

# Rothamsted Repository Download

## A - Papers appearing in refereed journals

Franklin, M. T., Course, J. A. and Clark, S. A. 1971. Population changes and development of *Meloidogyne naasi* in the field. *Nematologica*. 17 (4), pp. 575-590.

The publisher's version can be accessed at:

- <https://dx.doi.org/10.1163/187529271X00297>

The output can be accessed at:

<https://repository.rothamsted.ac.uk/item/96yw9/population-changes-and-development-of-meloidogyne-naasi-in-the-field>.

© Please contact [library@rothamsted.ac.uk](mailto:library@rothamsted.ac.uk) for copyright queries.



## POPULATION CHANGES AND DEVELOPMENT OF *MELOIDOGYNE NAASI* IN THE FIELD

BY

MARY T. FRANKLIN, SYBIL A. CLARK and JANET A. COURSE

Rothamsted Experimental Station, Harpenden, Herts., England

Experiments show that soil samples, especially those taken between October and April, yield more larvae if they are incubated for a week at 20°C than if they are processed on the day they are taken. Samples taken in autumn after a host crop and kept at 0°C for a week before incubation yield more larvae than incubated, non-chilled samples. The numbers of free larvae in field soil are few during winter, increase greatly as the soil temperature increases in spring, decrease sharply when the host roots are invaded, remain low during summer and show a moderate increase when some of the newly formed eggs hatch in late summer. In the absence of a host crop numbers increase in spring and decrease gradually during summer. The rate of development of *M. naasi* in host roots depends on temperature and is the same in barley and ryegrass. The amount of energy in the form of heat required for development is approximately the same throughout the year but the time taken varies greatly.

### ESTIMATING SOIL POPULATIONS OF *MELOIDOGYNE NAASI*

Root-knot nematodes, *Meloidogyne* spp., occur in soil as eggs containing larvae in different stages of development, or as free larvae. Egg masses may be enclosed in the roots where they developed, attached to the root surface, or loose in the soil. To estimate the numbers of *Meloidogyne* spp. in infested soil two methods are commonly used. Either the free larvae are extracted and counted in water suspension, or a host (indicator) plant is grown in the soil and the nematodes that invade its roots are counted or the galling assessed. Whereas the first method gives the number of larvae free in the soil, the second estimates the number of larvae that invade the roots of the test plant, including free larvae and some that hatch from egg masses during the test. Neither method includes all the individuals potentially able to attack roots because the first ignores unhatched larvae in egg masses and, with the second, all larvae may not have hatched and some hatched larvae may have failed to enter the roots when the test ends, which must be before a second generation arises. Motsinger (1964) found that elutriation recovered 73% of larvae added to soil but indicator plants recovered only 48%.

Extracting egg masses of *Meloidogyne* from soil by a flotation process as for *Heterodera* cysts is impractical because egg masses differ greatly in size and shape and may be embedded in roots. They are difficult to distinguish in floated debris because soil particles often adhere to them and, partly for this reason, many egg masses do not float (De Guiran, 1966). Dickson & Struble (1965) stained debris floated from soil with phloxine B, which colours the egg mass matrix bright



red, but we found the method tedious and inaccurate, especially with soil containing much organic matter.

In temperate regions the ratio of larvae in eggs to those free in soil depends on the time of year and whether or not a host plant is present. Szczygiel (1966) estimated populations of *M. hapla* in strawberry fields in Poland through the year by extracting larvae from soil and from incubated strawberry roots and found three peaks, the smallest in April-May, the largest in August-September and one nearly as big in November-December. The peaks were not directly related to soil temperature or moisture but to the growth of the strawberries and the life cycle of the nematodes. Strawberry roots proliferate during spring, grow little during the fruiting period in June and early July but again grow vigorously after fruiting until the end of September. Because the larvae invade the young roots during spring few occur free in the soil in June and early July. The progeny of the first generation produce the large peak in August-September and, entering the roots growing actively at this time, produce a second generation giving the second peak at the end of the year. In December few eggs hatch and any larvae that invade roots are unlikely to mature before the following spring.

Stein (1969) found similar seasonal fluctuations in numbers of *M. hapla* larvae in field soils planted with lettuce. There were few free larvae in the field during winter but plants grown in soil removed to the greenhouse were heavily galled.

When estimating numbers of root-knot nematodes in soil by counting free larvae, the month when the soil is sampled is important. If samples are taken when the infestation is mainly egg masses, the soil must be so treated that larvae in eggs hatch and are extracted together with the free larvae. While soil is drying, larvae continue to develop within eggs protected by the matrix of the egg mass, but free larvae are killed (Wallace, 1968). In dry soil the larvae in egg masses cannot hatch, but soon do when the dry soil is moistened. De Guiran (1966) got thirty times more larvae from soil first dried and then wetted in a mistifier than from samples of the same soil elutriated without previous drying. To estimate the total potential infestation of soil in this way, duplicate samples are necessary, one for extracting free larvae, the other for extracting larvae from egg masses after drying. De Guiran suggests alternatively that free larvae may be extracted by elutriation and the sludge remaining in the apparatus may then be put in a mistifier so that larvae in egg masses can hatch and be counted. This presupposes that larvae in the egg masses are ready to hatch or will become so during the extraction process.

Watson & Lownsbery (1970) tested the effects of a range of temperatures and of drying on the hatching *in vitro* of egg masses of *M. naasi*. They found that most larvae emerged after exposing egg masses for 7 weeks at 6°C and 12°C for alternate 24 hr periods followed by 5 days at 21°C. Keeping the egg masses at 6 or 9° for 6 weeks and then transferring them to 21° also gave a good hatch. Drying stopped hatching.



We tried to find a method of treating soil samples to extract the most *Meloidogyne naasi* larvae by inducing those in egg masses to hatch and extracting them at the same time as the free ones.

### Materials and Methods

Whitehead & Hemming (1965) found the best method of extracting active nematodes from soil was to spread sieved soil thinly on Scottie tissue supported in a wire basket in a shallow tray containing water just deep enough to saturate the soil. After a suitable time, when the nematodes have moved into the water, the suspension is concentrated and transferred to a shallow counting dish. We adopted this method because it is simple and extracts only active and, presumably, infective larvae.

