SELECTION FROM A FIELD POPULATION FOR VARIABILITY IN MELOIDOGYNE INCOGNITA ON SWEET POTATO

By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

INTRODUCTION

One of the primary objectives of the sweet potato breeding program at the Oklahoma Agricultural Experiment Station is the production of sweet potato varieties resistant to the root-knot nematode, Meloidogyne incognita (Kofoid and White) Chitwood. Root knot is recognized as a major disease of sweet potato in Oklahoma and throughout the southern United States. Knowledge concerning the nature of resistance, host-parasite relations, and the limits of variability in the pathogen are considered basic to an intelligent approach in such a breeding program.

Several different types of host-parasite reactions associated with root-knot resistance in sweet potato have been demonstrated in previous work at the Oklahoma Agricultural Experiment Station (4, 5, 13). These are: no galling to trace amounts on the host, moderate to severe root tip necrosis, generally failure of nematodes to reach mature stages, little or no reproduction by the nematode, and, in several instances, reduced numbers of eggs where reproduction does occur.

Dooley (5), working with populations of M. incognita from several locations within Oklahoma, obtained evidence of physiologic specialization in the nematode as expressed on different sweet potato lines. Evidence for physiologic specialization in this nematode species, with hosts other than sweet potato being used as differentials, has been presented by several investigators (7, 9, 10, 11, 15, 17). Dooley did not explore the possibility of selecting, within a

given isolate, physiologic races. Knowledge of such a possibility is important not only in determining the limits of variability in this nematode but also in relation to the likelihood of differential selection of more virulent races through repeated testing of resistant sweet potato lines in a given field. Riggs and Winstead (14) have demonstrated that by repeated growing of certain root-knot resistant tomato lines with a given nematode population, it was possible to obtain races of the nematode to which the tomato lines were susceptible.

The present study was initiated primarily to determine the possibility of selecting from a field population of M. incognita, by means of resistant sweet potato lines, races which might more readily attack resistant sweet potatoes. Evidence relative to such a phenomenon was obtained from studies of gross pathology, details of host-parasite interactions, and nematode morphology.

REVIEW OF LITERATURE

Since the transfer of root-knot nematodes from the genus Heterodera to the genus Meloidogyne and the species concept proposed by Chitwood (2), several investigations have been made relative to morphological and physiological variation in Meloidogyne spp. Chitwood himself noted that these species showed considerable morphological variation; however, he felt that similarities within species were sufficiently consistent to provide a sound basis for species differentiation. Allen (1) found considerable variation in perineal patterns of M. incognita acrita Chitwood females originating from single-egg-mass populations. He also found evidence of variation with respect to host plant reaction in this nematode. Dropkin (6) found less variation in perineal patterns of single larval families of M. incognita than in so-called wild populations. This variation was considered insufficient to invalidate the usefulness of perineal patterns in identification. Taylor, et al. (17) observed that females of each species and subspecies of the genus Meloidogyne have perineal patterns which vary around a typical pattern distinct from that of each other species or subspecies. Variation in perineal patterns and larval lengths were noted by Riggs and Winstead (14) in populations of M. incognita incognita, M. incognita acrita, and M. arenaria arenaria (Neal) Chitwood selected by repeated culturing on a resistant tomato line (Hawaii 5229). Perineal pattern variation was not considered sufficient to exclude any of the new strains from the parent type. Larvae of each of the new strains were, however, significantly shorter than those of each of the parent populations. Each of the

selected nematode populations was considered a new physiologic race on the basis of its ability to attack the tomato line (Hawaii 5229) which was resistant to the parent populations.

In an attempt to resolve the confusion resulting from observed variation in the perineal patterns of M. incognita incognita and M. incognita acrita, Triantaphyllou and Sasser (18) investigated the extent of variability in the morphology of the perineal patterns and the host specificity of these 2 nematodes. Since the 2 subspecies were not always readily separable on the basis of morphology or physiological behavior, they recommended that the 2 be considered as one species to be designated as M. incognita.

Ample evidence of physiologic specialiaztion within species of Meloidogyne is available from a variety of investigations in addition to those already mentioned. Physiologic races of M. incognita acrita have been reported by Sasser and Nusbaum (16) with tobacco and cotton as hosts, by Martin (11) with cotton as host, by Dropkin (6) using soybean varieties, by Lider (10) working with grapes, and by Dooley (5) with sweet potato as the host. Goplen, et al. (9) were able to select 3 races of M. incognita acrita, 2 of M. javanica Chitwood and 2 of M. hapla Chitwood through the use of 5 different alfalfa varieties.

MATERIALS AND METHODS

In the present work the recommendation of Triantaphyllou and Sasser (18) that Meloidogyne incognita acrita be consolidated with and considered as M. incognita is followed. Further comment on this point is presented later as part of the section on results.

Twenty-two single egg masses of M. incognita were selected at random from 2 root systems of the resistant sweet potato variety Nemagold which had been grown in soil infested with this nematode at the Perkins Farm of the Oklahoma Agricultural Experiment Station. Each of these egg masses was transferred for increase to a Rutgers tomato seedling which was then transplanted to steam-sterilized, sandy loam soil in a 6-inch pot. Each of these pots was placed on the greenhouse bench on an inverted 6-inch pot with adequate space between pots to reduce the possibility of contamination from pot to pot. Throughout this investigation precautions were taken at all times to prevent chance mixtures of nematode cultures. After 6 months, because of space limitations, 4 of the 22 single-egg-mass isolates were chosen at random for further work. Each of these 4 isolates, designated respectively as B, C, D, and E, originated from the same root system of a Nemagold plant. An isolate, designated as A, and considered representative of the field population at the Perkins Farm, was used to compare with the 4 isolates from Nemagold. Isolate A originated as a single-egg-mass culture and has been maintained in the greenhouse since 1950. All nematode isolates were increased and maintained on

Rutgers tomato. These 5 isolates (A, B, C, D, and E) were tested against 4 root-knot resistant sweet potato lines, namely, Nemagold, Heartogold, Tinian, and NC-172. The variety Allgold was used as a susceptible control.

All experiments, unless otherwise indicated, were repeated 4 times. Rooted sweet potato cuttings, 2 per 4-inch pot, were planted in steamed, sandy loam soil to which had been added, just prior to planting, 2 g per 4-inch pot of nematode inoculum in the form of chopped, galled tomato roots. There were 3 pots per treatment, thus making 6 plants of each sweet potato line in each test against each nematode isolate. Plants were exposed to nematode inoculum for 30-33 days at greenhouse temperatures ranging from 72.9° to 76.6° F at night and 74.5° to 86.8° F during the day.

After 30-33 days, each root system was carefully removed from the soil, washed, and rated for galling and necrosis. A rating of 1 was used for no galling, 2 for a trace, 3 for moderate, 4 for severe, and 5 for very severe. From these data a gall index was calculated for each sweet potato line against each nematode isolate. For necrosis a rating of 1 was used to denote whole-root necrosis associated with severe galling, a rating of 2 for trace to moderate root tip necrosis, and 3 for severe root tip necrosis.

Root systems of the 2 plants in each pot were chopped into pieces approximately 5 mm in length; the pieces were thoroughly mixed and a 500 mg sample was taken for staining and microscopic examination. Root pieces were stained in lactophenol plus acid fuchsin according to the method of McBeth, et al. (12) with the modification that 1 ml instead of 5 ml of acid fuchsin stock solution was used per 100 ml of lactophenol.

Cleared, stained roots were crushed between microscope slides and examined under a dissecting microscope to determine the number and relative development of nematodes present. Developmental stages of the nematodes were classified according to the method of Christie (3) as illustrated in Fig. 1. Group A includes second stage larvae to the stage where larvae still possess a more or less conical tail. Group B includes larvae from the stage where they have acquired a more or less hemispherical posterior end terminated by a spike to the stage where they are about to complete the final molts. Group C includes females from the stage where they have completed the molts to the stage where they are almost fully grown. Group D includes females that are fully grown but have not laid eggs. Group E includes egg-laying females.

To determine the relative size of giant cells induced in sweet potato root by each of the 5 nematode isolates, measurements of giant cell diameters were made with an ocular micrometer on fixed and stained root cross sections. Galled roots of only one variety, Allgold, were used in this study. Representative root pieces infected with each of the nematode cultures were killed and fixed in Craf III reagent. After dehydration in the Dioxan series and embedding in paraffin, microtome sections 14 μ in thickness were stained with safranin and fast green (15). For each nematode isolate, measurements were made on 100 random giant cells.

These same sections were also used to determine the number of giant cells produced per mature female nematode. Counts were made from 30 mature females for each isolate.

