RESISTANCE TO ROOT-KNOT NEMATODE (MELOIDOGYNE INCOGNITA) IN THE SWEET POTATO (IPOMOEA BATATAS) AND (IPOMOEA TRIFIDA) AND HYBRIDS INVOLVING THESE SPECIES

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CHAPTER I

INTRODUCTION

The sweet potato (*Ipomoea batatas* Lam.) is a hexaploid morning glory. It has been cultivated as human food for 5,000 years (6). Root-knot nematode is one of the common diseases of the sweet potato. Breeding for resistance to this disease has been one of the objectives of the sweet potato breeding program at the Oklahoma Agricultural Experiment Station.

Many research workers have tested the reaction of sweet potato lines to nematodes and found some are resistant. Struble et al. (23) reported that root knot resistance can be obtained from any combination of parents from resistant x resistant, through susceptible x susceptible.

Although the information of whether sweet potato lines are resistant or susceptible has been ascertained by screening test, nothing has been done concerning resistance in the wild species (*Ipomoea trifida*). The characteristics and extent of resistance in *I. trifida* has not been reported.

I. trifida was discovered by Nishiyama in Mexico in 1955 and published in 1961 (18). He described K123 (one of the clones of *I. trifida*) as being very similar to the sweet potato. It is a hexaploid and is compatible with the sweet potato. It has non-enlarged roots, a twining vine, with hairy stems, with flowers and leaves similar to the sweet potato. Moreover, it has the same homologous genome constitution 2n = 90 chromosomes and a common genetic system of self-incompatibility as the sweet potato. He suggested that *I. trifida* is the original form from which the modern sweet potato was derived.

Clones of *I. trifida* had been established in the Oklahoma State University greenhouse, from seed obtained from Nishiyama. Certain characters of this wild species has been studied by H. B. Cordner, Department of Horticulture. When the sweet potato was crossed with *I. trifida*, the roots of the F_1 plants were enlarged but only a few were enlarged. When the F_1 was backcrossed to the sweet potato, many individuals were produced which developed enlarged roots. Backcrosses to *I. trifida* return to non-enlarged condition but retain some sweet potato characteristics such as skin color, carotene content of the root, etc. On this basis, we might raise the question as to whether or not the two species have any gene in common. This study is to investigate: 1) the level of resistance to root-knot nematode in *I. trifida*, especially selfed lines and hybrids of the clone X 1-1; 2) to compare the greenhouse resistance index with the laboratory egg mass index.

CHAPTER II

REVIEW OF LITERATURE

As early as 1889 at least two species of *Ipomoea* had been reported as hosts of root-knot nematodes (1). The sweet potato was recorded as a host of this disease in 1911 (3). Studies on the sweet potato varietal reaction to root-knot nematode have been reported (19, 24, 25). It has been confirmed that sweet potato varieties range from very susceptible to highly resistant to this disease (7, 8, 15).

Prior to 1939 the nature of resistance to root-knot nematodes in plants had attracted few investigations. Steiner (22) stated that plants may resist namatodes either by some chemical or mechanical means. Tyler (24) defined the resistance as an ability to obstruct the invasion of parasites, but she did not define resistance in a plant as its condition of being an unsuitable host. With the work of Barrons (2), a clearer concept that resistance in plants could not be defined merely as a failure of larvae to enter the roots, but rather the failure of invading larvae to survive after entering. He found no significant difference between the mean number of larvae present in resistant and susceptible plants, when both were equally exposed to infection.

Christie (5) demonstrated "suitable" or "unsuitable" rather than susceptible or resistant hosts and pointed out many plants that are highly unsuitable are invaded as freely as the more suitable ones.

With regard to the nematode infection, it is obvious that second-stage larvae, except for the adult male, is the only form of the root-knot nematode that migrates freely in the soil and invades a host to initiate infection. In most instances the larvae enter near root tips, or through existing breaks in the epidermis.

