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Effect of Inoculum Density on Reproduction of Reniform Nematode *Rotylenchulus Reniformis* and on Root Development of Sweet Potato Cultivars

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EFFECT OF INOCULUM DENSITY ON REPRODUCTION OF
RENIFORM NEMATODE ROTYLENCHULUS RENIFORMIS AND
ON ROOT DEVELOPMENT OF SWEET POTATO CULTIVARS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Plant Pathology and Crop Physiology

by
Alice Karikurubu
B.S., Universite de Louvain, Belgique, 1977
Fall, 1984

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ABSTRACT

The effect of inoculum density on reproduction of the reniform nematode, Rotylenchulus reniformis, and on root development of sweet potato cultivars; W-152, Centennial, Porto Rico and Goldrush was studied under greenhouse conditions. The inoculum density had no significant effect on the root weight of the cultivars tested. The number of mature females on root systems was higher at the highest inoculum level and the number of eggs per female was significantly higher at lower densities. The number of eggs produced per root system at different inoculum densities was not significantly different on the cultivar Goldrush but was higher on the other cultivars as inoculum density was increased. Maximum eggs per root system was lowest on Goldrush and highest on Centennial.

Literature Review

Many nematodes require living organisms for food and for completion of their life cycle and are known as obligate parasites. Some cause diseases on the hosts they parasitize; however, some nematodes are free-living organisms inhabiting soil and water.

Plant parasitic nematodes are generally found in or on the roots of their hosts, or they are found in the stems, leaves and in the seeds of the plants. Because of their feeding habits, some cause severe damage to agricultural crops. The infected plants show many different symptoms associated with injuries caused by nematodes on roots as well as on above-ground parts of plants. For instance, the plant parasitic nematodes are responsible for excessive root branching, they induce the formation of root galls, root lesions, root cracking and predispose the roots to root rots. Sometimes, the host plants show a stunted growth and may have necrotic lesions or distortions of leaves and stems.

Although plant parasitic nematodes can cause diseases on plants by themselves, some of them play a major role in disease complexes involving certain other pathogens such as fungi and bacteria. Moreover, some plant parasitic nematodes are known to be the vectors of virus diseases.

Because of a great concern about economic losses of crops due to plant parasitic nematodes, several methods of control have been developed including cultural practices, biological control, resistant varieties and use of chemical agents.

The reniform nematode, Rotylenchulus reniformis, is a plant parasitic nematode found on a large number of cultivated plants and fruit trees in many tropical and subtropical countries (1, 15, 17, 29). It was first described from Hawaii (18) where it was found on the roots of cowpea plants growing in pineapple fields. The work done by Linford and Yap in 1940 (18) indicated that the reniform nematode infected the roots of 68 host plants in Hawaii. In addition, the reniform nematode has been found on a large number of cultivated plants and fruit trees in many tropical and subtropical areas including several places in the southern part of the United States of America (1, 15, 17). For instance, the reniform nematode constitutes a serious pest of cotton in Louisiana (2, 7), Alabama (21) and Texas (17), and has been reported on sweet potatoes in Louisiana (3, 20).

The reniform nematode (19) is an obligate plant parasite with only the females infecting the roots. The young females, which assume a C-shape when at rest and have a strong stylet, a sclerotized head and a narrow tail, enter the roots of host plants to about half their body length and the posterior part remains outside the roots.

During their feeding on the host, the females enlarge until the posterior part of their body swells and becomes kidney-shaped. After fertilization, the females secrete a jelly like substance around their body where they deposit their eggs. After the eggs hatch, the larvae molt twice to develop into males and females.

The reniform nematode causes heavy damage to sweet potatoes by causing root rotting, chlorosis, cracking of the fleshy roots and field losses (13, 20). Furthermore, Clark and Wright (13) reported that this parasite affected the quality by reducing the size of individual sweet potatoes and by causing the fleshy roots to crack in infested field plots.

