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TO THE ROOT-KNOT NEMATODE MELOIDOGYNE INCOGNITA.

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FACTORS INFLUENCING THE RESISTANCE OF COTTON TO THE
ROOT-KNOT NEMATODE MELOIDOGYNE INCOGNITA

by

Kenneth Carl Ellis

A Dissertation Submitted to the Faculty of the

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In Partial Fulfillment of the Requirements
For the Degree of

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THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my
direction by Kenneth C. Ellis
entitled Factors influencing the resistance of cotton to root knot
nematode, Meloidogyne incognita.
be accepted as fulfilling the dissertation requirement of the
degree of Ph.D.

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Jenneth C. Ellis

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ABSTRACT

Root-knot nematode susceptible cotton (Gossypium hirsutum var. Deltapine Smooth Leaf) and root-knot nematode resistant cotton (G. hirsutum var. Cleve wilt) were used in a series of studies aimed at determining the mechanism of nematode resistance in cotton. The factors, which could influence resistance, investigated in this study were attraction, penetration, egression of larvae from resistant root tissue, the influence of plant age on larval penetration and morphological development, morphological development and sex ratio, the histology of nematode infected resistant and susceptible root tissue, transference of resistance through grafting, and the effect resistant and susceptible plants have on each other when grown in close proximity.

It was found that there was no difference in attraction or penetration between the resistant and susceptible cotton varieties. Egression of larvae from infected roots was not observed in either variety. Penetration and morphological development was less in older plants in resistant and susceptible cotton. However, development in the resistant cotton was more retarded at any age when compared to the susceptible cotton. The sex ratio of nematodes in resistant cotton was not

statistically different from that of the resistant variety. Histological studies showed only subtle differences between the syncytia and surrounding tissue in resistant and susceptible cotton. Transference of resistance through grafting was unsuccessful and the effect resistant and susceptible plants have on one another was inconclusive.

It was concluded that the mechanism of resistance is probably due to the presence of a toxin or growth inhibitor which is inherent in the cells of the resistant host, and that resistance or susceptibility are biochemical reactions that occur on a cellular level in response to stimuli by the nematode.

INTRODUCTION

The southern root-knot nematode Meloidogyne incognita Chitwood, is a serious pest of cotton, Gossypium hirsutum L., in both the southeastern and southwestern United States. Each year this nematode causes substantial losses to the cotton industry by reducing the per acre yield of the cotton crop. In 1967 the reduction in yield of cotton, caused by the nematode, amounted to 1.6% of the total crop or a dollar value loss to the economy of \$18,000,000. In this same year the fungus Verticillium albo-atrum Reinke and Berth., caused a 4.4% reduction in cotton yields (27). The cost of damages caused by this nematode in complex with other plant pathogens is not known, but may be estimated to also be in the millions of dollars. If losses from the direct damage are added to the costs of inefficient use of soil amendments, and the increase in water required by plants parasitized by this nematode, annual loss figures become astronomical.

Chemical control of this nematode is relatively simple, and even though it is used rather extensively in some areas today, adds greatly to the cost of producing an acre of cotton. Cultural control, while economical, is in most cases complicated and difficult because there are too

many susceptible hosts and few or no non-hosts that can be substituted as a cash crop.

More efficient and economical nematode controls methods are needed for today's farmer to produce profitable crops. A partial answer to this problem would be to develop cotton varieties which are highly resistant to attack by the root-knot nematode.

Some progress has been made in this direction, by the selection of moderately nematode resistant varieties such as G. hirsutum var. Auburn 56 and more recently G. hirsutum var. Bayou, by cotton breeders in the southeastern United States. These varieties while adapted to the eastern areas are not effective in the western United States.

To date the mechanism of root-knot nematode resistance is unknown. Nematode resistance has been defined by Rohde (34) as a set of characteristics of the host plant which act more or less to the detriment of the parasite. Rohde states that resistance may be variable, ranging from complete to slight, and is measured by the ability of the parasite to survive and is not necessarily related to plant growth.

Some of the characteristics of resistant cotton plants that act to the detriment of root-knot nematodes could be, (a) reduced attraction, (b) less penetration of root-knot larvae, (c) egression of larvae from

resistant root tissue, or (d) failure of the root-knot larvae to establish a nutritive relationship with the cells of resistant plants.

Dropkin, Davis, and Webb (11) observed that one of the resistant reactions evidenced in tomatoes resistant to M. incognita and M. hapla was a reduction in larval penetration. Minton, Donnelly, and Shepherd (29) working with a number of species of vetch (Vicia spp.) and 5 species of root-knot nematodes (Meloidogyne spp.) showed that fewer M. incognita acrita penetrated resistant vetch V. sativa L var. Warrior than a susceptible vetch V. dasycarpa Ten. var. 'Auburn Woolypod.' Liao and Dunlap (24) observed that root-knot nematodes (Meloidogyne sp.) would penetrate Lycopersicon esculentum Mill., roots but not the roots of L. peruvianum L. They indicated that there appeared to be no difference in the morphological structure of the roots of these two tomato species. They suggested that a chemical inhibitor may be present or the resistant tomato was incapable of attracting the nematodes. Barrons (2) found no difference in the number of root-knot larvae that penetrated resistant or susceptible bean plants, but suggested that the production of a toxic substance by the resistant plant could account for the failure of root-knot larvae to develop. Minton (28) observed that resistance in cotton G. barbadense L. was attributed to conditions within the roots that prevented or delayed larvae

development and not a failure of nematodes to enter the roots. No morphological differences in the roots could be associated with resistance.

Other authors (7, 8, 21) have made similar observations that penetration is in some instances an important factor in the resistance of plants to root-knot nematodes.

Hansen, Lownsbery, and Hesse (18), Lavalley and Rohde (23), and Grundbacher and Stanford (17) indicated that attraction is probably not important as a mechanism of nematode resistance. However, since resistance in plants is rarely ever the result of a single mechanism (19) it appears logical to assume that attraction might be an important factor in at least some cases of resistance.

Resistance to the root-knot nematode might be due to the direct action of the resistant host on the nematode. In many cases resistant plants show a hypersensitive reaction to invading nematodes. Pi and Rohde (30) have shown that M. incognita larvae that penetrated resistant tomatoes were surrounded by necrotic cells. They further suggest that chlorogenic acid, a known phytoalexin, could be responsible for the necrosis. Similar observations concerning necrosis in resistant tomatoes have been reported by other investigators (7, 8, 10, 33). In their work with sweet potato (Ipomea batatas L.) Giamalva, Martin, and Hernandez (14) suggested that resistance to root-knot nematodes was due to root necrosis. Brodie, Brinkerhoff,

Struble (4) showed that resistance in cotton seedlings to M. incognita acrita was associated with three kinds of reactions; root necrosis, retarded gall development, and failure of the majority of the nematodes to reach maturity.

The resistance of peach root stocks (Prunus persica L.) to root-knot nematodes appears to be by other mechanisms. Malo's (25) histological studies showed that the penetration and initial development of the larvae was the same in resistant and susceptible peach root stocks. He found that after 8-10 days the newly formed giant cells as well as the nematodes became surrounded by suberized tissue in the resistant plants.

Another mechanism of root-knot resistance has been demonstrated by Riggs and Winstead (33) and Dean and Struble (7). Riggs and Winstead showed that some factor(s) in resistant L. esculentum causes root-knot larvae to die within 96 hours after they penetrate the roots of the host. Dean and Struble used the same resistant host and showed that invading larvae died and could not be detected two weeks after invading the resistant plants. These investigators noted that the same reaction was prevalent in resistant sweet potato, although they observed a longer time for the nematodes to disappear (7).

Another mechanism of resistance to root-knot nematodes is prevented or delayed larval development.

This phenomenon has been reported in cotton (4, 28), peach root stocks (25), and tomato (10, 21).