Soil used for experiments 1 and 2 was collected early in March from an unploughed field where barley was galled by *M. naasi* the previous year. The soil was stored at 4°C until needed, sieved through a coarse (6 mm) sieve and thoroughly mixed. The larvae were extracted at room temperature (18°C) from 300 ml samples and counted in aliquot samples of well mixed suspension.

### Experiment 1

To find out how long it takes for all the larvae to emerge from soil samples, larvae from six replicates were counted after 1, 2, 7, 14, 21 and 28 days. Few larvae emerged after the second day (Fig. 1). Of the total extracted approximately 60% emerged in 24 hr and 83% in 48 hr. For this soil, taken in March when the larvae in the over-wintered eggs hatched readily, a two-day extraction period was adequate and 7 days unnecessarily long.

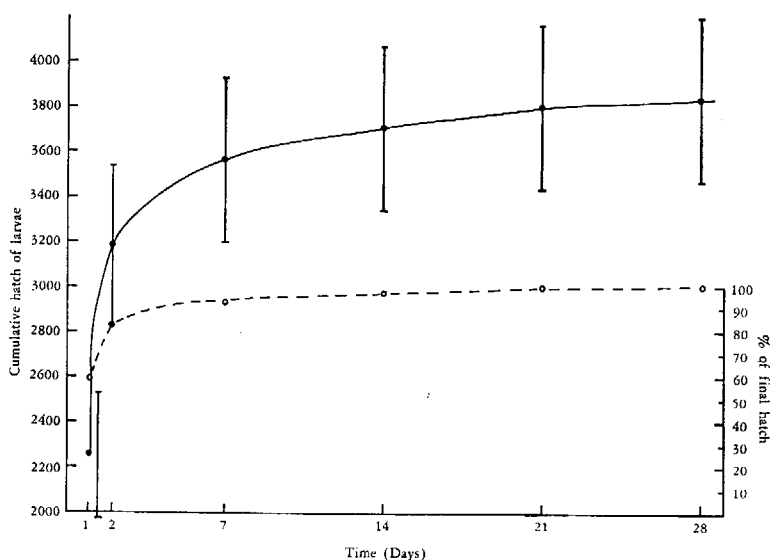


Fig. 1. Cumulative counts of larvae extracted from 300 ml of soil for 28 days at room temperature  
 ●—●: Percentage of final hatch ○—○: Means of six replicates with standard deviations.



*Experiment 2*

To test the effect of incubation on the hatching of *Meloidogyne* eggs in soil, bulk samples (3 kg) in polythene bags were stored at 15°, 20°, 25°, and 30° and sub-samples removed after 1, 2, and 3 weeks. Larvae were extracted from triplicate 300 ml sub-samples at room temperatures and counted after 24 hr and 72 hr.

The most striking result is that storage at 30°, for as little as a week, greatly decreased the number extracted (Tables I and II). This temperature is unlikely to be lethal but probably makes the larvae very active which uses up their stored food and shortens their life span. The high temperature may also encourage harmful microorganisms.

TABLE I

*Larvae emerging from 300 ml soil after incubation at four temperatures for three periods of time. Counted 24 hr after setting up: means of three replicates*

Incubation period		Incubation temperature				Comparison at different temperatures
weeks		15°	20°	25°	30°	
1	Mean	2160	3037	2100	693	15° vs 20°*
	S.E.	94	420	150	42	15° vs 25° n.s.
						20° vs 25°*
						25° vs 30°***
2	Mean	1690	2335	1218	237	15° vs 20° n.s.
	S.E.	365	365	369	1	20° vs 25°*
						15° vs 25° n.s.
						25° vs 30°***
3	Mean	1959	2720	1230	94	15° vs 20° n.s.
	S.E.	283	326	266	13	15° vs 25° n.s.
						20° vs 25°***
						25° vs 30°***
Comparison of times of incubation		n.s.	n.s.	1 vs 2**	1 vs 2***	
				1 vs 3***	1 vs 3***	
				2 vs 3 n.s.	2 vs 3***	

\* = probability 0.03-0.04

\*\*\* = probability 0.01

Van Gundy *et al.* (1967) found that the body contents of larvae of *M. javanica* stored at 30° for 4 days decreased to 40% and after 16 days to 0%. Storage at 25° for 16 days decreased them to 4%. Movement was little affected at either temperature after 4 days, but after 16 days was 25% at 25° and 0% at 30°.

In our experiment most larvae were extracted after incubation at 20°, with no significant difference between periods of storage. The effects of continuing extraction for 72 hr are in Table III which suggests that storage at 30° delays hatching or lessens mobility, but the experiment was not continued long enough to find whether all viable larvae had been extracted. The optimum temperature for hatching our population of *M. naasi* may be lower than for the one from



Illinois used by Siddiqui & Taylor (1970) who reported a massive hatch at 27°C. However, this was after treatment of egg masses for 15 min in 0.26% sodium hypochlorite (to disperse the eggs) which softens the egg shell.

TABLE II

*Larvae emerging from 300 ml soil after incubation at four temperatures for three periods of time. Counted 72 hr after setting up: means of three replicates*

Incubation period		Incubation temperature				Comparison at	
weeks		15°	20°	25°	30°	different temperatures	
1	Mean	2693	3125	2224	983	15° vs 20°	n.s.
	S.E.	231	442	133	109	20° vs 25°	n.s.
						15° vs 25°	n.s.
						25° vs 30°***	
2	Mean	2543	2741	1527	732	15° vs 20°	n.s.
	S.E.	320	548	336	41	20° vs 25°	n.s.
						15° vs 25°*	
						25° vs 30°* 2% level	
3	Mean	2617	3184	1593	503	15° vs 20°	n.s.
	S.E.	264	537	228	73	20° vs 25°***	
						15° vs 25°***	
						25° vs 30°***	
Comparison of incubation periods		n.s.	n.s.	n.s.	2 vs 3*	1 vs 2*	

TABLE III

*Effect of temperature and period of storage on numbers of larvae extracted from soil. Number emerging in 72 hr as percentage of number at 24 hr.*

Storage, weeks	15°	20°	25°	30°
1	125	103	106	142
2	150	117	125	309
3	134	117	130	535

### Experiment 3

To plan for the next year's crop it is sometimes necessary to estimate nematode numbers in soil after harvest. An experiment was done to find whether chilling before incubation would increase the number of larvae extractable from post-harvest samples. Soil infested with *M. naasi* was collected during November 1968 when the temperature 18 cm deep had ranged from 6-9°C during the previous 2 weeks. Batches of soil were given the treatments listed in Table IV and the larvae were extracted for 24 hr and counted.

