RESISTANCE AND SUSCEPTIBILITY TO ROOT-KNOT NEMATODES IN TOMATO AND SWEET POTATO

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INTRODUCTION

The economic importance of root-knot nematodes can hardly be over-emphasized. Their wide distribution, extensive host range and the special difficulties involved in their control are some of the factors that make them serious crop pests. Steiner, in a recent paper (28), estimates that nematodes cause an annual crop loss in this country of between four and five hundred million dollars. He further points out that some twenty species of nematodes are considered to be major crop pests and, of these, the root-knot nematodes are the most serious.

The use of resistant varieties for the control of plant disease is always a desirable practice when such varieties are available. In the case of root knot, control by other means is difficult and the value of resistant varieties is increased.

Resistance in plants to root-knot nematodes, however, has not always been properly evaluated to assure maximum benefits from the use of resistant varieties or species. The term "resistant" applied to a plant on the basis of symptomatology does not convey an adequate conception of the effect of the parasite on the host or of the effect of the host on the parasite. A plant may become severely galled when exposed to root-knot nematodes and yet markedly retard development of the worms (7). Furthermore, a plant may allow normal development and reproduction of root-knot nematodes without showing the typical galling of roots (27). Clayton (10), for example, found that "resistant corn", which showed few or no galls on the roots, was no better in a rotation for controlling root knot than was susceptible tobacco.

In view of the situation outlined in the foregoing paragraphs, it seems clear that any evaluation of resistance must take into account host-

parasite relations. It is unsafe to assume that a plant which does not become galled will therefore serve to reduce a population of nematodes in the soil. Conversely, it should not be assumed that a plant which does become galled cannot be used in a rotation to depress the amount of soil infestation.

The present work was begun with the aim of gaining a better understanding of resistance and susceptibility to the root-knot nematode, Meloidogyne incognita (Kofoid and White) Chitwood, in certain lines of tomatoes. Later the problem was expanded to include a few sweet potato lines. It was planned to work with known resistant and susceptible plants and to seek an explanation of the processes whereby some plants are injured very little by root-knot nematodes while others are severely injured. This led to a study of host-parasite interactions including the effect of the host on the parasite and the effect of the parasite on the host.

The problems involved in this study are of primary interest to the nematologist, plant pathologist, and plant breeder.

There is evidence that information on the nature of resistance in one plant to one nematode species may apply, in a general way, to other plants and other nematodes. Species of Meloidogyne (7), Heterodera schactii Schmidt (13), and Rotylenchulus reniformis Linford and Oliveira (17), at least, appear to be affected in a similar manner by resistant plants.

REVIEW OF LITERATURE

The work of Christie and Albin (6), Christie (7) and Christie and Havis (8), demonstrated that Heterodera marioni (Cornu) Goodey comprised several races, each differing from the others in some measure with regard to the plant species and varieties which they could successfully parasitize.

Chitwood (3) compared the morphology of several populations of H. marioni and concluded that the group constituted a genus apart from Heterodera. Meloidogyne was recognized as the earliest, valid, generic name. Chitwood recognized five species and one variety in this genus.

The life cycle of H. marioni has been clarified by Christie and Cobb (5). The specimens used in their study were from a population that had been maintained in culture on tomatoes in the greenhouse for a year or two. That life cycle is given here in some detail since it must be kept in mind in an evaluation of host-parasite relations, particularly with reference to the influence of the host on the parasite.

A larva spends its first stage within the egg. The first molt occurs while the larva is still within the egg and before it has attained its maximum length. Following the first molt of the ovic larva, there is a period of further growth before it hatches from the egg. The second stage ends with the second molt, which occurs in the tissues of a host plant.

There is no third stage of either male or female since two cuticles are loosened simultaneously, representing the second and third molts. Since the female molts a fourth time almost immediately following the third molt, the fourth stage of the female is very short.

In the life cycle of the male, however, there is a longer interval between the third and fourth molts, resulting in a definite fourth stage.

It is in this stage that metamorphosis of the male occurs. In this metamorphosis the male is transformed from a relatively short and broad form, similar to that of the female before the second and third molts, to the slender elongate form of the mature male. Upon completion of metamorphosis the male molts a fourth and last time and is mature. Both the metamorphosis and the final molt of the male occur while the animal is still within the sac-like structure which comprises the loosened cuticles of the second and third molts. At maturity the male emerges from this sac. It is then free to migrate through root tissues or soil.

According to Christie and Cobb, males and females cannot be distinguished until the end of the second stage. By this time both sexes have increased considerably in width but very little in length. After the second and third molts, which terminate the second stage, the male begins metamorphosis and the female loses the caudal spike. The female, upon completion of the final molt, increases greatly in both length and width and gradually assumes the pear-shaped form characteristic of the mature female.

It is generally assumed at present that the males have no function in reproduction. It has been demonstrated that females lay viable eggs without mating (30).

When the maximum size of the female is attained, egg laying begins. The eggs are extruded from the vulva of the female, and they accumulate around the posterior end of her body. A gelatinous matrix is extruded with the eggs, and the matrix with the contained eggs may rupture the cortex and the epidermis of a root. In this case the egg mass becomes situated externally on the root where it is visible macroscopically as a whitish mass about the size of, or slightly larger than, a mature female. The egg mass yellows with age and eventually becomes reddish or dark brown. Often the egg mass does

not rupture the epidermis but is contained within the root tissue.

Except for the mature males, the preparasitic, second-stage larva is the only form of the root-knot nematode that migrates freely in the soil. This is the only form that enters a host plant to initiate infection. It has been repeatedly observed that the larvae enter roots close to the tip, although this is not considered the only portal of entry.

The initial stages of infection by root-knot nematode larvae have been described by Linford (19). The nematodes begin their feeding on root tissues before they make an entrance to a root. They penetrate epidermal cell walls with their stylets and feed on the cell contents. Several cells may thus be penetrated by a single larva before it finally enters the root. Entrance is effected by use of the stylet, either to make an opening through a cell or between cells. Once inside a root, a larva travels intercellularly for the most part and migrates through the tissues for some time, stopping here and there to puncture and feed upon a cell. Eventually it stops migrating and takes a permanent position in the root.

All the molts of a root-knot nematode except the first one, which occurs within the egg, take place after the animal has taken this permanent position.

When a larva assumes its final position, it usually lies with its head in the plerome, and often the posterior end extends into the periblem. This, however, is not always the case, as sometimes the entire body of the parasite lies in the plerome parallel to the longitudinal axis of the root (4).

The feeding of a root-knot nematode after it becomes established in a final position has been studied by Linford (16). As in the earlier stages, the parasite punctures cells and sucks cell contents through the hollow buccal stylet. The nematode remains in a fixed position but swings its head

from side to side and feeds first on one and then on another of the cells within reach of the stylet. Linford has photographed the saliva expelled from the stylets of nematodes in nutrient solution. Cells around the head of a nematode feeding in plant tissue are profoundly influenced by some agent in the salivary secretion.

The tissue changes induced by the feeding of root-knot nematodes in tomato have been described by Christie (4). Twenty-four hours after inoculation cortical cells are seen to be enlarged; this condition is not necessarily confined to cells lying immediately adjacent to the path taken by a worm. Cells of the pericycle and endodermis near the path of a larva may also show slight enlargement. In some cases mitotic activity in the apical meristem is suppressed and forward growth of the root tip is slowed or stopped, particularly if several larvae enter at about the same time.

After a nematode has assumed a permanent position in the root, provided that this occurs a short distance behind the apical meristem, as is frequently the case, the cells in the perome immediately around the head of the parasite remain undifferentiated for the first 48 to 60 hours. At the end of this period these cells enlarge somewhat and some of them may divide with the formation of a very thin cell wall which soon disappears. The original cell walls also gradually disappear, and the cell contents of adjoining cells fuse, resulting in the formation of a giant cell. Surrounding cells are gradually appropriated by the giant cell by a dissolution of the separating walls.

This assimilation of surrounding cells continues for 10 to 20 days. The process gradually slows and eventually stops. Usually from three to six giant cells are formed in each gall. This giant cell formation usually occurs in the central cylinder but may occur in the cortex.

The first cells to be used in the formation of a giant cell are often members of a row of cells in the plerome that normally would have differentiated into part of a xylem vessel; thus, certain of the xylem vessels are interrupted. Others are not interrupted but are pushed out of alignment around the region of giant cell formation.

Christie observed, as have others, that lateral roots are formed with abnormal frequency in the region of a gall. The presence of the parasite seems to stimulate mitotic activity in the pericycle which results in the formation of a layer of small-celled parenchyma tissue not found in normal roots. It is in this tissue that the increased number of lateral roots have their origin. Christie further states that as the gall grows older some of these parenchyma cells become differentiated into short, misshapen, reticulate xylem elements with no definite organization.

