RESEARCH NOTE/NOTA INVESTIGATIVA

SUSCEPTIBILITY OF DIFFERENT ACCESSIONS OF *CROTALARIA JUNCEA* TO *BELONOLAIMUS LONGICAUDATUS*

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ABSTRACT

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Sunn hemp (*Crotalaria juncea*) has been used as a cover crop in rotation with various vegetable crops, providing benefits such as nitrogen fixation, weed control, and nematode suppression. The sting nematode (*Belonolaimus longicaudatus*) is one of the most damaging plant-parasitic nematodes in Florida. The host status of different accessions of sunn hemp to the sting nematode was evaluated in the greenhouse. The experiment was arranged in a randomized complete block design with a factorial arrangement of treatments. Factors were accessions of *C. juncea* plus corn (*Zea mays*) as a sting nematode-susceptible control with or without the sting nematode. Plant height, growth stage (number of leaves), fresh biomass of shoots, dry mass of roots, leaf area, and the reproduction of the sting nematode were recorded. Of 11 accessions tested, PI 322377 was the only sunn hemp accession that supported a greater population of sting nematodes than the best treatments (accessions without nematode reproduction). All of the other sunn hemp accessions suppressed sting nematode populations in comparison with corn.

Key words: Belonolaimus longicaudatus, cover crop, *Crotalaria juncea*, cultural control, organic farming.

RESUMO

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A crotalária (*Crotalaria juncea*) tem sido utilizada como cultura de cobertura em rotação com várias espécies vegetais, provendo benefícios como a fixação de nitrogênio, controle de plantas daninhas e supressão de nematoides. O 'sting nematode' (*Belonolaimus longicaudatus*) é um dos fitonematoides que causa maiores prejuízos no Estado da Flórida. A susceptibilidade de diferentes acessos de crotalária ao esse nematoide foi avaliada em casa-de-vegetação. O experimento foi conduzido em delineamento de blocos casualizados em arranjo fatorial. Os tratamentos foram diferentes acessos de *C. juncea* mais o milho (*Zea mays*) como testemunha suscetível, sendo essa planta submetida ou não à inoculação com *B. longicaudatus*. Foram avaliadas as seguintes variáveis: altura de plantas, estádio de desenvolvimento (número de folhas), massa fresca de parte aérea, massa seca de raiz, área foliar e a reprodução donematóide. Dos 11 acessos avaliados, PI 322377 foi o único acesso de crotalária a apresentar maior população do nematoide quando comparado aos melhores tratamentos (acessos sem reprodução do nematoide). Todos os outros acessos suprimiram as populações de *B. longicaudatus* em comparação com o milho.

Palabras-clave: Belonolaimus longicaudatus, cultura de cobertura, *Crotalaria juncea*, controle cultural, agricultura orgânica.

Belonolaimus longicaudatus (sting nematode) is one of the most damaging plant-parasitic nematodes in Florida, infesting fields that are used to grow a wide range of crops (Crow and Han, 2005). Initially, affected fields show localized growth inhibition with plants exhibiting foliar chlorosis, and eventually, the damage can extend across the whole area. The sting nematode injures root tips, decreasing the absorption of water and nutrients by the plants (Noling, 2012).

While the use of nematicides to control the sting nematode is the primary control method used by growers, dependence on a single method can result in reduced nematicide efficacy due to microbial degradation of the active ingredient or environmental contamination (Rousidou *et al*., 2013). To avoid such adverse effects, alternative approaches for the management of plant-parasitic nematodes can be used, and the integrated use of multiple methods can improve the control of these pests.

