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Progression of Root-knot Nematode Symptoms and Infection on Resistant and Susceptible Cottons

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Abstract: Progressive development in cotton root morphology of resistant A623 and susceptible M-8 cotton (*Gossypium hirsutum* L.) lines following infection by the root-knot nematode *Meloidogyne incognita* was studied in glass front boxes. Symptom development and radicle growth were observed; degree of galling, gall and egg mass diameter, and number of eggs per egg mass were recorded; and root segments were examined histologically. Small cracks caused by *M. incognita* appeared in the root epidermis and cortex soon after the cotyledons expanded on day 4. The cracks were longer and wider and extended through the cortex when the first true leaf became visible at day 8. Galls had formed on taproots by this time. When exposed to *M. incognita,* A623 had faster radicle growth (22%), fewer and smaller cracks in the root epidermis and cortex, fewer and smaller root galls, one-twelfth as many egg masses, and one-fourth as many eggs per egg mass as M-8. Root cracking, galling, and giant cell formation are major effects of *M. incognita* that may predispose cotton roots to pathogens resulting in synergistic interactions and diseases.
Key words: giant cell, *Gossypium hirsutum, Meloidogyne incognita,* multinucleated cell, resistance, root cracking, root galling, root-knot nematode, seedling disease, southern root-knot nematode.

Root galling and stunting of cotton (*Gossypium hirsutum* L.) by the root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood have been reported extensively in the literature. *M. incognita* increases the incidence and severity of cotton seedling diseases (1,7,12,20). In addition, Fusarium wilt of cotton, caused by *Fusarium oxysporum* Schlect f. *vasinfectum* (Atk) Synd. & Hans., was greatly increased in both seedlings and mature plants in the presence of *M. incognita* (8,11,18).

The mechanism by which *M. incognita* predisposes cotton plants to disease has not been fully explained. *M. incognita* may transport the Fusarium wilt fungus into roots or the fungus may invade through wounds made when *M. incognita* enters the roots (19). Stress and debilitation of seedlings caused by *M. incognita* damage may be major factors in the predisposition, and *M. incognita*-induced giant cells may attract and (or) be more easily parasitized by fungi than normal cells (2). *M. incognita* may induce production of a translocatable factor or factors that can predispose plant parts distant from the site of *M. incognita* infection (17). Root splits are caused by *M. incognita* (9,14), and the splits may be avenues through which the *F. oxysporum* f. *vasinfectum* and other disease-causing organisms invade roots (14).

The objectives of this research were to characterize the development of *M. incognita*-induced plant symptoms on cotton cultivars resistant and susceptible to the nematode and to identify those effects that would most likely predispose cotton plants to diseases.

MATERIALS AND METHODS

Glass front plastic boxes, 10 cm from front to back × 30 cm wide × 47 cm deep, were filled with methyl bromide-fumigated Wickham sandy loam soil (65% sand, 24% silt, 10% clay, 1% organic matter), a fine loamy, mixed thermic typic Hapudult. Two seeds of Auburn 623 RNR (A623), a root-knot nematode resistant breeding stock (15), or M-8, a susceptible doubled haploid of Deltapine 14 cultivar, were planted in each box. Seeds were germinated in petri dishes, and seeds with radicles just emerging were placed in holes 7.5 cm apart and 3.7 cm deep next to the glass front of the boxes and covered with soil. Twenty-four hours after planting (day 0), 2,000 *M. incognita* juveniles in 0.5 ml sterile water (0.5 ml sterile water only for checks) were pipetted into the soil 5 mm directly below

