THE INFLUENCE OF SOIL TEMPERATURE ON SURVIVAL OF LARVAE AND EGGS OF MELOIDOGYNE INCOGNITA IN THE FIELD AND IN THE LABORATORY

By

DONALD WARD DICKSON

Bachelor of Science

Austin Peay State College

Clarksville, Tennessee

1963

Submitted to the Faculty of the Graduate School
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
August, 1965

THE INFLUENCE OF SOIL TEMPERATURE ON SURVIVAL OF LARVAE AND EGGS OF MELOIDOGYNE INCOGNITA IN THE FIELD AND IN THE LABORATORY

Thesis Approved:

F. B. Struble
Thesis Adviser

John E. Thomas

Hoda G. Hauser

J. H. Boggs
Dean of the Graduate School

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Professor F. Ben Struble, thesis adviser, for his untiring assistance and helpful criticism during the course of this investigation and for his assistance and criticism in the preparation of the manuscript.

Dr. Walter W. Hansen and Dr. John E. Thomas have my appreciation for their constructive criticism during the final preparation of the manuscript.

Special pleasure is taken in thanking Dr. Robert D. Morrison for the suggestions and assistance in preparing certain data statistically. My thanks also go to Dr. Lester W. Reed and Gerald Laase for suggestions and assistance with soil moisture determinations.

Further, the author wishes to express his deepest appreciation to his wife, Gloria, who typed the manuscript.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES
(Continued)

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The root-knot nematode, Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949, occurs throughout the southern United States and is an important parasite on a wide range of economic plants.

Prior to the taxonomic revision of the root-knot nematodes by Chitwood, (5) this group was considered as a single species, Heterodera marioni (Cornu, 1879) Goodey, 1932. In 1949, Chitwood (5) transferred the root-knot nematodes to the genus Meloidogyne and described 5 species and one subspecies. These advances in taxonomy have helped to eliminate much of the earlier confusion regarding host range and pathogenicity of root-knot nematodes.

Since the recognition that there are several species of Meloidogyne, some of the species have been studied intensively and there is a considerable amount of literature on their taxonomy, life history, biology and control. In spite of these very important and necessary studies, there remain, in our knowledge of the life cycle of these nematodes, important gaps which have not received the attention they merit.

It is well recognized that because of the many factors operating in the soil environment, it is difficult to obtain reliable data on the effect of any single factor as it influences nematode survival or behavior.

One of the important aspects of the life history of M. incognita which has not been resolved is whether the nematode overwinters in the

larval stage, the egg stage or both in the absence of a host. It is generally recognized that M. incognita is limited geographically to temperate areas having relatively mild winters. This nematode cannot survive the cold winters of the northern United States. It is apparent that temperature is one of the most important factors governing the occurrence of this nematode and the severity of the disease it causes. Because of the economic importance of M. incognita, it is important that we know and understand how or in what form it overwinters.

The present investigation was undertaken to determine whether M. incognita overwinters in Oklahoma in the larval stage, the egg stage or both. To accomplish this objective studies were made of (1) the seasonal trend of populations of larvae and eggs in the field; (2) the ability of eggs, in masses, from a greenhouse population to overwinter; (3) the ability of larvae from a field population to overwinter and (4) the tolerance of larvae and eggs in the laboratory to cold temperature in moist and in dry soil.

CHAPTER II

REVIEW OF LITERATURE

One important aspect of the life history of Meloidogyne sp., which is at present completely open to question, is whether the organism overwinters in the larval stage, the egg stage or both. It is rather surprising that this important aspect of the life history of root-knot nematodes has not been fully explored especially since the overwintered eggs, as well as larvae, could serve as potential sources of inoculum in the spring.

Early reports on survival of root-knot nematodes are difficult, if not impossible, to interpret because of the uncertainty as to the species involved. In addition, there are conflicting points of view on survival of root-knot nematodes among many of the early reports. These conflicts evidently were the result of a failure to recognize that there were several species of root-knot nematodes instead of just one. Several workers reported on the effect of temperature on survival of root-knot nematodes prior to their taxonomic revision in 1949 by Chitwood (5). In 1934, Newhall (22) stated that a period of 24 hrs with the air temperature below -15° F greatly reduced the infestation of root-knot nematodes from peat soil in New York. Cunninghan (6) however, reported that winter conditions on Long Island, New York did not noticeably reduce root-knot nematode infestations. In 1946, Kincaid (17) found that root-knot nematodes from the South failed to survive the winter in Indiana and Wisconsin. In England, Franklin (14) found that

root-knot nematodes could survive at least 16 months in the absence of a host during which time occassional freezing occurred. In field studies on the overwintering of root-knot nematodes, Bessey (2) found in November that larvae were abundant in and around galls, while mature or partly mature nematodes, as well as eggs, were dead. He stated that it was probably the larval stage that overwintered the organism in the absence of a host. Studies on the effect of freezing on root-knot nematodes were made by Tyler (30). She found that repeated freezing at 32° F did not kill larvae or eggs, but at a constant temperature of -4° F all stages were killed in 2 hrs.

In 1959, Bergeson (1) was among the first to investigate throughly the influence of temperature on survival of different species and populations of the genus, Meloidogyne. He demonstrated, in the laboratory, that eggs were more resistant than larvae to cold temperatures. His results strongly suggest that the chances of Meloidogyne sp. persisting when soil temperatures are low may depend on its stage of development. It was suggested by Bergeson that M. incognita acrita Chitwood, 1949 may overwinter in the egg stage in moderately cold regions. Bergeson was the first to show that 50° F was the optimum temperature for long-time survival of both eggs and larvae which at this temperature survived for more than a year. He also reported that 32° and 40° F were usually lethal to larvae in 7 and 14 days respectively. Eggs at these same temperatures survived for approximately 2½ months and 6 months respectively.

In a study which compared the temperature response of 3 populations of M. javanica (Treub, 1885) Chitwood, 1949 and a population of M. hapla Chitwood, 1949, Daulton and Nusbaum (7) found that eggs from the popula-

tions of M. javanica were all non-viable after 12 days at -2° C. Eggs of the M. hapla population were greatly reduced in numbers, however, many eggs were still viable. In field experiments where they exposed egg masses of M. javanica and M. hapla to winter conditions in North Carolina only the latter species survived. Nusbaum (23) also found in North Carolina that eggs of M. hapla more readily overwinter than populations of M. incognita, M. arenaria (Neal, 1889) Chitwood, 1949 or M. javanica. No comparative tests using larvae were run by Daulton and Nusbaum or Nusbaum in these experiments.

The ability of M. hapla to overwinter in a colder climate was further substantiated by Sayre (27) in southern Canada. He found that while a population of M. hapla was drastically reduced, those of M. incognita and M. javanica were completely wiped out by the winter temperatures.

Another important aspect of survival is the effect of moisture on root-knot nematodes. Whether larvae, eggs in masses or both survive various adverse moisture conditions is not known. In earlier studies on flooding as a method of controlling root-knot nematodes, Brown (3) concluded that eggs survived approximately 22 months although he was not able to demonstrate the surviving form. In 1933 Tyler (30) found that root-knot nematodes could tolerate almost any moisture conditions. She stated that larvae survived for long periods in dry soil apparently because the nematodes were not so active as to deplete their store of reserve energy. Tyler (30) and Godfrey et al. (16) both attributed long survival of root-knot nematode egg masses and larvae in unspecified dry soil to the soil atmosphere being nearly saturated with water vapor.

Godfrey and Hoshino (15) showed that 100 per cent relative humidity

was required for root-knot nematode survival. As the humidity decreased, survival decreased, and the length of the exposure period increased the injurious effect. There was a rapid killing of all forms at any humidity below 100 per cent though a longer time was required to kill eggs in masses than free larvae. Daulton and Nusbaum (8) also found that eggs in masses failed to survive any relative humidity other than 100 per cent. Dropkin et al. (10) found that eggs placed in osmotic concentrations of .3 M NaCl which equals 15 atmospheres pressure or wilting coefficent, were inhibited from hatching. These results were similar to those of Linford's (18) which showed that eggs placed in soil at the wilting coefficent were inhibited from hatching; however, embryo development continued and this allowed rapid hatching when the eggs were later transferred to water.

Peacock (25) showed that eggs of Meloidogyne sp. survived 5-10 days at 100 per cent saturation (moisture holding capacity) but were not affected by 10 days at 20 per cent moisture (62 per cent of saturation). Numbers of larvae were reduced in soil held at 100 per cent saturation for 10 days but were not affected by 10 days in soil at 20 per cent moisture.

Some of the latest work done on the effect of moisture on root-knot nematodes was by Daulton and Nusbaum (8). They showed that there was a greater and more rapid reduction in egg viability in wet soil (20.4 per cent moisture content) than in dry soil (3.4 per cent moisture content). In wet soil, 12 days exposure was required before there was a decrease in viability.

CHAPTER III

MATERIALS AND METHODS

Field Studies

In the spring of 1963 experiments were begun in the field at Perkins, Oklahoma to determine the seasonal trend of numbers of larvae and eggs of *M. incognita* in the soil. Two plots were selected in 2 different root-knot nematode infested fields and the plots were planted with the very susceptible sweet potato variety, Allgold. The plots, designated as Plot I and Plot II, were approximately 20 x 15 ft and were planted with Allgold sweet potato slips. These plots were left undisturbed at harvest. Soil samples were taken from Plot I at 2-4 week intervals from 0-6, 6-12, 18-24 and 24-30 in. levels with a 4-inch-diam auger. The soil temperatures at 6, 12, 24 and 30 in. levels were recorded continuously by a 4-pen recording soil thermograph at the site of Plot I.

