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Damage Potential and Reproduction of *Meloidogyne incognita* Race 3 and *M. arenaria* Race 1 on Kenaf[1]

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Abstract: The effects of *Meloidogyne incognita* race 3 and *M. arenaria* race 1 on growth of kenaf cv. Everglades 41 was determined under greenhouse conditions. Seedlings of kenaf were inoculated with initial population densities (Pi) of 0, 625, 1,250, 2,500, 5,000, and 10,000 eggs/plant and placed on greenhouse benches in a randomized complete block design. Plant growth and nematode reproduction were assessed 6 and 12 weeks after inoculation. Growth suppression of kenaf in response to increasing Pi was observed 6 weeks after inoculation. Severe damage was observed by all Pi levels of *M. incognita* and *M. arenaria* at 12 weeks after inoculation. Plant height, basal stem diameter, and fresh and dry shoot weights had a negative linear relationship to $\log_{10}(Pi + 1)$ for both *M. incognita* and *M. arenaria*. Plant height was reduced 25%, basal stem diameter was reduced 19%, and dry shoot weights were reduced 64% at the highest inoculum rates of *M. incognita*. Similar reductions were observed for *M. arenaria*. Greater levels of root necrosis were observed with *M. incognita* than *M. arenaria* at similar inoculum levels. High reproductive factors (Rf) were observed on kenaf for both *M. incognita* (48 – 1,804) and *M. arenaria* (257 – 4,240), with the highest Rf values occurring at the lowest Pi. The host status of kenaf renders it unsuitable for use in rotation systems with other susceptible crops.

Key words: gall index, greenhouse, *Hibiscus cannabinus,* kenaf, *Meloidogyne arenaria, Meloidogyne incognita,* plant height, reproduction, root necrosis, root-knot nematode, yield.

Kenaf (*Hibiscus cannabinus* L.), a short-day and rapidly growing plant (7), is cultivated throughout much of tropical America and Asia for the soft bast (phloem) fiber found in the stem (12,16,17,19). Kenaf is being considered as a potential new crop in the coastal plain of the southeastern United States (10). The stem and leaves of kenaf can be used for forage, silage, biomass production, and fiber extraction. Fiber from the bast and once (xylem) is of sufficient quality to make a high-grade newsprint pulp (3). Although previous attempts to produce kenaf commercially in the southern United States as a supplement to tree wood pulp were abandoned as economically unsound, efforts have been renewed because of its potential use for making specialty papers, such as high-quality filter paper. This plant most likely will be planted in fields previously cropped in cotton (*Gossypium hirsutum* L.) and peanut (*Arachis hypogaea* L.) in the southeastern United States. Kenaf and cotton are taxonomically close relatives in the Malvaceae family.

Root-knot nematode, *Meloidogyne* spp., is one of the major problems associated with the successful cultivation of kenaf (7, 10,18). Root-knot nematodes have been reported to cause 20% to 60% yield losses on kenaf (4,10,13,15). Earlier research (4, 6,10,14,18,20,21) demonstrated that kenaf is susceptible to three major root-knot nematode species: *M. incognita, M. javanica,* and *M. arenaria.* In greenhouse tests, kenaf was susceptible to all host races of *M. incognita* (20,21), to *M. arenaria,* and to *M. javanica* (10). In field tests, severe galling and yield losses occurred when kenaf was infected by *M. incognita* (1,22). Only at higher initial population densities of *M. incognita* (5,000/500 cm^3 soil) were plant height, stalk diameter, and root and stem weights significantly reduced (4).

Meloidogyne incognita and *M. arenaria* are commonly found in agricultural soils of Georgia in association with cotton and peanut, which are hosts for *M. incognita* and *M. arenaria,* respectively. Soybean, a host for both species, also contributes to their widespread distribution. Although reports have shown that both *M. incognita* and *M. arenaria* were damaging to specific kenaf

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cultivars, the damage potential of *M. incognita* race 3 and *M. arenaria* race 1 from Georgia on kenaf has not been determined. The object of this research was to examine the damage function and reproduction of both *M. incognita* race 3 and *M. arenaria* race 1 on kenaf cv. Everglades 41 under greenhouse conditions.

