Host Differences Among Florida Populations of *Belonolaimus longicaudatus* Rau[1]

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Abstract: Three populations of *Belonolaimus longicaudatus* from Gainesville, Fuller's Crossing, and Sanford, all in Florida, were tested for parasitism on *Citrus jambhiri* (rough lemon), *Arachis hypogaea* ('Early Runner' peanut), *Fragaria* sp. ('Florida 90' hybrid strawberry), and *Lycopersicon esculentum* ('Rutgers' tomato). The three populations were found to be three different physiological races because: (i) the Fuller's Crossing population reproduced well on and caused injury to rough lemon and tomato but not strawberry; (ii) the Gainesville population reproduced well on and caused injury to peanut, strawberry and tomato but not on rough lemon; (iii) the Sanford population reproduced well on and caused injury to peanut, reproduced well on tomato, but neither reproduced upon nor caused injury to strawberry or rough lemon. Morphologically, females of the Sanford population have a lower "c" value and a greater number of tail annules than those of the Gainesville and Fuller's Crossing populations. Females and males of the Fuller's Crossing population had higher "a" values than those of the other two populations. *Key Words: Belonolaimus longicaudatus,* Sting nematode, Physiological races, Tomato, Strawberry, Citrus, and Peanuts.

The sting nematode, *Belonolaimus longicaudatus* Rau, is a recognized pathogen of many crops in Florida and certain other states of the southeastern USA. Its long host list has representatives from most families of flowering plants, including such important crops as corn, turfgrasses, cotton, peanut, citrus, strawberry and most vegetables.

Some interesting facts suggest that physiological races may occur within *B. longicaudatus*. Owens(5, 6) reported it pathogenic to peanuts in Virginia which was verified by Christie *et al.* (1). With fumigation trials conducted in North Carolina, Sasser and Cooper (9) demonstrated 3- to 4-fold peanut yield increases on soils where *B. longicaudatus* was probably the primary pathogen. No reports have yet been published on losses of peanut production in Florida and Georgia due to the parasitism by this pest. On the other hand, Perry and Norden (7) reported that a population of *B. longicaudatus* in a field near Gainesville, Florida, "failed to attack peanut."

DuCharme and Suit (3) and others recovered the sting nematode from the soils of unthrifty citrus trees in Florida. Standifer and Perry (11) established the pathogenicity and described the pathological effects of *B. longicaudatus* on grapefruit.

This study was designed to determine whether host-range differences in populations of the parasite exist and, if so, to investigate morphological differences. Populations of the parasite were collected from three sites; Gainesville, Sanford, and Fuller's Crossing, all in Florida. These were chosen because the Gainesville population was reported not to attack peanut, and the Fuller's Crossing population had been reported to attack citrus.

MATERIALS AND METHODS

Populations of *B. longicaudatus* were collected from the following locations in Florida: (i) a corn field at the Tobacco Unit, Florida Agricultural Experiment Station, Gainesville, called population "G"; (ii) corn at the Central Florida Experiment Station inoculated with specimens from a field near Sanford,

Received for publication 8 September 1969.

[1] Portions of a Ph.D. thesis presented by the senior author to the Graduate School, University of Florida. Florida Agricultural Experiment Station Journal Series Paper No. 3457.

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called population "S"; (iii) and a citrus grove near Fuller's Crossing, called population "FC." Populations were increased by filling individual greenhouse flats with the respective infested soil and then planting each to sweet corn (*Zea mays* L. var. 'Silver Queen') and bean (*Phaseolus vulgaris* L. var. 'Contender'). Two rough lemon seedlings (*Citrus jambhiri* Lush.) were planted in the flat containing the Fuller's Crossing soil about one month later.

Rough lemon (*C. jambhiri*), peanut (*Arachis hypogaea* L. var. 'Early Runner'), strawberry (*Fragaria* sp. hybrid var. 'Florida 90'), and tomato (*Lycopersicon esculentum* Mill., var. 'Rutgers') were tested as hosts for each of the three populations of sting nematodes. A Kanapaha fine sand soil was fumigated with methyl bromide in an airtight box for 48 hrs at the rate 0.908 kg/0.768 m^3, aerated a minimum of 4 weeks, then used to fill the number and type of greenhouse pots required for each experiment.