The length of the life cycle of root-knot nematodes is known to vary greatly with the temperature and the host plant. However, it is usually stated in the literature that under conditions favorable for the growth of the organism the length of the life cycle is 25 to 30 days. Tyler (31), using tomato as a host, obtained the shortest life cycle at 27°C. This temperature gave a complete life cycle, from larva to larva, in 25 days. Full development of the females was reached in 16 days, and an additional nine days were required for the development and hatching of eggs. At 16.5°C., under otherwise similar conditions, the life cycle was lengthened to 87 days, with 56 days required for the full development of females and 31 days required for the development and hatching of eggs. A complete life cycle did not occur below 14.3°C. or above 31.5°C. Godfrey and Oliveira (14) found the life cycle of Heterodera marioni to vary from 19 days on cowpea to 35 days on pineapple.

Apparently very little attention was given to the nature of resistance in plants to root-knot nematodes before 1939. In 1925 Steiner (26) wrote that resistance in plants to plant parasitic nematodes means that the plant opposes the entrance of nematodes by some mechanical or chemical means. In 1941 Tyler (33) defined resistance with special reference to root-knot nematodes as the ability of plants to obstruct the invasion of parasites.

With the work of Barrons (2) in 1939, it became clear that resistance in plants to root-knot nematodes could not be regarded as a simple failure of the larvae to enter the roots. Barrons had observed slight swellings on the root tips of Alabama No. 1 and Alabama No. 2 beans (Phaseolus vulgaris L.), but these swellings, he observed, did not persist and develop into galls. These observations seemed to indicate that nematodes were entering the roots of resistant plants but were failing in some way to produce the typical symptoms of root knot.

To investigate this possibility, Barrons compared resistant and susceptible plants with respect to the number of larvae that were found present in the roots after a short period of exposure to infection. He used 24 different kinds of resistant plants (varieties and species) and six different kinds of susceptible plants. It was decided arbitrarily to count the number of nematodes present in the terminal centimeter of a given root; from 20 to 39 roots were studied from each variety or species. The study included highly resistant plants such as Crotalaria spectabilis (L) Roth, rye, oats, and wheat. With regard to the number of larvae present per root tip, there was no significant difference between the means of susceptible and resistant plants.

The nature of resistance to Heterodera schactii in potato varieties has been studied by Gemmel (13). He compared resistant with susceptible

varieties with respect to the number of cysts produced, the size of the cysts, and the number of larvae produced per cyst.

Gemmel did not take data on the number of nematodes that entered the roots of the plants in the original period of infection. Therefore, the reduced number of cysts borne on resistant plants could be explained either as a failure of larvae to enter in large numbers or as a failure of larvae to survive after entering. The reduced size of cysts from resistant plants and the reduced number of larvae that these cysts produced can be explained, however, only as an inhibitory effect of the host on the parasite.

Christie and Albin (6) were concerned primarily with the problem of races of root-knot nematodes, but from their work came valuable information as to the nature of resistance in plants to these parasites. For example, in one experiment several populations of nematodes were tested on Persian clover. Examinations of the root systems were made 30 days after the seeds were planted in infested soil. Nematodes of one population were found to be entirely absent from the roots when the examination was made and there was no indication of galling. The root systems of plants inoculated with another population were beaded with small swellings but again no nematodes were found. Roots of Persian clover inoculated with a third population were found to harbor few nematodes; of those present, most were dead, and none had developed past the second stage.

McBeth (21) tested eighteen grasses for resistance to root-knot nematodes. In these tests roots were stained and examined microscopically. Nematodes were found to reproduce on several of the grasses, in some cases with the formation of only very small galls on the roots. Digitaria eriantha var. stolonifera Stapf. allowed development and egg laying by the parasites but the roots of this plant showed no trace of swellings.

In an experiment designed to test the influence of various host plants on various populations of root-knot nematodes, Christie (7) found that resistant plants prevented, in some measure, the development of nematodes within their roots. Some of the plants which were resistant to one or another of the populations of root-knot nematodes were Orange Little Stem sweet potato, Coker 100 cotton, Lantana camera L., and Pelargonium graveolens (Thunb.) L.'Hérit. In some cases larvae, after gaining entrance to the roots, failed to reach the second molt. In other instances few females ever reached the egg-laying stage and those that did were small and laid few eggs. In a plant only slightly resistant there was merely a lengthening of the period of development with no appreciable effect on the number of egg-laying females or the number of eggs laid per female.

The problem of the nature of resistance in plants to root-knot nematodes has been discussed by Christie (9). In his view, resistance is due to failure of the tissues of the host plant to respond properly to the stimulus of infection. In an elaboration of this hypothesis, Christie points out that nematodes are sedentary parasites and are able to feed only upon a few cells that are within reach of their heads. If the salivary secretion, which a nematode injects into surrounding cells, fails to change their normal development and differentiation, the parasite is soon surrounded by cells that either are too thick walled to be penetrated by the stylet or are so highly vacuolated as to be of no value as a source of food.

In the hypothesis of Barrons (2) giant cell formation was considered necessary to furnish the developing nematode with a continuing supply of food. Resistance was postulated to be due to the synthesis by the plant of a substance which neutralizes the giant cell inducing agent in the salivary secretion of the nematode. Christie (9), however, reports that Machmer has

observed egg-laying females in Pelargonium graveolens which had not induced giant cell formation. These nematodes were situated in small outgrowths from the bases of propagative cuttings where the surrounding tissue was composed of thin-walled parenchyma cells. In the roots of these same plants only very small galls had formed. Some of these galls contained no trace of a parasite; others contained dead or only partly developed nematodes. Christie points out that all plants are not necessarily resistant for the same reason. He considers it possible that some plants may be quite resistant or even immune to the entry of nematodes.

Resistance to root-knot nematodes in Lycopersicon peruvianum Mill. has been reported by several workers (1, 24, 11, 22, 34, 12). Five species of tomato were tested for resistance to root-knot nematodes by Bailey (1). Included in these tests were 95 commercial varieties, several selections from experimental laboratories, and 420 seed-lots of Lycopersicon esculentum Mill. from the United States Bureau of Plant Industry, Division of Plant Exploration and Introduction. Bailey reported no resistance from these plants with the possible exception of three lots of the 420 seed-lots of L. esculentum. Also tested were L. glandulosum, L. hirsutum H.B.K. and L. pimpinellifolium Dunal. No resistance was found from these sources. From 11 seed-lots of L. peruvianum, 299 selections were made. Twenty of those selections had remained free of galls after growing for six months in infested soil. It is interesting to note that Bailey did not use the term "resistance" in his paper but used "tolerance" instead.

The information available seems to indicate that resistance in at least some selections of L. peruvianum is controlled by two dominant genes (34).

Frazier and Dennet (12) stained and examined roots of tomatoes with

resistance from a L. peruvianum source. Nematodes in these plants were immature whereas nematodes in susceptible tomatoes grown under the same conditions contained numerous well-developed females. These writers reported that some cell necrosis was observed where nematodes were present in resistant plants. No counts were made of the number of nematodes present in resistant and susceptible plants but "many" nematodes were observed in resistant plants. The writers pointed out that this evidence supported the view of Barrons (2) that "resistance" was not a resistance to the entry of nematodes but a resistance to gall formation.

Liao and Dunlap (15) have described what they refer to as arrested invasion of L. peruvianum roots by root-knot nematodes. They observed that the larvae began penetration of roots but most of them either died before getting their bodies completely inside a root or failed to pass beyond the peripheral layers of cells.

It has been shown that L. peruvianum may be quite resistant to one species of Meloidogyne and quite susceptible to another. L. peruvianum was not infected by M. incognita, was heavily infected by M. arenaria (Neal) Chitwood and was moderately infected by M. incognita var. acrita Chitwood and M. hapla Chitwood (29). These authors did not indicate their criterion of infection.

Varieties of sweet potatoes have been tested for resistance to nematodes by Weimer and Harter (35) and Poole and Schmidt (23). In both of these papers Yellow Jersey, Red Jersey, Big Stem Jersey, and Porto Rico were reported resistant.

Christie (7) checked several sweet potato varieties for the number and state of development of nematodes in their roots three weeks and six weeks after inoculation. He found differences between varieties in the number of

nematodes that developed to maturity in this period. In this experiment Christie checked either 50 nematodes per plant or all the nematodes a plant harbored. Data was recorded for two plants of each variety. With two exceptions every plant checked yielded at least 50 parasites. The two plants of the variety Orange Little Stem, a mutant originating from Yellow Jersey, yielded only six and eight nematodes respectively. Christie apparently viewed this as evidence that fewer larvae entered the roots of Orange Little Stem than entered the roots of other varieties.

MATERIALS AND METHODS

To ensure a continuous supply of inoculum throughout the period of the work, a greenhouse bench was filled with nematode infested field soil. Susceptible tomato plants, var. Marglobe, were kept growing in the bench at all times to maintain a population of nematodes in the soil and to provide inoculum in the form of galls or egg masses. Nematodes from the root systems of tomatoes and sweet potatoes grown in the bench were identified as Meloidogyne incognita.[1]

In the tomato experiments, the variety Marglobe served as a nematode susceptible standard. The resistant plants were introductions of Lycopersicon peruvianum or hybrids with L. peruvianum parentage. The resistant tomatoes referred to in this paper are designated by the following numbers:

43-1-1 (L. peruvianum x L. hirsutum) x L. esculentum. The history of this hybrid is given by Frazier and Dennet (12). In their paper it was designated HES 3386. An individual selection from HES 3386 was designated 43-1.[2] The plants used in the present work were from seeds produced on an individual of 43-1 growing in nematode infested soil in the greenhouse in 1950.

