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Scottish Crop Research Institute, Invergowrie,
Dundee DD2 5DA, U.K.

THE INFLUENCE OF TEMPERATURE AND STORAGE TIME ON EGGS OF FOUR SPECIES OF *MELOIDOGYNE*

by
Z. A. STEPHAN

Temperature is the most important climatic factor determining the geographical distribution of many species of root-knot nematodes (*Meloidogyne* spp.). Survival of the eggs of *Meloidogyne* may be reduced in cold (<5°C), or warm soils (>35°C) (Taylor and Sasser, 1978). As part of a study of the biology of *M. ardenensis* Santos, a root-knot nematode found in Scotland, the effect of temperature on egg viability and hatching was studied. For comparison eggs of *M. hapla* Chitwood, a species also found in temperate regions, and of *M. incognita* (Kofoid *et* White) Chitw., and *M. javanica* (Treub) Chitw., two species from warmer parts of the world, were tested. Daulton and Nusbaum (1961) observed that the optimum temperature for egg-hatch of *M. javanica* varied for different populations, and therefore I used several populations of each species in the experiments.

Materials and Methods

Eggs of the four species of *Meloidogyne* were separated from their gelatinous egg-masses by agitating the roots of infected tomato plants in 0.5% sodium hypochlorite solution (chloros) (Hussey and Barker, 1973) for 4 minutes and collecting the eggs released on a 25 μm mesh sieve. The eggs were rinsed with water for 8 minutes to remove any residual chloros. The species and populations tested were: *M. ardenensis*, a single population from Scotland; *M. javanica*, two populations from Iraq and Jordan; *M. incognita*, Race 1 and 2 from USA and Nigeria respectively; *M. hapla*, populations from Canada and England, and two from USA, one with 17 and one with 45 chromosomes.

The first experiment measured the rate of egg-hatch of each of the populations at nine temperatures from 5-30°C. This was done by placing approximately 100 eggs per replicate in syracuse dishes in incubators. The larvae that hatched were counted and removed every 24 hours for a period of 7 days.

The second experiment tested the effect of storage for 30 or 60 days at different temperatures (1-30°C) on the viability of eggs of *M. ardenensis* and of the four populations of *M. hapla*. Pots (5 cm diameter) were filled with 100 g sterile loam soil, watered to field capacity and approximately 100 eggs added. The pots were placed in plastic bags, sealed to prevent loss of moisture and stored in incubators. At the end of the storage period the pots were placed in water baths at 18°C for the *M. ardenensis* or 20°C for the *M. hapla* and planted with tomato cv. Rutgers seedlings. After 45 days the roots of the tomato plants were assayed for galls.

The third experiment tested the effects of low (−5 or −22.5°C) and high (37°C) temperatures for 1, 3 or 7 days on the viability of egg-masses of *M. ardenensis*, *M. javanica* and the four populations of *M. hapla*. This was done by treating groups of four egg-masses in water in syracuse watch glasses. After treatment the watch glasses were maintained at 18°C for *M. ardenensis*, 22.5°C for *M. hapla* or 25°C for *M. javanica* and the number of hatched larvae determined after 14 days. Untreated egg-masses at the same temperatures served as controls.

In all three experiments there were four replicates per treatment. All results were subjected to an analysis of variance and significant differences among means were determined by Duncan's multiple range test.

Results

The percentage of the eggs hatching over 7 days for the different populations of *Meloidogyne* is shown in Figs. 1, 2, 3 and 4. Overall the eggs of *M. ardenensis* hatched less readily than those of the other species but the proportion hatching was similar for temperatures between 5 and 22.5°C (Fig. 1). In contrast eggs of *M. javanica*, *M. incognita* and *M. hapla* hatched most readily at temperatures between