Cultural control is perhaps the most sustainable method for the management of plant-parasitic nematodes, but its adoption requires careful planning. The aims of cultural control of nematodes are to limit proliferation and dissemination and to give the crop better conditions for development. In this context, the most important tool is crop rotation with poor hosts or non-hosts in areas in which plant-parasitic nematodes are already present (Crow *et al*., 2001). An example of a plant that can be included in a rotation to suppress plant-parasitic nematodes is the pearl millet (*Pennisetum glaucum*) hybrid 'TifGrain 102'. 'Tifgrain 102' has been demonstrated to reduce the population of sting nematodes as well as lesion nematodes (*Pratylenchus brachyurus*) and root-knot nematodes (*Meloidogyne javanica*) (Timper and Hanna, 2005).

A plant species that can be used in crop rotations with the aim of reducing the population density of certain nematodes is sunn hemp (*Crotalaria juncea*). This is a tropical leguminous cover crop native to India with rapid biomass production and the ability to fix nitrogen. Sunn hemp is also a non-host or poor host for many plant-parasitic nematodes including root-knot and reniform nematodes (Wang *et al*., 2001, 2002). When incorporated into soil, sunn hemp residues release compounds that are toxic to many plant-parasitic nematodes (Wang *et al*., 2004; Hinds *et al*., 2013).

The differential susceptibility to sting nematode of varieties or genotypes of a particular crop has been reported previously for bermudagrass (*Cynodon* sp.) (Pang *et al*., 2011a, 2011b). Although the characteristics that confer tolerance to plant-parasitic nematodes are genetically determined, the degree of tolerance can vary and can be influenced by environmental conditions. Sunn hemp is considered to be a good off-season cover crop for annual strawberry production due to its reported resistance to the sting nematode. However, it likely that sunn hemp genotypes may differ in their performance in different regions, and may differ in their ability to suppress the sting nematode. To test this hypothesis, we evaluated the susceptibility of 11 sunn hemp accessions from the germplasm repository of the US Department of Agriculture for growth and biomass production in the greenhouse and for their suitability as hosts for *B. longicaudatus*.

The experiment was conducted from April 1 to July 5, 2014, in a greenhouse located at the University of Florida in Gainesville (29°38'12.06"N and 82°21'42.93"W, and 28 m altitude). Soil, collected from a certified organic field at the Plant Science Research and Education Unit (PSREU) located near Citra, FL, was used to fill black 11.4-L pots. The soil was a Candler sand series (USDA, 1979) with more than 97% sand, a pH of 7.4, and 0.72% organic matter. Before the start of the experiment, the soil was pasteurized by heating for 1 wk in a drying oven at 61°C to kill any nematodes present.

The experimental design was a randomized complete block with a factorial arrangement of treatments (12 × 2) and five replications. The first factor consisted of 11 accessions of sunn hemp (Table 1; USDA-ARS Plant Genetic Resources Conservation Unit, Griffin, GA) and one corn cultivar (Nothstine Dent, organic; Johnny's Selected Seeds, Winslow, ME). The corn was used as a susceptible control to verify the effectiveness

Table 1. Accessions of sunn hemp (*Crotalaria juncea*) evaluated for susceptibility to the sting nematode (*Belonolaimus longicaudatus*).

Accession[z]	Country of origin	Cultivar or other designation
PI 207657	Sri Lanka	-
PI 219717	Myanmar	-
PI 250485	India	K679
PI 250486	India	K680
PI 250487	India	K681
PI 314239	Former Soviet Union	COL NO 524
PI 322377	Brazil	IRI 2473
PI 337080	Brazil	H&L 0468
PI 391567	South Africa	T'ai-yang-ma
PI 426626	Pakistan	Sanni
PI 468956	United States	'Tropic Sun'

[z]USDA ARS – Plant Genetic Resources Conservation Unit, Griffin (GA).

of the sting nematode inoculation. The second factor was inoculation with sting nematodes or no inoculation. Because only a limited number of seeds of the sunn hemp accessions were obtained, initially the seeds were planted in trays on April 8, 2014. Fifteen days after sowing the seeds, a single seedling was transplanted into each pot. All weeds that emerged in the pots were removed to ensure that any increases in the sting nematode population were due only to the plant accession growing in the pot. The pots were irrigated daily and an organic fertilizer was applied weekly (Neptune's Harvest Fish & Seaweed Fertilizer - Nitrogen 1.70%; Phosphorus P_2O_5 3.4%; Potassium K_2O 0.68%; Gloucester, MA).