Christie (5) demonstrated that root-knot nematodes are sedentary parasites, once inside the root, they remain in the same place. The larvae therefore can feed on only a few cells throughout the remainder of their life. When the stimulation of the salivary secretion of the nematode cannot cause normal giant cells, the animal will soon be surrounded by thick walled or highly vacuolated cells which resist puncture by the stylet of the nematode. Even if the puncture could be effected, the contents of these cells would be of little value as a source of food. The nematode would soon die of starvation. Nematode development and maturation was delayed in less suitable hosts. If a plant is slightly, to moderately resistant it is often evidenced as necrosis at the region of invasion thus suggesting a failure of a stimulating effect of nematodes on cells of the surrounding tissue. The actual result is death of adjacent cells.

Barrons (2) also suggested that resistance may be due to the plant synthesizing certain chemicals which tend to neutralize the giant-cell-stimulating effects of the salivary secretion of the parasites. Christie (5) considered if numerous larvae entered a small area in the root simultaneously, the amount of inhibitor chemicals in the root probably would be insufficient to neutralize the saliva of all the larvae. Under such circumstances it would be possible for a few nematodes to undergo at least partial development.

Riggs and Winstead (21) studied root-knot nematode resistance in tomato and suggested some by-product of host-parasite interactions toxic to parasitic nematodes. This they concluded might be one of the factors responsible for the early death of the larvae.

The host-parasite reactions to root-knot nematode in sweet potato has been studied (4, 8, 9, 20, 23). A resistant reaction was generally characterized by little or no galling, failure of most of the nematodes to reach maturity, reduced size of egg masses and varying degrees of necrosis of root tips of fibrous roots. Giamalva et al. (12) suggested that sweet potato had two types of resistance to Meloidogyne: one associated with root necrosis and another with reduced galls and no necrosis. He pointed out that resistant reactions were significantly different among nematode species and for varieties x species interaction.

Cordner et al. (7) tested 1,400 F_1 progenies of sweet potato and found that about 50 per cent were resistant to root-knot from crosses of resistant x resistant, about 30 per cent from resistant x susceptible and 10 per cent from susceptible x susceptible. Struble et al. (23) tested 4,343 sweet potato clones and seedlings and reported the percentage of resistance to be about the same as that reported by Cordner et al. (7). Although the investigations made by the above workers were quite comprehensive, the inheritance of resistance to root-knot nematode in sweet potato is still not well defined.

CHAPTER III

MATERIALS AND METHODS

Five genetically diverse kinds of seedling individuals were used in this study.

1) Ipomoea batatas (sweet potato): 185 x 106.

2) Ipomoea trifida (wild species): X 1-1 selfed and X 1-1 x X 2-3.

3) F_1 hybrid between selections of Ipomoea trifida and Ipomoea batatas: X 1-1 x 183.

4) F_1 species hybrid backcross to Ipomoea batatas: K_2 x 183.

5) F_1 species hybrid backcross to Ipomoea trifida: K_1 x X 1-1.

The above lines have been used in the sweet potato breeding program at Oklahoma Agricultural Experiment Station. The sweet potato lines are identified by numbers and are designated as parent-185, parent-106, etc. Ipomoea trifida is identified by a prefix X, plus numbers, such as X 1-1, X 2-3, etc. K is used to identify F_1 hybrids between I. trifida and I. batatas. Those are designated as K_1, K_2, etc., while K_1 = K123 x 161 and K_2 = K123 x 183. It must be mentioned that K123 identifies the collection number of Ipomoea species by Nishiyama and does not identify F_1 hybrids between I. trifida and I. batatas. The reaction of sweet potato lines to root knot had been determined over a period of several years by Struble and Cordner.* Parent lines 106, 183,

*This work was conducted by F. B. Struble, Department of Botany and Plant Pathology, and H. B. Cordner, Department of Horticulture, Oklahoma State University, Stillwater.

185 and 161 were recognized as resistant to *Meloidogyne incognita*.

The seed of the test lines were scarified with a needle and planted in a porous medium in greenhouse flats, watered and allowed to germinate. At the same time, two flats of steam-sterilized sandy soil were inoculated with southern root-knot nematode (*Meloidogyne incognita*) by means of chopped tomato root galls. In order to incubate the nematodes, the flats were placed on a greenhouse bench heated by means of an electric cable to maintain a favorable temperature (85°F) and at the same time kept moist for two weeks.

