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Influence of Inoculum Density, Host, and Low-temperature Period on Delayed Hatch of *Meloidogyne javanica* Eggs[1]

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Abstract: Most eggs of *M. javanica* hatch within several days when incubated in water. Those that do not are said to show delayed hatching. Several experiments were conducted to determine the effect of specific conditions on the percentage of eggs with delayed hatch. Six initial inoculum densities ranging from 100 to 20,000 eggs per pot did not influence egg hatch within a 45-day incubation period. In a 60-day test, the percentage of eggs hatching after more than 20 days was low for egg masses removed from carrot and okra and high for those from pepper and bean. Increasing exposure to cold temperature (8 C) from 7 to 30 days tended to delay hatch.

Key words: diapause, *Meloidogyne javanica,* nematode, quiescence, survival, temperature.

In previous studies (1,5), eggs of *Meloidogyne arenaria* and *M. incognita* hatched within several days when incubated in water. Of the unhatched eggs, only about 10–25% hatched after 25 days incubation, even when stimulated by temperature shock and root diffusates. These delayed hatching eggs were considered to be in "diapause," a latent period of the life cycle that occurred even under favorable conditions for the nematode (1). The percentage of eggs in diapause within egg masses depended on female age and host plant and could be increased by low-temperature treatment (4 C)(2,3). On the other hand, the number of *M. incognita* eggs that survived the winter was negatively correlated with population density found in final fall harvest, and some of the eggs in the fall entered dormancy, which lasted throughout the winter (6). The eggs that survived in winter may be those in diapause and quiescence only. Therefore, the objective of the present work was to evaluate the influence of inoculum density, different hosts, and different periods at low temperature (8 C) on egg hatch of *M. javanica.*

Materials and Methods

M. javanica populations were maintained on susceptible tomato plants for about 45 days under greenhouse conditions.

Inoculum density: Plastic pots with 1 liter of autoclaved soil (50% clayish yellow-red latosol, 25% sand, and 25% cow manure) were infested with 100, 500, 1,000, 5,000, 10,000, and 20,000 eggs per pot. Three 8-day-old tomato seedlings were transplanted in each pot. Forty days after soil infestation, the tomato roots were washed and the largest egg masses were selected for the hatching test. Twenty egg masses per replicate were placed in a nylon and cotton cloth supported by a plastic screen in a Baermann funnel (11.5 cm in diameter and 12.0 cm from top to base). The water reservoir in the funnel was increased twice each day to compensate for evaporation. The funnels were drained and second-stage juveniles counted at 2- to 4-day intervals until 90 days. There were six replicates per inoculation level.

Host: Four plants of carrot (*Daucus carota,* cv. Brasília, 8 days old), bean (*Phaseolus vulgaris,* cv. Rio Negro, 1 day old), pepper (*Capsicum annuum,* cv. Yolo Wonder, 16 days old), and okra (*Hibicus esculentus,* cv. Seleção Piranema, 8 days old) were planted in plastic pots with 2 liters of the same soil infested with 8,000 eggs of *M. javanica.* Sixty days after transplanting, 11 egg masses removed from the roots were tested for hatching as before. There were four replicates in the test.

Low-temperature period: Twenty egg masses from 3-month-old tomato roots were placed in each glass vial (2-cm diameter × 4-cm height) with 10 ml of water.

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These vials were placed in a container covered with a black plastic bag and kept within an incubator at 8 C for 7, 15, and 30 days. After the cold treatment, these egg masses were incubated in the funnels as described in the previous tests for egg hatch. In another similar test, 30 egg masses were removed from 45-day-old tomato roots and treated for 7, 14, and 28 days at 8 C in the dark. There were five replicates plus the control without cold treatment in the above two tests.

Counts of juveniles collected from Baermann funnels were used to estimate egg hatch. Eggs hatching before 20 days were considered to be normal, and those hatching between 20–90 days were considered to be delayed. The percentage of eggs exhibiting delayed hatch was calculated as the number of hatched juveniles after 20 days divided by the total number of hatched juveniles multiplied by 100.

RESULT

Inoculum density: Most of the eggs hatched within the first 20 days in the Baermann funnels. The mean percentage of eggs that hatched between 20 and 90 days of incubation ranged from 0.27 to 1.44% and did not differ among treatments.

Host: The number of juveniles that hatched from 11 egg masses during the first 20 days of the incubation period differed among hosts (Table 1). The number of hatched eggs was low in egg masses removed from bean, high from those grown on okra, and intermediate for those formed on carrot and pepper. The numbers of juveniles that hatched after 20 days were not different among the treatments. The percentage of eggs with delayed hatch was low in the eggs from carrot and okra and high in those from pepper and bean.

Low-temperature period: Within the first 20 days, the number of hatched eggs was inversely related to the time of exposure to 8 C (Table 2). Between 20 and 90 days, the number of eggs hatching with no exposure to 8 C was greater than that of cold-treated eggs in the first test, but not in the second test. There was a trend towards an increase in the percentage of eggs showing delayed hatch with increasing exposure to cold temperature in the two tests.

TABLE 1. Numbers of juveniles hatched from 11 egg masses before and after 20 days in the Baermann funnel. The egg masses were removed from four different host plant roots 60 days after inoculation of *Meloidogyne javanica*.

