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Title: STUDIES OF NORTHERN ROOT-KNOT NEMATODE (MELOIDOGYNE HAPLA, CHITWOOD 1949) BIOTYPES AND SOME FACTORS ASSOCIATED WITH THEIR BIOLOGY AND PATHOGENICITY

Abstract approved: Redacted for Privacy

Dr. Harold J. Jensen

Root-knot nematodes were among the earliest plant-parasitic nematodes to be recognized. Before 1949, they were considered as the one species Heterodera marioni (Cornu 1887) Goodey 1932. In 1949 Chitwood completed a taxonomic study of the root-knot nematodes and placed them in a separate genus Meloidogyne. Economically they are one of the most important group of nematode pests and their distribution is cosmopolitan.

As in many other parasitic organisms, biotypes or physiological races exist within various species of root-knot nematodes. Most frequently pathogenic characters on given hosts have been used in making distinctions of biotypes. Meloidogyne hapla, Chitwood 1949 is a root-knot nematode species found mainly in the temperate regions. It is widely distributed in the northwestern USA on numerous hosts, but

it is also known that some populations of this nematode have different host preferences.

In the biotype study, 14 of the 15 populations of M. hapla were collected from northwestern USA; one population came from eastern USA. These populations were exposed to various hosts in the greenhouse at temperatures averaging 73.4 F day and 62.6 F night. Five biotypes were established through pattern of infection on various hosts. The resistance expected of two graminaceous plants, corn and oat, was broken by two M. hapla biotypes.

A test was made to cross males of M. hapla biotype Five (Quincy #1) and females from M. hapla biotype Three (Ontario). Larvae that hatched from eggs of this "bisexual union" did not show a physiological action different from larvae that hatched from eggs of the female parent alone. A few larvae from the "two parents" and from the female parent alone, penetrated roots of lettuce (Iceberg) but did not develop into egg laying females. It did not appear that root-knot nematode males altered the physiological character of the larvae.

Tests were made to determine the influence of host age on resistance and susceptibility to M. hapla (biotype Five, Quincy #1). Results indicated that in a resistant alfalfa (65-298), newly germinated seedlings showed evidence of resistance to infection. This resistance increased with age in one-week and two-week seedlings. In susceptible alfalfa

('Lahontan'), newly germinated seedlings, one-week and two-week seedlings were equally disposed to infection.

Tests were made to determine the influence of pH ranges 4.7, 5.9 and 7.8 on resistance and susceptibility of plants inoculated with M. hapla (biotype Five, Dayton #1). All plants (resistant and susceptible alfalfa and susceptible Rutgers tomato) grew best in pH 7.8, good in pH 5.9 and poorly in pH 4.7. These pH ranges did produce marked differences in plant growth but did not affect their basic nematode resistance or susceptibility. Influence of pH on nematodes appeared to be indirect via the host plant in which the nematodes thrive.

Studies of Northern Root-Knot Nematode (*Meloidogyne hapla*, Chitwood 1949) Biotypes and Some Factors Associated With Their Biology and Pathogenicity

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

STUDIES OF NORTHERN ROOT-KNOT NEMATODE (MELOIDOGYNE HAPLA, CHITWOOD 1949) BIOTYPES AND SOME FACTORS ASSOCIATED WITH THEIR BIOLOGY AND PATHOGENICITY

INTRODUCTION

Root-knot nematodes, Meloidogyne spp. Goeldi 1887, were among the earliest plant-parasitic nematodes to be recognized. This was so because the below-ground symptoms of root-knot disease, which show as root galls, are characteristic and easy to see. Root-knot nematodes have been given different scientific names since they were classified by Berkeley in 1855 (7). Before 1949, the root-knot nematodes were considered as one species, Heterodera marioni (Cornu 1879), Goodey 1932. Then Chitwood (9) published a revision of this group (root-knot nematodes), removed them from Heterodera and established five species and one subspecies (or variety) within the genus Meloidogyne. Other workers (12, 31, 32, 59) have since described other species, and the genus Meloidogyne now has 20 known species and three subspecies.

From an economic standpoint, root-knot nematodes are considered one of the most important of nematode groups. Their extended distribution through the tropics, subtropics and temperate regions makes them one of the most widely distributed and common agricultural pests. Hosts of Meloidogyne spp. are estimated at over two thousand plants and include forage crops, small grains and weed plants. Although

Meloidogyne spp. have such a wide host range, a single species may have specific preferences for some hosts that differ from that of another species. This was the basis for the technique which Sasser proposed in 1954 as a method of identifying different species of root-knot nematodes by host reactions, a method which obviated critical morphological study of these nematodes.

Different species of root-knot nematodes differ in their ability to attack given hosts. But when members of a single species of root-knot nematode collected from several locations differ in their ability to attack given hosts, such differences are primarily physiological. This was how the concept of physiological races within a species of a nematode arose. The occurrence of physiological races (biotypes) within a species was probably known as far back as 1939 when Sherbakoff (48) reported considerable root-knot injury to cotton, Gossypium hirsutum L., grown on land previously planted to cotton. But he observed no injury to the cotton grown on land previously planted to tomatoes, Lycopersicon esculentum Mill, even though the tomatoes had been severely injured by root-knot nematodes.

It is thought that the work of Christie (10), Christie and Albin (11), established the existence of several "races" or "strains" of the root-knot nematode. They tried to show that differences in host parasite relationships of different "races" may be manifested in at least two ways: (1) a plant may be susceptible to one race and resistant to

another; or (2) a plant may be susceptible to each of the two races but the type of root galling produced by one race may differ from that produced by the other. But since these men worked at the time when root-knot nematodes were considered as one species, Heterodera marioni, they may have actually worked on different species rather than races of one species. In recent years several workers (2, 17, 25, 36, 44, 55) have shown that different populations of certain species of a root-knot nematode vary in their ability to attack specific host plants. Such biotypes or physiological races have been shown for Meloidogyne arenaria (44), M. hapla (25), M. incognita (2, 29, 34, 44, 55) and M. javanica (25).

A study is made to further discover biotypes in M. hapla, the so-called 'northern' root-knot nematode. M. hapla is normally a temperate type of root-knot nematode found in many parts of United States of America (USA), Canada, Europe, Australia and portions of Africa. It causes much damage to vegetable crops (26), including brassica, carrot, cucumber and lettuce, melon, parsnip, potato and tomato. Effort is directed toward distinguishing populations of M. hapla from locations in the states of Oregon, Washington, Idaho and Indiana. Studies of this nature would provide informations for the existence of biotypes only for those locations where the nematode populations were collected. As more information of this nature becomes available it would help in the control of these pests through cultural methods.

One important area of study being considered in nematology today is the mating relationship between male and female root-knot nematodes. Little has been done in this area. A study is made to further investigate the roles of males in reproduction. Other factors which may affect host-parasite interactions are age of a plant and soil pH. It is generally believed that older plants may become more resistant to nematode infection. A study is designed to investigate at what time a young seedling starts building up noticeable resistance to infection. Some work has been done on the influence of pH on host-parasite interactions. So far, however, there is little agreement on the part played by soil pH. The influence of pH is covered in a study.

LITERATURE REVIEW

Use and Definitions of Some Terms

In the literature relating to nematode diseases of plants in general and root-knot in particular many terms are used. Majority of these terms have been borrowed from related sciences and used with more or less diverse meanings according to the circumstances. Thus there may not be agreement between authors as to the precise meanings of certain terms. In addition concepts change as additional information is obtained. Terms used in the study of races in nematodes are so overlapping that a reader is often confused where one term ends and the other begins. Some of these terms are biotype, biological race, physiological race, etc. In this study the above terms are taken to mean the same thing. For example, a new race of nematodes (or biotype) distinguished by its specific physiological characteristic such as pathogenicity is a physiological or biological race. It is even a pathotype too. According to the circumstances a worker may apply one term or the other. Some of the terms that are defined below were first studied in nematology texts (8, 51) and others were found in Webster English dictionary or in a dictionary of biology (1). The author has simplified these terms to make their use in this study better understood.

Race and Associated Terms

Race: a class or kind of individuals with common characteristics or habits.

Biotype: a subdivision of a race with distinguishing peculiarity.

Biological race: a segment of a nematode species which differ from the rest of the species in some physiological characteristics.

Physiological race: existence within a particular species of a number of forms which show differences in physiological characteristics.

Population and Associated Terms

Population: individuals inhabiting a specific unit of substratum (substratum = layer beneath surface soil).

Pure population = pure culture: population contains a single species only; not necessarily asceptic.

Pure line population: population derived from a single parent or set of parents (male and female parents).

Isolate (as a noun): something separated from another substance so as to obtain pure or in a free state.

Terms Concerning Host-Parasite Interactions

Host plant: plants in which a parasitic species can reproduce.

Infection: invasion and establishment of a parasitic relationship within the host proper.

Immune plant: a plant which, under conditions favorable for infection, is not invaded by larvae of the root-knot nematode.

Evidence for existence of immunity in a plant must necessarily rest on negative findings of parasite in that plant. Therefore there can be some doubt that a plant is really immune. In practice it means that parasites (in this case nematodes) were not found in the plant after a thorough examination.

Resistant plant: a host plant in which there is reduced rate of reproduction of the parasite.

Susceptible plant: a host plant in which reproduction of a parasite is normal.

Terms Concerning Reproduction

Parthenogenesis: development of embryo without fertilization of the egg by spermatozoa. Parthenogenesis is diploid if development goes on with unreduced number of chromosomes; haploid if chromosome number has been reduced by half.

Race Evolution

On practical grounds a new race is said to arise when it is distinguished by its ability to attack a certain plant usually considered resistant or immune to other individuals of the same nematode species. However, a new race while it breaks through and attacks plants immune

to the same nematode species may, at times, start to lose its ability to sustain attack on a plant usually considered very susceptible to that same nematode species. The evolution of new races is one of probable genetic changes which accordingly alter certain physiological characteristics formerly possessed by members of that species. The alteration is a full gain for a new race if plants formerly immune to members of its species are attacked by it. But at the same time the new race should sustain its attack on those plants known to be very susceptible to members of that species. It is not a full gain for a new race if its physiological change is such that its ability to attack plants known to be very susceptible to members of that species begins to wane. Findings in this study lead us to believe that (1) physiological changes could make nematodes gain something, (2) gain and lose something, (3) lose something without necessarily making additional gains. In other words, a new race could arise through progressive and/or retrogressive physiological actions on given hosts.

Biotypes

The existence of races in plant parasites is of great significance in pathology. For example, where there exists a number of races of a plant pathogen, which differ in their pathogenicity towards varieties of a host plant, breeding a resistant variety is complicated. When no suitable resistant varieties are available for some crops, crop rotation as a cultural control method becomes less practicable for those

crops. As new races show up in plant pathogens, differences in pathogenicity to given hosts are continually produced. A case, for example, is Puccinia graminis tritici, a fungus which causes black stem rust of wheat, one of the most serious of all plant diseases. More than 300 physiological races of this fungus have so far been identified. Davison and Vaughan (15) differentiated five races of bean-rust fungus, Uromyces phaseoli var. phaseoli. In bacteria and viruses, races are frequently encountered. There are virulent and avirulent races of the bacteria, Pseudomonas marginalis and Erwinia carotovora. Patino and Zaumeyer (38) showed a new strain (or race) of tobacco streak virus from peas.

In nematodes there are various genera in which races are known or suspected to occur. As in other pathogens, pathogenic characters have been used frequently in making distinctions in race studies. Several workers (5, 6, 24, 46, 49, 58) have found races in the stem nematode, Ditylenchus dipsaci (Kuhn 1857) Filipjev 1936. Some species of the cyst nematodes Heterodera Schmidt 1871, have been studied (14, 20, 27, 39, 47) and evidence of races found. Some other genera in which races were noted include burrowing nematode, Radopholus similis (Cobb 1893) Thorne 1949 (19); the citrus nematode, Tylenchulus semipenetrans Cobb 1913 (3), and the root-lesion nematode Pratylenchus penetrans Cobb 1917 (37).

The work of Christie, (10) and Christie and Albin (11) may have

had the objective of finding race or races within a single species of a root-knot nematode. But since the 'single root-knot nematode species', Heterodera marioni, with which they worked was later divided into five species of root-knot nematodes by Chitwood (9), one would assume that Christie and Albin might have worked on different species of root-knot nematodes, rather than the races within a single species.

Martin (34) tested isolates of Meloidogyne incognita Kofoid and White 1919, and Meloidogyne incognita acrita Chitwood 1949, on seven varieties of cotton and found differences ranging from no infection to severe parsitism. The nature of galling also varied significantly between different isolates of both nematodes. With the cotton variety Delta pine 15, seven isolates of M. incognita produced mature females. Similarly, with M. incognita acrita, four isolates failed, while five developed to maturity.