Six lines of seedlings were transplanted into the inoculated soil. Two replications were established in the two flats. Each flat contained 60 plants which consisted of 6 rows of 10 plants each. This included 10 plants each of the 6 lines. The second flat included the same kind and number thus providing the replicate treatment. Two seedlings of nematode susceptible tomato variety Sioux were also set between rows of *Ipomoea* hybrids to verify the effectiveness of the soil inoculation.

Approximately two months later, the plants were removed and the roots examined macroscopically for evidence of the presence of nematodes. They were classified either as resistant, intermediate or susceptible, based upon the amount of root damage attributable to the nematode. Factors considered in the evaluation included abnormal enlargement of roots, root-tip necrosis or small dark areas indicating presence of nematodes and egg masses easily seen macroscopically.

Plants were rated; 1 for resistant, 3 for intermediate and 5 for susceptible. A resistance index was then derived by adding the three items and dividing this total by the total number of observed plants. This provided a weighted resistance index.

Sections of the root systems, rated for resistance, were also used for determining the stages of nematode development. A random sample of five small pieces, each 1.5-2.5 cm of each root system showing nematode injury, was taken, stained with acid fuchsin and cleared in lactophenol. The technic was essentially the same as that described by McBeth, Taylor and Smith (16), except 1 ml. instead of 5 ml. of acid fushsin stock solution was used per 100 ml. of lactophenol.

Cleared root sections were squashed between microscope slides and a dissecting microscope used to determine developmental stages of nematodes. The number of nematodes per centimeter of root was ascertained.

From the data on total number of egg masses per plant an index of nematode injury was also obtained. The root samples for each plant were classified either as resistant, intermediate or susceptible. The classification was based upon the number of egg masses per sample of roots; 0-5 number of egg masses per plant was assigned to the resistant class while 6-15 number of egg masses was classed intermediate and more than 16 as susceptible. The number of plants in each root-knot class was multiplied by the assigned score of each class (resistant, 1; intermediate, 3; susceptible, 5), the total added, and the sum divided by the total number of observed plants. An egg mass index was derived by the same methods used to derive the resistance index.

CHAPTER IV

RESULTS

The present study of resistance to root-knot nematode (*Meloidogyne incognita*) in the sweet potato, in a wild species and in related hybrids was based on the greenhouse seedling test followed by laboratory observation.

It has been previously noted that the resistance reaction in sweet potato is characterized by little or no galling of roots, failure of most nematodes to reach maturity, reduced size of the egg mass and varying degree of necrosis of tips of fibrous roots. These features are used to identify the degree of resistance or susceptibility of lines presently unknown.

The result of the counts of nematodes per centimeter of root is presented in Table I. Stage of development of nematodes in each plant were classified into A, B, C, D and E classes, according to their development. This classification was based on categories used by Christie (4), as shown in Figure 1. Group A includes larvae from the stage where they have begun to grow to the stage where they still possess a more or less conical tail. Group B includes larvae from the stage where they acquired a more or less hemispherical posterior end, terminated by a spike, to the stage where they are about to complete the final molt. Group C includes females from the stage where they have completed the molts to the stage where they are almost, though obviously not quite, fully grown. Group D includes females that are fully grown or almost fully grown but have not yet laid eggs. Group E includes egg-laying females.

TABLE I

NUMBER OF NEMATODES PER CENTIMETER OF ROOT OF CERTAIN *IPOMOEA BATATAS* AND *IPOMOEA TRIFIDA* SELECTIONS CLASSED IN EACH OF THE DEVELOPMENTAL STAGES