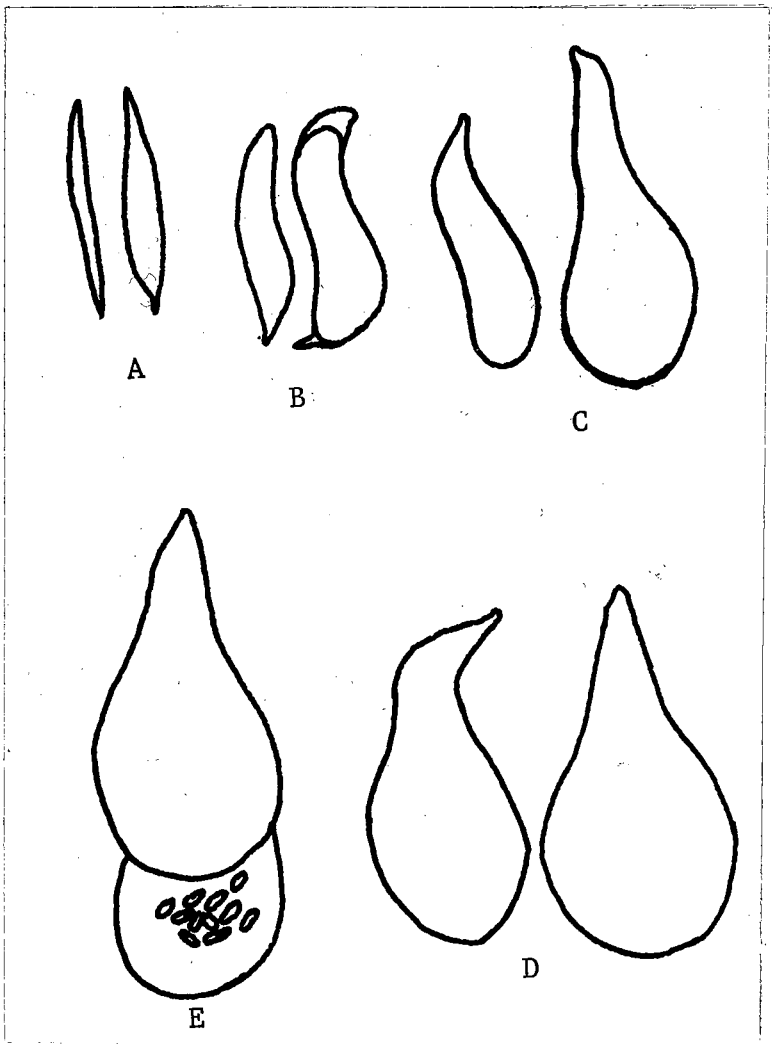


Fig. 1. Classes into which nematodes were grouped according to the amount of development they had undergone. (After Christie, 1946)

RESULTS

Host-Parasite Reactions

In most respects certain of the nematode isolates reacted differently on the susceptible sweet potato variety than did other isolates. Data on reactions of the sweet potato lines and nematode development and reproduction are presented in Table I. These data are means from 4 tests; variation between tests was not statistically significant. It will be noted that with isolates A, B, and C on Allgold moderate to severe galling resulted; with isolates D and E there was very severe galling. Representative effects with these isolates on Allgold are shown in Fig. 2. There were on Allgold more immature and more mature nematodes with isolates D and E than with isolates A, B, or C. In addition more egg masses were produced on this variety by isolate D or E than by isolates A, B, or C. Statistically, nematode isolates A, B, and C were shown to be different from isolates D and E with respect to the number of immature nematodes, the number of mature nematodes, and the number of egg masses produced on Allgold. Isolates A, B, or C were not different one from the other nor were isolates D or E different from one another.

Each of the resistant sweet potato lines reacted to each of the nematode isolates in a fashion typical of resistant lines as defined in previous investigations (4, 5, 13). Galling on these lines ranged from none to trace amounts. Dead root tips occurred on infected plants in trace to severe amounts. Also the relative number of mature nematodes and the number of egg masses were low on

these lines. Isolates D and E differed from A, B, or C on Nemagold in that more nematodes matured and more egg masses were produced.

Further evidence that isolates A, B, and C caused a different reaction on Allgold than did isolates D or E was obtained from studies of giant cells induced by the nematodes. These data are presented in Tables II and III. The differences in giant cells are illustrated in Fig. 3. The more pathogenic isolates, D and E, consistently induced in the host larger giant cells than did isolates A, B, or C. Giant cell measurements were made on Allgold roots only because on the resistant sweet potato lines giant cells were either not produced or occurred rarely.

TABLE I

HOST-PARASITE REACTIONS WITH EACH OF 5 ISOLATES OF MELOIDOGYNE INCOGNITA ON EACH OF 5 SELECTED SWEET POTATO LINES

Sweet Potato Line and Nematode Isolate	Galling Index[a]	Root Necrosis[b]	Nematode Development[c] Immature	Mature	Egg Masses
			No.	No.	No.
ALLGOLD					
A	3.1	1	93.2	209.8	77.8
B	4.0	1	76.5	211.0	84.5
C	3.9	1	87.8	188.8	84.5
D	4.8	1	149.5	273.0	98.3
E	4.8	1	175.8	274.2	102.3
NEMAGOLD					
A	1.0	2	50.5	15.8	12.1
B	1.1	2	44.8	4.8	7.3
C	1.2	2	29.8	6.0	5.5
D	1.5	3	49.8	26.5	17.6
E	1.7	3	47.8	21.5	21.0
HEARTOGOLD					
A	1.1	2	37.3	5.3	6.6
B	1.5	2	46.0	10.8	3.5
C	1.5	2	31.3	2.0	1.2

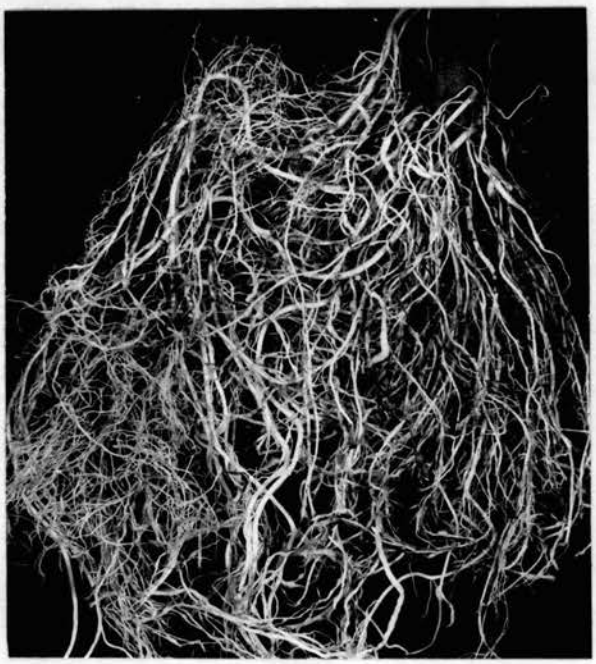
Table 1. Continued

Sweet Potato Line and Nema-Isolate	Galling Index[a]	Root Necrosis[b]	Nematode Development[c] Immature	Mature	Egg Masses
D	1.6	2	66.0	14.3	6.0
E	2.2	3	48.8	12.8	10.8
NC-172					
A	1.2	2	26.5	6.5	5.8
B	1.2	2	23.0	4.5	3.0
C	1.5	2	31.3	2.0	4.3
D	1.5	3	35.8	14.3	10.1
E	2.5	3	21.8	8.5	9.3
TINIAN					
A	1.1	2	28.3	19.0	8.8
B	1.5	2	36.3	14.3	11.5
C	1.4	2	48.5	7.3	7.8
D	1.8	2	42.0	18.0	11.6
E	1.7	3	29.0	22.0	13.0

[a]1 indicates no galling; 2 indicates traces or slight of galling; 3 indicates moderate galling; 4 indicates severe galling; and 5 indicates very severe galling.

[b]1 = Whole root necrosis associated with severe galling;
2 = Trace to moderate root tip necrosis;
3 = Severe root tip necrosis

[c]Immature nematodes are groups A, B, and D; mature nematodes are D and E groups (After Christie, 1946).



A

B

Fig. 2. Reaction of Allgold roots to M. incognita isolates A and D. A) Isolate A, B) Isolate D. Note that A did not severely parasitize Allgold while D caused considerable galling and necrosis.

TABLE II

GIANT CELL SIZE WITH EACH OF 5 ISOLATES OF M. INCOGNITA ON ALLGOLD SWEET POTATO WITH STATISTICAL SIGNIFICANCE AS SHOWN BY DUNCAN'S MULTIPLE RANGE TEST

Nematode Isolate	Giant cell size in each of indicated test periods: 1	2	3	Mean	Statistical Significance[a]
	μ	μ	μ	μ	
A	45.90	73.63	76.52	65.35	
B	56.43	78.35	90.99	75.36	
C	59.74	82.16	88.36	76.72	
D	77.44	111.82	105.23	98.16	
E	84.44	112.29	112.67	103.13	

[a]Means not enclosed in the same bracket are significantly different from one another at the 5% level.