Van der Linde, (29) worked on M. incognita acrita from different locations in South Africa and found marked variability in growth and reproduction when tested on the same host variety. Lider (28) also found evidence of racial differences in the ability of M. incognita acrita to produce galls on vines. Colbran (13) reported distinct physiological races for M. arenaria (Neal 1889), Chitwood 1949, M. incognita and M. javanica (Treub 1885) Chitwood 1949. Goplen, Stanford and Allen (25) tested the virulence of 20 populations of Meloidogyne spp. on five

varieties of alfalfa. Existence of races were found in M. incognita acrita as well as in M. javanica.

Dropkin (17) studied the host-parasite interaction between soybeans and four Meloidogyne spp. as a basis for establishing a bioassay method for distinguishing races. Both galling and egg mass production varied as parasite or host was changed. A California population of M. incognita acrita was distinguished from a Maryland population of M. incognita acrita by differential behavior on soybean varieties.

Riggs and Winstead (41) developed new races arising by selection from within nematode species. They recovered females of Meloidogyne incognita incognita, M. incognita acrita, and M. arenaria arenaria, which had matured on resistant Hawaii 5229 tomatoes. Larvae from the females were transferred to other plants of this resistant variety and in this way they obtained populations of new races which were as virulent in the resistant tomatoes as the parents were on the susceptible varieties. Triantaphyllou (53) also reported similar results with M incognita acrita on root-knot resistant tobacco, and was able to confirm the work of Riggs and Winstead on tomatoes.

Evidence for the existence of races in M. hapla, the northern root-knot nematode are as yet not many. Colbran's work (13) in Australia included this species and he indicated finding distinct physiological races. The work of Goplen, Stanford and Allen (25) also included M. hapla and they reported finding rwo races. It is thus clear

that races in species of plant parasitic nematodes and other pathogens are by no means new nor a rarity.

Races of nematodes are of importance to the plant breeder who is trying to produce plants resistant to nematode attack and to the neamtologist who relies to some extent on the pathological reactions of the plant to identify the nematode. Successful nematode control by crop rotation also depends on knowing which crops a particular nematode will attack. A consideration of races in host-parasite relations is therefore important, especially in control problems.

BIOTYPE STUDIES OF POPULATIONS OF MELOIDOGYNE HAPLA

Preface

Existence of biotypes within species of animals is an indication of continuous evolution of living organisms. The evolution of living organisms is reflective of changes in their respective physical and biotic environments. Meloidogyne hapla, for example, is found on numerous hosts in various locations in temperate climates. In response to specific local environment, the physiological actions and host relationships of the parasite may change. When such physiological changes occur in a species, they are evident in the pattern of infection of various populations of that species on host plants. M. hapla is widely distributed in northwestern USA on numerous hosts. It has been noted that some populations of this nematode have different host preferences. Thus, some emphasis of this study is devoted to relating various populations of M. hapla to various host crops.

Materials and Methods

The 15 populations of Meloidogyne hapla used in this study came from four states of the United States of America as follows: Idaho, one population: Indiana, one; Oregon, nine; Washington, four. Except for two populations in which source materials were galled plant roots, all others came from infested soils. Where two or more populations were collected from one general area or locality, such populations

were designated by the same arabic numeral, but having different subscripts in the English alphabet; for example, 1a, 1b, etc. These 15 populations are given in Table 1.

Levels of these populations were further increased on a highly susceptible tomato variety--Stokesdale Certified Michigan Lot No. 4048. Where infested soil was the source material, it was thoroughly mixed, and, if considered necessary, added to steamed soil (three parts loam to one part sand). Two-week Stokesdale tomato plants grown in autoclaved soil, were transplanted into soils containing these populations. Where infected roots were the source materials, such roots were minced, mixed with suitable quantities of steamed soil and planted with two-week Stokesdale tomato plants. Since this was merely to provide for population increase, the amount of steamed soil mixed with each source material and the sizes of cans used depended on the degree of infestation (if soil) or infection (if plant root). Infested soils were first assayed by the Bearmann Funnel Technique, and noting the number of root-knot larvae per quart of soil; infected roots were scored by visual observation.

The tomato plants were grown for an additional 60 days at an average daylight temperature of 23 C (73.4 F) and average night temperature of 17 C (62.6 F). At the end of this period, all populations had infected the Stokesdale tomatoes and mature females had produced egg masses. These increased populations were then regarded as parent populations.

Table 1. Designations and locality sources of populations of Meloidogyne hapla used in biotype study.

Population number	Locality source	Description
1	Corvallis, Oregon	gardenia plant grown in Botany greenhouse (galled roots)
2a	Dayton (#1), Oregon	Green Vista Farms grown with barley, clover, lilies and wheat (infested soil)
2b	Dayton (#2), Oregon	field #8, grown with bushbeans (2 yrs) and carrots (2 yrs) (infested soil)
3	Huntingburg, Indiana	field of strawberry, variety "Ozark Beauty" (galled roots)
4	Madras, Oregon	field grown with barley, potato (infested soil)
5	Ontario, Oregon	field grown with potato, sugar beet (infested soil)
6	Prineville, Oregon	field grown with alfalfa for 5 yrs (infested soil)
7a	Quincy (#1), Washington	field grown with potato (infested soil)
7b	Quincy (#2), Washington	field grown with alfalfa for 5 yrs (infested soil)
7c	Quincy (#3), Washington	field grown with alfalfa for 5 yrs (infested soil)
8	Redmond, Oregon	field grown with alfalfa and grain (infested soil)
9	Rock Creek, Oregon	field grown with alfalfa (infested soil)
10	Umatilla, Oregon	field grown with alfalfa, barley (infested soil)
11	Wilder, Idaho	field grown with corn, grain, sugar beet (infested soil)
12	Woodland, Washington	field grown with cabbage, carrots (infested soil)

A pure population from each parent population was established in the following manner: five egg masses were picked from the root system of a tomato plant grown in each parent population source. Egg masses from each population were surface sterilized in ten percent solution of Clorox for one minute to free them from other micro-organisms, a procedure adopted from Loewenberg et al. (30) and Tyler (57), and washed five times with distilled water. Again two-week old Stokesdale tomato plants grown in steamed soil were removed and washed. Five surface sterilized egg masses were placed, one on the root system of each of five tomato plants, which were then replanted in #2-1/2 inch cans containing steamed soil. For convenience, all cans with eggs from the same population are referred to as a series. There were, therefore, 15 pure populations series representing the 15 parent populations. These plants grew for 60 days in controlled greenhouse temperatures of 23 C (day) and 17 C (night). Meanwhile, perineal (tail) patterns of three to four mature femlaes from each population were examined with the oil immersion objective of a compound microscope. The characteristic features of the perineal patterns established these populations of root-knot nematodes to be the species Meloidogyne hapla.

At the end of 60 days mature females in the 15 pure populations had produced eggs. A tomato plant or plants from each pure population series were removed from soil and washed in tap water. Egg

masses were collected, surface sterilized in ten percent solution of Clorox and rinsed five times in distilled water. Then egg masses from each pure population were inoculated to 13 plants, representing seven plant groups (families), (Table 2). The following procedures were adopted: each of two test plants grown in 4-inch pots of steamed soil received two pure population egg masses except in strawberry, where each plant received four egg masses and was planted singly in each pot. Each treatment (except strawberry) comprised a pot containing two test plants and four egg masses and this was replicated four times. The soil used had an average pH reading of 5.9 and the temperature averaged 23 C (day) and 17 C (night). All crop plants were grown from seed; the strawberry was grown from cuttings.

The seeds and strawberry cuttings were surface sterilized in ten percent solution of Clorox for 1-1/2 minutes. In addition to sterilizing the seeds, this concentration of Clorox solution softened the seed coats, thereby hastening seed germination. Seeds of the two alfalfa varieties, corn, muskmelon, oat and watermelon germinated quicker than the other seeds. They were therefore planted soon after sterilizing and then inoculated. Lettuce and marigold seeds were placed on damp filter paper in a Petri dish. After seeds had swollen (due to imbibition of water) and had produced radicles, approximately 1 mm long, they were planted and inoculated. Cotton and okra seeds were placed in beakers of distilled water and were planted when they had

Table 2. Sources of plant materials.

Family	Scientific name	Common name	Horticultural variety	Obtained from:
Compositae	Lactuca sativa L.	lettuce	Icegerg	Northrup, King & Co.
	Tagetes erecta L.	marigold	African Double Mix	Chas. H. Lilly Co.
Cucurbitaceae	Citrillus vulgaris Schard.	watermelon	Dixie Queen	Northrup, King & Co.
	Cucumis melo Naud.	muskmelon	Hale's Best	Northrup, King & Co.
Gramineae	Avena sativa L.	oat	Lee (c·1·2042)	Harold G. Marshall (Research Agronomist) Pennsylvania State University
	Zea mays L.	corn (sweet)	Golden Cross Bantam	Seed Laboratory, Oregon State University
Leguminosae	Medicago sativa L.	alfalfa (resistant)	65-298	O. J. Hunt (USDA), University of Nevada, Reno
	Medicago sativa L.	alfalfa (susceptible)	'Lahontan'	O. J. Hunt
Malvaceae	Hibiscus esculentus L.	okra	Emerald	Joseph Harris Co., Inc.
	Gossypium hirsutum L.	cotton	McNair 1032	J. N. Sasser, North Carolina State University, Raleigh
Rosaceae	Fragaria ananassa Duch.	strawberry	Northwest	Ralph Garren, Horticulture Dept. Oregon State University
Solanaceae	Lycopersicon esculentum Mill.	tomato	Rutgers	Northrup, King & Co.
	Capsicum frutescens L.	pepper	Calif. Wonder	Northrup, King & Co.

produced radicles 1 mm long, while pepper and tomato were transplanted as two-week seedlings. These treatments were necessary considering that percentage germination of the different seeds varied. Okra and cotton were particularly poor in germination and by presoaking them only viable seeds could be selected from a lot.

All plants were examined after 60 days $\pm$ one day from date of inoculation. Roots of plants were washed free of soil and appropriately scored. Scoring symbols and their meaning are given in Table 4; infection ratings are as described for Figure 2. Egg mass production in host plant was the most important consideration; egg mass production was verified by using the binocular dissecting microscope at 50x magnification (= 12.5 x 4). Large roots of corn, oat and strawberry were submerged in water in a photographic tray, spread out and examined with an illuminated magnifier. Root-knot galls, when seen, were removed from the root system and examined with the binocular microscope for nematode and egg contents. When roots were moderately or heavily infected, no further examination was made. But if staining a root was desired for further study, the technique employed was the one described by McBeth, Taylor and Smith (35) in 1941. In all other cases, a root sample was removed from various parts of a root system, stained and cleared for more critical study. If roots were seen with abnormalities of questionable cause and suggesting root-knot symptoms, these were included in the sample. Comparative

Table 3. Reactions of selected hosts with Meloidogyne hapla compiled from literature. R = resistant; S = susceptible.

Plant	Variety	"Status" with M. hapla	Previous workers
lettuce	Iceberg	S	(21, 22)
marigold	African Double Mix	R	(22)
muskmelon	Hale's Best	S	(22, 52)
watermelon	Dixie Queen	R	(21, 22, 42, 43, 52)
oat	Lee	R	(22)
corn (sweet)	Golden Cross Bantam	R	(21, 22, 43)
alfalfa (resistant)	65-298	R	O. J. Hunt (correspondence)
alfalfa (susceptible)	'Lahontan'	S	O. J. Hunt (correspondence)
okra	Emerald	R	(22)
cotton	McNair 1032	R (	J. N. Sasser (correspondence)
tomato	Rutgers	S	(11, 21, 22, 40, 42)
pepper	California Wonder	S	(42, 43)

Table 4. Scoring symbols and explanations for plants infected with Meloidogyne hapla in biotype study.

Symbols	Nematode	Host Reactions
0	No larvae found in roots.	negative
1	Larvae found in roots but none developed into egg laying females	galls rare
2	Egg laying females rare; larvae may be common	galls few
3	Egg laying females and egg masses are common	galls common
4	Egg laying females and egg masses very abundant	galls very abundant
	Strawberry only	
+++	Egg laying females and egg masses are abundant	galls abundant
++	Egg laying females and egg masses are common	galls common
+	Egg laying females and egg masses are trace	galls trace

Figure 1. Response of lettuce (Iceberg) roots to Meloidogyne hapla (biotypes Three and Five)

(a) Roots not infected by M. hapla (biotype Three) from Ontario, Oregon

(b) Roots infected by M. hapla (biotype Five) from Dayton #1, Oregon.

Figure 2. Infection ratings for roots of pepper (California Wonder) inoculated with three M. hapla biotypes.

(a) Roots inoculated with M. hapla (biotype Three) from Ontario, Oregon. Few larvae went into roots. Rating = 1

(b) Roots inoculated with M. hapla (biotype Four) from Prineville, Oregon. Egg laying females and egg masses rare in roots. Rating = 2

(c) Roots inoculated with M. hapla (biotype Five) from Dayton #1, Oregon. Egg laying females and egg masses common in roots. Rating = 3

(d) Roots inoculated with M. hapla (biotype Five) from Quincy #1, Washington. Egg laying females and egg masses very abundant in roots. Rating = 4.

