Dedicated to My Parents and Teachers

COMPARATIVE STUDIES ON THE BIOLOGY AND MORPHOLOGY OF THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* (KOFOID AND WHITE, 1919) CHITWOOD, 1949

BY

YI YI MYINT

B.Ag. (BURMA)

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Doctor of Philosophy, of the Faculty of Science,
University of London

Imperial College Field Station
Ashurst Lodge
Sunninghill
Ascot, Berks.

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ABSTRACT

The isolates of root-knot nematode, Meloidogyne incognita from different countries (Burma, Ecuador, El Salvador, Nigeria, Barbados and U.K. greenhouse population) were used in these studies and their biology and morphology were compared. Single eggmass cultures of each isolate were raised on tomato. The influence on hatching, development, and survival by different environmental factors, such as temperatures, hosts, relative humidities was determined. For isolate B (Burma), E (Nigeria) and G (I.C.I.), maximum hatch occurred at 25°C, but for isolate A (Burma), C (Ecuador) and F (Barbados) it was at 30°C. Hatching at 20°C was low for all isolates. Life cycles were studied under fluctuating temperature in the greenhouse and at 27°C constant temperature. On the whole, life cycle from J_1 to J_2 stage took 40-45 days; this is longer than previous results in the literature. Second stage juveniles survived for 2 months in sterilised soil and invaded the roots after the period.

None of the isolates reproduced on cotton. Three resistant varieties of tomato, Rossol, Fiesta and Ronita were tested against all the isolates. Ronita was found to be susceptible to two of the isolates, from Ecuador and El Salvador. Tomato, eggplant, okra, tobacco, hot pepper, lettuce and corn were tested in a host range study and two biotypes were differentiated by their pathogenicity to corn. Hot pepper (Capsicum annum) was found to be resistant for all isolates. Biological

adaptation was observed after several generations with two isolates. Morphological studies were done after continuous generations on the same and different hosts. There was no host effect from single juvenile inoculation; the perineal patterns of the isolates were similar to the parents. Measurements of juvenile in larval length, tail length, medium bulb, were different between isolates.

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GENERAL INTRODUCTION

The first record of the root-knot nematode was made by Berkeley in 1855 (Christie, 1959). He found root-knot nematodes on cucumber roots from a greenhouse in England. Within a few years, root-knot nematodes were found in different places on many crops but they were described under different names; for example Cornu (1879) described Anguillula marioni in France, Goeldi (1887) named Heterodera exigua on coffee in Brazil, Treub (1885) described Heterodera javanica on sugarcane in Java, and Neal (1889) described Anguillula arenaria from peanut in Florida. But the name Heterodera marioni was widely accepted by the most workers for root-knot nematodes and until 1949 all root-knot nematodes were known under the one species, H.marioni. In 1949, Chitwood transferred the root-knot nematodes from Heterodera to Meloidogyne and separated them into 5 species and one sub-species by identifying the pattern of concentric rings, or annulations, the perineal pattern around the anus and vulva. During the past 2-3 years, root-knot nematodes have been described from different hosts in many parts of the world, ranging through Europe, America, Africa, South America, Russia, India and Japan, and many studies of the perineal pattern and its variability have been done by different workers.

Whitehead (1968) reviewed the early taxonomic work on the genus and gave detailed descriptions of

the 23 species known to him. He studied the possibility of using measurements of certain features of the perineal patterns for identifying species. After that Franklin (1972) reviewed Meloidogyne which then included 32 species. For identification of the different species she used the length of juveniles and the ratio of the body length to width. Recently, Esser, Perry and Taylor (1976) listed a total of 35 species in their compilation. They separated Meloidogyne incognita and Meloidogyne acrita according to the perineal pattern and larval rectum shape.

Chitwood (1949) originally separated the species M.incognita into M.incognita var incognita and M.incognita var acrita. These were considered to be separated by not only the perineal pattern but also by the shape of the rectum. M.incognita acrita usually has relatively coarse striae and M.incognita incognita has fine (close together) and wavy striae; M.incognita acrita has an undilated rectum, whereas M.incognita incognita has a dilated rectum.

A lot of work has been done on the morphological variation within the species, M.incognita; between the isolates, generations, and from different hosts (Triantaphyllou and Sasser, 1960; Dropkin, 1953; Netscher, 1973; Priest and Southands, 1971). Dropkin (1953) studied M.incognita acrita and found that patterns of the offspring from a single larva were less variable than from the general population. On the other hand Triantaphyllou and Sasser (1960) found that within single eggmass cultures of isolates of 12 populations the complete range of patterns could be found even after 12 generations, and they demonstrated that the host had no effect on the patterns.

Netscher (1973) studied the variation of the larval length on the generations of a clonal strain of M.incognita. He found that there was variation of the mean larval length from one generation to the next.

The life cycle of Meloidogyne spp has been the subject of numerous investigations. Godfrey et al. (1933) first reported that the entire life cycle took 24 days in cowpea roots at the optimum temperature. According to Tyler (1933), the minimum time required for the life cycle of the root-knot nematode from larva to larva in tomato roots was 25 days at 27°C. Tarjan (1952) studied 4 species of Meloidogyne infecting snapdragon (Antirrhinum majus) roots and found no basic differences between the nematode species in regard to their development. The influence of the environment on development and sex differentiation of the root-knot nematode was determined by Davidie and Triantaphyllou (1967). They found that M.incognita larvae reached adult stage within 10 days at 35°C, and 50 days at 15°C; males increased in heavily infected roots and the effect of the environment slightly altered sex differentiation of the 2nd larval stage. However, life cycles of the root-knot nematode can be affected by the host (Godfrey and Olivera, 1932; Tarjan, 1952), nutrition (Bird, 1970; Oteifa et al 1962), and fluctuating temperatures (Bird, 1959; Dropkin, 1963).

Peacock (1959) studied the effect of larval invasion of Meloidogyne on tomato roots grown in culture solution and showed that the life cycle took almost 32 days. On soybean, Ibrahim and Massoud (1974) described the "life

cycle" of M.javanica to be 30 days, which was from J_2 to oviposition but the actual life cycle would have been approximately 35-40 days. Other work has reported the effects of γ rays and α rays on the hatching and infectivity of the M.incognita (Ishibashi and Taguchi, 1965), and Bird (1972) studied the embryogenesis of M.javanica influenced by the temperatures.

Most of the work on the influence of environmental factors has been done with temperature for root-knot nematodes. It has been shown to be an important factor which influences the ability of the root-knot nematode to penetrate, and develop within a host, and also its survival outside the host (Tyler, 1933; Godfrey et al., 1933; Thomason, 1961; Dropkin, 1963; Bergeson, 1959).

Biological Races

When biological variation occurs frequently within a nematode species, it has generally suggested that biological or physiological races are present. This assumes that they are morphologically identical. Biological races are very common in plant parasitic nematodes. Biological races are generally detected by variation in host preference and all observations on physiological differentiation refer to pathogenic behaviour. Sturhan (1971) reported that the origins of genetic variability are mutations, which generally will arise spontaneously but may even be induced in nematodes by influences of the host plant. Biological races differ in fecundity and reproductive potential (Olthof, 1968). Golden (1967) indicated that

geographical isolates of Heterodera glycines differed in morphology and biology. Attempts to distinguish biological races on morphological grounds will generally fail because morphology is often strongly influenced by the host plant and morphological characters are not usually genetically linked with physiological characters.

The potato root nematode Globodera rostochiensis was shown to exist as pathotypes by their ability to break the resistance of resistant varieties of potato (Jones, 1957). Webster (1964) assumed that there was a genetic difference between some races. Sturhan (1971) confirmed that pathotypes or biological races may be heterogenous genetically and only phenotypically similar with respect to their pathogenic abilities. He suggested that this may be due to different alleles, different genes or different gene combinations. Stone (1972) later showed that in fact two separate species existed within the Globodera rostochiensis group which are differentiated morphologically.

There are a large number of reports on the biological races of M.incognita as differentiated by their pathogenicity to various hosts (Allen, 1952; Martin, 1954; Dropkin, 1959; Riggs and Winstead, 1959; Goplen, 1959; Sasser, 1966 and others). Southards and Priest (1971) tested 17 separate collections of M.incognita from 6 hosts (tomato, tobacco, cowpea, watermelon, cotton and pepper) and found 6 races. Dropkin (1959) recommended that host-parasite interaction between soybean varieties and Meloidogyne spp could be used as a bioassay method to distinguish between races of root-

knot nematodes. Biologically different populations of a species have been described as biological races, biotypes, pathotypes or physiological variations; they all appear to be synonymous and there is no clear distinction made in the literature, the terms often being freely interchanged.

Cotton is one of the hosts of M.incognita. Martin (1966) studied the response of resistant and susceptible varieties of cotton to Meloidogyne populations in the field and a wide range of root-knot resistance was observed, Sasser (1966, 1972) has shown the effect of different hosts, including cotton, on world-wide isolates of root-knot nematodes, and found that some isolates attacked cotton but some did not. He concluded that physiological races may be in existence among the isolates of M.incognita.

Populations are known also in parthenogenetic species in other genera of nematodes such as Meloidogyne spp (Triantaphyllou, 1971; Dao, 1970); Aphelenchus avenae (Evans and Fisher, 1970c); Tylenchulus semipenetrans (Van Gundy, 1958) and Heterodera trifolii (Mulvey, 1958a). Most of the Meloidogyne spp are considered to be parthenogenetic, M.carolinensis is the only species which reproduces by amphimixis.

It is evident from the above reports that the populations of the same Meloidogyne species from different locations frequently show differences in behaviour; plants attacked in one region are not attacked in another. If root-knot nematodes are to be controlled effectively in one area using resistant crop or non-host crops, the extent of physiological variation of the nematode population in that area

should be known. Differences in reproduction rate, speed of development and degree of infestation among races or pathotypes on the same host are not unusual.

In these studies, I have attempted to find out the nature and extent of variation amongst isolates of M.incognita morphologically and biologically; to determine the influence of geographical origin, physical environmental factors, and host on the hatching, viability, development, pathogenicity and morphology on the different populations.

As my own country, Burma, is a cotton growing country, I have also tried to evaluate different cotton varieties against different M.incognita isolates (including two from Burma).

SECTION I GENERAL MATERIALS AND METHODS

(a) Single eggmass culture

The root-knot nematode, Meloidogyne incognita, populations used in this work originated from different parts of the world, Burma, Ecuador, El Salvador, Barbados, Nigeria and England. Identification was determined by examining perineal patterns of the populations. Single eggmass cultures of each isolate were initially established on 10-day old tomato seedlings (var Moneymaker) raised in heat sterilised soil in 8" diameter plastic pots. When mature, eggmasses from each isolate were sub-cultured into several pots of tomato, so that stock pure cultures of each isolate would be available whenever needed. To avoid cross infection of nematodes among the cultured pots, pots were individually placed on plastic saucers. The plants were kept on a bench in a glasshouse at an ambient temperature of 20 $\pm$ 8°C. Each of those isolates was designated as follows:-

Isolate	Country of origin	Original host
A	Burma	Egg plant
B	Burma	Tomato
C	Ecuador	Tomato
D	El Salvador	Tomato
E	Nigeria	Okra
F	Barbados	Okra
G	England (I.C.I.)	Tomato

(b) Larvae as inoculum

Tomato plants from pure cultures were taken from the pots and gently washed free of soil. Mature, brown eggmasses were picked off roots with fine forceps. Hatched larvae were collected by placing the eggmasses on small nylon sieves (40 mesh) in distilled water in watch glasses for 3-7 days at 30°C and larvae were collected each day. The number of larvae available was estimated by counting the number in 3 one ml. aliquot samples from the larval suspension. This was done in a Hawskley 1 cc. counting slide. After this count, the concentration of juveniles was readjusted so that a known number could be introduced to the plant, according to the inoculum density required. By use of a syringe larval suspensions were inoculated into the holes which were made alongside the roots of a plant.

(c) Root-knot rating

The index value used in the experiments was as follows:-

0 - free from galls

1 - trace of galls; less than 5 galls

2 - very slight galling; 5 to 25 galls

3 - moderate galling; 26 to 100 galls

4 - heavy galling; galls numerous, mostly discrete

5 - very heavy galling; galls numerous, and large

The rating system was modified from the method described by Daulton and Nusbaum (1961).

(d) Counting nematodes

To estimate numbers of nematodes in root systems, roots were cut into small pieces, mixed thoroughly and weighed; 1 gm. of root was stained for 2 minutes in boiling 0.1% acid fuchsin lactophenol. After clearing in clear lactophenol for at least 24 hours, roots were pressed between two glass plates held together by clips and examined under a stereoscopic microscope etc.

SECTION II BIOLOGICAL STUDIES OF 7 ISOLATES OF *M.incognita*

A INFLUENCE OF TEMPERATURE ON HATCHING, DEVELOPMENT, LIFE CYCLE AND INVASION OF *M.incognita* ISOLATES

INTRODUCTION

The influence of various environmental factors upon development and sex differentiation of the *Meloidogyne* genus has been extensively reported. Temperature has been shown to be an important factor which influences the ability of the root-knot nematode to penetrate and develop within a host (Thomason, 1961; Dropkin, 1963; Davide and Triantaphyllou, 1967). Temperature effects on the life cycle of root-knot nematodes and upon resistance expression in some host plants have been studied by Bird and Wallace (1965), and Holtzman (1965). Generally, the life cycle of *M.incognita* and *M.javanica* from egg to adult females takes 30 days at 25 to 28°C on a good host (Wallace, 1966). The first moult occurs in the egg and the infective 2nd stage juveniles hatch and penetrate mainly the tips of the host roots. After penetration 2nd stage juveniles settle mostly in vascular tissue where they cause the production of transfer cells upon which they feed and develop to maturity. Third and fourth stage larvae appear within 2-4 days still surrounded by the cuticle of the 2nd stage juvenile. There is no feeding at 3rd and 4th stages because the stylet is absent

until the final moult occurs. After the 4th moult, the stylet reappears and a fully developed reproductive system is present.

Bergeson (1959) studied the effect of temperature on the rate of egg hatching of M.incognita and found that rate was good at 21°C, 26°C, 32°C respectively, but at 16°C hatching rate remained nearly constant. Bird and Wallace (1965) showed that the optimum temperature for hatching of M.javanica was between 25°C and 30°C. Similar observations have been reported by Wallace (1965). The only work on two populations of M.incognita is that of Dao (1970) who studied the influence of temperature ranges and gradients on biological activities including hatching, penetration and life cycle. He observed that hatching, gall formation and reproduction were abundant and rapid at 25°C and 30°C for a Venezuelan population and 20°C for a Netherland population. Wallace (1971) reported that the optimum temperature for embryonic development of M.javanica was 15°C and for hatching was 30°C. Bird (1972) observed that the optimum temperature for embryogenesis in M.javanica lay between 25 and 30°C. It was slightly more rapid at 30°C (9-10 days), but more eggs completed development at 25°C (11-13 days). The purpose of this experiment was to determine the influence of temperature on hatching, development, life cycle and invasion of the M.incognita isolates.

MATERIALS AND METHODS

1. The development of juveniles within the egg

Infected tomato roots were dissected in distilled water. Eggs in the two-celled stage were picked by pasteur pipette; a very narrow pipette was used with a tiny aperture so that eggs could be easily picked up individually. Eggs were surface sterilized with 0.5% hibitane solution for 5 minutes, followed by three washes in sterile distilled water. Twenty eggs were placed in each solid watch glass containing distilled water and kept in a 25°C incubator. Three replicates for each isolate were used, and eggs were observed every 2 days over a 14-day period.

2. Hatching of eggs

Cultures of each isolate were established from single eggmasses on tomato (var, Moneymaker) plants in the glasshouse. Polythene rings, approximately 4 mm. thick and 2 cm. diameter were covered at one end with 45 μ aperture nylon cloth. Each ring was placed in a solid watch glass to which was added 1 ml. of distilled water. Eggmasses of intermediate age were picked up by fine forceps from the infected roots. One eggmass was placed on each nylon sieve and the solid watch glass then covered with a glass square. On hatching, larvae moved down through the nylon sieve into the water and were counted. The water in the watch glass was changed daily after counting. The experiment was done at different temperatures

of 20, 25, 30°C and also at ambient temperatures (25 ± 8°C) in a glasshouse. Temperature records in the glasshouse were obtained by means of a thermograph and also a minimum/maximum thermometer. In this experiment, eight replicates were set up at each temperature. The experiment was run for 14 days until no more hatching was obtained. The number of unhatched eggs was determined by placing the remaining eggmass in 2% NaOCl solution for about thirty minutes and then teasing out eggs in distilled water and counting.

3. Invasion and development within the roots of tomato

Small polythene tubes measuring 2 x 6 cm., with holes made on both sides to allow drainage, were used in the invasion experiment. The tubes were filled with sterilised sand and 10-day old tomato (var Moneymaker) seedlings were transplanted to each tube. A total of 200 freshly hatched juveniles were inoculated in each tube. The experiment was set up at different constant temperatures of 20°C, 25°C, 30°C and ambient temperature 23 ± 5°C. The tubes were immersed in sterilised sand in plastic trays. Two plants for each isolate at each temperature were removed after 2, 4, 6, 8 and 10 days. As the seedlings in constant temperature cabinets were grown in the dark, in order to have the similar conditions, the tray, of ambient temperature, was covered with black plastic sheet. The roots were washed gently to free from sand and stained in 0.5% cotton blue lactophenol. The larvae within the roots were counted directly after staining in cotton blue lactophenol.

4. Life Cycle

For the life cycle experiment, each pot containing a 10-day old tomato plant was inoculated with 500 freshly hatched juveniles for each isolate. Two replicates were set up and the inoculated plants were kept at 27°C in a controlled temperature room and at ambient temperature 23 ± 5°C. Recording times were taken at 5-day intervals. At the termination of each period, plant roots from inoculated plants were washed free from soil and stained in cotton blue lactophenol and stained roots were cleared in pure lactophenol. To determine the numbers of eggs/eggmass, five eggmasses from each plant were taken. The experiment was terminated 45 days after inoculation.

RESULTS

1. Development within the egg

The development of J_1 to J_2 was rapid in isolate C and D, completed within 10 days (Table 1). Isolate A, B, E and F were completed within 12 days. Among them 15% of isolate F was discarded because eggs did not produce juveniles. The majority of the isolates reached J_2 stage within 12 days. Isolate C and D stopped hatching after 10 days' exposure but the total percentage hatch was similar for all the isolates.

Table 1 Percentage of Eggs in which Juveniles Developed to J_2 Stage over a 12-day period at 25°C [1)]

M. incognita Isolates	Total % Development		
	Days		
	8	10	12
A	22	43	90
B	18	66	95
C	26	95	95
D	32	94	94
E	20	72	92
F	8	36	85
G	10	40	90

1) Mean of 3 replicates

2. Hatch

Maximum hatch from isolates B, E, G occurred at 25°C, and isolates A, C, D, F at 30°C (Table 2). There was less hatch at ambient temperatures for all isolates apart from isolate D. Maximum hatch from all of the isolates occurred at both 25°C and 30°C within 8 days. Isolates A, C, D and G stopped hatching after 12 days at 30°C. At 20°C, all of the isolates started hatching after 3 days' incubation and rate of hatching was slow compared to higher temperatures. With the exceptions of isolates F and G the amount of hatch of the isolates differed significantly at all the temperatures. The hatching of isolates F & G was not significantly different at 20°C from that at ambient temperatures. At ambient temperatures, isolate D had a significantly greater egg hatch compared to other isolates.