63 L. peruvianum, P. I. 126946.

64 L. peruvianum, P. I. 128651.

Allgold sweet potato was used as a susceptible standard for the sweet potato experiments. The resistant sweet potatoes were:

Orlis--a mutant from Yellow Jersey selected by O. H. Elmer in Kansas.

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[1]This identification was made by A. L. Taylor, Division of Nematology, U. S. Department of Agriculture.

[2]This selection was made by H. B. Cordner, Horticulture Department, Oklahoma Agricultural and Mechanical College, in 1948.

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

Oklahoma 46--a seedling from the cross Oklahoma 29 x Orlis.

Allgold and Oklahoma 46 were included in field trials for nematode resistance in sweet potatoes in 1950.[3] These two lines appeared in three series with four replicates of each line in each series. Each replicate consisted of fifty plants. Each plant in the field was rated according to the amount of galling and the number of egg masses on its roots. On the basis of these ratings Allgold was quite susceptible and Oklahoma 46 quite resistant. In addition to the field ratings, nematodes were counted in a sample of roots obtained from each replicate. The samples were taken into the laboratory, and each root was sliced on a power-driven slicing machine. Each slice, a cross section of a root, was about 1/10 inch in thickness. The cut surfaces of each slice were examined, and the nematodes counted. Since examinations were made with the unaided eye, only those nematodes that were mature or nearing maturity were included in the counts. In 6,293 gm. of sampled roots from Allgold, the average number of nematodes per 100 gm. of root was 30.17; 6,775 gm. of Oklahoma 46 roots contained 0.10 nematodes per 100 gm.

Observation of Orlis growing on nematode infested soil in the field in 1949 indicated that this plant had a high degree of nematode resistance. No information on resistance and susceptibility in Oklahoma 29 was available when the present work was begun.

In order to study nematodes in whole roots the staining technique of McBeth and Taylor (20) was used. Whole root systems or a sample of roots

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[3]These tests were conducted by F. Ben Struble, Department of Botany and Plant Pathology, and H. E. Cordner, Department of Horticulture, Oklahoma Agricultural and Mechanical College.

were washed thoroughly in running water then boiled from one to four minutes in a solution of lactophenol and acid fuchsin. The staining solution was then poured off, the roots rinsed in water to remove excess stain, and cleared in lactophenol. Twenty-four hours were sufficient for clearing but if it were desired to examine roots sooner it was found possible to speed clearing by again boiling the roots for a few seconds in the clear lactophenol. As soon as the solution cooled, roots were examined. If single small roots were to be stained it was found that they could be removed with forceps from the hot staining solution, allowed to drain briefly, transferred to warm lactophenol and examined immediately. The time roots were boiled in the staining solution depended upon the size of the roots, the larger roots requiring a longer period. Ordinarily, boiling was continued until a slight reddening of larger galls could be detected.

To study tissue changes induced in root tips invaded by larvae, the root tips were killed and fixed in Bouin's fluid, and infiltrated and imbedded by the N-butyl alcohol method (25). Sections were stained with safranin-O in 95% ethyl alcohol and counterstained with fast green in 100% ethyl alcohol.

It was planned first to determine the relative numbers of larvae that enter the roots of resistant and susceptible tomatoes. This was to be accomplished by exposing the plants to some form of inoculation, removing the plants, staining their roots, and counting the number of nematodes present in a sample from the root system of each plant.

Several inoculation techniques were tried before a successful method was found. In the early attempts, plants were set directly into the infested soil in the greenhouse bench. At first the plants were left in the bench until they were removed for examination. Later, they were given a compara-

tively short period of exposure to infection in the bench, then removed and transplanted to autoclaved soil in pots.

Further attempts to find a suitable method for inoculation involved the use of chopped nematode galls for inoculum. At first the inoculum was applied directly to the plants in pots; later it was mixed with soil in a large container, left for a few days to allow for hatching and dispersal of larvae, then sieved out of the soil. This soil was then used to pot plants which were to be inoculated. In all the inoculation experiments in which chopped galls were used for inoculum, the plants were given an initial, short period of exposure to infection, then their roots were washed thoroughly in running water, after which they were transplanted to autoclaved soil in pots.

None of the methods for inoculating plants so far described were satisfactory. Examinations of the stained root systems of these plants disclosed that in every case extensive root decay had occurred, and it further appeared that this condition was worse on resistant than on susceptible plants. Isolations from roots of several plants yielded Rhizoctonia sp. Because of this decay of root systems, it was impossible to interpret certain observations. For example, it was observed that there were fewer nematodes in the roots of resistant tomatoes a few days after inoculation than there were in roots of Marglobe. The question of how to account for this difference then arose. Conceivably it could have been due to a difference in the number of nematodes that originally entered the plants, or to a difference in the number that survived after entering. If the latter were true, it was desired to know whether fungus decay of roots or factors in the plants themselves were responsible for the elimination of nematodes from the root systems.

To obtain more information on these problems, a technique was needed which would give reasonably uniform infection in a known, short interval of time, and which would avoid the problem of root decay incited by fungi.

It seemed that the technique used by Christie (4) for studying the development of root-knot nematode galls would serve this purpose. The method consists of inoculating plants in Petri dishes with a water suspension of nematode larvae. In these experiments, plants as large as could be fitted into a Petri dish were used. These plants had considerably branched root systems about two inches in length. Single plants were inoculated by pipetting a larval suspension over the entire root system of each plant as it lay in the bottom of the dish. The root system was then covered with sand, soil, or vermiculite and watered. A cover was placed on the dish and the plant left in the dish for 24 or 48 hours. At the end of the period of exposure to infection the plant was floated free of the substrate, its root system was carefully washed in running water, and it was either transplanted to autoclaved soil in a pot or stained for examination.

Experiments of this general nature were repeated many times varying the period of exposure, the substrate used to cover the root systems, the temperature, and the number of larvae applied. By use of this method, fungus decay of roots was avoided. Consistent infection, however, could not be obtained. In one experiment it was possible to demonstrate nematodes in all inoculated plants, but in several attempts to repeat this experiment under as nearly the same conditions as possible no more than 50% of the inoculated plants ever became infected.

The method that finally proved to be satisfactory was described by Christie (7). Small tomato plants or sweet potato cuttings were set into autoclaved soil in 2-inch pots. The plants were allowed to grow in these

until they had become pot-bound and a network of roots had formed at the interface between the walls of the pot and the soil mass. The soil mass containing a plant was then dislodged and removed intact from the pot. Five ml. of a suspension of nematode larvae in water was applied with a pipette to the sides and bottom of the soil mass. The plant was then returned to the pot and left for 24 or 48 hours. At the end of this period, the plants were again removed from the pots, their root systems carefully washed in running water, and they were transplanted to autoclaved soil in 4-inch pots. Washing the root systems served to remove those nematodes which had not already entered roots. By virtue of this method, the time of entry of nematodes into the roots of any plant was known to within 24 or 48 hours depending upon which period of exposure to infection was used.

To obtain the large numbers of larvae that were required to inoculate plants by this method, the following procedure was used. Root systems of Marglobe tomato plants heavily laden with egg masses were immersed in water in a large dish and manipulated until most of the egg masses became dislodged and sank to the bottom of the dish. The roots were then removed from the dish and the contents of the dish including debris and egg masses were poured over a 10-mesh screen. Some of the egg masses were collected in a container, but most were trapped in the coarse material held by the screen. A stream of water from the tap was run through the material on the screen dislodging the egg masses and allowing them to pass into the container.[4]

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[4]Later it was learned that Allgold sweet potato served as a better source of egg masses than tomatoes. Nematode infected roots of Allgold sweet potato do not become as severely galled as Marglobe tomato roots and practically all of the egg masses on small roots are situated externally where they can be easily dislodged.

To accomplish further separation, the egg masses were poured into a large graduate cylinder. The egg masses quickly settled to the bottom and the water with suspended debris was poured off. The egg masses were then placed on a piece of cheesecloth suspended by means of a nichrome wire support in contact with water in a glass funnel. Attached to the stem of the funnel was a short piece of rubber tubing which was fitted with a pinch clamp. The funnel apparatus was left at room temperature for the larvae to hatch. Twice daily the pinch clamp was opened and the larvae were collected and stored in a refrigerator at 5°C to 7°C until a sufficient supply was accumulated.

In several experiments it was desired to count all the nematodes present in the root systems of the plants. To accomplish this, the intact root system of a plant was floated out in a dish of lactophenol. A small portion of the root system was cut off and placed in lactophenol in a syracuse watch glass. With the aid of a dissecting microscope and a pair of finely pointed needles, every nematode that could be found in this portion of the root system was dissected out of the root tissue and counted. This process was repeated until the entire root system of the plant had been examined.