Sting nematode inoculum was collected from strawberry plants growing in Dover, FL (28° 0'56.38"N, 82°14'0.26"W, altitude 24 m). The strawberry plants were in the reproductive stage and were collected with the surrounding soil, transplanted into 3.8-L pots, and maintained in a greenhouse for later nematode extraction and inoculation. The sting nematode inoculum was extracted from the soil with the strawberry plants using the decanting and sieving method (Flegg, 1967), and quantified by counting the nematodes in five, 10-mL aliquots on a counting slide (Hawksley and Sons Limited, Lancing, Sussex, UK). Inoculum contained females, males, and juveniles.

Twenty-five days after sowing, the plants were inoculated. Four holes (1-cm-diam. × 2.5-cm-deep) were made by pushing a 0.5-cm-diam. dowel into the soil beside the plant in each pot to be inoculated. Ten milliliters of a suspension containing 200 sting nematodes was evenly distributed among the four holes, which were then sealed.

Evaluations of plant height, plant growth stage, leaf area, fresh mass of shoots, and dry mass of roots were performed. Plant height was measured from the surface of the soil to the insertion of the last completely expanded leaf at 0, 15, 30, and 60 d after the inoculation (DAI). Additionally, the growth stage of the plants was evaluated by counting the number of leaves completely expanded at 15 and 60 DAI.

The shoots were carefully removed from the pots and weighed to obtain the fresh mass at 60 DAI. The entire leaf area of each sunn hemp plant was then measured using an area meter model LI-3100 (LICOR, Lincoln, NE). To obtain the dry mass of roots, the rinsed roots were placed in a drying oven with forced air circulation for 72 hr with a mean temperature of 65°C.

At 60 DAI, sting nematodes were extracted from a 100 cm^3 subsample of soil from each pot using the centrifugal flotation technique (Jenkins, 1964) and the population density was quantified by counting the number of specimens in each

Table 2. Height of sunn hemp (*Crotalaria juncea*) accessions with and without sting nematode (*Belonolaimus longicaudatus*) at 0-60 d after inoculation (DAI).

	Height (cm)			
Accession	0 DAI	15 DAI	30 DAI	60 DAI
PI 207657	8.51 ± 1.37	25.11 ± 3.12	71.13 ± 6.78	110.74 ± 11.41
PI 219717	9.93 ± 1.20	25.29 ± 3.23	59.14 ± 7.99	102.36 ± 7.04
PI 250485	11.23 ± 1.50	31.14 ± 3.95	75.50 ± 8.66	107.44 ± 10.39
PI 250486	10.98 ± 1.17	23.96 ± 2.62	75.27 ± 7.07	123.25 ± 11.67
PI 250487	11.57 ± 1.82	25.05 ± 3.69	72.24 ± 8.44	112.01 ± 7.87
PI 314239	9.30 ± 0.66	24.00 ± 2.49	58.43 ± 9.12	93.21 ± 7.96
PI 322377	11.84 ± 1.47	24.19 ± 2.83	70.03 ± 7.74	127.76 ± 12.90
PI 337080	9.43 ± 1.63	21.46 ± 3.53	63.49 ± 7.53	118.77 ± 13.43
PI 391567	12.41 ± 1.29	28.41 ± 2.74	71.18 ± 6.68	151.51 ± 12.85
PI 426626	10.19 ± 1.03	20.28 ± 2.70	50.61 ± 7.59	113.03 ± 11.86
PI 468956	8.49 ± 0.62	15.69 ± 0.75	39.73 ± 2.47	115.88 ± 8.99
	Height (cm)			
	0 DAI	15 DAI	30 DAI	60 DAI
With Sting Nematode	10.13 ± 0.55	23.79 ± 1.63	62.44 ± 4.18	115.86 ± 4.89
Without Sting Nematode	9.55 ± 0.69	22.66 ± 1.53	59.57 ± 4.15	114.73 ± 6.66

Confidence interval ($P \leq 0.05$).

sample with a microscope.