TABLE III

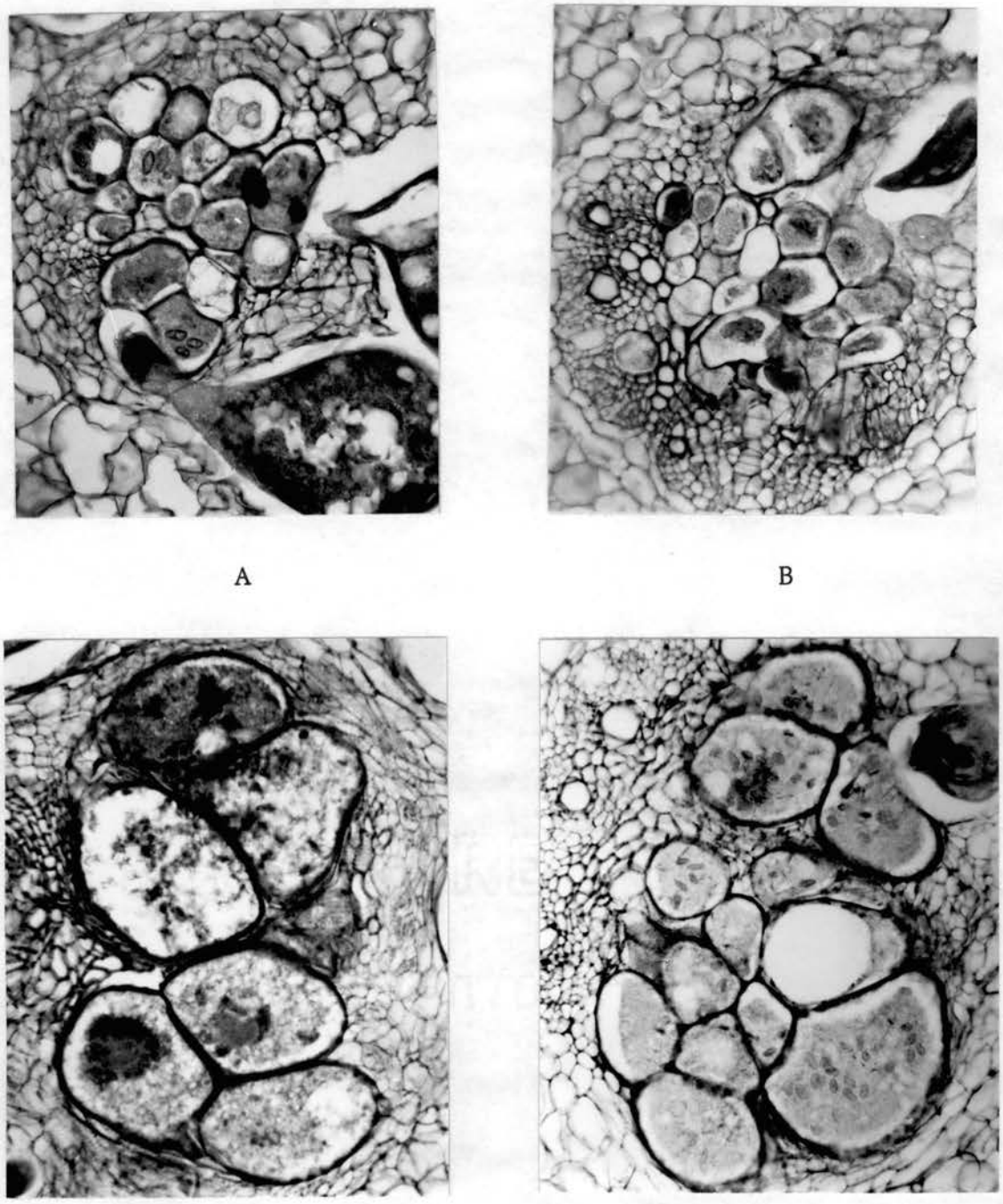
NUMBER AND SIZE OF GIANT CELLS PER MATURE FEMALE NEMATODE ON ALLGOLD ROOTS

Nematode Isolate	Mature Females Examined No.	Mean No. of Giant Cells 40-60 μ diam.	60 μ and above diam.
A	30	5.13	1.76
B	30	4.46	1.67
C	30	4.33	1.17
D	30	1.60	4.50
E	30	2.40	4.50

TABLE IV

VARIABILITY IN PERINEAL PATTERNS OF NEMATODE ISOLATES OBSERVED FROM A SAMPLE OF 20 MATURE FEMALES FROM ALLGOLD ROOTS

Nematode Isolate	Type of Perineal Pattern: Acrita	Acrita-Incognita	Incognita
A	17	3	0
B	14	6	0
C	17	3	0
D	19	1	0
E	18	2	0



A B

C D

Fig. 3. Relative size of giant cells produced on Allgold roots by nematode isolates A, B, D, and E (140X). A) Isolate A, B) Isolate B, C) Isolate D, D) Isolate E. Note smaller size of giant cells with A and B. Isolate C giant cells were comparable to those with A or B.

Variation in Morphology of Nematode Isolates

Since nematode isolates D and E were found to differ in pathogenicity from isolates A, B, and C, an attempt was made to determine whether there were also morphological differences between isolates. Perineal patterns were the only morphological character studied. Results from a study of 20 perineal patterns per isolate are presented in Table IV. Perineal patterns of more pathogenic isolates were not different from those of the other isolates.

Evidence from this study lends support to the work of Triantaphyllou and Sasser (18) from which they concluded that subspecies M. incognita acrita should be combined with and recognized as M. incognita. It will be noted in Table IV that while the majority of the patterns observed for each isolate were typical of acrita, there were always some which were intermediate between acrita and incognita. These intermediate type patterns tend to make difficult the separation of acrita from incognita as readily as might be desired.

Reaction of Selected Subisolates from Nematode Isolates D and E

To determine if virulence or pathogenicity of the nematode cultures could be further increased, subisolations by means of single egg-mass cultures were made from nematode isolates D and E on Nemagold. These subcultures were designated as D-1, D-2, and E-1, E-2, and E-3. They were handled, maintained, and tested as had been the parent cultures as described in the section on Materials and Methods. Data on host-parasite reactions with each of these 5 subisolates on each of the 5 sweet potato lines are presented in Table V. These data are from a single test with each sweet potato line replicated 6 times. In general these subisolates tended to be more pathogenic as measured by the relative amount of galling produced than were the parent cultures from which they originated. Mean galling indexes of these subisolates are shown graphically compared with their parent isolates in Fig. 4.

Among these subisolates certain of them, particularly D-2 and E-3, were highly pathogenic on resistant sweet potato lines. These effects, galling and stunting of the root systems, are shown in Fig. 5, 6, 7. These subisolates, also caused severe stunting of top growth in both resistant and susceptible sweet potato lines as illustrated in Fig. 8, 9, 10, 11. The effects of subisolate E-3 on top growth, though not shown in the illustrations, were essentially the same as for subisolate D-2.

Measurements of 100 giant cells from Allgold roots infected with each of the subisolates revealed that there was an increase in giant cell size as compared with the parent isolates (Fig. 12).

Giant cells were readily found in Nemagold roots infected with subisolates D-2 and E-2. As previously pointed out giant cells were rarely found in this variety infected with the parent isolates D and E.

TABLE V

HOST-PARASITE REACTIONS WITH EACH OF 5 SUBISOLATES FROM PARENT ISOLATES D AND E OF MELOIDOGYNE INCOGNITA ON EACH OF 5 SELECTED SWEET POTATO LINES

Sweet Potato Line and Nematode Subisolate	Galling Index[a]	Root Necrosis[b]	Nematode Development[c] Immature	Mature	Egg Masses
			No.	No.	No.
ALLGOLD					
D-1	5.0	1	16.7	74.7	75.3
D-2	5.0	1	12.0	65.0	46.6
E-1	5.0	1	1.3	49.3	40.7
E-2	5.0	1	5.0	24.7	27.4
E-3	5.0	1	15.3	103.7	55.3
NEMAGOLD					
D-1	3.3	2	4.0	6.3	5.7
D-2	3.5	3	16.3	21.3	15.3
E-1	2.7	2	6.7	17.0	23.6
E-2	2.0	2	6.7	9.0	10.3
E-3	3.7	3	7.4	46.7	43.7
HEARTOGOLD					
D-1	3.0	2	8.7	3.0	1.3
D-2	3.0	3	37.7	1.0	1.0
E-1	2.7	2	9.7	16.3	15.7
E-2	2.7	2	8.7	11.7	7.0

Table V. Continued

Sweet Potato Line and Nematode Subisolate	Galling Index[a]	Root Necrosis[b]	Nematode Development[c] Immature	Mature	Egg Masses
E-3	3.0	2	7.7	18.0	16.4
NC-172					
D-1	3.3	2	7.0	6.7	7.7
D-2	3.7	2	19.7	18.0	15.3
E-1	2.7	2	9.7	16.3	15.7
E-2	2.7	2	8.7	11.7	7.0
E-3	3.0	2	7.7	18.0	16.4
TINIAN					
D-1	2.3	2	7.3	20.0	23.0
D-2	3.0	3	20.0	23.3	25.7
E-1	2.7	2	7.0	24.0	13.4
E-2	3.0	3	7.7	24.0	27.6
E-3	2.7	2	9.3	21.3	21.7

[a]1 indicates no galling; 2 indicates slight or traces of galling; 3 indicates moderate galling; 4 indicates severe galling; and 5 indicates very severe galling.

[b]1 = Whole root necrosis associated with severe galling;
2 = Trace to moderate root tip necrosis;
3 = Severe root tip necrosis

[c]Immature nematodes are groups A, B, and C; mature nematodes are D and E groups (After Christie, 1946).