Lines	Replication	Nematodes per cm. of root in each of stated developmental stages*					Total
		A	B	C	D	E	
X 1-1 self	1	0.00	0.08	0.15	0.13	0.23	0.61
	2	0.11	0.15	0.17	0.25	0.07	0.76
Average		0.06	0.12	0.16	0.19	0.15	0.69
X 1-1 x X 2-3	1	0.22	0.65	0.41	0.33	1.30	3.03
	2	0.04	0.27	0.27	0.48	0.24	1.29
Average		0.13	0.46	0.34	0.41	0.77	2.16
X 1-1 x 183	1	0.29	0.75	0.65	0.59	1.21	3.49
	2	0.33	0.23	0.53	0.41	0.11	1.63
Average		0.31	0.49	0.59	0.50	0.66	2.56
K_1 x X 1-1	1	0.12	0.77	0.79	0.70	1.48	3.85
	2	0.15	0.68	0.67	0.85	0.63	2.98
Average		0.14	0.73	0.73	0.78	1.06	3.42
K_2 x 183	1	0.15	0.40	0.36	0.52	0.54	1.97
	2	0.18	0.62	0.45	0.66	0.73	2.64
Average		0.17	0.51	0.42	0.59	0.64	2.32
185 x 106	1	0.19	0.20	0.11	0.27	0.25	1.01
	2	0.25	0.53	0.74	0.38	0.13	2.05
Average		0.22	0.37	0.43	0.33	0.19	1.53

*The classification of developmental stages was based on categories used by Christie (4), shown in Figure 1.

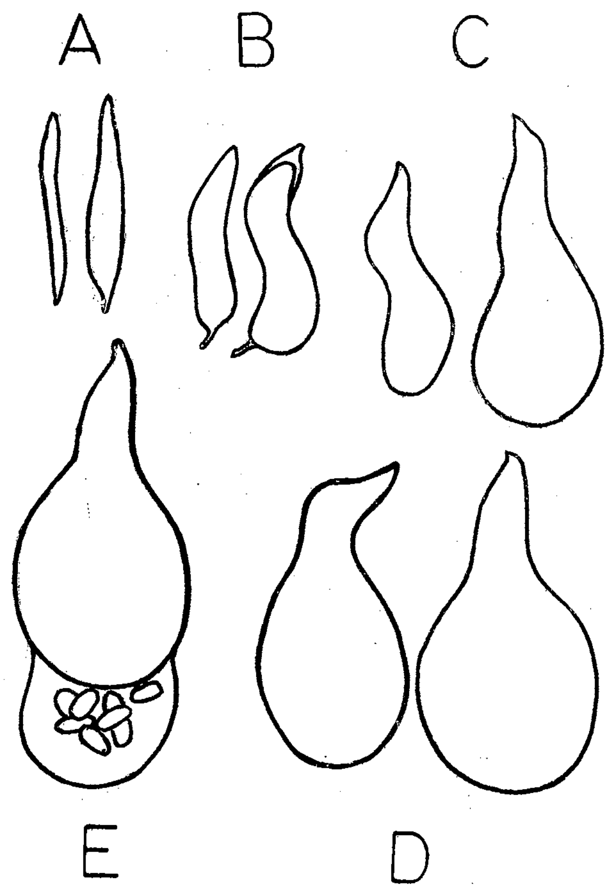

Figure 1. Classes of Nematodes Grouped According to Stage of Development [After Christie (4)].

Plants of X 1-1 selfed and 185 x 106 had the lowest infection count of larvae within the roots. Under microscopic investigation, only a few nematodes were found to have reached egg-laying maturity. Root-tip necrosis was observed. In such necrotic tissues they were very heavily stained with acid fuchsin, and the nematodes rarely developed beyond the "C" stage.

Although the total count for plants of X 1-1 x X 2-3 cross were also low, when only the egg-laying "E" stage was considered, it was higher than those of the X 1-1 x 183 and K_2 x 183 lines. This may have been due to the fact that two plants in one replication were very highly susceptible. Most of the X 1-1 x X 2-3 plants had moderate levels of necrotic root tips, the galls were slightly enlarged over those of the selfed plants of X 1-1.

In comparison of F_1 species backcross to sweet potato or to the wild species, plants of K_1 x X 1-1 cross showed higher levels of invasion by nematodes than K_2 x 183 plants. This was contrary to rating from visual judging of root damage. The characteristic kind of root damage to plants of the K_2 x 183 parentage was large galls, some fibrous roots were entirely necrotic, indicating invasion of nematode infected roots by secondary organisms, while galls on plants of the K_1 x X 1-1 were smaller and the necrotic areas were found only on root tips.