3. Invasion and development

Percentage of invasion was low in this experiment for all isolates. Invasion started 2 days after inoculation at 25° and 30°C with most of the isolates, but at 20°C and ambient temperature in the glasshouse, very little or no invasion was observed until 4 days after inoculation (Table 3, Fig. 1). For isolates B, E and G, penetration was highest at 25°C. The percentage invasion for most of the isolates was highest 10 days after inoculation. The maximum percentage of invasion (16%) was observed for isolate B at 25°C and the lowest percentage (6.9%) of invasion was observed for

Table 2 Percentage Egg Hatch of M.incognita Isolates at Different Temperatures[1)]

Isolates	Temperatures			
	20°C	25°C	30°C	Ambient
A	28	38	60	14
B	34	68	41	16
C	25	40	77	21
D	21	54	74	43
E	21	61	48	16
F	20	48	63	21
G	20	74	57	22

1) Mean of 8 replicates

L.S.D. (Isolate) 1% = 3.442

(Temp) 1% = 2.061

Table 3 Invasion of Tomato Roots by 7 M.incognita Isolates at Different Temperatures

20°C

Isolates	Days				
	2	4	6	8	10
A	0	10	24	32	38
B	0	2	12	32	38
C	0	2	14	21	32
D	0	12	16	20	29
E	0	8	22	29	32
F	0	6	14	28	34
G	0	4	12	20	38

25°C

Isolates	Days				
	2	4	6	8	10
A	4	15	32	39	52
B	6	18	36	44	56
C	4	12	25	36	42
D	0	4	12	29	42
E	1	4	26	34	48
F	0	10	21	31	48
G	2	12	20	31	54

Table 3 (continued)

30°C

Isolates	Days				
	2	4	6	8	10
A	6	20	26	38	54
B	6	12	28	40	49
C	4	20	34	39	52
D	8	12	29	39	56
E	0	6	18	23	44
F	6	14	26	37	58
G	0	10	18	28	48

Ambient Temperature

Isolates	Days				
	2	4	6	8	10
A	0	8	18	29	32
B	1	8	20	31	34
C	1	10	21	38	48
D	4	10	28	40	50
E	0	2	14	28	32
F	0	9	20	32	45
G	0	8	16	22	36

Fig(1). Percentage invasion of *M.incognita* isolates on tomato roots at 20° and 25° C

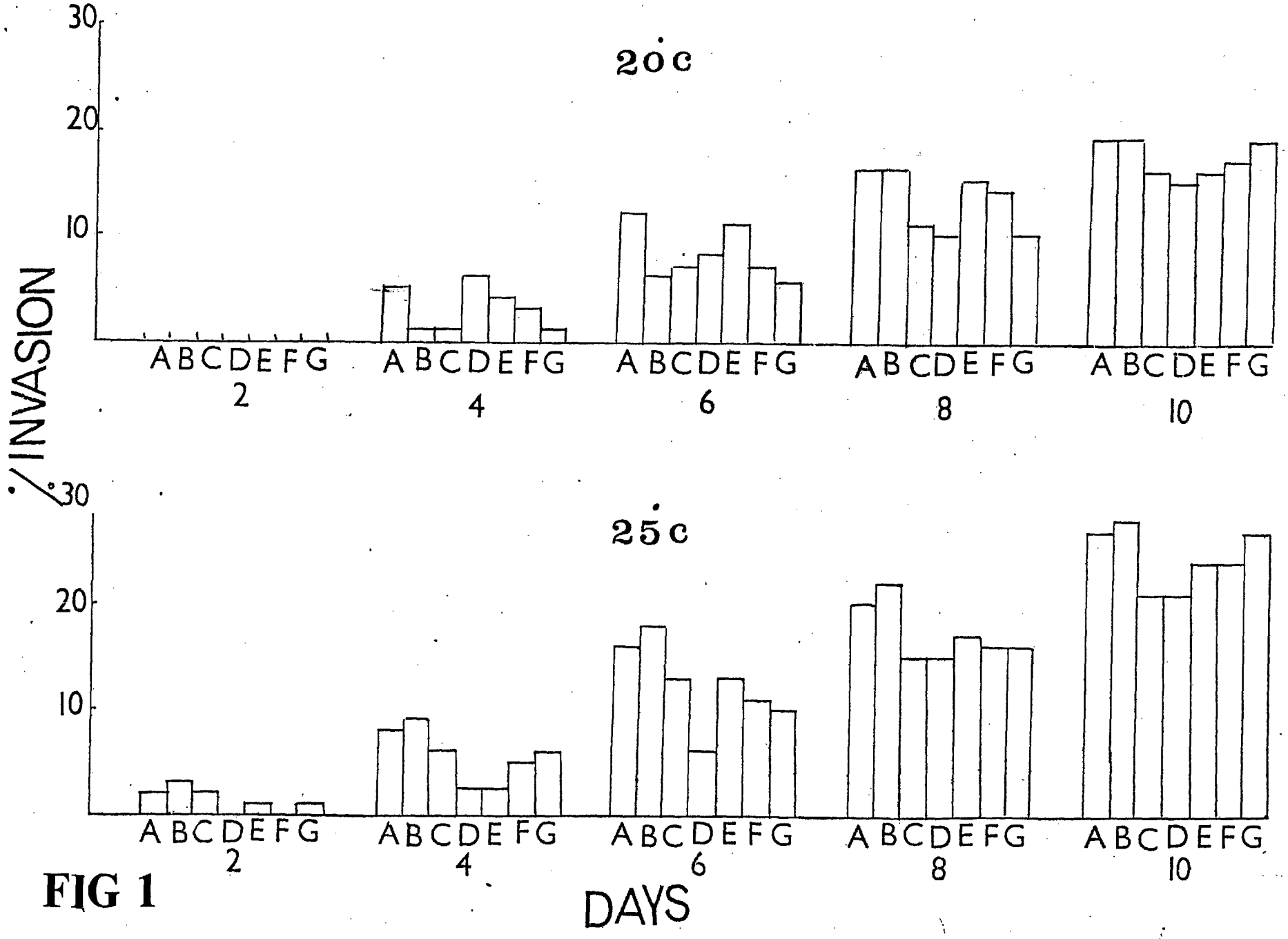


FIG 1

Fig (2). Percentage invasion of M.incognita isolates on tomato roots at 30° C and ambient temperature

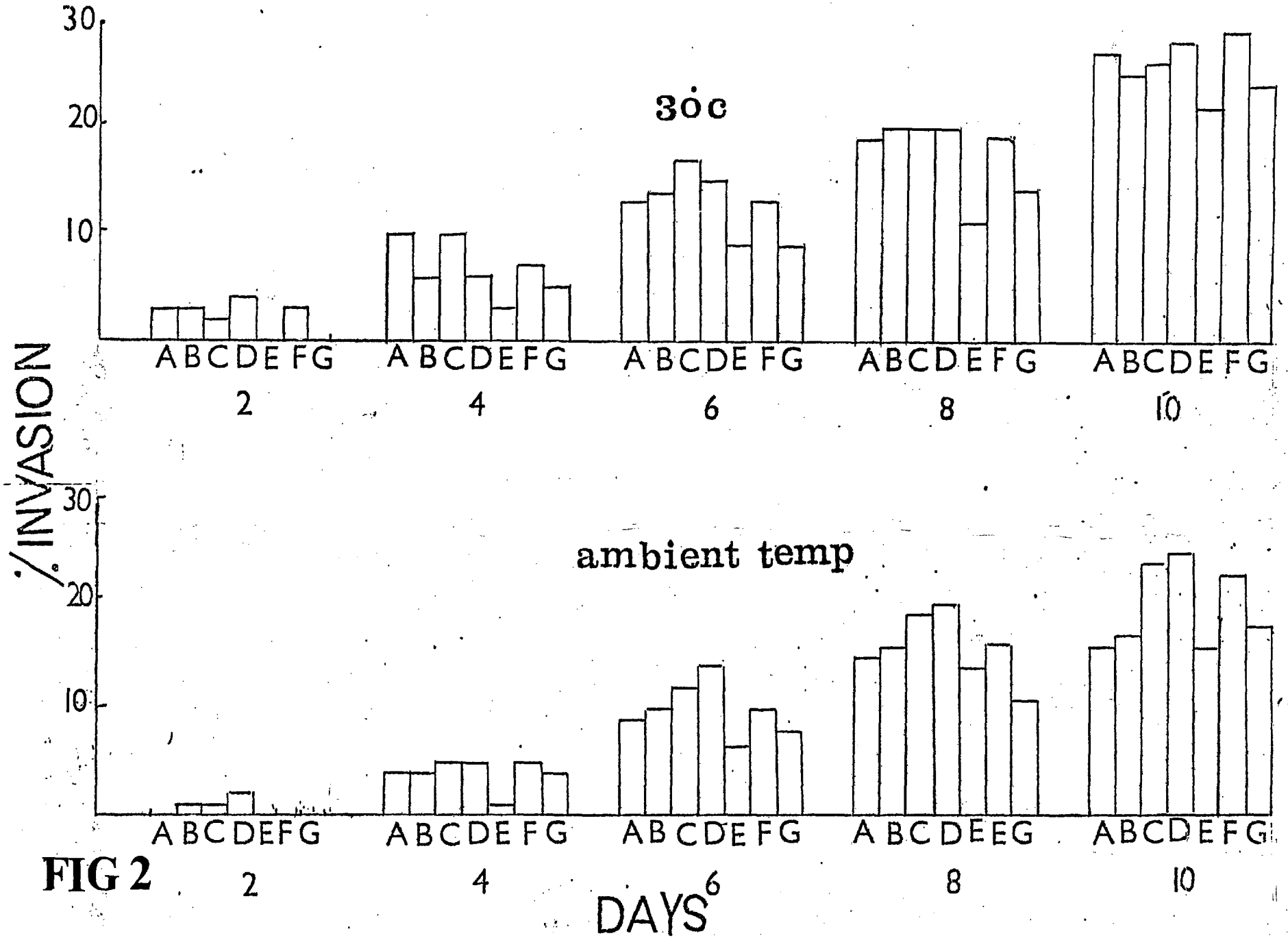


FIG 2

isolate C at 20°C. At higher temperatures the majority of the isolates reached the 3rd juvenile stage 10 days after penetration.

4. Life Cycle

The majority of larvae reached the adult stage within 20 days after inoculation at 27°C, and after 25 days at ambient temperature (Table 4). The periods of development of isolate A, B, C, E, F and G were similar at both temperatures; only isolate D differed.

Isolates A, B, C, E, F and G oviposited 25 days after inoculation at 27°C whereas D oviposited 30 days after inoculation. Eggmasses containing J_2 stage of isolates A, B, C, E, F and G were observed 40 days after inoculation at 27°C. The life cycles of those six isolates were completed within 40 days ($J_2 \longrightarrow J_2$ stage) at 27°C but at ambient temperature it was 45 days long. The life cycle ($J_2 \longrightarrow J_2$) of isolate D was 45 days at 27°C and 40 days at ambient temperature.

All isolates except D produced more eggs in the controlled temperature room at 27°C than in ambient temperature (Table 5). Males were observed in isolates C, D, and G at both temperatures at the end of the experiment. Males were present especially amongst large eggmasses. The sex ratio was approximately 20:1 females to males, in those three isolates in both conditions (Table 6). The males examined from the three isolates were observed to have either 1 or 2 testes.

Table 4 Development and Full Life Cycles of 7 Isolates of M.incognita at 27°C and Ambient Temperature in Tomato Roots

Isolates M.incognita	Temperatures	J_2	J_3	J_4	Adult	Adult with eggs in egg-masses	J_1 in egg	J_2 in egg
A	27°C	5-10	5-15	10-30	15-40	20-30	35-40	40-45
	Ambient	5-10	5-20	10-25	20-40	25-35	40-45	45
B	27°C	5-10	5-15	10-30	15-40	20-30	35-40	40-45
	Ambient	5-10	5-20	10-25	20-40	25-35	40-45	45
C	27°C	5-10	5-15	10-30	15-40	20-30	35-40	40-45
	Ambient	5-10	5-20	10-30	20-40	25-35	40-45	45
D	27°C	5-10	5-15	10-35	15-40	25-35	40-45	45
	Ambient	5-10	5-20	10-30	20-40	20-30	35-45	40-45
E	27°C	5-10	5-15	10-30	15-40	20-30	35-40	40-45
	Ambient	5-10	5-20	10-30	20-40	25-35	40-45	45
F	27°C	5-10	5-15	10-30	15-40	20-30	35-40	40-45
	Ambient	5-10	5-20	10-30	20-40	25-35	40-45	45
G	27°C	5-10	5-15	10-30	15-40	20-30	35-40	40-43
	Ambient	5-10	5-20	10-30	20-40	25-35	40-45	45

Table 5 Numbers of Eggmasses and Eggs/Eggmass of Isolates at 27°C and Ambient Temperature[1)]

Isolates	No of Eggmasses		No of Eggs/Eggmass	
	Temperature		Temperature	
	27°C	Ambient	27°C	Ambient
A	290	105	378	293
B	341	174	394	268
C	380	238	408	320
D	126	281	289	352
E	252	149	369	268
F	340	186	432	302
G	332	202	382	353

1) Mean of 5 replicates

Table 6 Number of Males and Sex Ratio of Isolates at 27°C and Ambient Temperature

Isolates	Number of Males		Sex Ratio ($O_+ : O^{\nearrow}$)	
	27°C	Ambient	27°C	Ambient
C	11	10	24:1	24:1
D	11	14	19:1	19:1
G	13	11	20:1	21:1

B INFLUENCE OF TEMPERATURE AND RELATIVE HUMIDITIES ON SURVIVAL OF M.incognita ISOLATES

INTRODUCTION

Some plant parasitic nematodes can survive without feeding for long periods as juvenile stages, adults or eggs. Survival can be affected by temperature, moisture, osmotic pressure, pH and other environmental factors.(Wallace, 1963).

Sansmyer (1955) mentioned that the temperatures required to kill all eggs of M.incognita acrita after exposure for 1, 2, 3, 4 and 5 hours was 46°, 45°, 44°, 43° and 42°C respectively. It has been shown that there were variable responses to temperatures between different Meloidogyne species (Thomason, 1957; Wallace, 1960). M.javanica (Daulton and Nusbaum, 1961) has a higher optimum temperature for reproduction and a greater tolerance to high temperature and less tolerance to lower temperature than either the subtropical or temperate isolates. Godfrey and Hoshino (1933) using relative humidities from 0 to 100%, found that eggmasses survived at 100% relative humidity but survival decreased rapidly with decreasing relative humidity. The physiological basis for survival in nematodes appears to be dependent upon their ability to store food reserves and upon their ability to function under different metabolic status. In the case of most parasitic forms, the infective stage is a resting

stage in the life cycle with reference to absence of feeding and growth, because it is dependent upon reserve materials for its continued existence. Van Gundy (1965) concluded that the infective stage acted as a bridge from one environment to another.

MATERIALS AND METHODS

1. Temperature

The soil used in this experiment was prepared by mixing sterilised soil, sand and peat at the ratio of 7:3:1. Plastic container cups with drainage holes were filled with 150 ml. of soil. A total of 1000 freshly hatched juveniles were added to each plastic container which were covered with foil paper. Containers were then placed in incubators set at 20°, 25°, 30°, 35°C and also in the glasshouse at the temperature range of $26 \pm 8^{\circ}$C. Four replicates were done at each temperature. Soil in each container was watered with 25 ml. of distilled water at 2-day intervals. After 2 months, survival of juveniles was determined by a bioassay method, 10-day old tomato plants (var Moneymaker) were planted to each container and all of the containers from each temperature were set up in completely randomized block design in the glasshouse. After 6 weeks the plants were removed and the roots were gently washed and damp dried with tissue paper and examined for gall formation. The extent of galling, a measure of juvenile survival, was rated on a 0-5 gall index scales (see Section I).

2. Relative Humidities

Freshly hatched juveniles were collected from watch glass after hatching from eggmasses on a small nylon sieve (page 17) by using a micropipette. With a pipette, the nematodes in a small amount of water were dropped onto a filter paper (No. 42 with pore size approximately 4 μ). The excess water was removed by vacuum suction. The filter paper with nematodes were placed in a desiccator. Different relative humidities were produced using different concentrations of NaCl by Solomon's modified method (Solomon, 1973) and measured with a hygrometer. Three replicates were used with each isolate. The filter paper was kept for 4, 8, 12, 24 hours at relative humidities of 50, 60, 70, 80, 90 and 100% respectively. At the end of each above period three replicates were removed and the filter papers containing nematodes were soaked for 24 hours in water in petridishes before nematodes were examined and counted. Since the difference between live and dead nematodes was sometimes indistinct, nematodes were considered alive if they showed activity, either spontaneously or upon touching.

RESULTS

1. Temperature

The survival of nematodes affected by temperatures was measured by root-knot indexing. From the observation, survival and viability of juveniles was best at

Table 7 Survival: Root-knot Index of Tomato seedlings planted into Infested soils kept at Different Temperatures

	ROOT-KNOT INDEX[1)]			
Temperature / Isolates	20°C	25°C	30°C	Ambient
A	2.25	3.0	1.75	2.0
B	1.75	2.87	1.5	2.0
C	2.38	3.25	1.5	2.25
D	3.0	3.38	1.62	2.62
E	2.25	2.5	1.62	2.12
F	1.75	2.25	1.5	2.25

1) Mean of 4 replicates

Index 0-5

L.S.D. .1% Isolates = 0.252

Temp = 0.2919

Table 8 Mean Percentage Survival of 7 Isolates of M.incognita over a 24-hour period at 6 Relative Humidities Level [1)]

Relative Humidities	50%				60%				70%				80%				90%				100%			
Length of exposure (hr)	4	8	12	24	4	8	12	24	4	8	12	24	4	8	12	24	4	8	12	24	4	8	12	24
Isolates A	6	0	0	0	8	0	0	0	20	0	0	0	44	29	0	0	48	25	18	0	48	45	44	32
B	9	0	0	0	9	0	0	0	21	0	0	0	38	25	0	0	52	26	19	0	61	53	39	28
C	10	0	0	0	12	0	0	0	21	0	0	0	45	37	0	0	57	31	17	0	62	52	41	25
D	13	0	0	0	12	0	0	0	20	0	0	0	42	26	0	0	64	32	25	0	66	46	37	26
E	9	0	0	0	11	0	0	0	15	0	0	0	33	25	0	0	44	26	13	0	60	52	51	25
F	11	0	0	0	15	0	0	0	13	0	0	0	27	23	0	0	39	28	19	0	63	49	40	20
G	13	0	0	0	17	0	0	0	15	0	0	0	31	28	15	0	40	33	15	0	74	72	66	25

1) Means of 3 replicates

25°C for all isolates with isolates A, C, D having the greatest number of nematodes surviving (Table 7). All isolates also survived at 20°C and survival of D was better than other isolates at 20°C.

