Rodriguez-Kabana and Pope, 1981) and reared on a diploid St. Augustinegrass (*Stenotaphrum secundarum* (Walt.) Kuntze) (FX-313) in 15-cm-diam. clay pots in a greenhouse with an average daytime temperature of 28 ± 5°C.

Morphological characterization: The nematode isolates were extracted by the modified Baermann method (Rodriguez-Kabana and Pope, 1981), and females and males of both isolates were handpicked arbitrarily from the water suspension with the aid of a stereomicroscope for morphological analysis. Specimens were processed using the method of Seinhorst (1966) with slight modifications. The following morphological characters were first examined to determine classification at the family and generic level: body size, length of stylet, shape of cephalic region and tail, and number of incisures in the lateral lines. Following that, additional selected morphological characters were examined from 20 females and males of the 2 nematode isolates (Geraert, 2011). The specimens were measured with an ocular micrometer using a compound microscope at ×1000 magnification with an oil immersion objective, except for total body length, which was measured at ×400. The characters measured included total body length, stylet, stylet cone and stylet shaft lengths, head height, tail length, tail width, anterior end to excretory pore distance, posterior end to phasmid distance, body width, spicule, and gubernaculum length. Ratios were calculated for the following: i) body length/body width, ii) body length/pharynx length, and iii) body length/tail length, tail/body width, stylet/tail, and percentage V (vulva position as percentage of body length).

DNA extraction and PCR amplification: Sting nematodes were extracted by the modified Baermann method (Rodriguez-Kabana and Pope, 1981), and single females from each isolate were handpicked and processed separately for DNA extraction with DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The extracted DNA was suspended in a 25 µl reaction volume containing 2.5 µl of 10× Standard Taq reaction buffer (100 mM Tris pH 8.3, 500 mM KCl, and 15 mM $MgCl_2$), 2 µl of 10× dNTPs (200 mM each), 0.8 units of 1 µl Taq DNA polymerase (Takara Bio, Shiga, Japan), 1 µl each of forward and reverse primers, 12.5 µl of HyClone water (Saiki, 1989) and 5 µl of DNA template. Two sets of primers were chosen for this study: (F) TW81 (5′–GTTTCCGTAGGTGAACCTGC–3′), (R) AB28 (5′–ATATGCTTAAGTTCAGCGGGT–3′) amplifying the ITS1; and 5.8-ITS2 rRNA (Vrain et al., 1992), and (F) D2A (5′–ACAAGTACCGTGAGGGAAAGTTG–3′), (R) D3B (5′–TCGGAAGGAACCAGCTACTA–3′) amplifying the D2/D3 expansion fragments of 28S rRNA (Cid Del Prado Vera and Subbotin, 2012). PCR cycling conditions for amplification were 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min, and a final step at 72°C for 10 min. Gene Amp PCR System 9700 (Applied Biosystems, Grand Island, NY) was used for all PCR assays. PCR products (10 µl) were resolved by electrophoresis on 2% agarose gel at 120 V for 45 min and then stained with SYBR Green II RNA Gel Stain (Lonza, Rockland, ME) for 45 min in the dark. PCR products (15 µl) were purified using High Pure PCR Products Purification Kit (Roche Applied Science, Manheim, Germany) following the manufacturer's protocol and stored at −20°C for further use.

Cloning and sequencing: Three PCR products per each isolate were processed for cloning by following standard methods (Sambrook et al., 1989). PCR purified products (4 µl) were ligated into the plasmid pCR2.1-TOPO using the TOPO TA cloning kit and used to transform *Escherichia coli* (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's recommendations. The transformed *E. coli* colonies were distinguished from wild type colonies by their white color, and 16 colonies from each PCR product were manually selected for analysis. These chosen colonies were cultured in 10 ml Luria–Bertani medium containing 20 µl of ampicillin and incubated at 37°C for 15 hr. Plasmids were extracted using PureLinkTM Quick Plasmid Miniprep Kit (Invitrogen Corp., Carlsbad, CA) following the manufacturer's protocol. The extracted plasmids were digested with *Eco*RI (Thermo Scientific, Pittsburgh, PA) to confirm successful cloning, and the plasmid DNA of three to six clones obtained from each nematode isolate was sequenced at the Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL. The sequences from the two sting nematode isolates infecting peanut were deposited to GenBank (acessions no. KF963097, KF963098 for ITS, and KF963199, KF963100 for D2/D3).