Materials and Methods

Seeds of Kenaf cv. Everglades 41, a cultivar which is being evaluated for production in Georgia, were germinated in vermiculite for 4 days. The seedlings then were transplanted into 15-cm-diam. plastic pots filled with methyl bromide-treated (1.7 kg a.i./m^3) loamy sand soil (84% sand, 8% silt, and 8% clay) and grown for 5 days before inoculation with nematodes.

Populations of *M. incognita* race 3, and *M. arenaria* race 1 were obtained from greenhouse cultures of cotton and peanut, respectively, and were increased on tomato cv. 'Marglobe' for 2 months before the experiment. Eggs were collected using an NaOCl blender extraction method (8). Egg solutions were adjusted to 10-ml aqueous suspensions for each inoculation level per plant and pipetted into depressions in the soil around the root system of each seedling. The six inoculum levels were 0, 625, 1,250, 2,500, 5,000, and 10,000 eggs per plant. Control plants received the same volume of egg-free root extraction filtrate. Each inoculum density was replicated four times, and pots were arranged in a randomized complete block design on greenhouse benches. All pots were watered gently after inoculation. Plants were watered twice a day with tap water and fertilized weekly with water-soluble N-P-K fertilizer (20-20-20). The greenhouse temperature was set at 27 °C, and supplemental light (photosynthetic photon flux density of 310 $\mu mol \cdot s^{-1} \cdot m^{-2}$ in the 400–700 nm waveband, 1.3 m from the greenhouse bench) was supplied for 14 hours/day. The experiment was repeated.

One group of plants was grown for 6 weeks after inoculation before harvesting. The other group was transplanted into 20-cm plastic pots 4 weeks after inoculation and allowed to grow for another 8 weeks before harvest (total growth for 12 weeks). For the 4-week transplant, the contents of the 15-cm pots were carefully transferred to 20-cm pots and additional greenhouse soil mix was added to each pot. This procedure was required to allow additional room for root growth during the final 6 weeks of the experiment.

At each harvest date, plant height was measured, and the shoot was cut at the base of the plant and weighed. The soil and root system was removed from each pot, and roots were separated gently from soil. A 500-cm^3 sample was taken from the soil of each pot. Second-stage juveniles (J2) were extracted from each sample by elutriation and centrifugation (5,9). After weighing, the root system of each plant was cut into 2-cm pieces and put into a 1,000-ml plastic bottle for NaOCl-blender extraction of nematode eggs (8). Both shoot and root systems were oven-dried at 60 °C for 5 days before determining dry weights. At the 12-week harvest, the basal stem diameters were determined, and gall index (0-10, 0 = no galling) and percentage root necrosis were rated.

Data from each harvest date were analyzed by analysis of variance, and means of shoot height, basal stem diameter, fresh and dry shoot weights, fresh and dry root weights, and nematode reproduction were separated with a Waller-Duncan test (P = 0.05). Linear regressions (11) were performed on plant height, basal stem diameter, fresh root weight, fresh and dry shoot weights versus $\log_{10}(x + 1)$ transformed initial population densities of nematodes.

Results

At 6 weeks after inoculation, dry shoot and dry root weights (g) of kenaf decreased in response to increasing Pi of *M. incognita* ($Y = 26.9 - 1.7 \log_{10}(x + 1)$, $R^2 = 0.29$, $P = 0.001$, and $Y = 14.4 - 1.4 \log_{10}(x + 1)$, $R^2 = 0.12$, $P = 0.05$, respectively). Similarly, a negative response to *M.*

arenaria was observed for shoot height (cm) and dry root weights ($Y = 176.9 - 4.5 \log_{10}(x + 1)$, $R^2 = 0.16$, $P = 0.05$, and $Y = 14.8 - 1.8 \log_{10}(x + 1)$, $R^2 = 0.27$, $P = 0.001$, respectively), but no relationship was observed for dry shoot weights. The reproductive factors (Rf) for both species of root knot were more than 100 for all inoculum levels (data not shown). No root necrosis was observed for any of the treatments at 6 weeks after inoculation.

