Effects of Temperature and Root Leachates on Embryogenic Development and Hatching of *Meloidogyne chitwoodi* and *M. hapla*[1]

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Abstract: At 20 C the duration of the embryogenic development of *Meloidogyne chitwoodi* and *M. hapla* was about 20 days. At 10 C the embryogenic development was 82–84 days for *M. chitwoodi* and 95–97 days for *M. hapla.* The effect of distilled water and root leachates of potato cv. Russet Burbank, tomato cv. Columbian, and wheat cv. Hyslop on the hatching of eggs of the two root-knot nematode species was investigated at 4, 7, 10, 15, 20, and 25 C (± 1 C). Cumulative egg hatch was no greater in root leachates than in distilled water, but temperature did significantly affect egg hatch ($P = 0.05$). Less than 1% of the eggs of both nematode species hatched at 4 C. The percent cumulative hatch at 10 C was significantly less ($P = 0.05$) than at higher temperatures for both nematodes and significantly more ($P = 0.05$) *M. chitwoodi* eggs hatched than did *M. hapla* eggs. At 15 C the percent cumulative hatch of both species was significantly lower ($P = 0.05$) than that at 20 and 25 C. The percent cumulative egg hatch of two species did not differ at 25 C, but was higher ($P = 0.05$) at 25 C than at 20 C. At 7 C the emergence of *M. chitwoodi* juveniles was about seven times ($P = 0.01$) greater than that of *M. hapla* in distilled water. *Key words:* Columbia root-knot nematode, northern root-knot nematode, juvenile, emergence. Journal of Nematology 15(1):123-127. 1983.

The Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden et al.) and the northern root-knot nematode (*M. hapla* Chitwood) are the two most economically important nematodes of this group in the Pacific Northwest (1,3,4). Recent studies (2) have shown that *M. chitwoodi* is able to reproduce at lower soil temperatures than *M. hapla* and that *M. chitwoodi* can invade potato (*Solanum tuberosum* L.) roots at 10 C (2). However, there is a lack of information on the effect of temperatures and root leachates on the egg hatch of these two species. A study was conducted to determine the duration of embryogenic development and the effect of six temperatures and three different plant root leachates on the emergence of *M. chitwoodi* and *M. hapla* juveniles.

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MATERIALS AND METHODS

A Washington State *M. chitwoodi* population from tomato (*Lycopersicon esculentum Mill.*) cv. Columbian and a Utah *M. hapla* population from lettuce (*Lactuca sativa* L.) were used in our experiments. Both nematode populations were reared on tomato cv. Columbian plants in a greenhouse to obtain inocula for experimentation.

Embryogenesis: Five single-cell eggs of each nematode species were enclosed in cavity slides filled with distilled water. The slides were placed in Petri dishes, lined with moist filter paper to retard evaporation, and stored in a growth chamber at 10 or 20 C (± 1 C). Phases of embryogenic development were recorded on a daily basis. There were four replicate dishes of each nematode at each temperature.

Effect of temperature on hatch: Ten egg masses (about 2,500–3,000 eggs) of each nematode species were removed from tomato roots, placed on a 200-mesh (75 μm) microsieve (2 cm d) and enclosed in a Petri dish. The Petri dish was partially filled with distilled water or a root leachate of either potato cv. Russet Burbank, tomato cv. Columbian, or wheat (*Triticum aestivum* L. em Thell) cv. Hyslop sufficiently to cover the egg masses. Root leachates were obtained from 2-month-old container grown plants by drenching the soil with 2 liters of distilled water and collecting the leachates that occurred during a 24-hour period. The leached root diffusates were centrifugated at 935 × *g* for 8 min to remove particulate matter and the supernatant liquid and stored at 4 C during the experiment. Dishes with eggs in distilled water and root leachates were maintained at 4, 7, 10, 15, 20, and 25 C (± 1 C), and each treatment was replicated four times. At 7 C egg masses were maintained only in distilled water. Distilled water and root leachates were changed daily, and counts of emerged juveniles were obtained daily until cessation of hatching, except those at 7 C which were terminated after 30 days. At the end of the experiment, all egg masses were ground in a glass tube and unhatched eggs were counted. Numbers of hatched eggs expressed as percent of the total initial egg population, were analyzed statistically using a split-plot in time analysis of variance.