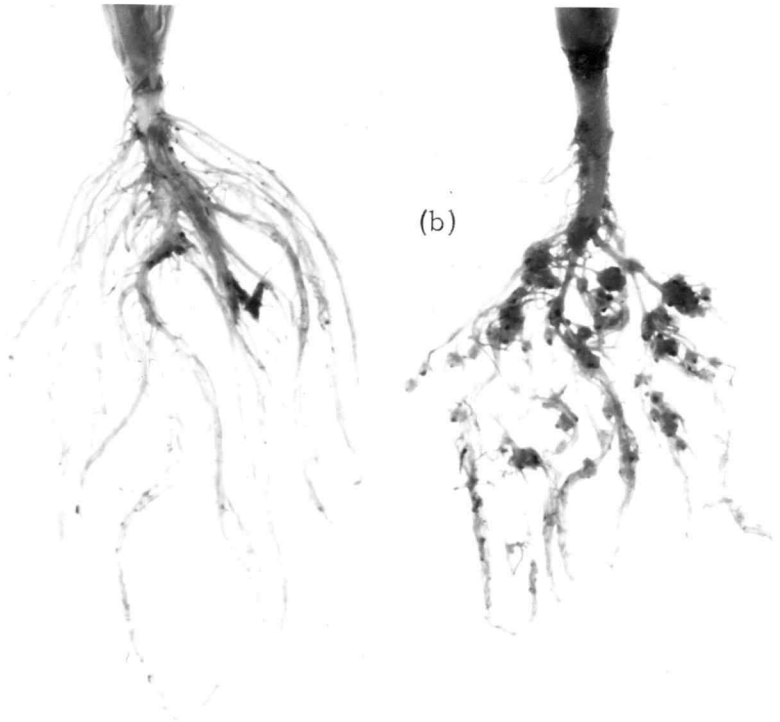


Figure 1

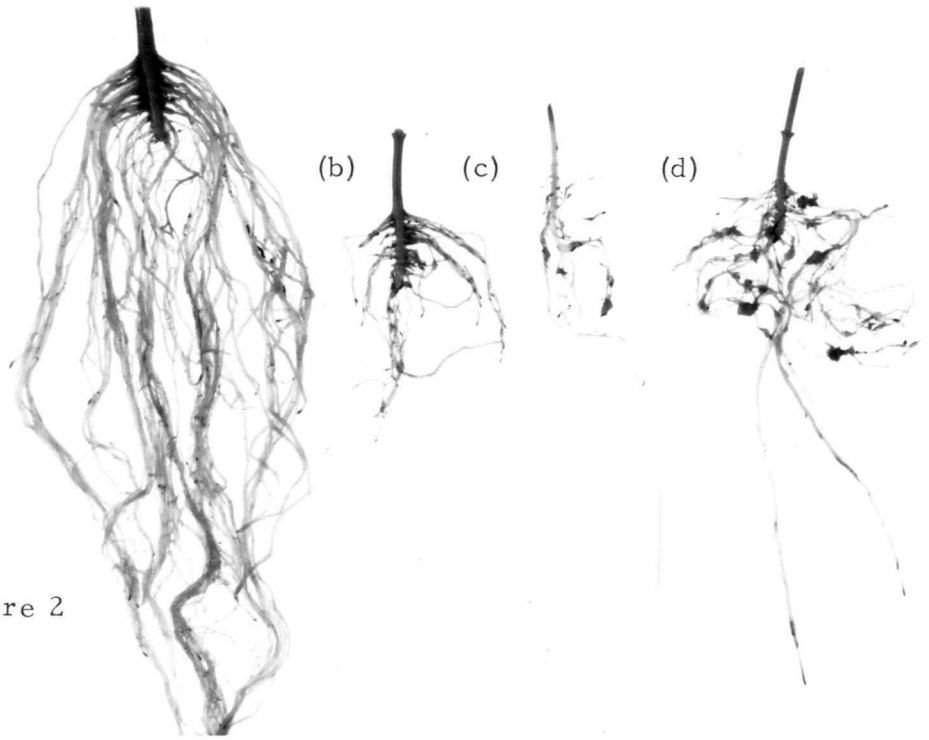


Figure 2

susceptibilities of different plants to the different populations of the northern root-knot nematode are given in Table 5.

It is not common that sweet corn, variety Golden Cross Bantam, is infected by Meloidogyne hapla. Its infection by population 5 (Ontario) was therefore of great interest. Histological preparations of the infected corn roots were made by standard methods of paraffin embedding after fixation as did Baldwin and Barker (4). Parts of infected corn roots were cut in several pieces approximately 1 cm long and fixed in formalin-acetic acid (FAA). The tissue was dehydrated with a tertiary-butyl alcohol series, embedded in Tissuemat (R) (Scientific Products, Minneapolis, Minnesota) and cut into 12 μ sections. The sections were mounted with Haupt's adhesive and four percent formalin and stained with Johansen's safranin and fast green.

Results

Results of this experiment are given in Table 5. Each number (or symbol) 0, 1, 2, 3 or 4, represents the mean of host reactions from four replicates. Other designations are explained in Table 4. The most striking differences in comparative susceptibility or resistance to the different populations were shown by the two alfalfa lines, corn and lettuce; muskmelon, oat, okra, pepper and watermelon. In the legume group the resistant alfalfa maintained its resistance to all populations except populations 3 (Huntingburg) and 8 (Redmond) (Figure 4).

Table 5. Rating of the susceptibility of plants to infection by Meloidogyne hapla. (Refer Table 4)

Plants	Population*														
	1	2a	2b	3	4	5	6	7a	7b	7c	8	9	10	11	12
Lettuce	3	4	4	3	2**	1**	2**	4	3	3	2**	3	3	3	3
Marigold	1	2	1	2	2	0**	1	1	2	2	1	1	2	2	1
Muskmelon	4	4	4	4	2**	4	1**	4	3	4	4	3	3	3	3
Watermelon	2	2	1	2	3**	3**	1	2	0**	1	2	1	1	1	1
Corn	0	0	0	0	2**	3**	0	0	0	0	0	0	0	0	0
Oat	0	0	0	0	3**	2**	0	0	0	0	0	0	0	0	0
Alfalfa (resistant)	2	2	2	3**	1	1	0**	2	1	1	3**	2	1	1	2
Alfalfa (susceptible)	3	4	4	3	2**	2**	2**	4	3	3	4	3	4	3	3
Cotton	1	1	1	1	1	0**	0**	1	1	1	1	1	1	0**	1
Okra	1	1	1	1	2	4**	0**	1	1	1	0**	1	1	2	1
Pepper	3	4	4	2**	2**	1**	2**	3	3	4	2**	4	3	3	3
Tomato	4	4	4	3	4	3	4	4	4	3	3	4	4	3	4
Strawberry	++	+++	+	+++	+	+	+	+	++	++	+++	+++	+++	++	++

*1 - Corvallis, Oregon
2a - Dayton (#1), Oregon
2b - Dayton (#2), Oregon
3 - Huntingburg, Indiana
4 - Madras, Oregon
5 - Ontario, Oregon
6 - Prineville, Oregon
7a - Quincy (#1), Washington
7b - Quincy (#2), Washington
7c - Quincy (#3), Washington
8 - Redmond, Oregon
9 - Rock Creek, Oregon
10 - Umatilla, Oregon
11 - Wilder, Idaho
12 - Woodland, Washington
** Extremes in host reactions

Figure 3. Single and multiple galls on susceptible alfalfa ('Lahontan') roots infected with M. hapla (biotype Five from Quincy #1), Washington.

(a) Single galls

(b) Multiple galls

Figure 4. Resistant alfalfa (65-298) attacked by M. hapla (biotype Two) from Redmond, Oregon.

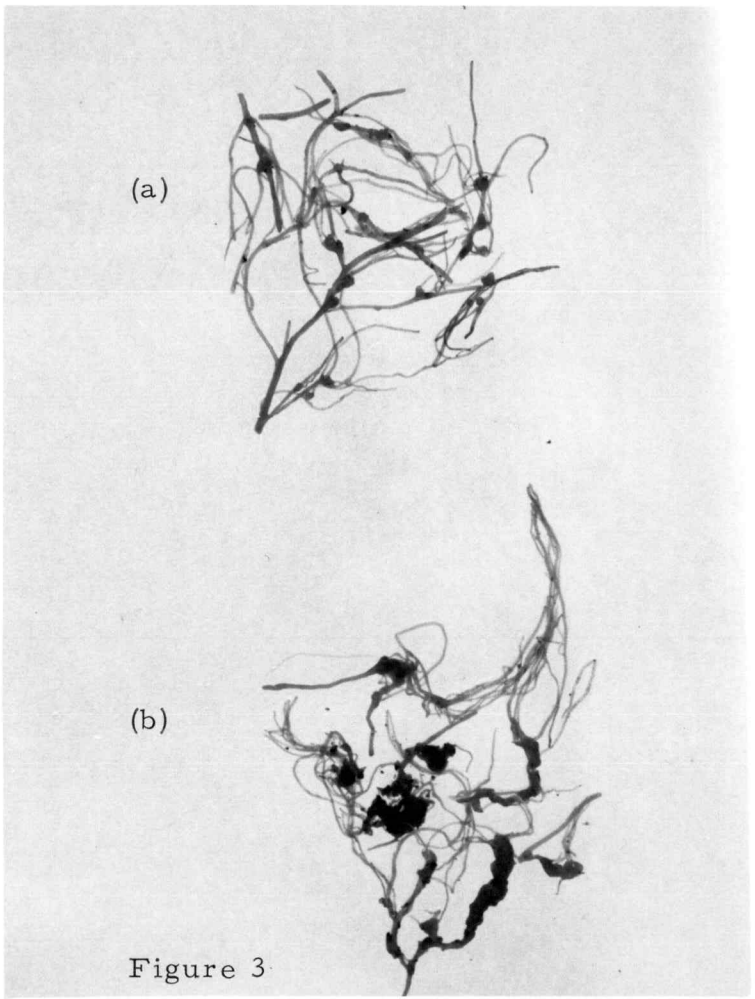


Figure 3

Figure 4

On the other hand, the susceptible alfalfa was susceptible to all populations except 4 (Madras), 5 (Ontario) and 6 (Prineville). The graminaceous plants, corn and oat, were immune to 13 of the 15 populations. Population 4 attacked corn very slightly, but its resistance was broken by population 5 (Figure 5). The reverse was true for oat; population 4 broke the resistance in oat (Figure 7), but population 5 attacked it very slightly. Lettuce was susceptible to all populations except 4, 5, 6 and 8. Of the two plants in the gourd family, muskmelon was susceptible to all populations, except 4 and 6; watermelon resisted all except populations 4 and 5. The resistance of Emerald okra was broken by population 5 (Figure 6). Populations 3, 4, 5, 6 and 8 did not do well in pepper. Each population of Meloidogyne hapla established its own pattern of infection on the given hosts. The criteria for differentiating these populations were therefore through results obtained from nematode-host interactions.

All populations produced egg masses to varying degrees on strawberry. Populations 2a, 3, 8 and 10 produced many (= +++); 1, 7b, 7c, 11 and 12 produced few (++), and 2b, 4, 5, 6 and 7a produced scanty (+) egg masses. These differences in egg mass production might have been used as a basis for differentiating the populations but the basis for evaluation was not the same as for the other plants.

On the basis of host reactions five biotypes were considered: Meloidogyne hapla from Madras (4), is a distinct biotype. For reasons

Figure 5. Roots of sweet corn (Golden Cross Bantam) attacked by M. hapla (biotype Three) from Ontario, Oregon.