By histological studies Crittenden (6) showed that in soybeans resistant to M. incognita one or more of the following abnormalities occurred: (a) no giant cells around the nematode's head, (b) small number of giant cells, (c) small size of giant cell area, and (d) the lack of cytoplasm in giant cells. Similar results were reported by Dropkin and Nelson (9). Minton (28) has reported that in some varieties of resistant cotton there is a reduction in hypertrophy and hyperplasia, with a corresponding reduction in tissue disorganization and gall formation and a failure of nematodes to mature.

Reynolds and Carter (31) have suggested another possible mechanism of resistance. In their work with M. incognita on alfalfa (Medicago sativa L.), they showed that 2 to 4 days after penetration root-knot larvae egressed from the roots of the resistant plants.

It appears, therefore, from the literature that the reaction of plants resistant to root-knot nematodes may be one of 5 forms: (a) reduced penetration (7, 8, 21, 24, 29), (b) hypersensitivity (3, 7, 8, 10, 14, 30, 33), (c) egression of larvae from roots (31), (d) retarded larval development (4, 10, 21, 25, 28), or (e) formation of suberized tissue around penetrated larvae and newly formed giant cells (25).

To further investigate the mechanism of resistance to nematodes some workers have tried to transmit resistance to susceptible plants through grafting. Forester (13) cross-grafted resistant nightshade to a susceptible tomato, and then infested the plants with Heterodera rostochiensis Woll. In the same experiment he infested susceptible tomato, that had been cross-grafted with resistant L. peruvianum with M. incognita. No differences in reaction were observed in the tomato-nightshade cross-grafts, but he did, however, observe that L. peruvianum scions decreased the susceptibility of the tomato root stocks. Forester concluded that there was basipetal movement of the resistance factor. Riggs and Winstead (32) also attempted to transfer root-knot resistance in the tomato, but were unsuccessful. They did not note any difference in reaction in resistant and susceptible tomatoes in which they had made reciprocal approach grafts. They concluded that the resistance or susceptibility factor(s) was inherent within individual cells in both the roots and tops of plants, and either was not translocated or did not cross the graft union. Chambers and Epps (5) were of the same opinion after cross-grafting resistant and susceptible soybean varieties and infesting the plants with Heterodera glycines Ichinohe, 1952. No differences in reactions were observed. They concluded that the factor(s) for resistance or susceptibility to H. glycines was not synthesized in one

part of the soybean plant and translocated to other parts, but is probably genetically inherent in all parts of the resistant plant. To add support to the fact that resistance is not generally translocatable, Golpen and Stanford (15) made reciprocal cross grafts with resistant and susceptible alfalfa plants and found no effect on the plants' susceptibility or resistance to root-knot nematodes.

Vigliorchio (37) cross-grafted sugar beets (Beta vulgaris L.) susceptible to Heterodera schachtii Schmidt, with Beta maritima L., a wild beet resistant to the nematode. His results indicate that both susceptibility and resistance could be transferred. His B. maritima root stocks became more susceptible when grafted to B. vulgaris scion and the B. vulgaris root stocks were more resistant when grafted to B. maritima scions. This study would, therefore, indicate that in some hosts the resistance factor(s) may be some product found or produced naturally in the above ground portion of the plant capable of being translocated to a grafted root system.

Statement of the Problem

The objectives of this study were to determine if the resistance exhibited by some varieties of cotton, to root-knot nematode, was due to, or influenced by: (a) attraction of nematode larvae to the roots, (b) penetration

of larvae into the roots, or (c) egression of larvae from resistant root tissue. It was of further interest to determine: (d) if the reaction to root-knot nematodes was changed when resistant and susceptible cotton plants were grown together, (e) if the rate of development and the sex ratio of larvae which had penetrated susceptible or resistant cotton plants differed, and (f) what if any were the histological differences between resistant and susceptible cotton roots infected with root-knot nematodes. The final objective of this study was: (g) to determine if resistance could be transmitted from a root-knot nematode resistant cotton plant to a root-knot nematode susceptible cotton plant by grafting.

Source of Resistant and Susceptible Cotton Varieties

Seeds of the highly root-knot nematode susceptible cotton variety Deltapine Smooth Leaf were obtained from the Department of Plant Breeding, University of Arizona and consisted of commercial seed. Seeds of the root-knot resistant cotton variety Clevevilt were obtained from the Cotton and Cordage Fibers Research Branch A. R. S., U. S. D. A. These two varieties were chosen as the best examples of resistant and susceptible material after many varieties and wild types had been screened.

Nematodes used in the experiments were a race of Meloidogyne incognita originally found on chiles in

Cochise County, Arizona. This race proved to be a very effective parasite of cotton.

Soil from infested chile fields was transported to the Plant Pathology Department's greenhouses and retained in steel bins. Chiles and tomatoes were planted in this soil to serve as host plants for the nematodes. Whenever larvae were required, a number of plants were removed from the bins and gently washed to remove as much dirt as possible.

Egg masses were removed by kneading the roots in cold water. The resultant water and egg mass suspension was passed through 20 and 60 mesh screens. Debris from the 20 mesh screen was discarded and the egg masses were washed from the 60 mesh screen directly onto two layers of facial tissue in a modified Baermann funnel. The modified Baermann funnel consisted of a rectangular 20.5 x 27 cm Nalgene dish fitted with a tray constructed of 20 mesh stainless steel wire. Distilled water was added to the dishes to the extent that it just wetted the facial tissue. Following 24 hours incubation, the stainless steel tray was removed and the water and nematodes discarded. The tray was replaced and more water added. The larvae harvested at the 24 hour intervals thereafter were relatively free of free living nematodes and oligochaetes. Egg masses treated in this manner continued to produce useable quantities of larvae for up to 5 days.

INVESTIGATIONS OF LARVAL ATTRACTION TO ROOTS OF RESISTANT AND SUSCEPTIBLE COTTON VARIETIES

Materials and Methods

Information has been presented in the literature review which indicates that susceptible and resistant cotton varieties may have different attractiveness due to some compound or compounds in the root exudates. To investigate this possibility in the resistant and susceptible cotton varieties used in this study, the following methods were used.

Plexiglass trays were constructed with a center chamber 2 cm long and 10 cm wide that could be placed in the middle of two end chambers that measured 12 cm long and 10 cm wide. The overall dimensions of the trays were 26 cm long and 10 cm wide. The trays were made leak-proof by applying lanolin to the removable side and end plates where they came into contact with the bottom of the tray. The sides and end plates were then bolted to the bottom of the trays with wing nuts provided for this purpose.

The center chamber was constructed by applying lanolin to the edges and bottom of the two end chambers of the tray and to the edges of the center chamber itself. Dialysis tubing (Van Waters and Rogers Company) was then cut into strips 11 cm by 3 cm and placed between the edges

of the end chamber halves and the center chamber. The end chambers and the center chamber were then bolted together tightly to ensure a leak-proof system. Twenty mesh white quartz sand was then added to the center chamber, and saturated with distilled water prior to planting.

Seeds of the root-knot nematode resistant cotton Cleve wilt, and the root-knot nematode susceptible cotton, Deltapine Smooth Leaf were surface sterilized in a 2% solution of Chloramine T ($1\text{-CH}_3\text{C}_6\text{H}_4\text{-4-SO}_2\text{NC1Na}\cdot 3\text{H}_2\text{O}$, Eastman Chemical Company) for 30 minutes. These seeds were then rinsed in sterile distilled water and transferred to steam sterilized growth pouches to which 30 ml. distilled water had been added. The seeds were incubated at 28-30 C until the roots had attained a length of 3 to 5 cm.

When the tap roots had grown to the desired length, the seedlings were transferred from the growth pouches to the center chamber of the attraction trays. Each chamber received two seedlings, either Cleve wilt or Deltapine Smooth Leaf. There were 4 trays, 2 each for the resistant and susceptible cotton varieties. Since each tray contained 2 end chambers to which nematodes could be added, the experiment could be replicated 4 times for each time the experiment was repeated. This experiment was repeated once.