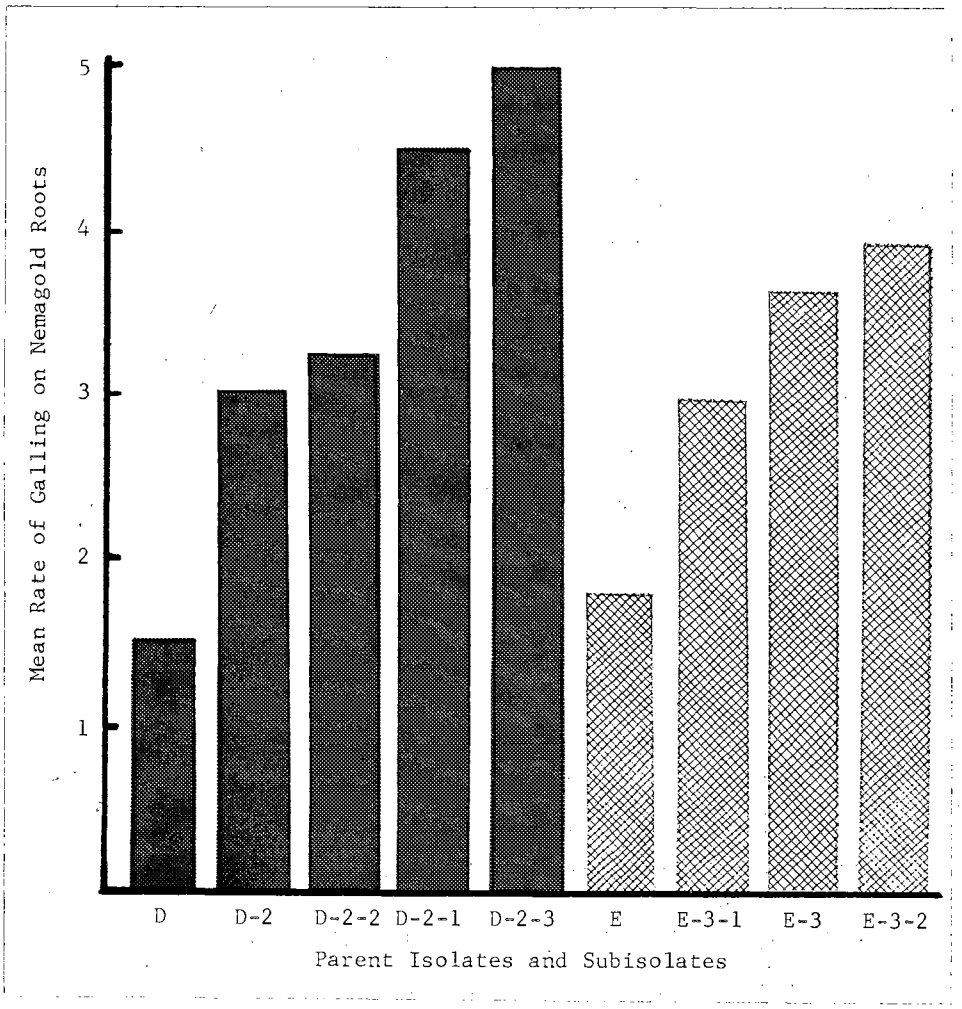


Fig. 4. Differences in pathogenicity on Nemagold as indicated by galling indexes of M. incognita subisolates and the isolates from which they were derived. Galling scores were as follows; 1 = no galling; 2 = trace galling; 3 = moderate galling; 4 = severe galling; 5 = very severe galling.

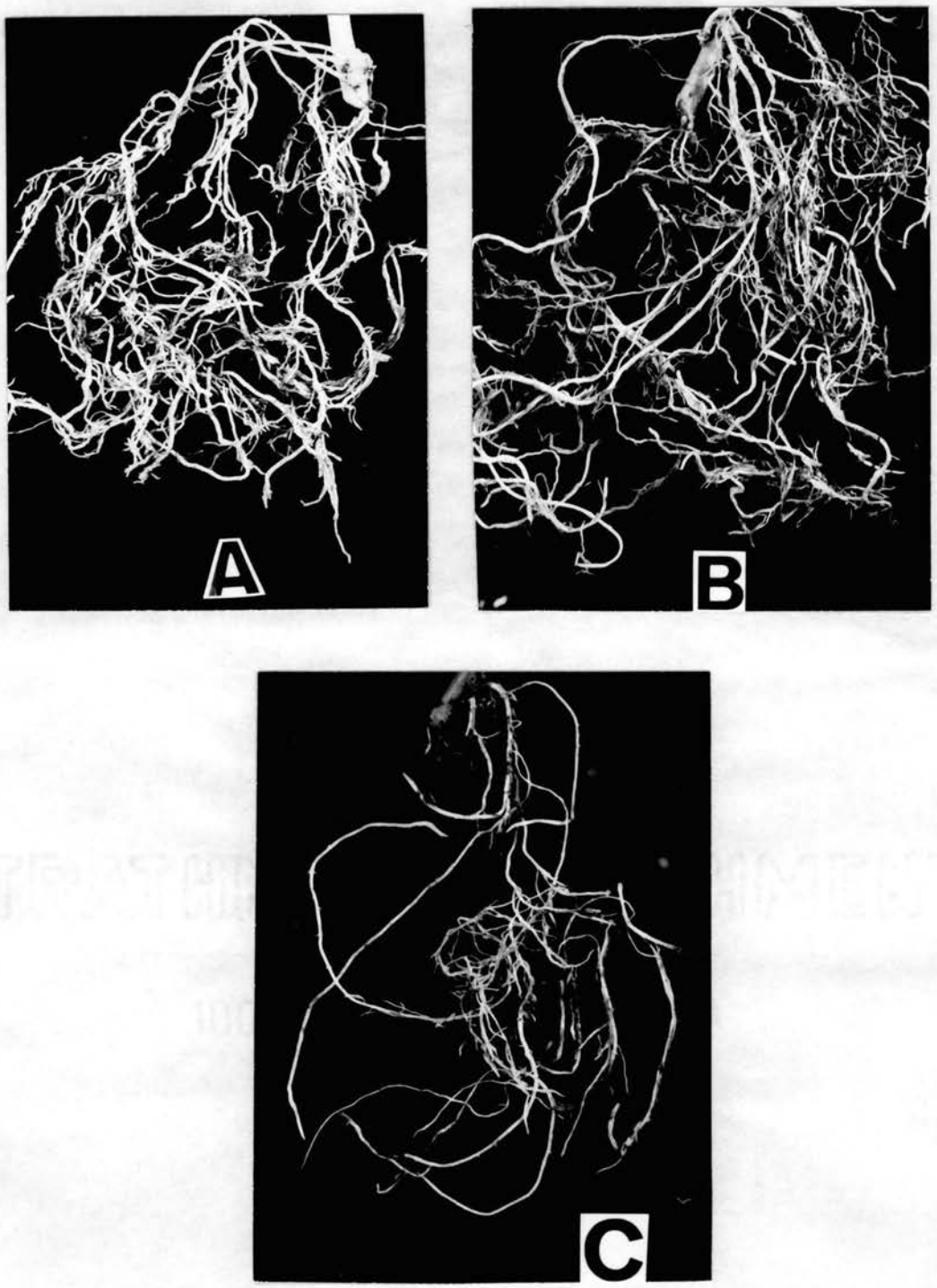


Fig. 5. Heartogold root systems showing the degree of disease damaged caused by M. incognita subisolates. A) Subisolate D-1, B) Subisolate E-1, C) Subisolate D-2.