RESULTS

Embryogenesis: Embryogenic development of *M. chitwoodi* and *M. hapla* was essentially the same as for other root-knot nematodes (5). Both species lay single cell eggs; *M. chitwoodi* eggs measured 83.0 μm (76–86) × 39.7 μm (38–42) and are significantly ($P = 0.05$) wider than *M. hapla* eggs which measured 81.5 μm (76–) × 37.7 μm (34–40) ($n = 16$). Egg segmentation through the six-cell stage required about the same time in both species: 77–100 hours and 75–97 hours at 10 C and 35–48 hours and 36–40 hours at 20 C for *M. chitwoodi* and *M. hapla*, respectively. Embryogenic development from egg deposition until the emergence of second-stage juveniles was similar for both species: 20–21 days for *M. chitwoodi* and 21–22 days for *M. hapla* at 20 C. At 10 C, however, embryogenic development of *M. hapla* was longer than that of *M. chiwoodi:* 95–97 days and 82–84 days, respectively.

Effect of temperature on hatch: The total number of eggs hatching for either nematode at 4 C was always less than 1% of the initial number of eggs. This was less ($P = 0.05$) than at other temperatures (Fig. 1A–H). Hatching of *M. chitwoodi* eggs occurred over 20, 20, 35, and 20 days in distilled water, potato, tomato, and wheat leachates, respectively (Fig. 1A–D). Hatching of *M. hapla* eggs occurred over 30, 15, 15, and 10 days in distilled water, potato, tomato, and wheat leachates (Fig. 1E–H).

The emergence of juveniles of both species extended for 60 and 70 days at 10 C and was longer than at other temperatures (Fig. 1A–H). The percent of cumulative hatch of each nematode species was significantly ($P = 0.05$) less at 10 C than at higher temperatures (Fig. 1A–H).

Between 60 and 70, 20 and 65, 25 and 65, and 30 and 65 days in distilled water, potato, tomato, and wheat leachates, respectively, the cumulative hatch of *M. chitwoodi* eggs was higher ($P = 0.05$) than that of *M. hapla* eggs. Between 30 and 70, 50 and 65, and 50 and 70 days in distilled water, potato, and wheat leachates, respectively, the percent cumulative hatch of *M.*