Treatment	Before 20 days	20–90 days	% of delayed hatch
Bean	284 c†	56 a	14.5 a
Pepper	683 b	117 a	16.0 a
Carrot	743 b	43 a	5.0 b
Okra	1,124 a	89 a	7.0 b

† Numbers in the same columns followed by the same letters show no difference by the Duncan test ($P = 0.05$).

DISCUSSION

In studies of arrested egg development, diapause is classified as being induced by endogenous factors and quiescence by unfavorable environmental factors (4). However, it is not easy to define a microenvironment favorable for the hatching of all eggs. Eggs in the interior of the egg mass may be quiescent, due to possibly lower oxygen levels than those reaching more external eggs. In the temperature experiment, eggs hatched continuously from the first day in funnels until the 19th, indicating that there was not a clear-cut separation of the two forms of dormant eggs. In the inoculum density test, however, all but a few eggs hatched within the first 20 days.

De Guiran (3) reported that high percentages of egg diapause were associated, to some degree, with plant resistance. This finding may partly explain the high percentage of delayed hatch of eggs removed from pepper and bean. Both hosts have a lower susceptibility to *M. javanica* as compared to okra and carrot (7). Also, bean is a plant with a short growing cycle of about 60 days. With an incubation period of 60 days, its cycle was almost complete when the experiment terminated. It is logical to

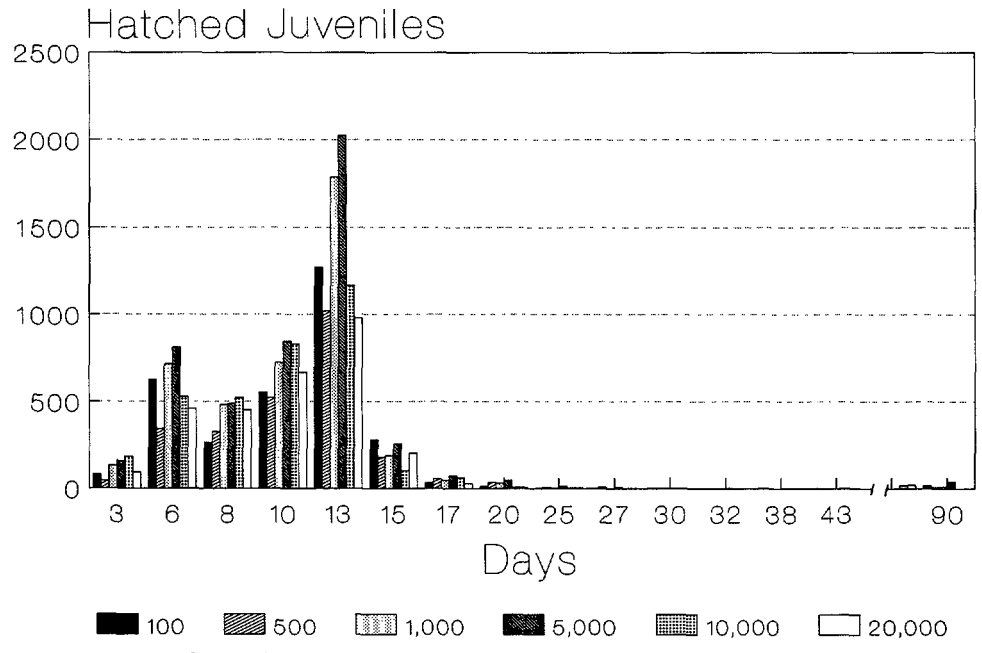

FIG. 1. Numbers of second-stage juveniles of *Meloidogyne javanica* hatched during 90 days in Baermann funnels from 20 eggs masses that were removed from tomato plants inoculated with six levels of eggs after 40 days incubation.

hypothesize that the bean plants close to the end of their growing period may release some substances inside the root tissue that impede normal reproduction of females, resulting in a high percentage of eggs unlikely to hatch during the winter. Similarly, the fact that the percentage of eggs with delayed hatch was higher in the first temperature test than in the second one may be attributed to different plant ages (3-month-old vs. 45-day-old). The explanation is in accordance with De Guiran's (2) report that older females produced more eggs in diapause. Possibly there are more older females in older plants.

Temperatures of 8 C for 30 days killed about 85% of the estimated number of eggs that would have hatched before 20 days, and 50-60% of the eggs that hatched only between 20-90 days. The results suggest that *M. javanica* eggs with delayed hatch are more resistant to cold temperatures than normal ones. It is also possible that *M. javanica* eggs may shift to quiescence and (or) diapause with the cold treatment.

TABLE 2. Numbers of hatched juveniles before and after 20 days in the Baermann funnels.

Treatment (days)	Before 20 days	20–90 days	% of delayed hatch
First test†			
0	1,768 a††	432 a	20.0 b
7	1,669 a	108 b	6.0 c
15	460 b	72 b	13.0 bc
30	241 c	173 b	44.0 a
Second test			
0	6,075 a	86 a	1.55 b
7	2,136 b	60 a	2.87 b
14	1,696 b	53 a	3.18 ab
28	987 c	43 a	6.46 a

† Twenty and 30 egg masses were respectively removed from 90-and 45-day-old tomato plants in the first and second tests, and treated for four periods at 8 C.

†† Numbers in the same columns followed by the same letters showed no difference by the Duncan test ($P = 0.05$).

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