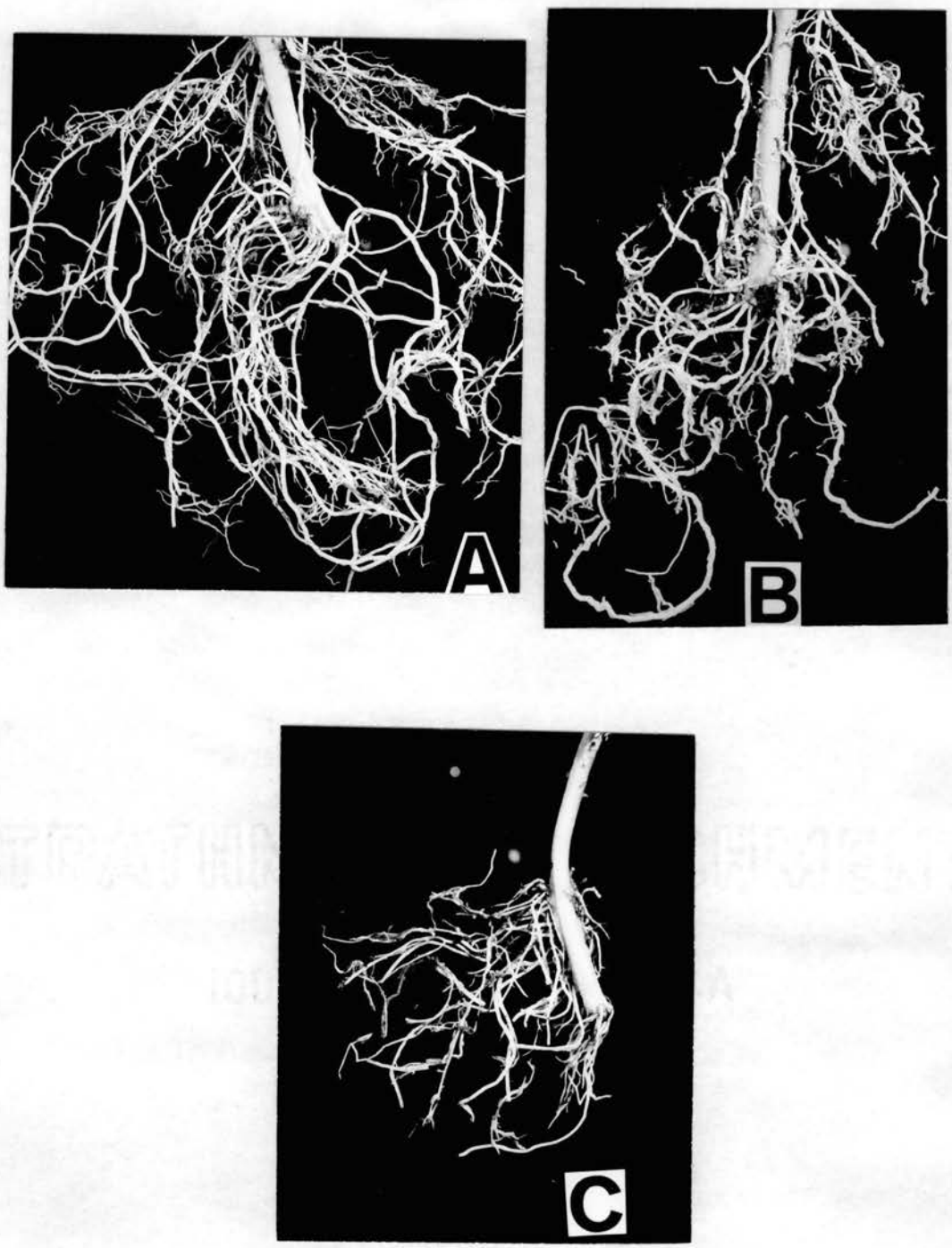


Fig. 6. Root systems of NC-172 showing the relative disease damaged caused by M. incognita subisolates E-1 as shown in A, subisolate E-3 shown in B, and subisolate D-2 shown in C.

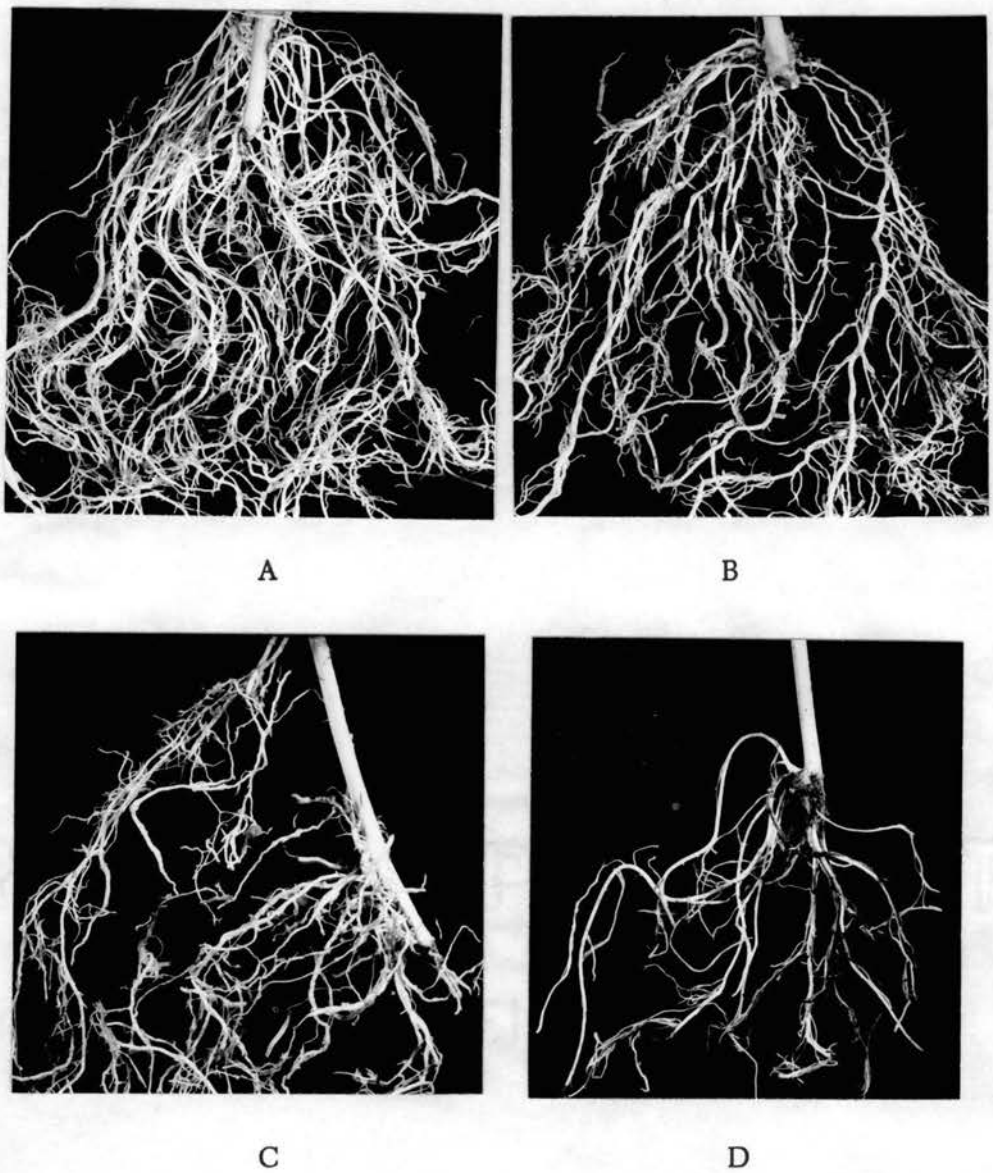


Fig. 7. Root systems of Nemagold exhibiting disease reaction to M. incognita subisolates E-1 as shown in A, D-1 as shown in B, D-2 as shown in C, and E-3 as shown in D. Reactions with isolates D and E were comparable to that shown with subisolate E-1.

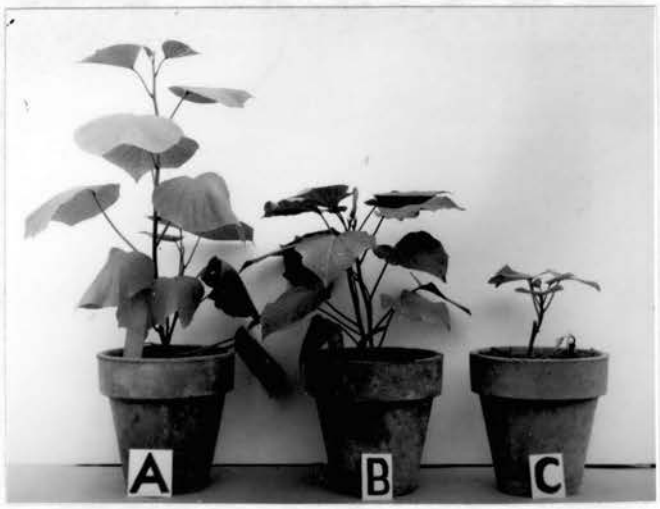


Fig. 8. Heartogold plants showing affects on top growth after inoculation with different subisolates of M. incognita. A) Subisolate E-1, B) Subisolate D-1, C) Subisolate D-2.

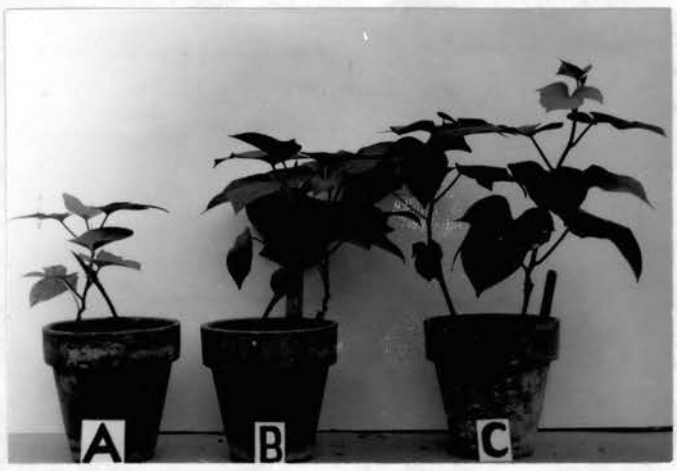


Fig. 9. Nemagold plants showing affects on top growth after inoculation with different subisolates of M. incognita. A) Subisolate D-2, B) Subisolate D-1, C) Subisolate E-1.

Fig. 10. Tinian plants showing affects on top growth after inoculation with different subisolates of M. incognita. A) Subisolate D-2. B) Subisolate D-1. C) Subisolate E-1.