Resistance to Rotylenchulus reniformis has been found within certain cultivars of soybeans, cotton and Irish potatoes. Rebois et al (22) showed that two soybean cultivars Pickett and Dyer were resistant to the reniform nematode. This resistance was characterized by the inhibition of female development on the incompatible host and by the inability of this nematode to induce the formation of giant cells in pericycle and phloem tissue of the resistant cultivars (24). The resistance in soybean to the reniform nematode was not necessarily the same resistance as for root knot nematode, but the cultivars resistant to the soybean cyst nematode (Heterodera glycines) were also resistant to the reniform nematode. Therefore, the authors (22) concluded that the same or linked genes for resistance

in cultivars Pickett and Dyer appeared to have been derived from Peking, a common soybean cyst nematode resistant parent and these genes were responsible for resistance to both nematodes. However, Birchfield (5) suggested that the resistance to the cyst nematode and to the reniform nematode was due to separate but probably linked genes since not all soybean cyst nematode resistant cultivars they tested were resistant to the reniform nematode. The studies of resistance in potato (Solanum tuberosum) to the reniform nematode (25) revealed high levels of resistance and tolerance. The genes for resistance to the reniform nematode seemed to be independent of the genes for resistance to races of potato cyst nematodes (Globodera rostochiensis). A high level of resistance to reniform nematode also has been found within certain selections of cotton (9). The histological responses of resistant and susceptible cultivars of cotton to the reniform nematode were studied by Carter (9). The nematodes developed equally in resistant and susceptible cultivars during 6 days after penetration. However, further development of nematodes in the resistant cultivars was inhibited because of the degeneration of the cells surrounding the feeding sites and because of the lignification of cell walls followed by cell necrosis and death of the nematodes. The reaction of different sweet potato cultivars to the reniform nematode was first described by Martin et al. (4). Working under greenhouse conditions, they reported significant

differences among sweet potato selections; although, none of these selections showed a high resistance to the reniform nematode. They found that of 24 sweet potato cultivars, Goldrush was the most resistant because it supported the least reproduction of this nematode and it was the least suitable for increase of the reniform nematode population of the cultivars tested. As Goldrush was very susceptible to root knot nematode, Melodogyne incognita, they concluded that the factors controlling resistance in sweet potatoes to Rotylenchulus reniformis may differ from those responsible for resistance to root knot nematode. Further studies on the reaction of some sweet potato selections to the reniform nematode (12) indicated that three sweet potato selections supported less reproduction of reniform nematode in the greenhouse than Goldrush (least suitable standard), while 26 selections supported greater reproduction than Centennial (highly suitable standard) and 13 selections were intermediate. Nevertheless, Goldrush was reported to be the most severely damaged under field conditions even though it had lower reniform nematode populations (13).

In summary, the reniform nematode is a serious pest of sweet potatoes and various measures of control have been used. Several sweet potato cultivars and selections have been tested for resistance to the reniform nematode in the fields and under greenhouse conditions, but neither immunity nor a high degree of resistance has been noticed up to

now. Chemical control is the most effective measure of control against the reniform nematode on sweet potatoes (16). Significant nematode population reduction and yield increase were recorded in areas treated with soil fumigants (12). In addition, the occurrence of cracks on the fleshy roots was significantly lower in fumigated plots (12). According to Birchfield and Martin (6) nematocide treatments on sweet potatoes against the reniform nematode resulted in significantly higher yields and better grades than the non treated plots.

INTRODUCTION

A number of publications (11, 16, 26, 27) dealing with certain nematode populations have reported that if a certain host plant is grown at different initial population densities, these nematodes tend to increase to similar final population levels. This phenomenon is called the ceiling level, and has been demonstrated with several species of plant parasitic nematodes, especially the cyst nematode (Heterodera S). The ceiling level has been observed by Chitwood and Feldmesser (11) and by Jones (16) in fields as well as in pot tests. Further investigation about this phenomenon has been done recently by Seinhorst (26, 27). The study of the ceiling level is of major interest because it may be used as a measure of the host status of a plant. (29) Efficient hosts are the plants on which nematodes can build up to high ceiling level while a poor host possesses a low ceiling level. Nonhosts are qualified as those plants on which the nematodes fail to reproduce at all. Preliminary studies indicated the possibility that sweet potato cultivars may have varying ceiling levels for reniform nematode reproduction (14).