At 12 weeks after inoculation, both *M. incognita* and *M. arenaria* caused severe damage to kenaf. Inoculation with the highest Pi of *M. incognita* decreased kenaf shoot height 26% (250 to 186 cm, Fig. 1A) and basal stem diameters 19% (20 to 16 mm, Fig. 1B) from the control. Fresh and dry shoot weights showed similar decreasing responses to increasing Pi of *M. incognita,* with fresh shoot weight decreasing 66% (217 to 73 g from control to highest inoculum rate, respectively) and dry shoot weight decreasing 71% (55 to 16 g from

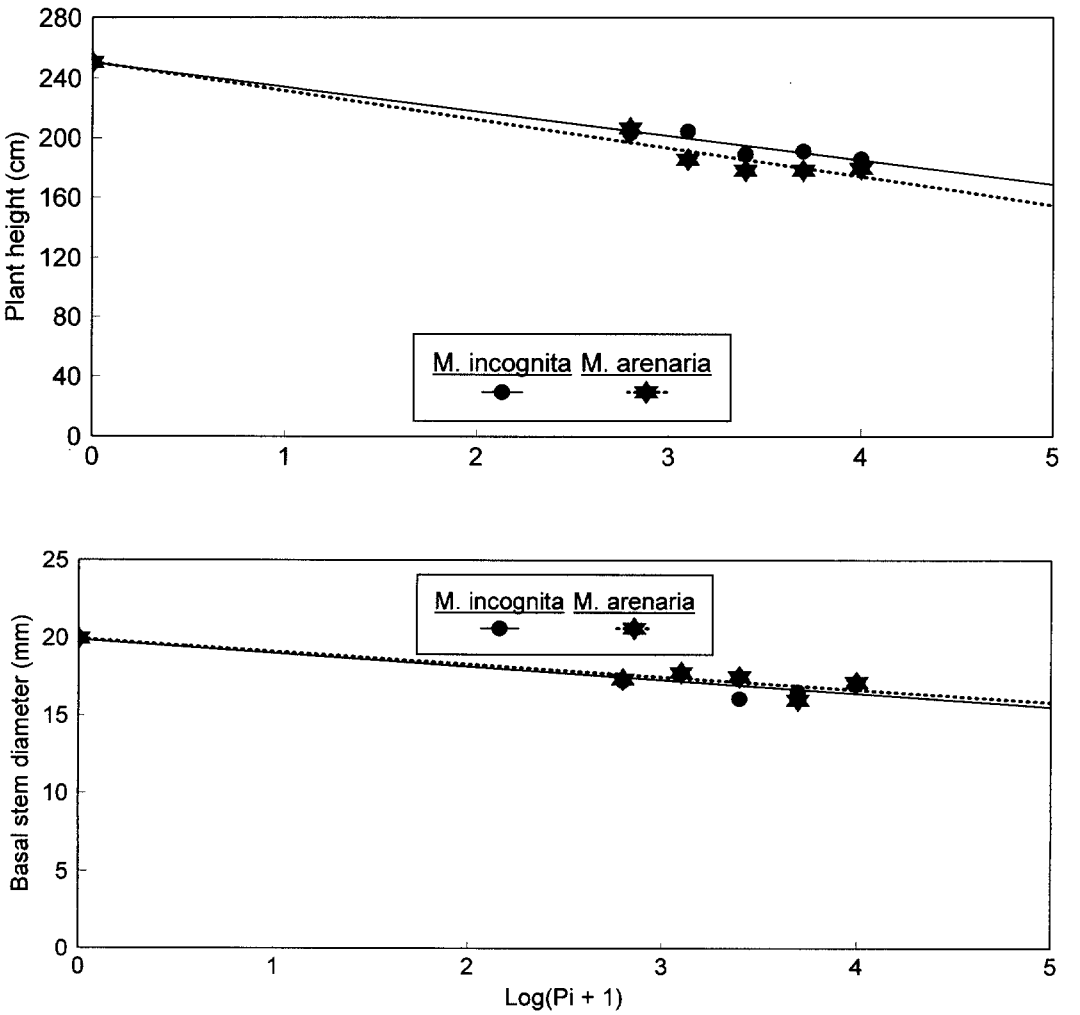

FIG. 1. Relationship between initial population densities of *Meloidogyne incognita* race 3 and *M. arenaria* race 1 to plant height (PH) and basal stem diameter (BSD) of kenaf cv. Everglades 41 at 12 weeks after inoculation in a greenhouse. Data are means of eight replications. $PH = 252.9 - 16.6 \log_{10}(Pi + 1)$, $R^2 = 0.49$, $P = 0.0001$ for *M. incognita,* and $PH = 251.1 - 19.3 \log_{10}(Pi + 1)$, $R^2 = 0.32$, $P = 0.01$ for *M. arenaria*; $BSD = 19.9 - 0.9 \log_{10}(Pi + 1)$, $R^2 = 0.57$, $P = 0.0001$ for *M. incognita,* and $BSD = 19.9 - 0.8 \log_{10}(Pi + 1)$, $R^2 = 0.61$, $P = 0.0001$ for *M. arenaria.*

