Resistance of Cotton to the Root-Knot Nematode, Meloidogyne incognita[1]

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Abstract: Cotton plants resistant to *Meloidogyne incognita* had roots characterized by fewer and smaller galls, and females that produced fewer egg masses containing fewer eggs than did susceptible plants. Many galls on resistant roots contained no nematodes at the time of examination. Penetration of the resistant cultivar was equal to that of the susceptible cultivar and independent of the number of nematodes in the inoculum. Fewer nematodes penetrated resistant or susceptible plants with eight leaves than those with fewer leaves. Reciprocal grafts of resistant and susceptible plants failed to confer resistance or susceptibility to the rootstock. *Key Words:Gossypium hirsutum,* attraction, penetration, host age.

The root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood causes heavy losses of cotton (*Gossypium hirsutum* L. and *G. barbadense* L.) throughout the cotton-growing areas of the world. Cultivars of cotton resistant to *M. incognita* are known, and attempts have been made to incorporate their resistance into commercially acceptable cultivars (5). Little effort, however, has been directed toward gaining a basic understanding of the mechanism of resistance. Brodie et al. (1) found that resistance in 'Auburn 56', a root-knot tolerant cultivar, was related to necrosis and death of infected roots, retarded gall development, and inhibition of larval growth. Minton (11) reported similar findings, and concurred with Brodie et al. that resistance in the cultivars tested could not be attributed to the failure of nematode larvae to penetrate. Resistance was not related to morphology, root wt, diam of roots, or no. of lateral roots.

Recently, Shepherd (13) announced the release of a noncommercial breeding stock of cotton, 'Auburn 623RNR', resistant to *M. incognita* and Fusarium wilt. This stock is a selection from a cross between 'Clevewilt-6-3-5' (*G. hirsutum*) and a wild *G. hirsutum* introduced from Mexico (14), both of which exhibit a high degree of resistance to *M. incognita.* Auburn 623RNR is described as a transgressive segregate for root-knot resistance, having a higher degree of resistance than either of the parent lines.

Our purpose was to examine certain factors which may determine the ability of root-knot nematodes to become established in susceptible or resistant cotton plants, and to assess those factors in relation to a possible mode of resistance. This paper reports observations on larval attraction and larval penetration as functions of plant age and inoculum level; and also the attempted translocation of resistant factor(s). A brief report of a part of these studies has been published (10).

MATERIALS AND METHODS

Clevewilt-6-3-5 (Clevewilt) was chosen as the resistant cultivar since relatively few pairs of genes control resistance and resistance is inherited in a quantitative manner (6). Seeds, obtained from R. Shepherd (USDA, ARS, Auburn, Ala.), were increased by self-pollination and bulk harvest. A single lot of 'Deltapine Smooth Leaf' (Deltapine) cottonseed was purchased from a commercial source and used as the susceptible cultivar. 'Bayou', an experimental cultivar derived from a cross between Clevewilt and Deltapine (5), was used as a moderately resistant cultivar.

Nematodes used in these experiments were derived from a single egg mass isolated from an infected chili pepper (*Capsicum frutescens* L.) plant growing near Elfrida, Arizona, in a field previously planted to cotton. The single-egg-mass isolate was maintained on chili peppers in the greenhouse. Inocula for all experiments consisted of freshly hatched, second-stage larvae obtained by the method of Lownsbery and Viglierchio (7). Pathogenicity of this isolate to Clevewilt, Deltapine, and Bayou was tested in the greenhouse. A suspension of 2,000 larvae in 20 ml of distilled

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water was added to the surface of soil in 15-cm pots containing single cotton plants in the one-leaf stage. After the water had been absorbed, the soil surface was flooded with an additional 100 ml of water. The experiment was conducted twice with 10 replicate pots of each cultivar. Plants were harvested 5 wk after inoculation, the roots stained (3), and the numbers of galls and egg masses counted.

Attraction of infective larvae to roots of resistant and susceptible cultivars was studied utilizing Viglierchio's procedures (15), except that the aluminum channel was replaced with one constructed of plexiglass. With this technique, larvae uniformly dispersed in sand are separated from the test plant by a dialysis membrane and the horizontal movement of nematodes in relation to the plant is measured by determining their distribution following a suitable period of incubation. Two seedlings with radicles 3-5 cm long were transferred to the plant chambers and the roots covered with 20-mesh quartz sand and irrigated with 20% Hoagland's solution. One wk later a suspension of 3,500 larvae was introduced into the nematode chamber along with sufficient 60-mesh sand to absorb excess moisture. The entire apparatus then was incubated at room temperature and ambient artificial light for 48 h. Larvae were recovered from successive 20-mm sections of sand by Baermann funnel and counted. The experiment was conducted twice with four replicates (migration chambers) per treatment.