The nematodes used as inoculum were isolated from the soil by using the Seinhorst elutriator method and inoculations were made within 12 hr. Each pot to be inoculated received 100 specimens (90 females and 10 males). Treatments were replicated either three or six times and equal numbers of uninoculated plants were maintained as controls. The pots then were placed in either a greenhouse or a plant growth room, with temperature maintained at approximately 27 C. The plants were fertilized and irrigated as required for good growth.

At the end of each experiment, a predetermined quantity of soil was taken from each pot, and processed for nematode counts using the Christie and Perry method (2). Tabulated data from all pathogenicity tests were statistically analyzed.

ROUGH LEMON: Rough lemon seeds were germinated in vermiculite. When the seedlings were about 25 cm in height, the root systems of 24 seedlings were washed with tap water, and one seedling was transplanted into each of 24 autoclaved 15-cm clay pots containing 1,000 cc of sterilized soil. The nematodes were added about 20 days later. Each treatment was replicated six times in a randomized block design.

After 6 months the roots were carefully extracted from the soil, washed and partially dried on paper towels. The fresh weight of each plant (root and shoot) was recorded and a 100 cc sample of the soil from each pot was processed for nematode counts. Each seedling was then replanted in an 8-liter porcelain crock containing the original 900 cc of soil plus 1,600 cc of sterilized soil (total 2,500 cc).

The experiment was terminated after about 14 months. Data on height, weight of the root system, weight of the foliage, dry weight of 10 leaves selected from below the four terminal leaves, number of leaves, and number of nematodes per 250 cc of soil were recorded.

PEANUT: Peanut seeds were germinated in the laboratory on paper towels until sprouts were about 5 cm long. Clay pots of 15-cm size were filled with 1,000 cc of sterilized soil, a shallow depression was made in the soil surface, and two sprouted seeds and 100 specimens of the parasite were placed in the depression which was carefully filled with soil and watered. Each treatment was replicated six times.

Weight of root and tops, and number of nematodes per 100 cc of soil were recorded after 5 months. The remaining soil (900 cc) was mixed with 300 cc of fumigated soil (total 1,200 cc), and returned to the original pot. Each pot was then replanted to one germinated 'Early Runner' peanut seed.

The test was terminated approximately 9 months after inoculation. The roots were freed from the soil, washed and fresh weights of the roots and tops were recorded. The

