THE NATURE OF RESISTANCE AND SUSCEPTIBILITY TO THE ROOT-KNOT NEMATODE (MELOIDOGYNE INCOGNITA VAR. ACRITA CHITWOOD) IN SWEET POTATO

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INTRODUCTION

One of the objectives in the sweet potato breeding program at Oklahoma A. and M. College is to develop root-knot resistant varieties. In the 5-year period from 1951 through 1955 nearly 3000 sweet potato seedlings have been evaluated for their reaction to root knot. In addition to these, several hundred varieties, parent lines and plant introductions, have also been screened during this period.

The method of evaluating resistance or susceptibility to root knot has consisted essentially of planting in root-knot nematode infested soil in the field and then observing at harvest the relative amounts of root injury attributable to this nematode. This method has left much to be desired in that consistent results are not obtained from year to year. That is, a given sweet potato line may vary over a period of years as to the degree of resistance or susceptibility attributed to it. These erratic results have been considered as due to the influence of varying environmental factors from year to year and probable human errors in judgment. Consequently, it has required in many instances a period of several years to establish what is considered a relatively true rating for many of these sweet potato lines.

If a method that would allow a more rapid and accurate evaluation of sweet potatoes to root knot were available not only would there be a considerable savings in time but also a greater degree of confidence in the results. The work of Dean (11) and Dean and Struble (12) indicated that there were certain consistent differences between root-knot susceptible and root-knot resistant sweet potatoes which might be utilized in evaluating sweet potato lines for their reaction to root knot.

The objective of the work reported herein has been to confirm and extend the work of Dean and to determine whether or not a method could be devised which would allow a more accurate and more rapid evaluation of sweet potatoes to root knot. The approach to this problem has been essentially two-fold: (1) to study and develop inoculation techniques so as to eliminate previously encountered difficulties in obtaining consistent infection, and (2) to investigate the several aspects of host-parasite relationships.

REVIEW OF LITERATURE

Prior to 1949 all root-knot nematodes were classified in the genus Heterodera. The work of Christie and Albin (9), Christie (6), and Christie and Havis (10) demonstrated that Heterodera marioni (Cornu) Goodey was comprised of several races each differing from the other in its host range.

Chitwood (4), on the basis of the comparative morphology of several populations of H. marioni, concluded that the group constituted a genus apart from Heterodera. Meloidogyne was considered the earliest valid generic name and Chitwood recognized 5 species and 1 variety in this genus at this time. Therefore, prior to 1949 the species of root-knot nematodes referred to in the literature is questionable.

The life cycle of Heterodera marioni has been presented in detail by Christie and Cobb (8). A summary of this life cycle is presented here to facilitate understanding of certain phases of the present investigation. During the life of an individual nematode there occurs a series of 4 molts. The so-called first stage of the larvae is spent within the egg where the first molt occurs before a larvae reaches its maximum length. During the second stage the larvae grows, hatches from the egg, migrates

through the soil, and enters a plant root. Here a second molt occurs which terminates the second stage. Larvae that do not find a suitable host never mature beyond the second stage. No true third stage of either male or female occurs since 2 cuticles are loosened simultaneously representing the second and third molts.

In the female a very short fourth stage results as the fourth molt occurs almost immediately after the third molt. Following the fourth molt the female is sexually mature and becomes pear-shaped. At this time she remains in a fixed position in the plant tissue.

In the male a longer fourth stage is found and it is during this stage that metamorphosis of the male occurs. Following the fourth molt the male emerges from a sac composed of the loosened cuticles of the second, third, and fourth molts. At this time the male is sexually mature and migrates freely through the root tissues or soil.

Initial stages of infection by root-knot nematode larvae have been described by Linford (16). He describes the larvae as feeding exteriorly for a time on possibly several different cells before actually entering the root. Usually the larvae are found to migrate along the root toward the young elongating zone or meristematic region before entering the host. Actual entry is accomplished through the use of the stylet which may break the cell wall or force an opening between the cells of the

epidermis. Generally, the larvae are found to migrate into the root between cells. Feeding is carried on during this migration in the root tissues in a manner similar to external feeding. Young epidermal and dermatogen cells at the point of entry of the larvae are killed as a response to the actual entry of the larvae and not by feeding alone. According to Linford this injury by larval entry also kills some cells within the root. Eventually the larvae stops migrating and takes up a permanent position within the root. Linford explains the cessation of elongation, the swelling of root tips, and the early hypertrophy of cells at a distance from the permanent feeding site as all due to feeding by the larvae during entry and migration.

Feeding by the parasite after it has assumed a permanent position in the root has also been described by Linford (15). The nematode obtains its food in this position by penetrating any of the giant cells within its reach with its stylet and feeding directly on the cell substances. By alternate feeding, destruction of these giant cells is avoided, thus perpetuating the food supply of the animal almost indefinitely. The head of the nematode in the final position in the root usually lies within the plerome and its posterior in the periblem. Sometimes the entire animal lies within the plerome parallel to the longitudinal axis of the root (5).

Christie (5) has described tissue changes induced by feeding of the root-knot nematode in tomato by infecting

tomato seedlings with H. marioni. The age of the galls in the roots in Christie's experiment was known within 24 hour limits in a series ranging from 24 hours to 40 days. The following effects on roots are described as occurring within 24 hours after infection: hypertrophy of cortical cells, slight hypertorphy of those cells of the pericycle and endodermis that lie near the path of the animal, stimulation of cell division in the pericycle, and an apparent inhibition of cell division in the apical meristem particularly if an appreciable number of larvae enter the root tip at about the same time.

Cells around the head of the parasite in the plerome are frequently found to remain undifferentiated for from 48 to 60 hours after the permanent position has been assumed by the animal. After 72 hours slight cell enlargement and wall disintegration of cells in the plerome begins and a giant cell starts to form. Nuclei of the cells of the plerome near the head of the parasite start to swell after 72 hours and after the giant cells are formed, these nuclei coalesce and disintegrate. Cells surrounding the giant cell from the central cylinder or cortex are assimilated during the first 10 to 20 days by a dissolution of the separating cell walls.

The nature of resistance in plants to root-knot nematodes received very little attention prior to 1939. Steiner (22) in 1925 wrote: " A plant may resist the attacks of nematodes either by some mechanical or chemical

means." Tyler (24) defined resistance, with reference to the root-knot nematode, as the ability of plants to obstruct invasion of the parasites. Barrons (1) in 1939 definitely established that resistance in plants to root-knot nematodes could not be defined as merely a failure of the larvae to enter nematode resistant plant roots. Barrons made a study of 30 plant varieties and species and counted root-knot nematodes in a terminal centimeter of no fewer than 20 roots of each plant variety or species. He found no significant differences between root-knot resistant and root-knot susceptible plants with regard to the numbers of nematodes entering the roots.