Radewald (20) made a histological study of nematode infection and recorded that many larvae died within 10 to 12 days after entering the roots of resistant sweet potato lines. Based on the fact that dead nematodes were found, it was not possible to demonstrate how many nematodes had died or when they had died, since the plants had been continuously exposed in nematode infested soil for more than 12 days.

Nematodes entered the roots of resistant plants as readily as they entered susceptible ones but with resistant lines, nematode development was retarded. For these reasons the total number of nematodes present in each root was not a precise factor in the evaluation. An egg mass index score seems to be a more reliable criteria of resistance to root-knot nematode. "Egg mass index" was defined on page 8 of the chapter on Materials and Methods and the results shown in Table II.

As a result of this egg mass index, plants of X 1-1 selfed and 185 x 106 appeared to be the more resistant. Dooley (10) found that in resistant sweet potato lines the egg mass size was reduced. Although few, slightly reduced size egg masses were found in these lines, there appears to be insufficient data to conclude any difference on this point. The egg mass index of plants of the X 1-1 x X 2-3 and X 1-1 x 183 lines was the same, yet there was slight difference between them. In X 1-1 x X 2-3 the development of nematode found in five root segments which came from the same plant were about the same, therefore to classify their reactions to the nematode appears to be more reliable than in the case of the X 1-1 x 183 line. The highest egg mass index was obtained from plants of the crosses K_2 x 183 and K_1 x X 1-1, the later was much greater than those of the other. It appeared that the most susceptible clones were those of F_1 hybrid species backcrossed to either I. batatas or I. trifida.

When the egg mass index was compared with the resistance index which is presented in Table III, the degree of resistance was in agreement, except for plants of the cross K_2 x 183. The low egg mass index score of the cross of K_2 x 183 may have been due to the following: during the experiment three plants died and one plant developed only one root. Of these four plants which were presumed to be high in egg mass three had died thereby making it impossible to count egg masses. Therefore, the actual number of susceptible samples was reduced. This might have caused the index to be lower than for plants of

TABLE II

NEMATODE EGG MASS INDEX OF CERTAIN *IPOMOEA BATATAS* AND *IPOMOEA TRIFIDA* SEEDLING LINES

Lines	No. of Obser-vations	Reaction Classes			Egg Mass Index*
		Resistant	Intermediate	Susceptible	
X 1-1 Self	20	18	2	0	1.20
X 1-1 x X 2-3	20	12	5	3	2.10
X 1-1 x 183	20	13	3	4	2.10
K_1 x X 1-1	20	10	6	4	2.40
K_2 x 183	16	8	7	1	2.13
185 x 106	20	18	2	0	1.20

*The values in this column were calculated according to the following formula suggested by H. B. Cordner:

$$\text{Egg Mass Index} = \frac{1}{\text{No. of observation}} \times [(1 \times \text{No. of Resistant plants}) + (3 \times \text{No. of Intermediate plants}) + (5 \times \text{No. of Susceptible plants})]$$

the K_1 x X 1-1 line. A rather strong correlation does exist between the factors, egg mass index and resistance index. As shown in Figure 2, the resistance index increased on the average by +1.154 units per egg mass index. The correlation coefficient, which was calculated as r = 0.8478 was significant at 5 per cent level. This tends to support the fact that sweet potato plant indexing in the greenhouse had considerable reliability for rapid evaluation of resistance to root-knot nematodes for the two species and their hybrids.

TABLE III

NEMATODE RESISTANCE INDEX OF CERTAIN *IPOMOEA BATATAS* AND *IPOMOEA TRIFIDA* SEEDLING LINES

Lines	No. of Obser- vations	Reaction Classes Resistant	Intermediate	Susceptible	Resistance Index*
X 1-1 Self	20	14	3	3	1.90
X 1-1 x X 2-3	20	10	3	7	2.70
X 1-1 x 183	20	7	6	7	3.00
K_1 x X 1-1	20	7	3	10	3.30
K_2 x 183	17	3	4	10	3.82
185 x 106	20	10	8	2	2.20

*The values in this column were calculated according to the following formula suggested by H. B. Cordner:

$$\text{Resistance Index} = \frac{1}{\text{No. of observation}} \times [(1 \times \text{No. of Resistant plants}) + (3 \times \text{No. of Intermediate plants}) + (5 \times \text{No. of Susceptible plants})]$$

Figure 3 shows the relation of resistance index and number of nematodes per centimeter of root. The calculated correlation coefficient r = 0.7841 was not statistically significant, in other words, these two factors were not correlated statistically. The mean difference of total number of nematodes per centimeter of roots also showed no significance at the 1 per cent level.