control to highest inoculum rate, respectively, Fig. 2). Kenaf root necrosis ratings (%) increased in a linear response to increasing Pi of *M. incognita* to a level at which for a Pi = 10,000, 66% of the total root system exhibited necrosis (Fig. 3A). The relationship of fresh root weights to increasing *M. incognita* Pi was best fit by a quadratic model, indicating that, relative to the control, root weights actually increased at lower inoculum levels but decreased 51% at the highest Pi (167 to 82 g from control to highest inoculum rate, respectively, Fig. 3B).

Inoculation with *M. arenaria* decreased kenaf shoot height 28% (250 to 180 cm, Fig. 1A) and basal stem diameters 18% (20 to 17 mm, Fig. 1B) from the control to the highest inoculum level. Fresh and dry shoot weights decreased linearly in response to increasing Pi of *M. arenaria,* with fresh shoot weight decreasing 57% (217 to 94 g from control to highest inoculum rate, respectively) and dry shoot weight decreasing 69% (55 to 17 g from control to highest inoculum rate, respectively, Fig. 2). Kenaf root necrosis ratings (%) increased in a linear response to increasing Pi of *M. arenaria* to a level at which 41% of the total root system exhibited necrosis for a Pi of 10,000 (Fig. 3A). As was observed for *M. incognita,* the relationship of fresh root weights to increasing Pi of *M. arenaria* was best fit by a quadratic model, indicating that root weights increased at lower inoculum levels but decreased 24% at the highest Pi (167 to 128 g from control to highest inoculum rate, respectively, Fig. 3B). Increases in root necrosis were higher for *M. incognita* then for *M. arenaria,* indicating that *M. incognita* was more pathogenic than *M. arenaria* to roots of kenaf cv. Everglades 41.

High reproductive factors were calculated from all of the inoculum levels of both *M. incognita* and *M. arenaria,* although higher Pi values resulted in lower Rf values in these greenhouse pot experiments. Final population densities (Pf) of *M. incognita* decreased in a linear response to increasing Pi (Fig. 4A), but no relationship was observed between Pf and Pi for *M. arenaria,* where final nematode densities averaged 287,000 eggs + J2/100 cm^3 soil. Nematode Rf values decreased with increasing Pi, but were consistently 3 to 4 times higher for *M. arenaria* than for *M. incognita* (Rf = 257 − 4,240 for *M. arenaria* as compared to 48 − 1,804 for *M. incognita,* Fig. 4B).