Christie and Albin (9) worked with different populations of H. marioni and, while they were primarily concerned with the problem of races of root-knot nematodes, they made some observations of interest with respect to the nature of root-knot resistance in plants. In one experiment they tested several populations of root-knot nematodes on Persian clover, Trifolium resupinatum L. and found that one nematode population was entirely absent from the roots of the clover. The roots of clover growing in another population were beaded and had slight swellings but no nematodes were found in them. Clover roots in a third population harbored a few nematodes; however, most of the nematodes were dead and none had molted a second time. Three-fourths of the nematodes found in the roots growing in a fourth population were

mature and had oviposited; however, the animals appeared ill nourished and egg output was very low. Christie (6) also found that resistant plants prevented root-knot development in some measure within their roots, and in plants only slightly resistant the period of animal development was merely lengthened; little effect was noted on the number of egg-laying females or in the number of eggs laid per female.

Christie (7), discussing the problem of the nature of nematode resistance in plants, believes resistance to be due to a failure of the host tissue to respond properly to the stimulus of infection. He points out that root-knot nematodes are sedentary parasites and once they become permanently located they are able to feed on only a few cells throughout the remainder of their life. Therefore, if the salivary secretion of the nematode fails to stimulate abnormal plant cell development, the animal is soon surrounded by thick walled or highly vacuolated cells which it cannot puncture with its stylet. If these cells could be punctured by the animal's stylet they would be of little value as a source of food.

Barrons (1) considered giant cells necessary to furnish a continuous food supply to developing nematodes and postulated resistance is due to the plant synthesizing a substance which neutralizes the giant cell inducing agent in the saliva of the nematode. Christie (7), however, reports that Machmer has found mature egg-

laying root-knot nematodes which had not induced giant cell formation in Pelargonium graveolens L'Her. These nematodes were located in small parenchyma outgrowths at the bases of propagative cuttings. In the roots of these plants only very small galls had formed, some galls contained no parasite, others contained only partly developed or completely dead nematodes. Christie, in conclusion, points out that all plants are not necessarily resistant for the same reason and that he still considers it possible that some plants may be highly resistant or even immune to the entry of nematodes.

Christie (6) observed the number and state of development of root-knot nematodes in the roots of 10 sweet potato varieties 3 and 6 weeks after inoculation. Two plants per variety and 50 nematodes per plant were sampled. If a sample of 50 nematodes were not present in each root system all the nematodes present were recorded. Examination revealed considerable differences between varieties with respect to the numbers of nematodes that developed to maturity in this period. One sweet potato variety faild to yeild 50 parasites and apparently Christie viewed this as evidence that fewer larvae entered the roots of this variety than entered those of the other 9 varieties.

Dean and Struble (12) studied root-knot resistance and susceptibility in tomato and sweet potato and reported that approximately one-half the number of larvae entered

the root systems of resistant tomatoes as entered those of susceptible tomatoes. The nematode involved in this study was identified as Meloidogyne incognitia (Kofoid and White) Chitwood. This nematode consistantly caused extensive necrosis in resistant tomato roots 48 hours after inoculation. After 2 weeks most of the larvae in the tomato roots had failed to develop as far as the second molt. No differences were found with respect to the numbers of larvae entering root systems of resistant and susceptible sweet potato lines and extensive necrosis of resistant sweet potato roots was observed several days after inoculation. A few nematodes in all resistant sweet potato lines were found to reach egg-laying maturity.

Shibuya (21) reports no statistically significant differences in the numbers of root-knot nematodes that originally enter roots of resistant and susceptible sweet potatoes.

Bingefors (3) reports a difference in the numbers of stem nematodes, Ditylenchus dipsaci (Kuhn) Filipjev, entering resistant and susceptible strains of red clover within 48 hours after inoculation. This difference in numbers of larvae per plant was not considered large enough to explain the differences found in field trials for susceptibility. Bingefors also points out that even though the inoculum used with his method of inoculation contained 2000-3000 nematodes per cc all plants were not actually infected.

Damage to sweet potatoes by root-knot nematodes has

been recognized for almost half a century (2). Sweet potato varieties resistant to these nematodes have been reported by Weimer and Harter (25), Poole and Schmidt (18), and others (8, 24).

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. These methods, in some instances, were roughly quantitative. Christie (6) described an inoculation method which involved the use of small plants grown in thumb pots. When roots were evident at the soil-pot interface, a few ml of a larval suspension was poured over them. The root mass was then returned to the pot for any desired period of inoculation, usually either 24 or 48 hours. After this time the root system was removed from the pot, carefully washed to remove soil and nematodes which had not entered a root, and transplanted to nematode-free soil. With this method a plant could be grown till the time the nematodes had completed a life cycle; the time of entry of the nematodes could thus be determined within the limits of whatever time had been used for inoculation. Dean (11) used this method as the most satisfactory of several tried.

Dropkin (13), using a pure culture of M. incognita var. acrita Chitwood developed an inoculation technique in which marked roots of tomato and cucumber seedlings

were exposed to counted numbers of larvae. Through the use of this technique it was possible to detect more subtle differences in infectivity of the 2 hosts than had previously been observed. Dropkin was of the opinion that with exposure of seedlings to a definite number of larvae there was less opportunity for environmental factors to affect results. With this technique, however, variable results were still obtained with respect to the numbers of nematodes entering seedlings.

MATERIALS AND METHODS

A pure culture of the root-knot nematode, Meloidogyne incognita var. acrita[1] Chitwood, was used throughout the present work. The culture was established from a single egg mass obtained from an Allgold sweet potato root and was propagated on roots of Allgold.

Four varieties or lines of sweet potato whose reaction to root knot was known were used in the several phases of this investigation. These were as follows:

Allgold - an open-pollinated seedling of Okla. parent 10 which originated in turn from a cross between a selfed seedling of Creole and an open-pollinated seedling of Triumph. Susceptible to root knot.

Orlis - a mutant from Yellow Jersey selected by O. H. Elmer in Kansas. Resistant to root knot.

Oklahoma 29 - a seedling from the cross (Triumph seedling x Creole seedling) x (Nancy Hall x Porto Rico). Resistant to root knot.