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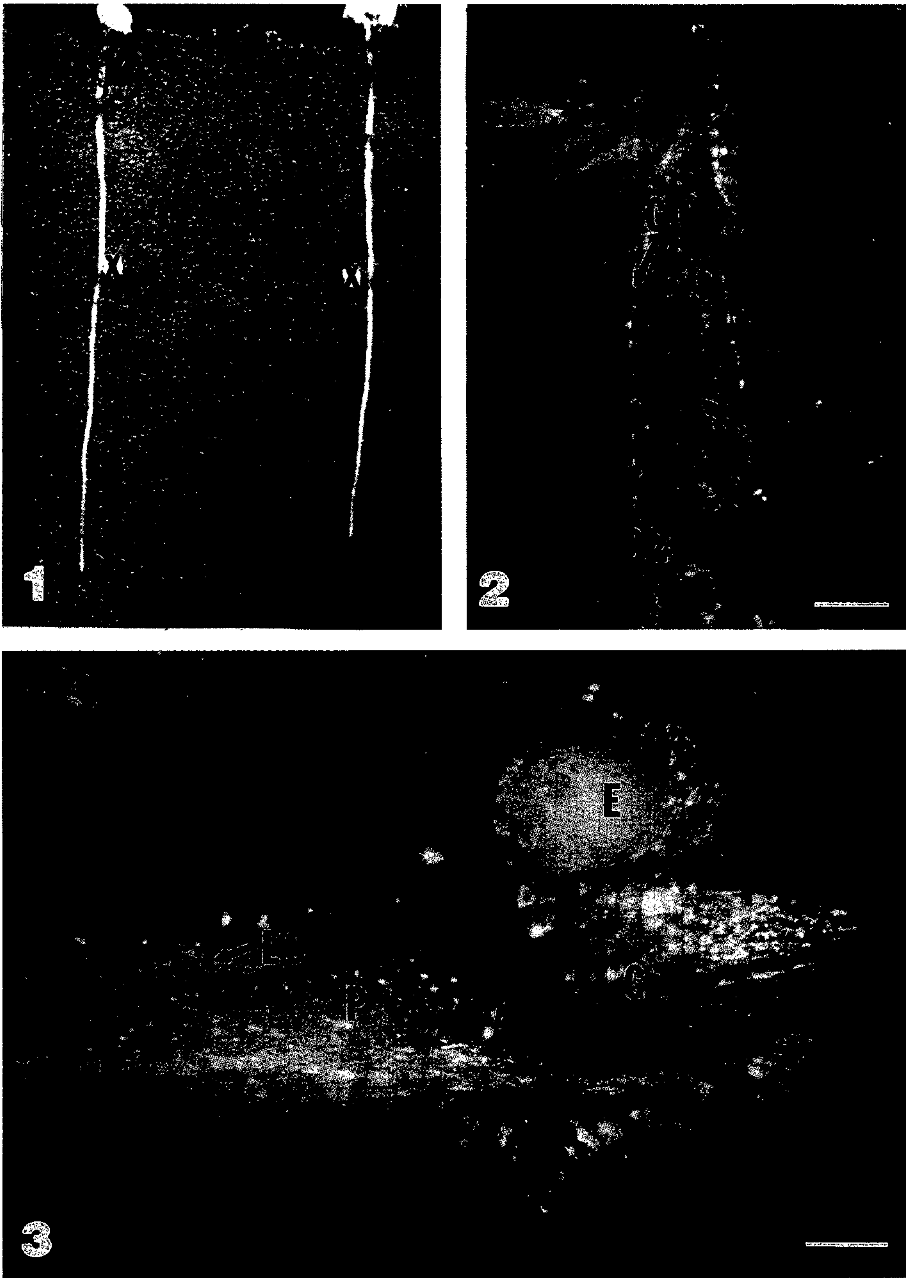
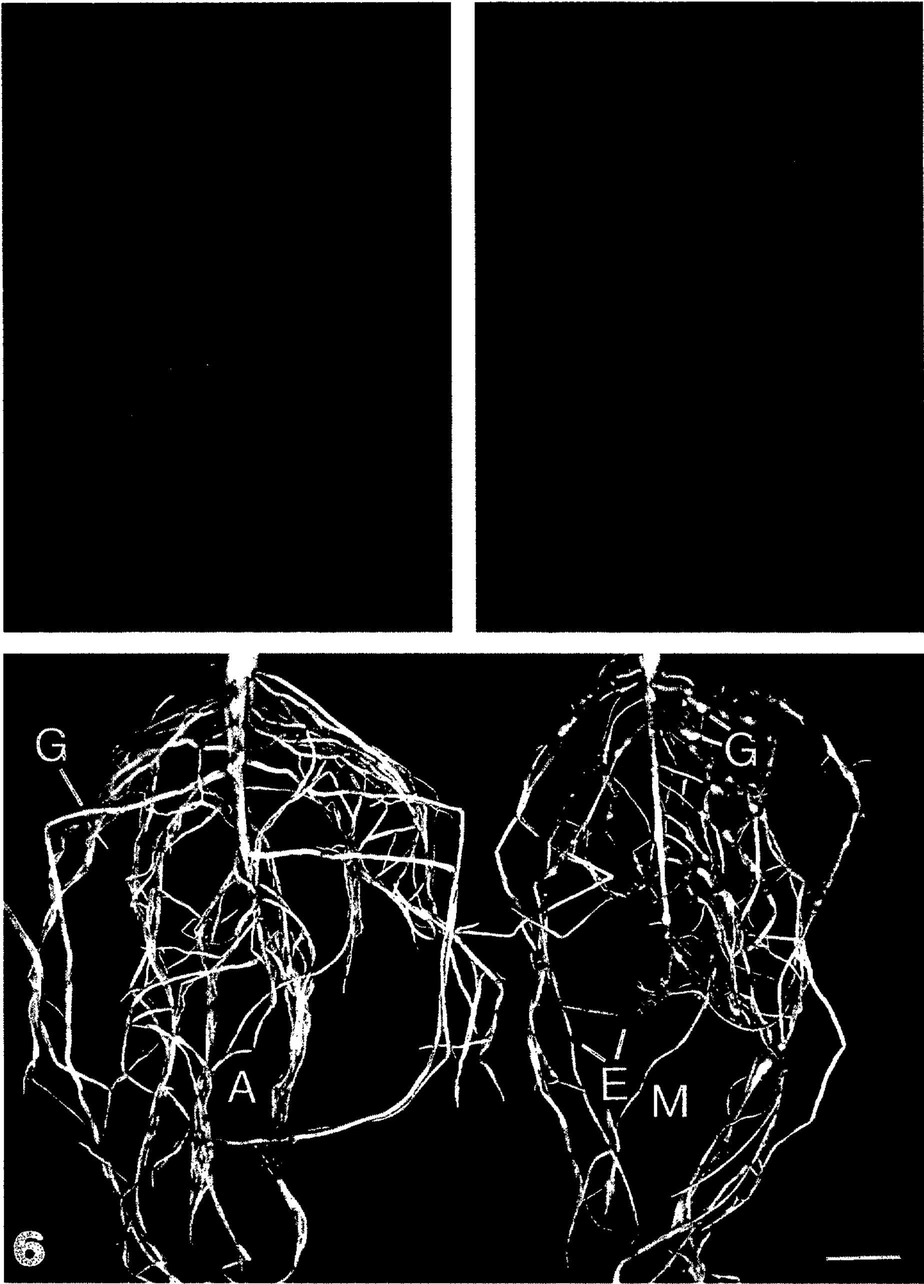