Plot II served only as a check with a different soil type and a different population of the nematode. Samples were taken from this plot at the 0-6, 6-12 and 12-18 in. levels. The samples were not taken deeper because of a clay hard pan which began at the 18-inch level.

In order to make a positive determination as to whether larvae or eggs were present in any given soil sample, it was necessary that reliable techniques be available for recovering single eggs, egg masses or larvae. The techniques must also be applicable for use with laboratory samples containing larvae and egg masses.

Caveness and Jensen's (4) and Minderman's (21) centrifugal-flotation techniques for recovering nematodes from small soil samples were evaluated because both appeared to offer a possible means for recovering larvae as well as single eggs. Preliminary tests, using controls containing known numbers of larvae and eggs, were made with the techniques to determine their reliability. From the results of these tests it was found that neither of these techniques was adequate for recovering root-knot eggs or larvae from large soil samples. Large soil samples, one-half pint, were necessary in this study; therefore, modifications of these techniques were necessary. The following modifications made possible an adequate recovery of single eggs as well as larvae from large soil samples. Larvae were recovered from field soil by taking one-half pint from each of the samples and thoroughly mixing this soil with 250 ml of water by stirring with an electric stirrer until the soil was suspended. The suspended soil was allowed to settle for approximately 10 sec and the supernatant was poured into a 250 ml centrifugation bottle. The samples were centrifuged simultaneously for 5 min in a Sorvall SS-3 automatic superspeed centrifuge with a GSA rotor. Timing began after a maximum of approximately 7,000 revolutions per minute, relative centrifugal force of 7,970 g's, had been reached. The resulting supernatant fluid was poured off with one smooth motion without disturbing the material at the bottom of the bottle. The first spinning of the samples eliminated material lighter than water.

Nematodes were recovered from the residue in the bottles by the addition of a $MgSO_4$ solution (215.2 g $MgSO_4$ made up to a liter with water) having a specific gravity of 1.20.

In a preliminary test sugar and $MgSO_4$ were compared as possible

recovery solutions. It was found that both gave approximately the same percentage recovery; however, the $MgSO_4$ solution yielded a cleaner nematode recovery because it precipitated clay particles. The $MgSO_4$ was also more economical, therefore, it was used throughout the experiments.

After the addition of $MgSO_4$, the contents of the bottles were stirred with an electric stirrer and centrifuged 5 min at approximately 5,000 revolutions per minute, relative centrifugal force at 4,080 g's. After spinning, the $MgSO_4$ containing the nematodes was immediately diluted by pouring it into 500 ml of tap water to avoid any harmful effects to the nematodes. The residue remaining in each bottle was mixed again with $MgSO_4$ and spun again. It had been determined that approximately 50 per cent of the nematodes recovered were from this second spinning. The resulting nematode suspension was combined with that previously collected. The combined nematodes from each sample were then poured onto a sieve with 30 μ openings. This sieve had been found to retain 100 per cent of the larvae while allowing water and clay particles to pass through. In preliminary tests measuring the reliability of the centrifugal-flotation technique for recovering nematodes, it had been observed that 25-50 per cent of the recovered larvae were retained on a sieve with 43 μ openings even with repeated sieving of the nematode suspension. Miller (20) reported using a sieve with 43 μ openings to recover nematode larvae, although the per cent retained on the sieve was not mentioned.

The nematodes collected on the sieve were washed into a beaker and allowed to settle 15-20 min. The excess water was decanted leaving approximately 15 ml of water which was poured into a Perspex counting dish and the larvae were counted.

The technique used to recover larvae was not suitable for the recovery of egg masses because the egg-mass specific gravity was too high. The sticky, gelatinous matrix surrounding the eggs had soil particles adhering to it which further increased the specific gravity. A modification of the technique for recovering larvae, however, made it possible to recover single eggs. The gelatinous matrix was dissolved, thus freeing the eggs, by adding a 250 ml solution of 12.5 per cent Chlorox (5.25 per cent sodium hypochlorite) (31) to one-half pint of soil and stirring with an electric stirrer for 30 min. The supernatant containing single eggs was decanted into the centrifuge bottles and the procedure as for recovering larvae was followed. This technique was used for the purpose of determining whether eggs occur singly or in masses in field soil. It was not used for any survival test because of damage to eggs from remaining in Chlorox for a long period of time. It was not possible to combine this procedure with the recovery of larvae because of an adverse effect of the Chlorox on larvae; within 5-6 min the Chlorox dissolved the body walls of the larvae and caused them to disintergrate.

The previously described techniques were satisfactory for recovering larvae and single eggs. It was determined, from controlled tests, that approximately 50-60 per cent of larvae and 80-90 per cent of single eggs added to soil could be recovered.

Because of the possibility of egg masses persisting in the soil it was essential to this study that a reliable technique be available for their recovery. Since such a technique was not available, one was developed. The details of this method are presented as a part of the Results of this paper.

Survival of eggs and some larvae from field experiments was deter-

mined by a bioassay method except for those eggs recovered from Plot II. A viability stain was used to determine survival of eggs from Plot II. This latter method is presented in the laboratory studies.

A bioassay test, which demonstrates that larvae are infective by the formation of galls on indicator plants, does not distinguish larval survival from egg survival. Therefore, in order to demonstrate whether larvae or eggs survived it was necessary to demonstrate the presence of either or both. All eggs from field samples, with the exception of eggs from Plot II, tested by the bioassay method were first recovered and identified as eggs. A direct count was used as a criterion of survival of larvae recovered from Plot I and Plot II. Larvae, placed in the field in the absence of eggs, were tested directly by the bioassay method.

Those samples to be tested by the bioassay method were transferred into 4-inch clay pots and placed in the greenhouse. Tomatoes, variety Rutgers, were transplanted into the pots and allowed to grow for only 35 days to avoid the production of a second generation of nematodes. The plants were removed form the pots, the roots washed and the extent of root galling, a measure of nematode survival and infectivity, was rated according to Daulton and Nusbaum's (7) infection scale. Experimental data were based upon mean index values from 0 to 100.

The scale was as follows:

Infection class	Index value	Description of index value
0	0	Free from galls.
1	1	Trace, less than 5 galls.
2	5	Very slight, trace to 25 galls.
3	10	Slight, 26 to 100 galls.
4	25	Moderate, galls numerous, mostly discrete.
5	50	Moderately heavy, galls numerous, many coalesced
6	75	Heavy, galls very numerous, mostly coalesced, root growth slightly retarded.
7	90	Very heavy, mass invasion, slight root growth.
8	100	Extremely heavy, mass invasion, no root development.

Laboratory Studies

Eggs and larvae were tested separately for survival in the laboratory to gain a more precise knowledge of the effect of cold temperatures on these forms. The nematode used in these studies had been maintained in the greenhouse since 1950 on Rutgers tomato grown in steamed soil. The population originated from a single egg mass taken from an Allgold sweet potato root grown in a nematode infested field at Perkins, Oklahoma. This nematode was originally identified as Meloidogyne incognita acrita, and the nematode was reidentified in 1958 when the population was repurified by picking and pooling 4 single-egg-mass cultures. In line with the suggestion of Triantaphyllou and Sasser (29) that the subspecies no longer be recognized, this nematode is now designated as M. incognita.

Experiments to determine survival of larvae or eggs at controlled conditions were conducted in a thermostatically controlled temperature chamber fitted with a hygrothermograph to record the temperature and relative humidity. The temperature variation in the chamber was no

greater than $\pm$ ½ degree and the relative humidity in the chamber was approximately 100 per cent.

Larval inoculum was obtained by placing egg masses on gauze strips supported by small screens attached to beakers containing enough water to wet the gauze but not submerge it. Larvae were harvested daily and stored at 49° F until enough of them were available for a test. Egg inoculum was obtained by picking egg masses from heavily infected tomato roots grown in the greenhouse.

A light, sandy-loam soil which had been steamed to eliminate the possibility of nematodes or other micro-organisms was used in this study. A moisture-retention determination was made to determine the field capacity and permanent wilting point of the soil using a pressure-plate apparatus as described by Richards and Fireman (26). With this method, soil moisture per cent, on a dry weight basis, can be read directly from the moisture-retention curve shown in Fig. 1.