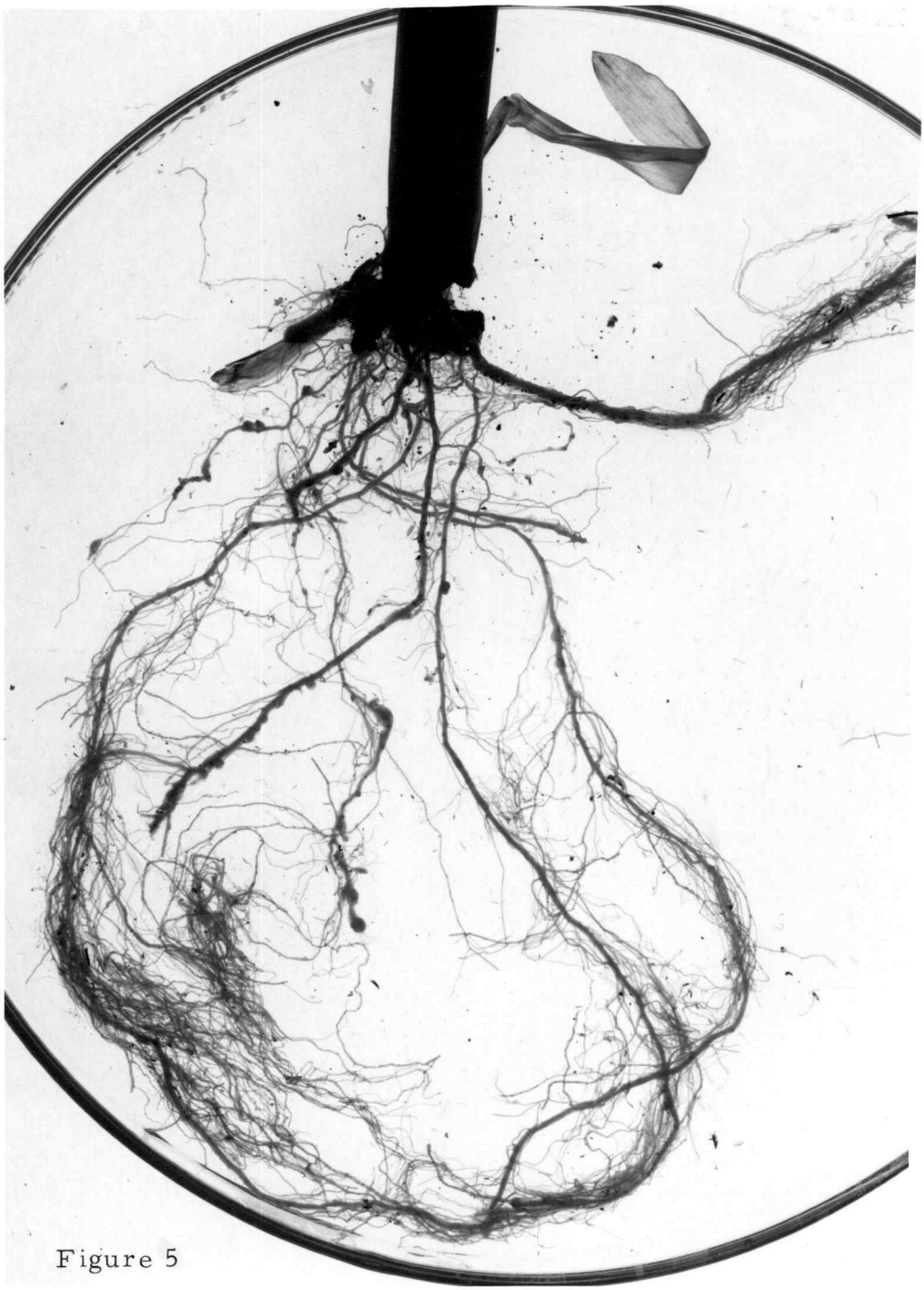


Figure 5

Figure 6. Roots of okra (Emerald) attacked by M. hapla (biotype Three) from Ontario, Oregon.

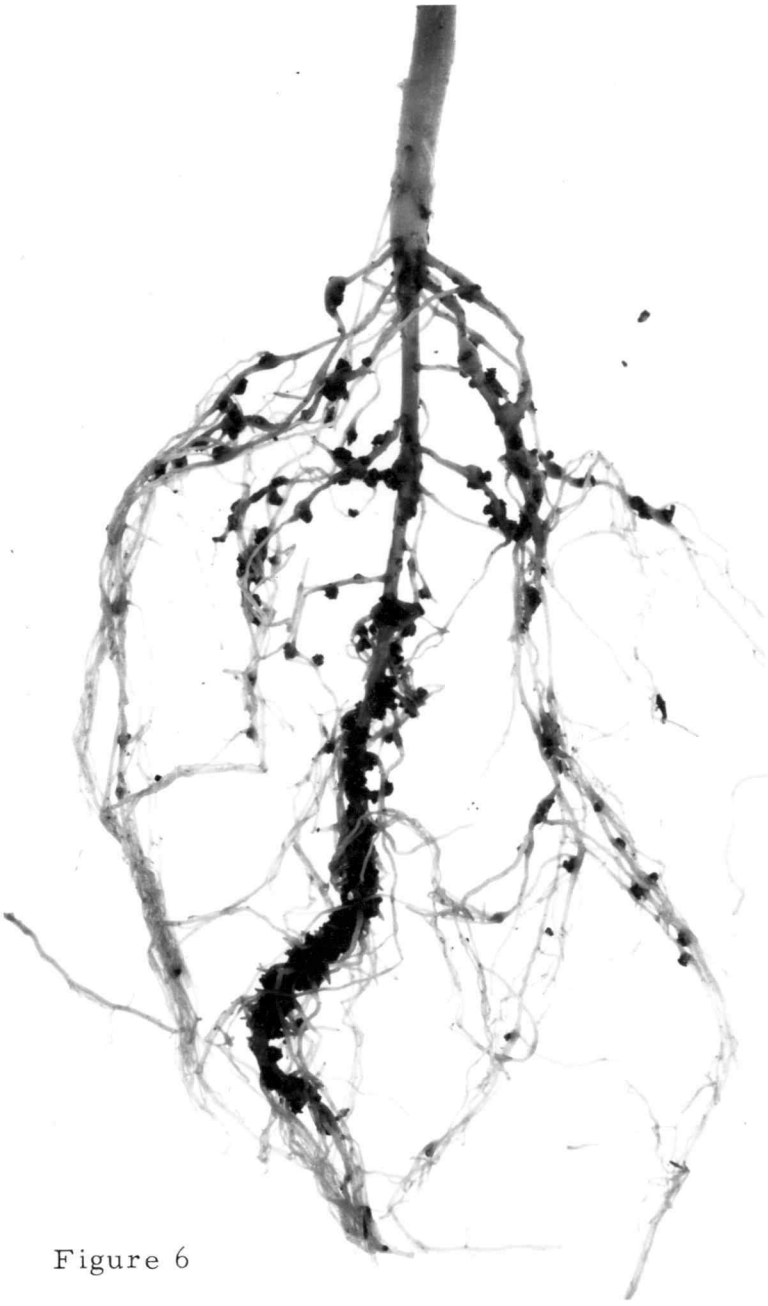


Figure 6

Figure 7. Roots of oat (Lee) attacked by M. hapla (biotype One) from Madras, Oregon (a) and (b) are portions of roots of two plants.

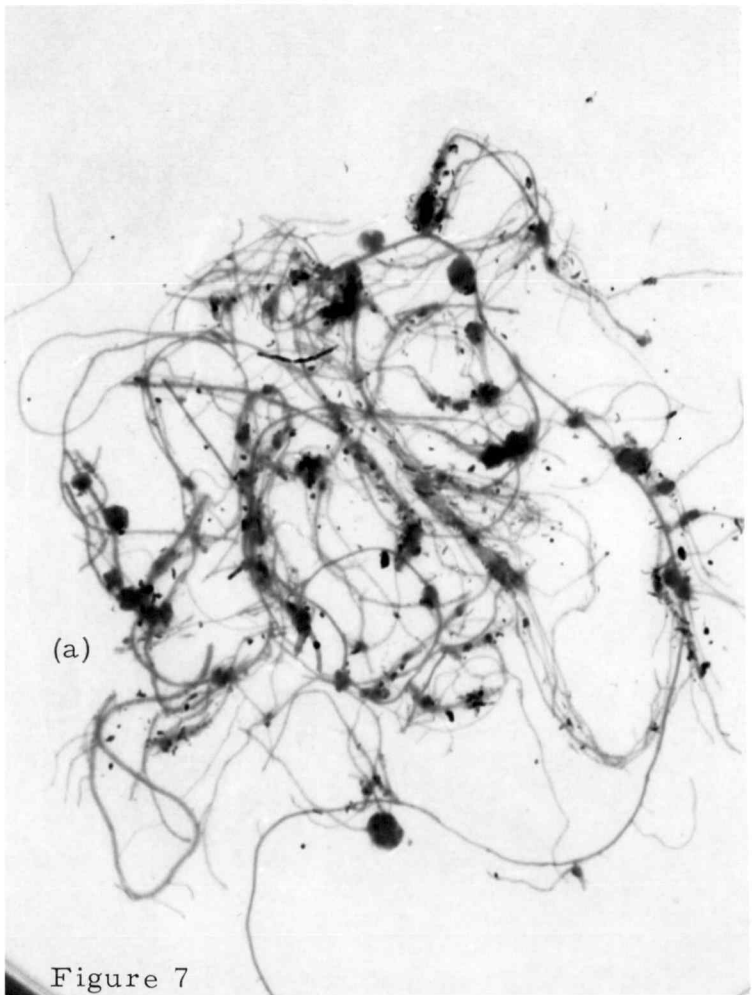
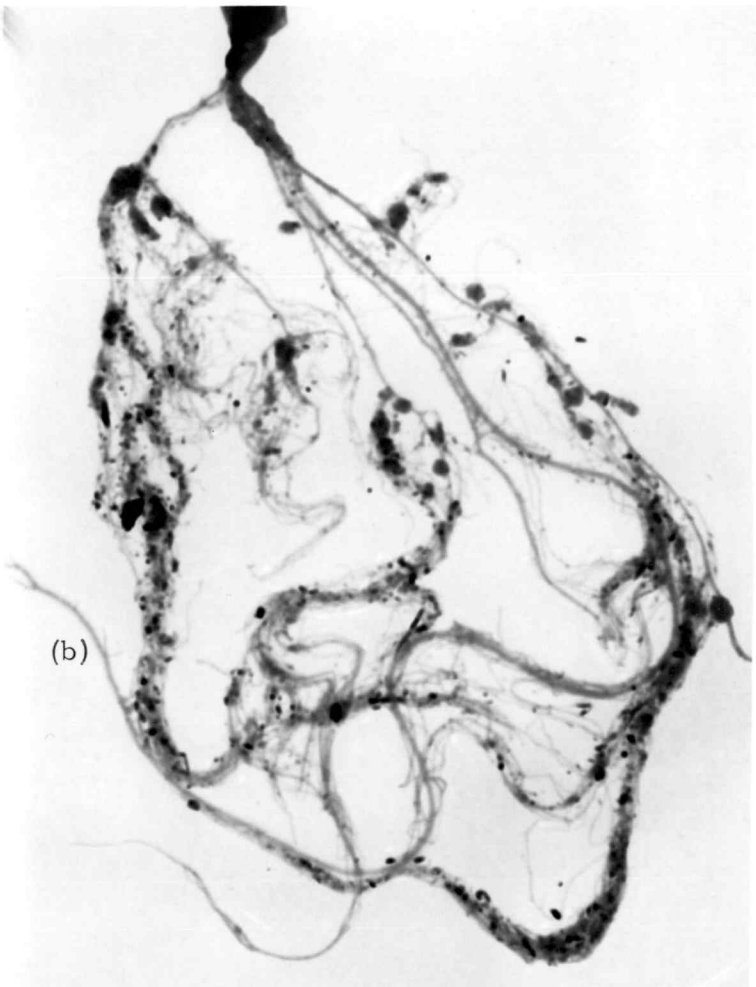


Figure 7



of simplicity, it would be called biotype One. Those from Huntingburg (3) and Redmond (8) were very close in their physiologic action and are therefore in one biotype group, = biotype Two. Ontario (5) = biotype Three; Prineville (6) = biotype Four. All others belong to a biotype group, = biotype Five. These are Corvallis (1), Dayton #1 and #2 (2a and 2B, respectively), Quincy #1, #2, #3 (7a, 7b and 7c, respectively), Rock Creek (9), Umatilla (10), Wilder (11), and Woodland (12).

In this last biotype group (= biotype Five), individual plant ratings plotted against corresponding plant, arranged in the same order, show graphs each of which is characteristic of a small letter v or its capital V joined to a capital letter W (Figure 9, a, b, c, d). This characteristic shape of the graphs differs from those of the other four biotypes, just as the graphs of these other four biotypes differ one from the other (Figure 8, a, b, c, d).

The infection of and reproduction on the strawberry, Fragaria ananassa variety Northwest was a further proof of the identity of this species of root-knot nematode. Elsewhere, except in Isreal (36) M. hapla is the only species known to infect strawberry. Strawberry was used as an indicator plant in this study and not for distinguishing the biotypes of M. hapla.