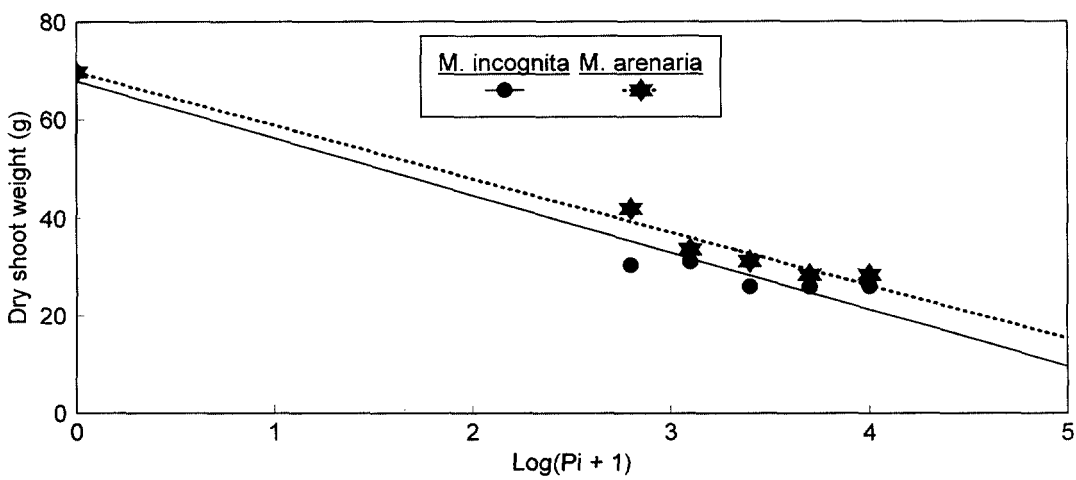

FIG. 2. Relationship between initial population densities (Pi) of *M. incognita* race 3 and *M. arenaria* race 1 to dry shoot weight (DSW) of kenaf cv. Everglades 41 at 12 weeks after inoculation in a greenhouse. Data are means of eight replications. DSW = 67.9 − 11.6 $\log_{10}$(Pi + 1), R^2 = 0.70, P = 0.0001 for *M. incognita,* and DSW = 69.7 − 10.9 $\log_{10}$(Pi + 1), R^2 = 0.49, P = 0.0001 for *M. arenaria.*