At a controlled temperature of 32° F, larvae and egg masses were subjected to 2 different moisture levels: (1) field capacity, 8.60 per cent moisture and (2) permanent wilting point, 3.76 per cent moisture. These moisture levels will be referred to as moist and dry, representing field capacity and permanent wilting point, respectively. The adjustment of the soil to the proper moisture levels was accomplished by first taking air-dried soil and dividing it into 20 kg lots. The soil was then placed in a large, double polyethylene bag and stored at 40° F for 48 hrs while the soil moisture was being determined on an oven-dry basis. The level of soil moisture was determined, and from this information the amount of water necessary to bring each lot of soil to the required moisture levels was calculated. The soil to be infested with egg masses was

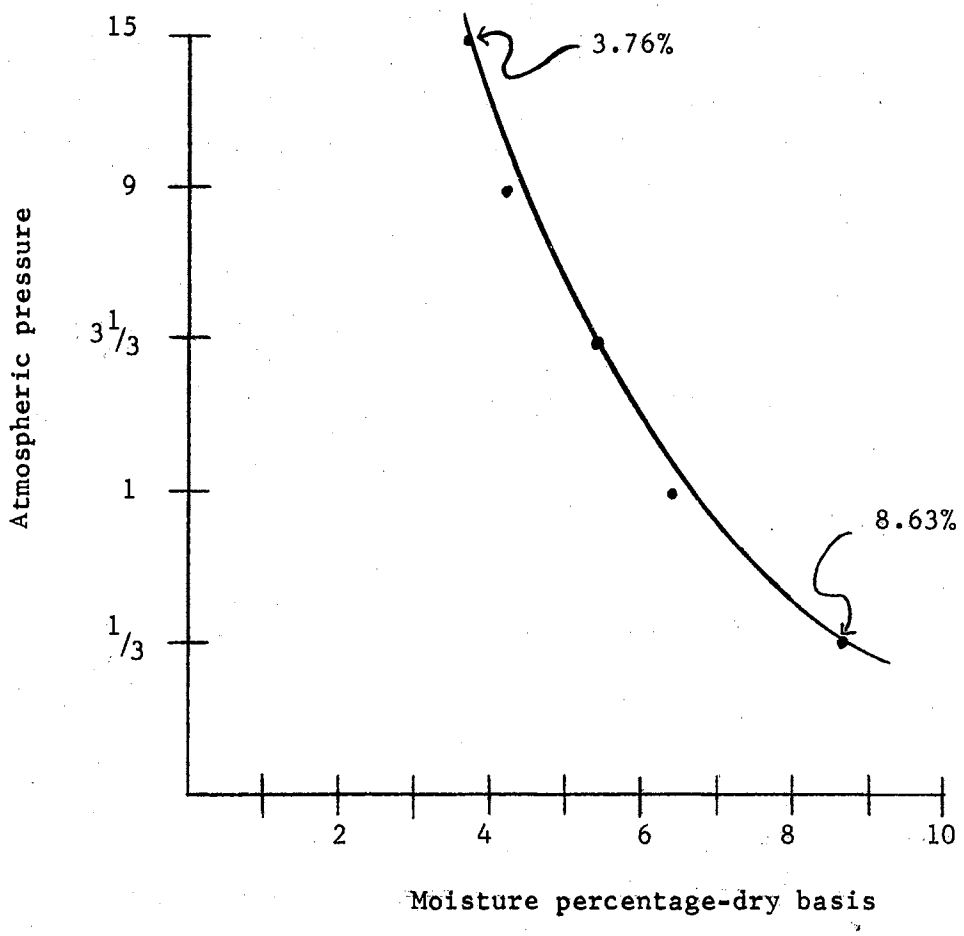

Fig. 1. Moisture-Retention curve for sandy-loam field soil.

adjusted to the desired level by adding the required quantity of distilled water and thoroughly mixing to insure a uniform distribution of moisture. The mixed soil was immediately placed in a double polyethylene bag and stored until used in the experiment. Soil to be infested with larvae was not adjusted to the desired moisture levels until the larvae were added and thoroughly mixed with the soil.

The inoculum, 6 egg masses grouped for uniformity in size or 2,000 larvae, was placed in one-half pint of soil contained in pint polyethylene bags (.15 mil thickness). According to specifications in Modern Plastic Encyclopedia (13) polyethylene is slightly permeable to water but allows CO_2 and O_2 exchange with the atmosphere. There was no significant water-loss from the samples during the experiments.

Survival of larvae and eggs was determined by phloxine B, which, according to Fenner (11), determines mortality by staining those individuals that are dead. This was confirmed by heat killing larvae and eggs. The larvae were recovered from the soil by the centrifugal-flotation technique. Approximately 3-4 ml of phloxine B stain were added to the larval sample and allowed to stand for 24 hrs. The stain was washed from the larvae by pouring onto a sieve with 30 μ openings and flushing with water. Larvae were washed from the sieve into a beaker and prepared for counting. The stained (dead) and the unstained (live) larvae were counted and the per cent dead calculated.

Egg masses were recovered by a sieving-staining method (procedure presented in Results). The gelatinous matrix surrounding the eggs was dissolved by Chlorox (10 per cent) for 6 min which made possible a count of the total eggs. The Chlorox had no noticeable effect on the eggs within this time limit. In preliminary tests comparing eggs freed by Chlorox

with eggs freed by a blendor no significant difference in viability was observed. In addition, it had been observed that eggs freed by Chlorox hatched sooner than eggs in masses. Similar observations were made by Loewenberg et al. (19) using 10 per cent Purex to break up egg masses. They stated that eggs were not damaged by this treatment. Phloxine B stain was added to the freed eggs which were then washed and counted as described for larvae.

Two types of controls were used in the laboratory studies. The first type of control was an infectivity and viability determination which was estimated by subjecting the inoculum to a bioassay test to determine infectivity and to phloxine B stain to determine viability. These tests gave a measure of the infectivity and viability of the inoculum before any population reduction due to time, temperature or moisture could occur. An adequate infection of the indicator plants or a low per cent dead as determined by phloxine B was an assurance that the nematodes used in the experiments were in good condition and capable of establishing infection. The second type of control was an exposure of larvae and eggs in soil to an optimum survival temperature, 50° F (1), alongside exposure of larvae and eggs to 32° F. The term control was used only in the second case.

CHAPTER IV

RESULTS

Technique for recovering egg masses from soil

As previously noted, there has been no technique for recovering egg masses of root-knot nematodes from soil. A suitable technique for assaying for egg masses was devised and has been reported (9). This method was essentially a sieving-staining process. One-half pint of soil was placed in a container and a strong stream of water was directed into the soil to dislodge and suspend the egg masses. The larger soil particles were allowed to settle 1-2 sec and the liquid was decanted onto a 60-mesh sieve. This procedure was repeated 4-5 times until the contents remaining in the container were freed of all debris. The material remaining on the 60-mesh sieve was washed into a beaker and 3-4 ml of 2 per cent aqueous phloxine B (C.I. No. 45410) was added, mixed and allowed to stand 5 min or longer. Phloxine B stains the gelatinous matrix surrounding the eggs a bright red, thus distinguishing egg masses from other material. The contents of the beaker were poured onto a 200-mesh sieve and washed briefly with water. The egg masses were then picked out. Using this technique for recovering egg masses from laboratory samples 90 to 100 per cent recovery has been obtained repeatedly. In addition matrices full of eggs, partially empty matrices and empty matrices of *M. incognita* have been consistently recovered from sandy-loam field soil samples at each of several depths.

Survival of Root-knot Nematodes Under Field Conditions

The seasonal effects on survival of the various stages of M. incognita was evaluated by comparing the relative numbers of the two forms, larvae and eggs, recovered from Plots I and II for 1 year.

The average daily maximum soil temperature from 1 June 1964 through 6 November 1964 and the average daily minimum soil temperature from 6 November 1964 through 1 June 1965 at the 6, 12, 24 and 30 in. depths of Plot I are presented in Tables I and II. The maximum temperature, 99° F, was recorded during August at the 6 in. depth. At this depth the temperature was above 92° F for 31 days from 21 June 1964 through 17 August 1964. The minimum temperature reached was 30° F recorded at the 6 in. depth during December. The average daily minimum varied considerably at the 6 in. depth from 16 November 1964 through 27 March 1965 with a total of 9 days below 32° F. There was a total of 100 days and 72 days when the temperature was below 40° F at the 6 and 12 in. depths, respectively.

The 24 and 30 in. depths provided a more favorable temperature range for nematode survival both during the summer and winter months. During the summer months the maximum temperature recorded at the 24 and 30 in. depth was 85° and 84° F respectively, while during the winter months the minimum temperature recorded for these depths was 40° and 41° F respectively. There was only a total of 3 days during the winter when the temperature went down to 40° F at the 24 in. depth.