The seedlings were incubated at 25 C and watered daily with Hoagland's nutrient solution (20%) one week after

transplanting, 3,500 newly hatched M. incognita larvae were added to each of the end chambers of the attraction trays. Distilled water was then added to disperse the larvae evenly over the bottom of the end trays. Enough 60 mesh white quartz sand was then added to the trays to make the level of sand in the center chamber and the end chambers equal. The sand in the end and center chambers was kept moist at all times.

Forty-eight hours after the nematodes were introduced, the sides and ends of the trays were removed and the sand divided into 6 sections, each 2 cm thick. These sections were then placed on a double thickness of facial tissue and placed on Baermann funnels. After 24 hours, 10 ml aliquots were removed from the Baermann funnels and the number of nematodes in each 2 cm section counted and recorded.

Data collected from this experiment were treated as a paired experiment and statistically analyzed by the "T" test. T values used were those compiled by Beyer (3).

Results

From the results shown in Table 1, there was no significant difference at the .05 level of probability between the attractiveness of the resistant Cleve-wilt variety and the susceptible Deltapine Smooth Leaf variety. Both varieties showed a definite attraction over a distance of from 0 to 8 cm. Because a larger number of nematodes

were recovered from the sections nearest the plants, the production of a repellant substance is also ruled out. The results obtained from the repeated experiments were in agreement with the original data.

Table 1. Number of M. incognita larvae attracted to the roots of susceptible or resistant varieties of cotton -- Average of four replications.

Distance from Membrane (cm)	Average no. of larvae recovered from 3,500 added	
	Susceptible	Resistant
	Deltapine Smooth Leaf	Cleviewilt
2	234	358
4	269	242
6	131	84
8	33	80
10	143	93
12	249	202
t-value 5%	N.S.	

INVESTIGATIONS CONCERNING LARVAL PENETRATION
INTO THE ROOTS OF RESISTANT AND
SUSCEPTIBLE COTTON VARIETIES

Materials and Methods

Preliminary investigations showed the cotton variety Clevevilt to have substantially fewer root-knot galls and mature females than the susceptible Deltapine Smooth Leaf variety. A possible reason for the reduced number of galls is that fewer larvae were able to penetrate the resistant variety than the number which penetrated the susceptible variety. To investigate this hypothesis the following experiment was developed:

Glass tubes 20 cm long and 0.5 cm inside diameter were cut from regular laboratory stock. One end of the glass tube was plugged with glass wool and the tube filled to a height of approximately 15 cm with sterilized 60 mesh white quartz sand.

Fifteen seeds each of Deltapine Smooth Leaf and Clevevilt cotton were treated as described in the investigation concerning host attraction. When the tap roots of the seedlings had grown to 4 to 5 cm in length, sufficient distilled water was added to sand in the tube to saturate it. Ten newly hatched M. incognita larvae were then placed in each tube. This was accomplished by drawing 10 of the second stage larvae into a capillary tube, counting them

with the aid of a dissecting microscope, and then depositing them in a drop of water on the top of the moist sand. After the nematodes were deposited, the capillary tube was again observed using the dissecting microscope to be certain that none of the larvae remained in the tube.

The cotton seedlings were then placed in the tubes so that the root tip just touched the top of the sand. More sand and water were added, at the same time to keep the roots and nematodes from dessicating, until the tube was full. This experiment was replicated 15 times and repeated twice.

The seedlings were incubated at 23 C for 48 hours. After that time, the seedlings were removed from the tubes. This was accomplished by removing the glass wool plug and immersing the whole tube in water for 3 or 4 minutes, thusly washing all the sand and remaining nematodes from the roots. After the seedlings had been removed from the glass tubes, the cotyledons were removed and the roots were stained 4 hours in a solution of 1:1 95% ethanol and glacial acetic acid to which 0.017 mg/ml acid fuchsin had been added. The roots were destained in a concentrated solution of chloral hydrate (12). The roots were removed from the chloral hydrate after 24 hours and placed in a clear lactophenol. With the aid of a microscope the number of larvae which had penetrated were recorded. This

study was treated as a paired experiment and a "T" test was used to analyze the data that were collected.

Results

The results from the penetration study appear in Table 2. In general penetration was low in both of the cotton varieties. Statistical analysis of these data showed that there was no significant difference in penetration between the two varieties. The results obtained from the repeated experiments were in agreement with the original data.

Table 2. Number of root-knot larvae added to and recovered from resistant and susceptible cotton varieties.

Rep. No.	Susceptible		Resistant	
	Deltapine Smooth Leaf		Clevewilt	
	Added	Recovered	Added	Recovered
1	10	0	10	0
2	10	3	10	5
3	10	1	10	2
4	10	2	10	0
5	10	1	10	1
6	10	1	10	0
7	10	4	10	0
8	10	0	10	2
9	10	0	10	0
10	10	0	10	0
11	10	0	10	2
12	10	1	10	0
13	10	0	10	0
14	10	0	10	0
15	10	0	10	0
Mean	10	.86	10	.80
t-value 5%	N.S.			

INVESTIGATION CONCERNING THE INGRESSION AND
EGRESSION OF ROOT-KNOT LARVAE IN
RESISTANT AND SUSCEPTIBLE
COTTON ROOTS

Materials and Methods

Among other possible explanations for resistance is that nematode larvae penetrate the plant, encounter unfavorable conditions for their development and egress from such roots. This hypothesis was investigated in the following manner:

Newly hatched M. incognita larvae were obtained and transferred to the root systems of the cotton plants as follows: Nematodes suspended in distilled water were pipetted into a 100 ml plastic petri dish in drops. With some practice, the size of the drop could be regulated so that approximately 10 larvae were in each. In this manner the exact number of nematodes in each petri dish was known. The nematodes were then suspended in distilled water. To each of these plates containing a known number of nematodes (an average of 200 ± 6), a previously germinated 3 day old cotton seedling was added. Sufficient 60 mesh white quartz sand was added to cover the root of the seedling. The plants were incubated at 25 C for 48 hours and every 48 hours thereafter for 16 days the plants were removed from the plates and the sand was placed on a modified Baermann

funnel. The dishes were washed and the plants replanted in fresh 60 mesh sand. Five plants were stained and the nematodes counted by the method previously described in the investigation of penetration. Water from the Baermann funnels was passed through 1.2 μ millipore filters (Millipore Company), to concentrate any nematodes that may have been present.

Results

The 2 day readings presented in Table 3 represent the number of larvae of the original inoculum of 200 that did not penetrate the cotton plant. After 4 days only one larva was recovered from either variety. The 6, 8, 10, 12, 14, and 16 day readings indicated that there was no egression of larvae from the plants.

In Table 4 is presented the number of nematodes observed in the stained roots of cotton plants subjected to the same treatment as those in Table 3. From the average 200 larvae in the inoculum only approximately 25% penetrated either of the 2 cotton varieties. There was a decrease in number of nematodes observed in both varieties at the end of 6 days. The nematode numbers remained relatively constant after this initial decrease throughout the remainder of the 16 day test period in both Deltapine Smooth Leaf and Cleve-wilt. However, the initial decrease in number of nematodes was greater in the resistant variety

Table 3. The number of M. incognita larvae recovered from sand technique used as an index of egression.

Rep. no.	No. nemas. in orig. inoc.		No. of nematodes r									
			2 days		4 days		6 days		8 days		10 day	
	DP ^{a/}	CW ^{b/}	DP	CW	DP	CW	DP	CW	DP	CW	DP	C
1	209	195	60	50	0	0	0	0	0	0	0	0
2	204	202	62	38	0	0	0	0	0	0	0	0
3	205	209	44	42	0	0	0	0	0	0	0	0
4	215	192	42	54	0	0	0	0	0	0	0	0
5	205	206	24	58	0	0	0	0	0	0	0	0
6	213	208	34	98	1	0	0	0	0	0	0	0
7	196	206	29	31	0	0	0	0	0	0	0	0
8	201	193	96	57	0	1	0	0	0	0	0	0
9	195	204	54	78	0	0	0	0	0	0	0	0
10	210	201	44	57	0	0	0	0	0	0	0	0

^{a/}Susceptible Deltapine Smooth Leaf.

