Resistance of *Cucumis* spp. to the Root-knot Nematode, *Meloidogyne incognita acrita*

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Abstract: The nature of resistance in *Cucumis ficifolius* and *C. metuliferus* to the root-knot nematode, *Meloidogyne incognita acrita*, was studied under greenhouse conditions. Although as many larvae penetrated the roots of these species as those of the susceptible *C. melo*, few developed to the adult female stage. Resistance in *C. ficifolius* and *C. metuliferus* was associated with hindrance of larval development beyond the second stage, delayed development of larvae to adults and stimulation toward maleness. Tissue necrosis or hypersensitivity was not associated with larval penetration. Comparisons of the histopathology of 26-day-old infections of *C. melo* and *C. metuliferus* roots showed no observable differences in the type of giant cell development in regions of roots associated with adult females. However, in *C. metuliferus* immature nematodes were associated with small giant cells which were limited to a few cells near the head of the nematode. *Key Words:* Resistance, Root-knot nematodes, *Meloidogyne incognita acrita*, *Cucumis ficifolius*, *Cucumis metuliferus*, *Cucumis melo*, Cantaloup.

Nematodes are serious pathogens of cantaloup (*Cucumis melo*) causing annual losses of over two million dollars in the United States (10). In South Carolina root-knot nematodes (*Meloidogyne* spp.) often cause extensive damage to melon crops.

Reports of resistance in *C. melo* to root-knot nematodes are scant. Thomason and McKinney (15) reported no resistance in 34 varieties of melons to *Meloidogyne incognita acrita*, *M. javanica* and to one population of *M. hapla*. Winstead and Sasser (18) found that the West India gherkin (*C. anguria*) was resistant to *M. incognita* and *M. arenaria*. Fassuliotis and Rau (7) also reported that the gherkin, *C. anguria* (PI-233646), was resistant to *M. incognita acrita*. Resistance was also found in *C. ficifolius*, *C. metuliferus*, *C. heptadactylus*, and *C. longipes* (6).

An intensive breeding program to incorporate root-knot nematode resistance in melons was begun at the U. S. Vegetable Breeding Laboratory near Charleston, South Carolina. However, interspecific crosses with *C. melo* were unsuccessful, usually resulting in fruit with non-viable seeds. Numerous attempts at embryo culture at frequent intervals following hand pollination were also unsuccessful. However, Dr. J. D. Norton at Auburn, Alabama, recently obtained a successful cross between *C. melo* (PI-140471) × *C. metuliferus* (personal communication).

The purpose of this investigation was to study the nature of resistance in two species of *Cucumis* to *M. incognita acrita* Chitwood under greenhouse conditions.

MATERIALS AND METHODS

The plant species studied were *C. ficifolius* (C-779), *C. metuliferus* (C-701), and *C. melo* ('Hale's Best Jumbo'). Seventy-five seeds of each of the three species were planted in washed builders' sand in a 20 × 25 cm flat and periodically watered with a commercial nutrient solution. Root-knot nematodes isolated from Irish potatoes grown in Wadmalaw Island, S. C. (14) were increased on 'Homestead' tomato (*Lycopersicon esculentum* Mill.). Egg masses were hand-picked from roots and larvae were hatched in water at 25 C. When the plants were at the two-leaf stage, approximately 15,000 larvae in 200 ml of water were added to the flat by distributing the larval suspension around each plant with a plastic wash

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bottle. This was followed by a gentle watering of the sand. Three days after inoculation seedlings were carefully removed and sorted according to species by the size and shape of the cotyledons. They were thoroughly washed and replanted again into washed sand in a flat. Roots of 10 plants of each species were stained with acid fuchsin in lactophenol (11) immediately after washing (3rd day) and again on the 9th, 16th and 26th days after the first day of inoculation. Root tips collected 3 days after inoculation were cut into approximately 1 cm lengths, pressed between microscope slides, and examined for larval penetration with a dissecting microscope at 30 ×. The nematode counts (N) were transformed to $\sqrt{N + 0.5}$ and analyzed using Duncan's Multiple Range Test. Older roots, 9, 16, and 26 days after inoculation, were dissected to remove embedded nematodes. These nematodes were mounted in clear lactophenol and examined at 250 ×. The stage of development and sex were determined using the figures published by Triantaphyllou and Hirschmann (17). In addition, roots of 10 plants of the 26-day collection of each species were macerated in Jeffrey's solution (5) and the number of females were counted following procedures previously described (6).

Histopathological comparisons of infected roots of *C. metuliferus* and *C. melo* were made 26 days after inoculation. Roots were placed in CRAF fixative for 24 hr, embedded in paraffin, sectioned at 10 μ and stained with safranin and fast green (8).

