THE NATURE OF RESISTANCE TO THE ROOT-KNOT NEMATODE (MELOIDOGYNE INCOGNITA ACRITA CHITWOOD) IN COTTON

By

BILL B. BRODIE

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

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Thesis Approved:

Lloyd A. Brinkerhoff
Thesis Adviser

Hoover Cothran

F. Ben Stubble

Dean of the Graduate School

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INTRODUCTION

The economic importance of the root-knot nematode in cotton production has been recognized for many years. The role of the root-knot nematode in increasing the severity of Fusarium wilt has caused more emphasis to be placed on breeding cotton for resistance to root knot. Successful attempts have been made in combining root-knot and Fusarium wilt resistance.

Breeding for resistance to root-knot nematode in Oklahoma is carried out in conjunction with the Fusarium wilt resistance breeding program. In the breeding nursery the testing of plants consists essentially of planting breeding material in infested soil and then selecting individual plants or progeny rows on the basis of disease symptoms and yield at the time of harvest. This practice has left much to be desired in that consistent results may not be obtained from year to year due to the influence of varying environmental factors.

To overcome such difficulties, it was realized that a method needed to be developed by which a more accurate and more rapid evaluation of cotton varieties to root knot could be obtained. With the use of such a method there should result a considerable saving of time with a greater degree of confidence in the results.

When breeding for resistance to root knot was started in Oklahoma, it was realized that more information was needed concerning the nature of resistance and the possibility of selecting for resistance

after artificial infection.

The purpose of this investigation has been to obtain a better understanding of host-parasite relationships and to determine whether or not an effective program of selecting for resistance to the root-knot nematode in seedlings could be carried out in the greenhouse and laboratory. The approach to this problem was essentially two-fold: (1) to develop an inoculation technique which would insure that all plants were uniformly infected with the nematode and (2) to determine any differentiating features that could be used in classifying resistance and susceptibility.

REVIEW OF LITERATURE

Several specific names have been applied to the root-knot nematode. Prior to 1949, what was considered to be the root-knot nematode was generally called Heterodera marioni (Cornu) Goodey. Many workers presented conflicting evidence as to the behavior and host range of nematodes classified as H. marioni. The work of Christie and Albin (7), Christie (5), and Christie and Havis (9) established experimentally that this species was not a single entity but was composed of several diverse forms.

Chitwood (3), on the basis of morphological studies, concluded that the root-knot nematodes constituted a genus apart from Heterodera. Meloidogyne was chosen as the earliest valid generic name. At this time, Chitwood recognized five species and one variety. The species causing root knot of cotton was classified as Meloidogyne incognita var. acrita Chitwood. Later, acrita was designated as a subspecies rather than a variety.

The life cycle of the root-knot nematode, then known as H. marioni, is given in detail by Christie and Cobb (8). A summary of this life cycle will be given to facilitate the interpretation of some of the results. In the nematode there are four molts during the process of maturation. The larva molts for the first time before it has attained its maximum length and while it is still in the egg. The second molt takes place soon after the larva has entered a suitable host. Larvae that do not enter a suitable host never develop beyond the second stage. There is

no true third stage since the second and third cuticles are loosened simultaneously.

In the male there is a distinct fourth stage, during which metamorphasis takes place. After the fourth molt, the male is sexually mature and migrates freely through the plant or soil.

In the female there is a very short fourth stage, as there is a very brief interval between the detachment of the second and third cuticles and the detachment of the fourth cuticle. After the fourth molt, the female is sexually mature and remains in a fixed position in the plant.

The external feeding and entry of the root-knot nematode has been presented by Linford (14). He reports that soon after the larva makes contact with the root it begins thrusting its stylet intermittently into the cells even in the relatively mature zone. The larva migrates down the root toward the root tip. After feeding on a cell or two, it advances to the young elongating zone or the meristimatic region. After a cell of the dermatogen or a young epidermal cell has been fed upon one or more times, its wall may break, allowing the head of the nematode to slip in. The larva then continues to penetrate into the root, feeding as it goes.

Christie (5) points out that after the larva has entered the root, it migrates intercellularly. Injury to the root through cell destruction is slight. When permanently located, the head of the larva usually is in the plerome near the beginning of the region of elongation.

Linford (15) also has given an account of the method of feeding of the root-knot nematode. He concluded that the root-knot nematode feeds by thrusting its slender stylet into a cell adjacent to its head,

apparently injecting saliva and then sucking out only part of the cell contents, then retracting its stylet. This process enables the nematode to feed on all cells within its reach, avoids early destruction of cells, and maintains conditions suitable for long continuous feeding in a small group of cells. Giant cell growth appears to keep pace with increasing requirements of the enlarging nematodes.

Christie (4) studied the development of root-knot nematode galls by infecting young tomato seedlings and timing the development of the galls. Christie's observations revealed that when a nematode enters a root of the host plant the immediate effects on the root cells are: hypertrophy of the cells of the cortical region; slight hypertrophy of the cells of the pericycle and endodermis lying near the path of the parasite; a stimulation of cell division in the pericycle; and frequently a suppression of cell division in the apical meristem.

During the first 48 to 60 hours after the larva has assumed its permanent position, the cells lying near the parasite remain undifferentiated. Within 72 hours these cells enlarge slightly and their walls disintegrate. Protoplasmic contents of adjacent cells coalesce to form a giant cell. The giant cell invades adjacent areas and other cells are absorbed after dissolution of their cell walls. Eventually, nuclear membranes break down and giant cell nuclei coalesce and finally disintegrate.

From this evidence, Christie postulated that it was highly unlikely that these changes are induced by mechanical injury, or through the removal of substances by the parasite, or by the stimulation of excretory products. Rather, it appeared probable that the tissue changes in the root are due to the stimulating action of some excretion expelled

through the stylet of the nematode.

Prior to 1939, nature of resistance to the root-knot nematode received very little attention. In earlier literature, resistance to nematodes has been considered as equivalent to the fact that the nematodes were prevented from entering the plant. Steiner (24) defined resistance as the ability of the plant to resist the attacks of nematodes, by either some mechanical or chemical means. Tyler (25) agreed with this definition and added that a resistant plant was relatively, but not absolutely free from nematodes when grown in infested soil. Moderately resistant plants might contain some nematodes and if the conditions were unfavorable for plant growth a severe attack might occur. Tolerance might occur when a plant was attacked but was able to avoid severe injury.

Barrons (1), in his investigation of Meloidogyne spp. in several species and varieties of plants, found that there was no general relationship between the extent of penetration of the nematodes and the resistance of a given plant. Some resistant bean varieties contained more larvae per root than susceptible varieties. He concluded that resistance was not dependent on prevention of penetration of the nematodes. Barrons proposed that resistance was due to the ability of the plant to starve the nematodes after they had entered it. He presented a theory according to which the resistance was dependent on the development of certain substances in the resistant plants which counteracted or neutralized the effect of the saliva of the nematodes. The saliva was thought to induce giant cell formation which made a convenient food supply for the developing nematodes.