When the soil was incubated for a week at 20°C after a week at 10°C, many more larvae were extracted than after storage at 10° and below, but the increase was three times greater when the soil was chilled at 0°C for a week before transfer to 20°C.



TABLE IV

*Numbers of larvae extracted in 24 hr from 300 ml soil, means of four replicates*

Soil treatment	Number of larvae	S.E. of mean
1. Extracted immediately	736 a*	6.3
2. Stored at 10° for 2 wks	639 b	8.3
3. Stored at 0° for 1 wk, then 10° for 1 wk	724 a	23.4
4. Stored at 10° for 1 wk, then 20° for 1 wk	3,421 c	173.3
5. Stored at 0° for 1 wk, then 20° for 1 wk	9,255 d	548.5

\* Values bearing different letters differ significantly from each other (2% level for 2 and 3, 1% for all others).

*Experiment 4*

To get more information on the effect of temperature, samples were collected in November 1969 from the same field as in November 1968. A cereal crop was not grown in 1969 but kale and turnips planted in August were lightly infested. Fewer larvae were immediately extractable than in the previous year, but treatment at 0° followed by 15° or 20° again gave a significant increase (Table V), possibly because it affected the few newly formed egg masses.

TABLE V

*Numbers of larvae extracted in 24 hr from 300 ml soil, means of four replicates*

Soil treatment	Number of larvae	S.E. of mean
1. Extracted immediately	606 a*	26.1
2. Stored at 0° for 1 wk, then 10° for 1 wk	631 a	19.7
3. Stored at 0° for 1 wk, then 15° for 1 wk	698 b	25.7
4. Stored at 0° for 1 wk, then 20° for 1 wk	758 b	25.1

\* Values bearing different letters differ significantly from one another. (1% level for 1 and 4, 2 and 4, 5% level for 1 and 3, 10% level for 2 and 3).

## SEASONAL CHANGES IN LARVAL NUMBERS IN FIELD SOIL

To follow changes in larval numbers throughout the year, soil samples were collected from an area in an infested field at monthly intervals for two years from March 1968. Spring barley was grown in 1968 and harvested in September; the young seedlings bore numerous root galls. The field was not ploughed during the winter of 1968-69 and was infested with couch grass (*Agropyron repens* (L.) Beauv.), a host of *M. naasi*. In 1969 the first cultivation was during May and the field was ploughed several times from then until a turnip-kale mixture was sown in August; this crop was lightly infested. Sampling was discontinued in February 1970.



Soil samples were taken with a 2.5 cm diam. auger 20 cm deep and divided into the upper 8 cm and the lower 8-20 cm. About 50 samples from each layer were mixed and passed through a 7 mm mesh sieve and four aliquots of 300 ml were taken. Two were processed immediately and the other two covered and kept in an incubator at 20°C for a week before processing. Larvae were extracted for 24 hr. Differences between numbers of larvae at the different depths were slight, so counts given are means for the two depths. Soil temperatures were obtained from the Agricultural Botany Field Laboratory of Reading University, on one of whose farms the infested field was situated. Figs. 2a & 2b show numbers of larvae and soil temperatures.

### *Results*

In 1968 the numbers of larvae extracted from 300 ml of soil during the 24 hr after sampling increased from 371 on March 11 to 6,444 on May 6, then decreased to 573 on June 4 and 255 on July 1. The decrease during May followed a massive invasion of the barley roots, first observed on April 25. Fewest larvae were extracted at the end of July (217 per 300 ml), then numbers increased gradually to 870 in October and remained near 600 until early March. This increase was probably from the generation on the 1968 barley crop, some of which probably hatched during the 24 hr processing of the soil at room temperature. In 1969, with no host crop, numbers of larvae free in the soil increased again as the temperature rose during April and May, to a peak of 7,360, and remained at nearly 5,000 through June and July, decreasing to 1,400 at the end of July and to 560 by the end of August. The large numbers free in the soil during the summer reflect the absence of a host crop.

Incubation of the soil samples made little difference to the numbers of larvae extracted from May till September but gave a large increase from October to April.

The threshold temperature for hatching in the field during spring seems to be about 10°, whereas in October, before the soil had been chilled, few free larvae were found although the soil temperature was 12-14°. However, incubation of the October samples at 20° without previous chilling increased the number of larvae extracted.

### RATE OF DEVELOPMENT IN HOST ROOTS

#### *In a field crop of spring barley*

Barley plants were removed at fortnightly intervals from the field from which the soil samples were taken, and their roots stained in cotton blue lactophenol. The first 100 nematodes found were dissected from the roots under the stereomicroscope and classified (Fig. 2c).

Plants dug on April 8, a month after sowing, had seminal roots 5-6 cm long but no larvae in them. Two and a half weeks later (April 25) the roots were twice as long and contained many larvae, most of which had entered recently and had not started feeding or were only partly within the tissues. There was no sign of



galling or of distortion of the stele, and most of the larvae were in the region of undifferentiated tissue immediately behind the root tips, one of which contained more than 100 larvae in 2 mm. Recently entered larvae were found until early June, an invasion period of about 6 weeks. On May 6 there was obvious galling and giant cells had formed: some larvae had begun to enlarge and the gonad