The objectives of this study were to: 1) define the ceiling levels for four sweet potato cultivars, 2) determine the relationship of root growth to ceiling level,

and 3) determine the reproductive capacity of individual nematodes at varying population densities.

Materials and methods

Four sweet potato cultivars: Centennial, Porto Rico, Goldrush and W-152 were selected based on unpublished and preliminary data (14). One vine cutting was planted in each pot filled with steam sterilized 1:1 sand-soil mixture. The plants were inoculated seven days after planting with different concentrations of reniform nematode eggs: 0 used as a control, 50, 500 or 5000 per 11 cm - diameter clay pot. For the first experiment, twenty replicate pots were used for each cultivar and concentration. Due to greenhouse space limitations, the different treatments were conducted sequentially. The plants were grown for 6-8 weeks at temperatures ranging from 80°F - 100°F. After 6-8 weeks, the plants were removed from the pots and were washed gently with running tap water to free roots of soil. Later, the roots were weighed and the total root system yield of each plant was recorded. The roots were cut into small pieces and placed in a petri dish containing water. The mature females on the roots were then counted with the aid of a stereoscopic microscope. The number of eggs per egg mass for each inoculum density on each cultivar tested was obtained by placing an egg mass into a drop of 0.525% sodium hypochlorite for 4 minutes. The eggs released were then counted with the aid of a stereoscopic microscope. Five egg masses were taken at random on each root system.

The method of Hussey and Barker (14, 30) was used for the extraction of eggs from the roots with 0.525% sodium hypochlorite.

The second experiment was set up in the same way as the procedures described above, except that only five plants for each inoculum density were used but all treatments were conducted simultaneously.

The data were analyzed by one way analysis of variance and Duncan's new multiple range test.

Results

The root weight of noninoculated Goldrush plants did not significantly differ from that of plants inoculated with different densities of the reniform nematode (Table 1 and 2). However, significant differences were recorded among the numbers of mature females per root system with a higher number occurring at the 500 inoculum level in test 1 and at higher inoculum level in test 2. The differences between eggs per female at different inoculum levels were not statistically significant in test 1, but in test 2 the number of eggs per female was significantly higher at low inoculum densities. The number of eggs per plant produced on Goldrush at different inoculum densities was not significantly different.

Porto Rico supported a significantly greater number of females and eggs per root system at the highest inoculum density, but its root weight was not significantly reduced.

(tables 3 and 4). The number of eggs per female was significantly higher at lower inoculum densities.

The effect of inoculum density was highly significant ($p < 0.01$) on root weight of Centennial cultivar which was reduced at higher densities in test 1 but not test 2. The number of eggs per root system was not significantly different among inoculum densities in test 1 (table 5); however, in test 2 (table 6) a significant increase in eggs per root system occurred with the 5000 inoculum density, but the number of the eggs per female was not statistically different.

Very few mature females were found on the root system of W-152 at low densities. The root weight was not affected; the number of eggs per female was statistically similar, but the number of eggs per plant was significantly higher at the 5000 inoculum density (tables 7-8).

Discussion

Different initial densities of the reniform nematode did not significantly affect root weight of the cultivars tested including Goldrush which is sensitive to reniform nematode damage in the field (13). The higher initial population levels in naturally infested fields (13). might have severely affected the root growth of Goldrush (13). Besides, stresses such as drought are more important in the field than in the greenhouse and they often make damage from root diseases more severe.

In spite of the extensive root systems and apparent availability of feeding sites for the nematodes, the number of mature females infecting the roots seemed to be dependent on the host. Some cultivars had few nematodes and a small number of eggs on the root system (Goldrush and W-152), whereas others supported a great number of mature females and eggs (Porto Rico and Centennial). These differences in infection and in reproduction of the reniform nematode on the cultivars tested may indicate the existence of differences in host status (16) among the cultivars.