Nemagold - Oklahoma 46, a seedling from the cross Okla. 29 x Orlis. Resistant to root knot.

The reaction of these sweet potato lines to root knot had been determined over a period of several years. These lines had been evaluated as part of the root knot trials regularly conducted at this Station in conjunction with

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[1]The identification of this nematode was confirmed by A. L. Taylor, Nematology Section, Agricultural Research Service, U. S. Department of Agriculture.

the sweet potato breeding program.[2] In this program the sweet potato lines are planted in soil uniformly infested with root-knot nematodes.[3] Sweet potato lines are rated on the basis of the relative amounts of injury - dead root tips, galling, roughening of potatoes - attributable to these nematodes at harvest. In addition samples of potatoes taken at harvest are sliced into sections approximately 1/10 inch in thickness; from these slices counts are made of the numbers of mature females in the root tissue. The rating for an individual sweet potato line is then determined as a composite of the field root index and the laboratory count. On the basis of these data sweet potato lines are categorized as resistant, intermediate or susceptible.

To study nematodes in whole roots the staining technique of McBeth, Taylor and Smith (17) was used. This consisted essentially of boiling washed roots in lactophenol plus acid fuchsin for 1 to 3 minutes depending upon the intensity of staining desired. The roots were then washed to remove excess stain and placed in lactophenol for clearing for a period of a day or two and

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[2]These tests were conducted by F. B. Struble, Department of Botany and Plant Pathology, and H. B. Cordner, Department of Horticulture, Oklahoma Agriculture and Mechanical College.

[3]Both *Meloidogyne incognita* and *M. incognita acrita* have been identified from sweet potatoes grown in this area. From limited survey work the latter species seems to predominate.

were then ready for observation. Roots to be examined immediately after staining were boiled carefully in lactophenol.

Examination of roots for nematodes was accomplished in either of two ways. One method was to place the roots along with a little lactophenol in a Syracuse watch glass and observe them as whole roots under the dissecting microscope or if nematodes were suspected and not seen the roots were gently teased apart with steel needles. With the other method roots were placed between thin pieces of 2" x 2" glass, gently crushed, and then examined. Especially for young roots the latter method was found to be more convenient and rapid. It is also believed that by using this method a more accurate count was possible when large numbers of larvae were in the roots.

To obtain the large numbers of larvae required to inoculate plants the following procedure was used. Allgold sweet potato roots that had grown approximately one month in soil infested with the pure culture of M. incognita acrita were harvested and washed. From these roots egg masses were picked off and placed on a double thickness of cheesecloth suspended over a petri dish containing water. The water level was maintained so that it just touched the cheesecloth. It was possible with the area involved to get several hundred egg masses on the cheesecloth at one time. Twice daily the resulting larval suspension in the water in the dish was carefully

poured off and stored in a 7° C refrigerator. Larvae were thus collected over a period of several days but in no instance were larvae more than 7 days old used for inoculation purposes. If necessary the larval suspension could be concentrated simply by allowing larvae to settle to the bottom of the container and pouring off the excess water on top.

Standardized larval suspensions were prepared for inoculation purposes by counting all the larvae in several 0.1 ml aliquots of the suspension and calculating the number of larvae per ml. The suspension was adjusted for the desired number of larvae simply by diluting with water or concentrating as noted above.

RESULTS

Determination of a suitable inoculation technique.

As previously noted Dean (11) had tried a variety of inoculation techniques using root-knot larvae and found each one unsatisfactory in one respect or another. One of the objectives of the present investigation was to determine experimentally an inoculation technique which could be relied upon to provide constant and consistent infection of sweet potato roots.

The inoculation method of Christie (6) as outlined in the Review of Literature was tested and abandoned because of the extreme variability encountered in the numbers of nematodes found in different roots of the same root system and also because many roots were not infected. A modification of Christie's technique was then tried. It will be recalled that as outlined in the original method a larval suspension was applied uniformly over those surfaces provided at the soil-pot interface. In an attempt to disperse the inoculum more uniformly around more roots, a larval suspension was injected at 1/2 in. intervals into the soil to a depth of about 1 1/2 in. around the base of plants in 2 inch pots. Injection was done with a syringe and a 16 gauge, 2 1/2 inch needle.

Examinations made at several time intervals following inoculation showed again that some roots were completely free of nematodes while others were heavily infected. This modification of Christie's technique did, however, increase the percentage of roots infected.

The next technique tried was one in which the substrate in which the roots were growing was reduced in volume by substituting 5/8 x 6 inch test tubes for pots. At the same time 4 different substrates were tried, these were: loam soil, sand, packed absorbent cotton, and ground vermiculite.[4] Rooted sweet potato cuttings were allowed to become established in tubes containing each of the substrate materials.

A standardized larval suspension was then injected, with a syringe and needle, into each of the growing media containing roots. The tubes were kept immersed in sand to provide darkness and to maintain a uniform temperature around the roots. At several periods following inoculation root systems from each of the substrates were examined for nematodes. It was found consistently that the percentage of roots infected in the vermiculite was higher than that in any of the other 3 media tried. Also this technique using vermiculite resulted in a higher percentage of infected roots than did the modification of

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[4]The vermiculite was ground in a Wiley mill with a 60 mesh retainer screen.

Christie's technique. The increase in roots infected was attributed to a more intimate contact obtained between nematodes and roots.

In the light of the experience up to this point, it was decided to test single root inoculations with vermiculite as the medium around the root. Sixteen cm lengths of 4 mm diameter glass tubing were pulled and broken in the middle so that from each length 2 pipette-like tubes resulted. These tubes were then filled with ground vermiculite (Fig. 1,a). Individual roots to be inoculated were labelled at their proximal ends with Scotch tape bands each 3 x 15 mm (Fig. 1,b). The plants were then supported against a small trellis in a pan 12 x 30 x 3 inches (Fig. 1). The plant roots rested on a layer of water-saturated vermiculite about 3/4 inch in thickness. At this time each of the tubes with vermiculite was injected with 1 ml of water containing a known number of nematode larvae. This resulted in a very viscous mass of vermiculite in the tube. Each inoculated tube was then carefully slipped over one of the labelled roots so that approximately 1 cm of the root tip was in the inoculum (Fig. 1,e). The final step was to cover the remaining roots and the tubes with dry vermiculite; capillary action provided sufficient moisture to this covering from the wet underlying vermiculite.