There was a seasonal change in the depths at which the greatest larval population density occurred (Tables I and II) in Plot I. Samples taken from Plot I during April 1964 from each of the 4 depths sampled, 0-6, 6-12, 18-24 and 24-30 in., yielded 384, 1,254, 2,035 and 1,203 larvae respectively. By May, the population was greatly reduced; 0, 282, 269 and 128 larvae were

TABLE I

A COMPARISON OF THE NUMBER OF LARVAE AND EGG MASSES OF MELOIDOGYNE INCOGNITA RECOVERED FROM FIELD PLOT I AS INFLUENCED BY SOIL DEPTHS AND HIGH TEMPERATURES

Sampling Date	Soil Depth	Soil Temperature		Recovery per ½ pint soil	
		Avg daily maximum between sampling dates	Total days above 92° F	Larvae	Egg Masses
	in.	°F	no.	no.	no.
2 July 1964	0-6	83.9	2	0	6
	6-12	76.9	0	0	5
	18-24	72.7	0	26	0
	24-30	72.1	0	26	0
17 July	0-6	91.4	12	2,022	9
	6-12	83.5	0	1,856	7
	18-24	79.0	0	102	0
	24-30	80.4	0	115	0
13 Aug.	0-6	92.4	31	294	6
	6-12	85.5	0	1,805	19
	18-24	82.4	0	179	12
	24-30	82.0	0	141	14
1 Sept.	0-6	82.0	31	486	13
	6-12	78.2	0	1,037	7
	18-24	77.8	0	3,571	11
	24-30	78.8	0	1,638	7
9 Oct.	0-6	76.3	31	268	7
	6-12	73.3	0	1,677	1
	18-24	73.9	0	3,533	12
	24-30	74.9	0	1,741	20
6 Nov.	0-6	64.4	31	141	0
	6-12	63.5	0	512	0
	18-24	65.0	0	3,264	0
	24-30	66.3	0	3,341	0

TABLE II

A COMPARISON OF THE NUMBER OF LARVAE AND EGG MASSES OF MELOIDOGYNE INCOGNITA RECOVERED FROM FIELD PLOT I AS INFLUENCED BY SOIL DEPTHS AND LOW TEMPERATURES

Sampling Date	Soil Depth	Soil Temperature: Avg daily minimum between sampling dates	Soil Temperature: Total days below 32° F	Soil Temperature: Total days below 40° F	Recovery per ½ pint soil: Larvae	Recovery per ½ pint soil: Egg Masses
	in.	°F	no.	no.	no.	no.
15 Dec. 1964	0-6	39.5	0	17	166	0
	6-12	43.5	0	8	653	1
	18-24	48.7	0	0	4,198	5
	24-30	50.9	0	0	10,483	6
21 Jan. 1965	0-6	35.1	4	47	38	3
	6-12	40.0	0	33	499	0
	18-24	44.1	0	0	2,624	0
	24-30	45.8	0	0	3,853	3
26 Feb.	0-6	35.8	9	78	30	1
	6-12	38.8	0	57	235	0
	18-24	42.6	0	3	2,001	1
	24-30	43.4	0	0	3,418	4
26 Mar.	0-6	36.3	9	99	17	0
	6-12	40.8	0	71	452	0
	18-24	42.8	0	3	1,036	2
	24-30	43.4	0	0	1,920	12
3 May	0-6	53.7	9	100	68	0
	6-12	55.6	0	72	77	0
	18-24	55.4	0	3	2,321	0
	24-30	54.9	0	0	3,354	2
1 June	0-6	67.7	9	100	12	0
	6-12	69.2	0	72	25	0
	18-24	68.0	0	3	282	0
	24-30	67.3	0	0	653	0

recovered respectively for each of the soil depths. These results were essentially the same for the 1965 season except that the reduction occurred later and the largest number of larvae surviving was at the 24-30 in. depth (Table II).

Approximately 50 days after the Allgold sweet potato plants were set in the 1964 season the greatest concentration of larvae was found at the 0-6 and 6-12 in. soil depths from mid-July through mid-August. In September, the concentration of larvae began increasing at the 18-24 and 24-30 in. depths and reached a peak in mid-December, while the concentration was greatly reduced in the upper depths. It appeared that the relative population build-up was directly correlated with the inoculum potential surviving through the winter at the various depths. Evidently the larger number of larvae appearing first in the upper depths is due to root growth in that area early in the season. Later, as the roots penetrate deeper into the soil, infection occurs, and results in a heavy inoculum increase at the lower depths. The sudden reduction of larvae at the 0-6 in. depth after August could be due to several factors: (1) reduction earlier in the possible number of infection sites preventing the completion of several life cycle, (2) depletion of larval food reserves due to conditions favoring activity and (3) dying of larvae due to high temperatures.

It was possible, after 2 July 1964 to recover single eggs from the upper depths by using Chlorox (12.5 per cent) to break up the gelatinous matrix surrounding the eggs. It was not until mid-August that single eggs could be recovered in this manner from the lower depths. Single eggs could be recovered from all depths through mid-December, although the number was greatly reduced. In January no

single eggs were recovered from the upper depth and only a few were recovered from the 24-30 in. depth.

With the sieving-staining technique, egg masses were first recovered from the upper depths beginning 2 July 1964, Tables I and II. It was not until mid-August that egg masses were recovered from all the depths sampled. There was a positive correlation between the occurrence of single eggs, egg masses and larvae. In every case, the appearance of single eggs corresponded to the appearance of egg masses, also the appearance of single eggs and egg masses occurred approximately 2 weeks before a population increase in larvae occurred. Matrices recovered in early November from all depths sampled were found to contain no eggs; this apparently was the result of a recent hatch. Samples were immediately taken again to determine if there were any matrices with eggs remaining in the field. Results from these samples emphasized the necessity for differentiating full egg masses, matrices with 50 eggs or fewer, and empty matrices. Thereafter, gelatinous matrices were characterized according to their condition. Data showing the condition of the matrices over a 1-year period at each of the soil depths sampled are presented in Tables III, IV, V and VI. Egg mass matrices were continuously recovered from all depths sampled through the winter and spring months. Using the bioassay test to determine the infectivity of the eggs contained in matrices, it was determined that no infective larvae hatched from the eggs recovered from the 0-6, 6-12 and 18-24 in. depths after 15 December 1964. Only the eggs recovered from the 24-30 in. level continued to hatch and induce galling on indicator plants after this date, although, in most cases, the number of galls produced were few and small in size.

TABLE III

COMPARISON OF THE CONDITION OF GELATINOUS MATRICES OF MELOIDOGYNE INCOGNITA RECOVERED OVER A 1-YEAR PERIOD AT THE 0-6 IN. SOIL LEVEL

Sampling Date	Soil Depth 0-6 in.			Root-knot Index Value[a]
	Matrices with eggs	Matrices with few eggs	Matrices with no eggs	
	no.	no.	no.	
2 July 1964	6	0	0	-
17 July	9	0	0	62.5
13 Aug.	6	0	0	7.5
1 Sept.	13	0	0	10.0
9 Oct.	7	0	0	10.0
6 Nov.	0	0	1	-
15 Dec.	1	0	0	17.5
21 Jan. 1965	3	1	2	0.0
26 Feb.	1	0	3	0.0
26 Mar.	0	0	1	-
3 May	0	0	2	-
1 June	0	0	0	-

[a]Refer to page 12 in Materials and Methods section for explanation of these values.

TABLE IV

COMPARISON OF THE CONDITION OF GELATINOUS MATRICES OF MELOIDOGYNE INCOGNITA RECOVERED OVER A 1-YEAR PERIOD AT THE 6-12 IN. SOIL LEVEL

Sampling Date	Soil Depth: 6-12 in.			Root-knot Index Value[a]
	Matrices with eggs	Matrices with few eggs	Matrices with no eggs	
	no.	no.	no.	
2 July 1964	5	0	0	-
17 July	7	0	0	62.5
13 Aug.	19	0	0	75.0
1 Sept.	7	0	0	10.0
9 Oct.	0	1	0	0.0
6 Nov.	0	0	1	-
15 Dec.	1	2	0	10.0
21 Jan. 1965	0	0	2	-
26 Feb.	0	0	2	-
26 Mar.	0	0	0	-
3 May	0	0	3	-
1 June	0	0	1	-

[a]Refer to page 12 in Materials and Methods section for explanation of these values.

TABLE V

COMPARISON OF THE CONDITION OF GALATINOUS MATRICES OF MELOIDOGYNE INCOGNITA RECOVERED OVER A 1-YEAR PERIOD AT THE 18-24 IN. SOIL LEVEL

Sampling Date	Soil Depth			Root-knot Index Value[a]
	18-24 in.			
	Matrices with eggs	Matrices with few eggs	Matrices with no eggs	
	no.	no.	no.	
2 July 1964	0	0	0	-
17 July	0	0	0	-
13 Aug.	12	0	0	62.5
1 Sept.	11	0	0	50.0
9 Oct.	12	0	0	17.5
6 Nov.	0	0	16	0.0
15 Dec.	5	2	2	5.0
21 Jan. 1965	0	0	1	-
26 Feb.	1	0	3	0.0
26 Mar.	2	1	1	0.0
3 May	0	1	6	7.5
1 June	0	0	6	-

[a]Refer to page 12 in Materials and Methods section for explanation of these values.

TABLE VI

COMPARISON OF THE CONDITION OF GELATINOUS MATRICES OF MELOIDOGYNE INCOGNITA RECOVERED OVER A 1-YEAR PERIOD AT THE 24-30 IN. SOIL LEVEL

Sampling Date	Soil Depth: 24-30 in.			Root-knot Index Value[a]
	Matrices with eggs	Matrices with few eggs	Matrices with no eggs	
	no.	no.	no.	
2 July 1964	0	0	0	-
17 July	0	0	0	-
13 Aug.	14	0	0	50.0
1 Sept.	7	0	0	75.0
9 Oct.	20	0	0	50.0
6 Nov.	0	0	9	-
15 Dec.	6	10	9	50.0
21 Jan. 1965	3	1	6	10.0
26 Feb.	4	3	12	10.0
26 Mar.	12	3	3	35.0
3 May	1	0	9	10.0
1 June	0	0	7	-

[a]Refer to page 12 in Materials and Methods section for explanation of these values.

Egg masses recovered from this depth during January and February gave index values of 10, while egg masses recovered from samples during late March gave an index of 35; this latter value represented a fair number of infective larvae hatching from the viable eggs. Viable eggs from the 24-30 in. depth continued to yield infective larvae through May, although no egg masses containing eggs were recovered from this depth after May.