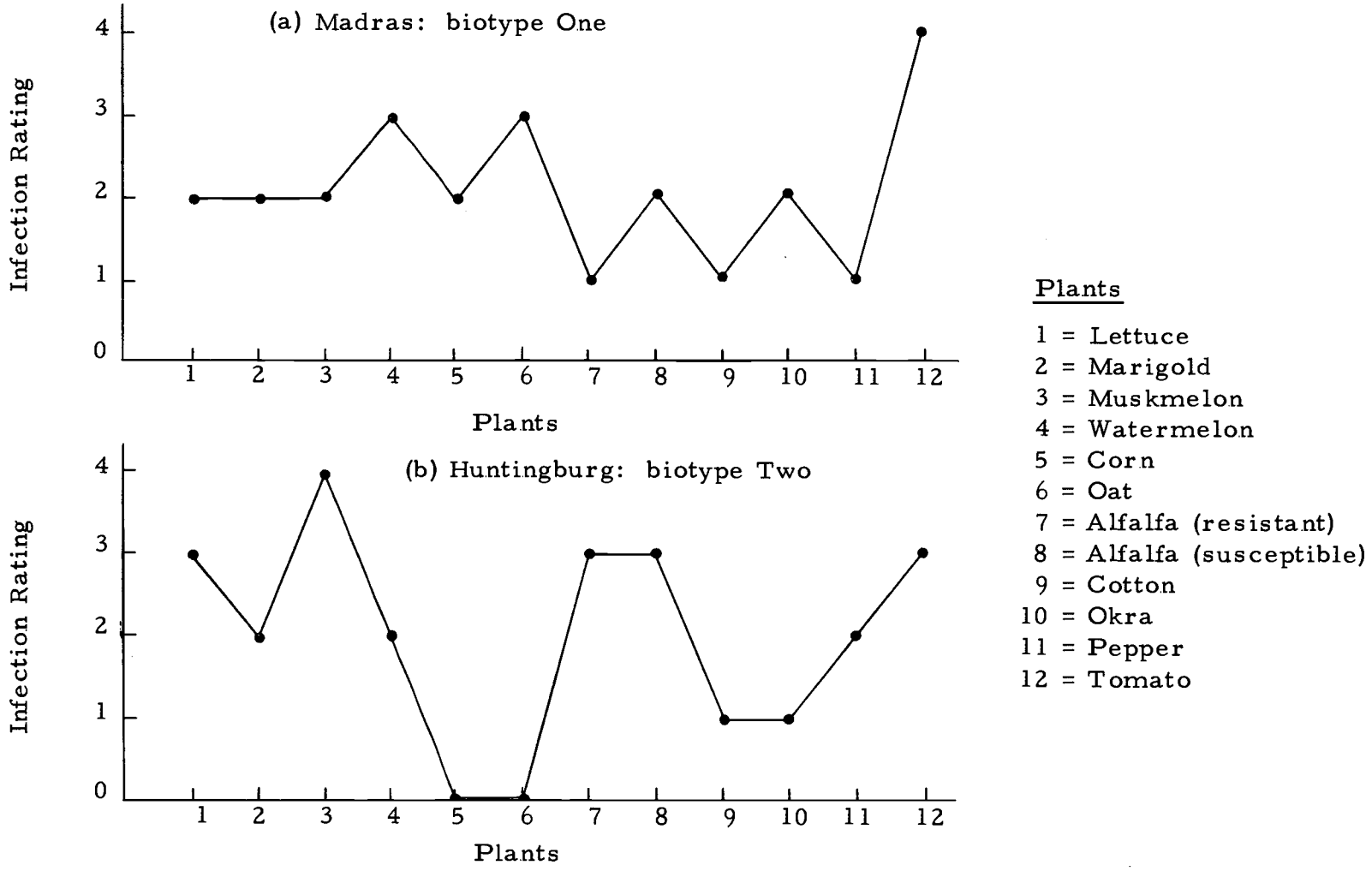
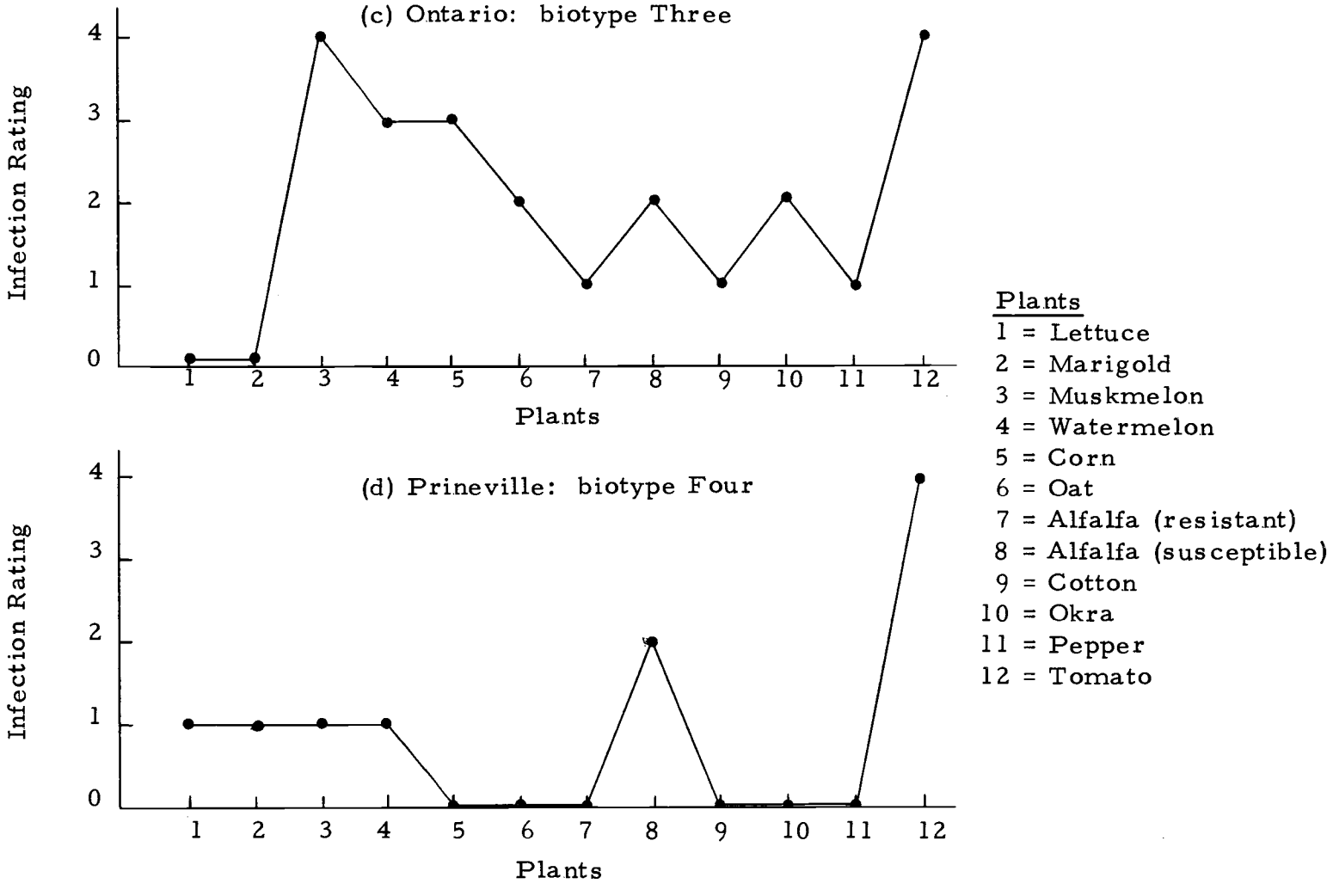


Figure 8. Graphs of M. hapla (biotypes One, Two, Three and Four) showing reactions with given host plants.


(c) Ontario: biotype Three
Infection Rating
0
1
2
3
4
1 2 3 4 5 6 7 8 9 10 11 12
Plants
(d) Prineville: biotype Four
Infection Rating
0
1
2
3
4
1 2 3 4 5 6 7 8 9 10 11 12
Plants
Plants
1 = Lettuce
2 = Marigold
3 = Muskmelon
4 = Watermelon
5 = Corn
6 = Oat
7 = Alfalfa (resistant)
8 = Alfalfa (susceptible)
9 = Cotton
10 = Okra
11 = Pepper
12 = Tomato

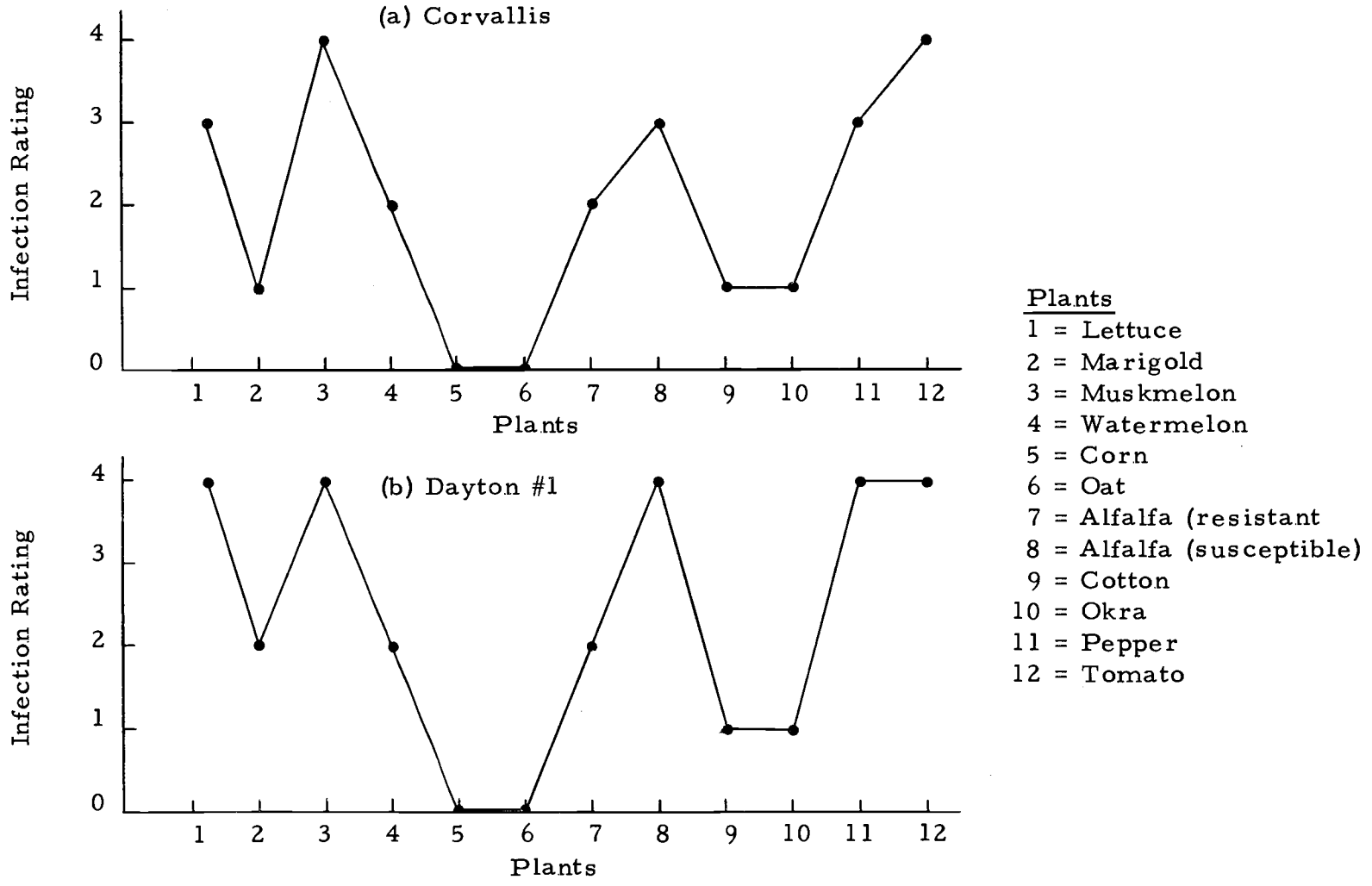
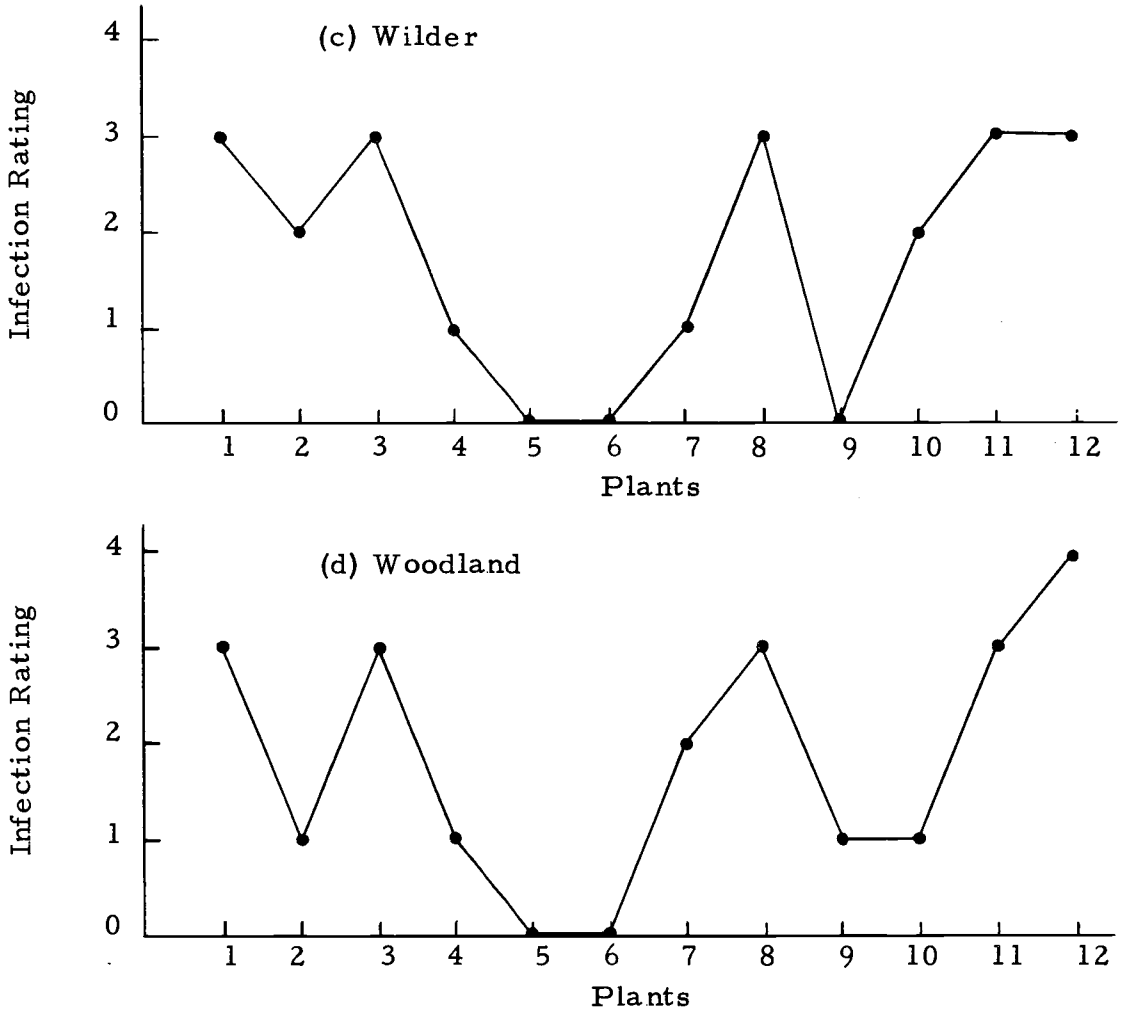


Figure 9. Typical V-W graphs of the biotype Five group showing reactions with given host plants.