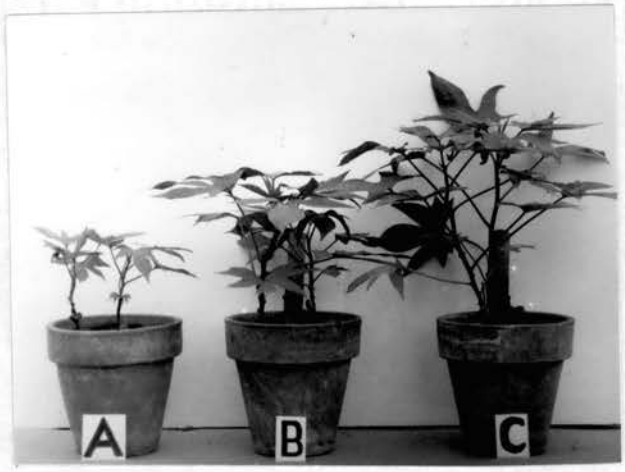


Fig. 11. NC-172 plants showing affects on top growth after inoculation with different subisolates of M. incognita. A) Subisolates D-2. B) Subisolate D-1. C) Subisolate E-1.

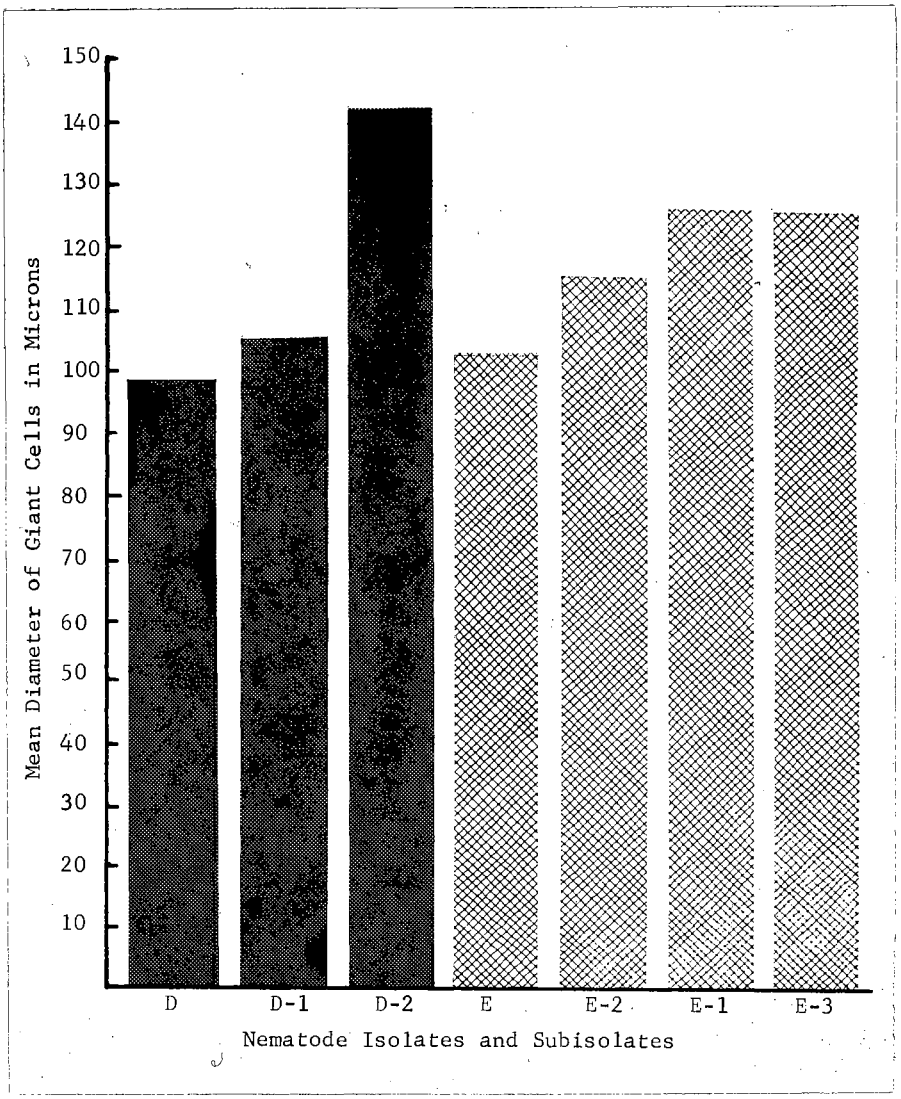


Fig. 12. Giant cell size on Allgold roots infected with M. incognita subisolates compared with those of parent isolates.

Reaction of Selected Subisolates from Nematode Cultures D-2 and E-3

Inasmuch as the nematode cultures D-2 and E-3 showed evidence of being more pathogenic than the parent cultures from which they were derived, further subselections were made from D-2 and E-3 to determine whether pathogenicity could be still further increased. Single egg mass isolates were made from cultures D-2 and E-3 on Nemagold. These subisolates were designated as D-2-1, D-2-2, D-2-3, and E-3-1, and E-3-2. These subisolates were maintained and tested as had been the parent cultures.

Host-parasite reponses with each of these 5 subisolate on each of the 5 sweet potato lines are presented in Table VI. In general these 5 subisolates reacted as being more pathogenic than the cultures from which they were obtained. Galling ranged from moderate to a very severe level even on previously resistant sweet potato lines. Necrosis on roots ranged from whole root necrosis, a typical susceptible reaction, to severe root tip necrosis. Numbers of nematodes, immature and mature, and numbers of egg masses found in roots of resistant sweet potato lines while generally not high tended to be higher than might be expected with a typical resistant reaction. With culture D-2-3 on Nemagold, mature nematodes and numbers of egg masses were as great as would be expected with a susceptible line.

On Allgold with cultures D-2-1 and E-3-1 the number of egg masses found was unusually low. There is a possibility that these cultures may have required a period longer than 30 days to reach a higher level of egg laying.

A comparison of the relative amount of galling induced on Nematold by cultures D and E and some of their subisolates is presented in Fig. 13. In Fig.

14 is shown a comparison of the relative rate of reproduction on Nemagold with cultures D and E and some of their subisolates.

Giant cells were regularly produced on Nemagold infected with cultures D-2-2 and D-2-3 (Fig. 15). In general these giant cells were smaller and fewer than those on susceptible Allgold.

TABLE VI

HOST-PARASITE REACTIONS WITH EACH OF 5 SUBISOLATES FROM PARENT ISOLATES D-2 AND E-3 OF MELOIDOGYNE INCOGNITA ON EACH OF 5 SELECTED SWEET POTATO LINES

Sweet Potato Line and Nematode Subisolate	Galling Index[a]	Root Necrosis[b]	Nematode Development[c] Immature	Mature	Egg Masses
			No.	No.	No.
ALLGOLD					
D-2-1	5.0	1	35.0	194.0	39.5
D-2-2	5.0	1	54.0	184.5	231.0
D-2-3	5.0	1	43.0	371.5	220.5
E-3-1	5.0	1	70.5	169.5	92.0
E-3-2	5.0	1	114.0	308.0	273.0
NEMAGOLD					
D-2-1	4.5	3	23.5	52.5	37.0
D-2-2	3.3	2	8.0	56.5	45.0
D-2-3	5.0	2	26.5	164.0	182.5
E-3-1	3.0	2	15.0	54.0	84.0
E-3-2	4.0	2	16.0	78.0	88.0
HEARTOGOLD					
D-2-1	3.3	2	26.0	74.0	10.0
D-2-2	4.6	1	78.0	18.5	13.3
D-2-3	3.0	2	30.0	25.0	26.0

Table VI. Continued

Sweet Potato Line and Nematode Subisolate	Galling Index[a]	Root Necrosis[b]	Nematode Development[c] Immature	Mature	Egg Masses
E-3-1	3.0	2	30.0	21.4	16.0
E-3-2	4.3	1	51.0	61.0	48.0
NC-172					
D-2-1	4.5	3	15.0	17.0	5.0
D-2-2	4.5	1	12.5	36.0	24.5
D-2-3	3.0	2	24.0	17.0	22.0
E-3-1	3.6	3	10.2	26.0	25.0
E-3-2	4.3	2	23.5	50.0	48.1
TINIAN					
D-2-1	3.5	3	8.0	44.0	8.0
D-2-2	3.5	3	29.0	28.5	18.0
D-2-3	4.0	2	18.5	53.0	83.5
E-3-1	3.0	2	6.5	45.1	51.0
E-3-2	3.3	3	27.0	60.0	63.0

[a]1 indicates no galling; 2 indicates traces or slight of galling; 3 indicates moderate galling; 4 indicates severe galling; and 5 indicates very severe galling.

[b]1 = Whole root necrosis associated with severe galling;
2 = Trace to moderate root tip necrosis;
3 = Severe root tip necrosis

[c]Immature nematodes are groups A, B, and C; mature nematodes are D and E groups (After Christie, 1946).