Christie (6) agreed with Barrons about the nature of resistance to Meloidogyne spp. He also pointed out that this did not exclude

those cases in which nematodes were prevented from entering the plants. Christie concluded that normally, however, the resistance was due to a failing, or very slow, reaction to the stimulant effect caused by the nematodes and, because of this, the nematodes were starved and their development retarded. Thus, Christie spoke of "suitable host" instead of susceptible plants in which the nematodes grew rapidly and reproduced. In a less "suitable host" the developmental period of the nematodes was prolonged. In some host plants this period might be prolonged so much that only very few individuals reached sexual maturity and reproduced. Such plants were classified as an "unsuitable host".

Sasser and Taylor (19) found that resistance to Meloidogyne spp. was associated with several phenomena. They listed these as follows: nematode might not penetrate the roots; a small number of larvae might penetrate, but develop weakly or not at all; or a great number of larvae might penetrate, but not develop in the plants.

Liao and Dunlap (13), in a study of the invasion of two species of tomato by nematode larvae of Meloidogyne spp., found that the resistant species Lycopersicon peruvianum (L) Mill. arrested the invasion of larvae. Only a few nematode larvae penetrated the root near the tip and these were located in the peripheral cells. Invasion was arrested when approximately one half of the nematode had become embedded in the growing point of the young rootlet.

Dean and Struble (10) investigated the penetration of larvae into roots of a resistant variety of tomato. After examining several stained root systems, they found that resistant tomato roots contained approximately half as many larvae as did those of the susceptible

variety. There was an extensive necrosis associated with resistance. The larvae in the resistant roots did not develop beyond the second stage.

Resistance and susceptibility to root knot in sweet potato was also studied by Dean abd Struble (10). They observed no differences with respect to the number of nematodes entering resistant and susceptible roots. An extensive necrosis caused by nematodes entering resistant roots was observed. A few female nematodes in all resistant sweet potato lines matured and layed eggs, but most larvae died and disappeared before reaching that stage.

Shibuya (20) studied the variation in resistance to Meloidogyne sp. in 20 varieties of sweet potatoes and found significant differences between varieties with respect to the development of the nematodes. These differences were correlated with known differences in resistance. The nematodes penetrated to the same extent in resistant and susceptible varieties, but in the former no giant cells were formed.

Radewald (18) demonstrated a significant difference with respect to the number of nematodes entering resistant and susceptible sweet potato lines in a given inoculation period. The biological significance of this difference was in doubt because of the great amount of variation observed within a variety. He concluded that resistance was manifested through the failure of the nematode to develop after entering and not through failure of larvae to enter the roots. Radewald concluded that resistance was due to a hypersensitive reaction on the part of the host.

Holston and Crittenden (12) observed in their investigation of resistance to M. incognita acrita in soybeans that the nematode popula-

tion in the roots of the resistant variety was as great as in the susceptible varieties; eggs were produced at the same time in both varieties. They considered the difference between the varieties to be a matter of tolerence rather than real resistance.

Bingefors (2) studied the nature of resistance to stem nematode in red clover and concluded that a susceptible red clover plant is one in which the nematodes are not only able to penetrate, but also to reproduce and increase their numbers. A plant is resistant if the nematodes do not reproduce even though they enter the roots.

The majority of the literature on root-knot nematode work in cotton is reported in conjunction with the Fusarium-wilt complex. In an investigation of varietal susceptibility to the root-knot nematode Fusarium-wilt complex, Miles (17) reported that eight upland varieties showed some resistance to root-knot nematode. He used the percentage of plants showing galls as a means of differentiating varieties for root-knot resistance. Upland wilt-resistant varieties, as a group, were found to be more resistant to root knot than wilt-susceptible varieties.

Smith (21) (22), in a study of the reaction of cotton varieties to Fusarium wilt and root-knot nematode, found that resistance to root knot was confined to wilt resistant varieties that had originated in the lighter coastal plain soils. Resistance was evaluated on the basis of relative percentage of roots showing galls.

In discussing the problems of breeding cotton for resistance to nematodes, Smith (23) stated that yields of the major cotton varieties planted on soil infested by both nematodes and the Fusarium wilt fungus could be improved by 25 per cent by incorporating into them the type

of resistance found in Auburn 56. Nematode resistance in this latter variety has been proven to be a superior type of resistance; the variety is also resistant to Fusarium wilt.

In a search by Smith for a better source of root-knot resistance, the most promising material found was a wild cotton, Gossypium barbadense var. darwinii Watt. From early crosses of this wild species, he concluded that root-knot resistance is inherited recessively and may be polygenic.

Techniques for inoculating plants with root-knot nematodes have been described by several investigators. The most commonly used methods have been to add galled roots, infested soil, or a larval suspension to the soil in which the test plants were to be grown.

Christie (5) describes an inoculation technique which involves the use of plants grown in thumb pots. When roots were evident on the inner surface of the pot, a few ml of larval suspension was poured over them. The plants were then returned to the pot for any desired period of inoculation.

Dropkin (11), working with M. incognita acrita, developed an inoculation technique in which roots of tomato and cucumber seedlings were exposed to a known number of larvae by placing larvae, in groups of 25, in close proximity to the root tip of growing seedlings that were germinated on filter paper and transferred to petri dishes containing agar.

Radewald (18), in studying the nature of resistance to root-knot nematode in sweet potato, developed a single-root inoculation technique. This technique involved the use of glass tubing 8 cm in length and 4 mm in diameter. These tubes were filled with vermiculite which

was previously ground and passed through a 60 mesh screen. Each tube was injected with one ml of water containing a known number of nematode larvae. This resulted in a very viscous mass in the tube. Each inoculated tube was carefully slipped over one root which had been labeled. The plants were then put in a pan containing vermiculite, and after a given inoculation period the tubes were removed and the plants were placed in pots containing nematode-free soil. He reported that 100 per cent of the roots inoculated were infected when this technique was used.

MATERIALS AND METHODS

For comparison of resistant and susceptible cotton varieties Auburn 56 and Stoneville 62 were used. Stoneville 62 is known to be very susceptible to root-knot nematodes, and is used as a susceptible check in the Fusarium wilt-nematode breeding nursery in Oklahoma. Auburn 56 is known to be highly resistant to nematodes and is also resistant to Fusarium wilt. This nematode-wilt resistant variety was developed by Smith and Tisdale of Alabama. The resistance of this variety to nematodes and wilt in Oklahoma has been confirmed in variety tests in a heavily infested field at Hollis.

In addition, several selections were tested from the nematode-wilt nursery at Hollis. Their origin is as follows:

9531 - a root-knot resistant variety developed by Smith and Tisdale of Alabama

M57-207 - a stormproof selection from 9531. Pedigree: H9531-2-4-B

M57-205 - a selection from bacterial blight resistant CR4. Pedigree: CR4-45-B

M57-206 - a selection from bacterial blight resistant CR4. Pedigree: CR4-52-2-B

609 - a selection from Cluster. Pedigree: Cluster 3-1-B

The population of M. incognita acrita used in the experiments in the present investigation was obtained from the nematode-wilt nursery at Hollis. A sample of soil was taken from this field and brought to the greenhouse at Stillwater. The soil was planted to Stoneville 62 cotton and at the end of 28 days, five egg masses were picked from a

single plant. The egg masses were surface sterilized in 1.5 per cent sodium hypochlorite solution and placed on steam sterilized soil from Hollis. This soil was planted to Stoneville 62 and the population was allowed to increase. All nematodes used in subsequent experiments were propagated on Stoneville 62 cotton.