Plants

1 = Lettuce
2 = Marigold
3 = Muskmelon
4 = Watermelon
5 = Corn
6 = Oat
7 = Alfalfa (resistant)
8 = Alfalfa (susceptible)
9 = Cotton
10 = Okra
11 = Pepper
12 = Tomato

Discussion

The use of host resistance or susceptibility to identify biotypes in nematode species is the basis of the most practical control measure available. In many instances, especially where large acreages of low value are involved, the use of resistant plants has been a common method for controlling the root-knot nematodes. Host resistance is built into the plant; it is economical and subject to fewer variables; neither crop nor soil residues are involved, and it is good for low value crops. The continued use of chemical methods for control does not diminish nor eliminate the need for more information regarding the comparative resistance or susceptibility of different plants to root-knot damage.

The existence of distinct biotypes of root-knot nematodes introduces new possibilities in the practical determination of the number of biotypes with which the plant breeder must contend. Equally important is the explanation of many apparent inconsistencies in experiments and field observations for which hitherto there seemed to be no logical reason. For example, Thomason and McKinney (52) found muskmelon Hale's Best variety, very lightly infected by M. hapla in contrast to the severe infection reported by Gaskin and Crittendon (22). Frequently crops regarded as highly resistant to root-knot and recommended for rotations in controlling this disease have failed to show the expected

resistance. The infection of corn by population 5 (Ontario) and oat by population 4 (Madras) are examples of such potential failures (Figures 5 and 7). Generally speaking, the existence of distinct biotypes in nematodes may aid in formulating more intelligent cultural control procedures when information is available regarding the identity and behavior of these biotypes. Such information will require (1) more biotype studies of various nematode species; (2) more research to uncover new sources of resistance in crop plants and related species; and to characterize this resistance as to the nematode and plant reactions; (3) resistance classification; by this I mean resistance of a plant at seedling stage and resistance at older or mature stage; (4) temperature is an important factor in plant resistance and should be specified for individual plants as a guideline for future investigators.

THE ROLE OF ROOT-KNOT NEMATODE MALES (BIOTYPE FIVE) IN INDUCING INFECTION AND MATURITY OF LARVAE (BIOTYPE THREE) IN A HOST PLANT

Preface

When an embryo of an animal develops without fertilization, this is parthenogenic reproduction (or simple parthenogenesis). Mulvey (45) suggested that parthenogenesis is of two types: diploid and haploid. The diploid type is commonest and occurs in some invertebrates including nematodes. Haploid type occurs in some Hymenoptera and Homoptera. Mulvey suggested that in the diploid type the egg with its diploid (unreduced) number of chromosomes is normally not fertilized, and, if fertilization occurred, the product should be a triploid and hence an abnormal zygote. The above discussion does not imply a genetic approach in this study. It is made to test Mulvey's suggestion that in nematodes an offspring starts life with hereditary material received from the female parent only. However, in the root-knot infected plants mature females are usually near the surface of the galls where they have developed. Eggs laid by the females are enclosed in a gelatinous matrix and one commonly finds one or more males in the gelatinous matrix. Why are the males there? Is their presence merely fortuitous? If their presence is more than fortuitous, then presumably they have some function, probably to fertilize the eggs laid by the females. If fertilization of eggs occurs, would the offspring of a bisexual union

differ physiologically from the parthenogenic offspring of the female? In this study, males from a different population were brought to fertilize the eggs laid by females of another population. The two populations used were (a) female larvae of population 5 (Ontario) obtained from tomato; (b) males from population 7a (Quincy #1), obtained from infected lettuce (Iceberg). Population 5 did not reproduce on lettuce in contrast to population 7a which infected and reproduced abundantly on lettuce (Table 5). Physiological characters inherited from the males could possibly permit infection and reproduction on lettuce by larvae arising from both parents. If so, the larvae should infect and reproduce on the lettuce, a physiological ability the female parents lacked. Moreover, this would show that the presence of males in the gelatinous matrix of eggs of root-knot nematodes is more than just fortuitous. But it is also possible that the male sperm (when introduced into a female) can enter and activate the egg into development without the union of sperm and egg nucleus taking place. Since no union of sperm and egg nucleus took place, no fusion nucleus is formed. Logically, embryonic development is still parthenogenic.

Materials and Methods

Two infected tomato plants (Stokesdale variety) that grew in pure culture from Ontario (population 5) were removed and washed in tap water. The infected roots were chopped into small pieces, mixed with

steamed soil and then put into a 6-inch pot. Four Stokesdale tomato seedlings (1 inch tall) grown in steamed soil for two weeks were transplanted into the 6-inch pot containing the soil mixed with infected tomato roots. The tomato seedlings were grown for another two weeks while larvae that hatched from eggs entered their root systems. Two of the four tomato plants were removed and their roots washed to determine the age of the larvae. Using a dissecting microscope (12·5 x4), these roots were shredded and larvae extracted. The larvae had developed to a stage where they still possessed more or less conical tails (spikes). Fifty mature males were picked from lettuce (Iceberg) infected with population 7a (Quincy #1). The males were washed in gentle running tap water for five minutes and transferred into a clean dish containing distilled water. The two remaining tomato plants were removed, washed in tap water and transplanted, one each, into a hole made in a damp steamed soil contained in a 4-inch pot. The 50 male root-knot nematodes were carefully poured down one hole containing a tomato plant. The hole was covered by gently pouring in steamed soil and then sprinkled with water. The other plant without males served as the control.

At six weeks from transplanting date (average temperature 23 C day, 17 C night), nematodes in each plant had laid eggs. Egg masses from both plants were removed and sterilized separately in ten percent solution of Clorox for one minute to remove other microorganisms

(Clorox (c), Clorox Company, Oakland, California, is a commercial bleach containing 5.25 percent sodium hypochlorite). Treatment procedure was adapted from Loewenberg et al. (30) and Tyler (57) who used the bleach to break up the egg sacs of Meloidogyne spp. Eight 4-inch autoclaved pots were filled with steamed soil to within one inch of the top. Each pot was planted with two seeds of lettuce (Iceberg) which were presoaked in damp filter paper for three days during which time radicles had started to emerge. Lettuce seeds in four pots were each inoculated with two egg masses from the tomato plants in which male nematodes were added; lettuce seeds in the remaining four pots were each inoculated with two egg masses removed from the tomato plant without male nematodes (control) (Figure 10). Seeds and their egg masses were covered with thin layer of steamed soil and watered. Placed in the greenhouse at an average daily temperature of 23 C, 17 C night, the plants were grown for 60 days. The lettuce plants were then removed from soil, washed and roots examined for galls and egg masses using 50x magnification.

Results

Results of this study are given in Table 6. In both treatments lettuce plants used had very tiny galls on their roots. However, very few galls per plant in both treatments actually contained undeveloped or underdeveloped larvae. No eggs were produced. Chi-square, an

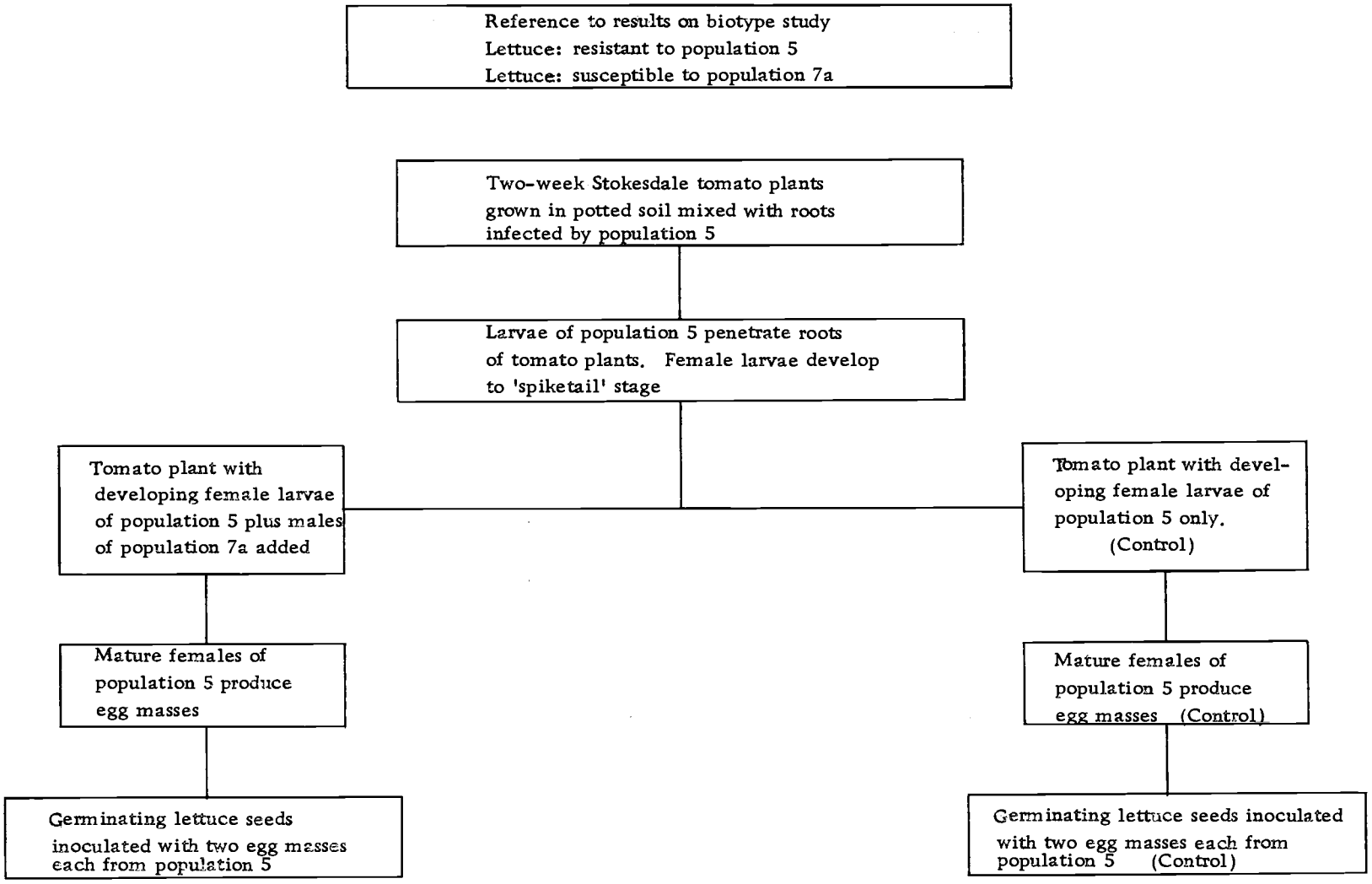


Figure 10. Schematic drawing illustrating a mating trial between males of population 7a (Quincy #1) and females of population 5 (Ontario).

index of dispersion (χ^2), was used to test the significance of gall numbers in the treatments. The χ^2 value or test statistic was not significant from the table value; Null Hypothesis (Ho) was therefore accepted.