The numbers of nematodes penetrating resistant and susceptible roots were determined at two levels of inocula in 100 X 15-mm diam petri dishes containing a single seedling, 60-mesh quartz sand, and sufficient distilled water (ca. 25 ml/100 g sand) to provide uniform moisture. Ten or 200 larvae were hand-picked and placed at the root tip in a small drop of water. After 48 h at 23 C, the roots were removed, washed, and stained (3) for microscopic examination. The experiment was conducted twice with 10 replicate seedlings of each cultivar.

Influence of the developmental stage of the plant on larval penetration was determined by germinating seeds at intervals to obtain plants in the cotyledonary stage or possessing two, four, six, or eight true leaves. All plants were inoculated simultaneously by placing the roots of each plant in shallow plastic saucers, each containing a suspension of 2,000 nematodes in 100 ml of distilled water. Sufficient dry, 60-mesh quartz sand was added to each saucer until the sand covered the roots and absorbed the water. Plants were held in the greenhouse for 48 h after which larval penetration was determined as described above, The experiment was conducted once with five replicate plants per treatment.

Reciprocal grafts were produced by inarching 4-wk-old plants growing in separate 15-cm pots of sterile soil. The grafts were allowed 14 days to heal and the unwanted rootstocks or scions were excised and discarded. Roots of the grafted plants were pruned, the plants repotted, and all but the terminal leaves removed to maintain a suitable shoot-to-root ratio. After a 2-wk period during which new root and shoot growth was produced, nematodes were introduced by pouring a suspension of 2,000 larvae on the soil. Four wk after inoculation, the numbers of galls and egg masses were counted on the stained and cleared roots. Non-grafted, but pruned and repotted, plants served as controls. The experiment was conducted twice with nine plants (replicates) per treatment.

RESULTS

Galling response and egg mass production on Deltapine indicated that cotton is a suitable host for the normal development and maturation of the isolate of *M. incognita* used in these studies. The greatest degree of resistance, as determined by the numbers of galls and egg masses per plant, was shown by Clevewilt, with Bayou having intermediate resistance. The mean numbers of galls and egg masses from two tests each with 10 replicates were: Deltapine 119.4, 52.7; Clevewilt 5.7, 1.2; and Bayou 34.9, 7.3. (All differences among varieties were significant at $P \geqslant 0.01$).

Galls on Clevewilt were smaller and contained fewer nematodes than those formed on either Deltapine or Bayou. Although some root systems of Clevewilt contained no nematodes, most of them contained a few adult females which had reached the egg-laying stage. We observed on Clevewilt, and to a limited extent on Bayou, galls in which no nematodes or nematode fragments could be detected after staining and dissection under a stereomicroscope at X 50 magnification. However, disruptions of the vascular system were observed. No attempt was made to determine the relative numbers of such galls.

Relative penetration of Clevewilt and Deltapine was independent of resistance and of

the numbers of nematodes in the inocula. The mean percentages of nematodes which penetrated Clevewilt and Deltapine were 24 and 19, respectively, at an inoculum level of 10 nematodes per seedling and 22 and 21% at an inoculum level of 200 per seedling. Differences between means of cultivars or levels of inocula were not significant ($P \geqslant 0.05$). Since penetration may reflect the ability of nematodes to locate host roots, these data support the observation that migration of *M. incognita* larvae to cotton seedlings was independent of their resistant status (Table 1). Attraction of larvae to stimuli emanating from the host was detected at distances up to 10 cm (horizontally) from the membrane partition; however, no significant differences ($P \geqslant 0.05$) were detected between the responses to Clevewilt and Deltapine.

Age of the plants at the time of inoculation affected the numbers of nematodes which penetrated cotton roots (Table 2). Penetration was inversely related to the developmental stage of the plant; but, with the exception of plants in the eight-leaf stage, the differences between Clevewilt and Deltapine were not significant when treatments of the same age- group were compared.

Attempts to transfer resistance to susceptible rootstocks by grafting resistant scions to them were unsuccessful (Table 3). Reciprocal grafts were executed with more than 90% success and growth of the plants following pruning and repotting was rapid. Infections on pruned and repotted but non-grafted Deltapine were uniform and comparable to those of grafted Deltapine. Rooted cuttings of Clevewilt retained their resistance. It appears, therefore, that any resistance factor(s) present in the shoots of Clevewilt are not capable of being translocated across a graft union. Similarly, resistant rootstocks remained resistant regardless of their scion.