Plant height means are shown with confidence intervals ($P \leq 0.05$). For the other variables, the data were subjected to analysis of variance by F test using the GLM procedure of SAS version 9.0 (SAS Institute Inc., Cary, NC) (SAS, 2002). When a significant treatment effect was obtained, the means were compared using the Fisher's Least Significance Difference (LSD) test ($P \leq 0.05$).

No differences in height of sunn hemp plants with or without sting nematodes were found ($P > 0.05$). Therefore, the plant height data was combined across inoculation treatments for analysis (Table 2). Plant height at the time of inoculation was greatest for accession PI 391567 (South Africa) although the height did not differ ($P \leq 0.05$) from PI 322377 (Brazil), PI 250485 (India), and PI 250487 (India). By 15 DAI, the mean height of the all sunn hemp accessions had increased by almost 132%. This was particularly apparent in the accessions PI 391567 (South Africa) and PI 250485 (India). At 30 DAI, PI 207657, PI 391567, PI 322377, PI 250485, PI 250486, and PI 250487 had the tallest plants and were taller by a factor of 2.85 since the previous evaluation at 15 DAI, while the shortest accessions had increased by only a factor of 2.49. At 60 DAI, PI 391567, a South Africa accession, had the tallest plants (Table 2). In addition to this accession, the accessions PI 250486 from India and PI 322377 from Brazil also had very tall plants; whereas accession PI 314239 from the former Soviet Union had the shortest plants. Interestingly, inoculation with sting nematodes had no effect on plant height when averaged over accessions.

At 15 and 60 DAI, no differences between the plants that were inoculated with the sting nematode and those that were not were observed (Table 3). Among the different accessions of sunn hemp, the accessions from Sri Lanka and the US (PI 207657 and PI 468956, respectively) were slow to develop, possessing fewer expanded leaves. At 60 DAI, plants of the accessions PI 250487, PI 391567, and PI 468956 were more developed than the other sunn hemp accessions (Table 3).

No differences were observed in the accumulation of fresh shoot mass or leaf area among the inoculated sunn hemp accessions (Table 4). The US accession PI 468956 produced the largest amount of fresh biomass and greatest leaf area of all accessions. Although they produced lower biomass and leaf area than PI 468956, PI 426626 (Pakistan), PI 391567 (South Africa), PI 322377 (Brazil), and PI 337080 (Brazil) were higher in biomass and leaf area than the other accessions.

Although all the accessions in the uninoculated control treatment numerically had a larger mass of roots at 60 DAI than inoculated accessions, only in three accessions of sunn hemp was this difference significant: PI 219717 (Myanmar), PI 426626 (Pakistan), and PI 468956 (US) (Table 5). The highest amount of root biomass was produced by PI 468956 (US), with intermediate amounts found in the accessions PI 219717 (Myanmar), PI 426626 (Pakistan), PI 322377 (Brazil), and PI 337080 (Brazil).

All of the sunn hemp accessions suppressed the sting nematode compared to corn (the susceptible control) (Table 5). The lowest sting nematode populations occurred with accessions PI 219717 (Myanmar), PI 250487 (India), PI 314239 (Former Soviet Union), PI 391567 (South Africa), PI 426626 (Pakistan), and PI 468956 (US), in which the number of sting nematodes recovered was zero. Sting nematode suppression by PI 322377 (Brazil) was not as great as with the other accessions, and the second Brazilian accession also supported low population densities of sting nematodes, comparable with those that supported no sting nematode population.