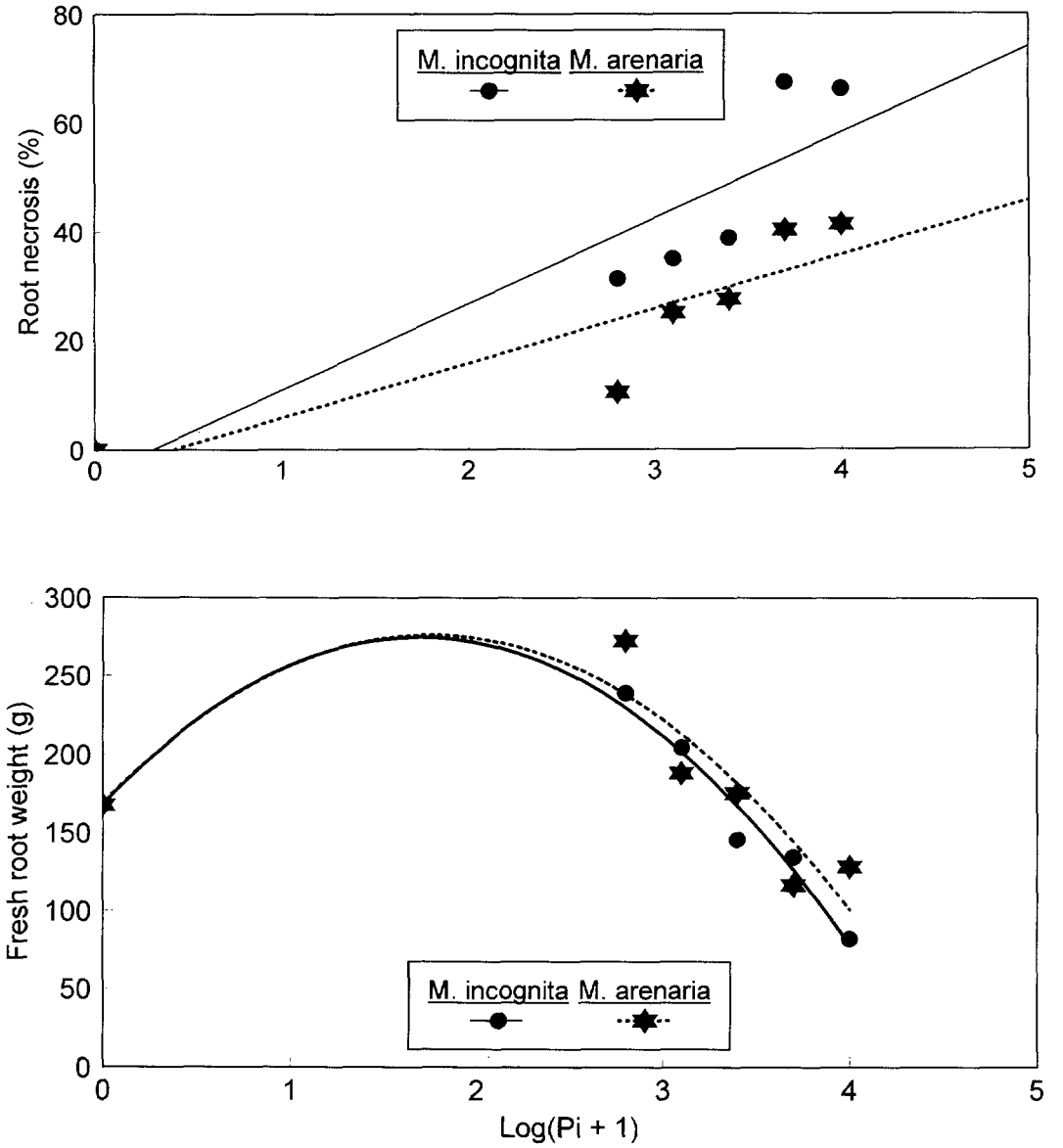

FIG. 3. Relationship between initial population densities (Pi) of *M. incognita* race 3 and *M. arenaria* race 1 to root necrosis (RN) and fresh root weight (FRW) of kenaf cv. Everglades 41 at 12 weeks after inoculation in a greenhouse. Data are means of eight replications. RN = $-4.6 + 15.8 \log_{10}(\text{Pi} + 1)$, $R^2 = 0.45$, $P = 0.001$ for *M. incognita*, and RN = $-4.2 + 9.94 \log_{10}(\text{Pi} + 1)$ $R^2 = 0.46$, $P = 0.0001$ for *M. arenaria*. FRW = $167.99 + 125 \log_{10}(\text{Pi} + 1) - 37(\log_{10}(\text{Pi} + 1))^2$, $R^2 = 0.52$, $P = 0.001$ for *M. incognita*, and FRW = $169 + 122 \log_{10}(\text{Pi} + 1) - 35(\log_{10}(\text{Pi} + 1))^2$, $R^2 = 0.35$, $P = 0.01$ for *M. arenaria*.

DISCUSSION

Both *M. incognita* and *M. arenaria* have tremendous damage potential on kenaf cv. Everglades 41. At the lowest inoculum level of 625 eggs per pot (approximately 50 eggs/100 cm^3 soil), both of these nematodes caused substantial growth suppression and root necrosis, and at the highest inoculum levels (10,000 eggs/pot, or approximately 650 eggs/100 cm^3 soil) profitable kenaf cultivation could not be considered. Previous greenhouse experiments reported 90% reductions in root weights