^{b/}Resistant Cleviewilt.

M. incognita larvae recovered from sand by the Baermann funnel
d as an index of egression.

No. of nematodes recovered															
2 days		4 days		6 days		8 days		10 days		12 days		14 days		16 days	
DP	CW	DP	CW	DP	CW	DP	CW	DP	CW	DP	CW	DP	CW	DP	CW
60	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0
62	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	42	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	54	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	58	0	0	0	0	0	0	0	0	0	0	0	0	0	0
34	98	1	0	0	0	0	0	0	0	0	0	0	0	0	0
29	31	0	0	0	0	0	0	0	0	0	0	0	0	0	0
96	57	0	1	0	0	0	0	0	0	0	0	0	0	0	0
54	78	0	0	0	0	0	0	0	0	0	0	0	0	0	0
44	57	0	0	0	0	0	0	0	0	0	0	0	0	0	0

eltapine Smooth Leaf.

wewilt.

Table 4. Nematodes observed in stained roots of root-knot resistance study.

Rep. no.	Number of days after i								
	2 days		4 days		6 days		8 days		10
	DP ^{a/}	CW ^{b/}	DP	CW	DP	CW	DP	CW	DP
1	33	41	87	87	49	29	34	14	21
2	41	7	51	5	1	28	31	0	11
3	47	87	60	38	4	14	50	0	15
4	37	39	49	40	45	31	1	5	28
5	48	45	61	33	33	14	33	6	37
Mean	41.2	43.8	61.6	40.6	26.4	23.2	29.8	5.0	22.4
"t" 5%	NS		NS		NS		2.85		

^{a/} Susceptible Deltapine Smooth Leaf.

^{b/} Resistant Cleve wilt.

ed roots of root-knot resistant and susceptible cotton plants in

Number of days after inoculation											
6 days		8 days		10 days		12 days		14 days		16 days	
DP	CW	DP	CW	DP	CW	DP	CW	DP	CW	DP	CW
9	29	34	14	21	35	31	4	13	18	36	5
1	28	31	0	11	2	29	8	14	4	38	11
4	14	50	0	15	16	32	17	3	14	8	0
5	31	1	5	28	11	33	9	27	5	35	0
3	14	33	6	37	10	21	5	47	2	44	0
16.4	23.2	29.8	5.0	22.4	14.8	29.2	8.6	20.8	8.6	32.2	3.2
NS		2.85		NS		9.3		NS		4.9	

ch Leaf.

than that observed in the susceptible variety. After 6 days an average of 67% of the total number of larvae which had penetrated the susceptible variety was observed, while only 18% of the total number of larvae could be found in the resistant variety.

INFLUENCE OF PHYSIOLOGICAL AGE OF PLANT ON PENETRATION AND DEVELOPMENT OF NEMATODES

Materials and Methods

To determine if the age of the plant influenced the penetration and development of root-knot larvae in cotton, and if so, if there was a difference between resistant and susceptible cotton varieties, the following experiment was conducted:

Seeds of the root-knot susceptible cotton variety, Deltapine Smooth Leaf, and a root-knot nematode resistant cotton variety, Clewewilt, were planted in 6 inch plastic pots filled with a 3:1 sand-soil mixture. The seeds were planted at intervals such that plants ranging from the cotyledon stage through the fourth true leaf stage were obtained. These plants were grown in a greenhouse where the temperature ranged from a day time high of 40 C to a night time low of 15.5 C. The plants were watered weekly with Hoagland's nutrient solution.

The experiment consisted of 2 parts. In the first part of the experiment 5 plants from each of the 5 physiological age groups were examined for differences in the number of larvae penetrated. In the second part of the experiment 5 plants from each of the physiological age groups were examined for morphological differences in

development. Four classes of development were recognized, adult females, developing larvae, infective larvae, and males.

When the plants had attained the correct physiological age, they were inoculated in the following manner:

The plants were removed from the pots and the root systems placed in 6 inch plastic saucers to which had previously been added 2,000 newly hatched M. incognita larvae. These larvae were suspended in distilled water. The roots were immediately covered with 60 mesh white quartz sand. The plants were incubated for 48 hours after which time they were removed from the saucers.

Root systems of the 5 plants for each physiological age group that were examined for differences in the number of larvae penetrating the resistant cotton variety compared with the susceptible cotton variety were immediately stained using the method previously described in the section on larvae penetration of root-knot nematode resistant and susceptible cotton.

The 5 plants from each physiological age group that were to be examined for morphological differences in development between the susceptible cotton variety and the resistant cotton variety were repotted in 6 inch plastic pots filled with a 3:1 sand-soil mixture. These plants were maintained under the same greenhouse conditions as stated previously in this section. After 28 days these

plants were removed from the pots, and washed free of soil. The root systems were then removed and fixed in F.A.A. for 24 hours. Following 24 hours the roots were stained by boiling them in lactophenol-acid fuchsin for 5 minutes (16). After staining, the roots were stored in clear lactophenol until they could be observed.

The number of root-knot larvae which penetrated the resistant and susceptible cotton plants at varying physiological ages was obtained by observing the root systems with the aid of a dissecting microscope and counting the number of larvae present. The root systems from which data were collected on the development of root-knot larvae in resistant and susceptible cotton plants of varying physiological ages were treated in the following manner:

With the aid of a dissecting microscope the root systems were observed and any nematodes present were dissected out. The nematodes which were dissected from the root tissue were placed in clear lactophenol in wells constructed on standard microscope slides with clear fingernail polish. The wells were constructed by taking a small brush and laying down a circle of fingernail polish on the surface of the slide. When the polish dried, a drop of clear lactophenol was placed inside the circle. In this manner nematodes dissected from the plant tissue were observed with the aid of a compound microscope. The nematodes were placed in one of the 4 stages of development

previously mentioned in this section on the basis of the number of cuticles present, gonad development, and presence or absence of a stylet.

The data collected from this experiment were analyzed by comparing computed "t" values with tabular "t" values at the .05 level of probability.

Results

It was found that there was a decrease in penetration in both the resistant and susceptible cotton varieties with age, but the difference between the two varieties was not statistically different at any of the physiological ages studied (Table 5). However, there was a difference in the development of the nematodes in the resistant and susceptible varieties. In the first and second true leaf stage of the susceptible variety, adults and developing females were found in much greater numbers than in the resistant variety.

Roots of both the resistant and susceptible cotton plants exhibited some necrosis. However, this reaction was more prevalent in the resistant variety. Galls containing no nematodes were found in the resistant variety but not in the susceptible variety. Due to the low numbers of nematodes observed in the roots of the resistant cotton variety 28 days after infestation body measurements for comparison between the resistant and susceptible varieties

Table 5. Penetration of resistant and susceptible cotton plants by M. incognita in relation to physiological age of the plant -- Average of five replications.

Physiological age of plant	No. of nematodes recovered from 2,000 added	
	Susceptible	Resistant
	Deltapine Smooth Leaf	Cleviewilt
Cotyledon	102	80
2 true leaves	52	49
4 true leaves	42	39
6 true leaves	37	55
8 true leaves	37	19

were not obtainable. Data presented in the section on nematode development and sex ratio indicate that measurements taken of a large number of nematodes show a difference in the development of nematodes in resistant cotton when compared to susceptible cotton.

NEMATODE INFECTION OF ROOT-KNOT RESISTANT AND SUSCEPTIBLE COTTON PLANTS GROWN TOGETHER

Materials and Methods

To determine if nematode susceptible and resistant plants grown in close proximity could influence either of their normal reactions to nematode infection, the following experiment was devised:

Seeds of susceptible cotton, variety Deltapine Smooth Leaf and the resistant cotton variety Cleve wilt were planted in sterilized soil in 6 inch plastic pots. The experiment consisted of the following treatments replicated 7 times: One plant each of Cleve wilt and Deltapine were planted together; 2 plants of Deltapine were planted together; and 2 plants of Cleve wilt were planted together.