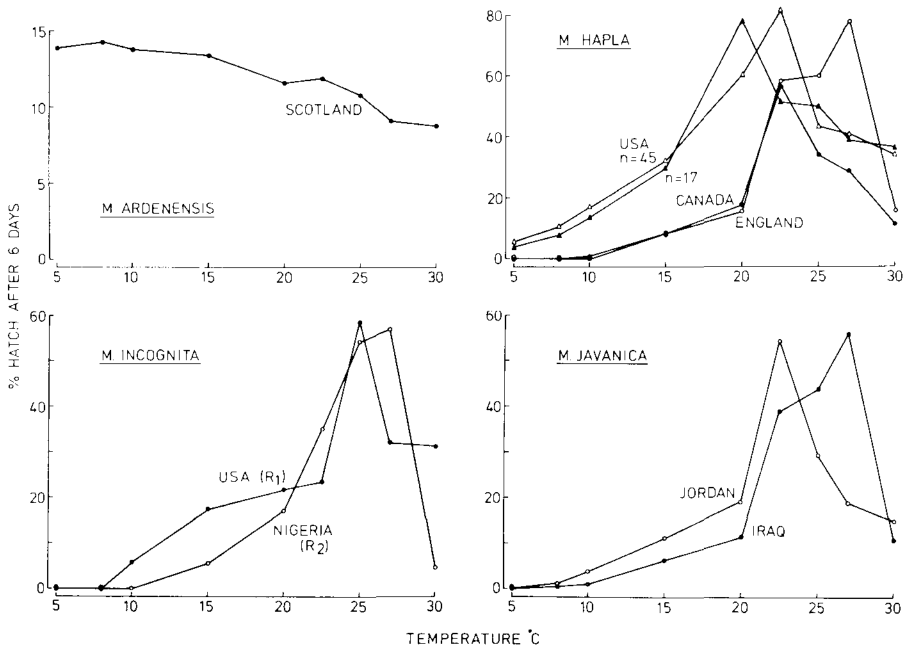

Fig. 1 - Effect of temperature on the egg hatch of four species of *Meloidogyne*.

20 and 27°C. The Jordan population of *M. javanica* appeared to be adapted to hatch at slightly lower temperatures than that from Iraq (Fig. 2). Similarly eggs of Race 1 *M. incognita* hatched more readily at 10-20°C and less readily at 27°C than did eggs of Race 2 (Fig 3). No eggs of *M. javanica* or *M. incognita* hatched at 5°C. Eggs of the two populations of *M. hapla* from North Carolina hatched at all temperatures between 5 and 30°C but eggs of the Canadian and English populations did not hatch at 10°C or less (Fig. 4). The percentage of eggs of *M. hapla, M. javanica* and *M. incognita* hatching ranged from 55 to 82%, whilst in *M. ardenensis* did not exceed 14.3%.

In the experiment which examined the effect of storage for 30 or 60 days at temperatures ranging from 1-30°C *M. hapla* subsequently produced many more galls on the roots of tomato than did *M. ardenensis* irrespective of the treatment applied (Table I). Galls were formed by *M. hapla* in all except those treatments where eggs were stored for 60 days at 25°C and 30 or 60 days at 30°C. However, storage for 30 or 60 days decreased the numbers of galls formed, the effect increasing as the storage temperature was decreased below 20°C.

Table I - *Effect of storage of infested soil for three durations (0, 30 or 60 days) at eight temperatures on the numbers of galls formed by* Meloidogyne ardenensis *at 18°C, and* M. hapla *at 21°C on tomatoes. Mean of four replicates.*

Temperature °C	Duration (days)					
	M. ardenensis			*M. hapla* (1)		
	0	30	60	0	30	60
1	17	30	60	74	24	9
4	17	0	0	74	31	15
10	17	8	4	74	39	20
15	17	5	0	74	47	22
20	17	0	0	74	57	33
22.5	17	0	0	74	58	34
25	17	0	0	74	20	0
30	17	0	0	74	0	0

(1) Mean gall number of 4 population of *M. hapla*.

M. ardenensis only survived and formed galls on the tomato roots in soils stored at 10 and 15°C for 30 days and 10°C for 60 days.

In the third experiment no eggs hatched after treatment at −22.5°C. At −5 or 37°C the numbers of larvae hatching after exposure for one day was significantly greater than those hatching after exposure for 3 or 7 days. Eggs of all three species survived storage at −5°C and +37°C, but the viability of the eggs was greatly reduced. *M. javanica* survived −5°C least readily and +37°C most readily and, as in the previous experiments, the eggs of *M. ardenensis* hatched less readily than those of the other species even in the untreated control. The four populations of *M. hapla* survived +37°C more readily than −5°C, there being little difference between the population (Table II and III).