2. Relative Humidity

The number of surviving juveniles declined with increasing length of exposure and decreasing relative humidity levels (Table 8). Survival was observed at 80% R.H. for 8 hours, at 90% R.H. for 12 hours and at 100% R.H. up to 24 hours. Among the isolates, the survival of isolate G was the highest at 100% R.H. at most different time intervals. Only a small percentage of juveniles were able to survive at 50% relative humidity for 4 hours only. At 50% R.H. for isolate A, survival was significantly less than other isolates. The juveniles of all the isolates at 80% R.H. level survived up to 8 hours, but not after 12 hours' exposure. At humidities 50%, 60% and 70% no survival was observed after 4 hours' exposure.

DISCUSSION

The results obtained from the experiment showed that the optimum temperature for hatching of isolates B, E and G was 25°C, and for isolates A, C, D and F was 30°C. Similar observation has been mentioned by Bergeson (1959) who showed that the optimum temperature for hatching of M. incognita was 26° to 32°C. He reported also that hatching decreased at

15°C and 21°C. From the observations, the ambient temperature was not suitable for most of the isolates except D. Though the origins of all the isolates were from the tropical countries where fluctuating air temperatures occur, the soil temperature remains more stable thus a particular hatching temperature requirement is probably necessary, as shown in this experiment.

At 20°C, percentage hatch was lower for all isolates and in all cases hatching was slower than at higher temperatures of 25°, 30°C. In addition to hatchings, Dropkin (1959) observed that M.incognita was relatively inactive at low temperature. Hatching is also affected by several environmental factors including soil moisture, pH, soil aeration, organic and inorganic chemicals in the soil water (Wallace, 1963, 1966, 1968a).

The low invasion rates in the experiment could be explained by the fact that the plants were grown in the dark and there was consequently poor growth of the host roots. The life cycle for all isolates was 40-45 days from J_2 to J_2 stage. Adult females were observed after 15 to 20 days and oviposition started between 20 and 30 days after inoculation. That stage was similar to that found by Triantaphyllou and Hirchmann (1960), who showed that adult females were observed 15 days after penetration of the roots and 6 days later eggs were laid.

From the observations, the number of females with eggmasses and their fecundity, were different between the isolates, even on the same host tomato plant. Similar observations have been made by David and Strubble (1962)

who reported that number of females with eggmasses and their fecundity were different on sweet potato with M.incognita.

Males only occurred with three of the isolates C, D and G. They were observed especially in mature big eggmasses. Davide and Triantaphyllou (1967) reported similar observations. They found that males were found in seriously damaged root-knots especially in the big eggmasses. Males, with one or two testes, were found in high infestations of root galls in big eggmasses. It has been assumed that males with two testes are converted from advanced stage females in adverse conditions (Triantaphyllou, 1960).

The results obtained from the survival experiment showed that the optimum temperature for survival of all isolates was 25°C. They were also able to survive at ambient temperature. At the temperatures of 20°C and 30°C, the survival was less than at 25°C.

The present observations agree with Daulton and Nusbaum (1961) who also showed differences in the survival of eggs among three isolates at 33°C. They found that the populations of M.javanica from Rhodesia had a greater tolerance to high temperature and lower tolerance to low temperature than the populations of the same species from Georgia and North Carolina and there was no survival at 35°C. Though most isolates were of tropical origin, their survival under experimental conditions was low at a constant temperature

of 30°C and they did not survive at all at 35°C. Under natural conditions the mean soil temperature could be lower than 30°C and the constant higher temperatures may be the reason for their poor survival.

Mai and Harrison (1959) reported that dry conditions may also depress the nematode activity; they showed that the rapid development of Heterodera rostochiensis occurred on potato roots in plots receiving regular irrigation but not in the non-irrigated plots. All nematodes can survive temporary periods of adverse conditions at certain stages of their lives; for example, Anguina tritici 2nd stage juveniles within the gall, Ditylenchus dipsaci 4th stage juvenile, and adult Hemicycliophora spp (Van Gundy, 1965). For many species, the eggs are more tolerant to unfavourable conditions than other life stages possibly because eggshell aids survival. Eggs within cysts of many Heterodera can survive for 1-8 years or even longer (Wallace, 1968).

Juveniles of root-knot can survive in the soil for several months without host using stored food reserve from the egg (Van Gundy,1965). During a period of starvation, food reserves are used up, so that their infectivity to the plants may be reduced (Golden and Shafer, 1960).

From my observations, the juveniles, after 2 months survival infected the tomato plants, although the infection was low compared with the normal infection to tomato plants with freshly hatched juveniles. The soil in the experiment was not allowed to dry out and it is unlikely that the nematodes were quiescent during the period.

If this was so, it suggests that the 2nd stage juveniles can survive in an active condition for 2 months on the food reserves without feeding and still remain viable.

Thomason and Lear, (1961) showed that the length of survival in field soil for M.javanica juveniles was greater than 6 but less than 12 months but he did not show at the percentage infectivity of the juveniles. The survival of the nematodes in terms of the infectivity to the host, pathogenicity, and fecundity has been largely ignored by other workers. Though the nematodes can survive in the soil, still alive, their viability is the most important aspect both in relation to biological studies and their survival as economic pests in the field.

From my observations, the survival of the juveniles of all isolates was lowest at 50%, 60%, 70% relative humidities as the exposure time was increased. As the humidity decreased, survival decreased, and also, as the length of the exposure increased, the survival decreased. Similar results were obtained with Heterodera rostochiensis by Hamblen and Slack (1959). They confirmed that if the soil moisture decreased, the proportion of white cysts of H.rostochiensis and the rate of juvenile emergence decreased. The viability of cysts of H.rostochiensis declined more rapidly in flooded and moist soil, although some encysted juveniles can survive for 8 months under such conditions (Lewis and Mai, 1960).

Godfrey and Hoshino (1930) studied the survival of unidentified spp of root-knot nematodes at different

relative humidities. They observed that at 50% relative humidity, juveniles survived for $3\frac{1}{2}$ minutes, eggs in eggmass for $2\frac{1}{4}$ hours, eggs for $1\frac{1}{4}$ hours and eggs in root tissue for $1\frac{1}{2}$ days. At 90% relative humidity, juveniles survived for 25 minutes, and eggs in the eggmass for $2\frac{1}{2}$ hours. Demeure (1976) has shown that eggs and juveniles in eggmass survived longer than free second stage juveniles in dry soil.

SECTION III RESISTANCE AND SUSCEPTIBILITY TO *M.incognita* ISOLATES ON COTTON AND TOMATO VARIETIES

A EVALUATION OF COTTON VARIETIES AS HOSTS FOR NEMATODE ISOLATES

INTRODUCTION

The root-knot nematode, *Meloidogyne incognita* attacks the cotton plant, throughout the cotton growing areas of the world. Furthermore, it has been found that other pathogens especially fungi such as *Rhizoctonia solani*, *Fusarium*, *Verticillium* interact with root-knot to cause serious damages (Reynolds and Hanson, 1957; Sasser, 1972). Atkinson (1889) first reported root-knot disease on cotton, at that time the name of the root-knot nematode was *Heterodera radicicola*. Sasser(1954), using single species cultures, identified according to the perineal patterns, found cotton to be susceptible to *M.incognita acrita* but not infected by *M.incognita incognita* *M.javanica*, *M.arenaria*, or *M.hapla*. According to Minton (1962), among the root-knot nematodes, only, *M.incognita incognita* and *M.incognita acrita* severely attack cotton.

Martin(1954) showed that the differences in parasitism on cotton among isolates of *M.incognita* and *M.incognita acrita* range from no parasitism to severe parasitism. Minton et,al(1960)studied the response of resistant and susceptible varieties of cotton to *Meloidogyne* populations

and a wide range of root-knot resistance was observed. Brodie et al. (1960) reported that resistance in cotton variety Auburn was associated with necrosis, death of infected cotton roots and inhibition of larval development.

Martin et al. (1960) established similarly that resistance to M.incognita acrita could be attributed to conditions within the roots that delayed larval development. Sasser (1966, 1972) has studied the effect of different root-knot nematodes, including world wide isolates within the M.incognita group and found that there was variation in their pathogenicity on cotton. Brodie and Cooper (1964) found that one isolate of M.incognita reached the egg-laying stage on the cotton variety Coker 100 WR within 30 days, whereas another isolate of M.incognita failed to develop beyond the second stage larvae. Jones and Birchfield (1967) reported that resistance to root-knot in cotton varieties of Bayou and Auburn 56 was related to failure of egg production in those cotton varieties.

The purpose of this study was

(i) to determine levels of resistance in cotton varieties to the different isolates of M.incognita

(ii) to determine whether any population of M.incognita could be separated into physiological races according to their ability to attack selected varieties of cotton

(iii) to evaluate the resistance level on some Burmese varieties to isolates of M.incognita from Burma and elsewhere.

MATERIALS AND METHODS

1. Comparison of 7 cotton varieties as Hosts for 7 Isolates of M.incognita

Single eggmass cultures of each of the 7 isolates of M.incognita were raised on tomato variety Moneymaker in the glasshouse. Seven varieties of cotton, Stoneville 7 (origin America), Mahlaing 5, Mahlaing 6, Wagale (local varieties from Burma), Delfos (U.S.A.), Malawi 637 (local variety from Malawi) and Makoka 74 (local variety from Malawi) were used in this experiment. The cotton seeds were grown in sterilised sand in a propagator for one week. After one week, uniform sized plants were selected and transferred into the mixture of sterilised soil in 4" plastic pots. One thousand freshly hatched larvae were inoculated onto each plant. The inoculated plants were kept in a completely randomized block design. Four replicates for each host and isolate were used, the pots were raised on upturned saucers to avoid contamination or cross-infection. The experiment was kept for two months in the glasshouse at ambient temperature of $20 \pm 6^{\circ}C$. At the harvesting time, the soil was washed with water gently from the roots and each root system was dried with tissue paper and checked for root-knots. The roots were stained in 0.5% acid fuchsin and cleared in pure lactophenol. The nematodes in the stained roots were then counted. (See General Materials and Methods).

2. Pathogenicity of 2 isolates of M.incognita on cotton varieties Makoka 74 and Wagale

Single eggmass cultures of isolates C and A were used as the test organisms and cotton varieties Makoka 74 and Wagale were used as hosts in this experiment. Uniform sized one-week old cotton seedlings were transplanted to 6" pots. Six replicates were done for each isolate on each host. One thousand freshly hatched larvae were inoculated to each plant and the inoculated plants were kept in a completely randomized block design. The experiment ran for 6 weeks. At the end of the experiment, top weight, root-weight and numbers of nematodes were taken. (See General Materials and Methods).

3. The comparative development to 2 isolates in roots of cotton varieties Makoka 74 and Wagale

M.incognita isolates C and A were used as test organisms and cotton varieties Makoka 74 and Wagale were used as the hosts. Four replicates for each host and isolate were done at every sampling. The plants were inoculated with 1000 freshly hatched larvae and harvested at 2-week intervals 4 times. The top weight, root weight, numbers of nematodes were taken. (See General Materials and Methods).

RESULTS

1. Comparison of 7 cotton varieties

Most of the cotton varieties appeared to be resistant to all isolates, but with some of them, though they were infected, only very tiny galls were observed. When they were dissected, some of the galls did not contain nematodes, especially those tiny galls which were woody and very hard. Empty galls were found in vars. Makoka 74 and Malawi 637 with isolates C and D. The sizes of the galls were 0.2 - 0.3 cm. in both cotton varieties. There were root-tip swellings on some of the varieties, especially Malawi 637 and Makoka 74 but only on those inoculated with isolates C and D. Only isolates C and D were found to invade roots of any of the cotton varieties (Table9). Neither of these isolates reached the egg laying stage but adult female stages were observed. Very few nematodes were observed in the galls. The roots of Makoka 74, Malawi 637, Wagale were necrosied with isolates A, C and D.

2. Pathogenicity on cotton

Isolate C attacked Makoka 74 at a very low level. None of the isolates attacked the Wagale variety. Mature females were observed on Makoka attacked by isolate C, but they produced very few numbers of eggs. At the harvesting time, 15-35 eggs were produced in some eggmasses. Second,

Table 9 Comparison of 7 Cotton Varieties as Hosts for Isolates of M.incognita

1. Number of nematode galls/root system 2 months after inoculation of nematodes [1)]

Cotton varieties	M.incognita isolates						
	A	B	C	D	E	F	G
Makoka 74	0	0	21 ± 8	20 ± 4	0	0	0
Malawi 637	0	0	28 ± 10	27 ± 10	0	0	0
Wagale	0	0	0	0	0	0	0
Stoneville 7	0	0	0	0	0	0	0
Delfos	0	0	0	0	0	0	0
Mahlaing 5	0	0	0	0	0	0	0
Mahlaing 6	0	0	0	0	0	0	0

1) Mean of 4 replicates

Table 10

Number of nematodes (♀'s and juveniles) per root system 2 months after inoculation [1)]

Cotton varieties	M.incognita isolates						
	A	B	C	D	E	F	G
Makoka 74	0	0	68±17	79±14	0	0	0
Malawi 637	0	0	39±12	23± 5	0	0	0
Delfos	0	0	0	0	0	0	0
Stoneville 7	0	0	0	0	0	0	0
Mahlaing 5	0	0	0	0	0	0	0
Mahlaing 6	0	0	0	0	0	0	0
Wagale	0	0	0	0	0	0	0

1) Mean of 4 replicates

Table 11 Comparison on the different assessments of root-knot nematode on 2 varieties of cotton[1)]

	COTTON VARIETIES					
	MAKOKA 74			WAGALE		
	M.incognita Isolates		Control (no nematodes)	M.incognita Isolates		Control (no nematodes)
	A	C		A	C	
Number of empty galls/root system	11	20	0	2	9	0
Number of nematodes/root system	6	27	0	0	2	0
Top weight (fresh)	26.9	26.1	35.9	28.8	33.2	38.3
Root weight (fresh)	21.5	22.8	30.9	22.0	26.2	32.2

1) Mean of 6 replicates

third and fourth stage juveniles were observed in Makoka roots(Table 11). Root-tip swellings and woody root-knots were found in Makoka roots. Necrosis occurred in some roots of vars. Makoka and Wagale. Nematodes of isolates A were observed in the roots of Makoka not in the galls. The root weights and top weights were significantly reduced (at 5% level) when compared to control plants(Table11).

3. Development in cotton

Two weeks after inoculation, the numbers of larvae per var Makoka root system attacked by isolate C averaged 20, but all of them were 2nd and 3rd stage juveniles (Table 12). Egg producing females were observed from isolate C in Makoka roots at the end of 8 weeks. They produced 20-38 eggs/eggmass but only a few females overall produced eggmasses (Table 12). Root-tip swellings were observed on both varieties of cotton. There were no root-tip swellings on control plants.

Table 12 Development of Root-knot nematode on 2 varieties of cotton [1)]

Time after inoculation	Nematode in roots	COTTON VARIETIES			
		MAKOKA 74		WAGALE	
		M.incognita isolates		M.incognita isolates	
		A	C	A	C
2 weeks	Juveniles	0	18	0	0
	♀'s	0	2	0	0
	♀'s + eggs	0	0	0	0
4 weeks	Juveniles	1	12	0	1
	♀'s	0	12	0	0
	♀'s + eggs	0	3	0	0
6 weeks	Juveniles	1	10	1	0
	♀'s	0	14	0	0
	♀'s + eggs	0	10	0	0
8 weeks	Juveniles	2	31	1	0
	♀'s	3	42	0	0
	♀'s + eggs	0	13	0	0

1) Mean of 4 replicates

B EVALUATION OF TOMATO VARIETIES AS HOSTS FOR 7 ISOLATES OF M.incognita

INTRODUCTION

Meloidogyne spp, causing root-knot disease of plants were polyphagous, parasitising many plants. However, some differences could be observed between different species of Meloidogyne in their behaviour towards a particular host. Tomato is one of the most important crops attacked by root-knot nematodes, mainly by the species M.incognita, M.javanica, M.hapla, thus, workers have tried to discover resistant varieties which can be grown commercially to reduce populations and improve yields. The tomato varieties which have been found to show resistance to Meloidogyne include Ronita, Rossol, Fiesta, Hawaii No. 7322 etc. (Victoria, 1971; Verma, 1969; Barham and Sasser, 1956; Dropkin, 1969; Thomason and Smith, 1957; Riggs and Winstead, 1959).

Steiner (1952) defined resistance to root-knot nematodes as the ability of the roots to resist penetration by the root-knot nematode. This study was undertaken to determine the nature of resistance using the resistance varieties "Ronita", "Rossol" and "Fiesta". The seeds of the former two varieties originated from Nigeria and the latter from El Salvador.

MATERIALS AND METHODS

The tomato seeds were sown in a propagator, and when the plants were 10 days old, the uniform sized ones were transplanted to plastic pots. Three resistant tomato varieties, Ronita, Rossol, Fiesta and one highly susceptible variety, Moneymaker, were tested. Four replicates were used and the plants were kept in the glasshouse at an ambient temperature of 22 $\pm$ 4°C. The plants were inoculated with 1000 freshly hatched larvae from each of the 7 M.incognita isolates and watered daily. After 6 weeks, the plants were removed from the soil and cleared in running water. The number of galls were then counted. (See General Materials and Methods).

RESULTS

Both isolates C and D attacked and produced galls on var Ronita but none of the other isolates produced galls on vars Ronita, Rossol or Fiesta. All isolates caused severe galling of var Moneymaker. The mean indices of root-gall value for Ronita attacked by isolates C and D were 2 and 1.5 respectively, compared with that of "Moneymaker" which was 4, but the sizes of the galls on var Ronita were small, 2 - 3 mm. in size compared to var Moneymaker. Root necrosis was observed on varieties Rossol, Ronita, Fiesta with isolates A, B, E, F and G but only Rossol and Fiesta with isolates C and D.

Table 13 Gall index for 4 varieties of tomato, inoculated with 7 isolates of M.incognita

Isolates	Tomato Varieties			
	Moneymaker	Ronita	Rossol	Fiesta
A	4	0	0	0
B	3.75	0	0	0
C	4	1.87	0	0
D	4.25	1.5	0	0
E	3.75	0	0	0
F	3.5	0	0	0
G	4.0	0	0	0

1) Mean of 4 replicates

L.S.D. 5% = .004

1% = .445

DISCUSSION

According to the observations, it was clear that some varieties of both cotton and tomato were resistant to some isolates of M.incognita while other varieties were not. Wallace (1963) suggested that nematodes may invade and then leave a resistant root whereas they tend to remain in susceptible roots.

Reynolds et al. (1970) mentioned that systematic response is evident when a nematode invades a resistant plant because he found that most larvae had left the resistant roots of alfalfa a few days after penetration. Christie (1946) observed empty galls without any nematodes on cowpea. Gall formation by surface feeding without entry of larvae of M.incognita and M.hapla were reported by Lowenberg et al. (1960). Empty galls were also described by Christie (1949) on Pelargonium, growing in root-knot infested soil and by McClure et al.(1973) in resistant Clevewilt cotton variety.

According to Minton (1962), M.incognita acrita reproduced on all varieties of cotton tested, whereas M.incognita incognita reproduced only in some varieties.