Sequence alignment and phylogenetic analysis: DNA sequences from both isolates were edited with BioEdit (Hall, 2013) to default parameters. Sequences were

aligned with CLUSTALX in MEGA5.2 (Tamura et al., 2011) with gap opening penalty set at 15, gap extension penalty set at 6.66 and delay divergent cut off set at 30% for both pairwise and multiple alignments. The alignments were further adjusted by eye. The aligned sequences of ITS region of rRNA, and D2/D3 expansion fragments of 28S rRNA from both peanut isolates were used in BLAST searches. Closely related *B. longicaudatus* sequences, including *B. euthychilus* and *B. gracilis* and different Florida population of *B. longicaudatus* that were previously deposited in GenBank (Gozel et al., 2006; Han et al., 2006) were obtained for comparison. The best fit models of nucleotide substitution were selected using jModelTest (Posada, 2008), and the maximum likelihood (ML) tree was constructed using MEGA5.2. *Tylenchorhynchus annulatus* and *T. leviterminalis* were included as outgroup taxa for ITS and D2/D3, respectively. Bootstrap values for 1,000 replicates were obtained. The alignments were deposited to TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S17416).

Population density changes, and yield suppression: Two sting nematode infested sites were chosen, one at the 35 Farms and another at the Brown Farm. The site at 35 Farms was delimited and flagged off in 2012 based on field symptoms during that year. The grower applied 1,3-D to the remainder of field in late winter 2013. The site at Brown Farm was delimited after visual symptoms began to be expressed on young peanut seedlings in 2013. The population densities of sting nematodes were followed at both sites from June to September 2013. The Virginia type peanut cv. Bailey (Isleib et al., 2011) was seeded at 35 Farms at the end of April, and the Runner type peanut cv. Georgia-06G (Branch, 2007) was seeded at Brown Farm on 19 March 2013. Peanut at both sites were seeded in twin rows spaced 42 cm on center on a 91-cm wide bed. The field design at 35 Farms included three replicates each with six twin rows, 22-m long × 1.8-m wide, and at Brown Farm there were five replicates each with five twin rows 15-m long × 1.8-m wide. Soil samples were collected using a cone-shaped sampling tube with a 2.5 cm opening every 2 wk from June to September at 35 Farms, and June to August at Brown Farm. At harvest, 10 peanut plants were chosen arbitrarily from each replicate. These were dug to remove roots, pods, and pegs and to retain as much of soil immediately surrounding them as possible. All samples were placed individually into plastic bags. Nematodes were extracted by centrifugal flotation method (Jenkins, 1964) and their numbers per 100 cm^3 of soil was counted.

At the Brown Farm, sting nematode infested and noninfested plots were delimited for estimating yield suppression. The nonsting nematode site showed no above-ground symptoms of nematode damage and to further ensure the site was free of sting nematodes, soil samples were taken from each of five locations and the nematodes extracted from 100 cm^3 of soil (Jenkins, 1964). The two sites were designed to contain five replicates, each 6-m long × 1.8-m wide. Immediately before digging, 23 August 2013, those vines to be harvested were marked by spray painting their foliage white. After the plants were dug with a peanut digger, those vines within a plot with white paint were collected by hand, placed in 6-m × 6-m tobacco sheets and dried in a peanut drying wagon to a moisture content of 10%. Once dried, the vines were thrashed by machine to collect the pods for determining weight. The mean pod weights from both sites were compared by a student's *t* test ($P \leq 0.05$).

Soil analysis: Soil samples were taken from both peanut farms and the percentages of sand, silt, and clay, and organic matters were determined following the Bouyoucos hydrometer method (Bouyoucos, 1936).

Infection of peanut in a greenhouse: The sting nematode isolates used were collected from 35 Farms and Brown Farm, Levy, Co., FL, and reared on St. Augustinegrass as described above. The treatments included the two nematode isolates, one peanut cv. Georgia-06G, replicated three times for the 35 Farms isolate, and twice for the Brown Farm isolate. Two replicates of a non-inoculated control for each treatment were included for comparison. The soil that was used for this trial was collected from the 35 Farms, steam pasteurized, and approximately 700 cm^3 of soil was added to each 15-cm diam. clay pot. Four peanut seeds were seeded 2.5-cm deep per pot. Plants were maintained in a completely randomized design in a greenhouse. The soil temperature at a depth of 4 cm was monitored and checked weekly using a Tidbit Data Logger (Onset HOBO Data Loggers, Bourne, MA). After the seedlings reached three true leaves they were thinned to one seedling per pot. Then 100 sting nematodes (mixed life stages) in a water suspension that had been extracted by the modified Baermann method (Rodriguez-Kabana and Pope, 1981) were pipetted into four 3-cm-deep holes around the plant stem. Plants were fertilized weekly and pesticide sprayed as needed. At harvest, 90 d after inoculation, plants were removed from the soil, and nematodes extracted (Jenkins, 1964) from 100 cm^3 of soil. Harvest of one of the three replicates of the 35 Farms isolate was delayed 1 wk to provide more time for the nematode to develop and reproduce. The nematodes were collected from the sample as described above. The reproductive factor (Rf) was calculated by the formula: Rf = final nematode density (P_f)/initial inoculum density (P_i).