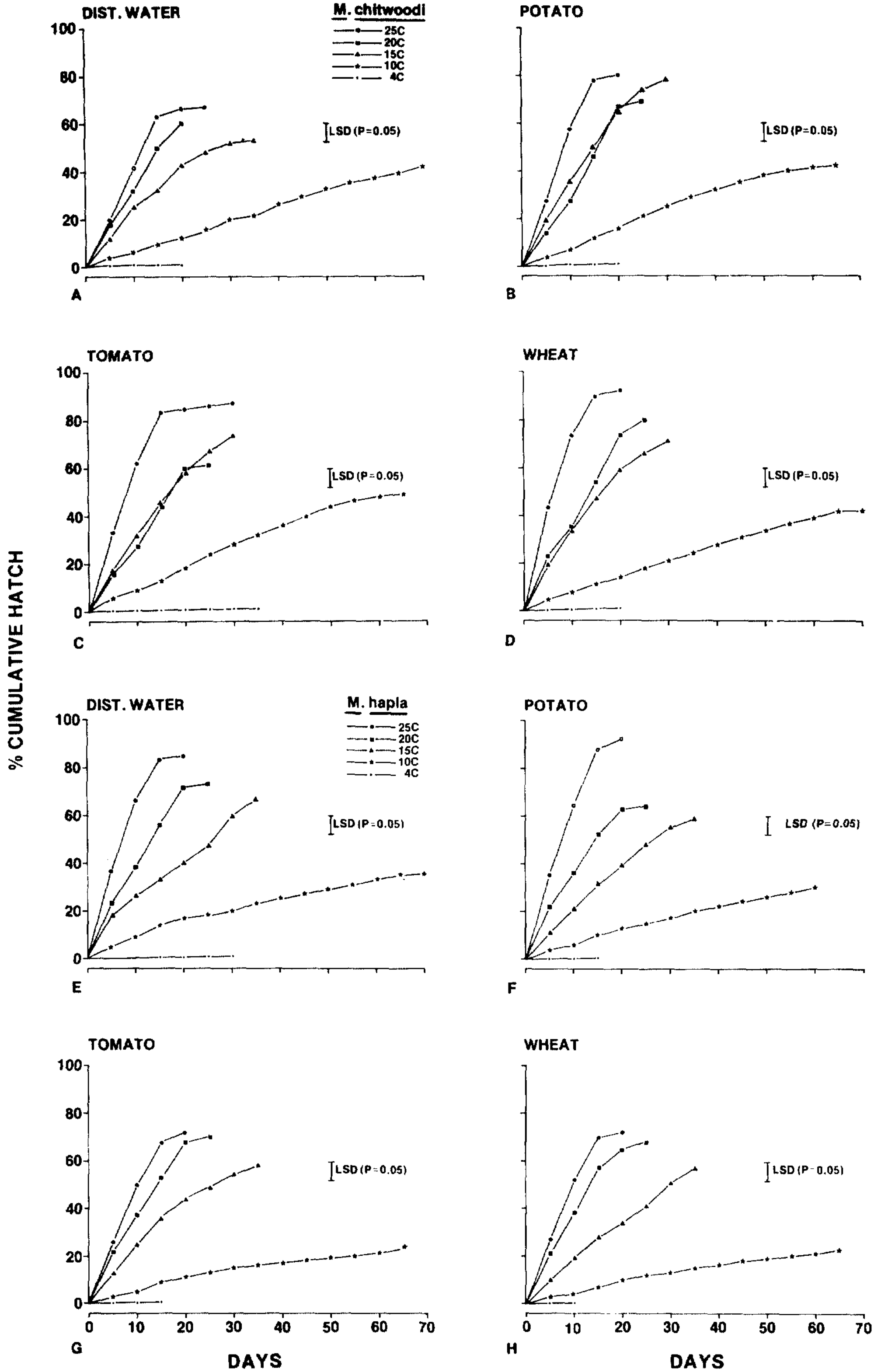

Fig. 1. Influence of temperature on the percent of cumulative egg hatch of *Meloidogyne chitwoodi* and *M. hapla* in distilled water, potato, tomato, and wheat root leachates. A–D) *M. chitwoodi*. E–H) *M. hapla*.

chitwoodi eggs was less ($P = 0.05$) than in tomato leachate. Between 50 and 70 days and 50 and 65 days, the percent cumulative hatch of *M. hapla* eggs in distilled water and potato leachate, respectively, was higher ($P = 0.05$) than in tomato and wheat leachates.

The duration of egg hatch of *M. chitwoodi* and *M. hapla* at 15 C was 30–35 days, about half that at 10 C. Cumulative hatch of eggs of the two species at 15 C was lower ($P = 0.05$) than at 25 and 20 C, except for *M. chitwoodi* which was not significantly different in potato and tomato leachates (Fig. 1A–D).

In distilled water at 15 C, cumulative hatch of *M. hapla* eggs was greater ($P = 0.05$) than that of *M. chitwoodi* eggs between 30 and 35 days. However, it was less ($P = 0.05$) than that of *M. chitwoodi* eggs in the leachates (Fig. 1A–H). Hatch of *M. chitwoodi* eggs in distilled water at 15 C was less ($P = 0.05$) than in leachates, but egg hatch in leachates of the different plants did not differ significantly (Fig. 1A–D). Egg hatch of *M. hapla* at 15 C in distilled water was higher ($P = 0.05$) than in leachates between 30 and 35 days (Fig. 1E–H), but there were no significant differences among the leachates in egg hatch (Fig. 1E–H).

Embryogenic development and egg hatch of *M. chitwoodi* and *M. hapla* occurred during 20 and 25 days at 20 C. The number of *M. chitwoodi* eggs hatching at 20 C was less ($P = 0.05$) than at 25 C in all leachates between 10 and 25 days; there were, however, no significant differences between the number of juveniles emerging in distilled water (Fig. 1A–D).