From my observations, all of the cotton varieties that I tested, except varieties Makoka 74, Malawi 637, were highly resistant to all isolates while vars Makoka 74 and Malawi 637 themselves were resistant to some extent. The mechanism of the resistance in those varieties could be due to the death and decay of larvae as a result of host toxicity or the

larvae may leave the roots after penetration because of the unsuitable food source.

From the pathogenicity of cotton experiment, the root-weights of inoculated plants were significantly reduced when compared with control cotton plants. In control plants, the root-systems were well developed whereas in the inoculated plants, the root-systems were very poor with necrosis.

Physiological races can be existent in populations within a species which attack in various ways on a given host, or change in their pathogenicity, development and behaviour on the host and reproduced differently (Riggs and Winstead, 1959; Triantaphyllou and Sasser, 1960).

Riggs and Winstead developed races of M.arenaria and M.incognita, on the resistant tomato variety Hawaii 5229; these B races were derived from the populations that had attacked this variety of tomato to only a very limited extent. Triantaphyllou and Sasser (1960) were able to develop this same process by repeating single eggmass cultures of M.incognita. Netscher (1970) found B races of M.incognita and M.javanica.

The previous workers, Victoria (1970) and Verma (1969), showed that Ronita variety was very highly resistant to Meloidogyne. But from my observations, the resistance of Ronita variety was broken by the 2 isolates C and D. Similar observations have been reported by Netscher (1976) on Rossol tomato. This variety was previously considered highly

resistant to root-knot nematodes. He assumed that the different reactions observed were due to genetic differences between Meloidogyne populations. My own observations agree with this work.

If a few galls are present on the roots of resistant plants, it is possible that races are able to develop. Generally, heavily infested soils should not be planted with resistant varieties because it is possible that certain nematodes belonging to a specialised race can be present.

SECTION IV COMPARATIVE STUDIES WITH DIFFERENT CROPS AS HOSTS FOR ISOLATES OF *M.incognita*

A HOST PREFERENCE OF *M.incognita* ISOLATES

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp) have a wide host range and they can parasitise more than 2000 hosts including crops belonging to the Graminaceae, Malvaceae, Solanaceae and others. (Raski, 1959; Christie, 1944, 1946; Sasser, 1954, 1966, 1972; Franklin, 1965; Golden, 1967; Martin, 1966; Whitehead, 1960, 1968; Singh, 1972; Linde, 1956). *M.incognita* is one of the most important root-knot species and many different crops have been reported as being highly susceptible to it. (Sasser, 1966, 1972; Winstead and Riggs, 1963; Goplen, 1959; Dropkin, 1959; Martin, 1954). Michell, *et al.* (1973) showed that host preference may exist for *M.naasi* and found differential reactions to the different hosts.

All root-knot nematodes were originally classified under the species *Heterodera marioni* (Cornu, 1879). Sherbakoff (1939) showed that populations of *Heterodera marioni* differed in their host preference. He reported considerable root-knot injury to cotton, grown on land previously planted

with cotton but observed no injury to cotton grown on land previously planted with tomatoes, even though the tomatoes had been severely injured by root-knot. Christie (1946) also found that populations of Heterodera marioni differed in their host preference relationships among the 14 populations of H.marioni. The work of Sherbakoff and Christie suggested that different species existed within the species H.marioni. This was confirmed by the work of Chitwood (1949) and by later workers, and the genus Meloidogyne consisting of a number of species was put into existence.

Sasser (1954), using different hosts found that some newly recognised species of Meloidogyne were more host specific than others and that for each species there were certain crops which were non-hosts. He concluded that when reactions were different, it constitutes evidence of pathogenic variation with the species being tested. Host specificity variation in M.incognita and M.incognita acrita has been reported by a number of investigators. Differences in host plant reaction (degree or type of galling) in populations of this nematode species have been observed by Allen (1952), Dropkin (1959), Martin (1954). In further investigations, variation has been found in the rate of reproduction and the ability of isolates to attack various cotton and soybean varieties. (Dropkin, 1953; Linde, 1956; Martin, 1954).

Linde (1956) found marked variability in the growth and reproduction of M.incognita acrita from different parts of South Africa when tested on the same host.

MATERIALS AND METHODS

A1 - Host-Range Study I

In this host-range study, tomato var Moneymaker (*Lycopersicum esculentum*), tobacco Nc 220 (*Nicotiana tabacum*), egg plant local var from Burma (*Solanum melongena*), Okra var Newera (*Hibiscus esculentus*), hot pepper local var from Burma (*Capsicum annuum*), sweet corn var Early king (*Zea mays* L) and lettuce (*Lactuca sativa* L) were used. The plants were grown in 6" pots and inoculated with 5 eggmasses to each plant for each of the 6 isolates. The temperature range in the glasshouse was 22-30°C during summer and 16-25°C during winter.

The plants were watered every day and bionutrient solution was added once a week. Two replicates were used and the plants were kept in the glasshouse for 40 weeks. Plants were replaced when required. After 40 weeks, plants were harvested and soil was washed from the roots. Nematode populations and their effect on hosts were estimated by a root-gall index of 0 - 5 (See Section I).

Eggs and females from each plant were fixed in T.A.F. solution for morphological studies (Section V); eggmasses from each isolate and host were kept in 0.3M NaCl solution for use in other studies.

A2 - Host-Range Study II

In this second host-range study, more detailed analysis of final nematode populations was made. Hosts used were tomato (Lycopersicum esculentum), eggplant (Solarium melongina), broadbean (Vicia angustifolia), cucumber (Cucumis sativa L) and lettuce (Lactuca sativa L).

A thousand freshly hatched larvae were inoculated to the different host seedlings for each isolate. The plants were placed in the glasshouse in a randomized block design at ambient temperature (20-26°C) for 3 months. After this period, the plants were harvested and roots were washed from soil and damp-dried on tissue paper. Nematode populations and the effect on hosts were determined by both root-gall index (see Section I) and detailed counts of nematodes on roots. The roots were first weighed, then chopped and mixed, and 0.5 g. of roots, randomly taken from each plant, were stained in 0.025% cotton blue lactophenol and cleared in pure lactophenol; number of nematodes, eggmasses and eggs per eggmass were counted directly under the microscope.

RESULTS

A1 - Host-Range Study I

There was a wide range of variation between hosts in their reaction to the different isolates. Tomato, egg plant, okra, tobacco, lettuce were hosts for all isolates but hot pepper was a non-host for all isolates with no gall formation or apparent host reaction. Nematodes of isolates C from Ecuador and D from El Salvador invaded corn roots but with the other 5 isolates there was no apparent invasion of corn roots as nematodes were not found within the tissues, although some root-tip swelling did occur. Tomato was the best host for all isolates and gall formation was greater than on other crops.

Only isolates C and D produced galls on corn (Table 14) and, though the galls were small compared to those on other hosts, eggmasses were produced. The sizes of the eggmasses on the galls from corn were small (0.2 - 0.25 mm.) compared to other hosts (0.2 - 0.4 mm.) but the numbers of the eggs per egg mass for the 2 isolates were similar. The number of eggs per eggmass was significantly different on corn between other crops. Both isolates C and D produced less eggs per egg-mass on corn than other crops (Table 15). The galls on corn plants produced by isolates C and D were smaller, being 0.2 - 0.3 mm. in size and the sizes of the galls were larger on tomato than on other crops for all isolates. Sizes of the galls on tomato were

Table 14 "Root-gall indices" of seven Isolates of M.incognita on seven different host plants after 40 weeks[1)]

Host / Isolate	Tomato	Tobacco	Eggplant	Okra	Lettuce	Sweetcorn	Chilli pepper
A	4.0	3.4	3.0	3.2	3.0	0	0
B	4.0	3.5	2.9	2.6	2.6	0	0
C	4.5	3.5	3.9	3.0	3.3	3.0	0
D	4.5	4.0	4.0	3.0	3.9	2.8	0
E	4.0	3.0	3.0	4.0	3.3	0	0
F	4.0	3.0	3.0	4.0	3.8	0	0
G	4.5	3.5	3.0	3.5	4.0	0	0

1) Mean of 2 replicates
Index 0 - 5

L.S.D. (Isolate) .05 = 0.255 (Host) .05 = 0.255
.01 = 0.439 .01 = 0.439

Table 15 Number of eggs/eggmass of seven Isolates of M.incognita on seven different host plants after 40 weeks[1)]

Host / Isolate	No. of eggs/eggmass						
	Tomato	Tobacco	Eggplant	Okra	Lettuce	Sweetcorn	Chilli pepper
A	345	319	278	340	319	-	-
B	382	302	310	294	334	-	-
C	419	345	370	318	329	248	-
D	430	322	345	339	379	259	-
E	348	299	315	316	318	-	-
F	380	245	339	320	279	-	-
G	392	331	346	349	345	-	-

1) Means of 2 replicates

L.S.D. (Isolate) .05 = 21.22 L.S.D. (Host) .05 = 24.99
.01 = 42.794 .01 = 53.42

0.3 - 0.9 mm. in diameter, on egg plant and tobacco they were 0.3 - 0.7 mm., on okra 0.2 - 0.5 mm., and on lettuce 0.3 - 0.6 mm. in diameter.

The root-knot index was also higher on tomato than on other hosts (Table 14). The value of root-knot index on tomato was 4.5 for isolate C, D and G and 4 for the rest of the isolates. There were highly significant differences in the root-gall index between isolates A, B and C on okra plant (Table 14). Root-knot index values for isolate C and D were similar on most hosts with the exception of tobacco and lettuce. The values of root-knot index for isolate C and D on tobacco were 3.5 and 4.0, while on lettuce the values were 3.3 and 3.9 (Table 14).

The roots of corn which were attacked by isolates A, B, E, F and G were observed to be necrosed although there was no gall formation except for some root-tip swellings.

A2 - Host-Range Study II

There was again, a wide range of host reaction with the different isolates. All the isolates caused severe galling on tomato, egg plant, lettuce and cucumber which were good hosts for all isolates. Bean was the poorest host for most of the isolates, except isolate B.

There were highly significant differences

in gall index not only between the isolates but also between the hosts (Table 16). Root-gall indices for all isolates on tomato were between 4 and 4.5 (Table 16). The root-gall index value was low on bean, between 2 and 3.2. Isolates C and D produced more galls on lettuce than on egg plant, cucumber and bean. The sizes of the galls on tomato for all isolates were 0.3 - 0.8 cm. in diameter. The root galls on bean were big, 0.4 - 0.7 mm. with isolates C, D and B, but very few in number and could be distinguished from nitrogen fixing galls. Isolates C from Ecuador and isolate D from El Salvador differed significantly from isolate F from Barbados on tomato, lettuce, cucumber and bean. There were no significant differences in gall index between isolates B, C and D on bean (Table 16).

The number of females on tomato were much higher than on crops (Table 20). Mean numbers of females produced by isolate C and D on tomato were 253 and 262 but on bean there were 145 and 89. For isolate F, the number of females on egg plant was much higher than other crops except tomato (Table 20).

The fecundity of the nematodes was different not only between the isolates but also between the hosts. All of the isolates produced the highest number of eggs on tomato, although the fecundity of isolate A was as high on cucumber as on tomato (Table 19). The sizes of eggmasses on tomato were larger than on the other hosts for all isolates. The number of eggs per eggmass was not significantly different on bean between isolate B (Burma), C (Ecuador) and D (El Salvador),

Table 16 'Root-gall indices' of six isolates of M.incognita grown on five different host plants for 3 months[1)]

Host / Isolate	Tomato	Eggplant	Lettuce	Cucumber	Bean
A	4.5	3.2	2.5	3	2
B	4	2.8	3	3.5	3.2
C	4.5	3	4	3.5	3
D	4.5	4	4.5	4	3
E	4.5	4	3.5	3	2.5
F	4	4	2	2.5	2

1) Means of 4 replicates

Index 0 - 5

L.S.D. (Isolate) .05 = 0.287 (Host) .05 = 0.22

.01 = 0.428 .01 = 0.518

but they were highly significant between isolate A (Burma), E (Nigeria), and F (Barbados) (Table 19).

There was a highly significant reduction in the root weights of all infected hosts, except bean, when compared to the control plants (Table 17).

Table 17 Root weight of 5 different hosts after 3 months inoculated with 6 isolates of M.incognita[1)]

Host / Isolate	Root wt. (g)				
	Tomato	Eggplant	Lettuce	Cucumber	Bean
A	7.5	6.2	3.7	3.4	8.9
B	6.8	5.9	4.5	4.2	9.5
C	7.0	6.9	5.5	3.0	9.8
D	9.0	7.2	6.5	5.2	10.0
E	7.4	6.5	4.2	4.9	8.5
F	6.6	6.9	4.0	3.0	9.0
Control	10.5	8.5	6.5	8.7	10.8

1) Mean of 4 replicates

L.S.D. (Isolates) .05 = 0.94
.01 = 1.754

(Host) .05 = 2.08
.01 = 3.99

Table 18 Eggmasses/g root of six Isolates of M.incognita on 5 different hosts grown for 3 months[1)]

	Nos. eggmasses/g root				
Host / Isolate	Tomato	Eggplant	Lettuce	Cucumber	Bean
A	143	123	97	75	49
B	120	29	89	143	68
C	189	148	131	121	54
D	182	179	111	191	69
E	191	162	101	101	59
F	137	169	69	59	40

1) Mean of 4 replicates

L.S.D. (Isolates) .05 = 23.114 (Host) .05 = 21.22
.01 = 41.207 .01 = 44.02

Table 19 Numbers of Eggs/eggmass of six Isolates of M.incognita on 5 different hosts grown for 3 months[1)]

Host / Isolate	No. of eggs/eggmass				
	Tomato	Eggplant	Lettuce	Cucumber	Bean
A	353	299	202	321	202
B	392	246	322	348	349
C	428	285	328	332	340
D	463	350	345	389	338
E	430	325	368	327	259
F	321	348	252	268	202

1) Means of 5 replicates

L.S.D. Isolates .05 = 24.2
.01 = 47.614

L.S.D. Hosts .05 = 22.8
.01 = 40.3

Table 20 Numbers of M.incognita females/g root of six isolates on 5 different hosts grown for 3 months[1)]

Host / Isolate	Tomato	Eggplant	Lettuce	Cucumber	Bean
A	223	129	148	159	92
B	181	89	152	168	162
C	253	98	192	160	145
D	262	182	262	265	89
E	259	169	159	173	88
F	192	172	92	92	91

1) Mean of 4 replicates

L.S.D. (Isolates) .05 = 21.618
.01 = 43.422

(Host) .05 = 18.99
.01 = 38.40

Table 20(a) Comparison of Host status on 3 month old plants by different assessment methods

1. On Tomato

Isolates	Root-knot index	Eggmasses/ g root	Eggs/ Eggmass	Females/ g root
A	4.5	143	353	223
B	4.0	120	392	181
C	4.5	189	428	253
D	4.5	182	463	262
E	4.5	191	430	259
F	4.0	137	321	192

Table 20(a) (continued)

2. On Bean

Isolates	Root-knot index	Eggmasses/ g root	Eggs/ Eggmass	Females/ g root
A	2.0	49	202	92
B	3.2	68	349	162
C	3.0	54	340	145
D	3.0	69	338	89
E	2.5	54	259	88
F	2.0	40	202	91

3. On Eggplant

Isolates	Root-knot index	Eggmasses/ g root	Eggs/ Eggmass	Females/ g root
A	3.2	123	299	129
B	2.8	39	240	89
C	3.0	148	285	98
D	4.0	179	350	182
E	4.0	162	325	169
F	4.0	169	348	172

Table 20(a) (continued)

3. On Lettuce

Isolate	Root-knot index	Eggmasses/ g root	Eggs/ Eggmass	Females/ g root
A	2.5	97	202	148
B	3.0	89	322	152
C	4.0	131	328	192
D	4.5	111	345	262
E	3.5	101	368	159
F	2.0	69	252	92

4. On Cucumber

Isolate	Root-knot Index	Eggmasses/ g root	Eggs/ Eggmass	Females/ g root
A	3.0	75	321	159
B	3.5	143	348	168
C	3.5	121	332	160
D	4.0	191	389	265
E	3.0	101	327	173
F	2.5	59	268	92

B INFLUENCE OF ORIGINAL HOSTS ON THE INVASION, DEVELOPMENT AND PATHOGENICITY BY CROSS INFECTION

INTRODUCTION

Plant pathogenic nematodes, being obligate parasites, depend on living roots of suitable host plants for their development and reproduction. The planting of susceptible crops on the same land year after year permits plant parasitic nematodes to build up high population levels in the soil, rendering it unfit for the production of these crops.

In the field, by culturing continuous generations of root-knot nematodes on the same resistant host for 4 or 5 generations, there was good reproduction on the resistant plant when compared with the original hosts (Riggs and Winstead, 1959). Netscher (1970) showed that by continuously growing resistant varieties of tomato in heavily Meloidogyne infested soil, resistance was broken by the 6th generation; biotypes had developed, populations were able to reproduce on resistant tomato, and a marked increase in the number of females occurred in that generation because of the adaptation to that variety.

Thirugnanam and Rangaswami (1967) did the cross infectivity and pathogenicity of 15 isolates of root-knot nematodes, M. incognita and M. javanica. They found the physiological races within species and concluded that the isolate obtained from the same plant would build up virulent population that could

reproduce even on the unsuitable host).

The purpose of the experiment was to determine whether the infectivity of Meloidogyne incognita was altered by different hosts and whether that alteration was substained when the population was introduced to its original host. The results of this work could be used to help when determining a crop rotation in a field which is infested by root-knot nematodes.