RESULTS

Tomatoes

Plants Inoculated in Pots with a Larval Suspension, Experiment 1

The tomato lines used in this experiment were Marglobe, 43-1-1, and 64. Each plant was inoculated with approximately 3,000 larvae and left in the inoculated pot for 48 hours before it was removed and transplanted to autoclaved soil. Two plants of each tomato line were removed and stained for examination after each period indicated in table 1. Also shown in the table is the number of nematodes in the entire root system of each plant.

A part or all of the nematodes in each plant were classified according to their state of development. This classification was based on the categories used by Christie (7), shown in figure 1. Classes A and B include all larvae up to the state at which the final molt is almost completed. Classes C and D include those nematodes that have completed all the molts but have not laid eggs. Class E includes only egg-laying females. The distinctions between A and B and between C and D are based simply upon differences in size and shape. Nematodes which had not reached the state shown for Class A in figure 1 were also included in that class. In Christie's work Sudan III was used to stain nematodes and according to him, that dye will not stain larvae that have undergone no development. In the present work acid fuchsin, which stains nematodes in all stages, was used.

Fewer larvae were found in the root systems of resistant plants than in susceptible plants two days after inoculation. The number of nematodes in Marglobe plants remained about the same throughout the experiment whereas the nematodes in 43-1-1 had disappeared completely by the end of 17 days after inoculation.

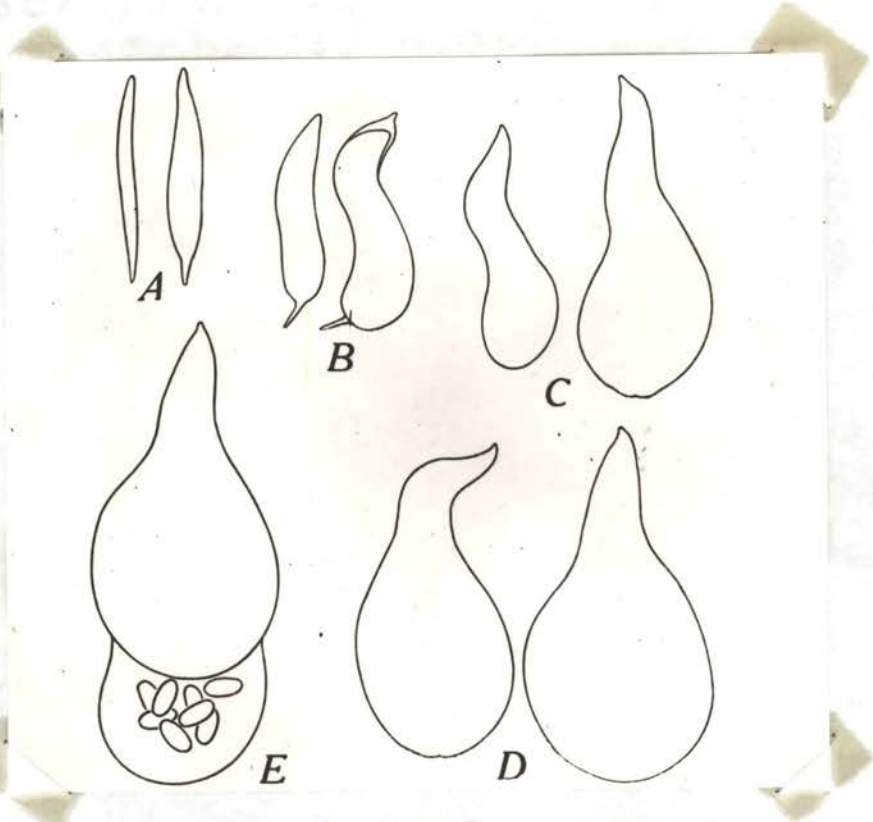

Fig. 1. Classes into which nematodes were grouped according to the amount of development they had undergone. (After Christie)

Table 1.--Numbers of nematodes in entire root systems of tomato plants at various periods after inoculation.

Period after inoculation	Replicate	Nematodes in each of stated tomato lines		
		Marglobe	43-1-1	64
Days	No.	No.	No.	No.
2	1	211	54	47
	2	189	27	25
6	1	74	2	10
	2	136	3	12
10	1	269	30	14
	2	165	21	6
17	1	147	0	-
	2	202	0	-
23	1	228	0	-
	2	180	0	-
28	1	199	0	-
	2	152	0	-
31	1	133	0	-
	2	129	0	-

Both of the 43-1-1 plants removed for examination ten days after inoculation appeared to be somewhat susceptible to nematodes. It is believed that these two plants differed genetically from the other plants of the same line with respect to nematode resistance. The numbers of nematodes in these two plants, 30 and 21, respectively, is considerably lower than the numbers in Marglobe plants removed at the same date but is not substantially different from the numbers in 43-1-1 plants removed 48 hours after inoculation. The nematodes present in the 43-1-1 plants at ten days after inoculation had undergone almost as much development as the nematodes in Marglobe at this same date. Although a few nematodes in Marglobe plants were placed in Class B (Fig. 1) and all nematodes in 43-1-1 were placed in Class A, no nematodes in either tomato line had advanced beyond the second larval stage and the difference in the stage of development of nematodes between lines was not striking.

Field tests for nematode resistance also have indicated genetic variation in the lines of resistant tomatoes used in this work.[1] In these tests the tomatoes were rated from one to five according to the amount of galling of roots. A rating of one was given to plants showing no galls and a rating of five was given to the most severe cases of galling found. The great majority of individuals in resistant lines used in the present work received ratings of one. Occasional plants were found, however, which were given ratings of three or four.

There seems to have been a tendency also for nematodes to disappear from the root systems of line 64 tomatoes. No plants of this line were available for study more than ten days after inoculation but the number and

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[1]These tests were made by E. E. Cordner in 1948 and 1949.

condition of the nematodes present at that time indicated that line 64 has the same type of resistance as 43-1-1.

No nematodes in resistant plants had developed beyond Class A at any date. Most of the nematodes in these plants had not developed at all and the appearance of many of them indicated that they were dead at the time that the plants were removed for examination. All nematodes in Marglobe plants removed less than ten days after inoculation were in Class A. The stage of development of 50 nematodes in each Marglobe plant from ten days after inoculation to the end of the experiment, 31 days after inoculation, is shown in table 2. Egg laying in Marglobe first began at some time between 23 and 28 days after inoculation. By the end of 31 days after inoculation, between 90 and 94 per cent of the nematodes checked were egg-laying females.

Table 2.--Stages of development of nematodes in Marglobe tomato at various periods following inoculation.

Period after inoculation	Replicate	Nematodes in each of stated classes					Total
		A	B	C	D	E	
Days	No.	No.	No.	No.	No.	No.	No.
10	1	40	10	0	0	0	50
	2	46	4	0	0	0	50
17	1	10	27	13	0	0	50
	2	12	29	9	0	0	50
23	1	0	7	11	32	0	50
	2	1	3	13	33	0	50
28	1	0	1	2	6	41	50
	2	0	2	1	13	34	50
31	1	0	1	2	2	45	50
	2	1	0	1	1	47	50

Plants Inoculated in Pots with a Larval Suspension, Experiment 2

The procedure followed in this experiment was essentially the same as described for the preceding experiment. The tomato lines used were Marglobe, 43-1-1, and 63. Approximately 10,000 larvae were used to inoculate each plant. All plants were exposed to infection for 48 hours except for two plants of each variety which were removed for examination 24 hours after inoculation. Table 3 gives the number of nematodes found in each plant at a given period after inoculation.

As in the preceding experiment there were fewer nematodes in resistant plants than there were in susceptible plants 48 hours after inoculation. At 24 hours after inoculation, however, no differences were indicated. It appears that resistant plants acquired about as many nematodes in 24 hours as they did in 48 hours. Marglobe, on the other hand, seems to have acquired at least twice as many nematodes in 48 hours of exposure to infection as it did in 24 hours of exposure.

The numbers in table 3 show that with the exception of two plants of the tomato 43-1-1 there was a definite tendency for nematodes to disappear from the root systems of resistant plants with the passage of time after inoculation. The two plants of 43-1-1 apparently did not have the degree of resistance found in most plants of that line. Not only had the nematodes failed to disappear from the root systems of these two plants but they had been only slightly retarded in their development as compared to nematodes in Marglobe at the same dates.

With the exception of three plants, no resistant plants at any date contained nematodes that were placed beyond Class A. One nematode in one of the line 63 plants removed 11 days after inoculation was placed in Class B. One 43-1-1 plant removed 11 days after inoculation contained a total of

222 nematodes. One hundred of those were checked for their state of development. Of those checked 62 were placed in Class A and 38 in Class B. One of the 43-1-1 plants removed 14 days after inoculation contained 62 nematodes, four of which were placed in Class B and the remainder in Class A.

Table 3.--Numbers of nematodes in entire root systems of tomato plants at various periods after inoculation.