FIGS. 1, 2. Cotton roots in a glass-front box with roots inoculated with *Meloidogyne incognita.* 1) Seedlings 1 day after inoculation of radicle tips. X = site of inoculation. Bar represents 4 cm. 2) *M. incognita*-induced crack (running from C1 to C2) extending through cortex (CO) of susceptible M-8 taproot 11 days after inoculation with *M. incognita* and showing galls (G) growing out through the crack. Bar represents 600 μm.

FIG. 3. Photomicrograph of M-8 root segment with *M. incognita*-induced crack. The large crack (running from C1 to C2) extends through the cortex exposing edges of the crack (EC), the pericycle (P) inside the crack, and a cavity (CA) between the pericycle and cortex formed by the expanding gall (G) which has a gelatinous egg mass (E) attached. Bar represents 400 μm.



FIGS. 4, 5. Photomicrographs of cross and transverse sections through galls from susceptible M-8 cotton roots containing adult *Meloidogyne incognita*. 4) Cross section showing anterior end (A) of adult *M. incognita* (R) adjacent to multinucleated, syncytial giant cells (S) containing dense cytoplasm. Bar represents 160 μm. 5) Transverse section showing M-8 root with anterior end (A) of adult *M. incognita* (R) adjacent to long syncytial cells (S) with multiple nuclei and dense cytoplasm. Bar represents 200 μm.

FIG. 6. Roots of *Meloidogyne incognita* resistant A634 and susceptible M-8 cottons both inoculated at planting. A634 roots (A) exhibit only a few small galls (G) and few egg masses while M-8 roots (M) exhibit many large galls (G) and egg masses (E) (egg masses stained red). Bar represents 15 mm.

the tip of each radicle through a 12.5-cm-long cannula with a hypodermic needle and syringe. The boxes were covered with black plastic held in place with plumber's tape and placed in a growth chamber regulated as 86 C for 14-hour days and 72 C at night. Water was added as needed. Roots were induced to grow against the glass fronts to facilitate root observation (Fig. 1) by tilting the boxes forward 15 degrees from vertical. Each box was a replicate, and four replicates of each treatment were arranged in a completely randomized design.

Radicle elongation, computed as millimeters per hour, and root-knot symptom development were recorded at 8-hour intervals for the first 20 days and then at daily intervals for the next 10 days. Roots were observed for *M. incognita* symptom development through the glass fronts of the boxes with a steroscopic microscope mounted on a freely adjustable extension arm.