MATERIALS AND METHODS

B1 - The Development of 2 isolates both from corn and tomato on Graminaceous hosts

Isolates C and D were selected because they were observed to react differently from other isolates, being the only two isolates that reproduced on corn. Eggmasses of isolates C and D cultured on both tomato and corn hosts from the previous experiment were maintained alive in 0.3M NaCl solution. Juveniles from those eggmasses were hatched in distilled water and inoculated to the hosts, tomato var. Moneymaker, sweet corn var. Early king, sorghum from Iraq, millet from Iraq and maize local variety from Malawi for each isolate. A total of 500 juveniles was used as inoculum from each isolate, for each host. Two replicates were taken at each sampling time and the inoculated plants were kept in completely randomized design on the bench in the glasshouse at a temperature range

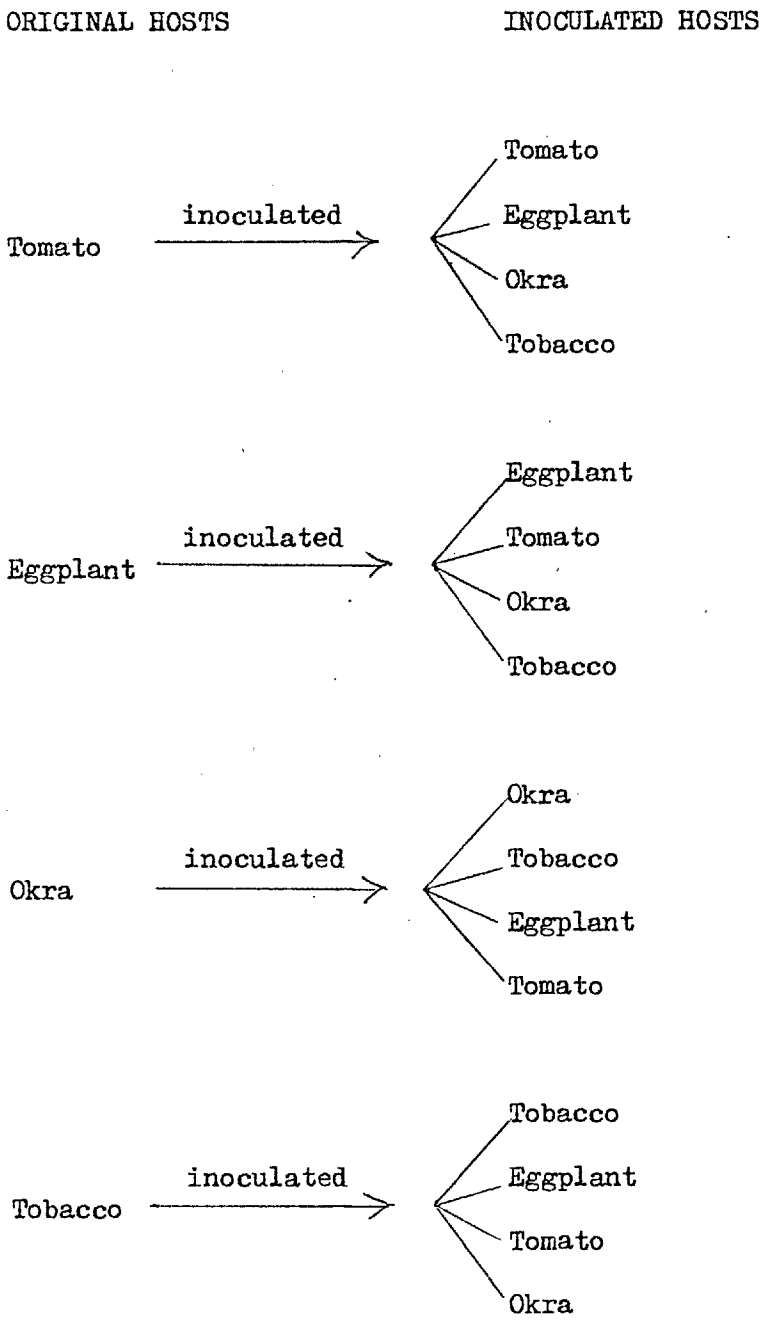
of 20-26°C. To determine the development and the pathogenicity, the plants were harvested at 2-week intervals over a total of 8 weeks. At each harvesting time, root weight, top weight and number of nematodes were taken.

B2 - Invasion of tomato and corn roots by juveniles of 2 isolates of M.incognita from tomato and corn cultures

In this cross-inoculation experiment, 10-day old plants of tomato and corn were used. Juveniles were collected from the eggmasses of isolates C and D that had been cultured on both tomato and corn plants for 40 weeks. 500 freshly hatched juveniles were inoculated to each plant in the experiment and root samples were taken at 2-day intervals up to 10 days. At each sampling, 4 plants from each host were harvested and the number of juveniles that had invaded the roots were counted under the microscope after staining with cotton blue lactophenol.

B3 - Pathogenicity of 2 isolates cross-inoculated on 4 different hosts

Eggmasses of isolate G from I.C.I. glasshouse culture and E from Nigeria cultured on okra, eggplant, tobacco, and tomato hosts from the host-range study no (1) were maintained alive in 0.3M NaCl solution. Juveniles from egg-masses were hatched in distilled water and 500 juveniles were inoculated to the same and different hosts from culture as follows:-



Four replicates for each were used, planted in pots containing sterilized soil mixture and kept in the glasshouse at 23 $\pm$ 4°C.

RESULTS

B1 - The development of 2 isolates both from corn and tomato on Graminaceous hosts

Isolates C from Ecuador and D from El Salvador attacked all hosts, but the infections on sorghum and millet were very low compared to other hosts (Table 21). As the root-galls on millet and sorghum were very tiny, the root index system was discarded in this experiment although females with eggmasses were produced on these hosts. Both isolates produced bigger galls on tomato root system than they did on corn. There was a significant difference in the number of eggs per eggmass depending on whether or not the inoculated host was the same as the original host (Table 22). The numbers of eggs per eggmass produced on tomato for isolate C from tomato and sweet corn were 329 and 269 respectively, and those for isolate D were 324 and 257 respectively (Table 22). All isolates produced less galls on sorghum, millet and maize (Table 21). There was no significant difference in the total number of females with eggmasses produced on tomato from either corn or tomato isolates, but nematodes originally from corn produced more females with eggmass on corn than the nematodes originally from tomato. The number of females on the 2nd corn host were even more than those produced on the 2nd tomato host (Table 21). The nematodes from the original corn host had the same infectivity on millet and sorghum hosts as those nematodes from

Table 21 Development of nematodes of isolate C on the graminaceous host inoculated from tomato and corn original cultures

Weeks after inoculation	Inoculated Host	ORIGINAL HOST TOMATO				ORIGINAL HOST SWEETCORN			
		Nos. of nematodes in roots				Nos. of nematodes in roots			
		J_2	J_3/J_4	♀	♀'s with eggmass	J_2	J_3/J_4	♀	♀'s with eggmass
2	Tomato	0	240	0	0	0	230	0	0
4		0	4	282	22	0	2	281	26
6		0	0	21	246	0	0	17	239
8		14	0	0	272	16	0	0	270
2	Sweet corn	0	211	0	0	0	262	0	0
4		0	0	262	21	0	0	198	28
6		0	0	19	168	0	0	21	190
8		11	0	0	217	12	0	0	287
2	Sorghum	0	54	0	0	0	39	0	0
4		0	0	58	7	0	0	60	8
6		0	0	15	68	0	0	12	62
8		0	0	0	78	0	0	0	71
2	Maize	0	79	0	0	0	70	0	0
4		0	0	137	17	0	0	120	16
6		0	0	11	144	0	0	11	132
8		0	0	0	167	0	0	0	129
2	Millet	0	28	0	0	0	30	0	0
4		0	4	35	0	0	0	36	0
6		0	0	4	36	0	0	16	41
8		0	0	0	59	0	0	0	61

Table 22 Development of nematodes of isolate D on the graminaceous host inoculated from tomato and corn original cultures

Weeks after inoculation	Inoculated Host	ORIGINAL HOST-TOMATO				ORIGINAL HOST-SWEETCORN			
		Nos. of nematodes in roots				Nos. of nematodes in roots			
		J_2	J_3/J_4	♀	♀'s with eggmass	J_2	J_3/J_4	♀	♀'s with eggmass
2	Tomato	0	207	0	0	0	200	0	0
4		0	0	208	19	0	0	205	18
6		0	0	38	259	0	0	34	210
8		20	0	0	233	29	29	0	216
2	Sweet corn	0	195	0	0	0	238	0	0
4		0	0	189	16	0	0	199	18
6		0	0	34	237	0	0	70	201
8		13	0	0	215	21	0	0	251
2	Maize	0	62	0	0	0	59	0	0
4		0	0	146	19	0	0	138	15
6		0	0	27	100	0	0	23	81
8		0	0	0	167	0	0	0	135
2	Sorghum	0	44	0	0	0	48	0	0
4		0	0	37	9	0	0	34	16
6		0	0	12	33	0	0	11	41
8		0	0	0	64	0	0	0	69
2	Millet	0	23	0	0	0	26	0	0
4		0	0	29	0	0	0	36	0
6		0	0	0	29	0	0	0	46
8		0	0	0	32	0	0	0	49

Table 23 Numbers eggs/eggmass produced on tomato and graminaceous hosts from juveniles originating from tomato and sweet corn after 8 weeks[1)]

Inoculated Hosts	Isolate C		Isolate D	
	Original Host		Original Host	
	Tomato	Sweet corn	Tomato	Sweet corn
Tomato	329	269	324	257
Sweet corn	271	290	269	279
Maize	246	219	192	194
Sorghum	166	188	137	153
Millet	122	125	98	98

1) Mean of 5 replicates

L.S.D. 5% (Origin) = 13.6
(Host) = 24.4
(Isolate) = 37.4

Table 24 Mean Root-weight at 2 week intervals of different hosts inoculated with juveniles of isolate C from tomato and sweet corn[1)]

(a) Root-weight (ISOLATE C)

Original Hosts	Inoculated Hosts	WEEK INTERVAL 2	4	6	8
Tomato	Tomato	12.5	14.6	19.6	24.4
	Sweet corn	16.5	19.8	21.3	29.2
	Maize	18.8	22.6	25.1	25.6
	Sorghum	13.2	16.5	21.2	33.7
	Millet	13.4	17.0	26.1	26.2
Sweet corn	Tomato	12.7	16.0	19.7	25.2
	Sweet corn	17.9	20.0	22.8	30.8
	Maize	19.1	26.3	28.6	26.0
	Sorghum	12.8	17.1	18.5	35.0
	Millet	12.9	17.6	21.7	27.4
Control plants	Tomato	12.1	17.8	24.8	26.6
	Sweet corn	17.6	21.5	23.1	30.3
	Maize	20.7	27.3	29.6	28.8
	Sorghum	12.8	18.1	18.6	20.9
	Millet	14.0	18.5	22.5	23.8

1) Mean of 2 replicates

L.S.D. 5% (Origin) = 6.4 (Host) = 4.5 (Isolate) = 18.4

Table 25 Mean Root-weight at 2 week intervals of different hosts inoculated with juveniles of isolate D from tomato and sweet corn[1)]

(a) Root-weight (ISOLATE D)

Original Hosts	Inoculated Hosts	WEEK INTERVAL 2	4	6	8
Tomato	Tomato	13.5	15.6	19.8	25.4
	Sweet corn	17.4	20.3	22.2	30.3
	Maize	17.5	23.7	25.9	27.0
	Sorghum	12.1	17.6	22.4	35.2
	Millet	13.1	29.6	22.0	26.8
Sweet corn	Tomato	13.7	16.4	23.1	27.9
	Sweet corn	17.0	21.6	23.5	30.3
	Maize	19.8	28.0	28.1	27.0
	Sorghum	13.1	18.1	22.0	31.7
	Millet	14.8	18.7	23.9	29.2
Control plants	Tomato	12.1	17.8	24.8	26.6
	Sweet corn	17.6	21.5	23.1	30.3
	Maize	20.7	27.3	29.6	28.8
	Sorghum	12.8	18.1	18.6	20.9
	Millet	14.0	18.5	22.5	23.8

1) Mean of 2 replicates

L.S.D. 5% (Origin) = 6.4 (Host) = 4.5 (Isolate) = 18.4

the original tomato host, but infectivity was significantly greater for nematodes from original corn host on tomato, sweet corn and maize.

Isolate D had a similar infection rate as isolate C on the graminaceous hosts. In fecundity, the numbers of eggs produced by the original tomato host when transferred to the 2nd host tomato were higher than those produced by nematodes that were transferred to sweet corn.

B2 - Invasion of tomato and corn roots by juveniles of 2 isolates of M.incognita from tomato and corn

More nematodes invaded in corn roots than tomato where the inoculated nematodes were from corn roots. Using populations from the original corn host, the number of nematodes that invaded the 2nd corn host was greater than those that invaded the 2nd tomato host. Two days after inoculation, the difference between the invasion rates by the juveniles into the corn and the tomato was equal in both cases. These differences were significant between the invasion rates of populations that originated from tomato and corn. For example, there was more invasion on corn if the nematodes had originated from corn, if the invasion rates were compared with tomato 6, 8 and 10 days after inoculation (Table 25).

Table 26 Comparison of invasion by isolate C cultured on tomato and sweet corn and inoculated onto 2 different hosts[1)]

Day-intervals	Original Hosts: Tomato		Original Hosts: Corn	
	Inoculated Hosts: Tomato	Inoculated Hosts: Corn	Inoculated Hosts: Tomato	Inoculated Hosts: Corn
2	27	14	16	29
4	51	33	40	40
6	112	77	83	103
8	268	186	178	198
10	299	223	212	235

1) Mean of 4 replicates

L.S.D. 5% Origin (O) = 12.415

Host (H) = 15.617

Isolate (I) = 27.414

B3 - Pathogenicity of 2 isolates cross-inoculated on 4 different hosts

Juveniles, originating from both tomato isolates E and G, had the same infectivity to fresh tomato plants (Table 26). Root-knot indices on tomato were between 4 and 4.5. With isolate G, the original host affected the pathogenicity of the nematodes on the different secondary hosts. Nematodes originating from okra produced more root galls on okra than on the other hosts (Table 26). Also nematodes originating from egg plant produced more root galls on egg plant than the other hosts. There was a highly significant reduction in the root weights of all infected hosts when compared to the control plants (Table 27).

DISCUSSION

A Host-Range Study

The fecundity on different crops was not the same among the isolates and the results obtained from the two host range experiments were similar. The evidence of the infectivity on corn showed that isolates of M.incognita used in these studies differed considerably in their pathogenicity.

Physiological races or biotypes or pathotypes can be found in populations within a species which

Table 27 Mean root-knot index of different hosts inoculated with juveniles of isolates E and G from the same and different original hosts[1)]

Inoculated Hosts	Original Hosts							
	Tomato		Egg plant		Okra		Tobacco	
	E	G	E	G	E	G	E	G
Tomato	4	4.5	4	4	4	4	4	4
Egg plant	3.5	3.5	3.8	4	3.5	3.5	3	4
Okra	3.5	4.5	3	3.5	3.8	4	3	3.0
Tobacco	4	4	3	3.5	3.5	3.5	3.8	3.8

1) Mean of 4 replicates

L.S.D. 5% = 0.954

1% = 1.994

Table 28 Mean Root-weight and Top-weight of different hosts inoculated with juveniles[1] of isolates E and G from the same and different original hosts[2]

(a) Root weight

Inoculated plants	Weight (g)								
	Original Hosts								Control plant
	Tomato		Egg Plant		Okra		Tobacco		
	E	G	E	G	E	G	E	G	
Tomato	35.4	31.9	37.8	36.4	34.4	38.9	38.9	40.3	46.4
Egg plant	44.4	43.4	41.8	37.2	37.2	41.4	39.2	39.4	47.2
Okra	35.2	46.4	33.4	39.4	39.4	39.8	38.4	39.2	39.9
Tobacco	34.2	34.9	34.7	37.2	37.2	31.8	31.8	30.4	43.4

(b) Top weight

Tomato	99.4	89.4	99.4	90.4	99.6	96.4	96.4	95.2	101.4
Egg plant	92.1	97.2	89.4	91.8	83.4	86.7	86.7	87.4	122.6
Okra	97.8	98.2	98.2	89.7	99.4	99.4	101.8	101.2	124.4
Tobacco	86.2	81.1	91.2	78.2	88.2	88.2	87.2	109.8	135.4

1) Number of juveniles inoculated per pot = 500

2) Mean of 4 replicates

react differently on a given host, or change in their pathogenicity, development and behaviour on the host (Allen, 1952; Dropkin, 1959; Sasser, 1954, 1966, 1972).

Races exist within the isolates if these are distinguished on the basis of their ability to reproduce differently on different hosts.

These results are similar to those obtained by Southards and Priest (1971), Allen (1952), Dropkin (1959) and Sasser (1972) showing that physiological races exist in M.incognita. The results obtained from the first experiment showed that two races or biotypes occurred in corn. There was also an indication that a third race, distinguished by its infectivity on bean, existed within the isolates.

None of the isolates produced galls or eggmasses on hot pepper and this host appeared highly resistant to all 7 isolates of M.incognita. Similar results have been observed on hot pepper (Capsicum annum) by Hare (1956). On corn roots, necrosis was observed for isolates A, B, E, F and G but no nematodes were found in the roots. Nematodes of these 5 isolates of M.incognita failed to reproduce on corn roots. Root tip swellings on corn were possibly due to invasion of nematodes although none were found in the roots. Migration of Meloidogyne juveniles from roots back into the soil after producing host reaction has been reported by Reynolds et al.(1970) and Escober (1975) on other hosts. Christie (1949) suggested that host resistance to root-knot was related to lack of larval entry, failure of giant cell formation and root tissue necrosis.

Root swellings on corn were only observed in the meristematic areas of root tips. Corn is considered to be a poor host for Meloidogyne spp (Baldwin and Baker, 1970) so it is used in crop rotation for control of Meloidogyne infections of other crops. But susceptibility may arise from natural selection of nematode biotypes adapted to corn (Sasser and Nusbaum, 1955). Baldwin and Baker (1970), Dropkin (1959), and Nelson (1957) reported differences in susceptibility among certain corn inbreds, which indicated that some may be more resistant than others to root-knot nematodes. It clearly shows that it is not possible to define with any certainty the host range of M.incognita because of the different populations that can exist within an area or country. In order to be safe in using corn in crop rotations to control M.incognita, it should be tested against local populations of the species.

The host status for Meloidogyne, as measured by populations of nematodes on the roots, can be assessed by different methods. Fox and Miller (1971) measured populations by their eggmass ratings in five roots. They concluded that the number of galls formed was not a reliable index of the reproduction of root-knot nematodes.

I measured the variability in pathogenicity between the populations not only by root-knot index rating, but also by number of females, root-weights, eggcounts, galls/g roots, and eggs/g roots. For all isolates, except isolate A, the number of eggmass per gram of root, eggs per eggmass and females per gram of root coincided with root-knot index especially

on tomato, but was also true for other hosts. Isolate A produced an equivalent number of galls, but the females produced fewer eggs.

To assess the host specificity of the root-knot nematode, the root-knot index, number of females per gram of root and fecundity are all important. For experimental work, all these assessment methods are possible though laborious; but for field experiments, root-knot indexing is the only practical method. From my experiment, no. of galls are related to other assessment methods, and in the field experiment with many more plants to examine, it is the quickest and most accurate. However, both the number of nematodes that develop to maturity and their fecundity are important because they show how much damage is being done to the crop and how much the population is being built up in the infested roots. For isolate A, its root-knot index was high but fecundity was low, but it must be pointed out that the plants were very heavily infested and had the most stunted growth. The nematodes did not have sufficient food as the root systems were reduced. So fecundity is not an accurate assessment of the pathogenicity in these older heavily infested roots. Also very few females are found inside the roots. Therefore, the number of females or eggmasses per gram of root or number of eggs per eggmass do not always accurately describe the infestation or damage suffered by the host.

B Influence of Original Hosts

In my experiments I set out to discuss whether nematodes were capable of becoming adapted to the same host after a period of one year and whether subsequently their ability to penetrate and infest new hosts was altered. The numbers of nematodes in the soil are affected by the types of crops which are grown, and their yields are reduced as the nematode population size increases. Crop rotation attempts to keep such populations to a level at which crop damage is reduced to a minimum. It is believed that by growing a non-host crop, the numbers of plant parasitic nematodes will be reduced, the number of years for this to occur depending on the initial population and the rate of population decrease.

In a crop rotation method, when determining the crop rotation for a particular area, it is important to identify which crops are attacked by the root-knot nematodes. Short-term crop rotation should be used so that the nematodes cannot adapt to the hosts which are grown in long term in the same field.