RESULTS AND DISCUSSION

The number of larvae entering the roots of the three *Cucumis* species did not differ significantly (Table 1). Development of *M. incognita acrita* was more rapid in *C. melo* roots than in the other two species. As early as 9 days after inoculation, larvae dissected from *C. melo* were more advanced than those recovered from *C. metuliferus* and *C. ficifolius*.

TABLE 1. Infectivity of *Meloidogyne incognita acrita* larvae to three species of *Cucumis* three days after inoculation.

Species	Average No.[a]	$\sqrt{N + 0.5}$[b]
C. ficifolius	6.51	2.31[a]
C. metuliferus	21.39	4.13[a]
C. melo ('Hale's Best Jumbo')	16.55	3.78[a]

[a] Average number of larvae from ten 1-cm root terminals from 8 plants.

[b] All infectivity data were transformed to $\sqrt{N + 0.5}$ prior to statistical analyses. Values followed by the same lower case letters are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Further lag in developmental rate was evident in the 16th and 26th day root collections (Fig. 1). Sixteen days after inoculation only 16% of the second-stage larvae in *C. melo* were sexually undifferentiated (Fig. 1-B) as compared with 44 and 41% for *C. metuliferus* and *C. ficifolius*, respectively. Forty-four percent of the larvae had developed to the saccate female stage in *C. melo* (Fig 1-E) in contrast with approximately 4% for the other two species. Twenty-six days after inoculation the same tendency in the developmental pattern was maintained. Seventy-eight percent of the nematodes in *C. melo* had developed to the saccate, adult female stage compared to 37 and 22% in *C. metuliferus* and *C. ficifolius*, respectively (Fig. 1-E). Approximately 20% of the total number of larvae from the roots of *C. metuliferus* and *C. ficifolius* were developing into males 16 and 26 days after inoculation (Fig. 1-F), whereas in *C. melo* the developing male population was 7 and 2% at 16 and 26 days, respectively. Increase in the proportion of males has been associated with environmental stresses which induce larvae to develop as males instead of females (16). At 26 days after inoculation, 20 and 29% of the nematodes within the roots of *C. metuliferus* and

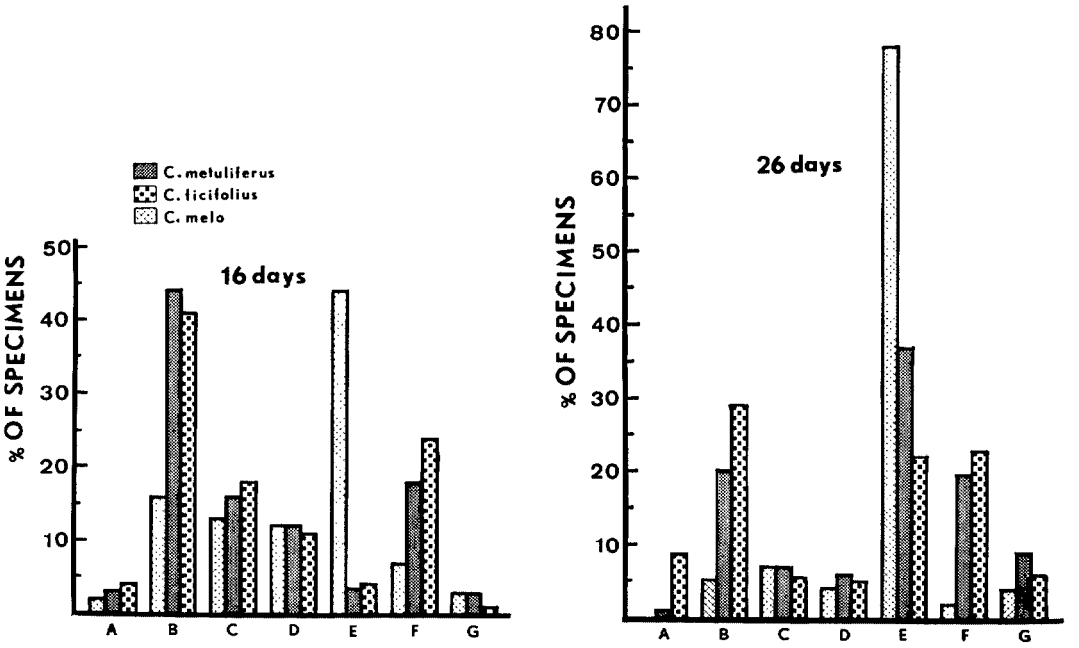

FIG. 1. Distribution of development stages of *Meloidogyne incognita acrita* on 3 *Cucumis* species 16 and 26 days after inoculation. **A.** Second stage infective larvae; **B.** sexually undifferentiated second stage larvae; **C.** Developing female larvae second through fourth stage); **D.** Molted fourth stage female larvae; **E.** Saccate adult females; **F.** Developing male larvae (late second through early fourth stage); **G.** Fourth stage male larvae.