DISCUSSION

Upland cotton cultivars moderately resistant to *M. incognita* have been produced (5) but have failed to gain general acceptance or widespread use. Among the various reasons that can be cited for this failure is the fact that many of the resistant cultivars have cultural characteristics which restrict commercial production to limited geographic areas. In addition, geographically isolated pathotypes of *M. incognita* (12) introduce the possibility that

TABLE 1. Distribution of *Meloidogyne incognita* larvae after a 48-h exposure to the roots of resistant (Clevewilt) and susceptible (Deltapine) cotton plants.

Distance from roots (cm)	No. of nemas recovered from sand[1]	
	Clevewilt[2]	Deltapine[2]
2	358 ac	334 a
4	242 a	269 ac
6	84 b	131 bc
8	80 b	33 b
10	93 b	143 bc
12	202 bc	249 bc

[1] Means of four replicates. Column means followed by different letters are significantly different. LSD, cultivars ($P = 0.05$) = 106.

[2] Clevewilt (= Clevewilt-6-3-5, a breeding line); Deltapine (= cultivars 'Deltapine Smooth Leaf').

TABLE 2. Penetration of resistant (Clevewilt) and susceptible (Deltapine) cotton plants by *Meloidogyne incognita* in relation to developmental stage of the plants.

Developmental stage	No. of nemas[1]		LSD .05 for cultivars
	Clevewilt[2]	Deltapine[2]	
Cotyledon	80 a	102 a	52
2nd leaf	49 a	52 b	25
4th leaf	39 a	42 b	22
6th leaf	55 a	37 b	72
8th leaf	19 b	37 b	18

[1] Means of five replicates. Column means followed by different letters are significantly different ($P = 0.05$).

[2] Clevewilt (= Clevewilt-6-3-5, a breeding line); Deltapine (= cultivars 'Deltapine Smooth Leaf').

TABLE 3. Numbers of galls and egg masses on rootstocks of cotton plants reciprocally grafted with resistant (Clevewilt) or susceptible (Deltapine) scions and inoculated with *Meloidogyne incognita.*

Scion	Stock	No. galls[1]	No. egg masses[1]
Clevewilt[2]	Deltapine	57.4 a	22.4 a
Deltapine[2]	Clevewilt	14.9 b	2.1 b
Deltapine	Deltapine	53.3 a	26.5 a
Clevewilt	Clevewilt	9.3 b	0.8 b
Non-grafted	Clevewilt	10.0 b	0.9 b
Non-grafted	Deltapine	50.6 a	22.1 a

[1] Means of nine replicates. Column means followed by different letters are significantly different ($P = 0.05$).

[2] Clevewilt (= Clevewilt-6-3-5, a breeding line); Deltapine (= cultivars 'Deltapine Smooth Leaf').

cotton cultivars selected for resistance in one cotton-growing area will not be resistant to the nematode strains of other areas. Ideally, therefore, breeding programs should be conducted in the area of intended production.

A severe limitation to this ideal is the traditional means of determining resistant individuals in large progenies resulting from crosses with resistant parents. Bioassay of infected roots sacrifices young greenhouse-grown cotton plants, and plants grown in the field cannot be rated for resistance until the seed has matured and is gathered (2). For these reasons, it is apparent that new approaches are warranted. Knowledge of the mechanism(s) of resistance could provide new criteria for breeding and selection based upon histological, cellular or biochemical details (8).

We have shown that the early stages of infection are unacceptable as a measure of determining resistance since both resistant and susceptible plants are penetrated readily. Resistance ratings based upon the total numbers of galls or an indexed rating of galled roots also have limitations. In the resistant cultivar, Clevewilt, galls frequently were found which did not appear to contain nematodes. Root systems with a high proportion of these "empty" galls, therefore, would be underrated for resistance. From this standpoint egg mass production would be a more desirable gauge. Cotton is unlike alfalfa resistant to *M. hapla* (4), since resistance in Clevewilt is not affected by age of the plant at the time of inoculation.

The failure of resistant scions to confer resistance to susceptible stocks suggests that resistance in Clevewilt is a local condition and is not due to factors freely mobile within the plant. In other studies (9), we have shown that larval development is inhibited in roots of Clevewilt and that there is an active lysis of those nematode larvae which enter but fail to reach maturity. We, therefore, advance the hypothesis that resistance in Clevewilt is a post-infection phenomenon acting at the histological or cellular level.

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