Population fluctuations, life cycle of root-knot nematode, *Meloidogyne ardenensis* in Cupar, Scotland, and the effect of temperature on its development

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Summary

The life cycle of *Meloidogyne ardenensis* Santos was studied on a hedge of *Lonicera nitida* in eastern Scotland and only one generation per year was observed. Females in the roots of the *Lonicera*, some with egg masses, survived through the winter and produced large numbers of eggs during the spring and early summer. Laboratory and glasshouse experiments showed that *M. ardenensis* was well adapted to cold conditions, females and eggs surviving —5° for one week, but was not adapted for warm conditions, larvae failing to develop to maturity when maintained at 20°. Different temperature optima were detected for the different stages in the life cycle. Females actively produced eggs when the temperature was between 7° and 13°, but little invasion of the roots occurred below 10°. Observations in the field and pots indicated the optimum temperature range for the development of the larvae in the roots was 15° to 18°.

Résumé

Evolution des populations et cycle biologique de Meloidogyne ardenensis *à Cupar, Ecosse et influence de la température sur son développement*

Le cycle biologique de *Meloidogyne ardenensis* Santos a été étudié, dans l'est de l'Ecosse, sur *Lonicera nitida* et on n'a observé qu'une seule génération par an. Les femelles contenues dans les racines de *Lonicera*, avec quelques masses d'œufs, survivent à l'hiver et produisent un grand nombre d'œufs pendant le printemps et le début de l'été. Des expériences au laboratoire et en serre ont montré que *M. ardenensis* était bien adapté au climat froid, les femelles et les œufs survivant à une exposition d'une semaine à —5°, mais que cette espèce n'était pas adaptée à la chaleur, les larves maintenues à 20° ne se développant pas jusqu'à maturité. Des températures optimales différentes ont été détectées pour les différents stades du cycle. Les œufs sont produits activement par les femelles entre 7° et 13° mais l'invasion des racines est très faible au-dessous de 10°. La température optimale pour le développement des larves dans les racines se situe entre 15° et 18°.

Meloidogyne ardenensis Santos, 1968 was first described from *Vinca minor* and other woodland plants in England. In 1968 it was found by Dr. P. Thomas on *Lonicera nitida* Wils in a farm garden near Cupar in Fife, Scotland (Franklin, 1978), and a range of plants were tested as hosts by Thomas and Brown (1981). *M. ardenensis* has also been described from Germany (Sturhan, 1976), but appears to be absent from more southern parts of Europe. From its known distribution it seems likely that *M. ardenensis* is adapted to cool, northern European conditions but its life cycle and environmental requirements have not been studied.

This paper reports a study of the life cycle of *M. ardenensis*, at Cupar, Fife, and of the effect of temperature on its development.

Materials and methods

The hedge of *Lonicera nitida* on which *M. ardenensis* was first discovered in 1968 had been removed, but another long established *L. nitida* hedge, about 200 m from the first, was also found to be infested. This hedge was 22 m long and five root and soil samples, one from each 4 m length of hedge, were collected each month starting in November 1981. The roots were washed, weighed and cut into small

pieces. Viable eggs were separated from their gelatinous egg masses by placing the roots in a 0.5% sodium hypochlorite solution (Hussey & Barker, 1973) for 4 mn after which the roots were agitated. The numbers of eggs released were then estimated using a Doncaster (1962) counting dish.

Nematodes in the soil were extracted by wet sieving, as described by Boag (1974), and collected on a 53 μm (300 mesh) sieve. The sievings, collected in a 150 ml beaker, were partly freed of soil particles before counting by adding three drops of 0.1 Separan (AP 273 Premier polymer) and stirring vigorously ; after 30-60 seconds the soil particles had settled on the bottom and the clear suspension was poured through a 25 μm (500 mesh) sieve to collect the nematodes. Each month the numbers of nematodes in five root samples and twenty soil samples were determined. The soil temperature at 10 cm depth was also recorded at each sampling.