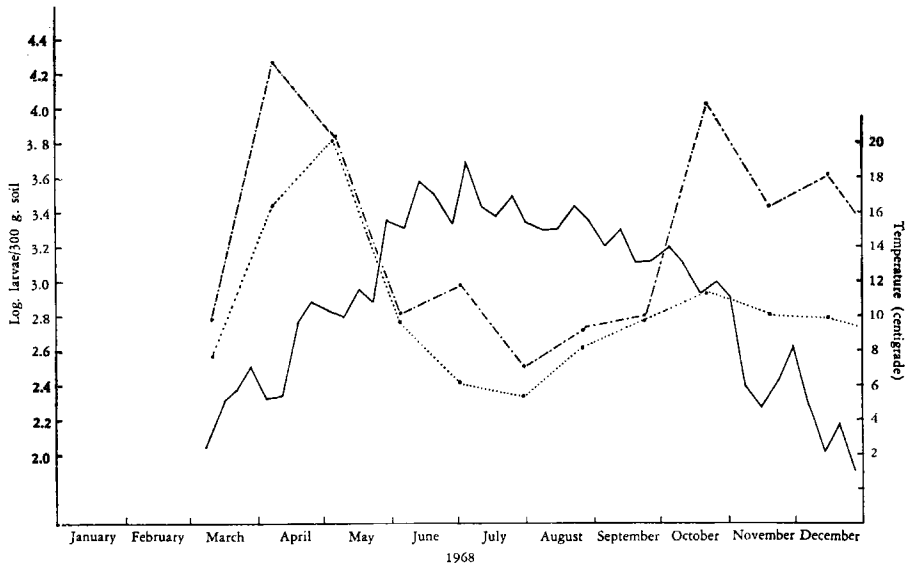


Fig. 2a. Larval counts and soil temperatures during 1968.

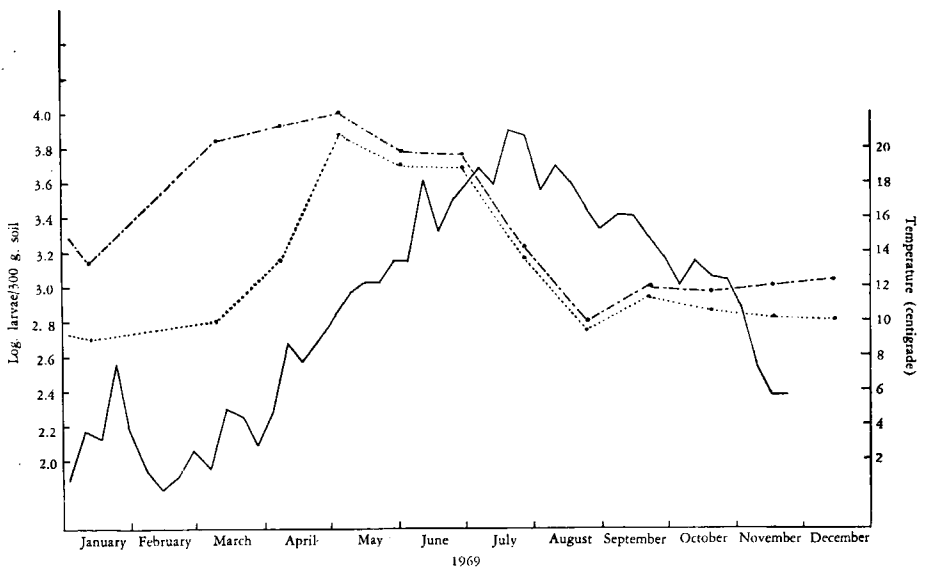
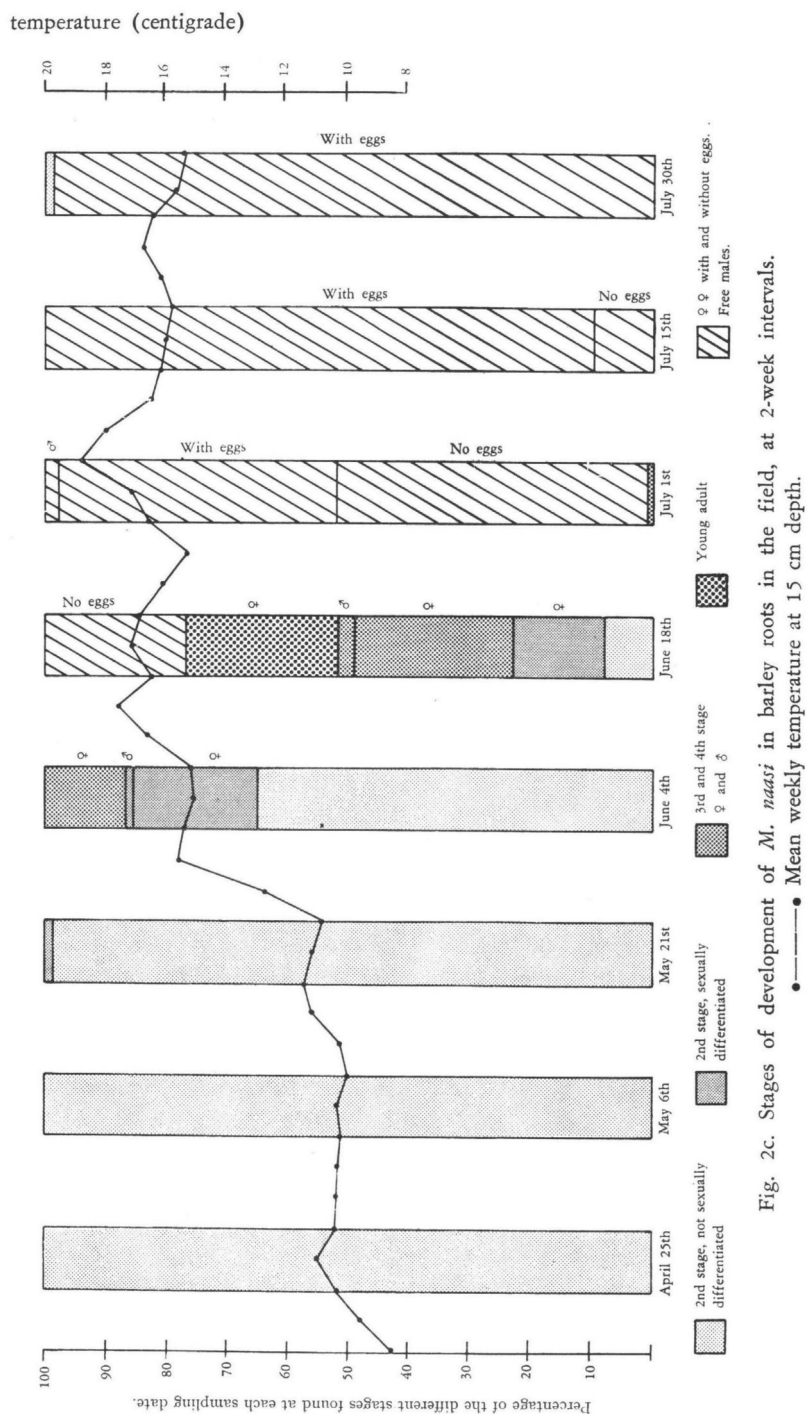