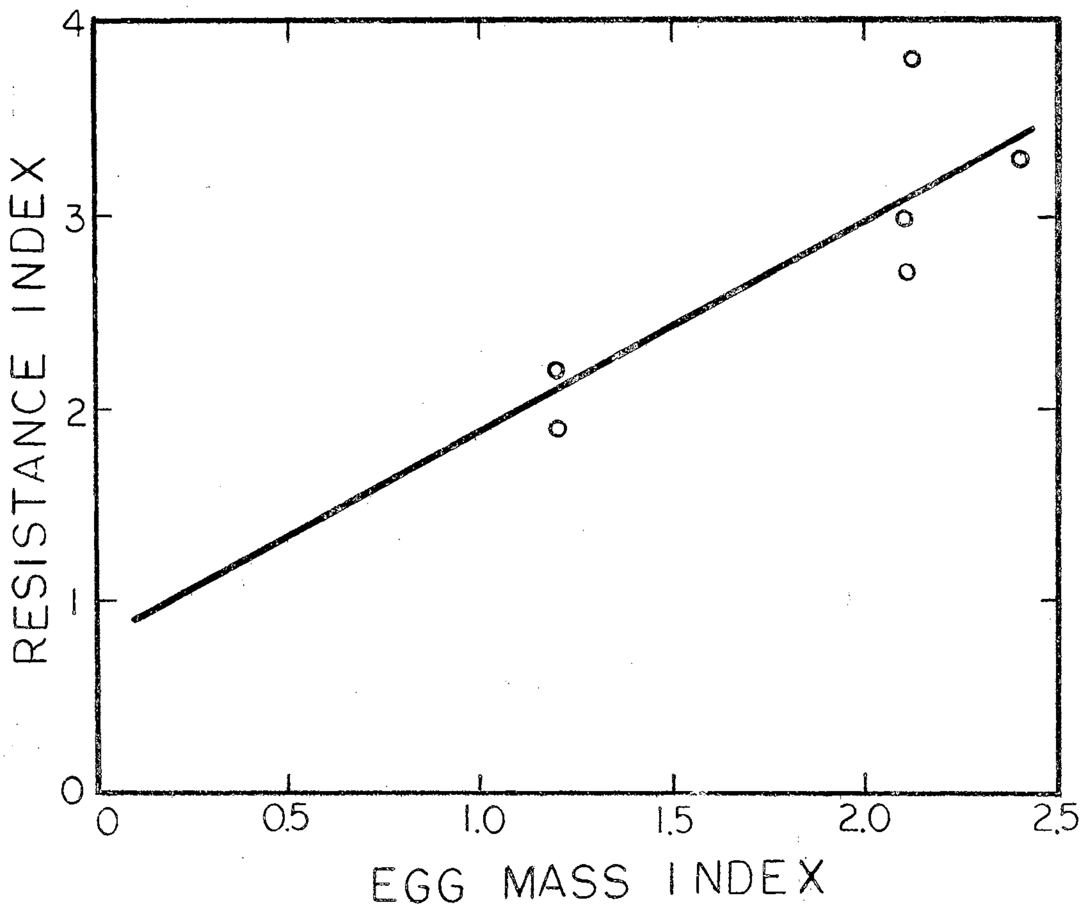

Figure 2. Scatter Diagram and Correlation Between Resistance Index and Egg Mass Index of Certain Lines of *Ipomoea batatas* and *Ipomoea trifida*. The Correlation Coefficient = 0.8478 The Regression Coefficient of Resistance Index on Egg Mass Index = 1.154