Period after inoculation	Replicate	Nematodes in each of stated tomato lines		
		Marglobe	43-1-1	63
Days	No.	No.	No.	No.
1	1	243	241	244
	2	213	221	319
2	1	751	254	241
	2	601	304	270
7	1	718	51	81
	2	623	217	44
8	1	657	45	73
	2	580	47	60
11	1	594	16	45
	2	702	222	55
14	1	640	62	8
	2	629	6	5

No nematodes in Marglobe tomato had developed as far as Class B until eight days after inoculation. The stage of development of 100 nematodes from each plant from this date on to the end of the experiment are given in table 4. A few nematodes in each Marglobe plant examined 14 days after inoculation had completed all the molts. No nematodes in any resistant plants ever reached the second molt. Most of the nematodes found in resistant plants seven or more days after inoculation had undergone no visible

development and many of them had obviously been dead at the time the plants were removed from the soil.

Table 4.--Stages of development of nematodes in Marglobe tomato at various periods after inoculation.

Period after inoculation	Replicate	Nematodes in each of stated classes					Total
		A	B	C	D	E	
Days	No.	No.	No.	No.	No.	No.	No.
8	1	70	30	0	0	0	100
	2	63	37	0	0	0	100
11	1	12	88	0	0	0	100
	2	26	74	0	0	0	100
14	1	4	90	6	0	0	100
	2	8	88	4	0	0	100

Necrosis of Tissue in Petri Dish Experiments

Attempts to inoculate plants in Petri dishes did not give consistent infection and was not used for studies on the number of nematodes entering plants or their rate of disappearance from the roots after entering. However, nematodes did enter some roots of both resistant and susceptible plants. Root systems of plants from these experiments were small enough to be mounted intact on a microscope slide under a 50 mm. x 23 mm. coverslip. Examination of such mounts disclosed that larvae entering root tips of resistant tomato plants had caused extensive injury to cells by the end of 48 hours after inoculation and had produced no swelling of root tips. Nematodes entering Marglobe root tips had injured a few cells in some cases but the injury was very light by comparison with that produced on resistant tomatoes. This difference is shown by a comparison of figures 2 and 3. It may also be noted that the marglobe root tip had begun to swell.

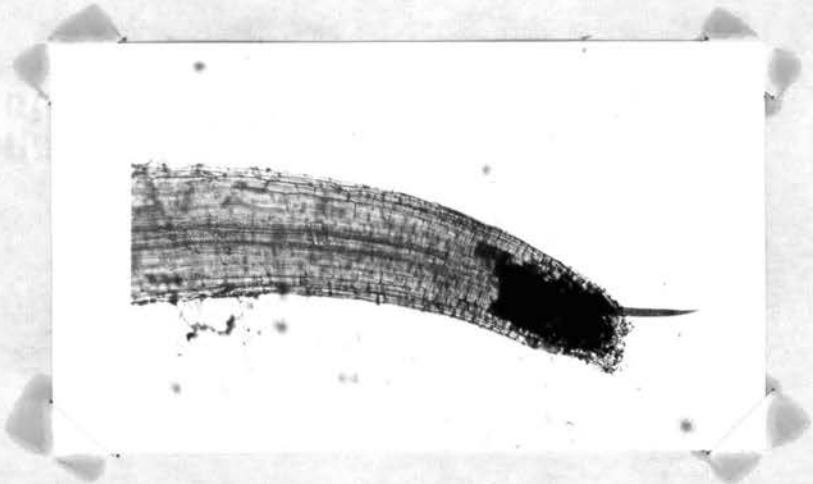

Fig. 2. Invaded root tip of tomato 43-1-1 two days after inoculation. The dark area surrounding the anterior portion of the nematode is necrotic tissue. (X78)

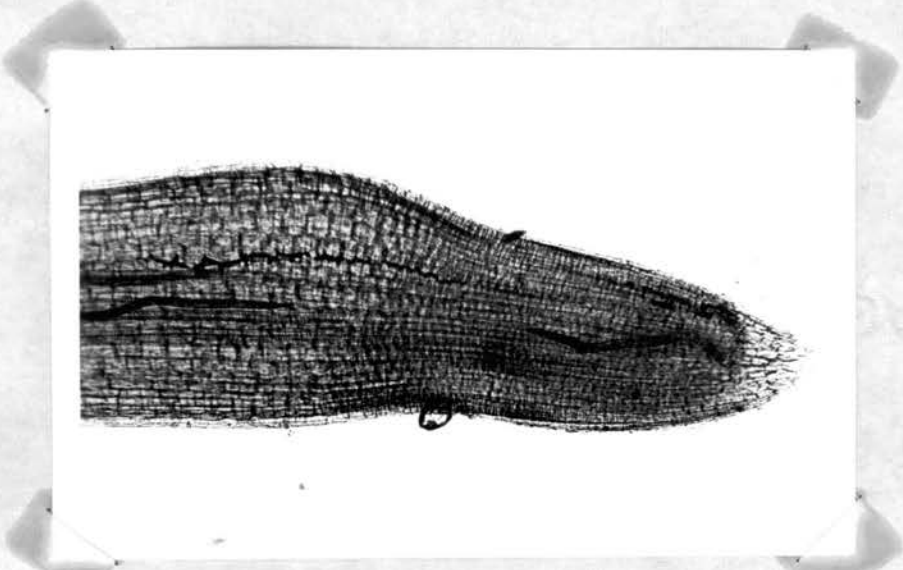

Fig. 3. Invaded root tip of Marglobe tomato two days after inoculation. Note small amount of cell injury and swelling back of root tip. (X78)

Tissue injury on resistant plants was easily discernible as a dark area in fresh, unstained root tips examined with the dissecting microscope at a magnification of 60 diameters. For this reason it was possible to pick out invaded root tips to be killed and fixed for cytological studies. Stained microtome sections of such root tips are shown in figures 4 and 5. The dark areas are due for the most part to collapsed cells which were very heavily stained by safranin-O. It seemed also that there may have been some sort of extracellular deposit on the cell walls of some cells which had been in contact with the body of a nematode. It could not be definitely decided, however, whether this was the case or whether all the heavily stained material consisted of collapsed host tissue.

Necrosis of Tissue in Pot Experiments

Nematode invaded root tips on resistant tomatoes from the pot experiments exhibited the same type of injury 48 hours after inoculation as that described for the Petri dish experiments. A comparison of root tips 48 hours and six or seven days after inoculation showed that in a few cases nematodes had entered root tips, killed the growing point and had subsequently died amid collapsed meristematic cells. This condition perhaps approaches that described by Liao and Dunlap (15) in which nematodes were said to have died before they had penetrated more than a few cell layers. In most cases, however, nematodes entered root tips, caused extensive damage to the immature tissues near the root tip, then migrated into mature tissue. Forty-eight hours after inoculation many nematodes were found that had passed into mature tissues and at this time cell damage surrounding the nematodes was very slight. A week or more after inoculation most nematodes were in mature root tissue and many were surrounded by dead cells. Many of the nematodes

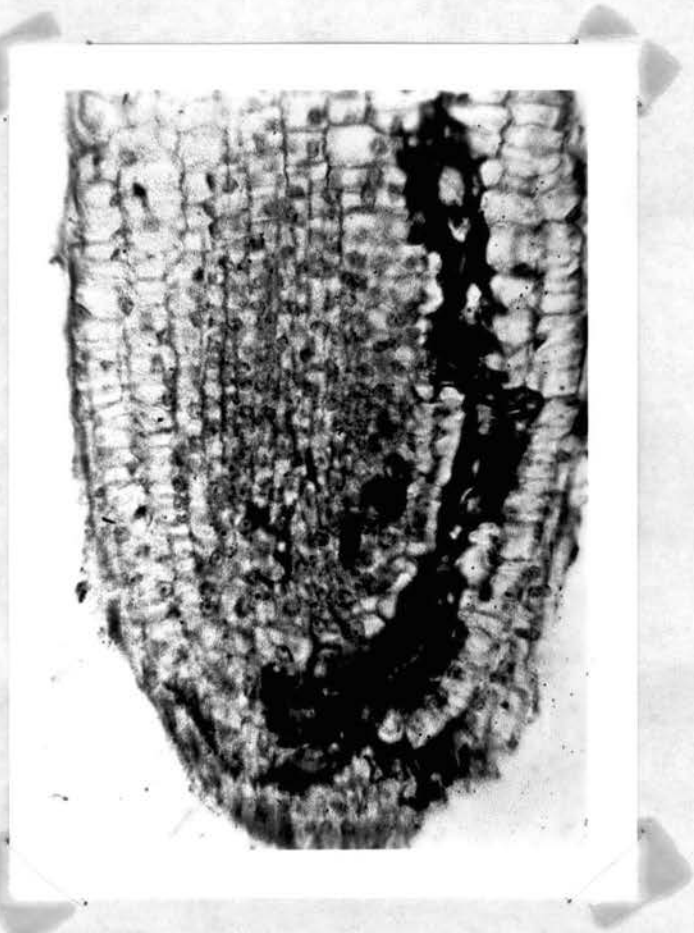

Fig. 4. Longitudinal section through invaded root tip of tomato 43-1-1 two days after inoculation. Note collapsed cells along path taken by a larva. (X325)