To obtain large quantities of nematode larvae to use for inoculations, the following method was used. Roots of Stoneville 62, which had been growing in soil infested with a pure culture of M. incognita acrita for approximately one month, were harvested and washed. Egg masses were picked from these roots and placed on a single layer of cheesecloth suspended over a petri dish. Water was added to the petri dish until the level of the water reached the cheesecloth. This procedure kept the egg masses moist at all times and permitted several egg masses to be hatched at one time.

The resultant larval suspension was poured off each morning simply by tilting the petri dish into a beaker. The egg masses remained on the cheesecloth and fresh water was added. The larval suspension was stored in a refrigerator held at 15 degrees C. This process was continued for several days. However, larvae held in water for more than seven days usually died or became very weak and were not used for inoculations.

To obtain a standardized larval suspension for inoculating seedlings several .1 ml aliquots of the suspension were mounted on a slide and placed under the microscope. The number of larvae was determined for each .1 ml aliquot, and from this the number of larvae per ml was calcuated. The larval suspension was concentrated by simply allowing the larvae to settle and afterwards pouring off the excess water. The suspension was diluted by the addition of tap water.

To observe nematodes in whole roots, the staining technique of McBeth, Taylor, and Smith (19) was used. This method involves the use of lactophenol plus acid fuchsin dye. The formula for the staining solution is as follows:

Lactic acid	20 ml
Glycerine	40 ml
Phenol	20 ml
Distilled water	20 ml
Acid fuchsin (stock solution)	5 ml

The staining procedure consisted essentially of washing the roots to remove excess soil, then boiling them in the staining solution for one minute. The roots were then washed in water to remove the excess stain. After removing the excess stain, the roots were placed in clear lactophenol for clearing. The clearing process requires from 4 to 24 hours, depending on the intensity of the stain desired.

Several methods have been devised for examining stained whole roots under the microscope. One method consists of placing the root in a Syracuse watch glass with a little lactophenol and then observing them as whole roots under the dissecting microscope. Or if the roots are thick and the nematodes can not be seen, the tissue may be gently torn apart with dissecting needles. Another method consists in placing the roots with a little lactophenol between two 3 in. x 1 in. microscope slides and gently crushing to render the nematodes more visible. The slides are then observed under the compound microscope. The latter method was proved more applicable in the present work since several roots could be mounted at one time and the nematodes could be counted more accurately.

DETERMINING A STANDARD INOCULATION PROCEDURE

Determination of a Suitable Inoculation Technique

One of the objectives of this study was to develop a suitable inoculation technique to evaluate the reaction of cotton seedlings to the root-knot nematode in a relatively short period of time. The first method to be tried was the single root inoculation technique described by Radewald (18) in his work with sweet potatoes.

To prepare the seedlings for inoculation, several seeds were allowed to germinate in a 12 in. x 30 in. x 3 in. pan filled with vermiculite. When the seedlings emerged, they were removed from the vermiculite and the tap root was excised just below the region of heavy root hair production. The seedlings were then transplanted to vermiculite where lateral roots developed within 24 hours. At the end of one week, the seedlings were removed and the vermiculite washed from them. Inoculation tubes containing ground vermiculite and 1 ml of larval suspension were carefully placed on two roots of each of ten seedlings of Stoneville 62 and Auburn 56.

Observations of the originally inoculated roots revealed that only 26 per cent of the roots were infected by the nematode larvae. Radewald reported that 100 per cent of the roots inoculated were infected when this method was used. This low percentage of infected roots of cotton was thought to be due to poor aeration in the inoculation tubes and possibly to mechanical injury to the roots during the process of inoculation.

It was then decided to attempt to develop a technique using vermiculite as a medium, but to allow for greater areation. This was accomplished by the construction of glass tubes 1 ¼ in. in diameter and 6 in. in length with a drainage hole in the bottom. The tubes were filled with vermiculite, in which the seeds were allowed to germinate. With this method, there was a very rapid drying of the vermiculite which resulted in poor germination and generally poor growth of the plants. Consequently, the method was abandoned.

The next method to be tried was a modification of Christie's (5) thumb pot inoculation technique and Radewald's method (18). This method employed the use of test tubes 1 ¼ in. in diameter and 11 in. in length (Fig. 1). The tubes were filled with vermiculite and placed in a rack so constructed that the root growth would take place in darkness. One seed was placed in each of the test tubes and covered with ½ in. of vermiculite. Water was added to the vermiculite in sufficient amounts to keep it moist but not waterlogged.

At the time of emergence, the seedlings were removed from the tube and the tap root was excised. The seedlings were then placed in the same tubes from which they were taken. When lateral roots had grown to 4 cm in length, which required about one week from time of planting, the tubes were inoculated with a larval suspension of known concentration.

To inoculate the roots without distrubing the plant, a hypodermic syringe equipped with a two inch eighteen gauge needle was used. The needle was pushed into the vermiculite until it rested near the side of a root tip. The larval suspension was ejected into the vermiculite with the needle in this position. Each root visible on the

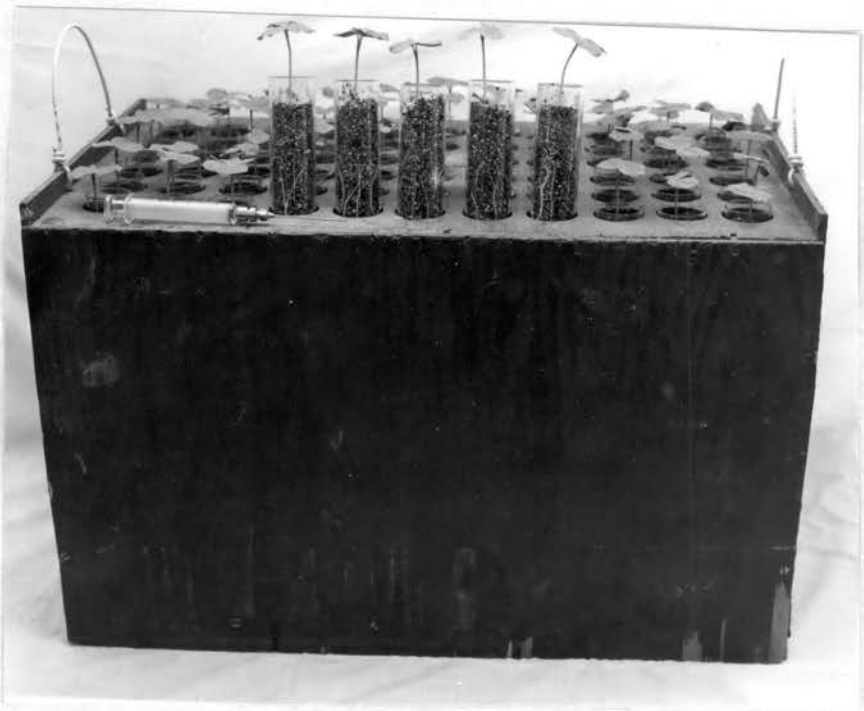

Fig. 1 (above) Equipment used for growing and inoculating seedlings. (below) Method of inoculating roots with hypodermic syringe in position for placing inoculum.

inner surface of the tube was inoculated in this manner. There was an average of five roots on each plant and each root was inoculated with 1 ml of larval suspension thus resulting in 5 ml of larval suspension to each test tube.

