韓國土壤動物學會誌 9(1-2) : 12~15 (2004)
Korean Journal of Soil Zoology

Characterization of Host Specificity and Reproductive Compatibility Among Different Isolates of *Belonolaimus longicaudatus* in Southeastern United States

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미국 동남부지역에 분포하는 *Belonolaimus longicaudatus* 개체군들간의 기주특이성과 생식교합능력의 비교

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ABSTRACT

Five isolates of *Belonolaimus longicaudatus* collected from different geographical locations and hosts in southeastern United States were tested to determine their host specificity and reproductive compatibility. Both the Georgia and Lake Alfred isolates reproduced well on cotton, bermudagrass, and citrus. On the other hand, the North Carolina and Gainesville isolates reproduced poorly on all crops. The Hastings isolate reproduced well on cotton and potato. The Lake Alfred and Hastings isolates showed greater host specificity to their original host of citrus and potato, respectively. Except for the Hastings isolate, all isolates reproduced poorly on potato, which clearly differentiated the Hastings isolate from others ($P \leq 0.05$). Hybridization test presented reproductive compatibility among five different isolates of *B. longicaudatus* by generating F_1 offspring. The Gainesville males showed comparatively poor ability to fertilize other females and generated a lower number of offspring. In contrast, the North Carolina males were attracted by all the other isolates of sting nematode, and produced the highest number of juveniles.

Key words : *Belonolaimus longicaudatus*, host specificity, hybridization, reproductive compatibility, sting nematode

INTRODUCTION

Belonolaimus longicaudatus (Rau 1958) is an economically importance plant parasite that attacks many varieties of agricultural crops from vegetables, and grasses to ornamental, and fruit trees (Cooper *et al.* 1959, Graham and Holdman 1953, Perry and Rhoades 1982). Since Christie *et al.* (1952) established pathogenecity of ectoparasitic nematode, especially *B. longicaudatus*, on strawberry, celery, and sweet corn, the significance of sting nematode in agriculture has increased.

The variation among different populations of *B. longicaudatus* was noted in host specificity as well as morphology, and in some isolates, host specificity or host range was determined to be greater than morphological variation (Abu-Gharbieh and Perry 1970, Robbins and Barker 1973). According to Robbins and Barker (1973), three North Carolina isolates of *B. longicaudatus* were clearly differentiated from a Tifton, Georgia isolate, and the Georgia isolate had larger host range than the North Carolina isolate. During that experiment, they also found that the reproductive rate of the Georgia isolate was significantly greater than that of the North Carolina isolates of sting nematode. Three morphologically similar Florida

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isolates of *B. longicaudatus* also responded differently to the different hosts (Abu-Gharbieh and Perry 1970).

In this study, five different isolates of *B. longicaudatus* collected from different geographical locations and hosts in southeastern United States were compared on different host crops to determine their host specificity. In addition, a hybridization test was conducted to examine reproductive compatibility or incompatibility among these isolates.

MATERIALS AND METHODS

1. Nematode isolates

Five isolates of *B. longicaudatus* were collected from different geographical locations and different hosts. Three isolates came from different locations in Florida; HA isolate from potato in Hastings; GV isolate from bermudagrass in Gainesville; and LA isolate from citrus in Lake Alfred. Others were GA isolate from cotton in Tifton, Georgia and NC isolate from corn in Scotland County, North Carolina.

2. Greenhouse culture of nematode

Each isolate was established on bermudagrass (*Cynodon dactylon* (L.) Pers.) in the greenhouse. Bermudagrass was grown vegetatively in 25 cm-diameter clay pots filled with pasteurized sandy soil (95.5% sand, 2.0% silt, and 2.5% clay). Nematodes were extracted by the Baermann method, and females and males were hand picked. The inoculation level for each was 100 to 200 nematodes per pot. The pots were maintained in a greenhouse at 25±5°C, and fertilized once a week with a 20-20-20 N-P-K soluble fertilizer, and watered every day.

3. Host plants

Peanut, cotton, bermudagrass, citrus, and potato, five hosts were tested for the host specificity of five isolates of *B. longicaudatus*. Peanut (*Arachis hypogaea* L. cv. Florunner), Cotton (*Gossypium hirsutum* L. cv. Sure grow 125), and Bermudagrass (*Cynodon dactylon* (L.) Pers.)