Results

Morphological characterization: The large body size (>1 mm long), long stylet (>60 μm), offset cephalic region, and cylindroid tail indicated that the specimens from both peanut fields were representatives of the subfamily

Belonolaiminae. The presence of a single groove-like incisure in the lateral field provided evidence that they belonged to the genus *Belonolaimus*. The following diagnostic characters of females provided further evidence of the species identification: the configuration of the lip region was constricted from the body in both isolates and ranged in height of 10–12 and 9.6–13 µm for 35 Farms and Brown Farm isolates, respectively; the stylet length ranged from 114 to 128 and 116 to 143 µm for the 35 Farms and Brown Farm isolates, respectively; and the average value of stylet/tail ratio was less than 1 for both isolates (0.92 and 0.8 from 35 Farms and Brown Farm isolates, respectively) (Table 1). Following the key prepared by Geraert (2011), both isolates were identified as representatives of the species *B. longicaudatus*. In addition, sclerotized opposed vaginal pieces were observed in both isolates. Both the peanut isolates showed a similar range in both male and female morphometric values (Tables 1,2).

Molecular characterization: The corrected sequences of ITS region of rRNA, and D2/D3 expansion fragments of 28S rRNA from both peanut isolates were used in BLAST searches. Closely-related *B. longicaudatus* sequences existing in the GenBank were obtained for comparison. The total amplified DNA of the peanut isolates, which include partial sequence of 18S rRNA, complete sequences of ITS-1, 5.8S rRNA, and partial sequence of ITS-2, was 982 bp after excluding the primer sequences. There was a three base pair (bp) insertion confirmed in the total amplified DNA of *B. longicaudatus* from the Brown Farm isolate (985 bp) compared to the isolate from 35 Farms. Both partial sequences of 18S rRNA were 40 bp. The ITS-1 was 464 bp and 5.8S sRNA gene was 167 bp for both sting nematode isolates. The total length of amplified DNA of D2/D3 was 787 bp for both isolates. A total of 1,253 and 792 aligned base pairs were obtained for ITS and D2/D3, respectively. Maximum likelihood trees were constructed for both ITS and D2/D3 (Figs. 1,2). The sequence analysis of the isolates of *Belonolaimus* from 35 Farms and Brown Farm indicated that they were evolutionarily closely related, and supported their placement in the same major clade including previously reported Florida isolates of *B. longicaudatus*.

Symptoms, estimations of population density changes, and yield suppression: The damage induced by sting nematode

TABLE 1. Morphometrics[a] of selected characters of *Belonolaimus longicaudatus* females attained from soil collected around peanut roots from 35 Farms and Brown Farm (n = 20) as compared with those reported in the original description of five species of *Belonolaimus*.