Additional information on population changes in larvae and eggs was obtained from Plot II. Essentially the same pattern of larvae and egg mass numbers was found as in the depths sampled in Plot I. Egg masses recovered from 0-6, 6-12 and 12-18 in. depths, 15 December, were tested by phloxine B stain to determine their viability. The eggs from these depths yielded 100, 65 and 63 per cent dead, respectively. No viable eggs were recovered from the 3 depths sampled after 15 December. The larval population reached a peak for all depths sampled, in December. The number of larvae recovered from the depths sampled was greatly reduced after this date.

Overwintering of Meloidogyne incognita Egg masses in Steamed Soil

In the fall of 1964, an experiment was set up in the field at Perkins, Oklahoma to determine the survival value of M. incognita egg masses. Twenty 12-inch clay tile were prepared as described by Sayre (27) and these were filled with steamed, sandy-loam soil. Ten egg masses for each sample were handpicked from tomato roots and placed in one-half pint of steamed, sandy-loam soil which was enclosed in a small nylon bag. These bags were placed at a depth of 3 in. and 9 in. in each tile.

To avoid a possible hatch due to high temperatures in the upper depths the experiment was set up in November. At the beginning of the experiment, at 24 days, and then at various intervals, 4 tile were selected at random, the nylon bags removed and the soil sampled by the sieving-staining technique. The recovered egg masses were then tested for nematode survival by the bioassay test.

Throughout the experiment, soil temperatures, at 6 in. and 12 in., were recorded continuously by a soil thermograph. Data on survival of egg masses are presented in Table VII. The average daily minimum temperature during the initial population determination (11 Nov. 1964 - 4 Dec. 1964) was 44.4° F at 6 in. and 47.6° F at 12 in. From the results of the bioassay test on the initial population it appeared that very few of the eggs hatched during the first 24-day period of exposure. The minimum temperature reached was 30° F recorded at 6 in. during December. The average daily minimum varied considerably at 6 in. from 11 November 1964 through 27 March 1965 with a total of 9 days below 32° F at 6 in. There was a total of 100 days and 72 days when the temperature was below 40° F at 6 and 12 in. respectively.

TABLE VII

SURVIVAL OF MELOIDOGYNE INCOGNITA EGGS IN EGG MASSES IN STEAMED SOIL IN THE FIELD UNDER WINTER CONDITIONS IN OKLAHOMA

Sampling Date	Soil Depth	Soil Temperature: Avg daily minimum between sampling dates	Soil Temperature: Total days below 32° F	Soil Temperature: Total days below 40° F	Root-knot Index Value[a]
	in.	°F	no.	no.	
Initial Population	0-6	-	-	-	
	6-12	-	-	-	59.4
17 Dec. 1964	0-6	40.4	1	9	36.4
	6-12	43.9	0	9	84.4
21 Jan. 1965	0-6	35.1	4	7	0.5
	6-12	40.0	0	33	56.2
26 Feb.	0-6	35.8	9	78	5.0
	6-12	38.8	0	57	50.0
2 April	0-6	39.5	9	0	0.6
	6-12	41.7	0	72	46.9

[a]Refer to page 12 in Materials and Methods section for explanation of these values. All values are means of 4 replicates.

The number of viable eggs, as indicated by the bioassay test, from the samples taken at 3 in. was reduced slightly during the first 13 days (Table VII). During the second period, 35 days, there was a sharp decline in the number of viable eggs at the 3 and 9 in. depths. The eggs at 3 in. were practically all dead by the second sampling date, 48 days. The eggs survived at the 9 in. depth at approximately the same level during the last 71 days of the experiment as they had during the first 48 days.

Winter Survival of Root-knot Larvae in the Field

In order to determine the effect of temperature on survival of larvae alone in the field it was necessary to eliminate the possibility of eggs hatching and giving rise to fresh larvae during the winter months.

Soil was taken from a 2 x 2 x 2 ft area in Plot I and placed on a bench in the greenhouse. The soil was sampled by the sieving-staining technique and the average of 5 replicates yielded 4 full egg masses and 3 empty matrices. Results with a bioassay test showed severe root galling of the indicator plants, thus indicating a large number of infective larvae and possible viable eggs present in this soil.

To induce the viable eggs to hatch, the soil temperature was raised to 78° F and the soil was allowed to dry for 9 days. Dropkin et al. (10) found that under dry conditions the eggs proceed to develop normally and when water was applied they hatched immediately. Water was added to the dried soil to induce the eggs to hatch. Six days later the soil was sampled for egg masses by the sieving-staining technique. An average of 4 empty egg matrices was recovered from each of the 4 replicates which indicated all the eggs had hatched. Larvae were recovered from the soil by the centrifugal-flotation method and from 4 replicates an average of 2,301 larvae per one-half pint was recovered. The soil was placed in a 2 x 2 x 2 ft wooden box 17 December 1964 and buried in the field. The bioassay test was used to determine nematode viability at each sampling date. The results of a bioassay test on the soil as it was placed in the field showed a higher index value than the initial bioassay test on

the soil as it was removed from the field. These results indicate that there was no loss of nematode infectivity resulting from the drying of the soil during storage. Soil samples, 3 replicates, were taken at 3-fifty day intervals from 0-6, 6-12, 12-18 and 18-24 in. levels. Throughout the experiment, the soil temperatures at 6, 12 and 24 in. were recorded continuously by a soil thermograph.

During the first 50-day period, soil temperatures declined steadily reaching 32° F and below 9 times at 6 in. (Table VIII). There was a total of 78 days at 6 in. and 58 days at 12 in. when the temperature was below 40° F. The temperature was more favorable for nematode survival at 24 in. where there was a total of only 3 days below 40° F. The numbers of larvae declined sharply at all depths sampled during this period as indicated in Table VIII. During the second 50-day interval the soil temperatures had warmed up considerably and there was a drastic reduction in the number of surviving larvae. At the completion of the experiment yields of larvae at each of the depths tested were 4, 273, 461 and 469, respectively. Apparently the warmer soil caused the larvae to become active and exhaust their food reserves.

TABLE VIII

SURVIVAL OF LARVAE OF MELOIDOGYNE INCOGNITA UNDER WINTER CONDITIONS IN OKLAHOMA AS INDICATED BY GALLS ON INDICATOR PLANTS

Sampling Date	Soil Depth	Soil Temperatures: Avg daily minimum between sampling dates	Soil Temperatures: Total days below 32° F	Soil Temperatures: Total days below 40° F	Root-knot Index Value[a]
	in.	°F	no.	no.	
Initial Population	-	-	-	-	83.0
12 Dec. 1965	0-6	36.3	9	45	5.0
	6-12	39.3	0	35	22.5
	12-18	-	-	-	20.0
	18-24	47.8	0	3	50.0
2 April	0-6	48.3	9	78	12.0
	6-12	40.9	0	58	29.2
	12-18	-	-	-	50.0
	18-24	43.5	0	3	61.0
15 May	0-6	61.3	9	78	3.0
	6-12	63.1	0	58	10.0
	12-18	-	-	-	20.0
	18-24	63.6	0	3	20.0

[a]Refer to page 12 in Materials and Methods section for explanation of these values. All values are means of 4 replicates.

The Effect of Temperature on the Rate of Egg Hatching in the Laboratory

The previous field experiments indicated that several viable eggs in masses remained in the field during the winter at the lower depths, particularly the 24-30 in. level. Therefore, the chances of M. incognita persisting in the field at this depth may depend on the effect of temperature on hatching. The following experiment was designed to find the effect on hatching of several temperatures representative of those which occurred throughout the winter in the lower soil depths. Four beakers with 5 egg masses each on cheese cloth strips over water were placed at each of the following temperatures: 40°, 40-50°, 50°, 50-60° and 60° F. Those placed at 40-50° and 50-60° F were changed from the lower temperature to the higher temperature every 24 hrs and vice versa. At intervals of 4 days for a 20-day period the nematodes were collected and counted. Water at incubator temperature was added to the beakers throughout the experiment. Results in Fig. 2 showed that by the end of the 20th day an appreciable hatch had occurred at 50-60° and 60° F, while hatch was restricted at 50° F and below. At this time, the unhatched eggs from each repicate were placed in Chlorox to break up the egg masses and free single eggs. Phloxine B stain was added to determine the number of viable, unhatched eggs which remained at the various temperatures. These results are given in Fig. 2. The per cent of dead eggs at each of the temperatures was: 40°--22.7 per cent; 40-50°--18.7 per cent; 50°--7.7 per cent; 50-60°--13.0 per cent and 60°--10.7 per cent.

These results indicated that hatchings at 40°, 40-50° and 50° F were partially arrested. Considerable hatch had occurred at 50-60° and 60° F, although there still remained appreciable numbers of unhatched, viable eggs.