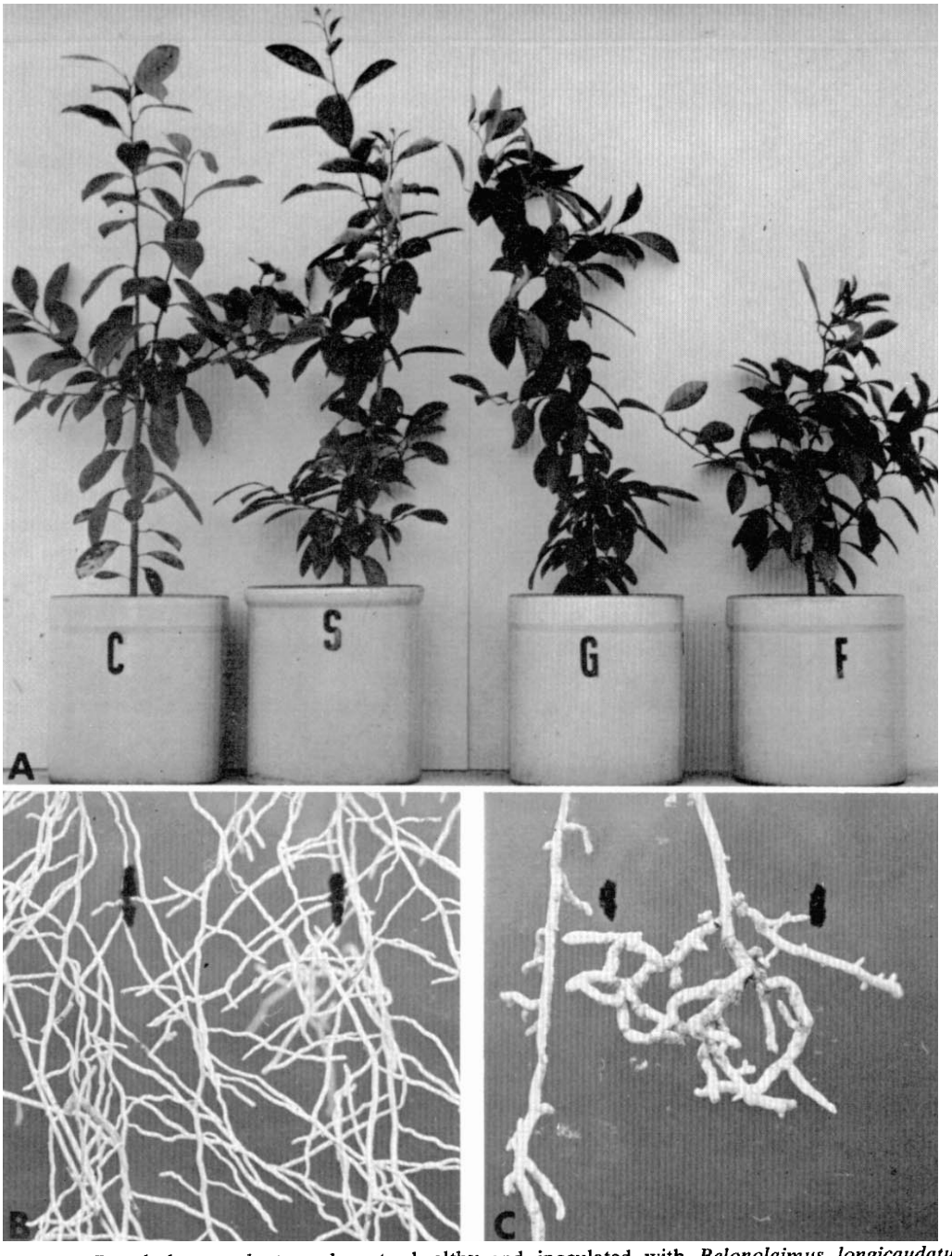

FIG. 1. Rough lemon plants and roots, healthy and inoculated with *Belonolaimus longicaudatus*. **A.** Comparison between representative plants inoculated with Sanford (S), Gainesville (G), and Fuller's Crossing (F) populations of *B. longicaudatus* and the non-inoculated check plants (C) fourteen months after inoculation. **B.** Roots of non-infested plants. **C.** Roots of plants inoculated with the Fuller's Crossing population.

TABLE 1. Effects of three populations of *Belonolaimus longicaudatus* on rough lemon seedings six months after inoculation with 100 specimens.

Nematodes	Fresh wt. of plant (g)	No. nemas per pot (1,000 cc soil)
Gainesville	85.50[a]	75
Fuller's Crossing	56.67[b]	5920
Sanford	73.83	18
Control	79.83	0

[a] Average of six replications.
[b] Significant at the 5% level.

number of nematodes in 100 cc of soil was determined.

STRAWBERRY: Strawberry 'mother' plants were grown in the greenhouse and runners were rooted in 1,500 cc of soil in 15-cm plastic pots. Twelve plants (one plant per pot) of a uniform size were inoculated with suspensions of each of the three sting nematode populations. Each treatment was replicated three times. The experiment was terminated after 4 months and fresh weights of foliage and of roots, and nematodes per 100 cc of soil were recorded.

TOMATO: Tomato seedlings were grown in 15-cm pots containing 1,000 cc of soil and twelve plants (one plant per pot) were chosen for the test. These plants were inoculated with the respective population, and treatments were replicated three times. The test was terminated after 5 months. Data on the weight of roots and tops and numbers of nematodes per 150 cc of soil were recorded.

MORPHOLOGY: Specimens of the three populations were isolated from soil around the roots of tomato plants from the above test. Permanent slides of females and males mounted in glycerin were prepared by routine methods (10). Morphological studies were made on 17 females of the Gainesville population, 19 of the Fuller's Crossing population and 16 of the Sanford population. Ten males from each population were studied.