Characters[b]	35 Farms	Brown farm	*B. longicaudatus* Rau, 1958	*B. gracilis* Steiner, 1949	*B. euthychilus* Rau, 1963	*B. nortoni* Rau, 1963	*B. maluceroi* Vera and Subbotin, 2012
L	2.23 ± 0.15	2.21 ± 0.10	2.2	2.15	1.85	1.85	1.6
	(1.98–2.75)	(1.98–2.39)	(2.0–2.6)	(1.40–2.46)	(1.43–2.09)	(1.60–2.12)	(1.4–1.8)
a	53.0 ± 3.5	55 ± 2.5	65.4	49	45	58	54
	(48.8–64.8)	(51–60)	(55.7–74.9)	(39–63)	(39–62)	(51–64)	(47–59)
b	11.8 ± 0.7	11 ± 0.6	8.4	6.7	5.7	8 9	6
	(10.4–14.7)	(10–12)	(7.3–9.9)	(5.1–9.8)	(5.2–6.1)	(6.9–9.3)	(7.2–11.3)
c	16.4 ± 1.9	15 ± 1.1	16.1	23	20	17	19
	(13.3–20.6)	(13–18)	(14.5–18.0)	(16–28)	(15–27)	(15–18)	(16.3–23.4)
v%	49.5 ± 2.0	50 ± 2.1	50	53	53	50	51
	(41.9–51.6)	(46–56)	(46–54)	(50–57)	(50–57)	(49–53)	(46–53)
Lip region	11 ± 0.7	11 ± 0.9	17.8	–	–	–	–
	(10–12)	(9.6–13)	(16.8–18.8)	–	–	–	–
Stylet	122 ± 4.5	128 ± 6.6	118	–	154	90	96
	(114–128)	(116–143)	(100–133)	(130–168)	(131–168)	(78–98)	(85–103)
Stylet cone	88 ± 4.2	93 ± 5.9	93	114	114	–	70
	(80–94)	(82–108)	(84–102)	(98–133)	(95–126)	–	(63–78)
Stylet shaft	31 ± 2.8	32 ± 1.8	34	39	40	–	25
	(27–36)	(28–35)	(28–39)	(25–43)	(35–45)	–	(23–28)
Tail length	136 ± 11.2	143 ± 11.6	140	78	88	108	83
	(116–171)	(120–160)	(117–163)	(53–134)	(56–148)	(84–130)	(50–96)
Tail/body width ratio	3.3.8 ± 0.3	3.7 ± 0.3	4.4	2.6	–	4.1	2.8
	(2.75–4.45)	(3.0–4.2)	(3.5–5.0)	(1.8–3.6)	–	(3.4–4.7)	(2.3–3.4)
Tail width	34 ± 2.0	32 ± 1.4	–	–	32.6	–	24
	(30–38)	(30–35)	–	–	(35–38)	–	(22–28)
Anterior end to excretory pore	227 ± 13.6	230 ± 12.8	215	–	235	195	–
	(200–249)	(194–248)	(184–233)	–	(195–251)	(170–206)	(146–195)
Stylet/tail ratio	0.92 ± 0.07	0.8 ± 0.08	0.81	1.76	–	–	1.2
	(0.81–1.08)	(0.7–1.1)	(0.68–1.0)	(1.33–2.31)	–	–	(1.1–1.8)
Body width	40 ± 1.6	38 ± 1.8	34	41	–	–	29
	(36–42)	(34–41)	(30–40)	–	–	–	(27–31)

[a] Measurements in µm except L in mm.
[b] L = total body length, a = total body length per body width, b = total body length per length of esophagus, c = total body length per tail length, V% = distance from anterior end to vulva of female as a percentage of total body length.

TABLE 2. Morphometrics[a] of selected characters of *Belonolaimus longicaudatus* males isolated from soil collected from peanut roots from 35 Farms and Brown Farm ($n = 20$) as compared with those reported in the original description of five species of *Belonolaimus*.

Characters[b]	35 Farms	Brown farm	*B. longicaudatus* Rau, 1958	*B. gracilis* Steiner, 1949	*B. euthychilus* Rau, 1963	*B. nortoni* Rau, 1963	*B. maluceroi* Vera and Subbotin, 2012
L	1.79 ± 0.12	1.70 ± 0.10	1.8	1.9	1.5	1.6	1.3
	(1.48–2.02)	(1.54–1.86)	(1.6–2.1)	(1.4–2.5)	(1.0–1.7)	(1.5–1.7)	(1.2–1.5)
a	43.1 ± 4.1	55 ± 5.2	64	52	50	59	54
	(54.6–6.01)	(48–66)	(55–74)	(44–61)	(39–59)	(54–62)	(50–58)
b	8.6 ± 0.7	9.8 ± 0.8	7.5	6.3	–	7.2	8.3
	(10.1–11.4)	(8.4–11)	(7.0–8.1)	(5.1–7.2)	–	(6.6–8.2)	(7.8–9.4)
c	13.6 ± 1.1	12 ± 0.7	15	17	18	16	17
	(11.8–17.4)	(10–14)	(13–17)	(13–29)	(14–25)	(15–17)	(14–22)
Lip region	10 ± 0.7	10 ± 0.9	–	–	–	–	–
	(9.6–12)	(8.8–12)	–	–	–	–	–
Stylet	113 ± 7.7	115 ± 5.2	120	137	–	87	90
	(96–126)	(105–123)	(111–132)	(99–154)	–	(84–95)	(83–98)
Stylet cone	83 ± 5.5	84 ± 5.0	–	–	–	–	–
	(72–94)	(76–92)	–	–	–	–	(59–72)
Stylet shaft	28 ± 2.9	28 ± 2.0	–	–	–	–	23
	(19–33)	(24–33)	–	–	–	–	(22–24)
Tail length	132 ± 12.0	135 ± 9.3	141	99	–	–	80
	(104–145)	(120–151)	(127–157)	(60.2–140)	–	–	(60–98)
Tail/body width ratio	3.97 ± 0.4	4.4 ± 0.6	–	–	–	–	1.2
	(3.02–4.45)	(3.8–5.9)	–	–	–	–	(0.9–1.5)
Tail width	23 ± 0.8	21 ± 1.8	–	–	–	–	18
	(20–24)	(19–24)	–	–	–	–	(17–20)
Anterior end to excretory pore	210 ± 14.1	204 ± 9.8	–	–	–	–	162
	(184–234)	(184–220)	–	–	–	–	(145–172)
Stylet/tail ratio	0.8 ± 0.08	0.8 ± 0.05	0.85	1.37	–	–	1.2
	(0.6–1.1)	(0.7–0.9)	(0.76–0.97)	(1.07–1.96)	–	–	(0.9–1.5)
Body width	33.5 ± 1.5	30 ± 2.2	–	–	–	–	24
	(30.4–37.6)	(24–35)	–	–	–	–	(23–26)
Spicules	47 ± 2.6	46 ± 2.7	43 45	–	39	30	
	(44–52)	(41–51)	(38–49)	(35–50)	(28–40)	(36–41)	(25–34)
Gubernaculum	16.3 ± 1.7	15 ± 1.3	17	16	–	16	13
	(13.6–20)	(13–18)	(15–18)	(14–18)	–	(14–17)	(11–15)