Fig. 2b. Larval counts and soil temperatures during 1969. — Soil temperature at 15 cm depth. ..... Larvae extracted in 24 hrs immediately after sampling. -.-.- Larvae extracted in 24 hrs following incubation at 20°C for one week.







cells had divided, but not sufficiently for their sex to be determined. The second stage larvae continued to grow for about 4 weeks with the soil colder than 12° (Fig. 2c). They then moulted and the adult stage was complete in about 2 weeks. Adult females continued to feed and lay eggs for 4-6 weeks. Galled roots taken at the end of July were chopped and kept in water for 3 weeks during which time a few larvae emerged, showing that in the field on spring barley one generation had taken approximately 12-14 weeks.

*In barley at controlled temperatures*

Infested plants with adhering soil were brought from the field on May 6 and planted in pots. One batch was kept at 20-25° and a second at 12.5°. Roots were removed at intervals and stained to determine the stage of development of the larvae. Adult females with eggs were first found on May 31 in roots kept at 20-25°, a month before adults were found in the field. In plants kept at 12.5°, adult females with eggs were first found on June 14, a little earlier than in the field where the soil temperature did not exceed 12.5° until May 25.

*In five hosts in pots*

Two tests with plants in pots were done to compare the rate the nematode develops in different host plants. Barley, wheat, ryegrass, cocksfoot (*Dactylis glomerata* L.) and sugar-beet were sown on April 28 in infested soil in 9 cm diam. pots sunk outdoors in sand. After 6 or 7, 8 and 10 weeks plants were lifted and the roots examined after staining in cotton blue lactophenol. There was no appreciable difference between the rates of development of the nematodes in any of the five host plants (Table VI).

TABLE VI

*Time from sowing for completion of nematode life cycle*

	Sown	Pre-adults	Females + eggs	Eggs with fully developed larvae
Barley	April	6 wks	8 wks	—
	August	4 wks	8 wks	11 wks
Wheat	April	6 wks	8 wks	—
	August	5 wks	7 wks	13 wks
Ryegrass	April	6 wks	8 wks	—
	August	6 wks	9 wks	13 wks
Cocksfoot	April	—	7 wks	—
Sugarbeet	April	6 wks	8 wks	—

A second series of pots was sown on Aug. 19 with barley, wheat, ryegrass or sugar beet and sunk in sand outdoors as before. The seed was pre-soaked to hasten germination and plants were dug and the roots stained at weekly intervals from the first to the fifteenth week after sowing. The results confirmed those of the April sowing (Table VI).



*In consecutive sowings of barley and ryegrass through the year*

To find the length of the life cycle under field conditions at different times of the year, barley and ryegrass were sown in an infested plot at monthly intervals and plants assessed at fortnightly intervals until mature females with eggs were found. The results were the same for both hosts (Figs 3 and 4).

In 1966 and 1967 embryonated eggs were first found at mid-July, in plants sown from January 31 to April 25 (ryegrass), and from January 31 to May 25 (barley); the shortest generation time during these months was 11 weeks. Barley, sown in June 1966 contained females with embryonated eggs 6 weeks after sowing and ryegrass 8 weeks after sowing. Any larvae that hatch from newly embryonated eggs during July or early August may produce a second generation by October because soil during August and September is warm enough, but Fig. 2a suggests that few hatch before the following spring.

The energy in the form of heat needed for nematodes to develop can be assessed from soil temperatures by summing the number of degrees above a chosen minimum (i.e. an assumed basal development temperature) recorded on each day of the period. This we did for barley and ryegrass, during the period from entry of larvae into the roots to production of eggs. Entry into the roots is taken to be at about the time that the seedlings are first seen above ground, referred to here as "germination". Values for the number of day-degrees above 10°C and above 12°C are illustrated in Fig. 5. Some of the values obtained may be excessive for two reasons. First, when the plants were growing in a lightly infested patch of soil, nematodes were not found in the roots on some sampling dates and there were too few plants to continue sampling until infested plants were found. An example (omitted from Fig. 5) is the ryegrass germinated on June 5, 1967: no infestation was found in plants dug on June 8 or 23 and only young stages on July 5 and 22, whereas barley sown on the same date contained females with eggs on June 23. Secondly, abnormally large day-degree readings may be recorded during a hot spell when the temperature exceeds that necessary for the nematodes to develop, without being hot enough to delay development, and a surplus of energy is received. Fig. 5 suggests that about 222 day-degrees above 10°C or about 137 above 12°C suffice for development of *M. naasi* in ryegrass and barley from invasion to egg formation.

*Discussion*

Several efficient methods of extracting active nematodes from soil have been described, but to assess the potential for damage of a soil infested with root-knot nematode, both free and unhatched larvae need to be counted. This is shown in Figs 2a and 2b by the big differences in numbers of free larvae whether or not a host crop was growing. In March, 370 larvae per 300 ml soil were extracted, whereas in May there were 6,400, about seventeen times as many. Most of the larvae extracted in May were present as eggs in March. Such fluctuations probably occur only in climates with a cold or dry season that prevents eggs from hatching.