The number of eggs per root system generally increased with increasing inoculum densities. However, this study showed a lack of significant differences among final nematode populations produced on Goldrush (tables 1, 2) and Centennial (table 5) inoculated with different inoculum densities. This fact suggests the possibility that these sweet potato cultivars may have different ceiling levels (16, 29) for reniform nematode reproduction under certain conditions. The number of mature females was high at the highest inoculum level and the number of eggs per female was significantly higher at the lower densities (tables 2, 3, 4, 5) but decreased with increasing inoculum density. This higher reproduction of individual females on the cultivars at low densities may explain the fact that the final populations were not significantly different at all inoculum levels.

In his report on the relationship between population increase and population density in plant parasitic nematodes, Seinhorst (29) pointed out the importance of the influence of external conditions on population increase. This remark may help us to explain why the same cultivars resulted in different numbers of eggs per female in the two different tests. The sweet potato cultivars were grown at different periods: test 1 was conducted during Fall-Winter season; while test 2 was during Spring-Summer period. Such differences in reniform nematode reproduction on the cultivars might be due to the changes in environmental conditions. Thus, further investigations on the effect of environment on the reproduction rate of the reniform nematode on sweet potato cultivars are needed.

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TABLES

Table 1: Effects of inoculum density on Goldrush cultivar (test 1)

Inoculum Density	Average of root weight in grams		Number of Females Root System		Eggs/Female		Eggs/Plant	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
0	10.17 b	4.66	0 b	0	0 c	0	0 b	0
50	14.56 a	4.86	7 b	4.87	72 a	6.18	1328 a	475.34
500	9.36 b	2.62	55 a	39.42	23 b	14.51	1184 a	1208.41
5000	9.995 b	3.83	11 b	2.28	71 a	0.89	1348 a	853.80

1. Each mean is an average of 20 replications with one pot chosen as one replicate.
2. The means followed by the same letter are not significantly different according to Duncan's new multiple range test at 0.05 level.

Table 2. Effects of inoculum density on Goldrush cultivar (test 2)

Inoculum Density	Average of root weight in grams		Number of Females Per Root System		Eggs/Female		Eggs/Plant	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
0	30.86 a	21.10	0 b	0	0 c	0	0 a	0
50	32.10 a	10.73	6 b	12.52	166 a	81.22	1376 a	3076.82
500	28.56 a	7.46	5 b	4.76	54 bc	15.70	2880 a	4656.17
5000	21.90 a	8.95	22 a	11.19	67 b	4.61	4208 a	3272.40

1. Each mean is an average of 5 replications with one pot chosen as one replicate.
2. The means followed by the same letter are not significantly different according to Duncan's new multiple range test at 0.05 level.

Table 3. Effects of inoculum density on Porto Rico cultivar (test 1)

Inoculum Density	Average of root weight in grams		Number of Females Per Root System		Eggs/Female		Eggs/Plant	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
0	15.63 b	5.23	0 c	0	0 c	0	0 c	0
50	17.17 b	5.45	4 c	9.83	303 a	79.14	4721 bc	9781.17
500	10.98 c	6.08	21 b	11.58	105 b	20.16	10952 ab	14374.94
5000	22.44 a	10.33	58 a	6.50	63 b	21.74	13740 a	14661.27

1. Each mean is an average of 5 replications with one pot chosen as one replicate.
2. The means followed by the same letter are not significantly different according to Duncan's new multiple range test at 0.05 level.