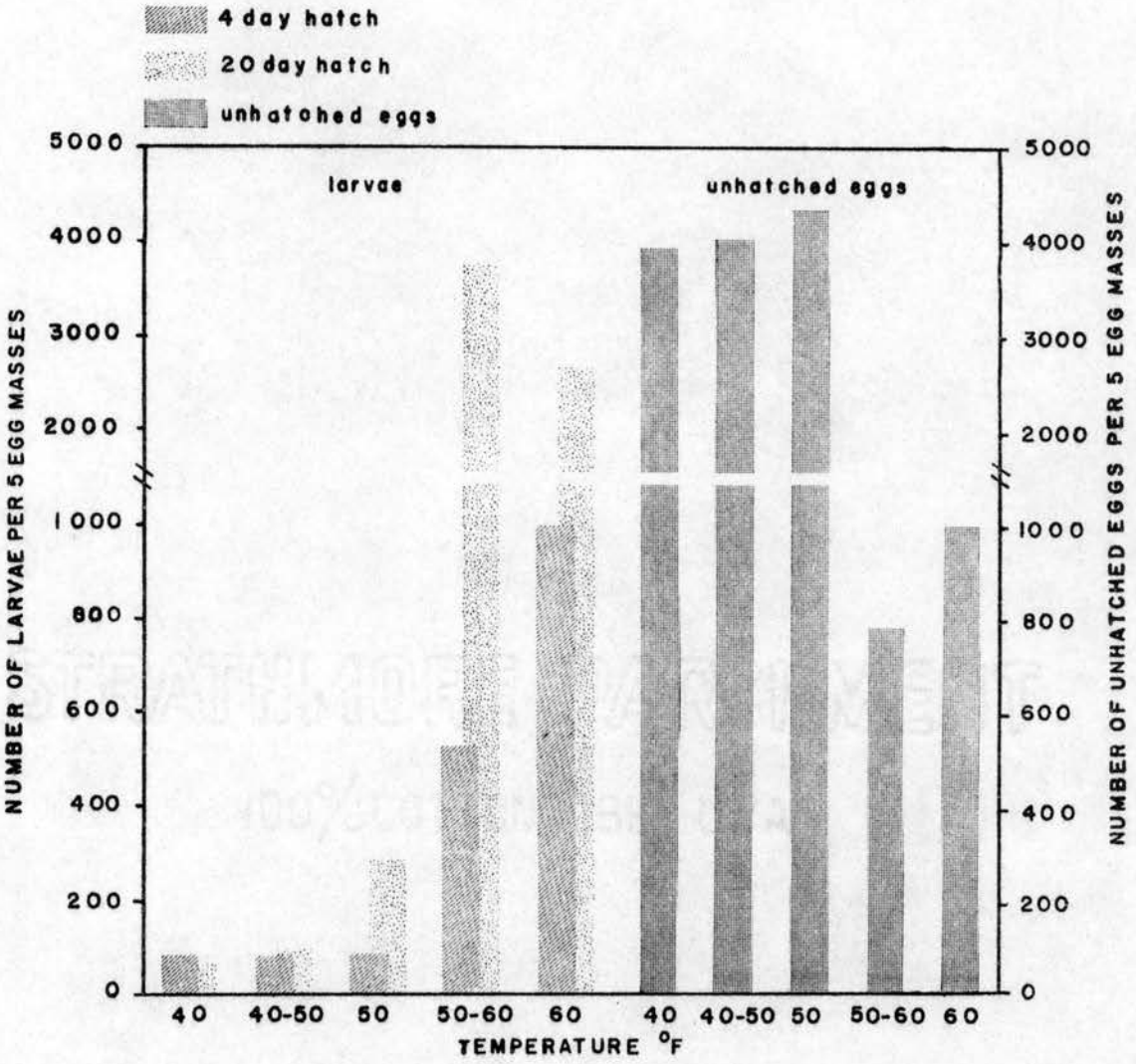

Fig. 2. Number of larvae hatched from 5 egg masses of Meloidogyne incognita after 4 and 20 days of storage in tap water at selected temperatures and the total number of unhatched eggs remaining after 20 days.

Initial Study of Survival of Larvae and Eggs in the Laboratory at 32° F in Moist and in Dry Soil

The objectives of this initial test were to (1) determine the effect of 32° F at 2 moisture levels, moist and dry as defined in Materials and Methods, on survival of larvae and eggs and (2) obtain information regarding effect of exposure time at 32° F for the 4 groups, larvae in dry and moist soil and egg masses in dry and moist soil, tested. The results of this test are recorded in Tables IX and X. The data in these tables disclose an extremely variable and inconsistent picture of nematode survival at the temperature and moisture levels employed. Because of this variation, the data were evaluated as positive or negative in regard to survival rather than attempting to draw definite conclusions from the magnitude of the numbers themselves. On this basis the following conclusions in regard to survival of larvae were made: (1) most larvae succumbed by the 28th to 35th day in moist soil and (2) survival in dry soil was very low with a complete kill occurring by the 15th to 18th day.

The experimental results on survival of eggs, Table X, approximated those obtained with larvae except that eggs showed more tolerance to the treatments. There was a sharp reduction in the number of live eggs in moist soil by the end of the 35th day, and a complete kill was indicated after 70 days. In dry soil, survival time of eggs was greatly reduced with a complete kill occurring by the 20th to the 35th day.

It was apparent from these results that the 2 moisture levels used were definitely a factor in determining the tolerance of larvae and eggs to 32° F.

TABLE IX

SURVIVAL OF MELOIDOGYNE INCOGNITA LARVAE AT 32° F IN MOIST AND IN DRY SOIL

Soil Moisture	Exposure Time	Dead Larvae as Determined by Phloxine B Stain				
		Replicates				
		I	II	III	IV	Avg
%	days	%	%	%	%	%
8.60	5	29.7	30.8	20.4	12.8	23.4
"	9	30.4	27.8	38.9	31.6	32.3
"	15	73.6	62.3	47.8	70.2	63.5
"	22	89.0	73.0	82.3	80.3	81.4
"	28	93.9	90.0	93.8	91.8	92.4
"	35	92.6	100.0	91.8	100.0	96.1
"Control[a] 10° F	35	5.2	7.7	10.7	2.5	6.5
3.76	4	27.5	36.5	28.6	41.1	33.4
"	9	89.9	75.0	76.0	76.0	74.2
"	15	100.0	98.5	100.0	100.0	99.6
"	18	100.0	100.0	100.0	100.0	100.0
"Control[a] 10° F	18	13.0	18.4	21.7	12.8	16.5

[a]Optimum temperature for survival

TABLE X

SURVIVAL OF MELOIDOGYNE INCOGNITA EGG MASSES AT 32° F IN MOIST AND IN DRY SOIL

Soil Moisture	Exposure Time	Dead Eggs as Determined by Phloxine B Stain				
		Replicates				
		I	II	III	IV	Avg
%	days	%	%	%	%	%
8.60	18	36.7	50.7	77.9	77.8	60.8
"	35	58.8	66.8	64.3	78.0	67.0
"	70	100.0	100.0	100.0	100.0	100.0
"Control[a] 10° F	70	14.8	10.3	11.9	4.6	10.4
3.76	3	58.0	68.5	54.7	78.8	65.0
"	20	92.1	79.8	83.4	93.0	87.1
"	35	100.0	100.0	100.0	100.0	100.0
"Control[a] 10° F	35	25.7	24.9	15.4	31.8	24.5

[a]Optimum temperature for survival

Survival of Larvae and Eggs in the Laboratory at 32° F in Moist and Dry Soil

On the basis of results from the previous test, it was obvious that time periods for sampling groups, larvae in dry and moist soil and egg masses in dry and moist soil, should be more frequent than those used in that test. Also, the samples should be concentrated around the 50 per cent kill of larvae and eggs. Three replicates of each group were set up at weekly intervals for a total of 3 weeks to minimize the possibility of error in laboratory technique. In addition to the samples which were to be tested for survival by the viability stain method, another series of samples was set up to be tested by the bioassay test. This provided information as to the infectivity of the surviving nematodes. Because the results obtained by the viability stain can be expressed quantitatively, only those data obtained by this method are included in this paper.

Due to the extreme variability of the organism in the preliminary experiment, statistical analysis was applied to the results from each group. Estimates of the effect of temperature treatment on survival of the 2 stages of the organism tested were made by comparing the total dead with the total number of nematodes exposed. The per cent dead larvae or eggs was then transformed into probits. According to Finney (12), under the assumption of a normal distribution of log tolerances, probit transformation achieves the linearization of the regression by metametric transformation of the percentage responses.

Using the abbreviated Doolittle method (24) to test for linearity of the 3 regression lines in each replicate it was found that of the total sum of squares for regressions the linear portions removed 90 per

cent or more for each replicate. That is, the linear portion removed 90 per cent or more of the error due to variation of the nematode or other uncontrollable factors. Since curvatures of the regression lines was very small, it appeared that the probit transformation adjusts the responses so that the response is practically linear with respect to log days survival.

Using the assumption of a linear response, as suggested by Finney (12), one can then look at the problem from a viewpoint of bioassay response where the response in probits is a linear function of days on test.

To substantiate the parallelism of the 4 regression lines representing the 4 groups (3 replicates per group), the sum of squares for the pooled regression coefficient was calculated. The analysis of variance presented in Table XI was used to calculate the error (pooled) sum of squares for the 4 groups. The F-ratio for mean squares was not significant at the 5 per cent level. It was concluded from the analysis of variance that there was no deviation from parallelism of the 4 regression lines representing the 4 groups.