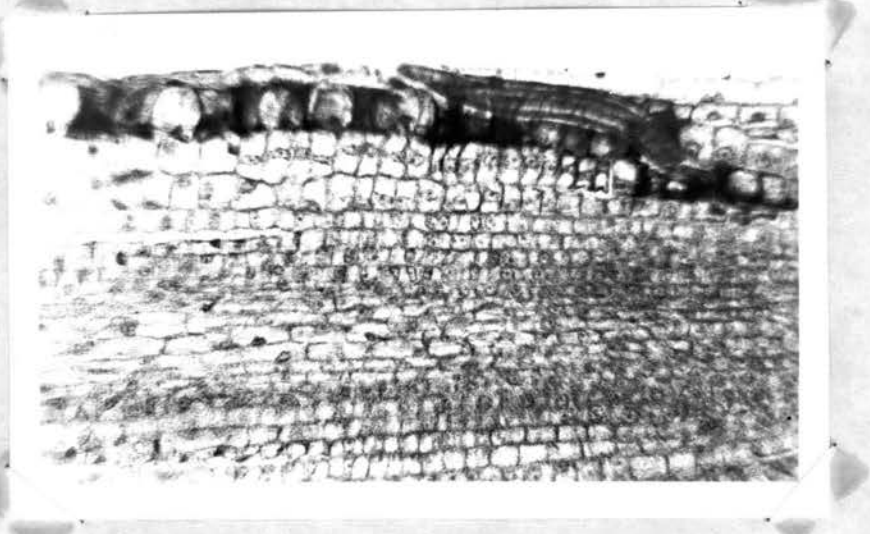

Fig. 5. Longitudinal section through root tip of 43-1-1 tomato showing nematode entering. The head of the nematode is toward the right; the tail was cut off in sectioning. The collapsed cells to the left of the nematode presumably were injured by the nematode before it entered the root. (X325)

found in this situation were dead and none showed any signs of development. The necrotic host cells were shrunken and very heavily stained with acid fuchsin. In many cases a necrotic area in a root was dissected out and mounted on a slide for study under high power of the microscope. Some nematodes imbedded in this disorganized tissue were visible along only a part of their length, the remainder of the worm being indistinguishable from the collapsed host cells. It seems clear that nematodes often die in roots of resistant tomatoes, disintegrate and disappear in necrotic host tissue. Some roots examined several days after inoculation were seen to have been invaded by a sufficient number of larvae to cause a general necrosis of the entire invaded area (Fig. 6). The ends of many roots had completely disappeared leaving a necrotic terminus in which one or two nematodes occasionally were still present. It is believed that in these cases heavy invasion by nematodes had caused the death of the roots which subsequently decayed and disappeared. Many nematodes undoubtedly are eliminated from the root systems of resistant plants in this manner. No evidence of fungus could be found in these roots and the condition was not present in the roots of Marglobe tomatoes.

The plants in these experiments were exposed to infection for only 48 hours. It seems likely that plants growing in heavily infested soil for a long period might suffer the loss of many more roots. When plants were grown in the infested soil in the greenhouse bench, it was observed that root systems of resistant tomatoes were more extensively decayed than those of susceptible plants. At that time it was presumed that the nematode resistant plants were more susceptible to Rhizoctonia root rot than were Marglobe tomatoes. It now seems probable that resistant plants were sustaining a considerable amount of root injury due to nematodes in addition

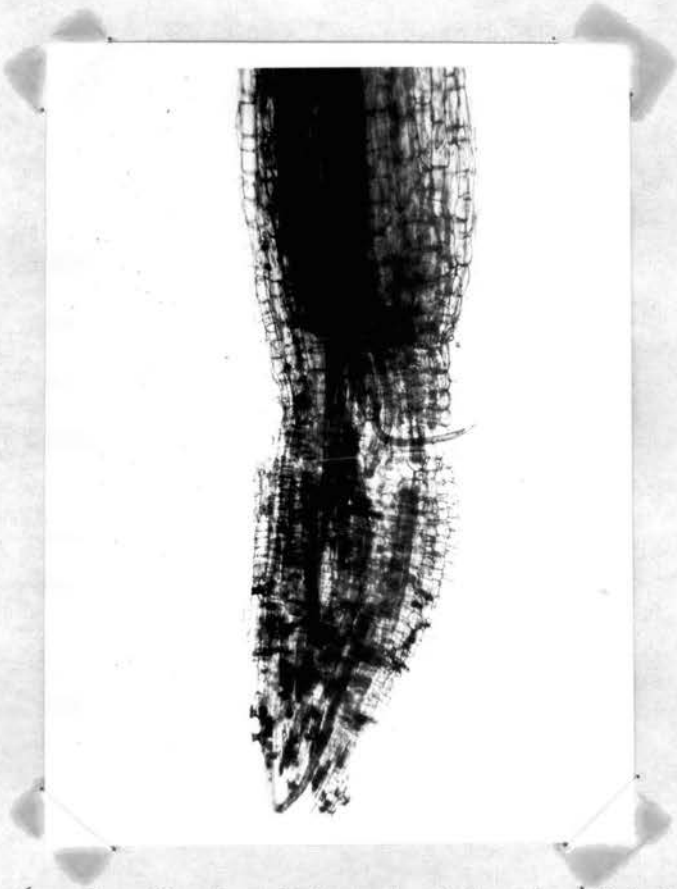

Fig. 6. Heavily invaded root of tomato 43-1-1 seven days after inoculation. All the tissue below the nematode at center was dead and disintegrating. (X78)

to the fungus decay.

Sweet Potatoes

Plants Inoculated in Greenhouse Bench

Cuttings of Allgold, Orlis, Oklahoma 29, and Oklahoma 46 sweet potatoes were placed in water and left until roots were just beginning to emerge. The plants were then set into the infested soil in the greenhouse bench in a Latin square design. Each plot contained two plants, making a total of eight plants of each sweet potato line. The plants were allowed to grow in the bench for five days after which they were removed and their roots sampled. Four roots were selected at random from each plant except that no roots less than 0.5 cm. long were used. The roots ranged from 0.5 to 10.4 cm. in length and all were unbranched. When the roots were stained and examined, the length of each root and the number of nematodes it contained were recorded. Average figures for these data are presented in table 5.

Table 5.--Nematodes in sweet potato roots exposed to infection five days.[a]

Sweet potato line	Root length	Nematodes	
		Per root	Per cm. of root
	cm.	No.	No.
Allgold	3.10	2.44	0.75
Orlis	3.21	2.19	0.68
Okla. 29	4.52	17.72	3.52
Okla. 46	4.72	5.41	1.14

[a]Each number in the table is an average for 32 roots.

Oklahoma 29 and Oklahoma 46, two resistant lines, were high in the number of nematodes they acquired while Allgold, a very susceptible line,

and Orlis, a very resistant line, were low. Thus it is seen that no relationship between resistance or susceptibility and the number of nematodes originally entering a root is indicated. The differences obtained appear to be related to root length rather than to resistance and susceptibility. As shown in table 5 the potato lines that averaged high for root length also averaged high for number of nematodes. Plants with longer roots not only acquired more parasites per root but more per unit length of root. To explain this it seems necessary to assume that the longer roots were longer not because they had grown faster, but because they were older and therefore had been exposed to nematodes for a greater period of time.

The very high number of nematodes recorded for Oklahoma 29 was due to the fact that the two plants in one particular plot were very heavily invaded. The number of nematodes recorded from this plot was more than eight times the average for the other three plots of Oklahoma 29. If the average figure for the other three plots is substituted for the very high figure obtained in the one plot, Oklahoma 29 is very similar to Oklahoma 46 with respect to the number of nematodes acquired. An analysis of variance indicated no significant differences between the means of different varieties with regard to the number of nematodes per plant. For purposes of this analysis the length of roots was disregarded.

The variation in this experiment was very large and small differences in the numbers of larvae entering different varieties could well have been missed. Since, however, the differences that were suggested were not related to resistance and susceptibility, no attempt was made to refine and repeat the experiment.

Plants Inoculated in Pots with a Larval Suspension, Experiment 1

In this experiment plants were inoculated with a larval suspension in pots, left exposed to infection for 48 hours, and transplanted to sterilized soil. The number of larvae applied per plant was not determined. The first plants were removed and stained for examination 16 days after inoculation. Plants were removed also at 22 days and 43 days after inoculation. Only one plant of each variety was removed at a given date. Because one Allgold plant died after inoculation, it was necessary to substitute another plant for examination at two different dates. The plant was removed from the pot for the 22-day examination and only a part of its root system was taken. Then the plant was re-potted and the remainder of the root system used for the 43-day examination. All the nematodes in each plant were counted and all nematodes were classified according to their state of development. This data is shown in table 6.

As in the tomato experiments the nematodes tended to disappear from the root systems of resistant plants with the passage of time after inoculation. Those nematodes that persisted were retarded in their development. Sixteen days after inoculation almost half of the nematodes in Allgold had completed the series of molts that occur in plant tissues. Two nematodes in Oklahoma 29, one in Orlis and none in Oklahoma 46 had reached this state of development. Only two egg-laying females were found in Orlis 43 days after inoculation. Both of these nematodes were considerably undersize and had laid not more than 25 or 30 eggs.