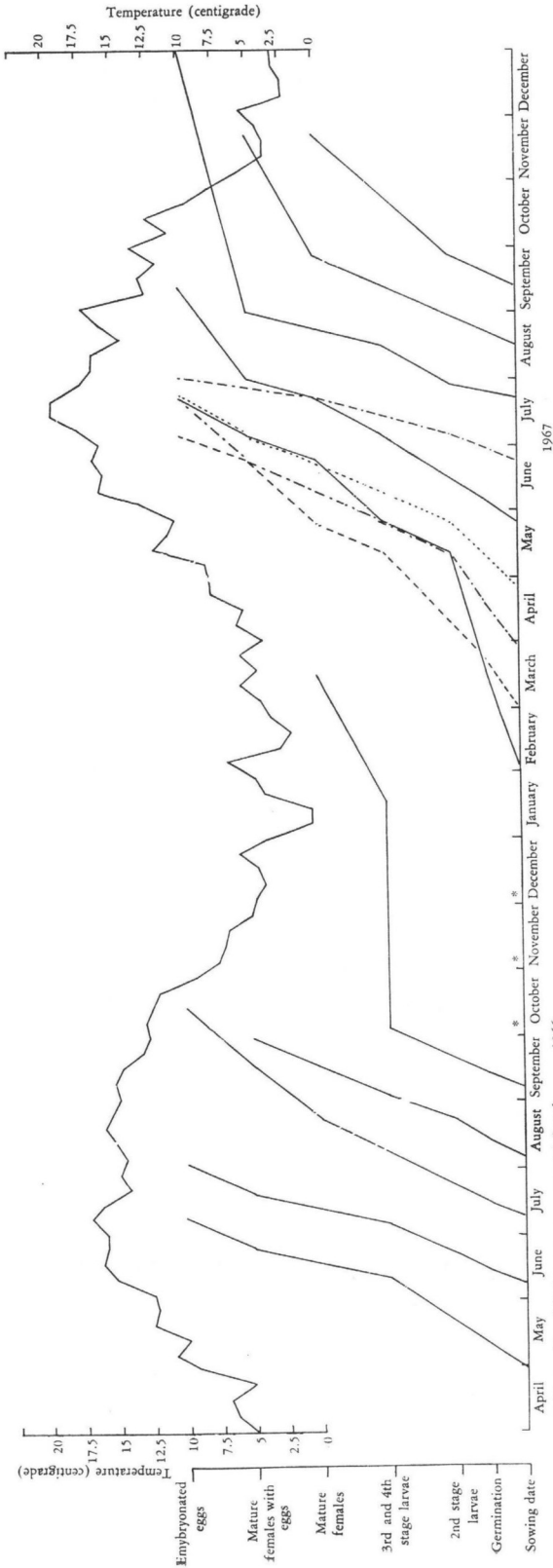


Fig. 3. The length of the life cycle of *M. naasi* in ryegrass under field conditions.

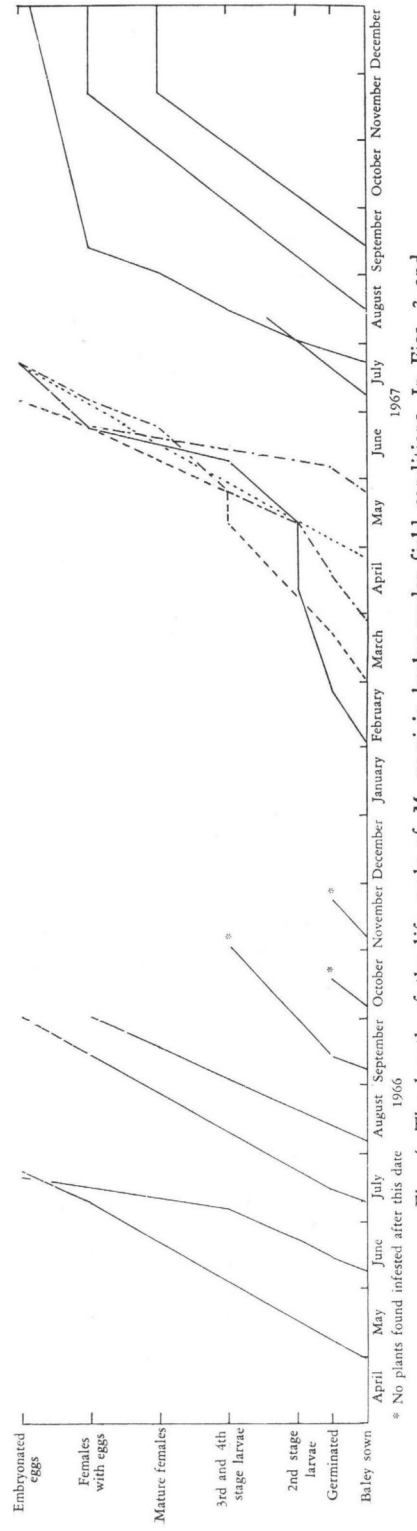


Fig. 4. The length of the life cycle of *M. naasi* in barley under field conditions. In Figs. 3 and 4 broken lines are used for clarity where the lines overlap.



Barker *et al.* in North Carolina (1969a) found most larvae in a mixed population of *M. incognita* and *M. hapla* in October and again in February. Their maximum count (per 300 ml of soil by Baermann funnel extraction) was about 420 during autumn or early spring, and minimum about 40 in January (i.e. one tenth),