The first trial with the above inoculation technique resulted in no infection which was attributed to too much water around the roots thus preventing adequate aeration. In the second trial, the water level was kept well below the roots and 100 per cent of the plants had infected roots.[a] Single-root counts revealed that infection varied considerably from root to root of a given plant. In an attempt to account for this variation, observations were made on samples taken from all the roots present at the time of inoculation rather than on single roots. In some instances, roots were observed that did not contain any nematodes. Such roots were considered to be escapes. Subsequently, samples from root systems rather than single were used in all determinations.

This inoculation technique has since been used repeatedly on susceptible and resistant plants and in no instance has infection failed to occur.

All of the equipment used for inoculating cotton seedlings with root-knot larvae is shown in Fig. 1. The rack in which the test tubes were placed is equipped to hold 66 test tubes. This rack can be moved easily without any damage to the plants.

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[a]The term infection is used here as indicating that the nematodes had entered the roots.

Determination of an Optimum Inoculation Period

After a suitable inoculation technique had been developed, it became important to determine the length of time that roots should be exposed to nematode larvae in order to obtain near optimum infection.

Fifteen plants each of Stoneville 62 and Auburn 56 were grown and inoculated by the test tube inoculation method. Each tube was inoculated with 5 ml of larval suspension containing approximately 600 larvae per ml. At the end of 24, 48, and 72 hours after inoculation, five plants of each variety were removed from the test tubes. The roots were thoroughly washed to remove excess vermiculite and stained. From each of these plants 3 roots were selected at random for observation under the microscope. Counts of larvae in stained roots revealed that more larvae had entered when the plants were exposed to the inoculum for 48 hours than when exposed for 24 hours (TABLE I). Holding the plants in the inoculating tubes for 72 hours did not increase the number of larvae entering over the 48 hour sampling period. Unless otherwise indicated, the inoculation period chosen for the remainder of the experiments was 48 hours.

Determination of an Optimum Concentration of Larvae

Fifteen seedlings of each, Stoneville 62 and Auburn 56, were grown and inoculated by the test tube method. Five plants of each variety were inoculated with each of the larval suspensions containing 150, 450, and 900 larvae per ml of suspension. Counts of nematodes entering at the end of a 48 inoculation period revealed that with a population of 150 nematodes per ml of suspension all roots of

TABLE I

COMPARISON OF NUMBER OF NEMATODES ENTERING RESISTANT AND SUSCEPTIBLE COTTON ROOTS AT 3 DIFFERENT INOCULATION PERIODS

		Hours After Inoculation					
		24		48		72	
Plant No.	Root No.	Sto. 62	A 56	Sto. 62	A 56	Sto. 62	A 56
	1	0	0	0	42	2	0
1	2	0	0	1	15	26	1
	3	13	6	3	29	4	24
		13	6	4	86	32	25
	1	0	0	18	27	40	9
2	2	6	1	36	25	4	2
	3	0	0	1	1	2	5
		6	1	55	53	46	16
	1	0	0	50	0	1	10
3	2	0	0	10	14	4	5
	3	0	0	0	7	0	6
		0	0	60	21	5	21
	1	0	0	13	10	0	23
4	2	0	0	26	1	29	12
	3	4	0	0	4	0	0
		4	0	39	15	29	35
	1	0	0	10	1	9	10
5	2	0	1	2	3	12	9
	3	0	0	1	2	8	8
		0	1	13	6	29	27
	Total	23	8	171	181	141	124

Analysis of Variance

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F-Value
Total	29	13623.20	---	---
Time	2	5513.10	2756.55	8.22*
Variety	1	16.13	16.13	0.05
Time x Var.	2	44.37	22.18	0.07
Error	24	8049.60	335.40	---

* Significant at the 1 per cent level of probability

TABLE II

COMPARISON OF NUMBER OF LARVAE ENTERING RESISTANT AND SUSCEPTIBLE COTTON ROOTS WHEN EXPOSED TO LARVAL SUSPENSIONS OF 3 DIFFERENT CONCENTRATIONS

		Concentration					
		150/ml		450/ml		900/ml	
Plant No.	Root No.	Sto. 62	A 56	Sto. 62	A 56	Sto. 62	A 56
	1	1	7	30	0	55	28
1	2	1	0	1	10	58	93
	3	1	0	0	12	10	40
		3	7	31	22	123	161
	1	1	7	7	32	54	28
2	2	0	0	12	27	19	46
	3	1	4	14	3	30	34
		2	11	33	62	103	108
	1	6	8	17	129	36	16
3	2	9	4	39	8	45	12
	3	3	2	48	26	47	27
		18	14	104	163	128	55
	1	4	3	15	7	15	54
4	2	0	5	32	9	83	3
	3	0	6	40	0	10	30
		4	14	87	16	108	87
	1	0	2	0	27	38	27
5	2	0	0	0	2	46	23
	3	0	0	6	2	53	9
		0	2	6	31	137	59
	Total	27	48	261	294	599	470

Analysis of Variance

Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F-Value
Total	29	81499.00	---	---
Concentration	2	49421.10	24710.55	19.59*
Variety	1	187.53	187.53	0.14
Con. x Var.	2	1629.57	814.78	0.64
Error	24	30260.88	1260.87	---

* Significant at the 1 per cent level of probability

some plants failed to become infected. However, when the population was increased to 450 or 900 nematode larvae per ml, 100 per cent of the plants had some roots which were infected (TABLE II). A population of 900 larvae per ml gave the most uniform infection. Therefore, this concentration was used in the remainder of the experiments unless otherwise indicated.

RESULTS

Relation of Resistance or Susceptibility to the Survival and Development of Nematodes in Cotton Roots

Previous studies pertaining to the nature of resistance to root-knot nematodes have indicated that resistance is due to a failure of the nematodes to survive or develop to maturity after entering a resistant plant. This has been demonstrated in sweet potato by Radewald (18), Shibuya (20), and Dean and Struble (10), in cowpeas by Barrons (1), and in tomato by Dean and Struble (10).

To test the survival and development of nematodes entering resistant and susceptible cotton roots, the following experiment was made. Forty seeds each of Stoneville 62 and Auburn 56 were germinated in $1\frac{1}{4}$ in. x 11 in. test tubes filled with vermiculite. Upon emergence, the tap root of each germinating seed was excised just below the root hair zone. When lateral roots were approximately 4 cm in length, each plant was inoculated with 5 ml of larval suspension containing 900 larvae per ml. The plants were allowed to remain in the test tubes for 48 hours and then removed, washed thoroughly, and transplanted to 4 in. pots containing steam sterilized soil. The plants were then grown for the time intervals after inoculation as stated in TABLE III. This method permitted the determination of the length of time that a given nematode had been in a given root to within 48 hours.

At each of the stated time intervals after inoculation, 5 roots from each of 5 plants of each of the two varieties of cotton were

examined for the presence of nematodes and their stage of development. The stage of development of the nematodes was determined from their shape and form as illustrated in Fig. 2, (5). Group A includes the stage in which the larvae have begun to grow to the stage where they still possess a more or less conical tail. Group B includes larvae that have acquired a more or less hemispherical posterior end terminated by a spike and those which are about to complete the final molts. Group C includes females from the stage in which they have completed all molts to the stage in which they are almost fully grown. Group D includes those females which were fully grown, but had not yet laid eggs. Group E includes egg-laying females.