C. ficifolius, respectively were still sexually undifferentiated second-stage larvae compared to only 5% in *C. melo* (Fig. 1-B).

Resistance in *C. metuliferus* and *C. ficifolius* apparently is associated with (a) hindrance of larval development beyond the second stage, (b) delayed development of larvae to adults, and (c) increased stimulation toward maleness. These effects were also reflected in the significantly fewer females recovered after maceration from the wild species than from *C. melo* (Table 2).

Unlike the extensive gall response associated with root-knot nematode infection in *C. melo*, the two wild species showed only slight swelling. No necrosis or hypersensitive reaction was observed associated with larval invasion. This contrasts with reports on resistance in tomato, tobacco, cotton, soybean and other plants (3, 4, 12, 13). However, Dropkin (2) found that the hypersensitive response was not entirely necessary for expression of plant resistance.

Comparisons of the histopathology of 26-day-old infections of *C. melo* and *C. metuliferus* showed no observable differences in the

TABLE 2. Recovery of female *Meloidogyne incognita acrita* from three species of *Cucumis* 26 days after inoculation.

Species	Average Root Knot Index	Average No.[a] Females/ Gram Root
C. ficifolius	2.0	17.8[a]
C. metuliferus	2.0	23.0[a]
C. melo ('Hale's Best Jumbo')	4.3	150.2[b]

[a] Values followed by the same lower case letters are not significantly different according to Duncan's Multiple Range Test at the 5% level.

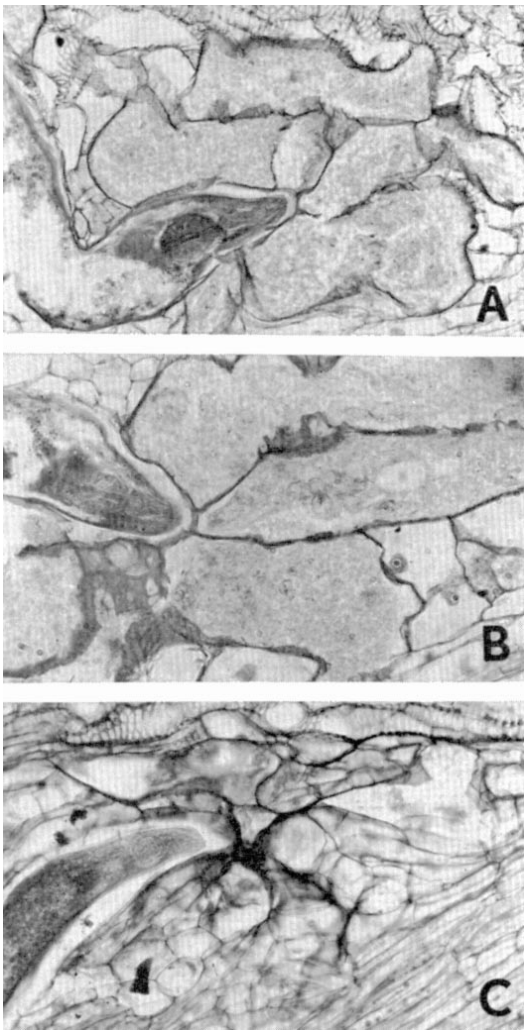

FIG. 2. *Cucumis* roots 26 days after attack by *Meloidogyne incognita acrita*. Successful host-parasite relationship with large giant cells. **A**. in *C. melo* 'Hale's Best Jumbo'; **B**. in *C. metuliferus*; and **C**. Unsuccessful host-parasite relationship in *C. metuliferus*. Giant cell formation is limited to a few cells around the head of the nematode.

type of giant cell development in regions of roots associated with adult females (Fig. 2-A and B). Giant cell formation was limited to the vascular cylinder and contained dense cytoplasm, and enlarged nuclei (9). In *C. metuliferus* both normal giant cell formation and small giant cells were found. In regions of root where giant cells were small, the nematodes were immature. In these cases cellular stimulation was limited to a few cells near the head of the nematode with a limited amount of hypertrophy and cell-wall dissolution (Fig. 2-C). This is consistent with the postulate that giant cell formation is necessary for nematode growth and development (1).

Wild species of *Cucumis* are important as potential multigenic sources for multiple resistance to nematodes, insects and other diseases. Norton's recent success in breaking the barriers to interspecific hybridization, will make it practical for breeders to develop root-knot nematode-resistant commercial melon varieties.

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