On day 31, plants were removed from the boxes and soil was washed from roots. Roots were rated for galling based on an index of 1–5: 1 = no galls or an occasional gall, 2 = light galling, 3 = moderate galling, 4 = heavy galling, and 5 = very heavy galling. Ten galls on lateral roots of each plant were chosen at random and measured at their greatest diameter. To determine normal root size, diameters were recorded for the ungalled part of the roots adjacent to and between the measured galls and the taproot. Ten egg masses were chosen at random from each plant, removed from the roots, and shaken in 1.05% sodium hypochlorite for 4 minutes. The freed eggs were washed in tap water and counted. When fewer than 10 galls and (or) 10 egg masses were found on a root system (A623 frequently had fewer than 10 of each) all galls and (or) egg masses found on a root system were measured.

Lateral root segments 6–10 mm long and containing galls, each with a single egg mass, were fixed in formalin–acetic acid–alcohol. Samples were embedded in paraffin, sectioned at 10 μm with a rotary microtome, stained with safranin, counter stained with fast green and orange G., and mounted as previously described (5).

Results and Discussion

Symptom development: During the first 48 hours after inoculation the inoculated radicles of M-8 and A623 elongated 2.7 and 3.3 mm/hour, respectively. Untreated radicles of both cotton lines elongated at similar rates which were 3.6 and 3.5 mm/hour, respectively. At day 3, cotyledons were completely expanded and lateral roots had been initiated. Fine longitudinal cracks appeared in the taproot epidermis of inoculated plants at day 4. Cracks were easily discernable through the root epidermis and partly through the cortex on day 5. At day 5, taproot elongation had slowed and lateral roots were developing rapidly. The first true leaves were barely visible at day 6 but became more pronounced by day 7. Gall formation was first apparent at day 7, and cracks in the radicles continued to increase in length, width, and depth. Many cracks extended completely through the epidermis and cortex by day 8. All plants had two true leaves by day 9, and gall formation on taproots was pronounced and cracks and galls were visible on lateral roots. More galls and cracks were evident on M-8 than on A623 roots at days 10–12. Many cracks in M-8 taproots at day 11 had galls emerging through them (Fig. 2). At day 12, A623 had a mean of five cracks per root system, 6 mm long × 0.1 mm wide, compared with 27 cracks, 18 mm long × 0.4 mm wide, on M-8. The cracks extended into lateral roots to a depth of approximately one-fourth of their diameter. Shorter cracks associated with individual galls located along a root at different intervals frequently ran together to form long continuous cracks. Often two or three cracks occurred on the same root segment, running parallel with each other and with the long axis of the root. Such cracks usually were spaced randomly around the periphery of the root.

At day 14, a gelatinous matrix became visible on the surface of some galls. The larger galls on M-8 appeared spongy and

some erupted at the surface with disorganized masses of undifferentiated cells similar to callus tissue. At day 15, eggs were deposited in the gelatinous matrices on both A623 and M-8 roots. Occasionally, chains of undifferentiated cells similar to callus tissue occurred on the surface of M-8 galls where the root tissue cracked. The few small galls on A623 roots at day 16 were firm and without callus cell proliferation. Both simple and large compound galls, the latter found mostly on M-8, had developed inside the vascular cylinder of roots causing large cracks by day 20.

By day 30, many galls, egg masses, and cracks in the cortex had developed on lateral roots of M-8 (Fig. 3). The gall tissue inside the vascular cylinder enlarged rapidly and created pressure inside the root which cracked the surrounding tissue (Figs. 2, C; 3, C). The cracks widened and lengthened as the underlying gall tissue increased in size exposing relatively large areas of the pericycle (Fig. 3, P).

Cavities frequently formed between the cortex and vascular cylinder when the enlarging gall (Fig. 3, G) separated the cortex from the ungalled, smaller diameter vascular cylinder (Fig. 3, CA). Such cavities commonly extended half the distance around the periphery of the vascular cylinder. Gall tissue which was initiated in the vascular cylinder underlying the cracks eventually filled the cracks and protruded through the openings.

Cross and transverse sections through root galls with adult nematodes revealed extensive abnormal cell and tissue development induced by the nematode (Figs. 4, 5). Metabolites and breakdown products from galled tissue and from the nematodes themselves may be involved in predisposing plants to other diseases (10).