Parallel regression lines were fitted to the data for the 4 groups tested (Fig. 3). The dosage response curve from these data indicates that eggs in masses were more tolerant to 32° F in both dry and moist soil than were larvae. While relatively short exposure periods were needed in all cases for a 50 per cent kill of eggs and larvae (Table XII); a long exposure period was required before 100 per cent kill was reached. In dry soil at 32° F, loss of viability of larvae and eggs was pronounced; 90 per cent were dead after 9 and 24 days, respectively. Larvae and eggs were better able to tolerate 32° F in

moist soil. At this latter condition, 90 per cent of the larvae and eggs were dead after 17 and 37 days, respectively.

The bioassay test confirmed the results obtained by the viability stain. A qualitative comparison of these 2 methods gave essentially the same pattern.

TABLE XI

ANALYSIS OF VARIANCE TESTING PARALLELISM OF THE 4 REGRESSION LINES REPRESENTING LARVAE AND EGGS OF MELOIDOGYNE INCOGNITA SUBJECTED TO 32° F IN DRY AND IN MOIST SOIL

Source of Variation	df	SS	MS	F-ratio
Total	96	3042.4374		
Mean	1	2972.4021		
Reps in Groups	8	1.5066		
Groups	3	30.9625	10.3208	
Time in Groups	29	66.3962		
Among Regression Coeff. (Adj.)	3	.0467	.0156	.402NS[a]
Residual	26	66.3495		
Error (Pooled)	55	2.1325	.0388	

[a]Non significant at the 5% level

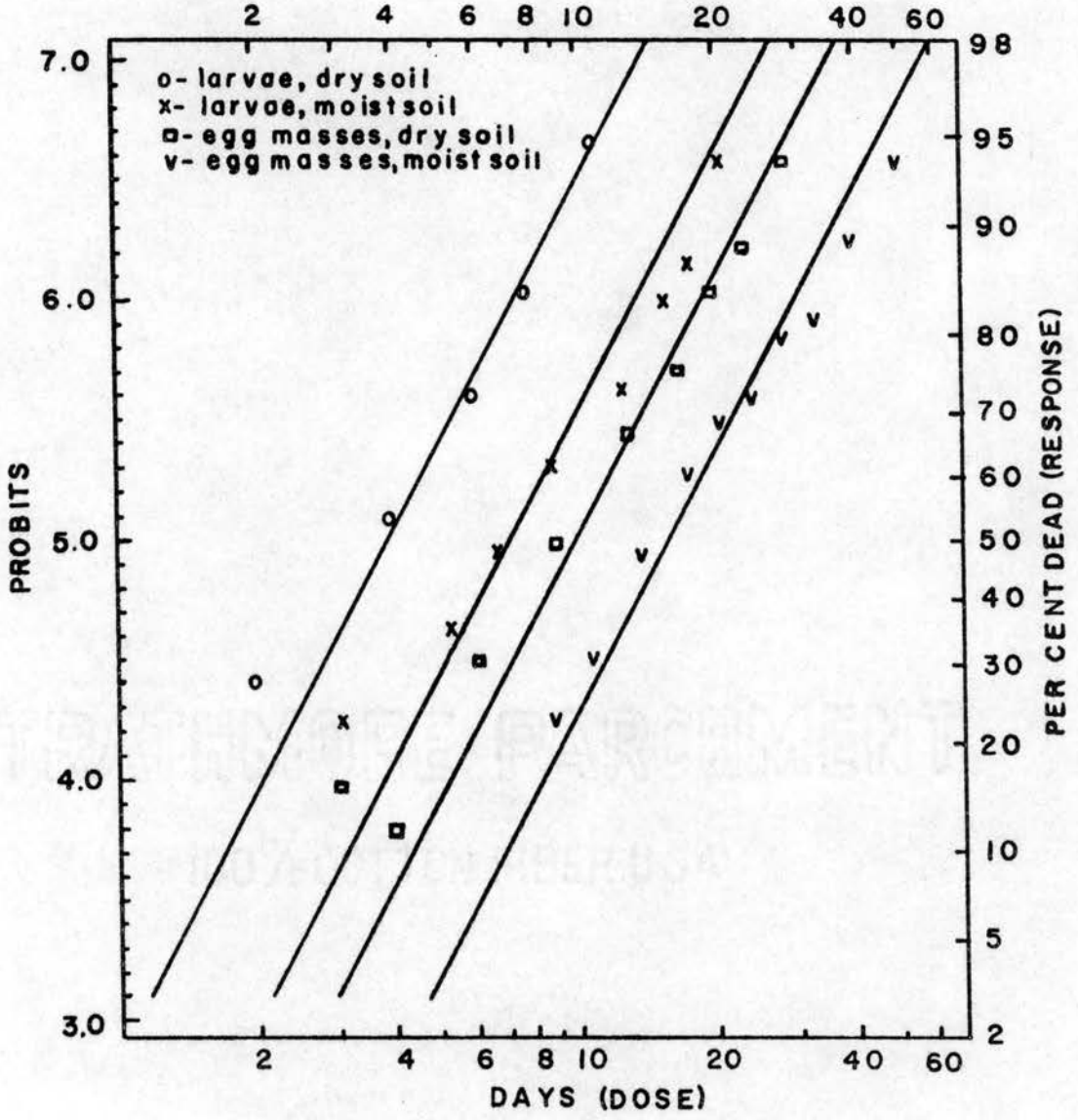

Fig. 3. Effect of 32° F on Meloidogyne incognita larvae and eggs in masses in both dry and moist soil. Per cent dead determined after each exposure period and plotted as dosage-response regression lines on probit paper.

TABLE XII

A COMPARISON OF THE TIME REQUIRED TO KILL 50, 90 AND 98% OF THE LARVAE AND EGGS OF MELOIDOGYNE INCOGNITA SUBJECTED TO 32° F IN DRY[a] AND IN MOIST SOIL

Conditions of test	Time for 50% kill	Time for 90% kill	Time for 98% kill
	days	days	days
Larvae in Dry Soil	4	9	15
Larvae in Moist Soil	7	17	27
Egg Masses in Dry Soil	10	24	39
Egg Masses in Moist Soil	16	37	60

[a]Dry soil - Permanent Wilting Point
Moist soil - Field Capacity

CHAPTER V

DISCUSSION

Previous investigators (1, 7, 27) have attempted to establish the survival value of different stages of several Meloidogyne sp. Because of limitations in available techniques, these investigators were able to bring to bear only indirect evidence as to the role of eggs in survival of these nematodes from season to season. With the modification of certain techniques reported here and the development of a new technique, i. e., the sieving-staining method for recovering root-knot nematode egg masses from soil, it was possible to recover and demonstrate, for the first time, any of the forms of M. incognita which are likely to occur in soil. While the techniques used were applied only to M. incognita, there seems no reason why they would not be equally useful as applied to other species of Meloidogyne.

That eggs of Meloidogyne sp. are laid in a gelatinous matrix and occur in masses is a well established fact. While it has been assumed that these egg masses persist in the soil in essentially their original form, there is apparently no direct evidence of this recorded. With the techniques used in this study, it has been possible to obtain direct evidence that eggs of M. incognita persist in masses either attached to root fragments or free in the soil. At no time was there evidence that the gelatinous matrix surrounding the eggs broke down and released the eggs individually into the soil. Individual eggs were not recovered from soil except when the matrix had been experimentally broken down.

Exhausted matrices, those in which all eggs had hatched, were readily recovered from soil particularly during the winter and spring.

During the course of this investigation it was demonstrated that M. incognita could overwinter in Oklahoma as larvae or as eggs in masses. Larvae, however, seemed to be the predominant overwintering stage. The trend over a 2-year period showed a drastic reduction in the larval populations at all soil depths investigated during early June. This may have been due to an increase in soil temperature at this time with a subsequent increase in activity of the larvae which results in a depletion of their food reserves and eventual death. This would tend to increase the importance of overwintered eggs in providing effective inoculum for the current growing season.

Up till June there was a sharp reduction in numbers of larvae in the upper soil levels, 0-6, 6-12 in. but considerable numbers persisted at the lower levels, 18-24 and 24-30 in. While it was not possible to determine the exact length of time that larvae persisted in field soil, it appeared that those at the 0-6, 6-12 and 18-24 in. levels had remained since December. This was supported by the fact that no viable eggs were recovered from these depths after 15 December. In addition, soil temperatures at 0-6, 6-12 and 18-24 in. were generally unfavorable for hatching. The fact that viable eggs continued to be recovered from the 24-30 in. soil depth and that the temperature range at this depth remained favorable for a slow rate of hatch indicates that larvae continued to emerge at a slow rate. There was no evidence that the eggs became dormant. It appears that several factors, including temperature and moisture during the winter, are important in determining the relative numbers of larvae and eggs which persist overwinter.

Another important aspect of survival as related to relative numbers of larvae or eggs present during winter months is soil temperature during the late fall. If favorable temperatures prevail in late fall, new nematode infections can occur and new egg masses continue to be produced. Results obtained by Thomason and Lear (28) on the influence of soil temperature on root galling and egg mass production showed that M. incognita acrita continued to produce a few egg masses at 15-16° C. In the present work, the average daily maximum temperature remained well above this through the first week in November, thus allowing possible late infections and production of egg masses. Apparently due to limited new infection sites and reduced larval populations very few new egg masses occurred late in the upper soil levels.