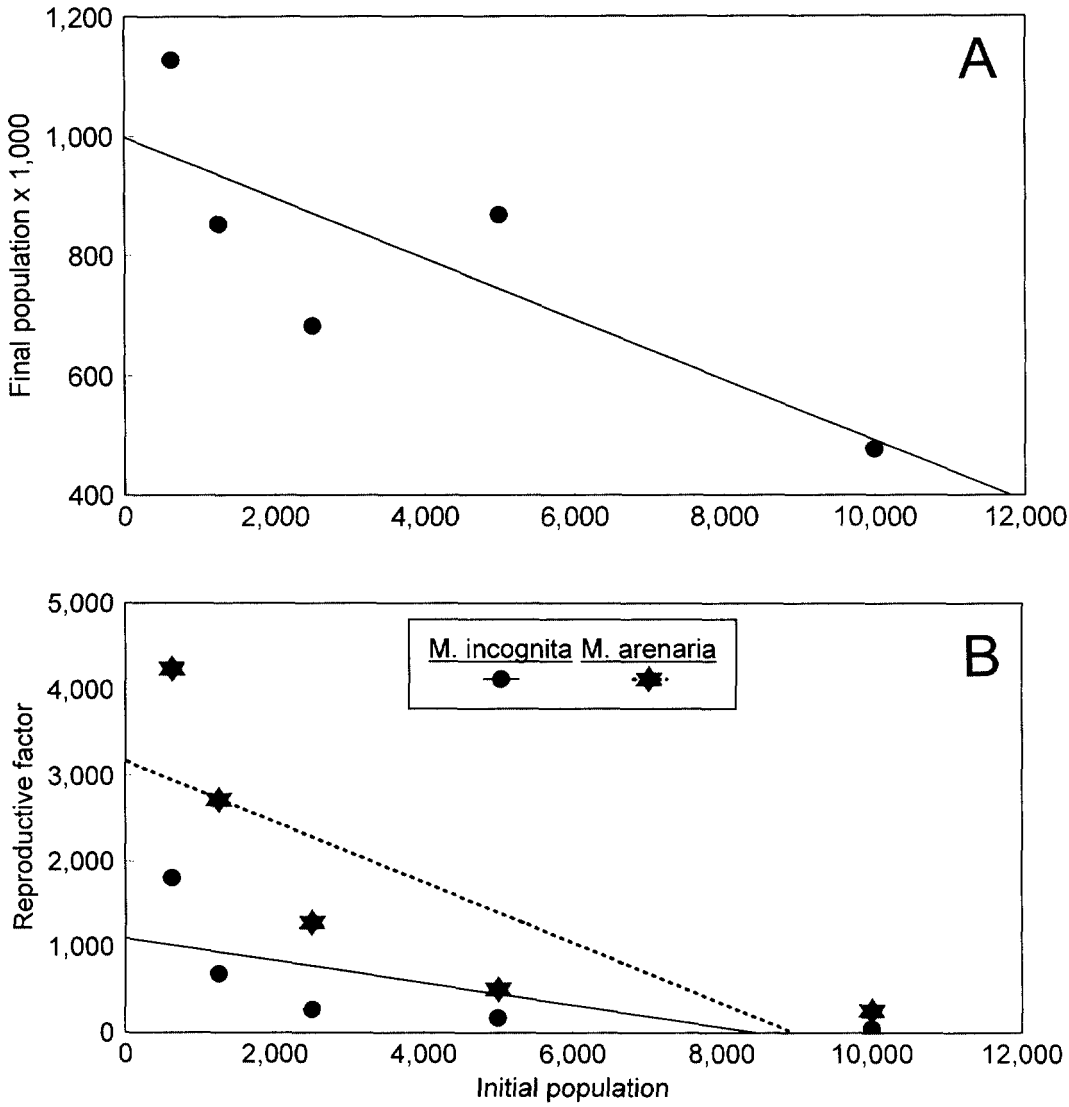

FIG. 4. A) Relationship between initial population (Pi) and the final population (Pf) of *M. incognita* race 3, and B) Reproductive factor (RF) of both *M. incognita* race 3 and *M. arenaria* race 1 on kenaf cv. Everglades 41 at 12 weeks after inoculation in a greenhouse. Data are means of eight replications. Pf = 998,582 − 50.6Pi, R^2 = 0.34, P = 0.01, RF = 1,099 − 0.13Pi, R^2 = 0.75, P = 0.0001 for *M. incognita*, RF = 3,169 − 0.35Pi, R^2 = 0.77, P = 0.0001 for *M. arenaria*.

(10) and 46% reductions in dry shoot weights (4) of kenaf 'Tainung 1' only 60 days after inoculation with *M. incognita*. At the 6-week observation in these experiments, plant growth parameters were not suppressed extensively by either *M. incognita* or *M. arenaria* at any inoculum level, but the reproductive factors of these two nematodes were more than 100 for all of the inoculum densities. Greenhouse experiments with *M. incognita* on kenaf 'Tainung 1' showed 90- to 180-fold increases in population densities after 60 days (4,10). Kenaf typically is cultivated for 180 or more days, so that even relatively low numbers of root-knot nematodes would likely build up to damaging levels before harvest. Dry shoot weights and basal stem diameters are most representative of grower yields, and the 50% to 60%

decreases observed in these parameters after 12 weeks indicates that cultivation of kenaf would not be profitable on land heavily infested with root-knot nematodes.

Our results indicate that the population of *M. incognita* race 3 was more pathogenic to kenaf than the population of *M. arenaria* race 1 used in these experiments. The higher level of pathogenicity was most apparent in root damage indices, whereas few or no differences were observed in the economically important shoot growth parameters. However, the plants were grown under ideal greenhouse conditions, with adequate water and nutrition supplied. It is likely that root damage would result in more shoot damage under stressful field conditions. Kenaf is susceptible to other species of root-knot nematodes although *M. javanica* is reported to be less pathogenic on kenaf than either *M. arenaria* or *M. incognita* (2). Reproductive factors were greater for *M. arenaria* than for *M. incognita,* probably because the greater levels of root damage caused by *M. incognita* suppressed nematode reproduction at 12 weeks.

Because both *M. incognita* and *M. arenaria* have high damage potentials and reproductive capacities on kenaf, more research is needed for the development of resistant or tolerant kenaf cultivars. Currently, chemical control methods may be required in nematode-infested soil. Crop rotation patterns will need intensive evaluation, not only in terms of the potential for reducing root-knot nematode numbers prior to a kenaf crop but also in terms of the extremely high residual population densities that can be expected following cultivation of kenaf. Rotating kenaf with peanut where *M. arenaria* is present, or cotton where *M. incognita* is present, could result in heavy crop losses. Research to determine the best potential areas for cultivation of kenaf in the southeastern United States also is needed.

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