The life cycle of *M. ardenensis* was also followed in 10 cm pots filled with infested soil and planted with a rooted cutting of *L. nitida* in December 1980. The pots were plunged in the soil adjacent to the hedge. Each month two pots were removed, the roots of the *L. nitida* washed free of soil and examined and dissected under a binocular microscope to determine the numbers of *M. ardenensis* present and their stage of development.

The effect of low temperatures on the hatch of groups of 100 eggs and of different storage temperatures for 30 or 60 days on the viability of groups of 1 000 eggs of *M. ardenensis* was determined in laboratory and glasshouse experiments. The effect of temperature on the rate of development was tested using plants growing in temperature controlled water baths. There were four replicates of each treatment.

Results

Population changes in the field

Eggs of *M. ardenensis* were present at each sampling, though they were comparatively few in number during the winter of 1980/81. In the spring of 1981 the numbers of eggs recovered from the roots increased dramatically as the females, which had overwintered in the roots, produced large numbers of new eggs. The numbers of eggs recovered gradually decreased during the summer. During the winter few of the eggs recovered were embryonated and few free larvae were obtained from the roots. During April the numbers of embryonated eggs and larvae recovered increased greatly, and then, as with total numbers of eggs, tended to decline during the summer. The numbers of second stage larvae tended to increase during September, October and November (Fig. 1).

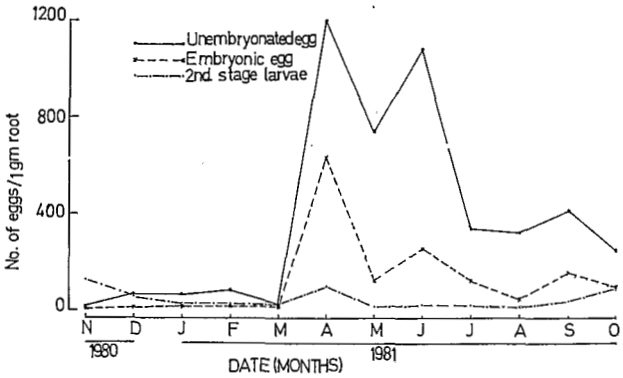

Fig. 1. Numbers of eggs and second stage larvae of *Meloidogyne ardenensis* obtained from roots of *Lonicera nitida* sampled at monthly intervals.

The numbers of larvae in the soil (Tab. 1) was small during the winter, increased during April and May, decreased slightly in June, and then remained high during July to October.

Dissection of the roots indicated that little invasion or development occurred during the winter when the soil temperature was less than 7°. However, all stages of development of *M. ardenensis*, from mature females to partially developed second stage larvae, were present in the roots during the winter. Many underdeveloped second stage larvae were first found in June (soil temperature 13°) and these developed into adults in late August and September. Some eggs were laid before November, and some new second stage larvae were found, but these mainly remained within the gelatinous matrix of the egg-mass.

Table 1

Numbers of second stage larvae of *M. ardenensis* recovered per 200 g soil during 1980-81

Month	*No. of larvae*	*Month*	*No. of larvae*
1980 November	7 ab	May	38 bc
December	3 a	June	24 bc
1981 January	9 ab	July	25 bc
February	4 a	August	47 c
March	6 a	September	51 c
April	29 bc	October	47 c

Values followed by the same letter are not significantly different at (P = 0.05) according to Duncan's multiple range test.

Table 2
Soil temperatures and development of *Meloidogyne ardenensis* in pots growing *Lonicera nitida*

Date of sampling	*Soil temp.*	*Root infection / plant*			*Mean nematode volume (1000 µm³)* **
		larvae	*female*	*egg-masses*	
17/ 2/81	4° (2.1) *	0	0	0	0
26/ 3/81	3.5° (4.3)	0	0	0	0
25/ 4/81	7° (7.1)	0	0	0	0
4/ 6/81	13° (13.8)	3	0	0	77
1/ 7/81	4.5° (14.9)	6	0	0	85
4/ 8/81	17.5° (15.8)	6	18	0	498
1/ 9/81	22° (17.7)	0	20	2	660
1/10/81	11.5° (12.8)	0	38	25	922
23/11/81	5° (5)	0	25	17	944

* Soil temperature between brackets are the monthly mean at 10 cm depth at SCRI.
** The volume of freshly hatched larvae is 23 (1000 µm³). The volume was calculated assuming the nematodes were cone shaped.