[a] Measurements in μm except L in mm.
[b] L = total body length, a = total body length per body width, b = total body length per length of esophagus, c = total body length per tail length.

on peanut was seen as large irregular patches at both farms (Fig. 3). Above-ground symptoms included severe stunting and chlorotic foliage, which indicated nutrient deficiency, whereas below-ground symptoms included abbreviated roots (Fig. 4A,B), and pods and pegs contained numerous small, round punctate-like necrotic lesions (Fig. 5A,B). These symptoms were observed at both peanut farms. Though peanut plants were severely stunted during the first 6 wk of growth, the symptoms on pods and pegs were most apparent in mid-July, ca. 110 d and 80 d after planting at 35 Farms and Brown Farm, respectively. The initial numbers of sting nematodes extracted from soil collected at 35 Farms averaged 44/100 cm^3 of soil on peanut cv. Tifguard in 2012, 39/100 cm^3 of soil on peanut cv. Bailey in 2013; and 28/100 cm^3 of soil on peanut cv. Georgia-06G at the Brown Farm in 2013. At 35 Farms, the number of sting nematode increased from mid-June until early August and decreased after their numbers peaked at 86 specimens/100 cm^3 of soil during the first week of August (Fig. 6). At Brown Farm, sting nematode averaged 30 specimens/100 cm^3 of soil from late June through late July, and increased to over 50 specimens/100 cm^3 in late August (Fig. 6). Other plant-parasitic nematodes found at both farms were root-knot, lesion and ring nematodes. The population densities of these nematodes in the sting nematode infested sites remained low throughout this study, except at Brown Farm where root-knot nematode second-stage juveniles increased to over 425 specimens/100 cm^3 of soil at the last sampling date, 23 August.

The average dried weight of peanut kernels harvested in plots from the nonsting nematode infested sites were increased 2.74-fold over those harvested in sting nematode-infested plots, resulting in an estimated yield suppression of 64%. No sting nematode was detected from any of the nonsting infested plots, but other plant-parasitic nematodes found were: 7 lesion, 42 root-knot, and 22 ring nematodes/100 cm^3 of soil.

Soil type and texture: The soil type was identified as Candler series (consists of very deep, excessively drained, rapidly permeable soil on uplands of Florida flat woods). The soil from 35 Farms contained 97.3% of sand, 1% of silt, 1.7% of clay, and 2.2% of organic matter, whereas the Brown Farm contained 94.7%

FIG. 1. Maximum likelihood tree based on internal transcribed spacer sequence of *Belonolaimus longicaudatus*. The sequences other than the two peanut isolates were downloaded from GenBank.

of sand, 2% of silt, 3.3% of clay, and 2.8% of organic matter.