Host suitability: A623 was a much poorer host of *M. incognita* than M-8. A623 had a mean of four egg masses per root system with 156 eggs per egg mass compared with 48 egg masses and 651 eggs per egg mass on M-8. Thus, 12 times more egg masses were found on M-8 than on A623 roots, and eggs per root system on M-8 were 31,248 compared with 624 on A623. Differences in *M. incognita* egg production on A623 and M-8 had been observed previously (Shepherd, unpubl.).

Mean galling indices were 4.5 for M-8 and 1.0 for A623. Mean diameter of root galls was 1.04 on M-8 and 0.74 on A623 roots which was 96 and 28% greater, respectively, than the diameter of their normal roots. The much greater galling index, the larger and more numerous galls, the slower radicle growth rate, and the larger root cracks on M-8 are evidence that root damage induced by *M. incognita* was much less on A623 than on M-8.

The number of egg masses on M-8 indicates that many *M. incognita* females matured and induced extensive syncytical tissue and gall formation in the vascular cylinder (Figs. 4, 5). This probably impaired vascular function. The impairment of vascular function by the nematode may have caused the slow radicle growth of M-8 and undoubtedly causes the characteristic stunting and general debilitation of plants that usually is associated with heavy *M. incognita* infestations in susceptible cotton.

Meloidogyne incognita effects that predispose plants to disease: The debilitating effects of *M. incognita* probably is one of the major factors in predisposing plants to disease. *M. incognita* and another parasitic organism may not need to be together in the same infection site for *M. incognita* to predispose cotton plants to other diseases. Overall debilitation of the plant may predispose the entire root system.

Cracks, such as those induced by *M. incognita* in M-8, also may play a significant role in predisposing cotton plants to diseases (Figs. 2, C; 3, C). Gossypol and related terpenoid aldehydes, described as phytoalexins, have been reported to provide antibiotic defenses against tissue invasion by micro-organisms (4). Gossypol and related terpenoid aldehydes are present in the epidermis and cortex but not in the vascular cylinder of roots (6). The *M. incognita*-induced cracks may compromise the root's defense against root-infecting pathogens by breaking the chemical barriers to in-

vasion in the cortex. Thus, by exposing large areas of the vascular cylinder, which is unprotected by phytoalexins, the cracks may provide extensive portals of entry through which disease organisms may enter (Fig. 3, P). Cavities between the cortex and central cylinder could further predispose plants to pathogens by providing favorable infection sites (Fig. 3, CA). Even if gall tissue fills the cracks replacing the normal epidermal and cortical tissue the root's defense mechanisms may be compromised (Fig. 3, G).

Meloidogyne incognita induced galls and giant cells may provide parasites with the energy they need for invading plant roots. Vascular wilt fungi must have a source of energy for development of a threshold level of inoculum potential before it can spread systematically in a plant (3). Many substances including free amino acids, amides, nucleic acids, N, P, and auxins increased in *M. incognita* galls and giant cell (13). Giant cells are more favorable substrates for fungal growth than normal cells (10). Therefore, root cracking (Figs. 2, C; 3, C), galling (Figs. 2, G; 3, G) and giant cells formation (Figs. 4, S; 5, S) probably are among the major effects of *M. incognita* that predispose cotton plants to disease and interact synergistically with disease organisms to increase the incidence and severity of the diseases. Small cracks in roots at the cotyledonary stage and galls at the appearance of the first true leaf predispose the seedlings to damage by other pathogens when seedlings normally are most vulnerable to diseases.

Potential for seedling and FW diseases in susceptible versus resistant cotton: Meloidogyne incognita-susceptible cotton planted in *M. incognita*-infested soil should have more infection sites for seedling disease and Fusarium wilt than resistant cultivars. Each generation of *M. incognita* on M-8 would increase infection exponentially, whereas infection sites on A623 should decrease with each generation. This is supported by a previous study (Shepherd, unpubl.) in which M-8 and Auburn 634, a resistant breeding stock (progeny of A623), were inoculated at planting with 10,000 *M. incognita* eggs each. At 40 days after planting, M-8 had 120,000 eggs per plant compared with 1,200 eggs per plant on A634 (Fig. 6). It is also supported by a field study in which the *M. incognita* density and plant stunting was reduced by planting A623 and increased greatly by planting a susceptible cotton (16).

Potential for using resistant cotton to control MI: Results of this and previous studies indicate that cotton cultivars with resistance to *M. incognita* similar to that in A623 would significantly reduce galling, root cracking, and plant debilitation, thereby eliminating much of its potential interaction with other pathogens. Such cultivars could greatly reduce economic losses in cotton caused by *M. incognita* directly or by interaction with other disease organisms.

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