Data from this experiment are given in TABLE III. The results show that approximately the same number of nematodes had entered the roots of resistant plants as had entered roots of susceptible plants, but that the development of the nematodes in resistant plants was definitely retarded. In the susceptible plants, some of the nematodes had completed the last molt within 12 days after inoculation. Nematodes that had completed the last molt were not observed in resistant material until 20 days after inoculation. Egg-laying females were observed in susceptible Stoneville 62 within 24 days after inoculation while they were not observed in resistant material until 30 days after inoculation. From 24 to 30 days after inoculation 85 egg-laying females were found in Stoneville 62 compared to 3 egg-laying females in Auburn 56. The 3 egg-laying females found in the resistant material were all on one plant from a sample of five plants. This indicates the likelihood of segregation for susceptibility in Auburn 56.

There was a constant necrosis and eventual death of the roots

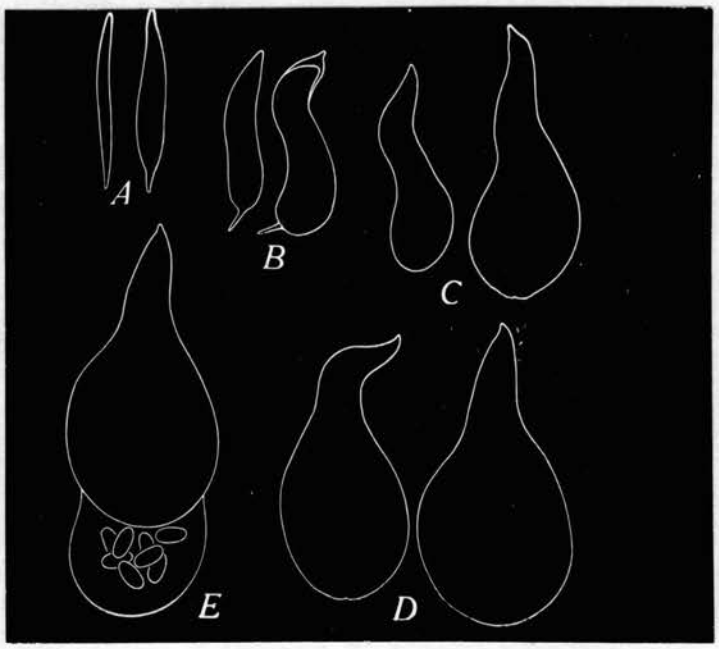

Fig. 2. Sketches of nematodes showing shape and size in each developmental group (after Christie).

TABLE III

COMPARATIVE SURVIVAL AND DEVELOPMENT OF NEMATODES IN TWO COTTON VARIETIES

Days after inoculation	Variety	Number of nematodes in each of the stated developmental stages: A	B	C	D	E
8	Stoneville 62	61	9	0	0	0
	Auburn	110	0	0	0	0
12	Stoneville 62	61	69	37	0	0
	Auburn 56	130	2	0	0	0
14	Stoneville 62	53	105	43	0	0
	Auburn 56	177	25	0	0	0
16	Stoneville 62	59	28	48	1	0
	Auburn 56	210	18	0	0	0
20	Stoneville 62	137	15	51	2	0
	Auburn 56	117	1	1	0	0
24	Stoneville 62	69	11	65	53	16
	Auburn 56	287	12	11	1	0
30	Stoneville 62	26	11	19	20	69
	Auburn 56	285	5	10	4	3

associated with resistant material. The infected roots started dying at the tip approximately 8 days after inoculation, and progressed toward the central root. At the time the roots started dying new lateral roots were being initiated from the tap root. At the end of 16 days, most of the infected roots of the resistant plants were dead (Fig. 3). In some instances, inoculated roots of resistant plants contained only a few nematodes and did not die but stopped growing.

Observations on the dead roots revealed that a large number of

larvae had entered but had not developed beyond the second stage. These larvae did not appear to have shrunk or to be deformed in any way. There were no distinct galls found on the resistant plants that possessed dead roots. However, some slightly swollen areas were observed on resistant plants. Further investigations of this type of reaction were carried out later; details are reported in a later section of this work. Extensive galling was observed on approximately 1 out of 5 supposedly resistant plants and was thought to be due to a segregation for resistance and susceptibility.

The susceptible plants showed no signs of dead roots but occasionally exhibited dead root tips which contained several second stage larvae. There was extensive galling associated with susceptiblity (Fig. 4). In some instances infected roots of susceptible plants stopped growing in length and produced several lateral roots from the galled region. Other cases were observed in which the infected roots did not stop growing in length, but laterals were produced from the galled region.

There was no indication of the nematodes disappearing in either resistant or susceptible plants even up to 30 days after inoculation.

Relation of Resistance or Susceptibility to External Appearance of Cotton Roots Infected with Root-Knot Nematode Larvae

One of the original objectives of this investigation was to determine a procedure by which cotton seedlings could easily and quickly be evaluated for their reaction to root-knot nematode in the greenhouse or laboratory. To do this, it was decided to investigate the possibil-

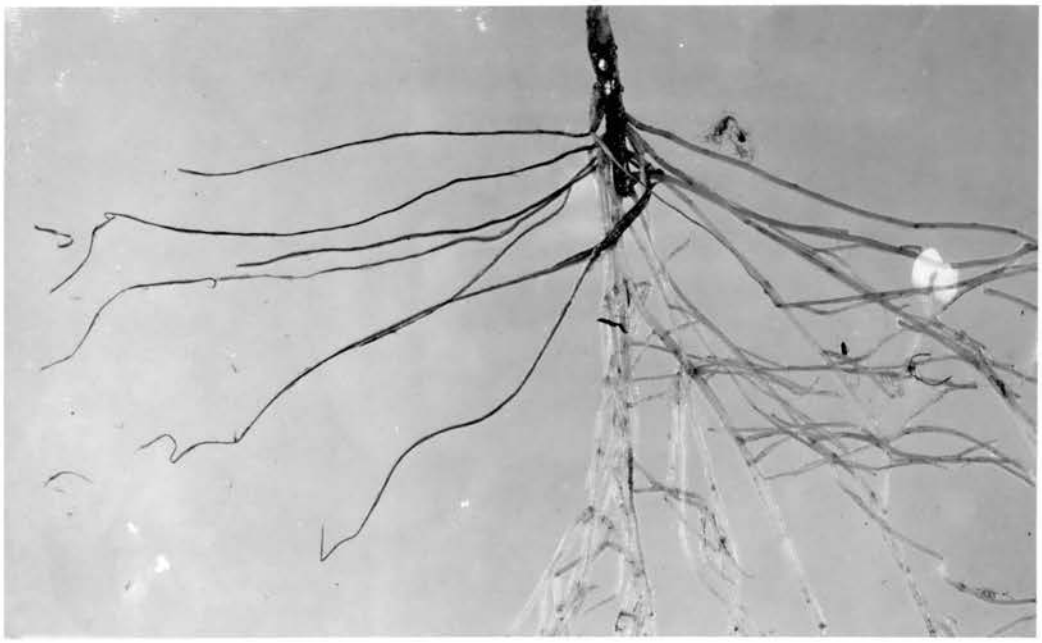

Fig. 3. Resistant Auburn 56 plant 16 days after inoculation showing dead infected roots on the left.