Infection of peanut in greenhouse: Punctate-like, isolated lesions were observed on pods and pegs of peanut plants inoculated with either peanut sting nematode isolates, whereas symptoms on root systems, such as root abbreviation and stunted plant growth, was much less severe as compared with that observed in the field. Only one plant inoculated with the Brown Farm isolate showed a severely abbreviated root system. The final population density was 95.2 sting nematodes/pot (13.6 sting nematodes/100 cm^3 of soil) with an Rf value of 0.95, and 15.4 sting nematodes/pot (2.2 sting nematodes/100 cm^3 of soil) and an Rf value of 0.15 from 35 Farms and Brown Farm isolates, respectively.

DISCUSSION

The peanut isolates were similar in their morphometrics and morphology to each other. All the range values of the morphometric characters of both isolates, except b ratio and height of lip region, were in agreement with those reported in the original description of *B. longicaudatus* (Rau, 1958). The ranges of the b ratio and height of lip region of both peanut isolates were greater and smaller, respectively than those reported in the original description (Rau, 1958). Similar variations in these characters have been reported previously (Abu-Gharbieh and Perry, 1970; Robbins and Hirschmann, 1974). Females from the peanut isolates shared the presence of opposed vaginal sclerotized pieces with

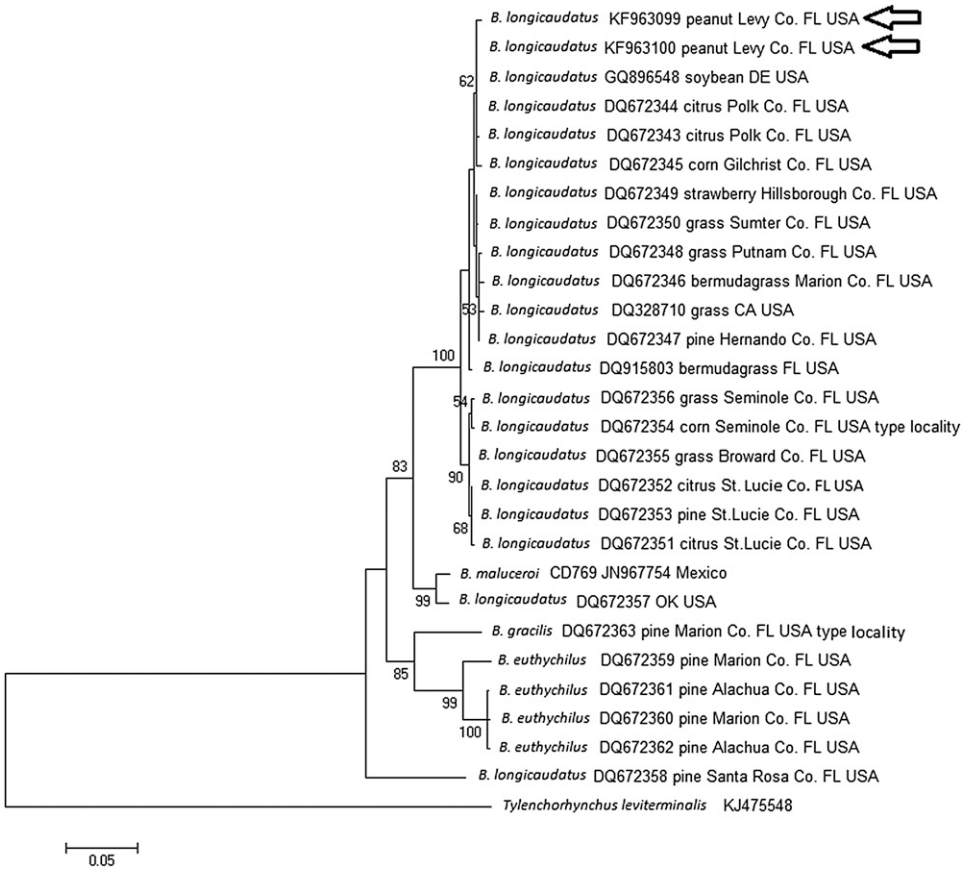

Fig. 2. Maximum likelihood tree based on D2/D3 expansion fragments of 28S rRNA from *Belonolaimus longicaudatus*. The sequences other than the two peanut isolates were downloaded from GenBank.

other Florida isolates (Lake Alfred and Gainesville) obtained from citrus and corn (Han et al., 2006), a North Carolina (Scotland Co.) isolate, and a greenhouse isolate from Tifton, Georgia (Robbins and Hirschmann, 1974), whereas *B. longicaudatus* collected from a peanut field in Severn, North Carolina lacked opposed sclerotized vaginal pieces (Robbins and Hirschmann, 1974). Although, the range of the stylet length of 35 Farms isolate was shorter than that of an isolate collected from Lake Alfred citrus (central Florida) (Duncan et al., 1996), the ranges of the stylet length of both peanut isolates were in agreement with those reported for other Florida populations. More accentuated differences were observed in total body length, tail width, anterior end to excretory pore distance and body width between the peanut isolates and central Florida isolates as compared with northern Florida isolates. The similarity in the morphological values between the peanut isolates and northern Florida isolates may indicate they originated from northern Florida rather than central Florida. The factors that cause these variations in morphological characteristics of *B. longicaudatus* are unknown.