Peanut and cotton: Seeds were germinated under a moist paper towel for 3 days and for bermudagrass, the stems of healthy bermudagrass were cut into 3 to 4 cm. Then, seedlings of peanut and cotton, and stem of bermudagrass were transplanted into 20 cm-d-clay pots filled with 1,200 g pasteurized soils. Two and half weeks later, 40 female and 20 male nematodes of each isolates were added with a pipette. All treatments were replicated five times for peanut and four times for cotton, and each block was completely randomized. The peanut and cotton plants were harvested after 75 days, and bermudagrass were harvested after 67 days

Citrus (*Citrus aurantium* cv. Sour orange): Two and half months old citrus seedlings were obtained from Lake Alfred citrus research center, and they were transplanted in 20 cm-d-clay pot filled with 1,200 g pasteurized soils. The five isolates of *B. longicaudatus* were inoculated on 8 to 12 cm long citrus seedlings 2 weeks later. For nematode inoculation, three 6 cm-deep holes were made about 3 cm apart from plant and 3 cm from base of plant stem. Sixty females and 20 males were added per pot. After 5 months, citrus was harvested.

Potato (*Solanum tuberosum* L. cv. Sebago): Potato tubers were planted into 25 cm-d-plastic pots to a 5 cm depth. When the stem shad grown 10 to 12 cm, the basal part around an inter-node was cut, and replanted into sandy soils in 20 cm-d-clay pots. After one and half weeks later, the entire plants were transplanted into 20 cm-d-clay pots. One week later from transplanting, nematodes were inoculated. Each pot was inoculated with 40 females and 20 males, and all treatments were replicated 5 times. After 67 days, potato was harvested.

4. Hybridization test

All isolates of *B. longicaudatus* were cultured on cotton (*Gossypium hirsutum* L. cv. Sure grow 125) for 2 months in a greenhouse, and different developmental stages of nematodes were extracted by the Baermann method. For the hybridization test, 10 nematodes for each of J4 females, J4 males, and adult males were picked from each isolate. Ten J4 females of the GA, LA, NC, HA, and GV isolates were combined with 10 J4 males and 10 adult males from each of the source isolates to give a total of 25 combinations. Experimental design was a completely randomized block (CRBD), and the test was repeated twice.

The host plant was 2-week-old cotton (*Gossypium hirsutum* L. cv. Sure grow 125) seedling that was germinated under a moist paper towel for 3 days at room temperature and then transferred to 4 cm-d-conetainers (Stewe and Sons, Corwallis, OR) filled with pasteurized sandy soil (95.5% sand, 2.0% silt, and 2.5% clay). The seedlings were placed at 28°C in a Florida Reach-In Chamber until inoculation. The nematodes were added to each by placing them in a 1 cm-wide × 5 cm-deep hole. After inoculation, the plants were returned to the Reach-In Chamber at 28°C, and harvested 45 days later.

Table 1. Comparisons of the final number of nematode per 100 g of soil among five isolates[a] of *Belonolaimus longicaudatus* in different hosts

Host plants	GV	HA	LA	GA	NC
Peanut (*Arachis hypogaea* L. cv. Florunner)	16.2±4.5 ab[b] (14-38)	22.2±9.6 b (11-22)	23.8±8.1ab (12-34)	28.4±5.0 a (24-37)	5.6±4.2 c (1-10)
Cotton (*Gossypium hirsutum* L. cv. Sure grow 125)	33.8±14.4 b (125-155)	375±108 a (225-475)	385±138 a (290-590)	387±194.5 a (220-610)	46.3±32.2 b (115-185)
Bermudagrass (*Cynodon dactylon* (L.) Pers.)	31±20.4 c (11-63)	50.4±17.4 bc (35-79)	196±209.2 ab (20-320)	227±136.4 a (80-435)	56.6±61.9 bc (18-162)
Citrus (*Citrus aurantium* cv. Sour orange)	26.2±25.3 ab (3-65)	4.2±26.9 ab (0-63)	90.6±88.5 a (0-229)	56.6±50 ab (9-120)	1.4±1.5 b (0-3)
Potato (*Solanum tuberosum* L. cv. Sebago)	7.3±6.8 b (0-13)	82.3±60 a (11-157)	27.5±23.4 b (10-61)	13.8±4.3 b (9-19)	6.8±5.3 b (0-13)

[a] The isolates of *B. longicaudatus* were collected from bermudagrass in Gainesville, FL (GV), potato in Hastings, FL (HA), citrus in Lake Alfred, FL (LA), cotton in Tifton, GA (GA), and corn in Scotland County, NC (NC).
[b] Same letters in a column denote no significant difference at P = 0.05.