After a sufficient inoculation period, initially 72 hours, the pan, containing the inoculated roots, was filled

Fig. 1. Materials used in the single root inoculation technique. a) Inoculation tubes filled with vermiculite. b) Scotch tape root labels. c) Syringe with needle. d) Larval suspension. e) Plants with roots that have been labeled and inoculated.

with water and left to stand approximately 5 minutes. The individual plants could then be lifted free of the vermiculite in the tubes. The Scotch tape bands adhered satisfactorily after this treatment; they, incidentally, also served to mark roots of plants transferred to pots of soil and grown for at least a month.

Examination of the original inoculated roots revealed that 100 per cent of them had been infected with nematode larvae. This inoculation technique has since been used repeatedly and in no instance with either susceptible or resistant sweet potato roots has infection failed to occur.

Determination of an optimum inoculation period.

After a satisfactory inoculation technique was devised, it then became important to determine how long roots should be exposed to nematode larvae in order to obtain a near optimum level of infection.[5] The single root inoculation technique was used to inoculate one root on each of 15 different plants of each of the 3 sweet potato lines, Allgold, Orlis, and Okla. 29. The inoculation tube for each root contained about 600 larvae and the temperature in the vermiculite during the time of the

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[5]The term infection is defined here simply to mean that nematodes had entered or were in the process of entering a root. Whether a disease relationship had been established could not be determined definitely.

experiment ranged from 21 to 23° C. Observations on 5 roots from each sweet potato line were made at 24, 48, and 72-hour intervals following inoculation. Counts of larvae in stained roots revealed that more larvae had entered roots of all 3 lines during the 48-hour inoculation period than had entered during the 24-hour period. Roots of all lines held in the inoculum for 72 hours showed a very small increase in larval numbers over those held for 48 hours. With the resistant line, Orlis, there was evident a considerable amount of necrosis on 3 of the 5 root tips. A small amount of necrosis was observed on 1 root of this line at the 48-hour sampling period.

The above experiment was repeated using a population of about 400, 600, 800 or 1000 larvae per inoculation tube. It was found that by using about 400 larvae per tube only a trace of necrosis appeared in Orlis in the 48-hour sampling period. Necrosis was quite evident at the end of 72 hours in both Orlis and Okla. 29 where 400 larvae had been used. It was also found that no matter how great the number of nematodes exposed to a given root that fewer entered in a 24-hour period than in a 48-hour period.

As a result of these experiments it appeared that 48 hours was an optimum inoculation time. This time, unless otherwise indicated, was used throughout subsequent experiments.

Relation of numbers of nematodes entering sweet potato roots to resistance or susceptibility.

One of the possible differences between resistant and susceptible plants as already mentioned is that nematodes may enter susceptible material more readily or in greater numbers than they do resistant material. While there was no real evidence of such a phenomenon in sweet potatoes from the work of Dean (11) principally because the numbers of plants with which he dealt were too few, the hypothesis seemed worthy of testing. Three separate experiments run at different times were used in an attempt to determine possible differences between lines with respect to numbers of nematodes entering.

In the first trial 5 vine cuttings from each of the 4 sweet potato lines - Allgold, Orlis, Okla. 46, and Okla. 29 - were rooted in tap water. Three roots on each of these plants were inoculated using the tube technique. For each root inoculated approximately 650 nematodes were used and the inoculation period was 48 hours. At the end of the inoculation period each root was removed, stained and observed for numbers of nematodes. An analysis of variance of the results showed that there were significant differences between lines at the 5 per cent level. Through the use of a hierachal design in the analysis of variance it was found that there was not significance between plants of the same line with respect

to the numbers of nematodes present at the end of the inoculation period. These data are presented in TABLE I. The multiple range test described by Duncan (14) was used to determine significant differences between lines. These data (TABLE II) show that at the 5 per cent level there were no discrete non-overlapping lines. However, Allgold, the susceptible line, was significantly different from Okla. 46 and Orlis.

In a second trial 20 vine cuttings of each of the sweet potato lines Allgold, Okla. 29, and Orlis were rooted in tap water and 5 roots on each plant of each line were inoculated with approximately 400 larvae per root. After the inoculation period of 48 hours, the 100 roots from each line were pooled, stained, and observed for the number of nematodes in each root. An analysis of variance of these results showed that there were significant differences between lines at the 1 per cent level (TABLE III). Since the roots for each plant in this experiment were not kept separate it was not possible to determine significance between plants. The multiple range test as applied to data from this trial showed that each of the 3 lines was significantly different at the 1 per cent level (TABLE IV).

In a third trial 4 roots on each of 7 plants of each of the lines used in trial 2 were inoculated as in trial 2. When final counts of larvae were made all roots were identified as to the plant from which they came. From an

TABLE I

ANALYSIS OF VARIANCE OF DATA ON NUMBERS OF NEMATODES ENTERING ROOTS OF FOUR SWEET POTATO LINES IN TRIAL 1.

Source of variation	d.f.	S.S.	M.S.	F.
Total	59	16,659.9		
Lines	3	13,530.7	4,510.3	3.14*
Plants in Lines	16	22,993.2	1,437.1	1.73
Error	40	13,136.0	828.4	

*Indicates significance at the 5 per cent level.

TABLE II

A MULTIPLE RANGE TEST SHOWING SIGNIFICANT DIFFERENCES IN FOUR SWEET POTATO LINES DUE TO NUMBERS OF NEMATODES ENTERING ROOTS. TRIAL 1.

Results:

Lines:	Allgold	Okla. 29	Okla. 46	Orlis
Means:	99.5	77.5	61.2	57.7

Note: Any two means not underscored by the same line are significantly different at the 5 per cent level. Any two means underscored by the same line are not significantly different at the 5 per cent level.

TABLE III

ANALYSIS OF VARIANCE OF DATA ON NUMBERS OF NEMATODES ENTERING ROOTS OF THREE SWEET POTATO LINES IN TRIAL 2.

Source of variation	d.f.	S.S.	M.S.	F.
Total	299	92,830.15		
Lines	2	18,734.13	9,367.07	37.55**
Error	297	74,096.02	249.48	

**Indicates significance at the 1 per cent level.

TABLE IV

A MULTIPLE RANGE TEST SHOWING SIGNIFICANT DIFFERENCES IN THREE SWEET POTATO LINES DUE TO NUMBERS OF NEMATODES ENTERING ROOTS. TRIAL 2.