The results obtained from the molecular analyses also corroborated both sting nematode isolates infecting peanut as *B. longicaudatus*. Han et al. (2006) reported size variations within the ITS-1 of *B. longicaudatus* obtained from different host and geographical locations that ranged from 428 bp to 468 bp. The peanut isolates, which had 464 bp, fit within this range. In addition, Gozel et al. (2006) reported the ITS-1 of *B. longicaudatus* isolated from pine with 464 bp, which

Fig. 3. Severely damaged peanut cv. Georgia-06G growing in field infested with the sting nematode at Brown Farm, Levy Co., FL, summer 2013.

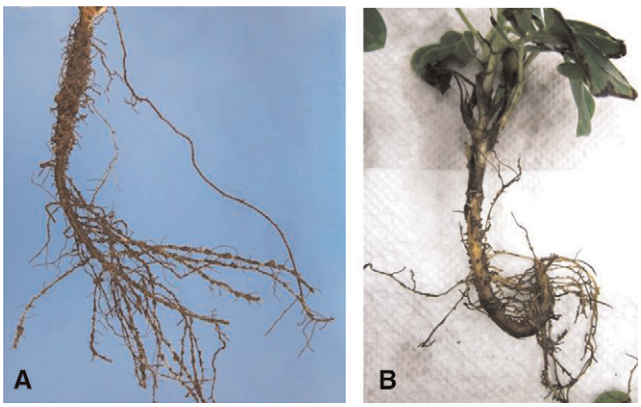

FIG. 4. Below-ground symptoms induced by the sting nematode on peanut. A) A close up of an abbreviated root system of peanut cv. Tifguard found at 35 Farms in summer, 2012. B) Abbreviated root system of an infected peanut cv. Georgia-6G found at the Brown Farm in summer, 2013.

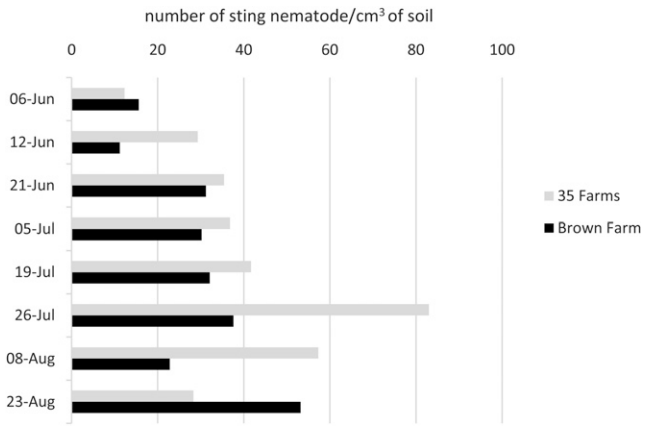

FIG. 6. *Belonolaimus longicaudatus* population density changes on peanut cv. Bailey at 35 Farms, and peanut cv. Georgia-06G at Brown Farm from June to August 2013.

was the same as that for the peanut isolates. Two of three pine isolates, however, were in more distantly related clades from the peanut isolates. Although the bootstrap values on the main branches for both ITS and D2/D3 were high, their values on the branch separating each Florida isolates were too low to support the inferred evolutionary relationships among them. Since *B. longicaudatus* is reported as a species complex (Gozel et al., 2006; Han et al., 2006), polymorphism may exist within the ITS region and D2/D3 expansion fragments. The placement of *B. longicaudatus* and *B. gracilis* within the clade that includes *B. euthychilus* in Figs. 1 and 2 also supports the existence of polymorphism in the genus *Belonolaimus*.