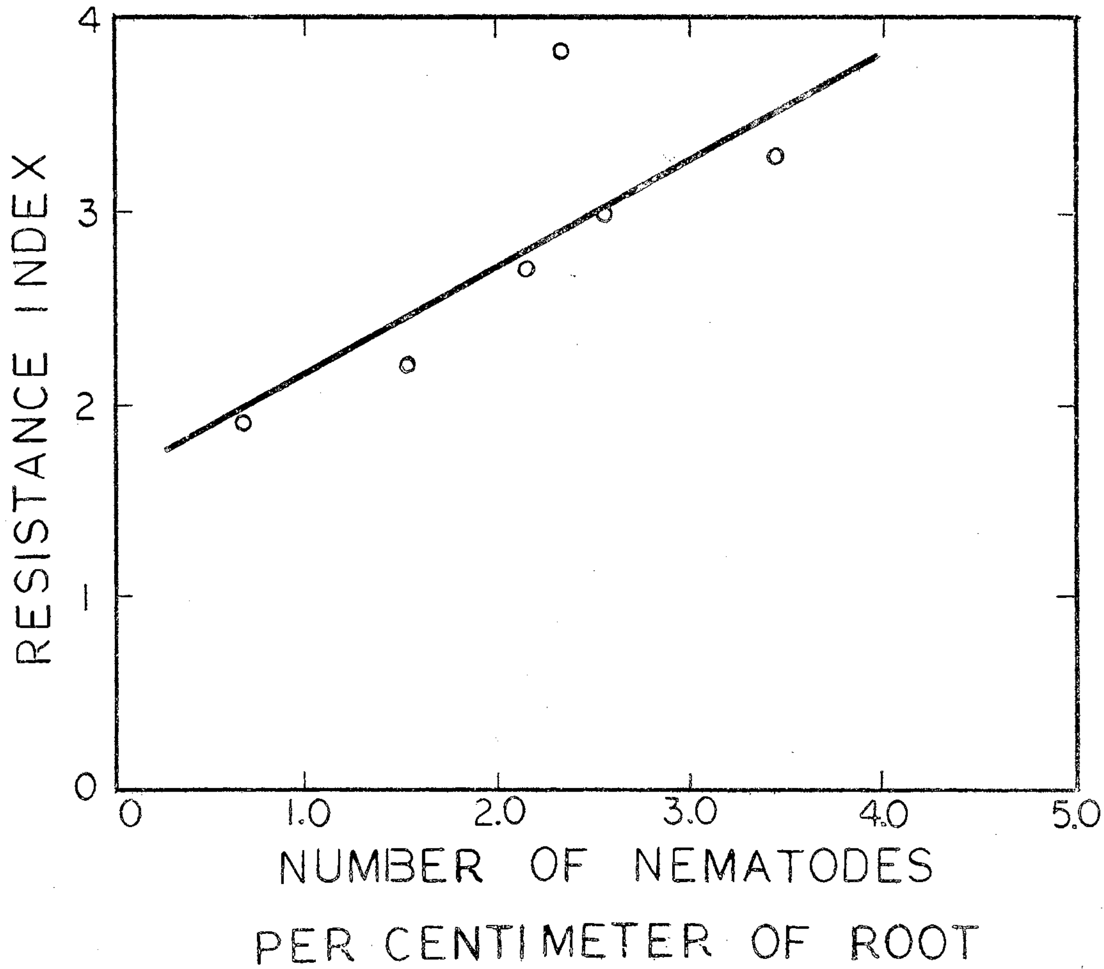

Figure 3. Scatter Diagram and Correlation Between Number of Nematodes Per Centimeter of Root and Resistance Index of Certain Lines of Ipomoea batatas and Ipomoea trifida. The Correlation Coefficient = 0.7841 The Regression Coefficient of Resistance Index on Number of Nematodes Per Centimeter of Root = 0.5974

CHAPTER V

DISCUSSION AND CONCLUSIONS

The results of this study indicate that *I. trifida* has nematode resistance. Seedlings of the clone X 1-1 x X 2-3 appear to be less resistant than the seedlings of the clone X 1-1 selfed. Either *I. trifida* or *I. batatas* was more resistant than the F_1 hybrid. The F_1 species hybrids backcrossed to *I. batatas* or to *I. trifida* were more susceptible. Since both the *I. batatas* and *I. trifida* are hexaploid, it did not appear possible with the limited number of plants to determine the genetic inheritance of resistance.