The evaluation of plant height and fresh mass of the sunn hemp accessions is important for understanding their potential for use as cover crops and as green manure. Greater biomass corresponds to better cover of the soil and possibly increased nematode suppression, because after termination of a sunn hemp cover crop, the nematicidal effect can continue due to the release of allelochemicals from the plant residues undergoing decomposition (Wang *et al.*, 2002). Differences in plant height may offer growers the possibility of choosing an accession with their preferred characteristics. For example, some farmers might prefer shorter cultivars that are easier to incorporate than tall cultivars.

The variation in height among accessions is consistent with earlier findings that the shorter accessions tended to be day neutral and the taller plants tended to be short-day (Cho *et al*., 2015). The greatest shoot and root biomass was found in accession PI 468956, developed by the US Department of Agriculture Natural Resources Conservation Service and the University of Hawaii Institute of Tropical Agriculture called 'Tropic Sun' (Rotar and Joy, 1983). This increased biomass is likely related to a longer vegetative stage (Table 3) since the day length in Gainesville at the time of the experiment was longer than that needed to stimulate the flowering of Tropic Sun, as this variety is a short-day plant (Abdul-Baki *et al.*, 2001; Cho *et al*., 2015).

In relation to root mass, the accessions with larger root systems may benefit the management of nematodes by accessing greater soil volumes and thus increasing interaction with plant-parasitic nematodes. The reason why the root mass of inoculated plants was less than for non-inoculated plants for several accessions despite having apparent immunity to the sting nematode is unknown. Since the inoculum source for the sting nematode was not

Table 3. Growth stage of different accessions of sunn hemp (*Crotalaria juncea*) subjected to inoculation with sting nematodes (*Belonolaimus longicaudatus*) or not inoculated.

	Growth stage of plants (numbers of leaves)			
	15 DAI		60 DAI	
Accession	With sting nematode	Without sting nematode	With sting nematode	Without sting nematode
PI 207657	3.0 b[z]	3.4 bc	30.0* b	30.8* b
PI 219717	5.2 a	5.4 a	26.0* c	27.2* d
PI 250485	4.6 a	5.0 a	21.6* d	23.2* e
PI 250486	5.4 a	5.2 a	26.4* c	27.6* cd
PI 250487	4.8 a	4.4 ab	34.4* a	35.6* a
PI 314239	5.4 a	4.6 a	24.4* cd	27.0* d
PI 322377	5.0 a	5.4 a	29.6* b	30.4* bc
PI 337080	5.2 a	4.8 a	26.2* c	28.0* bcd
PI 391567	4.4 a	5.4 a	37.2* a	38.0* a
PI 426626	4.6 a	5.0 a	30.2* b	30.8* b
PI 468956	3.2 b	3.0 c	36.0* a	36.2 a
Fcalc.	0.84		0.19	
CV (%)	19.04		7.81	

[z]Means followed by different lowercase letters in columns differ significantly by Fisher's LSD test ($P \leq 0.05$). No differences were observed for these variables between plants inoculated and non-inoculated with sting nematode.
*Plants in reproductive stage.

Table 4. Fresh mass of shoot and leaf area of sunn hemp (*Crotalaria juncea*) accessions subjected to inoculation of sting nematode (*Belonolaimus longicaudatus*).

	Fresh mass of shoot (g)		Leaf area (cm^2)	
Accession	With sting nematode	Without sting nematode	With sting nematode	Without sting nematode
PI 207657	43.4 de[z]	51.8 de	498 de	575 cd
PI 219717	43.4 de	42.8 def	556 def	624 cd
PI 250485	31.2 e	37.6 ef	347 e	458 d
PI 250486	49.0 de	47.6 def	605 de	550 cd
PI 250487	56.4 cd	49.6 def	630 cde	551 cd
PI 314239	32.0 e	30.2 f	389 e	318 d
PI 322377	75.4 bc	56.4 cde	915 bcd	637 cd
PI 337080	80.4 b	62.6 bcd	1071 bc	965 c
PI 391567	86.2 b	74.8 bc	932 bcd	710 cd
PI 426626	80.0 b	81.6 b	1297 b	1515 b
PI 468956	126.0 a	119.2 a	2570 a	2716 a
Fcalc.	0.69		0.48	
CV (%)	27.47		39.83	

[z]Means followed by different lowercase letters in columns differ significantly by Fisher's LSD test ($P \leq 0.05$). No differences were observed for these variables between plants inoculated and non-inoculated with sting nematode.

