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Reproduction of Four Races of *Meloidogyne incognita* on *Hibiscus cannabinus*

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Abstract: The feasibility of cultivation of kenaf (*Hibiscus cannabinus*) in the United States is receiving a multifaceted evaluation. Among the factors being evaluated is kenaf's susceptibility to nematodes. In this investigation, four races of *Meloidogyne incognita* reproduced extensively on each of the several kenaf genotypes examined in greenhouse tests. Some genotypes of kenaf, however, demonstrated limited resistance to certain races of *M. incognita.*

Key words: Hibiscus cannabinus, host suitability, kenaf, *Meloidogyne incognita,* nematode, races, resistance, root-knot nematode.

Kenaf (*Hibiscus cannabinus* L.) is a rapidly growing plant (2) cultivated throughout much of tropical America (6,10,12) and Asia (15). The plant produces bast (phloem) and once (xylary) fibers of sufficient quality to make a very high grade newsprint pulp (1). Because of their length and strength, the fibers are also valued for certain other speciality uses.

Kenaf and cotton (*Gossypium* spp.) are taxonomically close relatives in the Malvaceae. In the continental United States, kenaf can be grown in much of the cotton belt. However, because the commercial value of kenaf depends only upon vegetative tissue accumulation and not on fruit set and reopening, the kenaf production area could extend considerably north of the cotton belt. There is, for example, interest in kenaf production for special paper manufacturing in the Pacific Northwest. The trade-off for a more northern production area is a shorter growing season with concomitant reduction in biomass accumulation.

Previous attempts to produce kenaf commercially in the southern United States as a supplement to tree wood pulp were abandoned as economically unsound. Recently, perhaps encouraged by the fact that the United States imports more than 60% of its newsprint (1) at an annual cost of more than four billion dollars (11), efforts at establishing kenaf as a crop in the United States have been renewed. An additional impetus to renewed interest in kenaf cultivation may be a growing realization that kenaf production does not involve destruction of old growth forests to furnish our paper pulp needs.

In 1986 the United States Department of Agriculture and private joint venture interests entered a cooperative agreement to determine the biological, technological, and economic feasibility of growing and processing kenaf in the United States (5). Much of that assessment has been completed and the findings appear favorable. However, kenaf's responses to pests, including nematodes, are little known.

Earlier studies (7,8,12,15,18,20) demonstrated that nematodes, especially root-knot nematodes (*Meloidogyne* spp.), would likely be significant pests of kenaf. Unfortunately, much of this earlier work was done without a commonly accepted definition of host resistance to nematodes and before host pathotypes of *M. incognita* were recognized (14).

With regard to measuring host resistance, two concepts are useful: host suitability and host sensitivity. Host suitability, a measure of the host's ability to support nematode reproduction, is used in this article. In many instances an inverse relationship exists between host suitability and host resistance. Host suitability is expressed objectively as the ratio of the number of nematode units recovered at the end of the test—the final nematode population density (Pf)—to the number of nematode units used to inoculate the plant—the initial population density (Pi).

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Meloidogyne incognita is widely distributed throughout the world and consists of four pathotypes or races (14). Under the archaic name *M. incognita acrita,* it was reported to parasitize cotton and kenaf (8). The belief that races 3 and 4 of *M. incognita* are synonymous with *M. incognita acrita* (16) would thus indicate that *M. incognita* races 3 and 4 are also pathogenic on kenaf. However, the pathogenicity of *M. incognita* races 1 and 2 on kenaf are not addressed by this logic and remain in question. The objective of this study, therefore, was to individually evaluate several kenaf genotypes for host suitability to each of the four races of *M. incognita.*

Materials and Methods

Kenaf genotypes included cultivars Cubano, Everglades 41, Everglades 71, Guatemala-4; breeding lines 19-117-2, 45-9, and 15-2; and plant introduction PI 318723.

The four races of *M. incognita* were separately maintained on 'Rutgers' tomato (*Lycopersicon esculentum,* Mill.) in a greenhouse, and the race designations were confirmed with differential host tests (14) before use as inocula in this study. Eggs for inocula were extracted from tomato roots 6–9 weeks after inoculation by sieving and sugar flotation (3). A small volume of each egg suspension was held in a petri dish on the laboratory bench and examined 48 hours after extraction to confirm egg viability.

Ninety six 3-liter plastic pots were filled with pasteurized potting soil mix (3:1:1 fine sand: medium grade vermiculite: Michigan peat moss) amended with 300 ml gypsum and 300 ml dolomite per 40 liters of soil mix. The pots were placed on a greenhouse bench and supplied with a watering tube that automatically delivered 200 μg of 15-16-17 NPK per ml deionized water. Five days after planting each pot with two seeds, the pots were thinned to one kenaf seedling per pot. Ten days after planting, each pot was infested with 4,000 eggs of one race by dispensing 25 ml of egg suspension evenly over the soil surface. The eggs were then watered into the soil with additional water. Three replicate pots were included for each kenaf genotype and nematode race tested.