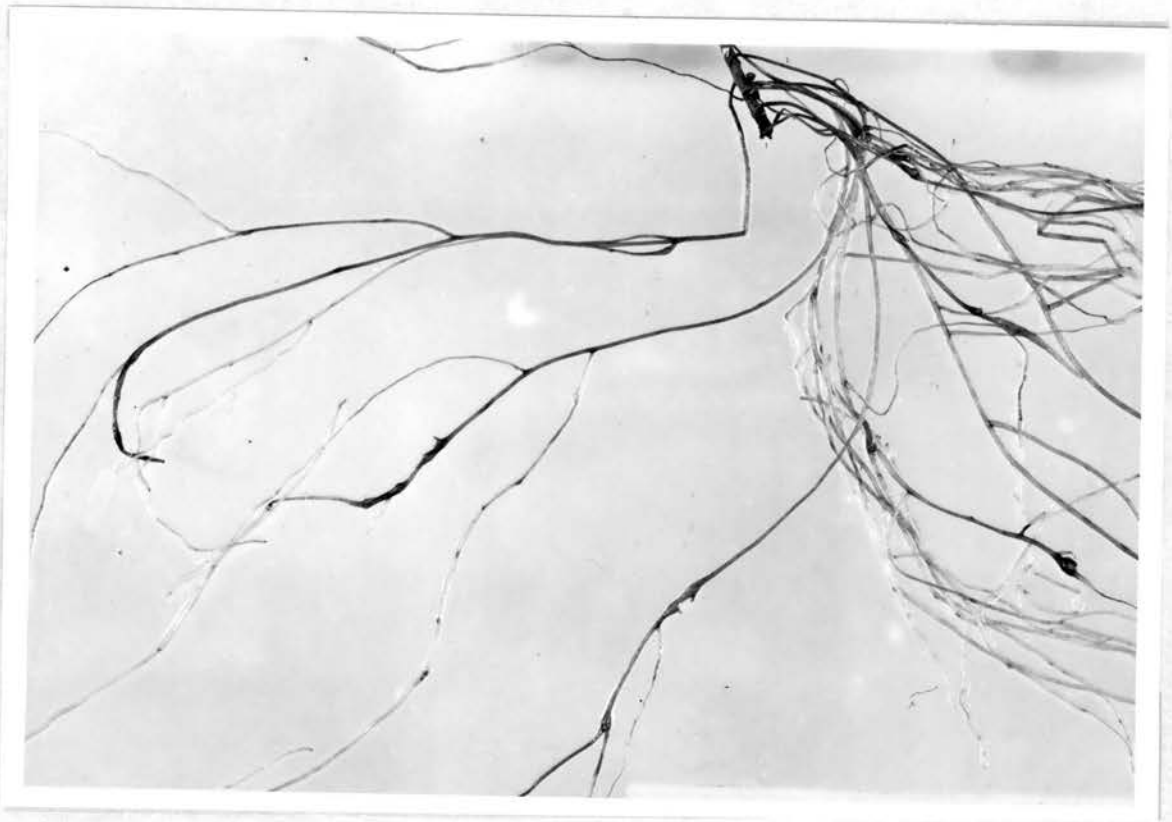

Fig. 4. Susceptible Stoneville 62 plant 16 days after inoculation showing production of lateral roots from distinctly galled roots at left.

ity of accurately determining resistance and susceptibility by macroscopic observations of roots which had been exposed to the root-knot nematode for a given period of time.

This experiment was set up in much the same way as the preceeding experiments. The tap root of each seedling was excised, and at the age of one week the seedlings were inoculated. Each plant received 5 ml of larval suspension which contained 900 larvae per ml. At the end of the 48 hour inoculation period the plants were removed from the test tubes, washed thoroughly and transplanted to 4 inch pots containing steam sterilized soil. The plants remained in the pots for 16 days after the date of inoculation and then were removed and washed free of soil. The root system of each plant was then carefully examined for the number and type of galls and the occurence of dead roots.

Data from this investigation are given in TABLE IV. The notes on severity of symptoms show that the Stoneville 62 plants exhibited much more galling when attacked by nematodes than did the Auburn 56 plants.

Of the 18 Stoneville 62 plants examined 16 days after inoculation, 2 plants, number 2 and 11, exhibited a resistant reaction. Each of these plants showed dead roots accompanied by little or no galling of the rest of the root system. Plant number 10 showed no symptoms and was probably an escape. The remaining 15 plants of Stoneville 62 were extensively galled. The galls were distinct and relatively large. Examination of stained roots under the microscope revealed that some nematodes had developed to the size to be classified in Group C (Fig. 6).

Of the 18 plants of Auburn 56 that were examined, 2 plants showed a normal susceptible reaction. This can be seen in plants number 7 and 8 in TABLE IV. Each of these plants showed distinct galls. Six of the

TABLE IV

SYMPTOMS ON COTTON PLANTS OF VARIETIES STONEVILLE 62 AND AUBURN 56 WHEN DETERMINED 16 DAYS AFTER INOCULATION

Plant No.	Description of root system	Number and description of galls
	Stoneville 62	
1	distinctly galled	24 galls of maximum size
2	4 dead roots; galls scattered	4 " " " "
3	distinctly galled	24 " " " "
4	scattered galling	4 " " " "
5	distinctly galled	100 " " " "
6	" "	" " " " "
7	" "	19 " " " "
8	scattered galling	" " " " "
9	scattered galling	" " " " "
10	no visible symptoms	no galls present
11	2 dead roots; galls scattered	7 galls not fully developed
12	scattered galling	5 galls of maximum size
13	distinctly galled	17 " " " "
14	" "	25 " " " "
15	" "	25 " " " "
16	" "	50 " " " "
17	" "	25 " " " "
18	scattered galling	5 " " " "
	Auburn 56	
1	1 dead root	no distinct galls; slight swellings
2	slight swelling of some roots	no distinct galls; slight swellings
3	no visible symptoms	no galls
4	4 dead roots	" "
5	5 dead roots	no distinct galls; swellings
6	1 dead root	" " " "
7	distinct galling	16 galls of maximum size
8	scattered galling	8 " " " "
9	slight swellings	no distinct galls
10	1 dead root	no distinct galls; swellings
11	3 dead roots	no galls
12	slight swellings	no distinct galls
13	2 dead roots	no galls

(TABLE IV cont'd)

Plant No.	Description of root system	Number and description of galls
	Auburn 56	
14	2 dead roots	no galls
15	no visible symptoms	" "
16	8 dead roots	" "
17	10 dead roots	no distinct galls; slight swellings
18	3 dead roots	no distinct galls; slight swellings

Auburn 56 plants exhibited a slight swelling of the roots. Observations (TABLE VI) of stained roots exhibiting this type of symptom revealed that the nematodes had developed to the size classified as Group B. Observation of roots showing swelling 30 days after inoculation showed that the nematodes did not develop beyond this stage (TABLE VI). With this type of symptom, the roots are not killed but stop growing. There was no evidence of lateral roots being initiated at these swellings, as was the case where there were distinct galls. This type of reaction, slight swelling rather than distinct galls, was considered as a resistant one, but of not as high a degree of resistance as in the case of the dead roots. Seven of the Auburn 56 plants showed dying of whole roots without any type of swelling. Detailed observations of these dead roots revealed that they were completely filled with nematode larvae that had not developed beyond the second stage (Fig. 5). This type of reaction is considered to be a highly resistant one.