It appears that late fall temperatures may be important in determining the relative numbers of larvae and egg masses that are present when temperatures drop or become unfavorable for further egg production. If late fall temperatures favor continued reproduction and hatch of eggs, larvae might be expected in large numbers. This assumption, however, most certainly depends on several factors any of which could determine the surviving stage. Factors which could play a definite role in determining whether larvae or eggs would be the surviving form are (1) severity of the winter in relation to soil temperature and moisture at various soil depths, (2) late fall temperatures determining late infections and (3) potential infection sites provided by host plants.

From the experiments reported here, it was evident that eggs in masses were more tolerant of unfavorable conditions, cold and dry or

cold and moist then were larvae. The results confirm those of other workers (1, 7).

If temperatures are severely reduced for long periods of time during the winter then it is likely that neither larvae nor eggs could survive. In Oklahoma, in the soil studied, root-knot nematodes maintain a maximum population within the 24-30 in. level. Larvae are able to maintain themselves quite well at this depth which provides the most favorable environment of the depths sampled. In the event of a severe winter it appears likely that M. incognita could maintain itself at this depth, then slowly become reestablished in the upper depths. If vertical migration does not occur, larvae or eggs surviving at this depth would do little damage to a host crop planted that year.

Bergeson (unpublished data) however, has evidence demonstrating that deep seated populations can migrate relatively rapidly up into the root zone of a newly planted host crop. If this is the case, then overwintering of larvae or eggs at the 24-30 in. depth certainly poses a problem in controlling this pest. Most certainly eggs would not be affected by any nematocide treatment available and migrating larvae would likely escape the most residual nematocide.

The results obtained in this study support the contention that decreases in nematode populations should reflect the severity of the preceeding winter. (Struble and Morrison unpublished data). Freezing temperatures over a 2-month period, especially under very dry conditions, should greatly reduce both larvae and eggs of M. incognita at depths affected. When these conditions are recorded, then it becomes feasible for a grower to eliminate root-knot nematodes by treating only those

levels where they are not greatly affected by the winter. The possibility of eliminating root-knot nematodes from the lower depths warrants further study, especially with respect to developing a practical method of applying nematocides to this area.

CHAPTER VI

SUMMARY

Two field plots infested with Meloidogyne incognita were sampled over a 2-year period to determine whether this nematode overwintered in the larval stage, the egg stage or both. Plot I and plot II, were sampled at 0-6, 6-12, 18-24 and 24-30 in. and 0-6, 6-12 and 12-18 in. depths respectively. Through the use of a modified centrifugal-flotation technique and the development of a new sieving-staining technique, it was possible to recover and demonstrate, for the first time, any of the forms of M. incognita which are likely to occur in soil.

From this investigation it was determined that M. incognita can overwinter in Oklahoma in both the larval stage and the egg stage. Larvae, however, seemed to be the predominant overwintering stage during the test conditions. The larval population was greatly reduced through the winter months in the upper 12 in. of soil. During the winter the maximum concentration of larvae remained at the 18-24 and 24-30 in. depths. Eggs of M. incognita were found to persist in masses either attached to root fragments or free in the soil. While eggs in masses were recovered during the winter from all the depths sampled, the only viable eggs recovered after mid-December were from the 24-30 in. level.

Field studies where larvae and egg masses were tested separately showed that eggs were more tolerant to the variable temperature of the

upper soil depths, although larvae and eggs were drastically reduced at the 0-6 in. level.

In the laboratory, eggs in masses were found to survive approximately twice as long as did larvae at 32° F in both moist and dry soil. The eggs survived 41 days in dry soil and 64 days in moist soil.

LITERATURE CITED

1. Bergeson, G. B. 1959. The influence of temperature on the survival of some species of the genus Meloidogyne, in the absence of a host. Nematologica 4: 344-354.

2. Bessey, E. A. 1911. Root-knot and its control. U. S. Dept. Agr. Bur. Plant Ind. Bull. 217. 89 p.

3. Brown, L. N. 1933. Flooding to control root-knot nematodes. J. Agr. Res. 47: 883-888.

4. Caveness, F. E., and J. H. Jensen. 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Helminthol. Soc. Wash., D. C., Proc. 22: 87-89.

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

6. Cunningham, H. S. 1936. The root-knot nematode (Heterodera marioni) in relation to the potato industry on Long Island. New York Agr. Exp. Sta. Bull. 667. 24 p.

7. Daulton, R. A. C., and C. J. Nusbaum. 1961. The effect of soil temperature on the survival of the root-knot nematodes Meloidogyne javanica and M. hapla. Nematologica 6: 280-294.

8. Daulton, R. A. C., and C. J. Nusbaum. 1962. The effect of soil moisture and relative humidity on the root-knot nematode Meloidogyne javanica. Nematologica 8: 157-168.

9. Dickson, D. W. and F. B. Struble. 1965. A sieving-staining technique for extraction of egg masses of Meloidogyne incognita from soil. Phytopathology 55: 497. (Abstr.)

10. Dropkin, V. H., G. C. Martin, and R. W. Johnson. 1958. Effect of osmotic concentration on hatching of some plant parasitic nematodes. Nematologica 3: 115-126.

11. Fenner, L. M. 1962. Determination of nematode mortality. Plant Dis. Rept. 46: 383.

12. Finney, D. J. 1952. Statistical Method in Biological Assay. Hafner Publishing Co., New York. 669 p.

13. Frados, J., Ed. 1964. Modern Plastics Encyclopedia 42(No. 1A): 461. McGraw-Hill, Inc., New York, N. Y.

14. Franklin, Mary T. 1937. On the survival of Heterodera marioni infection out-of-doors in England. J. Helminth. 15: 75-76.

15. Godfrey, G. H., and Helene M Hoshino. 1933. Studies on certain environmental relations of the root-knot nematode, Heterodera radicicola. Phytopathology 23: 41-62.

16. Godfrey, G. H., Juliette M. Oliverira, and E. B. H. Gittel. 1933. The duration of life of the root knot nematode, Heterodera radicicola, in soils subjected to drying. Soil Sci. 35: 185-195.

17. Kincaid, R. R. 1946. Soil factors affecting incidence of root knot. Soil Sci. 61: 101-109.

18. Linford, M. G. 1941. Some soil-moisture relationships to the root-knot nematode. Phytopathology 31: 862. (Abstr.)

19. Loewenberg, J. R., T. Sullivan, and M. L. Schuster. 1960. The effect of pH and minerals on the hatching and survival of Meloidogyne incognita incognita larvae. Phytopathology 50: 215-217.

20. Miller, P. M. 1957. A method for the quick separation of nematodes from soil samples. Plant Dis. Reptr. 41: 194.

21. Minderman, G. 1956. New techniques for counting and isolating free living nematodes from small soil samples and from oak forest litter. Nematologica 1: 216-226.

22. Newhall, A. G. 1934. Root-knot nematode population in New York reduced by cold winter. Plant Dis. Reptr. 18: 111.

23. Nusbaum, C. J. 1962. Winter survival of four species of Meloidogyne in North Carolina. Phytopathology 52: 23. (Abstr.)

24. Ostle, B. 1963. Statistics in Research. Iowa State Univ. Press, Ames. 585 p.

25. Peacock, F. C. 1957. Studies on root knot nematodes of the genus Meloidogyne in the Gold Coast. II. The effect of soil moisture content on survival of the organism. Nematologica 2: 114-122.

26. Richards, L. A., and M. Fireman. 1943. Pressure-plate apparatus for measuring moisture sorption and transmission by soil. Soil Sci. 56: 395-404.

27. Sayre, R. M. 1963. Winter survival of root-knot nematodes in southwestern Ontario. Can. J. Plant Sci. 43: 361-364.

28. Thomason, I. J., and B. Lear. 1961. Rate of reproduction of Meloidogyne spp. as influenced by soil temperature. Phytopathology 51: 520-524.

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

30. Tyler, Jocelyn. 1933. The root-knot nematode. Calif. Agr. Exp. Sta. Cir. 330 (Revised 1944).

31. Tyler, Jocelyn. 1938. Egg output of the root-knot nematode. Helminthol. Soc. Wash., D. C., Proc. 5: 49-54.

VITA

Donald W. Dickson

Candidate for the Degree of

Master of Science

Thesis: THE INFLUENCE OF SOIL TEMPERATURE ON SURVIVAL OF LARVAE AND EGGS OF MELOIDOGYNE INCOGNITA IN THE FIELD AND IN THE LABORATORY

Major Field: Botany and Plant Pathology

Biographical:

Personal Data: Born in Charlotte, Tennessee, December 9, 1938, the son of John H. and Allie Belle Dickson.

Education: Graduate from Dickson Elementary School, 1952, and Dickson High School, 1957, Dickson, Tennessee; attended Murray State College, Murray, Kentucky, 1957-1959; received Bachelor of Science degree from Austin Peay State College, Clarksville, Tennessee in August 1963; entered Oklahoma State University, Stillwater, Oklahoma in September 1963; completed requirements there for the Master of Science degree in August, 1965.

Professional experience: Employee of Ford Motor Company Glass Plant, 1959-1961, Nashville, Tennessee; Graduate research assistant in the Department of Botany and Plant Pathology, Oklahoma State University 1963-1965.

Professional Organizations: Member of the American Phytopathological Society, Phi Sigma, Beta Beta Beta, and Associate member of Sigma Xi.

Date of Final Examination: August, 1965.