Population changes in pots

This life cycle, in which there appears to be one generation per year, was confirmed from the pot-grown plants, in which possible confusion between overlapping generations was avoided (Tab. 2). No larvae were detected in the roots until June and these had developed into females by August. The first egg masses were found in early September, but the proportion of females with egg masses had greatly increased by October.

Studies in pots at constant temperatures in water baths (Tab. 3) showed that tomato roots were not invaded by *M. ardenensis* at or under 10°. At 14° it took approximately 90 days for females with egg-masses to develop and at 18° development from egg took 60 days. At 20° roots were invaded by *M. ardenensis* and in plants examined 30 days after inoculation slightly developed larvae were found. However, in plants examined 50 and 60 days after inoculation no larvae were detected.

The effect of temperature on the hatching of eggs, separated from the egg mass, was tested in water by exposing them to temperatures between 4° and 20° for seven days. The numbers of larvae hatching were found to be similar at all temperatures between 4° and 20° (Tab. 3). In another study (Tab. 3) egg masses, in water, exposed to -5° for seven days still contained viable eggs. When these egg masses were transferred to water at 15° a few larvae hatched over the following seven days and, although hatching appeared to have been decreased, 43% of the unhatched eggs were found to be viable when tested with New Blue R stain.

Table 3
Effect of temperature on the development time (egg to egg) of *Meloidogyne ardenensis* on tomato and hatching of eggs in water. Results are means of four replicates

Temperature	*Development duration (days)*	*% egg-hatch*
4	—	13.9 a
8	—	14.3 a
10	none	13.8 a
14	90	—
15	—	13.4 a
18	60	—
20	0	11.6 a
25	none	10.8 b

Values followed by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test.

Eggs freed from their egg mass and added to soil survived and were able to infect tomato roots after 60 days at 10°. At 15° eggs remained viable for at least 30 days. However, tomato was not infected when it was grown in soil which had previously been stored at 4° for 30 days (Tab. 4).

Table 4

Effect of storage of infested soil for three durations (0, 30 or 60 days) at six temperatures on the numbers of galls formed by *Meloidogyne ardenensis* on tomatoes at 18 °C. Mean of 4 replicates

Temperature °C	*Duration (days)*		
	0	*30*	*60*
4	17	0	0
10	17	8	4
15	17	5	0
20	17	0	0
25	17	0	0
30	17	0	0

Discussion

Our results indicate that in Scotland *M. ardenensis* has only one generation per year. Unlike *M. naasi* Franklin, 1965, the only other *Meloidogyne* spp. found in Scotland (R.M. Stewart, pers. comm.) there was no obvious dormant period or chilling requirement (Franklin, Clark & Course, 1971). In pots, on tomato, *M. ardenensis* completed its life cycle in 60 days at 18° and development from female to female was continuous.

Our experiments showed that *M. ardenensis* is well adapted to Scottish conditions and unsuited to warmer conditions, failing to develop successfully at 20°. Females of *M. ardenensis* inside roots were the main overwintering stage. In experiments these females withstood -5° for seven fays and in the field most females survived to the spring when they commenced laying eggs. A few eggs were laid in the autumn and larvae hatching from them also overwintered in the egg masses although the numbers surviving intially decreased. *M. hapla*, also found in temperate regions, overwinters mainly as egg masses and as second stage larvae and, in Canada, Sayer (1964) and Steplan (1980) found similar reductions in viability during the first part of the winter.

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Although *M. ardenensis* is adapted to surviving Scottish winters (in 1979, an exceptionally cold winter, soil temperatures at 10 cm at Invergowrie did not fall below — 4°) it has a relatively slow rate of development. Eggs hatched well at 4° but little invasion of plant roots occurred until the soil temperature was 13° (Tab. 3). As a result adult females did not develop before September and these laid few eggs before the onset of winter. This slow rate of development probably confines *M. ardenensis* in Scotland to perennial plants and ensures its absence as a pest in areas where arable agriculture is practised.

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