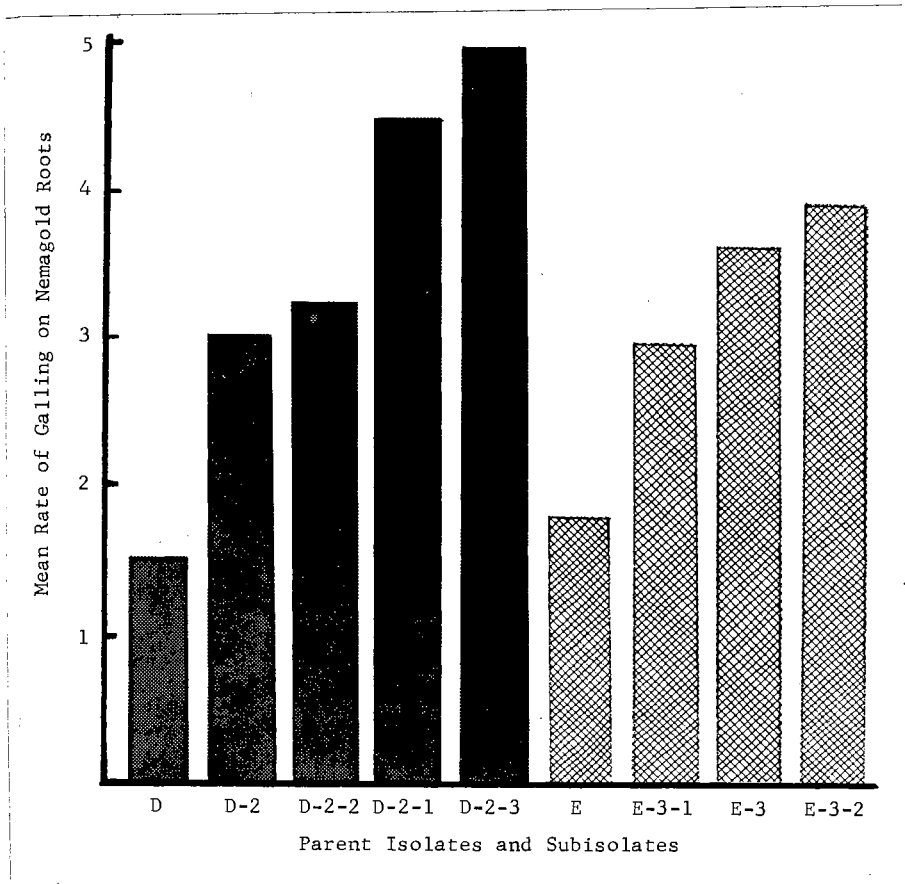


Fig. 13. A comparison of the rate of galling produced by the subisolates and their parents on Nemagold.

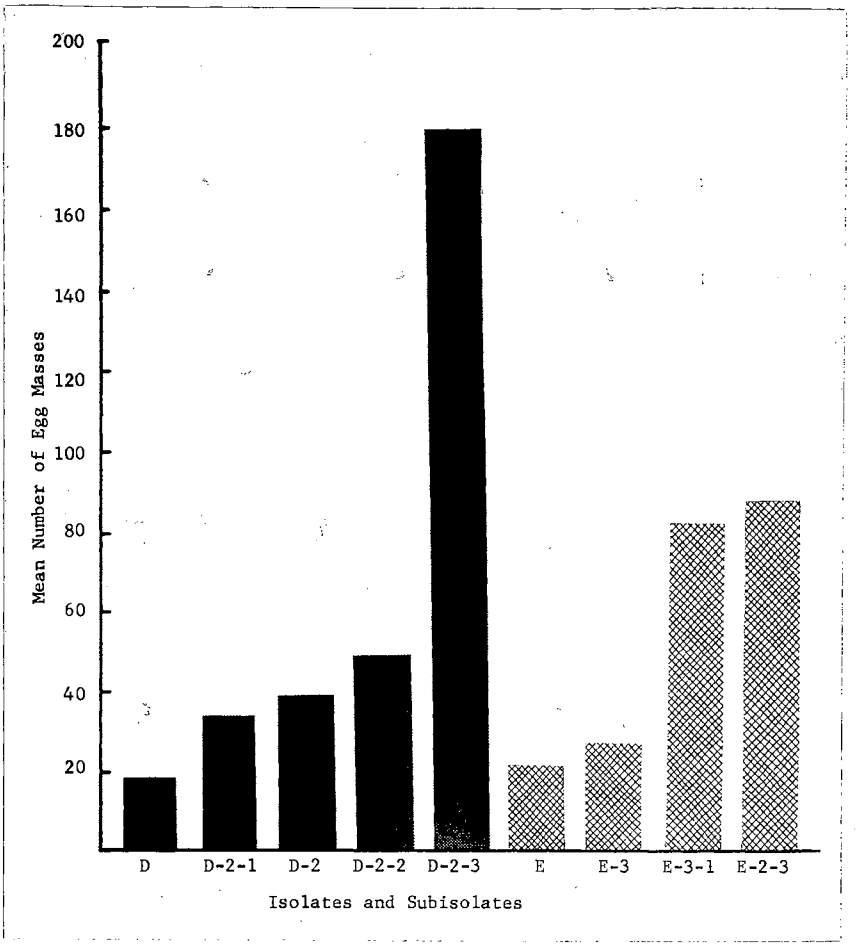
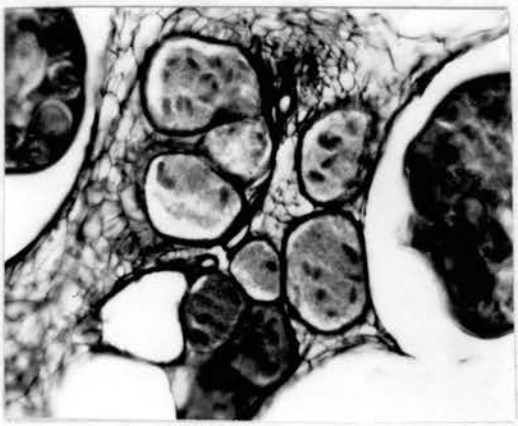
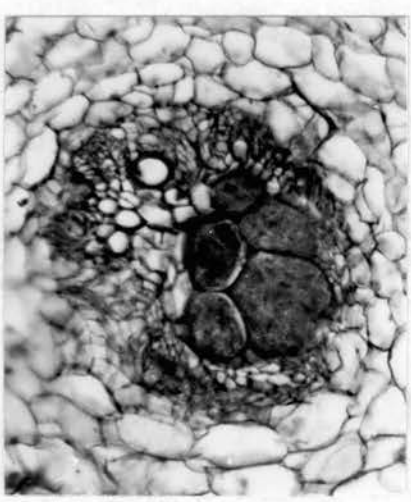


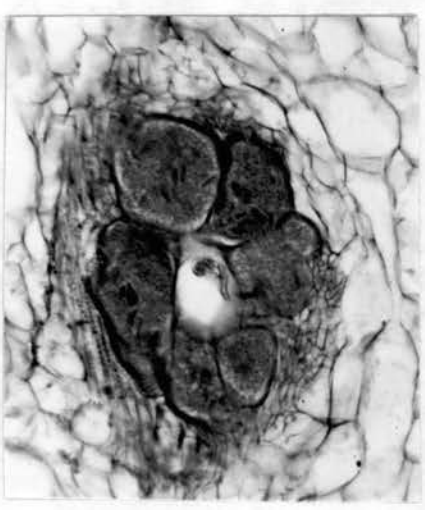
Fig. 14. Reproduction with nematode isolates and subisolates on Nemagold.



A



B



C

Fig. 15. Nemagold roots showing giant cells produced by parent isolate D-2 and its subisolates D-2-2 and D-2-3 (140X). A) Isolate D-2, B) Subisolate D-2-2, C) Subisolate D-2-3.

Relation of Nematode Variability to Time Required for Completion of Life Cycle

Since certain nematode isolates had been demonstrated to vary in their ability to induce disease, the possibility was considered that these variant isolates might also be different with respect to the time required to complete their life cycles. Nematode culture used in this test were isolates B and E and subisolates D-2 and E-3. Sweet potato lines inoculated with these cultures were Allgold, Nemagold, and Tinian. Greenhouse temperatures during this test varied from 80°-86°F during the day and from 70°-79°F during the night. Root systems of 6 plants of each line inoculated with each of the cultures were stained and examined at each of the following time intervals following inoculation; 15, 20, 25, 30, and 35 days.

Results from this test are shown in Fig. 16. Since the results with Tinian were essentially the same as those with Nemagold, only results from the latter are presented. No mature nematodes from any culture on any of the hosts were found in 15 days. Only a relatively few nematodes were found to reach maturity in 20 days except in the case of culture D-2 on Allgold. With this latter culture on Allgold reproduction in 20 days was comparable to levels reached by other cultures on Allgold in 25 days. Although data past 20 days with D-2 on Allgold are not available because the remainder of the plants were killed, the results suggest that in addition to being more pathogenic this nematode culture also matures at a greater rate on Allgold.

While the numbers of mature females found on the resistant lines were not as great as those found on Allgold, there was evidence that culture D-2

reached a higher rate of reproduction earlier, 25 days, than did the other cultures, 30-35 days, on the resistant lines.