Baldwin and Baker (1970) reported although corn was previously thought to be a reliable non-host suitable for use in a rotation, they found that the population of M.incognita increased with corn in the rotation. As corn was used frequently in rotations, M.incognita mutants had reproduced rapidly on the corn varieties. Sasser and Nusbaum (1955) made similar observations that corn-tobacco rotations

became ineffective for controlling the nematodes as corn became susceptible to strains of the root-knot nematodes. From my observations, the infectivity to sorghum, maize and millet was less when compared to the tomato and hybrid sweet corn. Similar results have been reported that there was less infection to sorghum, maize, barley and oat. (Sasser, 1954, 1966; Linde, 1956).

Dean et al. (1953) and Graham (1964) reported on resistant tomato and tobacco that resistant reactions were sometimes accompanied by damage to roots and these at least temporarily limited growth.

From the cross infection experiment, the infection of isolates C and D on corn, after it was cultured on corn nearly one year, increased. Similar observation has been reported by Sasser and Nusbaum (1955) that corn was non-host previously grown in the field to the original population but later root-knot nematodes adapted to corn when it was cultured for several years.

SECTION V MORPHOLOGICAL STUDIES ON 7 ISOLATES OF *M.incognita*

INTRODUCTION

The taxonomy of the root-knot nematodes was first reviewed by Chitwood (1949), who separated the species according to their posterior cuticular patterns and identified five species and one sub-species of *Meloidogyne*. In 1968, Whitehead gave detailed descriptions of the 23 species of *Meloidogyne* known to him. He studied a great number of male characters, as well as those of female and 2nd stage juveniles in order to determine methods for the identification of *Meloidogyne* species which depended to a lesser extent on the perineal pattern. He found that the length of the second stage juvenile was a suitable character to differentiate some species of *Meloidogyne*. Franklin (1972) reported that the larval characteristics and the head characters of males were easier to define than posterior cuticular patterns because they can normally be measured. In the region of the tail terminus, the cuticle is ridged and furrowed in a finger-print like pattern. This area includes the tail terminus, phasmids, lateral fields, anus and vulva which is surrounded by cuticular folds making a pattern for the different *Meloidogyne* species. However, Sasser reported that the patterns of individuals and populations within a species often vary.

The species M.incognita was first recognised by Chitwood (1949) who divided it into two sub-species, M.incognita incognita and M.incognita acrita, according to their posterior cuticular patterns. He found that the post-anal region of M.incognita had a distinct whorl, however, M.incognita acrita had no whorl but had folded striae. Taylor et al. (1955) emphasised the importance of shape and the spacing between striae on the arch of the posterior cuticular pattern. He distinguished M.incognita acrita as showing an arch which was formed by widely spaced wavy striae and M.incognita incognita with striae which were closely spaced and wavy to zig-zag. Whitehead (1968) identified the M.incognita type pattern as having a high dorsal arch, a rounded shape and closely spaced striae, but acrita type had a flattened dorsal arch, smooth and wider striae.

Dropkin (1959) studied the variables of the posterior cuticular patterns of two species, M.incognita and M.arenaria and found that, although the general characteristics of the pattern were inherited, the coefficients of variability of pattern shape within the two lines derived from single larvae of M.arenaria were about 20% and, in one M.arenaria population, 40%. He also reported that the progeny of a single female M. incognita may vary greatly, but special characteristics of an isolate were often inherited. There was no evidence that the patterns were affected by host species and less variation after successive, continuous generations (Triantaphyllou and Sasser, 1960). There is much variation between populations of the same species (Whitehead, 1968) and the morphology of the nematodes

can be varied by environmental factors such as temperature, geographical origins, quality and quantity of nutrition and hosts. (McClure and Viglierchio, 1966a; Bird and Mai, 1967, 1967a; Webster and Hooper, 1968; Evans and Fisher, 1969, 1970b).

Triantaphyllou and Sasser (1960) suggested that, though the perineal pattern was affected by age of the female, the variation could be the result of the rate of development of the nematode, the position in the host tissue and other factors. Franklin (1972) showed that the value of the measurements in perineal patterns was limited because of variation in size of females of a given species.

Netscher (1973) has observed the morphological differences between progeny from single larval inoculations of M.javanica with several generations. He found that the mean length of juveniles varied considerably from one generation to the next. The comparative morphology of 16 isolates of M.incognita was studied by Priest and Southards (1971). They found that there were differences in some morphological characters, especially in measurements of body length, tail length and the distance from the stylet base to the dorsal oesophageal gland orifice.

The purpose of these experiments was to study the variability of morphological characters within and between the isolates of M.incognita as affected by the different original hosts and to determine if any morphological differences that may exist between isolates were related to differences in their biological behaviour.

MATERIALS AND METHODS (GENERAL)

(a) Extraction

Eggs of mature eggmasses were hatched on a small 40u nylon sieve in water in a watch glass for 24-48 hours and the juveniles in suspension from the watch-glass were collected. Live females for measurement were taken out from the infected roots with fine forceps and kept in sterile distilled water. The eggs were separated from the eggmasses by teasing in 2% NaOCl solution for 15 minutes and double washing in distilled water before measuring.

(b) Processing and Mounting

Before processing and fixing in T.A.F., the nematodes were relaxed in hot water. The dead nematodes, in water suspension, were concentrated by decanting off supernatant and by drawing off the remaining excess water with a fine pipette. The nematodes were kept in the fixative for at least 72 hours before processing.

For processing, the fixed specimens were transferred through a series of lactophenol-glycerol solutions with increasing amounts of glycerol until at the end the nematodes were left in pure glycerol (Baker, 1958). All the operations were carried out at 60°C on a hot plate, and the whole process took about 1-1½ hrs.

Nematodes were picked at random and mounted in anhydrous glycerol, coverslips were supported by pieces of glass wool and slides were sealed with glyceel. (Goodey, 1963; Hooper, 1970).

(c) Drawing and Measurement

A drawing tube attachment (Camera lucida) for the Wild M20 and M12 was used to draw all the specimens. Drawings of the specimens were measured with a flexible thin lead fuse wire. In each case, 5 females, 20 juveniles and 20 eggs were measured, also 5 males when present. Measurement of stylet length, body width and median oesophageal bulb length were made at a magnification of 1000X, while those of body length were made at a magnification of 200X. Cuticular patterns around the vulva were cut with a sharp needle and trimmed, then stained in cotton blue lactophenol and mounted in pure lactophenol.

A MORPHOLOGICAL VARIATION AS AFFECTED BY THE HOST

Females, juveniles, eggs and males (if present) from host-range study I (Section IV) were used for the study of morphological variation as affected by the host after continuous generations. Measurements were taken for all isolates from each crop and compared to the original measurements of each isolate. The type of the perineal patterns of the females was also drawn.

B SINGLE LARVAL INOCULATION STUDIES ON AGAR AND IN SOIL CULTURES

Single larval inoculation for reproduction without males in aseptic root cultures of the root-knot nematode was first found by Tyler (1933). Then Dropkin (1953) attempted studies on the variability of the posterior cuticular pattern in pure lines of two Meloidogyne spp, M.incognita acrita and M.arenaria. He compared the types of the posterior cuticular patterns of original females and their progeny and found that there was less variability in single larva families of M.incognita acrita. Netscher (1971) tried single larva inoculation by using artificial media cultures and observed that the mean length of juveniles varied considerably from one generation to the next but there was overlap in the mean length of juveniles. I attempted single larva inoculation to find out whether the morphological characters were stable and consistent in successive generations of the different M.incognita isolates.

Additional Materials and Methods

1. Single larval inoculation on agar plates

Freshly hatched juveniles from all isolates were used for single larval inoculation. Tomato seeds var. (Moneymaker) were washed for a few seconds with 70% pure alcohol and

transferred into sterilised distilled water and rinsed 3 or 4 times. Then the seeds were stirred in 2% NaOCl for ten minutes and rinsed three times in sterilised distilled water before transferring them into petri dishes containing sterilised filter paper. Three days later the seeds germinated and were then transferred into a petri dish containing modified Whites medium (Goodey, 1963). Then 2 days later, roots of each seedling were inoculated with a single 2nd stage juvenile. Before inoculation, juveniles were surface sterilised with 0.5% hibitane solution for 5 minutes and then rinsed in sterilised distilled water 3 times. After that, the juveniles were placed to the tip of the tomato root. Petri dishes containing single nematode and tomato seedlings were kept in a 27°C controlled temperature room. All procedures were done in sterile conditions in an attempt to avoid contamination from fungi and bacteria.

2. Single larval inoculation in soil

Freshly hatched juveniles from isolates B and C were used for single larval inoculation. Ten day old, tomato seedlings (var. Moneymaker) were selected and transferred into the mixture of sterilised soil in 3" plastic pots. Single juvenile was inoculated onto each plant by using a small syringe. The inoculated plants were placed in the glasshouse at the ambient temperature of 24 $\pm$ 4°C. Before starting the experiment, the measurements of the parents (juveniles, females, perineal pattern) were taken for each isolate. For the next generation, the inoculum was used from the previous generation and the measurements of the nematodes, number of eggs production were taken at each generation.

RESULTS

From my observations there was no host effect on the morphology characters after 40 weeks cultured on the same host in the glasshouse. The perineal patterns and length of juveniles slightly varied in some cases but statistically they were not highly significant (Table 31). Though the perineal patterns of each isolate from different hosts varied in some cases, basically they were the same. For isolates C (Ecuador) and D (El Salvador), the perineal patterns from corn host slightly differed from the perineal patterns from other crops. On the perineal patterns of females from corn host there were numerous broken striations on the outer surface of the arch which the isolates from other hosts did not have.

Among the isolates, isolate B was the shortest and isolate F was the largest in larval length. The lengths of the larvae of all isolates from original cultures differed from each other. Isolates C, D, F and G were not significantly different in larval length but there were significant differences from isolates A, B and E. There were significant differences in some of the characters, i.e. tail length, female body width and length, female median oesophageal bulb and female stylet lengths (Table 29a). But the body width of the juveniles did not differ significantly between the isolates.

From the observations, basically, the perineal patterns of isolates A, B, E, G and F were Meloidogyne

incognita type with a high arch, and close, wavy striae. However, isolates C and D had flat arches and striations were (in some cases) close. The shape of the perineal pattern was more or less square shape, though in isolates A, B, E, G and F it was more of an oblong shape (Fig. 4).

2. Single larva inoculation

From the single larval inoculation experiment, the results with agar plates were discarded because of fungus contamination. Only the results from the glasshouse experiment were taken. From the first generation of isolate C, eggmasses produced 320 eggs/eggmass whereas isolate B had eggmasses with average 290 eggs/eggmass. In the second generation, isolate C produced eggmasses with 350 eggs/eggmass, and in the third generation 360 eggs/eggmass (Table 45). Isolate B produced 320 eggs/eggmass at the second generation and 340 eggs/eggmass at the third generation.

There were males produced in the second and third generations of isolate C; numbers produced in the third generation were greater than those in the second generation. Males were always observed in large mature eggmasses. There was no significant difference in measurements of males between the generations (Table 44), and no male production was observed with isolate B.

There was no significant difference in the measurements of all characters including larvae, females between the generations in both isolates, and the range of measurements was smaller (Table 42, 43). The perineal patterns of C and B of the continuous generations were similar to those of the parents (Fig. 5).

DISCUSSION

From my observations, it was clear that there was morphological variation between the isolates but no differences within isolates from the hosts. Similar observations on morphological variation between populations have been reported by Whitehead (1968), Sasser (1953), and Bird (1967). As the isolates originated from different parts of the world, the geographical factors could be an influence on the morphological characters, they could have become adapted especially to the hosts and temperatures of those countries. Temperature has been correlated with the morphological characters of the nematodes (Sasser, 1956; Triantaphyllou, 1968; Evans and Fisher, 1970b). Though the perineal patterns were not identical from the different hosts, their basic characters were similar. This agrees with results obtained by other workers (Dropkin, 1953; Sasser, 1953; Triantaphyllou and Sasser, 1960) who showed that there was no evidence of the influence of the hosts on the morphological difference of Meloidogyne.

In the first generation the patterns were similar to the original patterns with little variation. In the third generation, the same isolate also showed a very similar type of perineal pattern, and variation was again small.

The number of males produced by the third generation was greater than those of the second generation. Males were observed in the big eggmasses. These observations were similar to those of the host range study. Males may

copulate with females and could possibly stimulate greater egg production and thus larger eggmasses. To distinguish the above isolates of M.incognita, the measurements of juvenile body length, tail length, body width, stylet length were more useful than the type of perineal patterns because the perineal patterns were not always constant except in isolates C and D.

Among root-knot nematodes, M.carolinensis has pure amphimictic reproduction, and M.arenaria, M.exigua, M.hapla and M.graminicola can reproduce both parthenogenetically and amphimictically. The rest of the Meloidogyne spp are parthenogenetic. (Triantaphyllou, 1971). As males with one testis are true males, amphimictic reproduction may occur in isolates C, D and G. The fecundity from the single larval inoculation of isolate C was better at the third generation with more production of eggs.

The perineal pattern types of isolate C and D were neither M.incognita incognita nor M.incognita acrita type. But they were between them because of the flattened arch with those striations. From my observations, two biological races occurred within the populations separated by their biological behaviour, but also by the production of males and their perineal patterns. If detailed work of morphological studies on those two isolates is done, new species may occur. The isolates C and D were similar in both morphology and biological characters.

Table 29(a) Original Measurements of M.incognita Isolates cultured on tomato before inoculation to different hosts in the host-range experiment

Isolates	Juvenile				Egg	
	Body length	Body width	Tail length	Stylet length	Length	Width
A	353.8 ± 17.7 (340 - 387)	12.95 ± 0.28 (12.02 - 13.4)	38.61 ± 2.11 (35.2 - 41.8)	11.47 ± 0.42 (10.5 - 11.98)	79.0 ± 2.84 (76 - 82.8)	37.9 ± 1.77
B	338.8 ± 11.5 (328 - 362)	12.19 ± 0.32 (11.8 - 12.24)	35.5 ± 1.33 (32.4 - 38.6)	10.38 ± 0.49 (9.8 - 11.4)	72.7 ± 2.45	33.4 ± 1.6
C	371.9 ± 22.5 (342 - 412)	13.11 ± 0.22 (12.4 - 13.8)	39.53 ± 1.95 (35.0 - 42.4)	11.85 ± 0.39 (10.9 - 12.6)	80.5 ± 2.03	38.4 ± 3.4

cont'd....

Isolates	Juvenile				Egg	
	Body length	Body width	Tail length	Stylet length	Length	Width
D	379.2 ± 15.8 (348 - 402)	13.61 ± 0.33 (12.8 - 13.98)	38.94 ± 1.75 (34.6 - 42.8)	12.18 ± 0.36 (10.9 - 13.0)	80.8 ± 3.20	38.9 ± 1.37
E	351.8 ± 14.7 (338 - 384)	12.82 ± 0.17 (11.9 - 13.2)	36.6 ± 1.4 (33.2 - 38.2)	10.93 ± 0.53 (10.1 - 11.9)	77.5 ± 2.41	35.4 ± 1.95
F	383.4 ± 15.3 (348 - 412)	13.56 ± 0.37 (12.4 - 13.86)	40.17 ± 1.66 (38.6 - 42.4)	12.45 ± 0.37 (11.2 - 13.)	82.4 ± 4.08	39.7 ± 1.85
G	381.3 ± 19.9 (338 - 408)	13.03 ± 0.28 (12.6 - 13.92)	39.66 ± 1.63 (37.2 - 43.4)	11.98 ± 0.43 (10.8 - 12.8)	81.6 ± 3.20	39.6 ± 1.75
L.S.D. 5%	17.9	0.11	0.91	0.76	3.4	2.5

Isolates	Female					
	Body length	Body width	Stylet length	Stylet base	Median bulb length	Median bulb width
A	535.8 ± 25.9 (502.2 - 586)	374.6 ± 15.9 (360 - 422.4)	14.34 ± 0.19 (13.8 - 14.68)	3.9 ± 0.24 (3.7 - 4.2)	45.6 ± 1.91 (43.2 - 48.2)	38.8 ± 1.59 (36.4 - 41.2)
B	552.8 ± 17.6 (522 - 582.9)	399.2 ± 16.62 (352.0 - 420.8)	14.04 ± 0.32 (13.4 - 14.78)	3.52 ± 0.2 (3.2 - 4.0)	42.0 ± 2.85 (40.8 - 45.8)	35.8 ± 1.69 (33.2 - 40.0)
C	572.8 ± 25.29 (539.9 - 601.4)	400.4 ± 16.6 (372.8 - 440.8)	14.80 ± 0.39 (13.9 - 15.3)	3.96 ± 0.04 (3.78 - 4.2)	51.8 ± 3.35 (48.2 - 53.8)	40.4 ± 3.53 (36.2 - 46.8)
D	570.4 ± 15.9 (542.2 - 602.8)	425.0 ± 24.26 (369.9 - 462.9)	14.84 ± 0.77 (14.0 - 15.8)	4.0 ± 0.06 (3.9 - 4.2)	53.2 ± 2.5 (49.8 - 56.4)	44.2 ± 3.79 (39.4 - 49.2)

cont'd...