The soil around the root system of each plant was infested with 2,000 M. incognita larvae approximately 2 weeks after germination. Twenty-eight days after inoculation the plants were removed from the pots and the roots were killed and fixed in F.A.A. and stained in acid fuchsin-lactophenol. After destaining in clear lactophenol, the number of galls and egg masses were recorded. The method of using galls and egg masses as an index of resistance is based on the fact that only mature females produce eggs and in resistant plants the number of larvae

reaching maturity is reduced as compared to the susceptible variety.

The data were analyzed by computing "t" values for the .05 level of probability.

Results

Data from this study indicate that there was no significant difference between the number of egg masses produced by the nematodes on the resistant cotton variety when grown alone or in combination with the susceptible cotton variety. However, egg masses produced by the susceptible cotton variety were significantly less when grown in combination with the resistant variety than when grown alone. The influence that resistant and susceptible cotton plants had on each other when grown together is shown in Table 6. The number of total galls produced on each of the resistant and susceptible cotton varieties was not found to be significantly different when the plants were grown together or when they were grown separately.

Table 6. The number of egg masses and galls observed on resistant (Deltapine Smooth Leaf) cotton plants when grown together with incognita larvae.

Rep. No.	Clevewilt and Deltapine				Deltapine and Deltapine			
	Egg Masses	Galls	Egg Masses	Galls	Egg Masses	Galls	Egg Masses	Ga
1	0	4	25	8	47	38	84	29
2	1	3	37	12	57	21	57	42
3	21	8	29	76	70	43	60	19
4	3	14	34	41	57	41	39	50
5	7	5	10	8	37	47	39	16
6	6	7	71	16	77	51	59	29
7	2	5	35	17	53	12	78	34
Mean	5.7	6.5	34.4	25.4	56.8	36.1	59.4	31
t-5%	NS	NS	5.8	NS				

masses and galls observed on resistant (Clevewilt) and susceptible (Leaf) cotton plants when grown together and inoculated with M.

Deltapine		Deltapine and Deltapine				Clevewilt and Clevewilt			
Plants	Galls	Egg Masses	Galls	Egg Masses	Galls	Egg Masses	Galls	Egg Masses	Galls
8	47	38	84	29	1	4	2	6	
12	57	21	57	42	0	2	0	3	
76	70	43	60	19	0	5	0	4	
41	57	41	39	50	0	0	0	7	
8	37	47	39	16	2	14	3	5	
16	77	51	59	29	0	7	1	15	
17	53	12	78	34	1	8	2	4	
4	25.4	56.8	36.1	59.4	31.2	1	5.7	1.1	6.2
8	NS								

INVESTIGATIONS TO DETERMINE THE INFLUENCE OF HOST
RESISTANCE ON THE SEX RATIO AND DEVELOPMENT
OF ROOT-KNOT NEMATODES

Materials and Methods

Another possible mechanism of cotton resistance to root-knot nematodes might be that unfavorable physiological or morphological conditions encountered by the nematode larvae in the resistant plants could be responsible for retarding larvae development or cause a large increase in the number of males. To investigate this hypothesis the following study was initiated:

Cleviewilt and Deltapine Smooth Leaf cotton seeds were planted in 6 inch plastic pots filled with a sterile sand-soil mixture 3:1. One seed was planted in each pot and enough seeds were planted to insure that each observation date could be replicated 7 times. The seeds were germinated and the plants grown in growth chambers at 29 C day temperature and 24 C night temperature. The day length was 15 hours. The plants were watered daily with Hoaglands nutrient solution.

When the plants had attained the true leaf stage, the roots were infested with 2,000 M. incognita larvae, as per the method described in the penetration versus physiological age study.

After 48 hours, the plants were carefully removed from the inoculation saucers and the roots were washed to remove larvae that were attached to the roots but had not penetrated. Eight days after inoculation and every 4 days thereafter for 24 days, 5 plants were removed from the pots, killed and fixed in F.A.A. solution for 24 hours, stained in boiling lactophenol-acid fuchsin for 4 minutes, and stored in clear lactophenol for clearing until they could be read (16).

Data were obtained by dissecting all of the nematode from the roots, counting them and determining what morphological stage of development they had attained. Morphological development was separated into 4 classes; adults, developing larvae, infective larvae, and males. These classes were determined by observing the nematodes with the aid of a compound microscope and recording the number of cuticles present, presence or absence of the stylet, gonad development, and the absence or presence of eggs. The sex was determined and the total body area of the nematodes was measured. Measuring was accomplished by projecting the image of the nematodes onto a piece of paper from a constant distance of 166.25 cm. The image was traced on the paper and then measured to the nearest 0.1 cm^2 with a compensating polar planimeter. From these tracings 50 were selected at random and were analyzed statistically for differences in mean body area.

Results

Table 7 shows the average number of nematodes that comprise each of the 4 classes, adult females, developing larvae, infective larvae, and males, at 8, 12, 16, 20, and 24 days after inoculation. This table also shows the average body diameter of the nematodes recovered at these dates. Table 8 shows what per cent each of the 4 classes represents of the average number of nematodes recovered. The data from Tables 7 and 8 indicate that larval development is retarded in the resistant cotton variety as compared to the susceptible variety. Statistical analyses of the data from Table 7 showed that the number of nematodes in each class, and the mean body area of the nematodes in the resistant cotton variety were significantly lower, at the .05 level of probability, than those of the susceptible cotton variety.

Table 7. Number and mean cross sectional area of nematodes comprising four developmental classes, adult females, developing larvae, infective larvae, and males, redovered from resistant and susceptible cotton plants -- Mean cross sectional area is an average of 50 nematodes. All others are means of five replications.

Days after inco.	Deltapine Smooth Leaf					Clevewilt				
	Ad. Fem.	Develop. larvae	Inf. lar.	Males	Mean ^a / cross sec. area (cm ²)	Ad. Fem.	Develop. larvae	Inf. lar.	Males	Mean ^a / cross sec. area (cm ²)
8	0	193	1	3	3.81	0	22	35	2	1.62
12	106	46	0.4	5	6.79	2	14	7	1	3.15
16	91	22	0	4	11.31	2	23	6	2	3.67
20	220	5	0	7	18.24	18	25	0	5	5.61
24	201	13	0	3	21.94	12	5	0	2	17.32

^a/Magnification factor = 80.

Table 8. The number of nematodes comprising four classes, adult infective larvae, and males, expressed as a per cent of resistant and susceptible cotton -- Average of five re

Day after inoc.	% of the total number recd				
	Adult females		Developing larvae		Infect
	Deltapine	Cleviewilt	Deltapine	Cleviewilt	Deltapine
8	0	0	97.96	37.3	.2
12	67.5	8.3	29.2	58.3	0
16	77.7	6.0	18.8	69.6	0
20	94.8	37.5	2.1	52.0	0
24	92.6	63.1	5.9	26.3	0

s comprising four classes, adult females, developing larvae, males, expressed as a per cent of the total number recovered from ble cotton -- Average of five replications.

% of the total number recovered					
Developing larvae		Infective larvae		Males	
Deltapine	Cleviewilt	Deltapine	Cleviewilt	Deltapine	Cleviewilt
97.96	37.3	.2	59.3	1.5	3.3
29.2	58.3	0	29.1	3.1	4.1
18.8	69.6	0	18.1	3.4	6.0
2.1	52.0	0	0	3.0	10.4
5.9	26.3	0	0	1.3	10.5

THE HISTOLOGY OF RESISTANT AND SUSCEPTIBLE COTTON PLANTS INFECTED WITH ROOT-KNOT NEMATODES

Materials and Methods

Material has been presented in the literature review that indicates that in some instances the histology of resistant plants infected with nematodes differs from susceptible plants infected with the same nematode. To investigate this possibility in root-knot nematode resistant and susceptible cotton, the following study was conducted: Deltapine Smooth Leaf and Cleve wilt cotton plants infested with root-knot nematodes were obtained by the method previously described in the section on sex ratio and development. This experiment consisted of 2 replications for each of the study dates of 8, 12, 16, 20, and 24 days after inoculation. On each of these dates, the root systems of 2 plants were killed and fixed in F.A.A. Selected portions of these root systems were embedded in paraffin, sectioned to 12 μ on a microtome, and stained with safranin and fast green (36). Slides obtained from this procedure were observed with the aid of a compound microscope and pictures were taken of selected sections. The camera used was a Nikon M-35 attached to a Nikon Model SUR-Ke Microscope. Kodak Kodacolor-X film was used.