The population densities of both Florida peanut isolated reached their peaks during August when the ambient air temperature in Levy Co. averaged 28°C. This temperature is consistent with that reported for sting nematode reproduction (Robbins and Barker, 1974; Han et al., 2006). The numerous single punctate-like lesions on peanut pegs and pods appeared to be caused by the nematode feeding deep within the tissue with its long stylet. The small size of these lesions appeared unique. They are easily distinguished from the much larger lesions caused by the endoparasitic nematode, *Pratylenchus brachyurus*, a well-known peanut pathogen (Dickson, 1998; Dickson and De Waele, 2005). Although some ring, lesion, and root-knot nematodes were extracted from the sting nematode infested sites, they were present in relatively low numbers. It seemed that sting nematode infection during early stages of peanut development allowed it to establish its niche before other plant-pathogenic nematodes began to increase. Yield of pods was reduced by sting nematode by an estimated 64% in this study. Others have also reported significant peanut yield reductions induced by sting nematode (Cooper et al., 1959; Sasser et al., 1960). They reported yield increases following soil fumigation ranging from 109% to 400%. However, because sting nematode damage was often distributed in widely scattered patches, these scientists suggested that overall suppression of peanut yields in large fields would be relatively low. Although sting nematode damage at both 35 Farms and Brown Farm occurred in scattered patches, their extensive size and the severity of damage recognized in these patches would suggest a moderate to high level of yield suppression. It should be pointed out that the grower at 35 Farms seeing the amount of sting nematode damage that occurred during the 2012 season chose to apply 1,3-D soil fumigant over the entire 200-ha field in 2013, whereas the grower at Brown Farm seeded bahiagrass across the entire sting nematode infested block following peanut harvest in 2013.

FIG. 5. Symptoms induced by the sting nematode on peanut pod and peg of peanut cv. Tifguard found at 35 Farms in summer, 2012. A) Punctate-like isolated lesions on affected pods. B) A close up view of an infected peg.

The economic damage threshold reported for most sting nematode on most crops is at or near the detection level (Crow and Han, 2005). For instance, Crow et al. (2000) reported that the economic threshold for sting nematode management on potato was 2 to 3 *B. longicaudatus*/130 cm^3 of soil. Dickson and De Waele (2005) reported the economic threshold level on peanut varied from 2 to 5 *B. longicaudatus*/130 cm^3 of soil.

Therefore, it seems that only a small number of sting nematodes are needed to induce damage on peanut.

The percentages of sand, silt, and clay from both peanut farms were consistent with those previously reported for sting nematode development (Brodie and Quattlebaum, 1970; Robbins and Barker, 1974). Although the initial population densities of sting nematode at 35 Farms and Brown Farm were similar, higher numbers were found later in the season at 35 Farms, where the Virginia type peanut cv. Bailey was grown. Virginia type peanut was reported to be more susceptible to *B. longicaudatus* than Runner, Bunch, and Spanish types (Miller, 1952; Holdeman and Graham, 1953). More investigations are needed to determine the host suitability of different types of peanut to sting nematode in Florida, especially with the numerous new peanut cultivars that are now available.

In a greenhouse study, Abu-Gharbieh and Perry (1970) reported that although peanut was a host for Gainesville and Sanford, FL isolates, neither supported large numbers of sting nematode 4 mon after inoculation. In their study, they used the peanut cv. Early Runner. In our study we also found that Georgia-06G, a runner type, supported low numbers of sting nematodes. Timper and Hanna (2005) compared reproduction of *B. longicaudatus* isolated from Tift Co., GA on different hosts. They reported peanut as a poor host of the Georgia isolate, which had a low Rf value of 0.3. This low value of Rf is in agreement with that of the Brown Farm isolate on peanut Georgia-06G. In general, reproduction of sting nematode isolates on peanut grown under greenhouse conditions has been low. Thinking that soil type was a factor, we used only soil collected from 35 Farms. In our study, however, most developing pods and pegs of peanut inoculated with both isolates showed sting nematode symptoms, and some root systems of peanut inoculated with the Brown Farm isolate were clearly stunted. Although it was evident that the nematodes were feeding, there was little nematode reproduction occurring. It is assumed that the greenhouse conditions were unsuitable for reproduction, the runner type peanut is a poor host, or there were other unknown conditions affecting the nematode's ability to reproduce. Because of the difficulty of collecting sufficient numbers of both peanut sting nematode isolates, only a few replicates were possible for evaluating the pathogenicity under greenhouse conditions. However, the symptoms induced by low densities of *B. longicaudatus* on peanut confirmed it as a pathogen.

Obviously, an important question is why these sting nematode populations increased on peanut to such a degree that they caused extensive field damage. Both farms have a history of long-term monoculture of peanut. This fact, plus the soil type being ideal for sting nematode development, may have provided an opportunity for sting nematodes to develop on peanut.

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