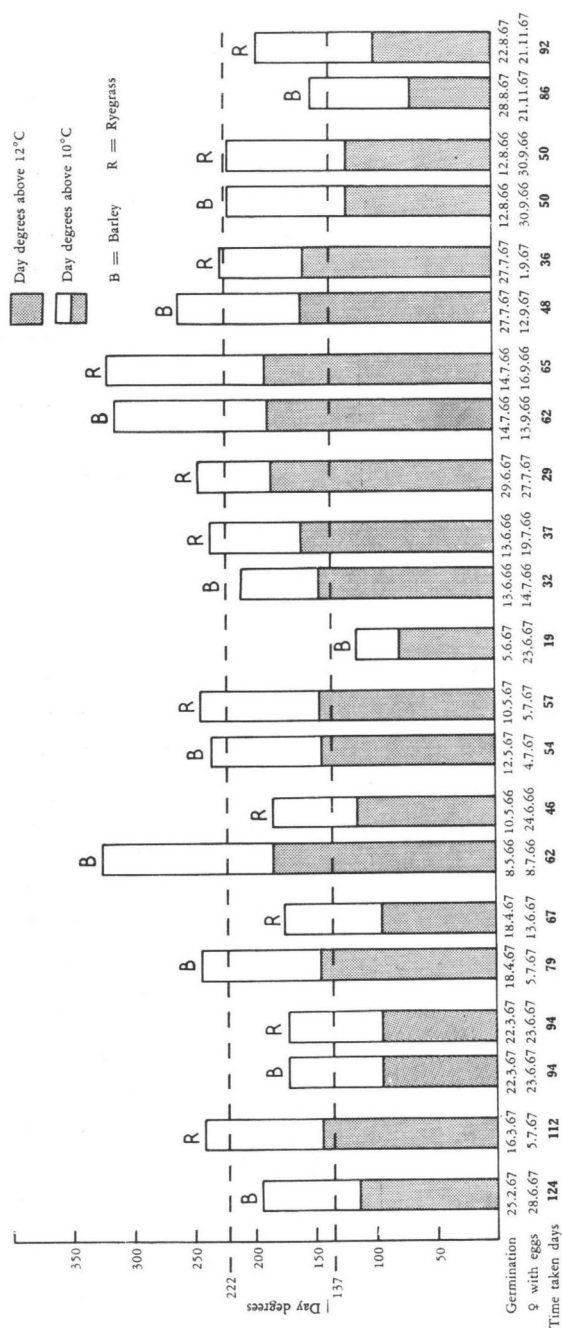


Fig. 5. Energy, as day-degrees, in the soil during development of *M. naasi* in separate sowings of barley and ryegrass from February to November. Dates at bottom give period of development; number of days taken are on bottom line.



suggesting that fewer were unhatched than in the English population, and also that the larvae of these species hatch soon after they have developed and do not first require a period of chilling. Elutriation of soil samples extracts only free larvae, so to get an estimate of the total numbers of larvae a method, such as the tray method, should be used to allow time for eggs to hatch.

To estimate numbers of *M. naasi*, soil samples taken in spring need no pre-treatment if they are extracted at room temperature for 2 to 3 days, but samples taken in autumn should be chilled at 0° for a week and then incubated at 20° for several days before extraction. Watson & Lownsbery (1970) had no hatch from fresh egg masses kept at 21° for up to 12 weeks but after storage for 7 weeks at 6° or 9° before incubating at 21° there was a moderate hatch. Allen *et al.* (1970) also found that larvae of *M. naasi* from northern California need to be chilled before they hatch readily. A period of 6 weeks at 40°F (4.4°) was effective.

When soil samples have to be stored before processing it is important that the storage temperature should be below 25°. Barker *et al.* (1969b) found that 16 weeks storage of soil samples at 30° or 36°C gave fewer *M. incognita* larvae than at 13-24°. This species appears to tolerate lengthy storage periods at 24° better than *M. naasi* does at 25°C.

Our observations on seasonal fluctuations in the field emphasize the importance in assessing larval numbers, of the time of year, presence or absence of a host crop and previous cropping. Such information is also useful when deciding what crop to grow in an infested field. If a host crop must be grown, the possibility of sowing before the soil reaches a temperature when massive hatching occurs should be considered, so that the plants may establish their root systems before they are invaded by larvae. For *M. naasi* in England the threshold for hatching is about 10°C (Figs. 2a, and 2b), a temperature usually reached in the top 20 cm of soil about the end of April or beginning of May in the southern part of England.

The results of the experiment with consecutive sowings confirm that there is only one generation on barley a year because, when larvae from the first generation hatch in July, the barley roots are senescent and unsuitable for invasion by nematodes. When winter barley is sown in October the soil is usually too cold for development to be completed in the same year. In California also there is only one generation a year on barley (Allen *et al.*, 1970). On perennial ryegrass, a second generation could develop by October. This plant has a main peak of root initiation during March and April and a second one during autumn. Root growth is slow in July but increases greatly in August and September (Williams, 1969), which would favour development of a second generation. In our experiments with ryegrass, plants sown early in July had female nematodes with eggs by mid-September and the eggs contained larvae by mid-October. When sown in late July, the following year, plants had females with eggs by early September but eggs containing larvae were not found till late December.

Temperature affects the rate the nematodes develop in the host roots, and the period for completing the life cycle in the field ranged from 6 weeks in July



to 24 weeks in plants sown at the end of January. Fig. 2c might be thought to suggest that the development accelerates when the temperature rises above  $15^{\circ}\text{C}$ , because below this temperature the larva remained at the second stage for 4 weeks and when the temperature rose above  $15^{\circ}$  the three moults leading to the adult stage took at most 2 weeks. However, Bird (1959) showed that second stage larvae of *M. javanica*, on tomato grown at temperatures fluctuating evenly between  $40.5^{\circ}$  in the daytime and  $11^{\circ}$  at night, feed and grow for 14 days, and then undergo three moults to become adult in 5 days. Siddiqui & Taylor (1970) also found that on wheat seedlings grown at  $26^{\circ}$  during the day and  $20^{\circ}$  at night *M. naasi* second-stage larvae feed and grow for 15-18 days, then undergo three moults and become adult in 3-6 days. The feeding period of the second stage larva of both species was three or four times longer than the non-feeding moulting stage at constant temperatures. This invalidates the suggestion made above that  $15^{\circ}$  is the critical temperature below which moulting does not occur; length of feeding period of the 2nd stage larvae is also important. Our experiment at controlled temperatures illustrated the effect of temperature on rate of development but this may be partly indirect through the plant. The production of giant cells by the plant, necessary for the nematode's feeding, may be slowed by cold. Experiments made by Jones (1950) with four species of *Heterodera* showed that, in this genus too, the rate of development of the nematodes is closely related to soil temperature and that the number of generations possible in a year depends on the length of the vegetative period of the host plant.

The amount of energy in the form of heat, measured as day-degrees above  $12^{\circ}$  in the soil, required to complete the life cycle is more constant through the year than is the length of the life cycle. For example, ryegrass on which the nematodes took the longest time to develop (112 days) received approximately the same number of day-degrees above  $10^{\circ}$  as that on which the nematodes developed in 29 days (Fig. 5).