Results

Comparisons of the effect of M. incognita larvae on resistant and susceptible cotton varieties are shown in Figures 1-10. In general, giant cells in the resistant cotton variety appeared to have thinner cell walls (Figures 4, 8, 10) than the giant cells of the susceptible variety (Figures 3, 7, 9). Another reaction observed in the resistant variety and not in the susceptible variety was the presence of large amounts of parenchyma tissue surrounding the giant cells (Figures 4, 10). In Figure 8, the giant cells of the resistant cotton appear to be more vacuolated than the giant cells of the susceptible cotton in Figure 7. This observation was made in a number of instances in the resistant variety but was not consistent enough to be considered a real difference between the reactions of the two varieties.



Figure 1. Cross section of a root of root-knot nematode susceptible cotton, Deltapine Smooth Leaf, 8 days after inoculation with M. incognita. (400X)

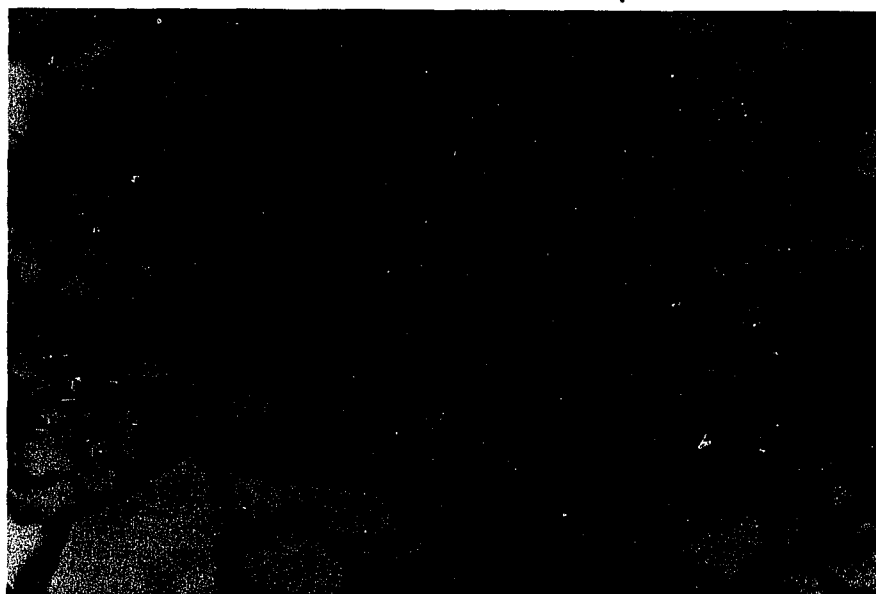


Figure 2. Cross section of a root of root-knot nematode resistant cotton, Clevewilt, 8 days after inoculation with M. incognita. (400X)



Figure 3. Cross section of a root of root-knot nematode susceptible cotton, Deltapine Smooth Leaf, 12 days after inoculation with M. incognita. (400X)



Figure 4. Cross section of a root of root-knot nematode resistant cotton, Clevevilt, 12 days after inoculation with M. incognita. (400X)



Figure 5. Cross section of a root of root-knot nematode susceptible cotton, Deltapine Smooth Leaf, 16 days after inoculation with M. incognita. (400X)

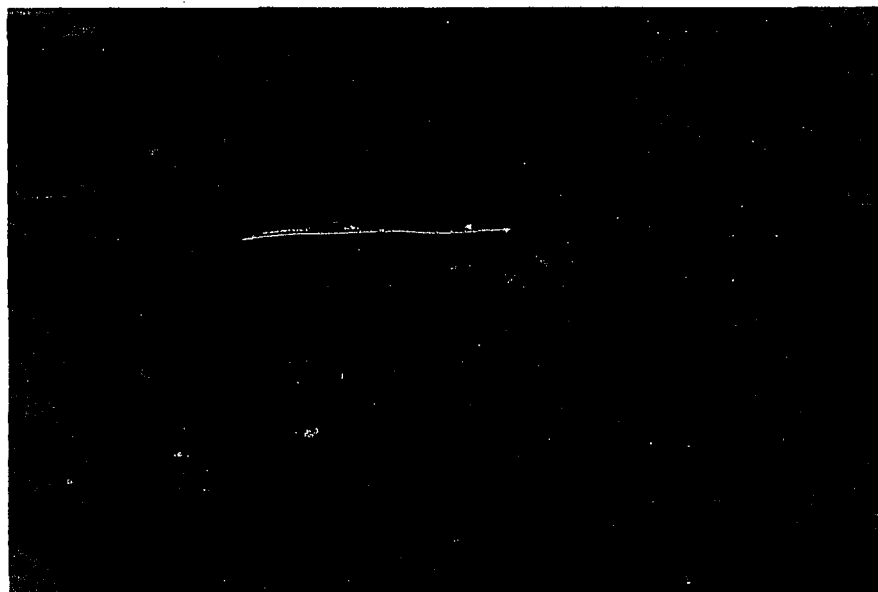


Figure 6. Longitudinal section of a root of root-knot nematode resistant cotton, Clevevilt, 16 days after inoculation with M. incognita. (100X)

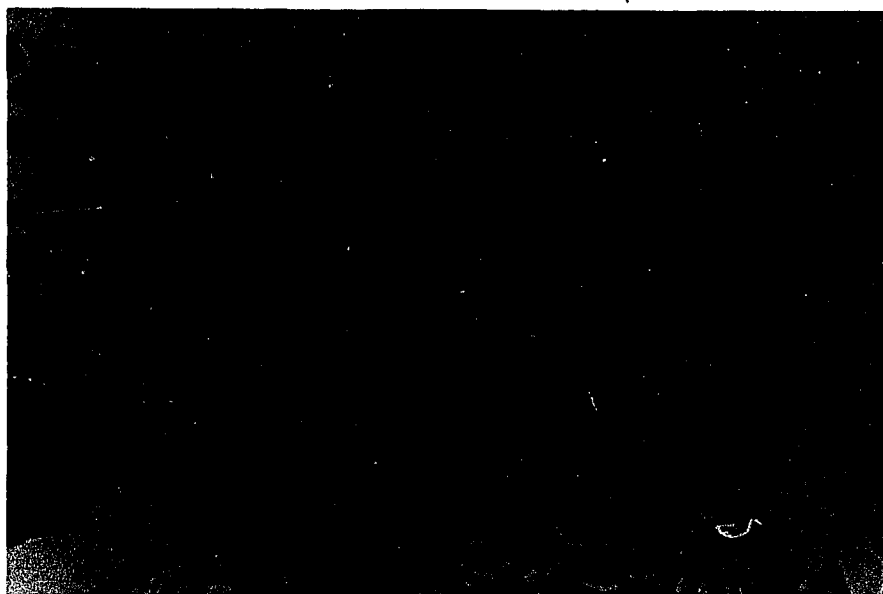


Figure 7. Cross section of a root of root-knot nematode susceptible cotton, Deltapine Smooth Leaf, 20 days after inoculation with M. incognita. (100X)



Figure 8. Cross section of a root of root-knot nematode resistant cotton, Cleviewilt, 20 days after inoculation with M. incognita. (100X)