RESULTS

ROUGH LEMON: Rough lemon seedlings attacked by the Fuller's Crossing population of *B. longicaudatus* were visibly shorter within 2 months and definitely stunted after 6 months when the weights of whole plants were 29% less than those of the non-inoculated plants (Table 1). The infected roots were quite short and thick (Fig. 1-C) resembling the "stubby root" syndrome described by Christie *et al.* (1). At 14 months the plants were "bushy," significantly shorter than the controls and with increased lateral proliferation. Fresh weights of foliage and roots were reduced 16.2 and 23.7% respectively (Table 2). The leaves of infected plants remained green but were more numerous, smaller, rougher in texture and weighed less than those of the control plants. Nematode increase at 6 months was 60-fold but by 14 months had risen to 111-fold (11,145 per plant). During the same 14 month period the Gainesville population had increased only

TABLE 2. Effects of three populations of *Belonolaimus longicaudatus* on rough lemon seedlings fourteen months after inoculation with 100 specimens.

Nematodes	Foliage fresh wt. (g)	Root fresh wt. (g)	No. of leaves	Dry wt. 10 leaves (g)	Plant height (cm)	No. nemas per pot (3,500 cc of soil)
Gainesville	84.00[a]	81.50	95	2.75	103	750
Fuller's Crossing	66.50	64.83	126[b]	1.65[c]	52[b]	11,145
Sanford	76.33	81.50	97	2.28	82	11
Control	78.17	83.83	97	2.73	84	0

[a] Average of six replications.
[b] Significant at the 5% level.
[c] Significant at the 1% level.

TABLE 3. Effects of three populations of *Belonolaimus longicaudatus* on peanut plants five months after the first planting.

Nematodes	Foliage fresh wt. (g)	Root fresh wt. (g)	No. nemas per pot (1,000 ml soil)
Gainesville	21.53[a]	8.81	76
Fuller's Crossing	21.65	9.62	133
Sanford	23.61	10.45	165
Control	24.33	9.55	0

[a] Average of six replications.

TABLE 5. Effects of three populations of *Belonolaimus longicaudatus* on strawberry plants four months after inoculation with 100 specimens.

Nematodes	Foliage fresh wt. (g)	Root fresh wt. (g)	No. nemas per pot (15,000 cc soil)
Gainesville	9.33[a]	2.63	393
Fuller's Crossing	11.17	3.80	7
Sanford	11.23	3.40	37
Control	11.73	3.90	0

[a] Average of three replications.

8-fold, a few roots were "stubby" but generally the plants appeared normal. The Sanford population neither reproduced nor caused visible symptoms on the plants.

PEANUT: In the first planting, foliage and root weights of peanut plants inoculated with either of the three populations of *B. longicaudatus* were not statistically different than the checks (Table 3). In all cases the numbers of nematodes recovered were low.

Four months after the second planting, the fresh weights of foliage of plants inoculated with the Fuller's Crossing, Sanford and Gainesville populations were 26, 32, and 33% less, respectively, than of control plants (Table 4). Plants inoculated with the Sanford and Gainesville populations were stunted and chlorotic but symptoms were more severe with the former. The foliage of plants inoculated with the Fuller's Crossing population appeared to be healthy in spite of the reduced foliage weight. Root weights of plants inoculated with the Gainesville and Sanford populations were 47 and 61% less than those of the control plants; these results were statistically significant. The roots, especially of the plants inoculated with the Sanford population, were greatly reduced, discolored, and exhibited extensive necrosis on roots, pegs and hulls (Fig. 2A, B). Roots of plants inoculated with the Fuller's Crossing nematodes were not significantly different from those of the control.

The numbers of nematodes recovered were about four, three, and two times as many as the original inoculum in the Gainesville, Sanford, and Fuller's Crossing populations, respectively (Table 4). Apparently, peanut plants were unable to support large numbers of either parasite, a situation comparable with strawberry plants described by Christie *et al.* (1), who suggested, as a reason, failure of

TABLE 4. Effects of three populations of *Belonolaimus longicaudatus* on peanut plants four months after the second planting (total of nine months).

Nematodes	Foliage fresh wt. (g)	Root fresh wt. (g)	No. nemas per pot (1,200 cc soil)
Gainesville	16.33[a]	3.00[b]	373
Fuller's Crossing	18.17	5.42	177
Sandford	16.67	1.87[b]	317
Control	24.50	5.69	0

[a] Average of six replications.
[b] Significant at the 1% level.