Discussion

Stephan and Trudgill (1982) observed that *M. ardenensis* was adapted to temperate conditions, and this study has shown that eggs and egg-masses of *M. ardenensis* were more tolerant to low temperatures than those of *M. incognita* and *M. javanica*. Only a small pro-

Table II - *Effect of sub-zero temperature (−5°C) at three exposure times (1, 3 or 7 days) on the egg-hatch of* Meloidogyne *spp. Means of 4 replicates.*

Species	% hatch after —5° (Days)		
	1	3	7
M. ardenensis	8.5 a (1)	3.9 b	3.0 b
M. hapla (England)	58.1 a	15.5 b	3.1 c
M. hapla (Canada)	30.9 a	13.8 b	2.2 c
M. hapla (n = 17)	63.0 a	7.7 b	1.0 c
M. hapla (n = 45)	38.7 a	12.3 b	1.3 c
M. javanica	4.1 a	0 b	0 b

(1) Data followed by same letter in rows are not significantly different (P = 0.05) according to Duncan's multiple-range test. All data were significantly different from control.

Table III - *Effect of high temperature (37°C) at three exposure times (1, 3 or 7 days) on the egg-hatch of* Meloidogyne *spp. Mean of 4 replicates.*

Species	% hatch after 37° (Days)		
	1	3	7
M. ardenensis	10.0 a (1)	0 b	0 b
M. hapla (England)	53.6 a	29.2 b	13.8 c
M. hapla (Canada)	29.9 a	14.0 b	3.5 c
M. hapla (n = 17)	63.3 a	28.5 b	2.7 c
M. hapla (n = 45)	30.2 a	12.3 b	3.1 c
M. javanica	84.9 a	71.1 ba	25.3 c

(1) Data followed by same letter in rows are not significantly different (P = 0.05) according to Duncan's multiple-range test. All data were significantly different from control.

portion of *M. ardenensis* eggs survived more than 24 hours at 37°C, whereas more eggs survived for 60 days at 10°C. There was a similar rate of hatching between 5 and 22.5°C but it was significantly greater than that in temperatures between 25 and 30°C.

The results also indicate that, depending upon their origin, populations within the same species may have different temperature optima for egg-hatch, and differ in their tolerance of high or low temperatures. Thus, eggs of *M. hapla* populations from Canada and England freed from their egg-masses failed to hatch at temperatures below 10°C, but their eggs were more tolerant of sub-zero temperatures than were those of two USA populations. Unexpectedly, eggs in egg-masses of the four populations of *M. hapla* were more tolerant of a short exposure, to +37°C than to −5°C. However, in the field egg-masses of *M. hapla* successfully survived in the USA and Canada (Nusbaum, 1962; Sayer, 1963) but eggs freed from their egg-masses did not (Stephan, 1980).

The results showed that the proportion of eggs of *M. ardenensis* hatching was small compared with that of other *Meloidogyne* species but a preliminary experiment indicated that tomato root exudates did not stimulate egg hatching. In contrast eggs of *M. hapla, M. javanica* and *M. incognita acrita* were stimulated in the presence of tomato roots (Viglierchio and Lownsbery, 1960). Thus eggs of *M. ardenensis* are adapted to survive temperate conditions but the reasons for their relatively low rate of hatch are unknown.

SUMMARY

The effect of temperature and duration of storage on the viability and hatching of eggs of a population of *Meloidogyne ardenensis* from Scotland was examined *in vitro*. For comparison eggs of four populations of *M. hapla* and of two populations each of *M. incognita* and *M. javanica* were also tested. In comparison to the other species only a small proportion of the eggs of *M. ardenensis* hatched over the duration of the tests but the rate of hatching was almost unchanged for temperatures between 5 and 22.5°C. Over-all eggs of *M. ardenensis* were more tolerant of cold and less tolerant of hot conditions than those of the other species. The eggs of the other species had relatively high temperature optima for hatching which differed both for the species and the populations within a species. Egg-masses of the four populations of *M. hapla* were more tolerant of a short expossure to +37°C than to −5°C.

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