The planted, infested pots were held on a greenhouse bench at 28–30 C for 63 days until harvest. At harvest, the main stems of the plants were cut at the surface of the soil and the soil was gently washed from the roots. The washed root systems were individually placed in plastic bags and sufficient 1% NaOCl was added to each bag to cover the roots. The bagged roots were held at 4 C for no more than 2 days until eggs could be extracted and counted. The eggs were collected by the NaOCl method (3), concentrated by sugar flotation and centrifugation, and counted under a macroscope to determine Pf. Nematode reproduction was determined by Pf/Pi, with Pi being a constant 4,000.

The roots from which the eggs were extracted to determine Pf were air-dried for 72–96 hours on a laboratory bench and the air-dry weights determined. Nematode reproduction was adjusted for the amount of root tissue from which the eggs were extracted by dividing the ratio of Pf/Pi by the air dry weight of the tissue from which the Pf value was obtained. The data were log-10 transformed and analyzed by analysis of variance followed by Fisher's protected LSD test (9).

Results

The average number of eggs recovered from each kenaf genotype × nematode race combination is shown in Table 1. It is apparent from this data that, with the possible exception of breeding line 19-117-2 and race 3, each of the kenaf lines tested is a suitable host for each of the four races of *M. incognita.* Furthermore, race 3 appears to be the least aggressive of the four races on all the genotypes collectively (Table 1). These observations are reflected most clearly by the relatively large *R* values of nearly every genotype × nematode race combination; however, genotypes Guatemala-4, Everglades-41, and Cubano were more suitable host for all races of *M. incog-*

TABLE 1. Mean number of eggs (×1,000) recovered from kenaf (*Hibiscus cannabinus* L.) genotypes separately infected with the four races of *Meloidogyne incognita*.

Genotype	Race 1	Race 2	Race 3	Race 4	Genotype means
Cubano	220 ± 67	176 ± 176	57 ± 49	170 ± 81	156 ± 110 a†
Everglades 41	169 ± 95	221 ± 149	98 ± 75	183 ± 44	168 ± 96 a
Everglades 71	97 ± 53	111 ± 88	294 ± 323	212 ± 73	134 ± 74 a
Guatemala-4	222 ± 121	211 ± 225	104 ± 79	185 ± 271	180 ± 169 a
PI 318723	69 ± 43	39 ± 21	85 ± 47	162 ± 239	89 ± 116 ab
19-117-2	53 ± 32	104‡	1	28	56 ± 47 b
45-9	60 ± 47	107	38	37	60 ± 37 ab
15-2	184 ± 193	286 ± 292	115 ± 116	86 ± 12	142 ± 189 a
			Race means		
	139 *x*	169 *x*	71 *y*	150 *x*	

Data are means ± standard deviation of three replications.
† Untransformed means followed by the same letter are not different based on the Fisher's protected LSD test ($P \leq 0.05$). Mean separation test based on analysis and means of transformed data.
‡ Blank space = insufficient data to complete standard deviation.

nita than were 19-117-2, PI 318723, and 45-9 (Table 2). When the *R* value was adjusted (Table 3) for the weight of root tissue from which the eggs were extracted (Table 4), two genotypes, 'Everglades-71' and PI 318723, had adjusted *R* values larger ($P \leq 0.05$) than the other genotypes tested (Table 3).

DISCUSSION

All four races of *M. incognita* reproduced prolifically on kenaf. In several instances, the nematode population increased 50-fold in 7 weeks. Unlike cotton, which is considered a poor host for races 1 and 2 of *M. incognita* (16), kenaf supports a high level of reproduction by these races. Although not substantiated by statistical analysis, it seemed that on Cubano and Guatemala-4, *M. incognita* races 1 and 2 reproduced more prolifically than did races 3 and 4 on these genotypes. Considering the close relationship between kenaf and cotton, it is curious that Races 1 and 2, which are not considered pathogenic on cotton, reproduced more prolifically on kenaf than did Races 3 and 4, which are recognized as pathogenic on cotton. Regardless, earlier reports (8,10,12) dealing with parasitism of kenaf by undetermined races of *M. incognita* are not discredited by this report.

The fact that all races of *M. incognita* are pathogenic on kenaf has serious implications regarding crop rotations. If kenaf is planted following cotton on which *M. incognita* was not observed, it cannot be concluded that the kenaf crop will also escape

TABLE 2. *R* values (Pf/Pi) of genotypes of kenaf (*Hibiscus cannabinus*) individually infected by the four races of *Meloidogyne incognita;* Pi was 4,000 in all cases.

Genotype	Race 1	Race 2	Race 3	Race 4	Genotype mean
Cubano	55	44	14	42	39 a†
Everglades 41	42	55	24	46	42 a
Everglades 71	24	28	30	53	34 ab
Guatemala 4	55	53	26	46	45 a
PI 318723	17	10	21	41	22 b
19-117-2	13	26	0.3	7	12 b
45-9	15	27	10	9	15 b
15-2	46	75	4	21	37 ab
			Race mean		
	34 *xy*	40 *x*	16 *y*	33 *xy*	

Data are means of three replications.
† Untransformed means followed by the same letter are not different based on the Fisher's protected LSD test ($P \leq 0.05$). Mean separation test based on analysis and means of transformed data.