Table 4. Effects of inoculum density on Porto Rico cultivar (test 2)

Inoculum Density	Average of root weight in grams		Number of Females Per Root System		Eggs/Female		Eggs/Plant	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
0	51.22 a	24.30	0 b	0	0 c	0	0 b	0
50	43.44 ab	12.88	8 b	11.87	318 a	32.64	1568 b	1028.55
500	26.32 b	13.66	20 b	13.75	69 b	12.15	3728 b	2329.36
5000	47.92 ab	7.36	99 a	71.29	79 b	23.13	23232 a	24809.45

1. Each mean is an average of 5 replications with one pot chosen as one replicate.
2. The means followed by the same letter are not significantly different according to Duncan's new multiple range test at 0.05 level.

Table 5. Effects of inoculum density on Centennial cultivar (test 1)

Inoculum Density	Average of root weight in grams		Number of Females Per Root System		Eggs/Female		Eggs/Plant	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
0	11.20 a	4.96	0 b	0	0 d	0	0 b	0
50	10.05 a	4.57	2 b	4.47	358 a	0.89	37284 a	72059
500	8.43 ab	5.06	125 ab	83.72	65 c	31.37	28284 a	21556
5000	6.05 b	2.93	177 a	181.30	125 b	30.43	29764 a	24290

1. Each mean is an average of 5 replications with one pot chosen as one replicate.
2. The means followed by the same letter are not significantly different according to Duncan's new multiple range test at 0.05 level.

Table 6. Effects of inoculum density on Centennial cultivar (test 2)

Inoculum Density	Average of root weight in grams		Number of Females Per Root System		Eggs/Female		Eggs/Plant	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
0	30.56 a	14.19	0 b	0	0 b	0	0 b	0
50	31.96 a	10.98	11 ab	21.18	93 a	22.06	3696 b	7777
500	31.88 a	11.64	10 ab	9.02	99 a	47.61	4752 b	4685
5000	24.60 a	11.28	39 a	37.91	90 a	16.17	40304 a	45358

1. Each mean is an average of 5 replications with one pot chosen as one replicate.
2. The means followed by the same letter are not significantly different according to Duncan's new multiple range test at 0.05 level.

Table 7. Effects of inoculum density on W-152 cultivar (test 1)

Inoculum Density	Average of root weight in grams		Number of Females Per Root System		Eggs/Female		Eggs/Plant	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
0	16.31 b	7.14	0 b	0	0 b	0	0 b	0
50	17.59 b	9.62	0 b	0	0 b	0	0 b	0
500	15.95 b	4.17	3 b	2	44 a	0.57	1288 b	1235
5000	26.96 a	11.17	17 a	3	59 a	20.55	5520 a	3232

1. Each mean is an average of 20 replications with one pot chosen as one replicate.
2. The means followed by the same letter are not significantly different according to Duncan's new multiple range test at 0.05 level.

Table 8. Effects of inoculum density on W-152 cultivar (test 2)

Inoculum Density	Average of root weight in grams		Number of Females Per Root System		Eggs/Female		Eggs/Plant	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
0	35.54 a	15.90	0 b	0	0 b	0	0 b	0
50	36.58 a	20.88	0 b	0	0 b	0	0 b	0
500	28.74 a	3.35	4 b	1.51	80 a	1.41	608 b	202
5000	28.54 a	10.86	67 a	10.86	71 a	32.38	15840 a	8807

1. Each mean is an average of 5 replications with one pot chosen as one replicate.
2. The means followed by the same letter are not significantly different according to Duncan's new multiple range test at 0.05 level.

VITA

Alice Karikurubu was born on March 1, 1953 in Rutongo City, Burundi. After completing her secondary school education, she entered L' Universite de Louvain, Belgique where she received a Bachelor of Science degree in Botany in september 1977.

After her graduation she worked as a research assistant in University of Burundi. She enrolled in Louisiana State University in Fall 1982. She is presently a candidate for the Master of Science degree in plant pathology.

EXAMINATION AND THESIS REPORT

Candidate: Alice Karikurubu

Major Field: Plant Pathology

Title of Thesis: Effect of Inoculum Density on Reproduction of Reniform Nematode Rotylenchulus Reniformis and on Root Development of Sweet Potato Cultivars

Approved:

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Date of Examination:

November 26, 1984