Table 5. Dry mass of root and number of sting nematodes in 100 cm^3 of soil of different accessions of sunn hemp (*Crotalaria juncea*) 60 d after inoculation with 200 *Belonolaimus longicaudatus* per pot.

	Dry mass of root (g)		
Accession	With sting nematode	Without sting nematode	Number of nematodes (100 cm^3 of soil)
PI 207657	0.65 Ab[z]	2.17 Ad	4.0 bc
PI 219717	1.48 Bb	4.67 Ab	0.0 c
PI 250485	0.49 Ab	1.52 Ad	3.4 bc
PI 250486	0.72 Ab	2.30 Acd	3.8 bc
PI 250487	0.95 Ab	1.58 Ad	0.0 c
PI 314239	0.42 Ab	1.68 Ad	0.0 c
PI 322377	1.90 Ab	3.50 Abcd	12.8 b
PI 337080	0.95 Ab	2.97 Abcd	7.8 bc
PI 391567	1.12 Ab	2.43 Abcd	0.0 c
PI 426626	1.19 Bb	4.46 Abc	0.0 c
PI 468956	5.11 Ba	8.52 Aa	0.0 c
Corn	-	-	60.0 a
Fcalc.	0.73		5.81
CV (%)	76.96		151.96

[z]Means followed by different lowercase letters in columns and with different uppercase letters in rows differ significantly by Fisher's LSD test ($P \leq 0.05$).

pure cultures, it is possible that a non-quantified soilborne pest or an unidentified pathogen was introduced during the inoculation process.

Higher numbers of sting nematodes were found in the accessions from Brazil. Reports from this country indicated that sunn hemp in Brazil does not effectively suppress some ectoparasitic nematodes (*Helicotylenchus dihystera* and *Mesocriconema ornatum*). As a result, some Brazilian farmers prefer to use other cover crops from this genus such as *Crotalaria spectabilis*, *Crotalaria breviflora*, and *Crotalaria ochroleuca* (Rosa *et al*., 2004; Lima *et al*., 2009).

The suppressive effect of sunn hemp on the sting nematode may be related to the presence of the pyrrolizidine alkaloids (PA) in the tissue of these plants (Thoden *et al*., 2009). While PA levels were generally low in the seeds of sunn hemp accessions compared with *C. spectabilis*, accessions ranged in their PA content from a low of 0.8 μmol/g with PI 391567 (South Africa) to a high of 3.8 μmol/g in PI 426626 (Pakistan) (Ji *et al*., 2005). Although these analyses were done on seed extracts and the results cannot be extrapolated to roots, Colegate *et al.* (2012) estimated that PA in immature 'Tropic Sun' roots, stems, and leaves comprised 0.05, 0.12, and 0.01% w/w compared with 0.15% w/w in seeds.

Apparently, the PA responsible for the nematicidal effect of sunn hemp are junceine, trichodesmine, and isohemijunceine, since monocrotaline, a toxic PA found in *C. spectabilis*, is not produced by sunn hemp (Ji *et al*., 2005). However, 'Tropic Sun' has more than 15 different alkaloids in its composition (Colegate *et al*., 2012) and nematode suppression could be related to the combined effect of several of these compounds.

In conclusion, the sting nematode did not affect the height, growth stage, fresh mass of shoot, and leaf area of sunn hemp plants. However, the sting nematode affected the dry mass of root of the different accessions of *C. juncea*. PI 322377 (Brazil) was the only accession that had a higher number of nematodes when compared with all other accessions. Despite this, all the accessions of sunn hemp suppressed the sting nematode, resulting in lower numbers of nematodes than with corn (the susceptible control).

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