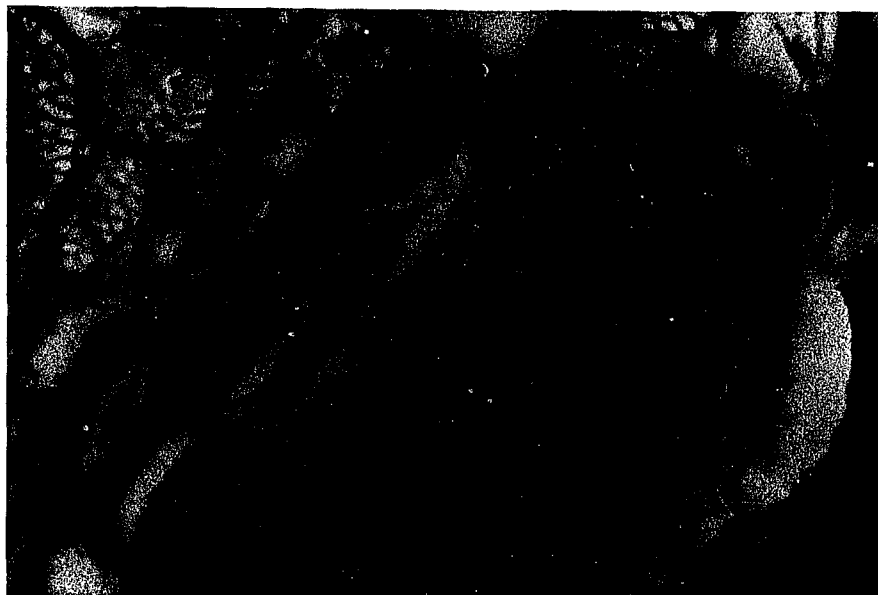


Figure 9. Longitudinal section of a root of root-knot nematode susceptible cotton, Deltapine Smooth Leaf, 24 days after inoculation with M. incognita. (400X)



Figure 10. Longitudinal section of a root of root-knot nematode resistant cotton, Cleve wilt, 24 days after inoculation with M. incognita. (400X)

INVESTIGATIONS TO TRANSFER ROOT-KNOT SUSCEPTIBILITY AND/OR RESISTANCE IN COTTON BY GRAFTING

Materials and Methods

To test the hypothesis that resistance could be transferred from a resistant plant to a susceptible plant the following study was performed:

Seeds of Deltapine Smooth Leaf and Cleve wilt cotton were planted in 6 inch plastic pots filled with a sand-soil mixture 3:1. One seed was planted in each pot. Forty seeds of each variety were planted. The plants were watered weekly with Hoagland's nutrient solution.

When the plants had grown for approximately 1 month, a technique of in-arch grafting was used to join 1 plant of Deltapine Smooth Leaf cotton to 1 plant of Cleve wilt cotton. Grafting was accomplished by removing a portion of the stems of the 2 cotton plants so that some cambial tissue was exposed. A sharp razor blade was used to accomplish this. After the cambial tissue was exposed, the 2 plants were bound together with plastic ribbon of the type used by florists to tie potted plants to support stakes. Leaves and petioles of the grafted plants, except for those at the growing tip, were removed to reduce transpiration. The grafts were allowed to heal for 14

days. This technique of grafting had an approximate degree of success of 90%.

When the grafts had healed, the root stocks and tops were separated in order to achieve the desired combination of resistant tops on susceptible root stocks and vice versa. For example, if a plant with a resistant top on a susceptible root stock was desired, the stem of the susceptible cotton plant was cut off above the graft union, and the stem of the resistant cotton plant was cut off below the graft union.

Due to the age of the plant when the graft unions had healed the root systems were extensive. To ensure a high probability of success of infection by the nematode larvae to be used as inoculum, the roots were pruned and the plants repotted in 6 inch plastic pots filled with a 3:1 sand-soil mixture. The plants were allowed to grow new roots, which took approximately 2 weeks. Following 2 weeks the soil around the root systems of the plants was infested with 2,000 M. incognita larvae per plant. Following 28 days the plants were removed from the pots, and washed gently to remove soil adhering to the roots. The root systems were removed and stained using the method previously described in the section on larvae egression from the roots of resistant cotton varieties.

The number of galls and egg masses observed on the roots of the grafted plants was used as an indication as to

whether the resistance factor was transferred. If the resistance factor was transferred to susceptible root stock from the resistant top, fewer larvae would mature in the susceptible root stock and, therefore, fewer egg masses would be produced when compared with plants which had both susceptible tops and root stocks.

The data collected were statistically analyzed by computing "t" values at the .05 level of probability.

Results

The data obtained from counting egg masses and galls of the reciprocal grafts are shown in Table 9. The difference between the mean number of egg masses (26.4) observed on the plants where both the root stock and top was the susceptible cotton and the mean number of egg masses (6.6) observed on the plants where the top was the resistant cotton and the bottom was the susceptible cotton, was nearly significant. The computed value of "t" was 2.75 while the tabular value of "t" was 2.77. The difference in the number of galls observed on these combinations was not significant.

The differences in the number of galls and egg masses observed on the combinations, susceptible tops on resistant root stocks, and resistant tops on resistant root stocks, were not significant.

Table 9. The number of galls and egg masses observed on reciprocal grafts of resistant and susceptible cotton.

Plants	CW ^{a/} on DP ^{b/}		DP on CW		CW on CW		DP on DP	
	Egg Masses	Galls	Egg Masses	Galls	Egg Masses	Galls	Egg Masses	Galls
1	24	103	1	18	0	5	70	19
2	0	4	1	2	0	3	7	9
3	3	13	2	8	3	16	14	24
4	5	72	0	5	1	8	15	7
5	1	18	0	3	Missing		26	17
Mean	6.6	42.0	.80	7.2	1	8	26.4	15.2
t-value 5%	N.S.		N.S.					

^{a/}Root-knot nematode resistant cotton.

^{b/}Root-knot nematode susceptible cotton.

DISCUSSION

Even though a wide variety of materials is produced by the roots of plants (1) the evidence for and against a chemical attractant is about equal (34). On the other hand, there is considerable evidence that some plants which are resistant to nematodes do produce a toxin or repellant (32, 38).

In this study it was found that larvae of M. incognita were attracted equally by the susceptible and resistant cotton varieties. It would also appear from these results that root exudates from resistant cotton plants have neither a repellant nor toxic effect on the nematodes over the time period during which the nematodes were exposed.

While it is the opinion of some (23) that attraction is not important as a resistance mechanism, penetration appears to play a greater role in resistance of plants to nematodes. Gaining entrance into a plant would constitute a major prerequisite to parasitism by such nematodes as the genus Meloidogyne. Plants whose morphology were such that for some reason or another their roots could not be penetrated by nematodes would exhibit the ultimate in resistance--immunity. This rarely if ever occurs, especially in cotton. Rohde (34) states that in general,

resistance shows up after infection. However, it is reasonable to conjecture that one of the reasons for apparent resistance could be that fewer nematodes penetrate the resistant host than the susceptible host. This phenomenon has been investigated in many hosts (2, 4, 7, 10, 11, 24, 28, 29, 31, 33), and at present the evidence for and against reduced larval penetration in the resistant host is approximately equal. In all cases where it was investigated, there was no apparent difference in root morphology between the susceptible and resistant host.

In this study it was shown that there was no difference in penetration of the resistant cotton variety as compared with the susceptible variety.

Penetration of cotton roots by nematode larvae was lower in the penetration study (approximately 17%) than it was in any of the other studies where penetration was recorded (approximately 30%). This may have resulted from using cotton seedlings which were rapidly elongating, and placing the nematode larvae in one zone instead of mixing them throughout the sand. In this case the portion of the root most susceptible to root-knot nematode attack, the area directly behind the root tip, would grow through the zone of highest nematode concentration before the total number larvae that would normally penetrate could do so. Even though penetration was lower than generally observed in other portions of the study, the data obtained should

be applicable since both the resistant and susceptible cotton varieties were treated in the same manner.