TABLE 3. *R* value (Pf/Pi), adjusted for the weight of the roots from which the eggs were extracted, for eight genotypes of kenaf (*Hibiscus cannabinus*) individually infected by the four races of *Meloidogyne incognita*).

Genotype	Race 1	Race 2	Race 3	Race 4	Genotype mean
Cubano	13 ± 3	11 ± 6	4 ± 3	10 ± 6	9 c
Everglades 41	22 ± 17	12 ± 8	9 ± 3	18 ± 5	15 bc
Everglades 71	7 ± 5	6 ± 1	15 ± 14	14 ± 6	21 ab
Guatemala 4	14 ± 11	11 ± 10	8 ± 5	14 ± 20	12 bc
PI 318723	33 ± 16	20 ± 10	29 ± 3	20 ± 21	26 a
19-117-2	8 ± 8	7 ± 4	2‡	29	10 c
45-9	13 ± 1	14	16	7	13 bc
15-2	15 ± 14	19 ± 18	2 ± 2	8 ± 7	11 c
			Race mean		
	16 *x*	12 *x*	11 *x*	15 *x*	

Data are means ± standard deviation of three replications.
† Untransformed means followed by the same letter are not different based on Fisher's protected LSD test ($P \leq 0.05$). Mean separation test based on analysis and means of transformed data.
‡ Blank spaces = insufficient data to compute standard deviation.

parasitism. Populations of *Meloidogyne incognita* race 1 or 2, present on cotton or weeds but undetected, could overwinter and multiply rapidly when kenaf is planted the following season. Additionally, a field infested with races 1 or 2 and planted to kenaf may exacerbate problems in subsequent plantings to cotton. Recent studies indicate that *M. incognita* races 1 and 2, although not reproducing extensively on cotton, may reduce cotton yield (13,17,19).

Of the kenaf entries evaluated, I believe only 19-117-2 could be considered resistant (Pf < Pi) to *M. incognita,* but that resistance is manifested only to race 3. Although the reproductive indices of the other races of *M. incognita* on kenaf breeding line 19-117-2 were greater than 1, they were among the lowest *R* values encountered. Regardless, when the *R* value exceeds 1, the nematode population is increasing and it is, therefore, doubtful that any kenaf genotype should be considered resistant to all races of *M. incognita.*

Because of the considerable variation that exists in the host suitability of the various genotypes of kenaf to the various races of *M. incognita,* I believe that there is sufficient extant genetic variability that crop improvement programs aimed at decreasing the suitability of kenaf to the *M. incognita* can be successful. Whether reducing host suitability will translate into improved crop yields on nematode-infested land is not known. Given the widespread distribution of *M. incognita* and the suit-

TABLE 4. Mean dry weight (g) of roots from which eggs were extracted for each genotype of kenaf (*Hibiscus cannabinus*) individually infected by the four races of *Meloidogyne incognita.*

Genotype	Race 1	Race 2	Race 3	Race 4	Genotype mean
Cubano	4.3 ± 0.5	3.6 ± 1.8	3.5 ± 0.3	4.4 ± 1.2	4.0 a†
Everglades 41	2.4 ± 1.5	4.8 ± 1.4	2.6 ± 1.2	2.5 ± 0.4	3.1 ab
Everglades 71	3.7 ± 0.8	5.3 ± 4.7	4.6 ± 5.6	3.8 ± 0.2	3.5 ab
Guatemala 4	4.8 ± 2.4	4.1 ± 1.0	2.9 ± 1.3	3.4 ± 1.6	3.8 a
PI 318723	0.7 ± 0.8	0.5 ± 0.2	0.8 ± 0.5	1.3 ± 1.2	0.8 d
19-117-2	2.0 ± 0.8	5.2 ± 4.7	0.1‡	0.2	2.4 bc
45-9	1.1 ± 0.8	1.9	0.6	1.3	1.2 cd
15-2	3.0 ± 1.1	3.8 ± 0.8	1.2 ± 0.9	2.1 ± 1.8	2.5 b
			Race mean		
	2.8 *xy*	3.7 *x*	1.9 *y*	2.6 *y*	

Data are means ± standard deviation of three replications.
† Untransformed means followed by the same letter are not different based on Fisher's protected LSD test ($P \leq 0.05$). Mean separation test based on analysis and means of transformed data.
‡ Blank spaces = insufficient data to compute standard deviation.

ability will translate into improved crop yields on nematode-infested land is not known. Given the widespread distribution of *M. incognita* and the suitability of kenaf to all of the four recognized races, crop improvement by breeding for tolerance (the ability to yield effectively upon challenge by the parasite) may be more productive than breeding for reduced host suitability (resistance).

Clearly, kenaf production in the cotton belt will be seriously challenged by *M. incognita*. It is important now to obtain information on the sensitivity of kenaf to nematodes, especially the *M. incognita*, under field conditions.

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