Results:

Lines:	Allgold	Okla. 29	Orlis
Means:	47.1	35.6	27.9

Note: Any two means not underscored by the same line are significantly different at the 1 per cent level. Any two means underscored by the same line are not significantly different at the 1 per cent level.

analysis of variance of the data obtained it was determined there was significance between lines at the 1 per cent level (TABLE V). There was no significance between plants within a line. The multiple range test as applied to these data showed Allgold as significantly different from both Okla. 29 and Orlis. Neither Okla. 29 nor Orlis was significantly different from one another.

In each of the 3 trials just outlined the susceptible line Allgold was shown to be significantly different from the resistant line Orlis with respect to the number of nematodes entering the roots in a given inoculation period. In one test Okla. 29, according to the multiple range test, reacted like Allgold, in another it reacted like neither Allgold nor Orlis, and in a third it reacted like Orlis. In the one test in which Okla. 46 was included it was found not to be different from Okla. 29 in response to number of nematodes entering the roots. From the data presented, then, it would seem that there are consistent differences with respect to the number of larvae entering roots only between the highly susceptible Allgold and the highly resistant Orlis.

Relation of resistance or susceptibility to the survival and development of nematodes in sweet potato roots.

Dean (11) had demonstrated that root-knot nematodes that had entered roots of resistant sweet potatoes failed

TABLE V

ANALYSIS OF VARIANCE OF DATA IN NUMBERS OF NEMATODES ENTERING ROOTS OF THREE SWEET POTATO LINES IN TRIAL 3.

Source of variation	d.f.	S.S.	M.S.	F.
Total	83	16,464.7		
Lines	2	3,963.9	1,981.95	12.8**
Plants in Lines	18	2,804.5	155.8	1.02
Error	63	9,696.3	153.9	

**Indicates significance at the 1 per cent level.

TABLE VI

A MULTIPLE RANGE TEST SHOWING SIGNIFICANT DIFFERENCES IN THREE SWEET POTATO LINES DUE TO NUMBERS OF NEMATODES ENTERING ROOTS. TRIAL 3.

Results:

Lines:	Allgold	Orlis	Okla. 29
Means:	44.2	30.9	28.8

Note: Any two means not underscored by the same line are significantly different at the 1 per cent level. Any two means underscored by the same line are not significantly different at the 1 per cent level.

to survive to maturity. Larvae in the sweet potato lines with which he was working reached varying stages of development according to the resistance or susceptibility of the line.

In order to confirm these observations the following experiment was set up. Cuttings of the 3 sweet potato lines - Allgold, Okla. 29, and Orlis - were rooted in water until a minimum of 4 roots on each plant had each reached at least 4 cm. in length. One root on each of 9 plants of each line was then inoculated with about 1000 larvae. In order to know more definitely the period in which infection had occurred, the inoculation period was reduced to 24 hours. Following inoculation, root systems of all plants were washed thoroughly and planted in autoclaved soil in 4 inch pots. The plants were then grown for the periods of time following inoculation as stated in TABLE VII. This method permits the determination of the time a given nematode has been in a given root to within 24 hours or the inoculation period.

Soil temperatures during the time these plants were growing ranged from 18° to 21° C. At each of the stated time intervals after inoculation the inoculated root on 1 plant of each of the 3 sweet potato lines was examined for the number of nematodes present and their state of development. These data are presented in TABLE VII. The state of development of the nematodes was determined from their size and form, the same method that Christie (6) had used.

TABLE VII

COMPARATIVE SURVIVAL AND DEVELOPMENT OF NEMATODES IN 3 LINES OF SWEET POTATOES

Period after inoculation	Sweet potato line	Nematodes in each of stated developmental stages: A	B	C	D	E	Total nematodes
Days		No.	No.	No.	No.	No.	No.
3	Allgold	40	0	0	0	0	40
	Okla. 29	32	0	0	0	0	32
	Orlis	39	0	0	0	0	39
6	Allgold	27	8	0	0	0	35
	Okla. 29	33	0	0	0	0	33
	Orlis	20	0	0	0	0	20
9	Allgold	12	13	1	0	0	26
	Okla. 29	11	1	0	0	0	22
	Orlis	16	0	0	0	0	16
12	Allgold	7	15	9	0	0	31
	Okla. 29	20	3	0	0	0	23
	Orlis	19	1	0	0	0	20
15	Allgold	4	19	17	0	0	40
	Okla. 29	18	2	0	0	0	20
	Orlis	4	0	0	0	0	4
18	Allgold	4	9	18	1	0	32
	Okla. 29	16	5	1	0	0	22
	Orlis	0	3	0	0	0	3
21	Allgold	2	1	17	6	0	26
	Okla. 29	11	11	3	0	0	25
	Orlis	0	0	0	0	0	0
24	Allgold	1	1	18	12	1	33
	Okla. 29	11	1	3	1	0	16
	Orlis	1	0	0	0	0	1
30	Allgold	0	0	8	8	19	35
	Okla. 29	6	2	3	3	1	15
	Orlis	2	0	0	0	0	2

Group A included the stage where the larvae had begun to grow to the stage where they still possessed a more or less conical tail. Group B included larvae that had acquired a more or less hemispherical posterior end terminated by a spike to those that were about to complete the final molts. Group C included females from the stage where they have completed molts to the stage where they are almost fully grown. Group D included those females which were fully grown but had not yet laid eggs. Group E included egg-laying females.

In this experiment the nematodes tended to disappear from the roots of resistant sweet potato lines with the passage of time following inoculation. Those nematodes that did survive in resistant roots were definitely retarded in their development. Begining with the period 18 days after inoculation and continuing through the 30-day period only 6 nematodes were observed in the Orlis roots as compared with 39 nematodes recovered from 1 root of this line 3 days after inoculation. Nematodes in Okla. 29 did not disappear as rapidly nor to the extent they did in Orlis. In Allgold the number of nematodes present 30 days after inoculation was essentially the same as that 3 days after inoculation. Fifteen days after inoculation nearly half the nematodes in Allgold had completed all molts while in either Orlis or Okla. 29 none of the nematodes had matured to this stage. Thirty days after inoculation

approximately half the nematodes in Allgold had oviposited while only 1 nematode in Okla. 29 had reached this stage. None of the nematodes observed in Orlis ever completed all molts.

A considerable amount of necrosis in inoculated roots was observed with Orlis throughout this experiment. Only dead, undeveloped nematodes were observed in areas of necrosis. Necrosis associated with nematodes was also observed in roots of Okla. 29 but it was usually neither as extensive nor as severe as that found in Orlis. Necrosis attributable to nematodes in Allgold roots was never observed in this experiment nor in any other observation made during the course of this investigation.