An inspection of table 6 suggests that a second generation of nematodes was encountered in Allgold 43 days after inoculation. The relatively high number of nematodes in Classes A and B and the very few in Class C with an increase again in Class D would seem to indicate that the most advanced of the second generation had not developed beyond Class C. The number of

Table 6.--Stages of development of nematodes in sweet potatoes at various periods after inoculation.

Period after inoculation	Sweet potato line	Nematodes in each of stated classes					Total
		A	B	C	D	E	
Days		No.	No.	No.	No.	No.	No.
16	Allgold	55	147	166	0	0	368
	Okla. 29	136	42	2	0	0	180
	Okla. 46	127	7	0	0	0	134
	Orlis	164	6	1	0	0	171
22	Allgold[a]	13	40	25	35	0	113
	Okla. 29	54	39	15	4	0	111
	Okla. 46	45	19	14	0	0	78
	Orlis	36	19	6	0	0	61
43	Allgold[a]	15	25	2	42	63	147
	Okla. 29	1	13	3	8	33	58
	Okla. 46	1	3	0	6	16	26
	Orlis	1	1	3	1	2	8

[a]The root system of this plant was divided at the examination made 22 days after inoculation. Part of the root system was removed from the plant and examined at that time, the plant was then returned to a pot and the remainder of the root system examined 43 days after inoculation.

second generation larvae seemed much lower than would be expected since the color of the egg masses on the plant indicated that they were quite mature. A possible explanation for this would be the large number of presumably predatory mites that were found in collapsed galls on this plant. These mites were not found on any other plant in the experiment.

Plants Inoculated in Pots with a Larval Suspension, Experiment 2

The same procedure used in the preceding experiment was followed here except that around 10,000 larvae were applied to each plant and the plants were exposed to infection for only 24 hours. The results of the experiment are given in table 7.

The results of the examination made immediately after inoculation would seem to indicate no appreciable differences between varieties with regard to the number of larvae that had entered the plants. The highest number was recorded for Oklahoma 46 and the lowest for Orlis. Both of these plants are very resistant.

Twenty-one days following inoculation the number of nematodes in resistant varieties seems to have been considerably reduced. Those present were retarded in their development. At this date no nematodes in resistant plants had progressed beyond the second larval stage whereas in Allgold almost 79% of the nematodes had completed all the molts.

Table 7.--Stages of development of nematodes in sweet potatoes at various periods after inoculation.

Period after inoculation	Sweet potato line	Nematodes in each of stated classes					Total
		A	B	C	D	E	
Days		No.	No.	No.	No.	No.	No.
1	Allgold	211	0	0	0	0	211
	Okla. 29	151	0	0	0	0	151
	Okla. 46	224	0	0	0	0	224
	Orlis	148	0	0	0	0	148
21	Allgold	2	53	217	22	0	294
	Okla. 29	67	44	0	0	0	111
	Okla. 46	60	21	0	0	0	81
	Orlis	11	0	0	0	0	11
31	Allgold	3	32	16	25	168	244
	Okla. 29	19	31	16	17	2	85
	Okla. 46	9	26	6	8	1	50
	Orlis	1	6	0	0	0	7

Thirty-one days after inoculation no nematodes in Orlis had reached the molting stage or if they had, they had subsequently died and were no

longer discernible. Only seven nematodes were found in this plant. One nematode in Oklahoma 46 and two in Oklahoma 29 had laid eggs. All three of these egg masses were very small compared to those on Allgold and the mature females were badly stunted. In this particular experiment, however, the egg masses on Allgold were smaller than usual for that variety.

Apparently no second generation nematodes were encountered in this experiment.

Necrosis in Sweet Potatoes

Examinations of root systems of resistant sweet potato plants 24 or 48 hours after inoculation revealed that nematodes entering root tips occasionally produced necrosis of meristematic cells similar to that described for resistant tomatoes. Tissue necrosis at this short interval after inoculation, however, was rare in sweet potatoes. Nematodes in resistant sweet potato roots did produce a very considerable amount of necrosis but this occurred later and usually involved mature tissues of the cortex. No sweet potato roots were examined between 48 hours and 16 days after inoculation.

Sixteen days after inoculation some nematodes in resistant roots were located with their heads in the vascular cylinder where giant cells had formed. The nematodes in this situation usually showed some signs of development although their development was retarded compared to nematodes in Allgold roots. Many other nematodes were located in cortical tissues where they had undergone no visible development. These parasites were usually surrounded by dead cells and in any case no giant cells had formed. The condition of many of the nematodes found in this area of the root left no doubt that they were dead at the time the roots were stained. Dead

nematodes were shrunken and distorted often having one or more prominent swellings and constrictions. Rarely could nematodes in this condition be dissected completely free of root tissue. Their bodies seemed to be cemented in some way to the surrounding plant cells and they usually disintegrated when touched with the dissecting needle. It was possible to dissect such nematodes out of a root if the surrounding cells were left more or less intact. A small piece of cortical tissue with the included nematode could be mounted on a microscope slide for closer observation (Fig. 7).

Not all nematodes surrounded by dead host cells were unquestionably dead themselves but none of them had undergone any development since entering the plant. Often a trail of dead cells were left behind a nematode migrating through cortical tissues and the nematode was to be found at the end of such a trail with more extensive cell necrosis around its head. Necrosis might include four or five cells in any direction away from the head of the animal. It was impossible to know in these cases whether the animal had punctured all the injured cells with the stylet or whether a diffusible substance from the body or salivary secretion of the nematode had affected these cells. Fortunately, in a few cases living nematodes in fresh roots of resistant tomatoes were observed under the microscope. These nematodes were observed to advance through the root tissues as far as the length of their bodies. They might then move backward to the original position and again advance in the same or a different direction. It was apparent that these larvae were not opening new passages through the tissue but were moving through channels which they had previously opened. If this type of behavior can be assumed for nematodes in sweet potatoes, injured cells at some distance from a nematode's head at the time of examination might easily have been penetrated by the stylet.

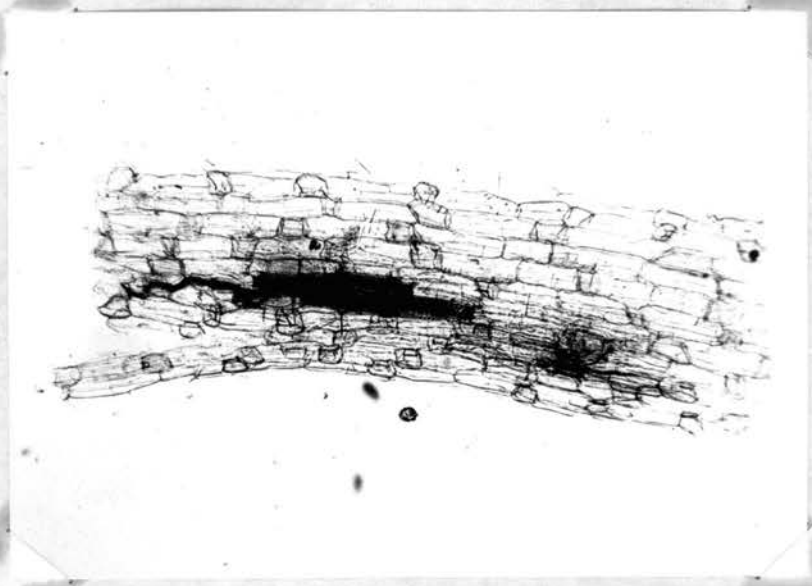

Fig. 7. Section of cortex and epidermis of Orlis sweet potato root containing a larva surrounded by dead cells. The body of the nematode extends out of the lesion toward the left. The head of the parasite is imbedded in the lesion. (X78)

Dead host cells were heavily stained with acid fuchsin and contained dark, granular cytoplasm. Nematodes amid these cells were found in all stages of disintegration. As in the resistant tomatoes, nematodes could be found which were recognizable along only a part of their length. When counts were made, under the microscope, only nematodes that could be definitely distinguished were included.

With respect to necrosis of host tissue and death of nematodes, Orlis and Oklahoma 46 presented about the same picture. In Oklahoma 29 there were more nematodes which had not produced the necrotic reaction in host tissue and more nematodes developed to maturity.

Abundant galls were formed on roots of Allgold and to a much lesser degree on Oklahoma 29. In these experiments swelling was almost absent from roots of Oklahoma 46 and Orlis.

The description given for plants at 16 days after inoculation applies about as well for 21 and 22 days after inoculation. At 31 and 43 days few nematodes were found surrounded by necrotic tissue. Most of the few nematodes remaining in resistant plants at these dates were located with their heads in the stelar region and usually had undergone at least some development.