The above experiment was repeated except that the plants were

TABLE V

SYMPTOMS AND AMOUNT OF EGG PRODUCTION ON COTTON PLANTS OF VARIETIES STONEVILLE 62 AND AUBURN 56 WHEN DETERMINED 30 DAYS AFTER INOCULATION

Plant No.	Description of root system	No. egg masses
	Stoneville 62	
1	extensive galling	23
2	" "	17
3	" "	10
4	" "	25
5	" "	25
6	" "	22
7	scattered galling	7
8	no visible symptoms	0
9	extensive galling	15
10	" "	10
11	" "	25
12	scattered galling	5
13	" "	3
14	" "	8
15	extensive galling	10
16	" "	17
17	" "	25
	Auburn 56	
1	extensive galling	14
2	3 dead roots; no galls	0
3	7 dead roots; slight swellings	0
4	4 " " " "	0
5	2 dead roots; 14 galls	13
6	3 dead roots; slight swellings	0
7	5 dead roots; 13 galls	5
8	scattered galling	2
9	2 dead roots; slight swellings	0
10	2 " " " "	0
11	4 " " " "	2
12	4 " " " "	0
13	no visible symptoms	0
14	4 dead roots; no galling	0
15	3 " " " "	0
16	no visible symptoms	0

Fig. 5. Auburn 56 root 16 days after inoculation showing developmental stage of the nematode. 125X.

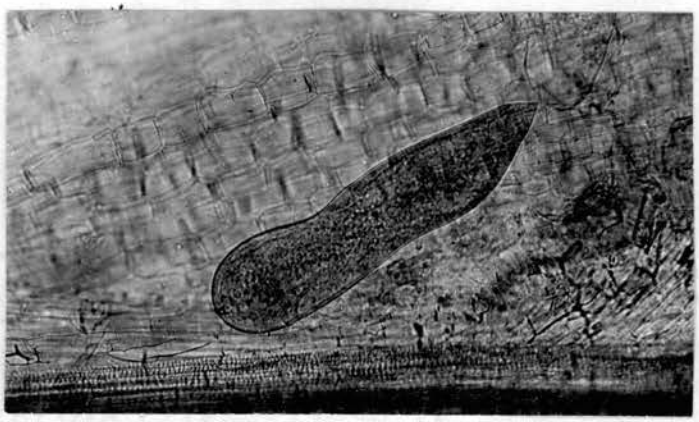

Fig. 6. Stoneville 62 root 16 days after inoculation showing developmental stage of the nematode. 125X.

TABLE VI

RELATION OF SURVIVAL AND DEVELOPMENT OF NEMATODES TO THE NUMBER OF GALLS AND DEAD ROOTS ON SUSCEPTIBLE AND RESISTANT COTTON

Variety	Aver. No. of galls per plant	Aver. No. of dead roots per plant	No. of nematodes in each developmental stage				
			A	B	C	D	E
Sixteen days after inoculation							
Auburn 56	1.8	2.5	44	9	0	0	0
Stoneville 62	16.2	0.3	5	48	29	0	0
Thirty days after inoculation							
Auburn 56	0.5	2.7	182	3	1	0	0
Stoneville 62	12.5	0.6	42	9	21	15	45

allowed to grow for 30 days after inoculation. An attempt was then made to correlate the amount of reproduction with resistance. Details from this investigation are given in TABLE V. The symptoms on the Stoneville 62 plants were much the same as at the 16 day sampling period. Each plant showed extensive galling accompanied by egg production. There was a large variation in number of egg masses per plant, ranging from three to twenty five.

In this test, Auburn 56 showed 6 of the 16 plants examined to contain mature females that had reproduced in the plant. However, the number of egg masses per plant was considerably less than found in susceptible Stoneville 62. Symptoms on the remaining Auburn 56 plants were characterized by dead roots or slight swelling on the roots, with

no egg production.

A summary of the detail observations of these two investigations are given in TABLE VI. These observations are based on ten roots taken at random from each variety at each sampling date. The studies on development and survival indicate that the nematodes in the resistant material had not undergone any further development in 30 days than they had in 16 days. It was also noted, in the resistant material, that there was an absence of egg production and that dead roots were much more prevalent.

These observations indicate a positive correlation between the type of galls and the stage of development of the nematodes. A positive correlation between the number of dead roots and the retarded development of the nematode was also observed. Thus, by making macroscopic observations only, resistance could be determined by the occurence of dead roots or by slight swellings that do not form distinct galls.

Application of Greenhouse and Laboratory Technique to Evaluation of Selections from Improved Varieties

On the basis of information accumulated from the previous experiments, it was believed that selections from improved cotton varieties could be evaluated for their reaction to root-knot nematode 16 days after inoculation. To test this assumption, five selections from the nematode-wilt nursery were used. These selections were made on the basis of earliness and yield of individual plants. Each selection was given a number and their pedigrees were unknown to the writer.

A total of 20 plants of each selection was grown and inoculated

in the $1\frac{1}{4}$ in. x 11 in. test tubes. Each plant received 5 ml of larval suspension containing about 900 larvae per ml. The plants were exposed to the larval suspension for 48 hours and then grown in steamed soil for 14 days. At the end of this time they were removed and brought to the laboratory for observation of symptoms and processed for nematode counts and development. A decision as to the reaction of the selections was made on the basis of the presence of necrotic roots, the relative amount of swelling, and the stage of development of individual nematodes.

Data from this test are given in TABLE VII. All selections showed some degree of resistance. The most highly resistant reaction was shown by selection M57-207, which is a selection from Alabama 9531. The most susceptible of the selections was M57-205, which is from CR-4. The degree of resistance or susceptibility is attributed to the fact that these selections were segregating for resistance. Most of the plants showed no galls, while some plants gave a susceptible reaction. All plants of Stoneville 62, which was used as a check, were galled.

After the selections had been evaluated macroscopically, the developmental stages reached by the individual nematodes were recorded. The results again revealed that in plants with necrotic roots and relatively little galling, the development of the nematodes was retarded. The study also revealed that there was a direct correlation between the type of galling and the rate of development of the nematodes.

These selections were evaluated from field data as being resistant to both root-knot nematodes and Fusarium wilt. The results from the foregoing investigation coincided with field evaluations in regard to resistance to root-knot nematodes. The selections were not evaluated for Fusarium wilt resistance in this study.

TABLE VII

REACTION OF 7 COTTON STRAINS AS DETERMINED BY LABORATORY TECHNIQUE

Strain or variety	Plants with galls	Galls per plant	Nematodes in each developmental stage[a]:			
			A	B	C	D
	Per cent	Av. No.	No.	No.	No.	No.
Auburn 56[b]	12.0	0.5	67	4	0	0
Alabama 9531	48.0	8.5	36	15	1	0
M57-207	23.0	5.6	46	9	0	0
609	50.0	6.5	24	12	0	0
M57-205	49.0	9.7	13	16	4	0
M57-206	50.0	16.3	45	17	0	0
Stoneville 62[c]	100.0	22.4	8	11	26	0

[a] Nematodes are total from 10 roots of each strain.

[b] Auburn 56 was used as a resistant check.