A second experiment to study survival and development of nematodes in the same sweet potato lines was set up in the same way as the first experiment. The only difference was that instead of inoculating only 1 root per plant, 4 roots per plant were inoculated. Two of these inoculated roots were taken at each of the time periods after inoculation as had been done previously and the remaining 2 were preserved for studies on pathological histology. In all respects except for a minor variation in numbers of nematodes the results with these roots with respect to observed survival and development of the nematodes were the same as those reported in TABLE VII.

Relation of resistance or susceptibility in sweet potato

roots to pathological histology.

Tissue changes induced in roots and development of nematodes in roots were studied in more detail in those roots noted in the previous section as having been preserved for studies on pathological histology. The roots were killed and fixed in formalin-acetic acid-alcohol. In some instances picric acid dissolved in absolue ethanol was also used as a killing and fixing agent. After dehydration in a tertiary butanol-ethanol series and embedding in paraffin, microtome sections 15 microns in thickness were stained for observation with safranin-O in 95 per cent ethanol and counterstained with fast green in clove oil. Sections selected for photographing were also stained with Mayer's hemalum (20) to accentuate cell wall detail.

Seventy-two hours after inoculation a considerable amount of necrosis was found in Orlis and Okla. 29 in the epidermal and outer cortical cells of the meristematic and elongation regions. No defects were observed in nematodes present in the resistant roots at this time.

Six days after inoculation necrosis was found in both resistant lines in the cortex, the pericycle, the meristematic region and the root cap; some hypertrophy was seen in the cortical regions (Fig. 2). In the resistant lines a few giant cells were found around the heads of live nematodes in the pericycle, while in Allgold at this time

many giant cells were developing in the same root area. On all roots sampled subsequent to the 6-day observation period excessive numbers of lateral roots were found proximal to the infected areas. Approximately 50 per cent of the nematodes in Orlis at the 6-day period were dead and in the roots of Okla. 29 most of the nematodes were still alive although some seemed partially shriveled and were probably dead.

Nine days after inoculation necrosis was apparent throughout the resistant roots in any region where nematodes had migrated and fed. If the meristematic regions of the resistant roots were not completely necrotic by this time, necrosis in the vascular regions usually had interrupted root growth (Fig. 3). No giant cells free of necrosis were found in Orlis in any roots sampled after the 9-day observation period. The giant cells found in this line previous to 9 days were always smaller than the ones found in Allgold at the same period. This does not mean that giant cells were never present in Orlis after 9 days but it is indicative that few survived due to necrosis. In Okla. 29 a few giant cells were found 9 days after inoculation but these were much smaller than the ones found in Allgold. The giant cells in Allgold at this time had enlarged considerably over those in the 6-day sample and in some instances were commencing to force vessels out of alignment. Cortical cells in the vicinity of the giant cells were hypertrophied and the

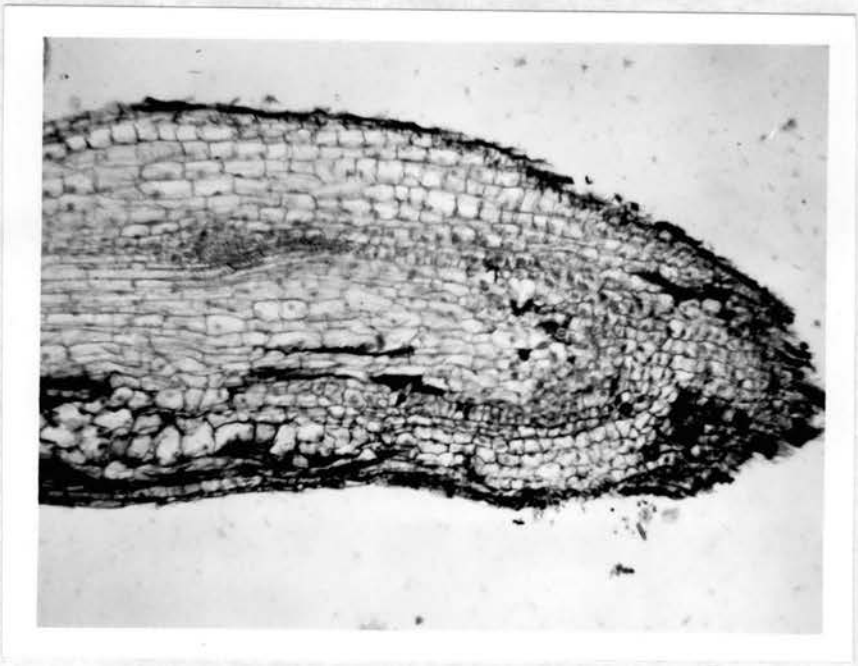

Fig. 2. Orlis root 6 days after inoculation showing necrosis in the cortex, pericycle, root cap and meristematic region. Note hypertrophy in cortex. 112 X.

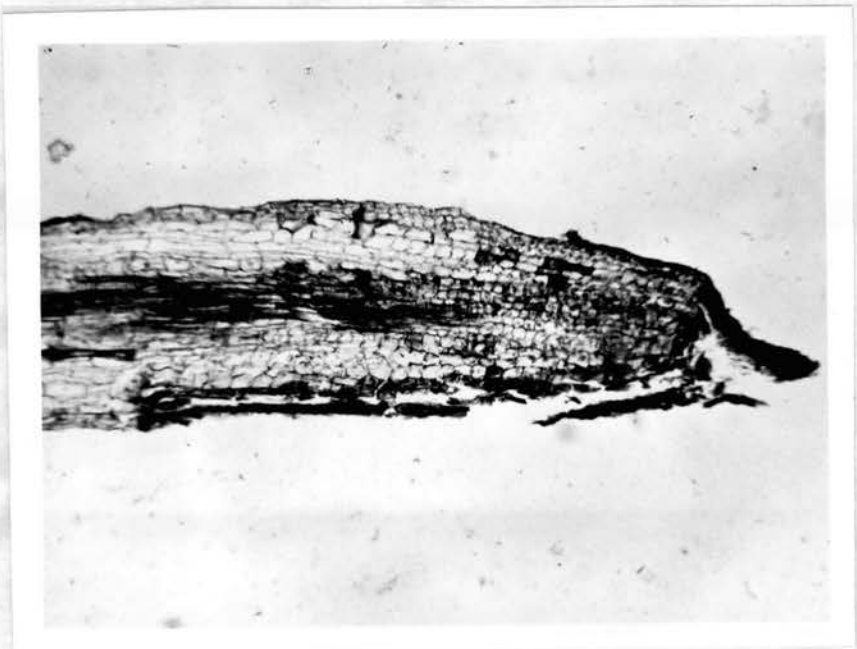

Fig. 3. Root tip of Okla. 29 nine days after inoculation showing general necrosis of the root. 110X.

ones adjacent to the giant cells were thin walled and undoubtedly in the process of disintegration and becoming a part of a giant cell. At this time approximately 50 per cent of all nematodes in Okla. 29 had disappeared. Most nematodes found in the resistant lines at the 9-day period had their heads embedded in the pericycle; those not in the pericycle were in the cortex of the region of elongation or maturation. Oftentimes it was evident these animals had migrated from the cortical tissues distal to these regions as obvious trails of necrotic cells led directly to the animal.