TABLE 6. Effects of three populations of *Belonolaimus longicaudatus* on tomato plants five months after inoculation with 100 specimens.

Nematodes	Foliage fresh wt. (g)	Root fresh wt. (g)	No. nemas per pot (1,000 cc soil)
Gainesville	72.33[a]	9.37[c]	8,552
Fuller's Crossing	62.67[b]	7.77[c]	10,190
Sanford	84.00	16.60	2,960
Control	86.33	17.83	0

[a] Average of three replications.
[b] Significant at the 5% level.
[c] Significant at the 1% level.

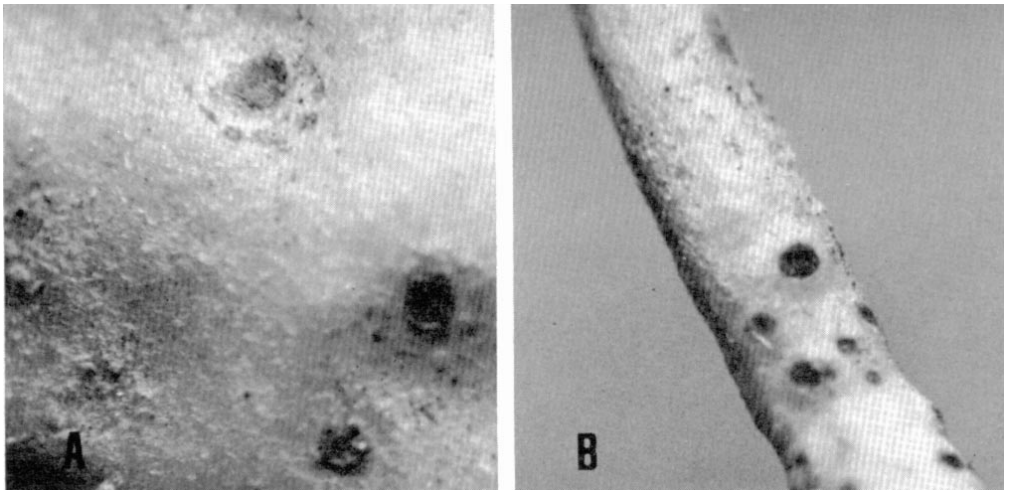

FIG. 2. Necrotic lesions on the hulls (**A**) and pegs (**B**) of peanut inoculated with the Sanford population of *Belonolaimus longicaudatus*.

the parasitized plants to produce new roots.

STRAWBERRY: During a 4 month period, strawberry plant growth was affected by the Gainesville population of *B. longicaudatus*. Although statistical analysis showed no significant differences from the control plants, the average weights of foliage and roots were reduced by 21 and 32.5% respectively (Table 5). The plants inoculated with the Gainesville population were stunted and the foliage was mildly chlorotic. The root systems were distinctly reduced and discolored and new roots were sparse and stubby compared to control plants. Nematode reproduction averaged 4-fold. Strawberry roots were not able to support high populations of this parasite probably because the plants failed to produce secondary roots necessary for nematode feeding.

The Sanford and Fuller's Crossing populations failed to reproduce and inflicted no detectable injury to the inoculated plants (Table 5).

TOMATO: Retardation of top growth of the tomato plants inoculated with the Fuller's Crossing population began to appear about two months after inoculation. At termination of the experiment, growth retardation was even more conspicuous with slight foliar chlorosis. The same type of symptoms appeared on the plants inoculated with the Gainesville population but to a lesser extent. The average fresh weight of foliage was 27.3, 16.3 and 2.7% less when inoculated with the Fuller's Crossing, Gainesville and Sanford populations, respectively than the control (Table 6). Reduction of the average weight of roots was 56, 47.5 and 7% in the same order.

Roots of the plants inoculated with the Fuller's Crossing and Gainesville populations showed the stubby root condition and were discolored and necrotic. No similar symptoms appeared on the roots inoculated with the Sanford population. The tomato plants supported extremely high populations of *B. longicaudatus* with an average increase of 102, 85, and 30 times with the Fuller's Crossing, Gainesville, and Sanford populations, respectively.

MORPHOLOGY: Average total lengths of females from the Gainesville, Fuller's Crossing, and Sanford populations were 2,097, 2,274, and 2,206 μ, respectively (Table 7), but these

TABLE 7. Morphological data[a] on the females and males of *Belonolaimus longicaudatus* from Gainesville (G), Fuller's Crossing (FC), and Sanford (S) populations.