Tyler (1933) measured the heat requirements of an unidentified species of *Meloidogyne* cultured on tomato both in soil and culture solution. The unit was one degree above  $10^{\circ}\text{C}$  acting for 1 hour and the normal range was 7,000-8,500 from invasion to egg-laying. In "day-degrees" this would be approximately 300-354. This is a bigger value than that obtained for *M. naasi* (230), but the requirement no doubt differs for different species.

We thank the Department of Agriculture of the University of Reading for permission to take soil samples from one of their farms, and Mr. R. J. Silver of the Agricultural Botany Department for supplying soil temperatures.

#### ZUSAMMENFASSUNG

##### *Veränderungen und Entwicklung der Populationen von Meloidogyne naasi im Freiland*

Zur Ermittlung der Anzahl Eier und Larven von *M. naasi* im Boden wurden Bodenproben untersucht. Ein Teil hatte eine Kältebehandlung zur Schlüpfaktivierung der Eilarven durchgemacht, die dann mit den freien Larven zusammen gewonnen werden konnten. Die meisten Larven ergaben



im Herbst entnommene Proben, die zuerst für eine Woche bei 0°C und dann für eine Woche bei 20° aufbewahrt wurden. Nach einer Lagerung bei 30° wurden viel weniger Larven gewonnen als bei einer Lagerung bei 15°, 20° oder 25°. Eine Untersuchung der jahreszeitlichen Schwankungen der Larvenzahlen im Boden zeigte eine Spitze Anfang Mai mit einer raschen Abnahme bei Anwesenheit einer Wirtspflanze und einen langsamen zweiten Anstieg im August bis zu einer Spitze im Oktober. Bei Fehlen einer geeigneten Wirtspflanze blieben die Zahlen den Juni und Juli hindurch hoch und nahmen dann allmählich ab. Proben, die eine Woche lang bei 20° gehalten wurden, ergaben immer mehr Larven als unbehandelte, besonders im April und im Oktober nach dem Anbau einer Wirtspflanze. Die Entwicklung von Larven in Gerste und Raygras entsprach dem Verhalten anderer Arten dieser Gattung. Einer langen Periode der Nahrungsaufnahme bei der I II folgt eine kurze Häutungsperiode ohne Nahrung, an die sich dann Nahrungsaufnahme und schnelles Wachstum der erwachsenen Weibchen anschließen. Im Freiland hängt die Entwicklung vom Eindringen der Larven in die Wirtswurzeln bis zur Eiproduktion der Weibchen von der Bodentemperatur ab. Im Juni und Juli war die Entwicklungszeit am kürzesten, aber die Anzahl der Tagesgrade oberhalb eines angenommenen Minimums von 10° oder 12° war das ganze Jahr hindurch ähnlich.

## REFERENCES

- ALLEN, M. W., HART, W. H. & BAGHOTT, K. (1970). Crop rotation controls barley root-knot nematode at Tulalake. *Calif Agric.* **24**, 4-5.
- BARKER, K. R., NUSBAUM, C. J. & NELSON, L. A. (1969a). Seasonal population dynamics of selected plant-parasitic nematodes as measured by three extraction procedures. *J. Nematol.* **1**, 232-239.
- , — & — (1969b). Effects of storage temperature and extraction procedure on recovery of plant-parasitic nematodes from field soils. *J. Nematol.* 240-247.
- BIRD, A. F. (1959). Development of the root-knot nematodes *Meloidogyne javanica* (Treub) and *Meloidogyne hapla* Chitwood in tomato. *Nematologica* **4**, 31-42.
- DICKSON, D. W. & STRUBLE, F. BEN (1965). A sieving-staining technique for extraction of egg masses of *Meloidogyne incognita* from soil. *Phytopathology* **55**, 497 [Abstract].
- GUIRAN, DE, G. (1966). Infestation actuelle et infestation potentielle du sol par les nématodes phytoparasites du genre *Meloidogyne*. *C.r. hebdomadaire Séances Acad. Sci. Paris, Sér. D.* **262** (16), 1754-1756.
- JONES, F. G. W. (1950). Observations on the beet eelworm and other cyst-forming species of *Heterodera*. *Ann. appl. Biol.* **37**, 407-440.
- MOTSINGER, R. E. (1964). Root-knot nematode population measurements in soil for use in a nematode advisory service. Ph.D. Thesis, Auburn Univ.
- SIDDIQUI, I. A. & TAYLOR, D. P. (1970). The biology of *Meloidogyne naasi*. *Nematologica* **16**, 133-143.
- STEIN, W. (1969). Auftreten und Vertikalverteilung infektionsfähiger Stadien von *Meloidogyne hapla* Chitwood im Jahresablauf. *Z. PflKrankh.* **76**, 263-269.
- SZCZYGIEL, A. (1966). Studies on the fauna and population dynamics of nematodes occurring on strawberry plantations. *Ekol. pol.*, serie A, **14** (34), 651-709.
- TYLER, J. (1933). Development of the root-knot nematode as affected by temperature. *Hilgardia*, **7**, 391-415.
- VAN GUNDY, S. D., BIRD, A. F. & WALLACE, H. R. (1967). Aging and starvation in larvae of *Meloidogyne javanica* and *Tylenchulus semipenetrans*. *Phytopathology*, **57**, 559-571.
- WALLACE, H. R. (1968). The influence of soil moisture on survival and hatch of *Meloidogyne javanica*. *Nematologica* **14**, 231-242.
- WATSON, T. R. & LOWNSBERY, B. F. (1970). Factors influencing the hatching of *Meloidogyne naasi*, and a comparison with *M. hapla*. *Phytopathology*, **60**, 457-460.
- WHITEHEAD, A. G. & HEMMING, J. R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Ann. appl. Biol.* **55**, 25-38.
- WILLIAMS, T. E. (1969). Root activity of perennial grass swards. In: W. J. WHITTINGTON (Ed.), *Root Growth*, Proc. 15th Easter School in Agricultural Science, Univ. Nottingham, 1968. London: Butterworths, 270-279.

Accepted for publication: July 1, 1971