Twelve days after inoculation all resistant roots were in a state of partial or complete necrosis and obviously no growth was occurring. The giant cells in Allgold at this time had reached about their maximum size. It was also obvious that in some instances xylem vessels as well as cortical cells had coalesced to become a part of a giant cell (Fig. 4). In all root samples of Orlis following the 12-day sampling period only a few live nematodes were found and these had undergone little if any development. The root tissues by this time were usually in a state of complete necrosis. In all root samples of Okla. 29 following the 12-day sampling period some nematodes were found which had undergone development. However, they were few in number and were much slower developing than those found in Allgold. In Okla. 29 scattered necrosis was always evident and complete necrosis of the

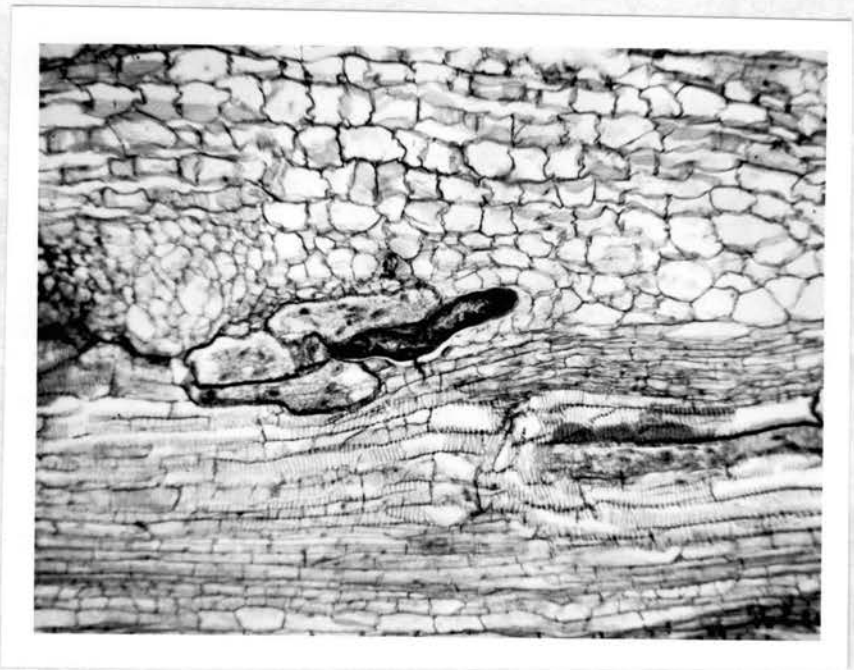

Fig. 4. Allgold root showing nematode, giant cells and interrupted vascular elements 12 days after inoculation. 92 X.

entire root tip was not uncommon.

Fifteen days after inoculation the giant cells in Allgold had reached their maximum size and in the remaining sampling periods the only noticable effect on the root tissue was a continued hypertrophy of the cells of the endodermis and cortex near the nematode. The state of development of the nematodes in Allgold in this experiment after 15 days closely resembled that of the nematodes in the experiments on survival and development and the results will not be repeated here.

A more detailed histological study of infected Orlis roots was completed at a later date to determine as accurately as possible the time when most of the nematodes perished in the resistant roots. With this information it was felt a sampling period could be selected more precisely to determine when the necrotic reaction in this resistant line occurred. Twenty-four Orlis roots were inoculated in a manner identical to that in the previous experiment and 3 roots were sampled from each plant at 24-hour intervals over a period of from 3 to 10 days inclusive.

The roots from the 8 sampling periods revealed that after 4 days the nematodes started to constrict, shrivel and die. This process was not found to be confined to a period of 2 or 3 days but was found to continue on through 10 days. The majority of the parasites, however, at the 10 day period were showing some signs of constriction when

they were killed and fixed. Only one nematode in all the roots examined in this experiment showed any signs of development and this nematode was found in the 10-day sample.

This experiment confirmed the time nematodes perished in resistant roots; previous experiments had revealed the rate of animal development in both resistant and susceptible roots. Consequently, the combined information indicated it should be possible to evaluate unknown sweet potato lines for nematode resistance in a minimum of 12 days after inoculation.

Application of laboratory techniques to evaluation of sweet potato lines.

On the basis of information accumulated to this point in the present investigation it was believed that the reaction of sweet potato lines to root knot could be determined in the laboratory in as short a period as 12 days after inoculation. To test this assumption on sweet potato lines other than those already used in this study, 16 lines whose reaction to nematodes was known from field data were used. The identity and reaction of these lines were unknown to the writer and remained unknown until the end of this experiment.

Two uniform roots on each of 2 plants of each of the 16 lines were each inoculated with about 400 nematodes.

The inoculation period was 48 hours. At the time the roots were removed from the inoculum they were carefully washed and observed for symptoms such as swelling or necrosis, and then each plant was potted in autoclaved soil and grown in the greenhouse for the remainder of the 12-day period. At the end of this period the roots were again observed for symptoms and processed for nematode counts and development. A decision as to the reaction of a sweet potato line was made on the basis of gross morphology of the roots, i. e., necrosis and relative amount of swelling, and the state of development of the individual nematodes. The total nematodes present in each root was not a deciding factor in the determination. Data from field observations were then referred to and a comparison with them was made (TABLE VIII). From these data it will be seen that in every instance, except one, the laboratory evaluation coincided with the field evaluation. The exception was the line P 94 which had been classified as intermediate in reaction to root knot on the basis of field data. This line was classified as susceptible in the laboratory. It has been previously pointed out that the intermediate category has been a difficult one to determine on the basis of several years field evaluation.