Discussion

Results of this experiment indicate that males of population 7a (Quincy #1) brought into close contact with developing female larvae of population 5 (Ontario) played no detectable role in increasing the ability of population 5 to infect lettuce. The lettuce plants still proved resistant to the larvae of both the test females and the control females of population 5. The reasons may be that (a) the males of population 7a did not fertilize the eggs of test females of population 5; (b) if the males of population 7a did fertilize the eggs, this did not improve the infection and maturity of larvae of the test females on lettuce. Larvae from both females (test females and control females) did "browse" on lettuce roots and small number did penetrate the roots causing gall formations. But these larvae did not develop into egg laying females. From standpoint of physiology, eggs laid by both sets of females were similar and the embryonic development of these eggs was apparently parthenogenetic. Larval actions on lettuce were also similar.

It is known that eggs of root-knot nematodes could develop parthenogenetically. Both Dropkin (16) and Tyler (56) showed that generations of root-knot nematodes can be produced without males. In crop

Table 6. The role of root-knot nematode males (biotype Five) in changing the maturity of larvae of Meloidogyne hapla (biotype Three) on lettuce.

Females plus males added		Females without males added (control)	
Galls	Egg masses	Galls	Egg masses
14 *	0	8	0
10	0	14	0
16	0	14	0
8	0	6	0
18	0	12	0
16	0	8	0
12	0	14	0
10	0	6	0
104	0	92	0

Grand total of galls in both treatments = 104 + 92 = 196.

$$\mu = \frac{196}{2} = 98$$

degree of freedom (d.f.) = (2-1) = 1

$$\chi^2 = \frac{1}{98} (104 - 98)^2 + (92 - 98)^2$$

$$\chi^2 = 0.735 \text{ (not significant)}$$

$$\chi^2_{1}, .05 = 3.84$$

* A pair of results for two plants in one pot; plants inoculated separately.

plants, however, some factors could sometimes promote this parthenogenetic reproduction. For example, (a) in some swollen underground parts of plants like the potato, carrot and sugar beet, mature females are usually completely embedded in the host tissue. It is very unlikely from the relative position of the females, that the males can reach them; (b) males may be absent in some populations at one time.

In this study, it is thought that in some populations of root-knot nematodes where males may occur, and where the relative positions of females are favorable, fertilization is possible with or without fusion of male sperm and the egg nucleus (ovum). Triantaphyllou (54) had reported seeing sperm nucleus in the ovum, an example of sperm and egg nucleus not fusing.

Results obtained in this study showed, as was stated earlier, that males in contact with females (for purposes of fertilizing eggs laid by females) caused no change in the physiology of the progeny. Probably fertilization that would have yielded the expected results did not take place. In the author's view this study raises some interesting postulations. These are, (a) Sex incompatibility: the two populations (5 and 7a) used in this study are different biotypes. It is not known whether different biotypes of a root-knot nematode species are actually compatible or not; (b) egg nucleus rejuvination: it is possible that a nematode species can undergo many generations of parthenogenetic reproduction. But after a while nuclear divisions in parthenogenesis may

begin to slow down, that it would require a male sperm to 'recharge' or 'rejuvenate' it, with or without fusion. The presence of males prior to time of 'slowed nuclear division' then serves no purpose; (c) hermaphroditic species: hermaphroditism is common among the free living nematodes for example, the Rhabditoids. Such phenomenon is not yet known, but may be possible, in root-knot nematodes. It is known that intersexes can and do occur in one species, Meloidogyne javanica. Should hermaphroditism become evident in a species of root-knot nematode, the presence of males becomes unnecessary in reproduction.

INFLUENCE OF HOST AGE ON RESISTANCE OR SUSCEPTIBILITY TO *MELOIDOGYNE HAPLA* (BIOTYPE FIVE)

Preface

Seedling host plants are susceptible to infection by species of root-knot nematodes (31). At this time roots are actively growing, and are attractive to nematodes. But a mature plant may become less susceptible to infection as a result of tissue thickening. A germinating seed of a susceptible plant has little, if any, provision for protection. This study was undertaken to answer the following question: is early resistance possible in the germinating seed of a resistant plant, that is, could there be advanced fortification or premunity in the seed?

Materials and Methods

In this experiment, resistant and susceptible lines of alfalfa were used. Alfalfa seeds or seedlings were inoculated with egg masses from population 7a (Quincy #1). Results obtained in the biotype study show that population 7a infected susceptible alfalfa severely, while infection of resistant alfalfa was light (Table 5).

Three Stokesdale tomato plants infected with population 7a were held at wilting point for a fortnight by letting them remain in dry soil condition. The plants were watered very lightly and sparingly. If infected plants are kept for a fortnight at wilting point and the egg

masses (sacs) are then put into water, the larvae hatch in large numbers. In dry conditions hatching is inhibited though embryos continue to develop (18). Egg masses removed from a plant were surface sterilized in ten percent Clorox for one minute and rinsed five times in distilled water. Sixty seeds each of a resistant and susceptible alfalfa lines were also sterilized in ten percent Clorox for one minute and rinsed five times in distilled water. Eight seeds of each alfalfa were sown (two seeds per pot) into four 4-inch pots containing steamed soil. The eggs were then placed close to the alfalfa seeds (two egg masses per seed). The inoculated seeds were covered with thin layer of soil (pH 5.9) and watered lightly. The remaining seeds of the two alfalfa lines were planted in two separate 5-inch cans containing steamed soil and left to germinate. After seven days (one week) from day of germination, eight alfalfa seedlings of each line were dug, washed in tap water and inoculated with two surface sterilized egg masses from another wilting tomato plant. They were then transplanted into 4-inch pots containing steamed soil. There were two seedlings in each pot with four replications for each treatment. On the 14th day from day of germination, another set of eight seedlings of each alfalfa were dug, washed, inoculated with egg masses from a third wilting tomato, and transplanted with same number of replications. Thus the three alfalfa series planted were inoculated as seeds (zero week), one-week and two-week seedlings.

Alfalfa is usually a deep-root feeder and the main root of a one-

week seedling often grows approximately one inch below the soil level. Some difficulty was experienced in transplanting young alfalfa seedlings and after some trial and error manipulations, the following technique was developed: round holes about one mm in diameter were made in the potted damp soils. The holes were sufficiently deep to take up entire length of the main roots of the alfalfa seedlings. Each seedling dug was washed in tap water and while still wet was applied against a clean 4-inch steel rod in such manner that the end portion of the root was extended from the lower end of the rod (Figure 11). The thin film of water on the root held it against the rod. Two surface sterilized egg masses were placed on the end portion of the root and then both seedling and rod were lowered gently into the hole. When the entire root had been inserted, the rod was pressed gently against one side of the hole whereupon the seedling was disengaged from the rod. On withdrawing the rod, soil was driven carefully from all sides of the hole thus covering the hole and leaving the plant in upright position. Transplanting was done in the cool evening hours. Plants grew for 60 days from dates of planting or transplanting. Daily temperatures in the greenhouse were recorded with a thermograph. These temperatures averaged 74 F day and 66.6 F night. Plants were scored for galls and the number of egg masses. Every egg mass, large or small, was considered as an egg mass. Every gall to which no egg masses were attached was shredded. If the gall contained a mature female, it (female) was broken

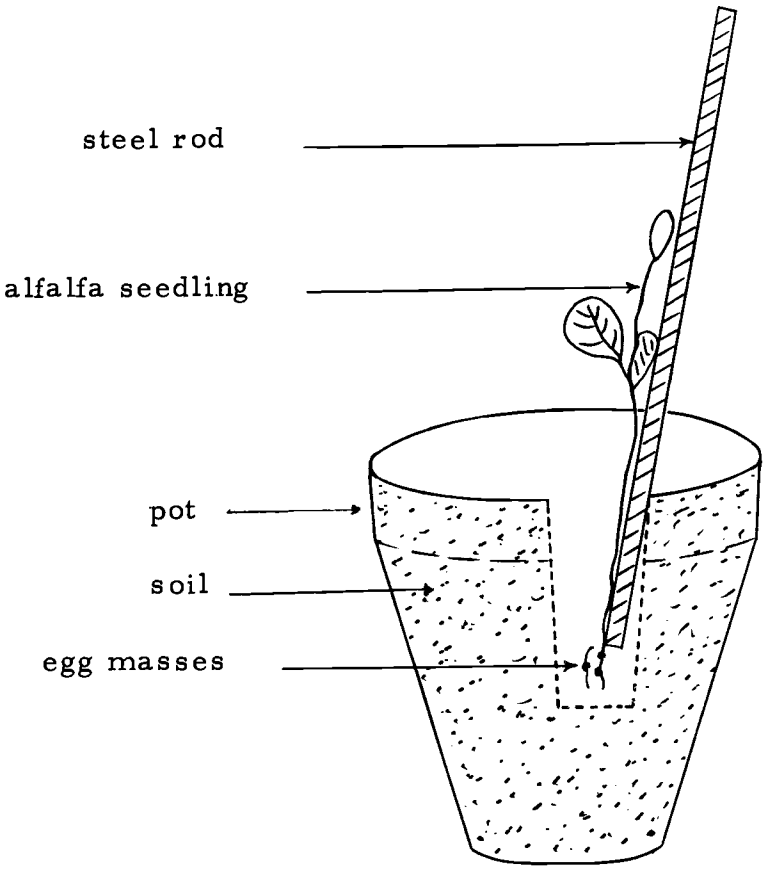


Figure 11. Inoculation and transplantation technique for alfalfa seedlings.

with dissecting needle. Eggs in a gravid female were also considered as a unit egg mass.

Results

Results of tests of the influence of host age are given in Tables 7 and 8. Chi-square (χ^2), an index of dispersion, was used to test Null Hypothesis (Ho), on the observed differences among samples. In the resistant alfalfa (Table 7), the Chi-square values for galls and egg masses indicated significant differences at the five percent level; therefore Null Hypothesis (Ho) was rejected. In the susceptible alfalfa (Table 8), Chi-square value for galls was significantly different at five percent level. Ho was therefore rejected. Chi-square value for egg masses in the samples was also significantly different at five percent level; Ho was accepted.

Discussion

In the resistant alfalfa, χ^2 values for galls and egg masses called for rejection of Ho. This means that observed differences in the seed inoculation (zero week), one- and two-week inoculations were simply not due to chance. Total number of galls formed and egg mass produced by mature females decreased considerably as the resistant alfalfa increased in age. It appears so when plants in one treatment are considered on overall basis. Larval penetration and consequent gall

Table 7. Influence of host age on resistance to infection by Meloidogyne hapla (biotype Five): I. Using resistant alfalfa (65-298).

Galls			Egg Masses		
0 week (seed)	1-week seedling	2-week seedling	0 week (seed)	1-week seedling	2-week seedling
3 *	2	0	1	1	0
0	3	0	0	2	0
2	3	0	0	1	0
3	0	0	0	0	0
4	0	0	3	0	0
1	0	0	0	0	0
4	0	0	0	0	0
7	2	0	5	1	0
24	10	0	9	5	0

Grand total = 34

$\mu = 11.3$

$\chi^2 = 25.72^{**}$

Grand total = 14

$\mu = 4.6$

$\chi^2 = 8.84^{**}$

$\chi^2_{2, .05} = 5.99$

* A pair of results for two plants in one pot; plants inoculated separately.

** Indicates a significant difference exists among the three values at the five percent level of significance.

Table 8. Influence of host age on resistance to infection by Meloidogyne hapla (biotype Five): II. Using susceptible alfalfa ('Lahontan').

Galls			Egg Masses		
0 week (seed)	1-week seedling	2-week seedling	0 week (seed)	1-week seedling	2-week seedling
14	89	28	45	97	20
65 *	29	57	81	39	42
41	62	61	26	54	56
43	56	24	65	62	28
52	25	91	75	22	111
63	38	35	59	43	41
21	27	89	24	31	103
35	28	17	28	23	22
334	354	402	403	371	423

Grand total = 1090

μ = 363.3

χ^2 = 6.723**

Grand total = 1197

μ = 399

χ^2 = 3.449

χ^2_{2} , .05 = 5.99

* A pair of results for two plants in one pot; plants inoculated separately.

** Indicates a significant difference exists among the three values at the five percent level of significance.

formation occurred readily after seed inoculation, but resistance to nematode reproduction was sometimes expressed equally by plants in the three treatments. For the resistant alfalfa, the highest number of galls produced was 24 on eight plants and this averaged 3 galls per plant. The highest number of egg masses was 9 for eight plants and this averaged 1.125 egg masses per plant. These values are very low infection ratings and showed that the resistant alfalfa retained its quality of resistance from the seed inoculation stage and apparently this resistance increased as the plant grew older. But the basic nature of resistance is unknown. Resistance could be expressed in a number of ways: (1) failure of larvae to penetrate a host plant due to mechanical barrier of the host's root cortex; (2) retardation or failure of larval development to maturity when a host plant produces very little giant cells or none at all; (3) probable post infection production of inhibitory materials, and (4) host production of repellants, etc.