One of the most unique resistance mechanisms suggested in the literature recently investigated (31) is that of egression of nematode larvae from resistant roots. Reynolds and Carter (31) have observed this phenomenon in root-knot resistant alfalfa. They have shown that M. incognita acrita enter both resistant and susceptible alfalfa varieties at the same rate. However, after 3 to 4 days there was a sharp decrease in the number of larvae in the resistant alfalfa. They determined that this decrease was due to an egression of larvae from the roots. It is generally accepted that after 72 to 96 hours in the host, root-knot larvae have in most cases initiated giant cell formation which in turn is taken as evidence of feeding. In the case of egression after 72 to 96 hours, this may indicate that the nematodes tried to initiate a host-parasite relationship but upon failing to do so immigrated from the plants. Data collected from this study indicate that this phenomenon does not occur in resistant cotton. It was found that nearly equal numbers of M. incognita penetrated both resistant and susceptible cotton varieties, but there was no egression from the roots of the resistant variety at any time up to 16 days. At this time, there were fewer nematodes in the resistant cotton than were in the susceptible cotton. There is some evidence that the

nematode larvae died or were killed and disintegrated in the resistant tissue. Dean and Struble (7) have observed a similar occurrence in sweet potatoes resistant to root-knot nematode.

The method of capturing egressing larvae in sand then extracting them on a Baermann funnel could have been too insensitive and could have influenced the results if the emerging larvae were weakened or in low numbers. To check this possibility, the experiment was repeated using a method of collecting egressing larvae in water, then concentrating them on a millipore filter. The results obtained from this experiment concur with the original design and it is concluded that there is no egression of M. incognita larvae from resistant cotton, and furthermore egression does not play a role in the resistance mechanism for this cotton. The observed decrease in the number of nematodes present in the resistant variety could be explained in 3 ways. It could have been possible that larvae in the resistant cotton did not stain well and were therefore overlooked in the counting process. However, plant material from both the resistant and susceptible tissues were treated exactly the same and no gross differences in the nematode counts, that could be attributed to technique, were observed in the susceptible plants. Furthermore, the same staining technique used in this experiment was used many times before and after on the

resistant cotton and no problems of the nematodes taking the stain were encountered. The second explanation is that with time the root volume increases logarithmically and infective larvae could be more easily overlooked in the large mass of roots examined. In the susceptible cotton, the nematodes were larger and easier to detect. The third explanation is that the nematode larvae in the resistant plants died or were killed and disintegrated or were absorbed by the plants. Larval disintegration and/or absorption and the observation that nematode development is retarded in the resistant plant would explain why even though resistant and susceptible plants are penetrated by essentially the same number of larvae, the number of larvae reaching maturity in the resistant plants is substantially lower.

It has been stated that the age of the plant influences nematode development (39). If this is so, is there more, less, or equal influence in resistant plants compared to susceptible plants? Jorgensen and Musselman (22) have shown that sugar beet plants are more susceptible to attack by Heterodera schachtii in the seedling stage than when they are older. The data obtained from this study show this to also be true of cotton. These data also show that the decrease in penetration by M. incognita larvae with plant age is apparent in both resistant and susceptible varieties. Decreases in penetration with age

could be due to a change in the physiology of the plant making the older root tissues less attractive, or to the hardening of the epidermal tissue of older roots making it difficult for larvae to penetrate. From the time the susceptible plants were about 2 weeks old until the end of the experiment, at which time the plants were 1 month old, the number of penetrating larvae remained relatively constant. However, this was not so for the resistant variety in which the number of penetrating larvae decreased steadily up to the last experimental date. As the resistant plants mature, there may be an increased production of some chemical substance which makes the resistant plant tissue less attractive than the susceptible tissue. Histological investigations of the two varieties do not show any apparent physical differences in the tissue of the two varieties.

Nematodes developed more slowly in older plants of both resistant and susceptible cotton. However, development was more retarded in older resistant plants than in susceptible plants of the same age. This retardation of development may be caused by a failure of the resistant plant tissue to respond to nematode secretions or it may be caused by production, by the plant, of a growth retardant or toxin in response to the stimulus of the invading nematode. Also, there is the possibility that a toxin or growth retardant is inherently present in resistant tissue but more so in older tissue. In the

author's opinion the presence of a growth retardant or toxin in resistant tissue, that increases with the age of the plant, is the most feasible explanation. If the resistant plant failed to respond to nematode secretion, it would be expected that there would be no giant cells formed, but this is not the case. If the plant produced a toxin or growth retardant in response to the stimulus of the invading nematode, penetration and development should be the same in resistant plants of any age. On the other hand if a toxin or growth retardant were inherent in the resistant tissue, but in greater quantity in older tissue, it might be expected that younger plants would show greater penetration and less retardation in the development of nematodes.

It was shown conclusively in an earlier experiment that root-knot larvae were attracted equally by resistant and susceptible cotton plants. Even so it was of interest to determine if root-knot susceptible and resistant cotton plants, when grown in close proximity, would have any effect on the normally observed reaction of one another. Mature root-knot resistant cotton might produce a toxin as a root exudate that would affect the attraction or penetration of root-knot larvae of a susceptible plant growing in close proximity. Rohde and Jenkins (35) showed that mature asparagus plants produced a toxin as a root exudate which made them resistant to Trichodorus christiei. They also

showed that T. christiei would not increase on the roots of a suitable host if they were intermingled with the roots of the asparagus. As indicated in Table 5, there was a decrease in the number of egg masses produced on the susceptible cotton when grown together with the resistant cotton. These results cannot be held as necessarily conclusive since the experiment was not repeated.

It has been shown in this study that development of nematodes in resistant plants is retarded at any stage when compared with development in susceptible plants. Development of the nematode method of determining the extent to which a host-parasite relationship, favorable to the parasite, has developed. Another means, especially with root-knot nematodes, of measuring the host-parasite relationship is to observe the total maleness of the population. It has been shown that under stress or poor nutrition (26) the number of males in a given population will increase. In the study of nematode development and sex ratio in resistant and susceptible cotton varieties, it was shown (Table 7) that the number of males recovered in the resistant plants was not significantly higher than the number recovered in susceptible plants. It was also noted, in the histological study, that the syncytia formed in the resistant cotton varied only subtly from those formed in the susceptible cotton. In all cases studied, some nematodes always developed into mature egg-laying

females. In view of these observations, the retardation in development of nematodes in the resistant host is probably due to something other than lack of a favorable nutritive relationship with the host. The mechanism of resistance could be due to the presence of an inhibitor, as discussed earlier, that prohibits enzyme secretions of the nematode from eliciting a favorable response from the host cells. Or, that particular substance(s) which causes the host cells to respond to nematode secretions and become a syncytium may be produced in too small a quantity to cause the reaction to occur.

From information presented in the literature review, transference of resistance from a resistant to susceptible plant through grafting has for the most part been unsuccessful. Data presented in this study (Table 9) show that neither resistance nor susceptibility could be conferred upon plants by grafting. These data indicate that resistance and susceptibility are both factors which are inherent in individual cells due to chemical substances which do not move across the graft union. Resistance or susceptibility are biochemical reactions which occur on a cellular level and furthermore probably only manifest themselves when stimulated by nematode injury or secretions.

CONCLUSIONS

From the observations and data obtained from the study, the following conclusions can be made:

1. There is no difference in attraction, of the root-knot larvae, between the resistant and susceptible cotton varieties.
2. Root-knot larvae penetrate susceptible and resistant cotton roots equally. However, there may be a slight decrease in penetration of the resistant host with an increase in plant age.
3. Larvae of M. incognita do not egress from the roots of resistant or susceptible cotton plants.
4. Development is greatly retarded in resistant plants as compared to susceptible plants.
5. Resistance or susceptibility cannot be transferred by grafting.
6. The mechanism of resistance in cotton is probably due to the presence of a toxin or growth inhibitor inherent in the cells of the resistant plant.

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