From the results presented and their agreement with previous data, it would seem that sweet potatoes can be evaluated for their reaction to root-knot with the techniques developed here. The approximate elapsed time

TABLE VIII

REACTION OF 16 SWEET POTATO LINES AS DETERMINED IN A LABORATORY EVALUATION

Sweet Potato Line	Nematodes[a] in each of stated classes: A	B	C	Total Nematodes	Reaction[b] to nematodes as determined in: Field	Lab.
	No.	No.	No.	No.		
Okla. 51	74	32	0	106	S	S
Okla. 52	58	49	0	107	S	S
Okla. 53	121	48	5	174	S	S
Okla. 54	36	35	19	90	S	S
P 69	52	27	0	79	S	S
P 79	43	46	7	96	S	S
P 81	47	51	0	98	S	S
P 94	61	26	0	87	I	S
4X66-2N	73	1	0	74	R	R
47X55-1C	41	0	0	41	R	R
51X66-6N	39	0	0	39	R	R
P 80	60	8	0	68	R	R
P 87	45	0	0	45	R	R
P 88	68	0	0	68	R	R
P 101	34	4	0	38	R	R
Okla. 55	72	7	0	79	R	R

[a]Nematodes in each class are total from 4 roots.

[b]R = Resistant; S = Suceptible; I = Intermediate.

involved in obtaining the data presented in TABLE VIII was 13 days as compared with 2 to 4 years to get data from the field trials. Whether or not the situation with regard to the intermediate category can be resolved remains for further investigation.

DISCUSSION

One of the stated original objectives of this investigation was to confirm the work of Dean (11). This has been done with respect to the developmental stages reached by nematodes in resistant and susceptible sweet potato lines. The fact that nematodes in resistant sweet potatoes generally do not survive to maturity has also been confirmed. While Dean had concluded that there were no differences between resistant and susceptible sweet potatoes with respect to the numbers of nematodes entering in a given inoculation period, the number of plants with which he dealt was probably too few to make a certain determination of this point. In the present investigation a considerable number of plants has been used in an attempt to determine this point. While at times it appeared that there were real differences between resistant and susceptible sweet potato lines with respect to the number of larvae entering in a given inoculation period, these differences were not consistent enough, except in the case of Allgold and Orlis, to warrant a conclusion that this character could be used to separate resistant from susceptible lines. Even in the case of Allgold and Orlis, observational evidence over a period of time indicates that this character would not in all cases be a reliable

one on which to base a judgment. While a real difference may exist between sweet potato lines with respect to numbers of larvae originally entering, the present indications are that it will require more refined techniques to detect them.

Christie (6) has presented data from which he concluded that certain sweet potato varieties were invaded by fewer nematodes than others. The roots of inoculated plants were not examined until from 3 to 5 weeks following inoculation. Evidence from the present investigation suggests that had Christie examined roots at an earlier time he would have found essentially as many larvae in resistant as in susceptible plants and that what was actually happening in his resistant material was that the nematodes were dying before the roots were examined. Since Christie does not mention having observed necrosis in his more resistant material it is also entirely possible that he was dealing with a different type of resistance than that encountered in the present work. He does note, however, that in certain varieties nematode development was retarded; this is in agreement with data from resistant lines in the present work.

Sasser (20) in a series of experiments to determine relative infectivity and development of different species of root-knot nematodes in several different plants found that generally resistant plants were not infected as "readily" as were susceptible plants. These determinations

were made after the plants had grown 20 days in infested soil. There again appears the likelihood that had Sasser made earlier observations he would have found little if any difference with respect to numbers of nematodes entering resistant or susceptible plants.

While it is still questionable as to whether or not there are real differences between sweet potato lines with respect to numbers of nematodes entering, it has been demonstrated in the present investigation that there are other differences which permit a relatively rapid evaluation of sweet potato lines whose reaction to root knot is unknown. As has already been pointed out 16 sweet potato lines whose identity and reaction were unknown to the writer were classified for root knot resistance with results that agreed in every case but one with those obtained over a period of several years from field evaluation. The laboratory evaluations were based principally on the state of development of the nematodes at a given time following inoculation and whether or not inoculated roots showed a necrotic reaction. These features had been found relatively constant in the work with known lines. A uniform level of infection was made possible through the use of the inoculation technique developed in the course of the present work.

As has been pointed out a difficulty still remains in distinguishing sweet potato lines whose reaction to root-knot appears intermediate on the basis of field

data. In the laboratory test the intermediate line appeared to react in a fashion very similar to that of susceptible lines. Only through further investigation can this problem of the nature of an intermediate type of resistance, if indeed such a type is a reality, be resolved.

From the results available in the present investigation it would seem that resistance to Meloidogyne incognita var. acrita consists essentially of a hypersensitive reaction on the part of the host. This reaction is expressed by necrosis in affected host tissues and by failure of the nematodes, obligate parasites, to survive or develop to maturity.

SUMMARY

Sweet potato lines known to be resistant or susceptible to root knot (Meloidogyne incognita var. acrita Chitwood) have been investigated with the objective of defining possible differential features in host-parasite relations and using these features to evaluate sweet potato lines presently unknown with respect to resistance or susceptibility.

A technique was developed using single sweet potato roots which permitted the use of a uniform level of inoculum and assured infection. Through the use of this technique it was possible to demonstrate statistically significant differences between resistant and susceptible sweet potato lines with respect to the number of nematode larvae entering in a given inoculation period. The biological significance of these differences, however, remains in question because observational evidence did not consistently support the conclusion that there were real differences. Real differences between resistant and susceptible sweet potato lines were demonstrated with respect to survival and development of nematodes once they had entered roots. Larvae in resistant roots generally failed to survive to maturity while those in susceptible roots usually survived and became sexually

mature. The observed rate of development of larvae in resistant roots was considerably slower than that in susceptible roots. A necrosis of root tissues was constantly associated with the presence of nematodes in resistant roots.

Sixteen sweet potato lines whose reactions to root knot were known only from field data were evaluated in the laboratory on the basis of a necrotic reaction in the roots and the state of development reached by individual nematodes 12 days after inoculation. The data from the laboratory evaluation agreed in all cases but one with the field data.

In the plant material used in this investigation resistance to M. incognita var. acrita in sweet potato is believed to consist essentially of a hypersensitive reaction on the part of the host.

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Master of Science

Thesis: THE NATURE OF RESISTANCE AND SUSCEPTIBILITY TO THE ROOT-KNOT NEMATODE (MELOIDOGYNE INCOGNITA VAR. ACRITA CHITWOOD) IN SWEET POTATO

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