As previously mentioned, the sweet potato has several types of resistance. The investigation of Gentile et al. (11) with nematode resistance variety Tinian and its progenies, showed either no root-tip necrosis or only a trace. This was not in agreement with the root-tip necrosis data secured by Dean and Struble (8). They suggested that this represents a different type of resistance. In addition, Giamalva et al. (12) found the variety Heartogold with relatively little root necrosis associated with *Meloidogyne incognita acrita*. They further suggested that this represents still another type of resistance. However, a discussion presented by Struble et al. (23) on these arguments seems to be reliable. They concluded that various types of resistance may have had different origins and may represent one or more genetically controlled mechanisms. Several host-parasite relationships associated with resistance in *I. batatas* and *I. trifida* were found to be similar to that of Dean and Struble (8). From this study, it appears to be premature to attempt to identify or define the type of resistance found in *I. trifida*.

It is obvious that resistance reactions have some modifications with varieties. Determining resistance of sweet potato lines when using only one factor is highly improbable, however, the egg mass index appears to be a very useful tool. If this is done, it is usually carried out in the laboratory where it becomes a rather complicated procedure and requires more time. Due to the restrictions of this method under greenhouse conditions, the present research was done by a more rapid but less accurate evaluation -- resistance index. The main advantage of this method was to allow a rapid evaluation of resistance to root-knot nematode with a considerable saving in time and yet with some degree of confidence in the results. It is suggested that this method is especially suitable to preliminary evaluations.

As shown in Figure 2, the linear relationship between the resistance index and the egg mass index expressed by correlation coefficient, approximates 0.85. This demonstrates that the present work has considerable accuracy in the expression of resistance. Since this work compared resistant parental sweet potatoes with the *I. trifida* and their hybrid and backcrosses, no evidence is at hand to suggest the effect of resistance compared with susceptible lines. As to the laboratory technique, some root segments of the proper length weighed too much. In these cases it would have been better to use the individual plant basis, that is count the entire root system and then give each plant equal weight. The effect of temperature and other environmental factors on host-pathogen interactions should also be evaluated (10).

Recently, Jones (14) compared Nishiyama's (17) K123 (*I. trifida*) with the sweet potato from the standpoint of morphological, compatibility and cytological information. Progeny of both crosses were studied and Jones (14) concluded that K123 was a contemporary of *I. batatas* and not a representative of some progenitor species. It, nevertheless, appears that *I. trifida* likely represents an early form of the sweet potato. From the present investigation, *I. trifida* has as much resistance to root-knot nematode as does the sweet potato. The clone X 1-1, when selfed, appears to be homozygous for resistance. Assuming that the present sweet potatoes have been cultivated and selected from the original species since 3,000 B. C. (6), it might be said that the origin of the character of nematode resistance in *I. trifida* was probably the result of natured selection while the resistance in sweet potato was probably obtained and purposefully perpetuated by man's selection. The work of Hsu (13) showed that the clone X 1-1 selfed was 14 per cent self compatible, thus inbreeding may be effectively used. It appears that these species will require extensive supporting data in repeated experiments before one could identify actual genetic inheritance patterns. The fact that the same resistant line may vary from test to test, suggests that further tests on different nematode species or with different populations of the same species may be necessary for more precise evaluation of resistance.

CHAPTER VI

SUMMARY

The investigation on the resistance to root-knot nematode (*Meloidogyne incognita*) in *I. batatas* and *I. trifida* and hybrids involving these species has been carried out. The results show that *I. trifida* has nematode resistance as does *I. batatas*. The clone X 1-1, when selfed, appears to be the more resistant. Both the F_1 species hybrid and the backcrosses were less resistant.

A method of rapid evalution of nematode resistance is suggested, named resistance index, which is considered to be suitable for exploratory evalution because of speed and simplicity. The degree of resistance judged by resistance index was in agreement with that by egg mass index with the exception of K_2 x 183. The results also indicate that this method affords a saving in time while also providing a satisfactory confidence. The correlation between resistance index and egg mass index has been shown in Figure 2. In this instance the expression of resistance shows a rather high correlation. No direct comparison of resistant and susceptible lines was undertaken. Further studies are suggested in order to complete this work.

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