In the susceptible alfalfa differences in the number of galls in the three sample treatments were significant. This resulted in the high value of χ^2 and required Ho be rejected. But such differences were mainly due to the considerable number of multiple galls, a coalesence of several single galls (Figure 3). During counting such multiple galls were regarded as 'single' galls although they bore several egg masses. It is important to note that the number of egg masses for the three treatments showed no significant difference and therefore Ho was

accepted (Table 8). Since egg mass production is a truer index of nematode welfare in a host plant, it is proper to say that the susceptible quality of the alfalfa was not affected significantly by age, that is, from seed to two-week old seedlings. Loos (31) found in Ceylon that tea plants were resistant to attack by many species of Meloidogyne after they matured into bushes, although young seedlings were attacked severely. Actually a mature susceptible plant could produce protective corky tissues in its older roots which could function as a resistant mechanism against nematode invasion; yet new emerging roots close to such older roots are subject to attack.

THE EFFECTS OF VARYING SOIL pH ON GROWTH, RESISTANCE OR SUSCEPTIBILITY OF PLANTS TO MELOIDOGYNE HAPLA (BIOTYPE FIVE)

Preface

Many plants tolerate a fairly wide range of soil pH's. But some plants often show a preference for a fairly narrow pH range through their abundance in certain localities or by better growth. Plants that thrive on acid soils, pH 4.8 or below, are acidiphilous; those usually found on soils with pH above 7 and up to 8.4 are alkaliphilous. Could not the presence or absence of host plants in a particular soil be a factor determining the levels of nematode infection? Besides plant growth, could pH interfere with the inherent resistance or susceptibility of a plant?

Materials and Methods

Three levels of soil pH were used in this study. These were pH 4.7, 5.9 and 7.8. Both 4.7 and 5.9 are in the acid range, while 7.8 is in the alkaline range. The soil with pH 5.9 was the supply source and from this beginning pH, the other two levels were prepared. After thoroughly mixing the soil source, a small sample (30 g) was analysed for extractable cations and cation exchange capacity. Calcium hydroxide, $Ca(OH)_2$, and aluminum sulphate, $Al_2(SO_4)_3$ were used in altering the pH of measured quantities of the source soil (pH 5.9). From

calculations, 13.2 grams of calcium hydroxide powder was added to a 25 lb. (or 11,350 gram) weight of source soil, and 50 grams of crystalline aluminum sulphate was added to another 25 lb. weight of source soil. In each case the soil plus the chemical compound added to it was moistened with water and thoroughly mixed in a cement mixer for 15 minutes. The mixed soil was then put in polyethylene bag, tied up firmly and left for ten days at room temperature (23 C). From time to time soil in each bag was shaken up to ensure more thorough reactions and an equilibrium. After ten days the pH of each soil was read with pH meter. Three readings for each soil were taken to obtain an average. Soil treated with aluminum sulphate gave pH 4.7; soil treated with calcium hydroxide gave pH 7.8. So three soil lots with three levels of pH were available for use, pH 4.7, 5.9 and 7.8. Two-week seedlings of Rutgers tomato and seeds of resistant and susceptible alfalfa were placed in 4-inch pots containing these soils. All seeds and seedlings were inoculated, each with two sterile egg masses from population 2a (Dayton #1). There were five replications of each treatment. Sixty days from date of inoculation, plants were examined for galls and egg masses. Final pH readings of the three soils were taken after plants were removed. Throughout the period of the experiment daily greenhouse temperatures were recorded with the thermograph. These temperatures averaged 74.5°F (day) and 66.8°F (night).

Figure 12. The effect of three pH ranges on growth of susceptible alfalfa ('Lahontan') inoculated with M. hapla (biotype Five)

pH 4.7, poor growth

pH 5.9, moderate growth

pH 7.8, excellent growth

Figure 13. The effect of three pH ranges on growth of susceptible tomato (Rutgers) inoculated with M. hapla (biotype Five)

pH 4.7, poor growth

pH 5.9, moderate growth

pH 7.8, excellent growth

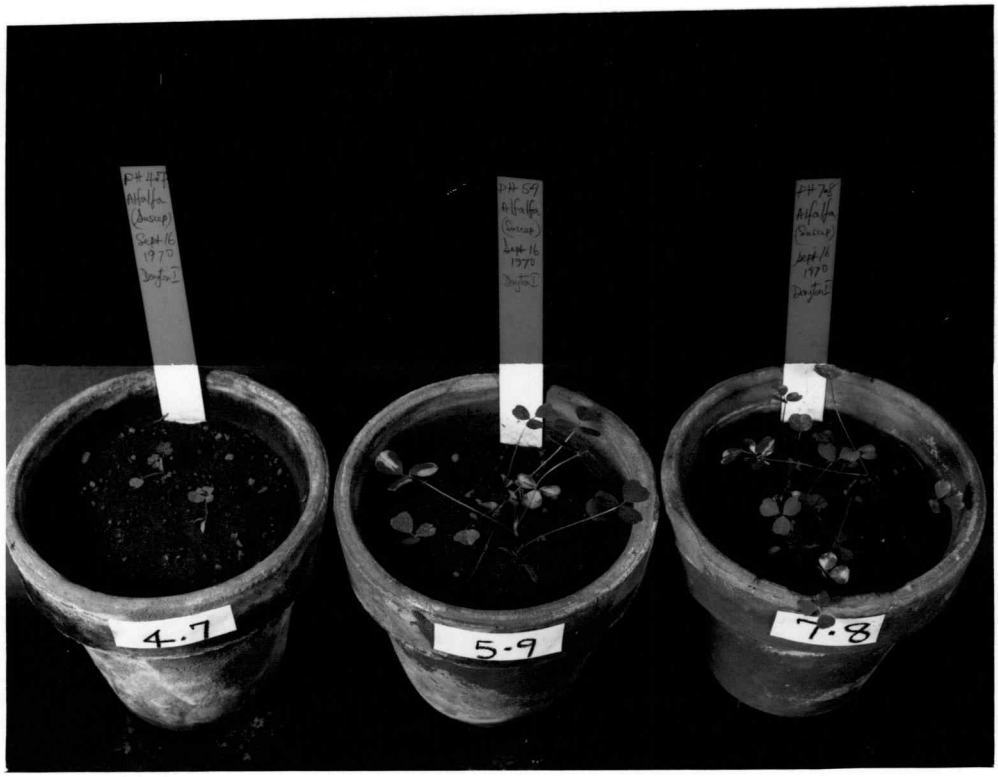


Figure 12

Figure 13

Results

Results of this experiment are given in Tables 9, 10 and 11. As in the host age experiment, Chi-square (χ^2) was used in testing the Null Hypothesis (Ho) on observed differences among treatments in the three pH ranges, 4.7, 5.9 and 7.8. In the resistant alfalfa, χ^2 values for galls and egg masses were not significant at five percent levels; therefore, Ho was accepted (Table 9). But in both the susceptible alfalfa (Table 10) and susceptible tomato (Table 11), χ^2 values for galls and egg masses were significant and in each case Ho was rejected. Both alfalfas and tomato showed by their growth a preference for higher pH's than lower pH (Figures 12, 13). In pH 4.7 plants were dwarfed and stunted. In pH 7.8 they grew abundantly, slightly less so in pH 5.9. The pH's of the soils after plants had grown in them for 60 days showed no significant variations. pH 4.7 rose slightly to 4.73; pH 5.9 came to 6.1 and pH 7.8 fell slightly to 7.77.

Discussion

Results obtained for the resistant alfalfa (Table 9) showed no significant differences occurred considering the number of galls formed and egg masses produced by nematodes in the three pH ranges. However, the total number of galls formed and egg masses produced were lower in pH 7.8. In general, the pH ranges used in the experiment

Table 9. Influence of pH on the welfare of Meloidogyne hapla (biotype Five) on resistant alfalfa (65-298).

Galls			Egg Masses		
pH 4.7	pH 5.9	pH 7.8	pH 4.7	pH 5.9	pH 7.8
2	1	2	0	0	0
9*	2	1	4	0	0
0	3	3	0	0	0
2	1	5	0	0	0
2	2	0	0	0	0
2	0	1	0	0	0
3	4	0	0	1	0
1	6	3	0	0	0
3	8	1	0	0	0
1	4	1	0	0	0
25	31	17	4	1	0

Grand total = 73

$\mu = 24.333$

$\chi^2 = 4.055$

not significant

Grand total = 5

$\mu = 1.666$

$\chi^2 = 5.200$

not significant

χ^2_{2}, .05 = 5.99

* A pair of results for two plants in one pot; plants inoculated separately.

Table 10. Influence of pH on the welfare of Meloidogyne hapla (biotype Five) on susceptible alfalfa ('Lahontan').

Galls			Egg Masses		
pH 4.7	pH 5.9	pH 7.8	pH 4.7	pH 5.9	pH 7.8
9 *	22	32	12	12	41
12	27	76	8	16	89
11	37	78	8	27	92
13	30	67	16	15	91
15	50	78	19	33	99
22	31	38	29	21	49
20	14	34	27	9	39
28	10	36	32	7	43
14	15	32	15	8	45
18	25	72	12	12	102
162	261	543	178	160	690

Grand total = 966

μ = 322

χ^2 = 242.739**

Grand total = 1028

μ = 342.666

χ^2 = 528.566**

χ^2_{2} , .05 = 5.99

* A pair of results for two plants in one pot; plants inoculated separately.

** Indicates a significant difference exists among the three values at the five percent level of significance.

Table 11. Influence of pH on the welfare of Meloidogyne hapla (biotype Five) on susceptible tomato (Rutgers).

Galls			Egg Masses		
pH 4.7	pH 5.9	pH 7.8	pH 4.7	pH 5.9	pH 7.8
29 *	47	31	38	60	27
24	44	59	35	53	62
32	58	36	39	67	55
15	53	60	19	62	71
21	60	37	26	76	28
32	21	26	30	30	23
23	51	36	26	66	39
26	39	45	34	48	39
23	21	55	18	25	48
28	35	43	21	41	39
253	429	428	286	528	431

Grand total = 1110

μ = 370

χ^2 = 55.497**

Grand total = 1245

μ = 415

χ^2 = 71.484**

χ^2_{2}, .05 = 5.99

* A pair of results for two plants in one pot; plants inoculated separately.

** Indicates a significant difference exists among the three values at the five percent level of significance.

caused no significant changes in the resistant quality of the resistant alfalfa such that any significant observable differences resulted in the number of galls formed and egg masses produced. The only significant differences were in the growths of the alfalfa plants in the different pH's. Risistant alfalfa (like the susceptible alfalfa) grew poorly in pH 4.7; best in pH 7.8 (Figure 12). If resistant alfalfa is able to tolerate a wide range of soil pH's, it did show in this experiment a preference for soils with pH above 7. The limit to which it could tolerate pH higher than 7.8 is unknown. Although the resistant alfalfa grew differently in pH's 4.7, 5.9 and 7.8, it did maintain its resistant quality, as demonstrated by the generally low degrees of infection for individual plants (Table 9).

Both the susceptible alfalfa (Table 10) and susceptible Rutgers tomato (Table 11) showed significant differences for the sums of galls and egg masses produced in the different ranges of pH. Like the resistant alfalfa, these susceptible plants grew poorly in pH 4.7, best in pH 7.8. Nevertheless, nematodes did infect and did produce egg masses on plants in all pH ranges. The amounts of infection in pH 4.7 were such that the susceptible plants were considered infected to the maximum carrying capacity of their small root systems. In Rutgers tomato especially, galling and egg mass production were generally high in all pH ranges. The tomato plants grown in pH 4.7 were infected severely considering their smaller root systems. Galls and

egg masses were, however, more numerous in plants that grew in soils of pH 5.9 and pH 7.8 (Figure 13). For the susceptible plants (tomato and susceptible alfalfa) the greater abundance of galls and egg masses in pH's 5.9 and 7.8 was, probably, not because these plants became more susceptible in the higher pH's than they were in pH 4.7. It could be that their larger root systems exposed greater areas for infection. If this is so, then the different pH ranges did not affect the basic susceptibility of the susceptible plants, although growth was visibly affected. Better growth produced larger root systems, hence larger areas were exposed to infection. It is not known whether aluminum would itself cause any depleting effect on plant growth.

On the other hand the influence of pH could affect the nematode itself such as in hatching of eggs and survival of larvae. But this experiment was not geared to show evidence in this direction. Actually, evidence for the influence of pH on plant nematodes is fragmentary and contradictory. For example, Loewenberg, Sullivan and Schuster (30) suggested that the optimum pH for hatching and survival of larvae of Meloidogyne incognita incognita in Heller's nutrient solution is 6.5. But Godfrey and Hagan (23) found that soils at a range of pH from 4.0 to 8.5 had little influence on the infestation of pineapple by a Meloidogyne species. Lownsbery (33) found no difference between population levels of Criconimoides xenoplax (ring nematode) in soil at pH 5 and 7.

Possible variations in the occurrence of plant parasitic nematodes in soils could be due to an indirect influence via the host plant rather than a direct pH influence on the nematode. Steiner (50) thinks pH may be unimportant in nematode biology, but there are not enough observations as yet to support such a view.

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