RESULTS AND DISCUSSION

All isolates of *B. longicaudatus* showed distinctive reactions to the five different host plants. Both the GA and LA isolates reproduced well on cotton, bermudagrass, and citrus (Table 1). On the other hand, the NC and GV isolates reproduced poorly on all crops. The HA isolate reproduced well on cotton and potato.

The LA and HA isolates showed greater host specificity to their original host of citrus and potato, respectively, whereas the GV isolate did not reproduce well on its original host of bermudagrass. Except for the HA, all isolates reproduced poorly on potato, which clearly differentiated the HA isolate from others ($P \leq 0.05$). Both the NC and GV isolates were differentiated from other isolates by lower number of nematodes produced on cotton ($P \leq 0.05$). There was no difference in dry or fresh root/stem weight among all hosts.

In host range test of Georgia and North Carolina isolates of *B. longicaudatus*, Robbins and Barker (1974) found that the Georgia isolate increased quickly with a higher reproductive rate than the North Carolina isolates. Abu-Gharbieh and Perry (1970) reported that Fuller's Crossing, Gainesville, and the Sanford isolates had different host specificity and reproductive rate on rough lemon, tomato, strawberry, and peanut. In the previous work, Georgia and Florida isolates do not reproduce well on peanut (Good 1968, Brodie *et al.* 1970). Later, however, both Georgia and Florida isolates were able to reproduce on peanut (Abu-Gharbieh and Perry 1970, Robbins and Barker 1974). In this study, Georgia and Florida isolates reproduced on peanut but the level of increase was comparatively low.

Table 2. Determination of compatibility or incompatibility among different isolates of *Belonolaimus longicaudatus* based on hybridization test after 45 days

Male[b] isolates	Replication	Female isolates[a] GA	HA	GV	LA	NC
GA	1	+[c]	+	m[d]	+	m
	2	+	+	++	++	+
HA	1	++	+	++	−	++
	2	+	++	+	−	−
GV	1	−	+	+	+	−
	2	−	+	+	−	+
LA	1	+	+	−	+	+
	2	+	++	+	+	+
NC	1	+++	+	+++	+++	+
	2	m	+	+	+	+

The isolates of *B. longicaudatus* were collected from cotton in Tifton, GA (GA), potato in Hastings, FL (HA), bermudagrass in Gainesville, FL (GV), citrus in Lake Alfred, FL (LA), and corn in Scotland County, NC (NC).
[a] J4 females (n = 10)
[b] Combination of male (n = 10) and male forth-stage juvenile (n = 10) of each isolate.
[c] + The number of juveniles is < 5; ++ The number of juveniles is between 5 to 10; +++ The number of juveniles is > 10; − No offspring (juveniles).
[d] Missing data.

The reproductive compatibility or incompatibility among different isolates of *B. longicaudatus* was determined by a hybridization test (Table 2). The existence of second-stage, or third-stage juveniles was especially indicative of reproductive compatibility, whereas absence of juveniles was assumed as incompatible. Hybridization test presented reproductive compatibility among different isolates of sting nematode except for the GA (female) × GV (male) and the LA (female) × HA (male). However, inter-population matings of the GV (female) × GA (male) and the HA (female) × LA (male) resulted in the existence of F_1 offspring, therefore, all five isolates of *B. longicaudatus* showed reproductive compatibility each other. The GV males showed comparatively poor ability to fertilize other females and generated a lower number of offspring. In contrast, the NC males were attracted by all the other isolates of sting nematode, and produced the highest number of juveniles. In a similar experiment, Robbins and Hirshmann (1974) showed that in interbreeding test between isolates they produced F_1 offspring, which were ranged from 0.7 to 32.3. The number of progeny was higher in North Carolina (female) × Georgia (male) than Georgia (female) × North Carolina (male), which was opposite results from this experiment. Even though North Carolina and Georgia isolates produced limited number of F_1 offspring, they failed to reproduce F_2 offspring (Robbins and Hirshmann 1974).

In this study the experiment was not completed to trace the reproduction of F_2 offspring followed by F_1 self-fertilization, however, the results proposed geographically isolated sting nematodes are not biologically separated species. Meanwhile, more variations among different isolates of *B. longicaudatus* were observed in the morphology and genetic characteristics (Han, unpublished data) as well as the host preference. Therefore, sting nematode isolates are not reproductively separated, but possess various characteristics that could be differentiated, which is a useful information to understand species and population structure of *B. longicaudatus* and further application for nematode management in agricultural system.

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