Nematodes		Total length	a	b	c	V%	Stylet length	Length Mesometa-rhabdion	Anterior-guiding ring[b]	Anterior-excretory pore	Spicule length	Length Guber-naculum	Length Gub. flexure	Tail length	No. tail annules
	♀	2097	52	7.5	17.2	51.9	124	36	60	223				119	68
G	♂	1712	49	6.5	14.8		115	32		215	53	19	7.2	116	
FC	♀	2274	64	7.9	17.6	50.0	118	33	55	217				131	82
	♂	1790	64	6.9	14.6		107	29		200	43	17	6.6	122	
S	♀	2206	59	7.9	15.0	49.8	113	31	54	224				141	94
	♂	1718	56	6.9	14.0		106	29		203	42	16	5.6	122	

[a] Average of 17, 19, and 16 females and 10 males from each of G, FC, and S populations respectively. Measurements in microns.

[b] Presence of a stylet guiding ring has not been reported for the sting nematodes. An inconspicuous guiding ring is located about the middle of the stylet.

measurements are not significantly different. In each case they ranged between the average lengths of *B. gracilis* (1,900 μ) and *B. longicaudatus* (2,500 μ) as indicated by Rau (8), but corresponded to measurements of *B. longicaudatus* reported by Graham and Holdeman (4). The average stylet lengths of the three populations were not significantly different (Table 7). They corresponded to lengths reported by Graham and Holdeman (4) but were much less than those reported for *B. gracilis* by Rau (8).

The average "a" value was much higher in the Fuller's Crossing population than that of the Gainesville population indicating a narrower body at the vulva of the former. The average "a" value of females of the Sanford population was intermediate (Table 7).

TABLE 8. Host differences between three Florida populations of *Belonolaimus longicaudatus*.

	POPULATIONS					
	Fuller's Crossing		Gainesville		Sanford	
HOSTS	Nema incr.	Host sympt.	Nema incr.	Host sympt.	Nema incr.	Host sympt.
Rough lemon	+	+	—	—	—	—
Tomato	+	+	+	+	+	?
Strawberry	—	—	+	+	?	—
Peanut	+	—	+	+	+	+

The females of the Sanford population had a lower "c" value than that of the Gainesville and Fuller's Crossing populations. This was due to differences in the tail lengths which averaged 119, 131, and 141 μ in the Gainesville, Fuller's Crossing, and Sanford specimens, respectively. The number of annules on the females tail corresponded positively with the tail length of the respective population (Table 7).

The average "a" values of the males followed the same trend as that of the females (Table 7). Average length of spicules and the gubernaculum flexure of the Gainesville population gave higher values than those of the other two populations.

DISCUSSION

The data presented indicate that populations of *B. longicaudatus* collected from different areas of Florida and from different hosts give differing reactions when inoculated to certain hosts growing under greenhouse conditions. In general the intensity of symptoms expressed by the host was correlated with parasite population increase. Population levels reached under the experimental conditions were determined by the particular host used and by the original source of the parasite.

The scope of the morphological studies was insufficient to warrant the naming of a new species for either of the populations in question. This study was initiated to determine whether the populations differed sufficiently to aid in the diagnosis of sting nematode disease in Florida. Further investigations of tail length, number of tail annules and stylet characteristics may provide means of morphologically determining which hosts might be attacked by a certain population.

Citrus is apparently attacked by *B. longicaudatus* in a few locations in Florida. The Fuller's Crossing population was collected from an orange grove in which the trees exhibit dieback, thinness of the top, small leaves and fruit and "stubby root." *B. longicaudatus* has been collected from other citrus plantings wherein no symptoms of injury are evident. It is probable that indigenous hosts support these populations which apparently do not attack citrus to a significant degree.

Of especial interest is the fact that, under greenhouse conditions, populations of this sting nematode from Florida fed upon peanuts and reproduced. This is in contrast to the fact that significant populations have not been found in peanut fields in Florida and the data reported by Perry and Norden (7). No explanation can be offered until further researches are conducted.

Table 8 summarizes the major differences among the 3 populations of the parasite.

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