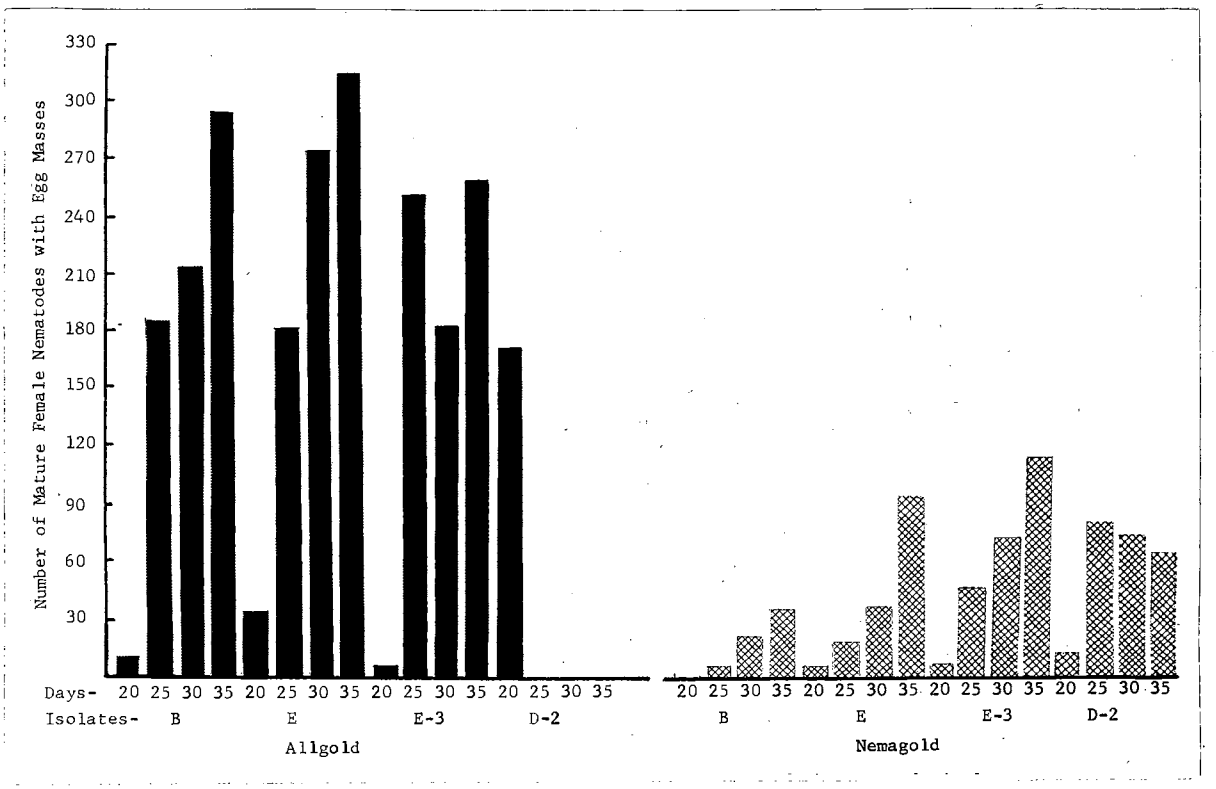


Fig. 16. Days required for the completion of the life cycle of some of the nematode isolates on Allgold and Nemagold sweet potato lines. Isolate D-2 killed all Allgold plants after 20 days.

DISCUSSION

It has been demonstrated in the present investigation that a field population of M. incognita may be composed of a number of physiological races. This fact is neither new nor unique since as was pointed out earlier physiological specialization in this nematode has been reported by several investigators using several different host species. However, a search of the literature has failed to reveal evidence, as presented in the present work, that pathogenicity of nematode isolates can be further increased by selection of single-egg-mass cultures from the more pathogenic isolates.

This evidence has a very important bearing on the field evaluation of sweet potato, and possibly other crops, for resistance to M. incognita. Since these more pathogenic races of this nematode are potentially present in the field the continued growing of supposedly resistant plants might operate to favor increase of the more pathogenic nematode races. In the present investigation no evidence has been obtained on the survival value of these more pathogenic races in field. Since some of the more pathogenic races were virulent enough to retard severely or kill infected plants, it would seem that these races may not survive or increase to the extent of a less virulent race. Further evidence on this point needs to be obtained.

Since all nematode selections in this investigation were made from the resistant sweet potato variety Nemagold no evidence is at hand to suggest the effect of resistant compared with susceptible varieties in screening for more

pathogenic nematode races.

Evidence presented suggests that the relative amount of root galling and reduction in top growth are more reliable criteria in evaluating for nematode pathogenicity than are factors such as the numbers of nematodes reaching maturity. Even with the most virulent nematode cultures isolated the number of adult females and egg masses on resistant sweet potato lines was still relatively low when compared with results from the susceptible sweet potato variety. This suggests some inherent factor or factors which still make varieties such as Nemagold appear partly resistant in spite of the susceptible response to root galling. The fact that certain nematode isolates induced giant cells which were relatively small and few in number in Nemagold roots as compared with the same isolates on Allgold again suggests a certain level of resistance.

Dropkin (7, 8) presented evidence that in soybean larger giant cells were associated with susceptibility and smaller giant cells with resistance to M. incognita and M. incognita acrita. It would seem that evidence from the present investigation confirms this even though in this case nematode isolates rather than host varieities were being tested.

Evidence that at least one of the more virulent nematode isolates reproduced at a higher rate and earlier than the less virulent isolates has been presented. This occurred on both Allgold and Nemagold. As far as can be determined, these observations have previously not been reported.

SUMMARY

It was demonstrated that physiological races of the root-knot nematode (Meloidogyne incognita) can be selected from a field population through isolates obtained from a resistant sweet potato line, Nemagold. These races differed in the response they induced on Allgold. Differences were noted in rate of galling, numbers of mature females with egg masses, and in the size of giant cells.

Subisolates from the more pathogenic of these races on Nemagold were demonstrated as more pathogenic than the parent nematode cultures. Certain of these subisolates attacked the resistant sweet potato lines and made them appear as susceptible. Severity of root galling and stunting of top growth appeared to be more reliable criteria of susceptibility to this nematode than did the numbers of nematodes that reached maturity.

There was evidence that the most pathogenic of the subisolates reached maturity in greater numbers about 5 days earlier on both susceptible and resistant sweet potato than did those less pathogenic.

The findings relative to the existence of highly pathogenic forms of M. incognita in a naturally occurring field population must be taken into consideration in evaluating sweet potato lines for reaction to this nematode.

LITERATURE CITED

1. Allen, M. W. 1952. Observations on the genus Meloidogyne Goeldi 1887. Proc. Helminthol. Soc. Wash. D. C. 19: 44-51.

2. Chitwood, B. G. 1949. "Root-knot nematodes" - Part I. A revision of the genus Meloidogyne Goeldi, 1887. Proc. Helminthol. Soc. Wash. D. C. 16: 90-104.

3. Christie, J. R. 1946. Host-parasite relationships of the root-knot nematode, Heterodera marioni. II. Some effects of the host on the parasite. Phytopathology 36: 340-352.

4. Dean, J. L. and F. B. Struble. 1953. Resistance and susceptibility to root-knot nematodes in tomato and sweet potato. (Abstr.) Phytopathology 43: 290.

5. Dooley, H. L. 1961. Reaction of sweet potato selections to different populations of Meloidogyne incognita acrita and to other species of Meloidogyne. Master's Thesis, Oklahoma State University. 35 p.

6. Dropkin, V. H. 1953. Studies on the variability of anal plate patterns in pure lines of Meloidogyne spp. The root-knot nematode. Proc. Helminthol. Soc. Wash. D. C. 20: 32-39.

7. Dropkin, V. H. 1959. Varietal response of soybeans to Meloidogyne - A bioassay system for separating races of root-knot nematodes. Phytopathology 49: 18-23.

8. Dropkin, V. H. and P. E. Nelson. 1960. The histopathology of root-knot nematode infections in soybeans. Phytopathology 50: 442-447.

9. Goplen, B. P., E. H. Stanford, and M. W. Allen. 1959. Demonstration of physiological races within three root-knot species attacking alfalfa. Phytopathology 49: 653-655.

10. Lider, L. A. 1954. Inheritance of resistance to a root-knot nematode (Meloidogyne incognita var. acrita Chitwood) in Vitis spp. Proc. Helminthol. Soc. Wash. D. C. 21: 53-60.

11. Martin, W. J. 1954. Parasitic races of Meloidogyne incognita and M. incognita acrita. Plant Disease Reptr. Suppl. No. 227: 86-88.

12. McBeth, C. W., A. L. Taylor, and A. L. Smith. 1941. Note on staining nematodes in root tissue. Proc. Helminthol. Soc. Wash. D. C. 8: 26.

13. Radewald, J. D. 1957. The nature of resistance and susceptibility to the root-knot nematode (Meloidogyne incognita var. acrita Chitwood) in sweet potato. Master's Thesis, Oklahoma State University. 51 p.

14. Riggs, R. G. and N. N. Winstead. 1959. Studies on resistance in tomato to root-knot nematodes and on the occurrence of pathogenic biotypes. Phytopathology 49: 716-724.

15. Sass, J. E. 1958. Botanical microtechnique. Third Edition. The Iowa State College Press, Ames, Iowa.

16. Sasser, J. N. and C. J. Nusbaum. 1955. Seasonal fluctuations and host specificity of root-knot nematode populations in two-year rotation plots. Phytopathology 45: 540-545.

17. Taylor, A. L., V.H. Dropkin, and G. C. Martin. 1955. Perineal patterns of root-knot nematodes. Phytopathology 45: 26-34.

18. Triantaphyllou, A. C. and J. N. Sasser. 1960. Variation in perineal patterns and host specificity of Meloidogyne incognita. Phytopathology 50: 724-735.

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