[c] Stoneville 62 was used as a susceptible check.

DISCUSSION

The results of the foregoing investigations tend to indicate a close similarity between the nature of resistance to M. incognita acrita in cotton and in certain other plants that have been investigated.

In all experiments with cotton reported herein it appeared that the nematode larvae entered the roots of the resistant plants as readily as they did those of the susceptible plants. Analysis of the data from the first two tests showed no statistical differences between the number of larvae entering resistant and susceptible plants. Consequently, after the first two tests of the present investigation data pertaining to the number of nematode larvae entering resistant and susceptible roots were not taken. Radewald (18) demonstrated significant differences between the number of larvae entering resistant and susceptible sweet potato plants. However, he did not consider these differences consistant enough to be of value in defining resistant and susceptible sweet potato lines.

Development of the nematode larvae was sharply retarded in the resistant material used in this study. There was a constant association of necrotic roots with resistant plants. In these necrotic roots the larvae did not develop beyond the second stage, or the stage at which they entered the roots. This retarded larval development was considered to be due to a hypersensitive reaction on the part of the host. As a result of this hypersensitivity whole roots died within 12

to 16 days after inoculation.

Radewald (18) reported in sweet potato and Dean and Struble (10) reported in tomato and sweet potato that the nematode larvae disappeared from infected roots of resistant plants. The larvae apparently died and disintegrated. From the present investigation there was no evidence of nematode larvae disappearing from infected roots of resistant cotton even after 30 days when the nematode had undergone no development since entering the host. Whether or not these larvae were dead in the necrotic roots could not be determined. However, they did not appear to be deformed in any way nor were fragments of nematodes ever observed. The failure of invaded roots of resistant cotton to recover from infection to the same extent as sweet potato and tomato may account for these differences.

Nematologists are generally agreed that root-knot nematodes feed on the contents of the giant cells, the formation of which is induced by the salivary secretion of the nematodes themselves. Barrons (1) suggested that resistance is due to certain chemicals within the roots of resistant plants that counteract or neutralize the giant cell producing effect of the salivary secretion of the nematode. From the data presented in TABLE V it seemed highly possible that this situation prevailed in cotton to some degree. As noted previously distinct galls did not develop in most of the resistant plants. The galls were reduced to slight swellings on the roots. That some chemical mechanism was operating to counteract the giant cell producing effect of the nematode seems to be a possibility for explaining this type of reaction of cotton resistant to root-knot. In most cases the larvae did not develop beyond the size to be classified in the B group as defined and illustra-

ted in the section on the survival and development of nematodes in cotton roots.

It is generally agreed that at or near the root tip is a favored point of entrance for root-knot larvae. It was noted in the present investigations that larvae entered at any point along the root of resistant cotton plants. The plants were inoculated when the roots were approximately 4 cm in length. The infected roots usually became necrotic at the tip resulting in the death of the whole root within 12 to 16 days after inoculation. However, in some instances slight swellings developed on infected roots of resistant plants and the roots did not die.

As previously mentioned, instances were observed in which a plant of resistant Auburn 56 material exhibited extensive galling and females developed to the egg-laying stage. This was thought to be due to segregation for resistance and susceptibility in Auburn 56. However, there is also the possibility of this reaction being due to different physiologic races of M. incognita acrita.

In two instances it was noted that seedlings of the susceptible variety Stoneville 62 exhibited a resistant reaction. Thus, there would appear to be a possibility of finding resistance in an established variety.

As already has been pointed out, 5 selections from the Fusarium wilt-nematode nursery were evaluated for their reaction to the root-knot nematode. The greenhouse evaluations, in which resistance was based principally on the occurence of necrotic roots and the type of galling, agreed with data obtained when the plants were evaluated by studying the stage of development of the individual nematodes. The

greenhouse evaluations also agreed with the evaluations from field data.

Through this investigation it was demonstrated that several differentiating features can be used to evaluate cotton seedlings for their reaction to the root-knot nematode. It would appear that resistance and susceptibility could be determined fairly accurately in 16 days after inoculation by using the occurence of dead roots and slightly swollen discolored roots as criteria on which to base resistance. These findings should be especially helpful to the breeder since seedlings could be screened in the greenhouse and saved for propagation.

SUMMARY

An effective technique for inoculating cotton seedlings with the root-knot nematode (Meloidogyne incognita acrita) was developed. Individual seedlings, with tap roots excised so as to stimulate lateral root development, were placed in glass tubes of vermiculite. Five days later one ml of larval suspension of a previously determined concentration was injected near the root tip of each lateral root by using an 18 gauge hypodermic needle. After specified inoculation periods the seedlings were removed, washed thoroughly, and transplanted to individual pots of sterilized soil in the greenhouse. A concentration of approximately 900 larvae per ml gave more consistant infection than 450 or 150 larvae per ml. An inoculation period of 48 hours gave more consistant infection than a 24 hour period.

Resistance to root-knot nematode in Auburn 56 and 5 Oklahoma cotton selections was found to be associated with a necrotic reaction of the invaded roots. Nematode larvae entered the roots of the resistant variety, Auburn 56, as readily as they entered the roots of seedlings of the susceptible variety Stoneville 62. Invaded lateral roots of resistant seedlings generally ceased growing within eight days after inoculation and death of the roots resulted within 16 days after inoculation.

In the resistant variety Auburn 56 the development of the nematode was much retarded. The nematode larvae usually failed to reach sexual maturity while the development of the larvae in the suscept-

ible variety Stoneville 62 was apparently normal.

The preceeding findings were applied in developing a simple screening technique with which to select resistant plants from seedlings that had been either artifically infected and then potted in steam sterilized soil or grown in infested soil for 16 days. Resistance was based on the presence of dead roots or slightly swollen necrotic roots.

The five Oklahoma selections were evaluated by both of the above mentioned methods, and were found to be segregating for resistance and susceptibility. All of the selections showed 50 per cent of the plants with galls excepting M57-207 which had only 23 per cent of the plants with galls. Stoneville 62 used as a susceptible check showed 100 per cent of the plants with galls compared to the resistant check, Auburn 56, which had only 12 per cent of the plants showing galls.

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VITA

BILL B. BRODIE

Candidate for the Degree of

Master of Science

Thesis: THE NATURE OF RESISTANCE TO THE ROOT-KNOT NEMATODE (MELOIDOGYNE INCOGNITA ACRITA CHITWOOD) IN COTTON

Major: Botany and Plant Pathology

Biographical:

Personal data: Born at Van Buren, Arkansas, April 25, 1934, the son of George E. and Carmel M. Brodie.

Education: Attended elementary school in Van Buren, Arkansas; graduated from Van Buren High School, 1951; received the Bachelor of Science Degree from Oklahoma State University with a major in Botany and Plant Pathology, May, 1956; completed the requirements for the Master of Science Degree, May, 1958.

Experiences: Born and reared on a farm; part time employee in the Department of Botany and Plant Pathology, Oklahoma State University, 1951-1955; research assistant in the Department of Botany and Plant Pathology, Oklahoma State University, 1955-1956; on a joint appointment with the USDA, ARS, Crops Research Div. and the Oklahoma Agricultural Exp. Sta., in the Department of Botany and Plant Pathology, with the rank of Instructor and Agent, 1956-

Date of Final Examination: April, 1958