DISCUSSION

Barrons (2) could find no evidence that resistant and susceptible plants differed with regard to the number of nematodes that entered their roots. Christie (7), on the other hand, presented data from which he concluded that some plants are invaded by only a very few nematodes. In Barrons' work the plants were continuously exposed to infection in infested soil until the time the roots were examined. Barrons counted the nematodes in the terminal centimeter of each sampled root. This method would be expected to reveal only those nematodes which had entered the root during the period of growth of the terminal centimeter. Therefore the nematodes observed by Barrons had been present in the roots for only a relatively short period and evidently no reduction in number had occurred in resistant roots due to death of nematodes. In Christie's work plants were exposed to infection for a period of only 24 hours then examined three to eight weeks after inoculation. In the light of the present research it seems possible that the reduced number of nematodes found by Christie in resistant plants was due not to a failure of the larvae to enter but to their death and disappearance after entering. This would seem particularly to apply to Christie's sweet potato experiments which have already been described. It may be remembered that Christie found few parasites in Orange Little Stem three weeks or six weeks after inoculation. In the present work, few nematodes were found in resistant sweet potatoes at about these same periods after inoculation but little difference could be noted between resistant and susceptible sweet potatoes one day after inoculation. One of the resistant sweet potatoes used in this work was Orlis which, like Orange Little Stem, originated as a mutation from Yellow Jersey. It seems probable that Orange

Little Sten and Orlis have the same genetic basis for resistance.

Fewer nematodes were found in resistant than in susceptible tomatoes 48 hours after inoculation provided that all plants had been exposed to infection throughout the 48 hour period. Twenty-four hours after inoculation, however, there were as many nematodes in susceptible as in resistant tomatoes. It seems that this situation could develop in either of two ways. Nematodes could have stopped entering resistant roots after 24 hours but could have continued entering susceptible roots throughout a 48 hour period or nematodes could have been both entering and leaving resistant roots in the second 24 hour period after inoculation. At present there seems to be no basis upon which to decide this point.

Because Barrons (2) observed that nematodes entered the roots of resistant plants but failed to produce any appreciable galling, he believed that the nematodes died after entering. He proposed the hypothesis that resistant plants synthesize a substance which neutralizes the giant cell inducing agent in the salivary secretion of the nematodes thus preventing the nematodes from obtaining proper nutrition. To explain occasional small galls on resistant plants, Barrons supposed that if a large number of larvae entered a root at about the same place, the plant might be unable to synthesize enough of the neutralizing substance in that area to prevent giant cell formation. The resulting gall would remain small, however, due to a continuing production of the neutralizing substance by the plant.

This hypothesis does not seem to explain the results obtained in the present work. In either resistant tomatoes or sweet potatoes, those few nematodes which were able to develop at all were often located in an area of the root in which other nematodes were not present. Actually when large numbers of larvae invaded a small area of the root, the result was a

generalized necrosis throughout the area.

Necrosis of root tissue as a result of invasion by root-knot nematodes has been mentioned in the literature. Frazier and Dennet (12) stated that some necrosis was observed around nematodes in tomatoes with Lycopersicon peruvianum parentage. The extent of this necrosis or its possible significance in relation to resistance was not discussed. Christie (9) states that if a plant is slightly to moderately resistant, entering larvae will often produce a small necrotic area at the region of invasion. The significance which Christie attributes to this observation is indicated by the following quotation.

> That larvae fail to develop in resistant plants because they kill the tissues on which they depend for food is an attractive theory but one that, on close scrutiny, does not appear to be adequate. It seems probable, however, that this reaction of the tissues is one of the factors that make some plants unsuitable hosts and that sometimes it may be largely responsible for the death of larvae.

It appears that a necrotic reaction of host tissue in response to the presence of nematodes should be considered of fundamental importance to the defense mechanism of the resistant plants used in the present investigation. It may be noted that in the sweet potato experiments nematodes with their heads imbedded in the central cylinder usually had failed to cause death of surrounding cells but had instead induced giant cell formation. These parasites usually were undergoing a retarded development whereas nematodes in the cortex had not produced giant cells and had failed completely to develop. A root-knot nematode in final feeding position in a root usually lies with the head in the vascular cylinder, but Linford (19) has demonstrated that migrating larvae feed in the cortex before assuming the final position. Many of the nematodes in resistant sweet potatoes died in the cortex before becoming established with their heads

in the central cylinder and in most cases these nematodes were surrounded by dead cells.

The necrotic reaction is as common in resistant tomatoes as in resistant sweet potatoes but there are differences between the two types of plants with regard to the time of development of the reaction and its location in the root. In the tomatoes there was extensive killing in the root tips within at least 48 hours after inoculation. At this time the reaction was confined largely to meristematic cells or immature cells immediately behind the apical meristem. Seven or more days after inoculation, nematodes in mature tissues were surrounded by necrotic tissue in most cases.

In sweet potatoes necrosis of meristematic cells soon after invasion was rarely observed. Sixteen days after inoculation many nematodes in cortical tissues had killed the surrounding cells. This injury was somewhat less generalized than in tomatoes. The dead cells were confined more directly to the area immediately surrounding a parasite.

At present it cannot be guessed how many nematode resistant plants respond to the presence of the parasite by death of cells. There is a possibility that this reaction is much more widespread than has been hitherto suspected. It is suggested that the reaction may have been missed in many plants because of a time factor. In the great majority of cases in which plants have been examined for nematode resistance, the plants were exposed to continuous infection until the time the plants were to be examined. Then all plants were removed and examined at about the same date. In some cases this may have been too early for the necrotic reaction to develop. If a longer period of exposure was used, nematodes in all stages of development were present. Some may not have been present long enough for the necrotic reaction to develop while others may have long since died and

disappeared. In the latter case, necrotic areas in the root may have been present but it would not have been evident that they were due to nematodes. In the present work if no sweet potato plants had been examined between 48 hours and 30 days after inoculation, it is not likely that the samll amount of tissue damage observed at these times would have attracted attention.

In addition to the matter of timing, fungus decay of root systems may have been a factor in obscuring observation of nematode incited necrosis in resistant plants. Several workers have reported difficulty in this respect. Bailey (1) and Frazier and Dennet (12) used a solution of copper oxide on soil in an attempt to reduce fungus decay of tomato roots. Frazier and Dennet reported that decay of root systems of resistant tomatoes (Lycopersicon peruvianum hybrids) was common. Possibly a part of this decay was produced by the nematodes themselves but it was impossible to separate the effects of the fungi from those of the nematodes.

A survey of the literature has shown how the concept of resistance to root-knot nematodes has changed from the early view that nematodes failed to enter resistant roots to the view that nematodes do enter, in most cases at least, but fail to survive after entering. Perhaps it may now be added that, at least in some cases, the failure of the parasites to survive is due to the fact that they kill the cells upon which they are dependent for food.

The point has been made that resistance in plants to root-knot nematodes must be evaluated in terms of host-parasite relations before such plants may be utilized most effectively. The present research has necessarily been of an exploratory nature, but the results indicate that plants with the type of resistance found in tomato lines 63, 64, and 43-1-1 and sweet potato lines Oklahoma 46 and Orlis, could be expected to grow in nematode infested

soil, to escape serious injury and, indirectly, to depress the soil population of nematodes thus affording some protection to a future crop.

SUMMARY

Nematode resistant tomatoes, lines 63, 64, and 43-1-1, have been compared with a susceptible tomato, variety Marglobe, with respect to host-parasite relations involving the root-knot nematode Heloidogyne incognita. Small plants growing in pots were inoculated with standardized larval suspensions. All larvae which had not entered the root systems in 48 hours were removed by thorough washing; the plants were then repotted in sterilized soil. After various time intervals, root systems were removed, washed, stained, and observed for the number and state of development of the nematodes. Entire root systems were used for determining the number of nematodes present. Wherever available 50 or one hundred nematodes per plant were checked for their state of development.

By the end of 48 hours after inoculation there were at least twice as many nematodes in the root systems of susceptible plants as there were in resistant plants. Twenty-four hours after inoculation, however, there were no appreciable differences.

The nematodes entering roots of resistant plants in the original infection period failed to develop as far as the second molt. About two weeks following inoculation most of the nematodes had died and disappeared from resistant roots. Nematodes in resistant roots were usually surrounded by necrotic host tissue. The number of nematodes in susceptible plants did not change with the passage of time after inoculation, at least up to a period of about a month, and in this period the nematodes developed to egg-laying maturity. Little or no cell injury was observed in susceptible plant material.

Resistant and susceptible sweet potatoes were studied using the same

methods as had been used with tomatoes. The variety, Allgold was used as a susceptible standard; Oklahoma 29, Oklahoma 46, and Orlis were the resistant lines used.

With respect to the number of nematodes entering sweet potato roots in the original period of infection, no appreciable differences were noted. As in the tomatoes, however, nematodes tended to disappear from resistant roots with the passage of time after inoculation.

Development of nematodes in resistant roots was sharply retarded but not so much as in resistant tomatoes. A few nematodes in all sweet potato lines reached egg-laying maturity. The few egg masses produced on resistant roots were small and the mature females were below normal size.

As in the tomatoes, nematodes produced necrosis of host tissue in resistant roots. This reaction, however, appeared somewhat later in sweet potatoes than it did in tomatoes.

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THESIS TITLE: RESISTANCE AND SUSCEPTIBILITY TO ROOT-KNOT NEMATODES IN TOMATO AND SWEET POTATO

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