Isolates	Female					
	Body length	Body width	Stylet length	Stylet base	Median bulb length	Median bulb width
E	547.8 ± 23.48 (520.2 - 588.9)	377.2 ± 33.1 (331 - 422.4)	14.12 ± 0.26 (13.8 - 14.4)	3.86 ± 0.1 (3.70 - 4.0)	48.2 ± 2.76 (44.2 - 52.8)	34.6 ± 2.26 (32.0 - 39.0)
F	610.0 ± 27.0 (544.4 - 648.8)	468.4 ± 28.59 (421.2 - 502.8)	15.08 ± 0.22 (14.0 - 15.6)	4.16 ± 0.04 (3.9 - 4.28)	60.0 ± 2.89 (56.2 - 62.8)	49.9 ± 2.82 (45.4 - 52.8)
G	583.0 ± 22.4 (549.9 - 630.8)	407.6 ± 23.24 (362.3 - 442.8)	14.84 ± 0.34 (14.4 - 14.6)	4.08 ± 0.05 (3.9 - 4.28)	53.46 ± 2.5 (48.4 - 58.4)	46.8 ± 1.83 (43.8 - 50.2)
L.S.D. 5%	20.0	22.4	0.91	0.08	2.8	2.4

TABLE 30 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Measurement of larval body width

Isolates / Host	A	B	C	D	E	F	G
Tomato	12.75 ± .14	12.11 ± .09	13.29 ± .10	13.77 ± .16	12.82 ± .21	13.16 ± .04	13.12 ± .12
Egg plant	12.97 ± .13	12.04 ± .06	13.75 ± .22	13.97 ± .07	12.74 ± .16	13.34 ± .09	13.08 ± .08
Okra	12.85 ± .08	12.08 ± .12	13.37 ± .11	13.9 ± .13	12.89 ± .18	13.4 ± .12	13.19 ± .17
Tobacco	12.93 ± .11	12.16 ± .17	13.69 ± .13	13.91 ± .18	12.92 ± .12	13.22 ± .06	13.11 ± .4
Lettuce	12.97 ± .17	12.16 ± .12	13.85 ± .07	13.74 ± .22	12.70 ± .14	13.19 ± .08	13.10 ± .13
Corn	-	-	13.84 ± .08	13.80 ± .27	-	-	-

TABLE 31 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Measurement of larval body length

ISOLATES / HOST	A	B	C	D	E	F	G
Tomato	358.6 ± 17.2	339.5 ± 18.8	386.75 ± 19.2	379.2 ± 12.06	359.5 ± 11.13	379 ± 16. 4	372.5 ± 11.5
Egg plant	355.6 ± 19.0	348 ± 20.05	386.25 ± 12.9	381.8 ± 12.41	362.25± 14.11	385.6 ± 12.28	375.2 ± 15.2
Okra	362.5 ± 15.4	338.9 ± 23.38	389.2 ± 11.9	381.2 ± 12.28	361.7 ± 13.54	377 ± 13.02	377.5 ± 16.1
Tobacco	362.6 ± 16.3	348.6 ± 11.6	387.3 ± 11.3	388.6 ± 12.87	368. ± 11.83	373.65± 13.15	389 ± 13.0
Lettuce	358.4 ± 17.7	348.0 ± 13.46	382.4 ± 19.1	388.1 ± 12.24	360.5 ± 12.03	372.45± 17.5	388 ± 11.07
Corn	-	-	383.5 ± 14.19	379.0 ± 13.58	-	-	-

TABLE 32 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Measurement of tail length

Isolates / Host	A	B	C	D	E	F	G
Tomato	39.1 ± .74	35.54 ± .06	38.54 ± .26	39.33 ± .55	37.1 ± .46	37.79 ± .28	37.2 ± .4
Egg plant	38.94 ± .45	35.66 ± .14	38.60 ± .11	39.25 ± .65	35.7 ± 6.5	39.44 ± .56	38.9 ± .5
Okra	38.95 ± .59	35.67 ± .13	38.41 ± .15	39.5 ± .66	35.68 ± 6.53	39.7 ± .27	38.4 ± .5
Tobacco	38.74 ± .44	35.77 ± .10	38.78 ± .17	39.65 ± .54	37.19 ± .47	39.59 ± .67	39.0 ± .5
Lettuce	39.01 ± .41	35.72 ± .32	38.79 ± .16	39.5 ± .57	37.18 ± .32	46.24 ± .47	38.2 ± .4
Corn	-	-	38.77 ± .18	37.9 ± .28	-	-	-

TABLE 33 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Measurement of stylet (larval stylet length)

Isolates / Host	A	B	C	D	E	F	G
Tomato	11.56 ± .18	10.37 ± .04	11.6 ± .24	12.31 ± .15	11.05 ± .1	11.82 ± .04	11.5 ± .09
Egg plant	11.51 ± .14	10.51 ± .12	11.97 ± .09	12.35 ± .15	11.08 ± .11	11.84 ± .08	12.0 ± .02
Okra	11.74 ± .12	10.06 ± .17	11.73 ± .23	12.37 ± .26	11.01 ± .16	11.95 ± .06	11.8 ± .04
Tobacco	11.76 ± .12	10.47 ± .18	11.86 ± .16	12.53 ± .26	11.19 ± .14	12.0 ± .17	11.4 ± .02
Lettuce	11.49 ± .12	10.55 ± .08	11.85 ± .15	12.42 ± .19	11.10 ± .18	11.95 ± .06	11.9 ± .04
Corn	-	-	11.88 ± .13	11.99 ± .15	-	-	-

TABLE 34 THE EFFECT OF HOST ON M.incognita ISOLATES

Female Body Length

Isolates / Host	A	B	C	D	E	F	G
Tomato	588.8 ± 21.4	548.2 ± 19.4	611.2 ± 22.4	602.2 ± 23.4	562.2 ± 30.2	579.2 ± 29.2	612.2 ± 32.4
Egg plant	542.4 ± 19.9	538.2 ± 19.2	594.2 ± 21.6	612.4 ± 24.6	548.2 ± 28.7	599.8 ± 30.2	599.2 ± 27.2
Okra	568 ± 19.4	544 ± 19.4	572.2 ± 19.4	614.3 ± 25.2	578.2 ± 26.8	592.2 ± 29.2	578.2 ± 22.2
Tobacco	578.8 ± 21.0	552.7 ± 21.6	566.2 ± 19.4	606.2 ± 19.4	590.2 ± 27.8	572.2 ± 29.2	599.8 ± 26.2
Lettuce	587 ± 20.4	568.2 ± 22.8	598.8 ± 18.2	599.4 ± 19.2	582.2 ± 27.2	578.2 ± 27.2	602.2 ± 27.8
Corn	-	-	578.7 ± 21.4	594.2 ± 19.2	-	-	-

TABLE 35 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Female Body Width

Isolates / Host	A	B	C	D	E	F	G
Tomato	429.2 ± 19.4	402.2 ± 18.4	440.2 ± 27.2	429.4 ± 26.4	410.2 ± 19.2	496.2 ± 26.2	426.2 ± 17.2
Egg plant	422.2 ± 18.4	412.2 ± 22.4	438.2 ± 26.8	442.4 ± 27.2	428.2 ± 22.4	410.2 ± 29.2	437.2 ± 17.8
Okra	434.2 ± 18.6	418.2 ± 17.2	444.4 ± 27.2	414.2 ± 26.9	435.2 ± 27.2	420.8 ± 27.2	440.2 ± 22.2
Tobacco	428.2 ± 26.8	419.2 ± 17.2	452.4 ± 28.2	427.2 ± 19.4	444.2 ± 26.2	430.2 ± 19.8	418.2 ± 20.2
Lettuce	429.2 ± 26.7	429.2 ± 26.2	442.4 ± 27.2	427.2 ± 23.2	424.2 ± 26.2	440.2 ± 27.8	419.2 ± 27.8
Corn	-	-	428.8 ± 26.2	404.2 ± 22.8	-	-	-

TABLE 36 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Female stylet length

Isolates / Host	A	B	C	D	E	F	G
Tomato	14.9 ± .58	14.9 ± .40	14.2 ± .50	15.9 ± .49	15.6 ± .44	14.8 ± .42	15.2 ± .49
Egg plant	15.4 ± .24	15.2 ± .44	14.4 ± .52	16.0 ± .42	15.4 ± .49	14.8 ± .42	15.4 ± .49
Okra	14.8 ± .52	15.0 ± .42	14.9 ± .50	16.2 ± .52	15.2 ± .44	15.0 ± .49	14.2 ± .48
Tobacco	14.7 ± .22	14.8 ± .48	14.9 ± .48	15.6 ± .62	15.4 ± .42	16.0 ± .50	15.2 ± .42
Lettuce	14.9 ± .28	15.2 ± .50	14.7 ± .42	15.4 ± .42	15.4 ± .42	15.9 ± .49	14.8 ± .42
Corn	-	-	15.0 ± .48	15.2 ± .44	-	-	-

TABLE 37 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Female stylet, basal knob width

Isolates / Host	A	B	C	D	E	F	G
Tomato	4 ± .2	4 ± .19	4 ± .2	4 ± .2	4 ± .2	4 ± .2	4 ± .2
Egg plant	4.1 ± .19	4 ± .18	4 ± .19	4 ± .2	4 ± .2	4 ± .2	4 ± .2
Okra	4.2 ± .19	4 ± .12	4 ± .19	4 ± .2	4 ± .2	4 ± .2	4 ± .2
Tobacco	4.0 ± .19	4 ± .19	4 ± .19	4 ± .19	4 ± .2	4 ± .2	4 ± .2
Lettuce	4.0 ± .19	4 ± .19	4 ± .19	4 ± .19	4 ± .2	4 ± .2	4 ± .2
Corn	-	-	4 ± .19	4 ± .19			

TABLE 38 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Female Median oesophageal bulb length

Isolates / Host	A	B	C	D	E	F	G
Tomato	44.2 ± 2.6	47.2 ± 2.2	58.2 ± 2.2	50.2 ± 2.2	44.2 ± 1.69	50.2 ± 1.99	58.2 ± 1.9
Egg plant	46.9 ± 2.4	48.2 ± 2.19	54.2 ± 2.4	52.4 ± 2.1	46.2 ± 2.2	48.2 ± 2.0	49.2 ± 1.90
Okra	47.2 ± 1.99	49.4 ± 2.22	52.2 ± 2.2	52.4 ± 2.1	46.2 ± 2.2	48.2 ± 2.0	50.2 ± 1.28
Tobacco	48.2 ± 2.28	49.4 ± 2.14	49.2 ± 2.2	51.8 ± 2.2	47.4 ± 2.2	50.2 ± 2.0	50.8 ± 2.0
Lettuce	49.2 ± 2.42	50.2 ± 1.99	55.6 ± 2.2	51.2 ± 2.2	44.2 ± 2.2	52.2 ± 1.99	49.2 ± 2.2
Corn	-	-	52.4 ± 2.2	51.2 ± 2.2	-	-	-

TABLE 39 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Female Median oesophageal bulb width

Isolates / Host	A	B	C	D	E	F	G
Tomato	38.2 ± 1.82	39.0 ± 2.01	37.2 ± 1.99	38.2 ± 1.99	38.9 ± 1.62	38.4 ± 1.22	38.4 ± 1.45
Egg plant	38.4 ± 1.80	38.9 ± 1.99	38.2 ± 1.92	39.0 ± 1.92	38.8 ± 1.67	38.2 ± 1.20	38.9 ± 1.82
Okra	37.8 ± 1.78	38.9 ± 1.92	39.2 ± 1.94	38.2 ± 1.92	38.6 ± 1.68	38.4 ± 1.28	39.2 ± 1.86
Tobacco	38.0 ± 1.90	39.2 ± 2.01	38.2 ± 1.94	37.9 ± 1.90	38.6 ± 1.68	38.6 ± 1.29	38.6 ± 1.78
Lettuce	38.2 ± 1.88	38.8 ± 2.11	38.2 ± 1.94	38.9 ± 1.79	38.2 ± 1.68	38.8 ± 1.22	38.4 ± 1.25
Corn			38.4 ± 1.92	38.2 ± 1.78			

TABLE 40 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Measurement of Egg Length

Isolates / Host	A	B	C	D	E	F	G
Tomato	82.6 ± 2.92	78.4 ± 2.2	81.7 ± 3.0	80.8 ± 3.0	74.2 ± 2.2	80.0 ± 1.9	82.0 ± 1.99
Egg plant	80.4 ± 2.48	80.2 ± 2.1	82.0 ± 2.8	81.2 ± 3.0	74.2 ± 2.2	82.0 ± 2	80.8 ± 1.99
Okra	82.4 ± 2.2	78.4 ± 2.0	82.8 ± 2.2	80.8 ± 3.2	72.2 ± 2.0	84.0 ± 2.0	82.9 ± 1.99
Tobacco	80.4 ± 2.19	76.4 ± 2.2	82.8 ± 2.6	80.8 ± 3.2	74.2 ± 2.0	85.0 ± 1.99	80.8 ± 1.92
Lettuce	82.0 ± 2.2	78.4 ± 2.2	82.0 ± 2.2	80.8 ± 3.2	73.2 ± 1.99	84.0 ± 1.99	82.0 ± 3.8
Corn	-	-	82.0 ± 2.2	81.2 ± 2.8	-	-	-

TABLE 41 THE EFFECT OF HOST ON THE MORPHOLOGY OF M.incognita ISOLATES

Measurement of Egg Width

Isolates / Host	A	B	C	D	E	F	G
Tomato	38.4 ± 3.0	34.0 ± 2.8	37.6 ± 2.2	39.2 ± 1.99	38.9 ± 1.44	35.7 ± 3.5	39.2 ± 3.2
Egg Plant	37.4 ± 2.9	36.2 ± 2.2	36.9 ± 2.2	38.2 ± 2.0	39.0 ± 1.6	36.8 ± 3.2	40.2 ± 3.2
Okra	36.4 ± 2.8	34.2 ± 2.8	37.9 ± 2.2	38.2 ± 2.2	38.4 ± 1.6	34.9 ± 3.2	41.2 ± 3.2
Tobacco	37.2 ± 2.4	31.2 ± 2.8	38.9 ± 2.2	38.9 ± 2.2	38.4 ± 1.7	35.7 ± 3.4	42.2 ± 3.2
Lettuce	37.8 ± 2.8	34.2 ± 2.8	38.6 ± 2.2	38.2 ± 1.99	31.7 ± 2.2	36.4 ± 3.4	47.2 ± 3.8
Corn	-	-	37.9 ± 2.1	38.6 ± 1.28			

Table 42 Morphological studies on three generations of Isolate B originating from single juvenile inoculation

	Juvenile				Female						Egg	
	Body length	Body width	Tail length	Stylet length	Body length	Body width	Stylet length	Stylet base	Median bulb length	Median bulb width	Length	Width
Original	340.8 ± 13.2	13.0 ± 0.38	35.8 ± 1.25	10.42 ± 0.52	568 ± 16.2	401.2 ± 17.8	15.0 ± 0.38	3.58 ± 0.2	42.8 ± 2.8	35.8 ± 1.72	73.4 ± 3.4	34.5 ± 1.7
1st generation (from single juvenile)	342.4 ± 12.5	13.2 ± 0.4	35.8 ± 1.3	10.5 ± 0.6	574 ± 16.0	400.8 ± 16.2	14.8 ± 0.4	3.52 ± 0.2	41.9 ± 2.7	35.2 ± 1.8	74.4 ± 3.0	35.2 ± 1.9
2nd generation	342.8 ± 12.8	13.4 ± 0.6	36.8 ± 1.3	10.5 ± 0.4	570 ± 15.4	400.2 ± 19.8	14.6 ± 0.6	3.56 ± 0.19	43.0 ± 2.4	36.0 ± 1.9	76.0 ± 3.0	36.0 ± 1.98
3rd generation	345.8 ± 12.6	13.4 ± 0.5	35.9 ± 1.4	10.6 ± 0.4	567 ± 16.0	402.9 ± 22.2	15.0 ± 0.4	3.54 ± 0.22	43.2 ± 3.0	35.9 ± 1.22	74.0 ± 2.98	34.0 ± 1.92

Table 43 Morphological studies on three generations of Isolate C originating from single juvenile inoculation

	Juvenile				Female						Egg	
	Body length	Body width	Tail length	Stylet length	Body length	Body width	Stylet length	Stylet base	Median bulb length	Median bulb width	Length	Width
Original	382.6 ± 22.4	14.0 ± 0.35	38.9 ± 1.76	12.89 ± 0.4	584 ± 16.0	430 ± 23.5	15.2 ± 0.88	4.8 ± 0.08	54.2 ± 2.6	44.9 ± 3.8	80.8 ± 1.9	39.9 ± 2.22
1st generation (from single juvenile)	386.4 ± 16.2	14.8 ± 0.4	38.9 ± 1.5	12.8 ± 0.2	568 ± 17.6	440 ± 19.8	15.2 ± 0.9	4.8 ± 0.07	55.6 ± 2.8	45.2 ± 3.2	80.2 ± 2.0	40.2 ± 2.2
2nd generation	388.6 ± 10.8	15.2 ± 0.2	38.6 ± 1.2	12.7 ± 0.19	588 ± 13.2	450 ± 10.8	15.2 ± 0.72	4.8 ± 0.07	55.6 ± 2.8	44.2 ± 1.8	80.2 ± 2.0	40.2 ± 1.8
3rd generation	386.6 ± 10.4	15.2 ± 0.2	38.6 ± 1.2	12.8 ± 0.2	586 ± 11.2	440 ± 10.2	14.9 ± 0.58	4.8 ± 0.07	55.2 ± 1.9	44.2 ± 1.22	78.9 ± 1.2	40.2 ± 1.28

Table 44

Measurement of Male (Isolate C)

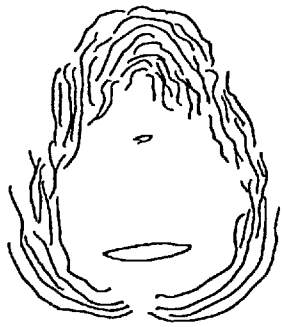
Measurement in μ / Generation	Body length	Body width	Tail length	Stylet length	Stylet base	Med bulb length	Med bulb width
2nd generation	1578 ± 99.2	24.2 ± 4.8	45.7 ± 3.8	25.6 ± 2.4	6.0 ± 0.4	46.0 ± 4.88	40.0 ± 2.9
3rd generation	1594 ± 111.2	25.4 ± 4.2	42.2 ± 3.7	26.0 ± 2.0	6.2 ± 0.39	48.6 ± 4.54	37.2 ± 2.42

Table 45 Numbers eggs/eggmass produced on tomato from isolate B and C at each generation

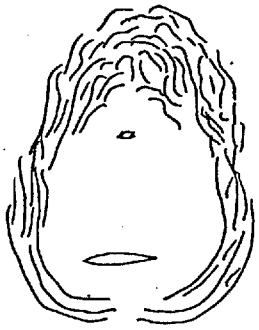
Generation	Isolates	
	B	C
1st	290	320
2nd	320	350
3rd	340	360

Fig (3). Perineal patters of M.incognita isolates A and B cultured on different hosts

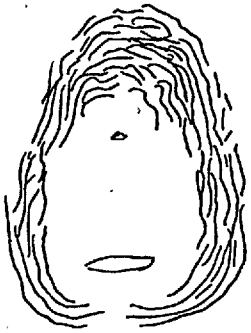
A



tomato
(origin)



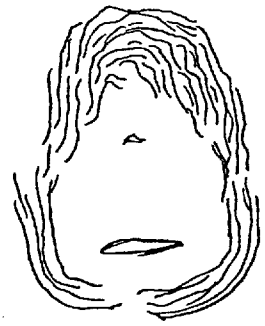
tomato



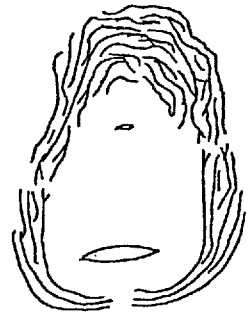
okra

tobacco

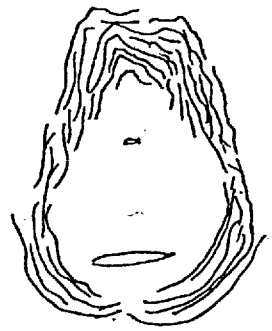
B



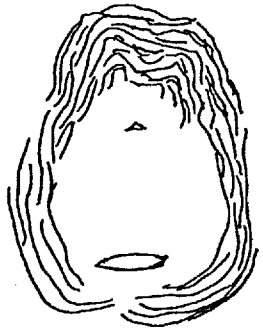
tomato
(origin)



tomato

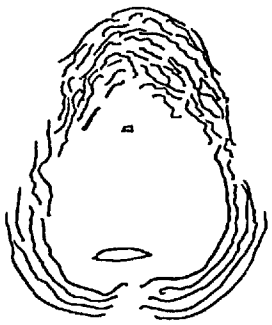


okra

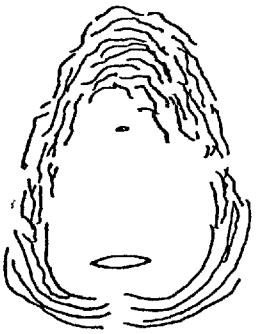


tobacco

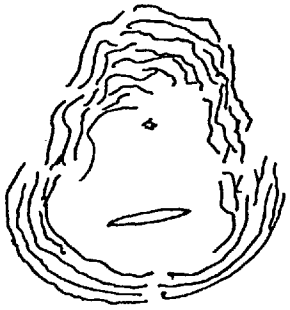
Fig.(4). Perineal patterns of 7 isolates of M.incognita from single egg mass cultures on tomato



A

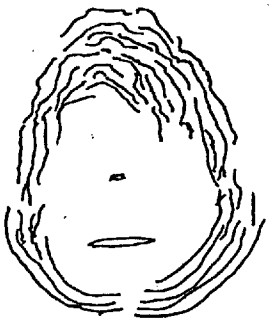


B



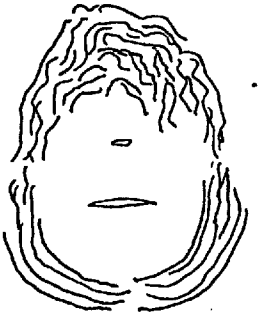
C

D



E

F



G

Fig(5). Perineal patterns of M.incognita isolates B and C from single larva inoculation of 3 generations on tomato

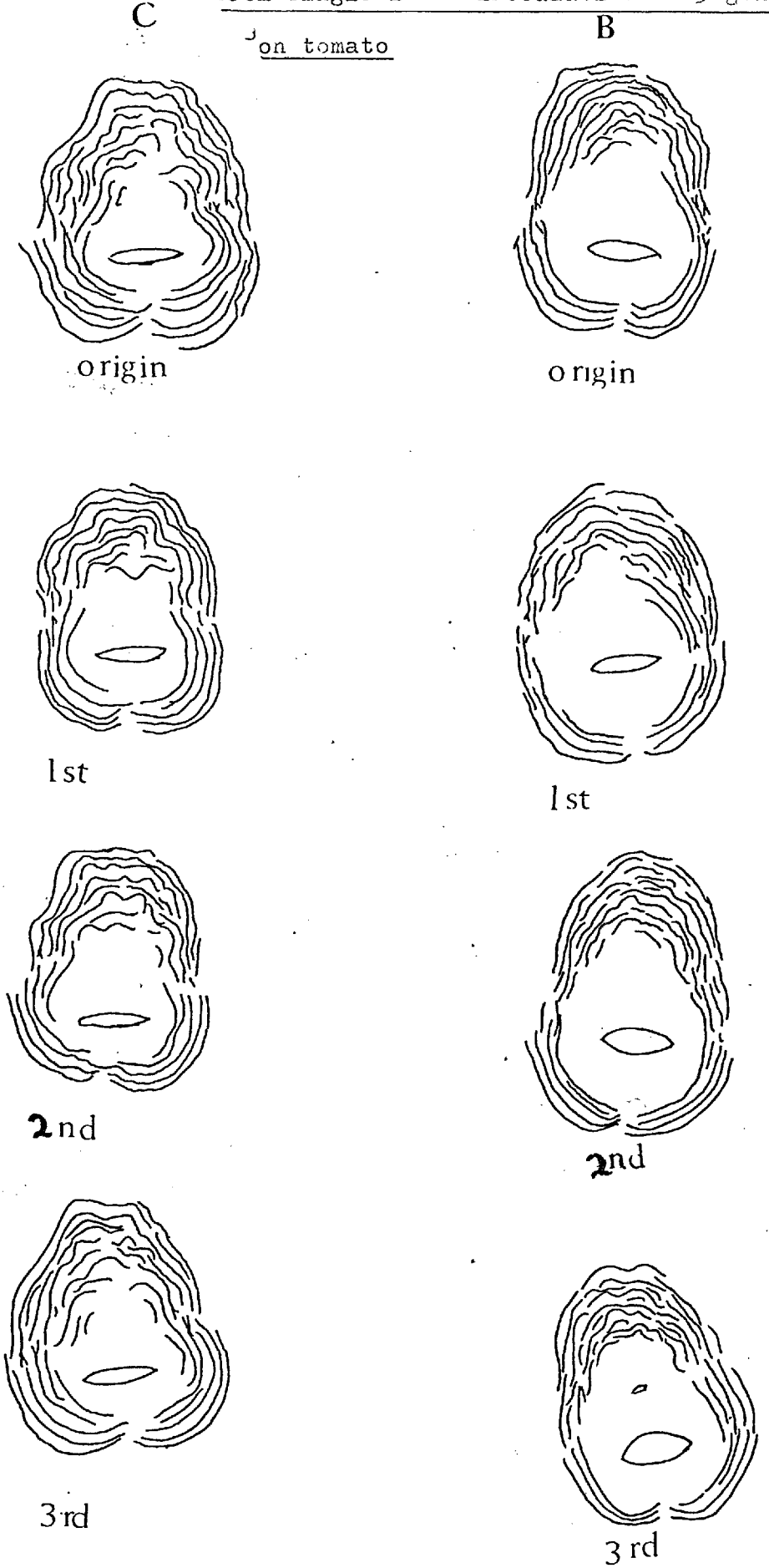
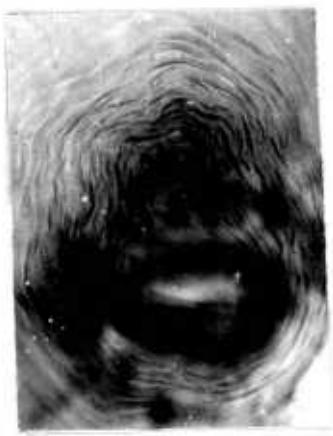
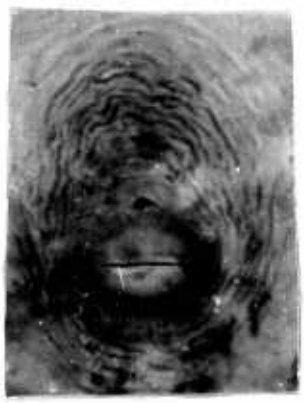


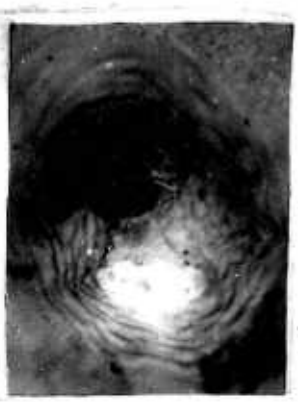
Fig (6). Perineal patterns of M.incognita isolates C,D,A, and B, from single eggmass cultures on tomato



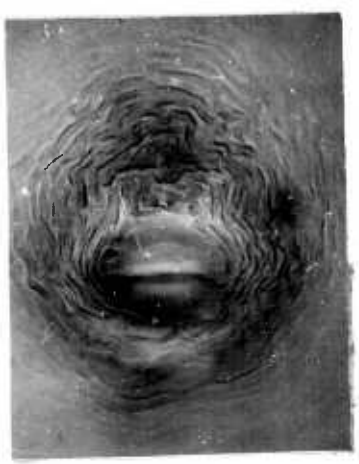
A



B



C



D

SECTION VI CYTOGENETIC STUDIES

INTRODUCTION

The cytology of the living things is a very important factor in the detailed taxonomy and biological behaviour of the species. The root-knot nematode has a complex taxonomy and biology because it has various types of reproduction. Most of them are parthenogenetic: only 6 species are with amphimictic reproduction. Most known species of the genus Meloidogyne can reproduce without fertile males.

The basic chromosome number of the genus Meloidogyne is n = 18; this has been observed in the amphimictic M.carolinensis and facultatively parthenogenetic M.graminicola, M.naasi, M.graminis, M.ottersoni and M.exigua. M.incognita, the most important species of root-knot nematode, should easily be distinguished from all other species on the basis of the behaviour of its chromosomes during maturation of the oocytes. M.incognita is known to be polyploid with a variable number of chromosomes (41-44).

If each race of M.incognita was found to have a constant chromosome number (lying between 41 and 44), then races could be more easily identified and afterwards their relationships between the different variation of chromosomes and the different pathogenic behaviour could be found out. Since chromosomes are one of the fundamental characters of life, they are obviously related with the specialised behaviour of living things.

It is hoped that if the number of chromosomes is known, the cause of the physiology races can be found without using complicated measurements or host range test.

MATERIALS AND METHODS

Single eggmass isolates of M.incognita were propagated on tomato (var. Moneymaker) in the glasshouse at the temperature of 25 $\pm$ 5°C. Young females with few eggs in the eggsac, old females with big eggmass and males (if present) were dissected out of the roots in physiological saline (2.67% sodium chloride) solution and were then processed for staining. For cytological studies, (1) Triantaphyllou (1966) and (2) Darlington (1963) methods were applied in this study.

1. Egg laying females and males were transferred to a clean slide. The head region of each female was dissected with a fine needle and the body contents including the whole reproductive organ were smeared on the surface of the slide. The smeared slides were then submerged in 1N Hydrochloric acid for 3 minutes and 3:1 alcohol acetic acid for 15 minutes. The fixed specimen was stained in 2% acetic orcein for 15 minutes and the excess stain was washed away by submerging the slides for a few seconds in 45% acetic acid. During mounting, a moderate pressure was applied to the coverslip to flatten the oocytes and spread the chromosomes.

2. A smear was done with the above procedure. The specimen was fixed in methanol for one minute and stained with Giemsa's stain for thirty minutes face down position. (Giemsa's solution was prepared with two drops of Giemsa's stain to each ml. of buffer distilled water).

RESULTS AND DISCUSSION

The specimens were stained with orcein orange colour. No chromosomes were observed, only the nuclei were deeply stained. It was confirmed that the stages were very young with only meiotic cell division and thus the chromosomes could not be seen in this case.

M.incognita is polyploid with 41-44 chromosomes (Triantaphyllou, 1971). Hackney (1974) successfully applied chromosome counts in order to identify different species of Meloidogyne. Triantaphyllou (1971) showed that one maturation division occurs which is a regular mitotic division and results in the formation of one polar nucleus and the egg pronucleus, both with the somatic chromosome number. In this case, no sperm is needed for activation of the oocytes.

Studies of nematode chromosomes have been done only in connection with gametogenesis. The chromosomes of later cleavage divisions and divisions of other somatic cells (hypoderms, epithelium of gonad) during post-embryogenesis are very small and have never been observed clearly. At the successive cleavage division, the chromosomes become smaller, and at the end of the blastula stage no discrete chromosomes can be distinguished in the very small metaphase plates of such divisions.

Few studies have been done on M.incognita chromosomes because this species is very difficult and complex with biological races. So there is a wide range of variation.

Triantaphyllou reported that much greater variation is observed in groups of nematodes in which polyploids occur in association with a parthenogenetic mode of reproduction.

GENERAL DISCUSSION

The purpose of this investigation was to see if there were any morphological and biological differences between the isolates (Burma, Ecuador, El Salvador, Nigeria, Barbados and U.K.I.C.I.); and if differences existed, how they differed in their pathogenicity to cultivated crops and whether they were biological races or new species. On the basis of the results, particularly from the host studies, it was hoped that some improvement could be made for selecting suitable crops for crop rotation in those areas.

Though a lot of work has been done on M. incognita, concerned with morphology, different biology, or specificity of hosts, further investigations on the morphology and biological behaviour was necessary to see if morphological characteristics can be correlated to physiological behaviour. The other problem with M.incognita is that it has a very wide host range, thus the physiological behaviour may be quite different between populations and hosts though we are dealing with the same species.

Various stages of the life cycles of those isolates which were tested differed quite considerably from those described by the previous workers especially the stages from adult through eggs to second stage juveniles. The results obtained from the hatching experiment were similar to those observed for the same species, M.incognita, by Bergeson (1959), Dao (1971),

and Dropkin (1959) who found that the optimum temperature for hatching lay between 25°C and 30°C. Hatch of isolate D from El Salvador was good in ambient temperature which was similar to the results on M. javanica found by Bird (1959) who observed that this species could hatch at an ambient temperature of 20-26°C very well.

The failure of penetration by the juveniles under different temperatures was probably caused by the use of unhealthy plants which were grown under dark conditions. If the plants are not grown under ideal conditions, there is less penetration of nematodes inside the roots (Wallace, 1966; Dropkin, 1968). Previous workers who tried to investigate the nutrition of plants required by plant parasitic nematodes found that less invasion was observed in the plant roots which had not enough or suitable nutrients of the plants required by the parasitic nematodes.

Males only occurred with isolates C, D and G especially in the big eggmasses. Males with one or two testes were observed. It was similar to those observations of Davide and Triantaphyllou (1967), who concluded that the occurrence of males in the mature big eggmasses were due to the adverse conditions undergone by the juvenile stage such as overcrowding and inadequate nutrients. Perhaps single testes males are fertile and copulate with the female which may lead to more eggs being produced, increasing the size of her eggmass.

The survival of all stages of all isolates of the nematode was equally good at constant temperatures of 20°C, 25°C, but at ambient temperature the survival was less. This result was similar to those of Daulton and Nusbaum (1962), Dropkin

(1962) who mentioned that the root-knot nematodes M.incognita have their own optimum survival temperature. From the survival point of view, nobody has done detail work of the viability of surviving nematodes of root-knot nematodes. Previously the only assessment of survival of M.incognita was shown simply by the root-knot index value (Dropkin, 1968; Bergeson, 1958; Daulton and Nusbaum, 1962). Further investigations of the assessment of survival should be done with root-knot nematodes.

The relative humidities, soil temperature, and the minerals in the soil influence the survival of root-knot nematodes. From my observations, the survival of the juveniles of all isolates were the lowest at 50%, 60% and 70% relative humidities as the exposure time was increased. As the humidity increased, survival increased, and also, as the length of the exposure increased, the survival decreased. These results were similar to those obtained from H.rostochiensis by Hamblen and Slack (1959).

As Burma is a cotton growing country, the resistance of cotton varieties including local Burmese varieties were tested. From the observations of tests against 7 isolates of M.incognita, all 7 cotton varieties tested were highly resistant to all isolates. Only isolates C and D reproduced on Makoka and Malawi 637 varieties, but in very low numbers.

From the tomato resistant variety experiments, one variety, Ronita, was attacked by the same 2 isolates that reproduced on cotton, and the other isolates did not attack the resistant varieties which were tested. Resistance of var. Ronita was broken by these two isolates. This observation of broken

resistance was similar to that obtained by Netscher (1976), who found populations of M.incognita in Senegal broke resistance of a similar tomato variety, Rossol. Moreover, from the host range study test, only isolates C and D attacked Zea mays (sweet corn). It can be concluded that 2 physiological races exist within the populations according to their behaviour on the hosts; isolates C and D, from Ecuador and El Salvador respectively, behaved very similarly on Zea mays, Gossypium hirsutum and Lycopersicum esculentum in each case different from all other isolates.

From the host-range study, the root-gall indices, numbers of females, numbers of eggs/eggmass, root-weight, and top weight were taken for the assessment of the pathogenicity. In the majority of cases, the root-gall index method coincided with the rest of the more elaborate and time consuming assessment methods, thus it can be concluded that the root-gall index method is the most useful, efficient and quickest method. Though other methods may give a more accurate assessment of populations, they are laborious and waste a lot of time and generally would not be suitable for field experiments.

From the graminaceous host experiment, the two isolates C (Ecuador) and D (El Salvador) attacked all graminaceous crops. But there was less reproduction in millet than other graminaceous hosts. Sorghum, maize and sweet corn were found as hosts of M.incognita by Linde (1956), Sasser (1952), and Baldwin et al. (1965). But millet has not been known previously as a host of M.incognita. The root galls on all graminaceous hosts were small compared to those on dicotyledonous hosts, presumably, those nematodes were unable to induce the formation of large galls.

From the cross-infection experiment, I found that nematodes that were transferred from the original host after a year to the same new host were more successful than those transferred from the original host to a different new host. So, it can be concluded that, the nematodes can adapt to the host if they are cultured for a long time. It is probably that there were genetical influences or changes to the nematode that occurred during that year when the population was allowed to build up under ideal conditions on the single original host.

From the morphological study, there was no effect of different hosts on the same isolates of the nematodes. Only in some cases, perineal patterns and the juvenile body length slightly varied but these differences were not shown to be significant. But there was a highly significant difference between the isolates in all the morphological characters that I tested on the same host. It could happen between the isolates because of the different original hosts. Whitehead (1968) reported that there was morphological variation between the populations though they were the same species.

Males were observed in isolates C (Ecuador), D (El Salvador), G (I.C.I.). These males with one or two testes were observed on the mature, big eggmasses. Triantaphyllou (1960) stated that, when they are under unfavourable conditions, most of the second stage juveniles differentiate as males and develop into adult males with one testis. When the conditions change from favourable to unfavourable, the second stage female juveniles either die or undergo sex reversal and continue their development to adult males which possess two testes. Though M.incognita is

parthenogenetic, amphimitic reproduction cannot be ruled out if there are males with one testis present.

Mutation may occur in some nematodes if there is a change of environmental conditions. If there is mutation within the species, certain types of new race or species or sub-species can arise. A special genetic constitution, considered to characterize a race may arise phynotypically, i.e. independently at different sites and at different times. Host specificity may be due to different genetic factors, and if new mutations appear independently one cannot assume that they are completely identical in different and geographically isolated populations. Biological races may be heterogeneous genetically and only phenotypically similar with respect to their pathogenic abilities, which may be due to different alleles, different genes, or different gene combinations.

Among the isolates, the behaviour of isolates C and D was different from the other isolates according to their infection of corn, Ronita variety of tomato and two cotton varieties; perineal types were also different from the others. The perineal types of these isolates are between M.incognita incognita and M.incognita acrita. According to the infection to the plants these isolates can be classed as biological races. Although differences in perineal patterns are consistent and easily recognised, I do not think that this warrants separating them into a new species, but, if further investigations on their morphology are done, new species may be found to occur in these and other populations of M.incognita. Stone (1971) separated out two patho-types of H.rostochiensis and showed that they were different

species by using morphological differences. They were previously assumed to be biological races, but by detailed morphological studies, females of H.pallida were distinguished from those of H.rostochiensis by their longer stylets, shorter anal vulval distance, small number of cuticular ridges between anus and vulva and the cream or white internal colours of the females.

From the single larval inoculation, the morphological characters of the offspring were similar to those of their parents. This was similar to work of Dropkin (1959) who observed that there was less variation after the continuous generation from single larval inoculation.

The failure of chromosome work was because of the polyploid type of chromosome in M.incognita. It was difficult to work out though different chromosome staining methods were used.

My work has shown that it must not be assumed resistant varieties are always resistant to all populations of M.incognita as I have shown that resistance can be broken down. So to choose a resistant variety is not always reliable without first doing field tests.

My work can be useful to explain why farmers have inexplicable problems with continuous infestation of root-knot nematodes and may help to improve their rotational systems. Work with M.incognita is difficult because the species has a very wide host range, biological races, and also have polyploid type of chromosomes.

In future, more investigations of chromosome

work, biology and morphology study within the populations should be done. This work has shown that M.incognita species or group is still not clearly defined. Considerably more work on cytogenetics morphology, and determination of the extent of pathogenic variation are required to gain a better understanding of this very economically important species and it is hoped that more workers in future will be able to concentrate their efforts on the root-knot nematodes.

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