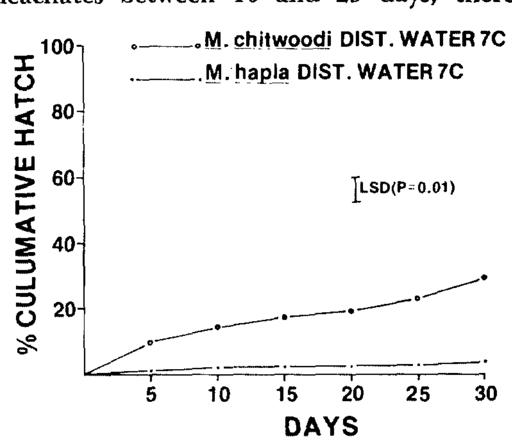

Fig. 2. Influence of temperature on the percent of cumulative egg hatch of *Meloidogyne chitwoodi* and *M. hapla* in distilled water over a 30-day period.

Hatch of *M. hapla* eggs in distilled water and potato leachate was less ($P = 0.05$) at 20 C than at 25 C between 10 and 25 days, but similar in tomato and wheat leachates during the same time period (Fig. 1E–H). Hatch of *M. chitwoodi* eggs in distilled water and tomato leachate was less ($P = 0.05$) than that of *M. hapla* between 15 and 25 days (Fig. 1A–H), but more ($P = 0.05$) *M. chitwoodi* eggs hatched than did *M. hapla* eggs in potato and wheat leachates at 20 C (Fig. 1A–H). Between 20 and 25 days, the number of *M. chitwoodi* eggs hatching in wheat leachate was more ($P = 0.05$) than those in distilled water, potato, and tomato leachates (Fig. 1A–D) at 20 C. Hatch of *M. hapla* juvenile was not significantly different in any of the media used (Fig. 1E–H).

The highest accumulative egg hatch for both nematodes occurred at 25 C (Fig. 1A–H). *M. chitwoodi* egg hatch was higher ($P = 0.05$) in tomato and wheat leachates and less ($P = 0.05$) in distilled water and potato leachate than *M. hapla* between 5 and 25 days (Fig. 1A–H). Between 5 and 25 days, hatch of *M. chitwoodi* eggs in distilled water was less ($P = 0.05$) than in the leachates; the effects of leachates on egg hatch, however, were not different from one another during the same time period (Fig. 1A–D). Hatch of *M. hapla* eggs in distilled water and potato leachate were greater ($P = 0.05$) than in tomato and wheat leachates between 5 and 20 days at 25 C (Fig. 1E–H).

A significantly ($P = 0.01$) greater number of *M. chitwoodi* eggs (30%) hatched than did *M. hapla* eggs (4%) between 5 and 30 days incubation at 7 C (Fig. 2).

DISCUSSION

Potato and tomato are both hosts of *M. chitwoodi* and *M. hapla,* and wheat is host of *M. chitwoodi.* The root leachates of these plants obtained by the method used in this experiment showed no effect on the hatch of *M. chitwoodi* and *M. hapla* eggs; its influence was erratic and changed at different temperatures. Hatch of *M. chitwoodi* eggs in distilled water was less ($P = 0.05$) than in root leachates at 15 and

25 C but was similar to the root leachates at 10 and 20 C. This indicates no persistant and stable action of the leachates on the hatch of *M. chitwoodi* eggs. The effect of the root leachates on the hatch of *M. hapla* eggs was also erratic and inefficacious. More (P = 0.05) *M. hapla* eggs hatched in distilled water than in tomato root leachate at 10, 15, and 25 C, and, in addition, the effect on egg hatch of leachate of tomato, a good host of *M. hapla,* was similar to that of leachate of wheat, not a host of *M. hapla,* at all temperatures.

The hatch of *M. chitwoodi* and *M. hapla* eggs was greatly influenced by temperature. *M. chitwoodi* egg hatch was greater (P = 0.05) than *M. hapla* at 10 and 7 C. At 7 C there were greater (P = 0.05) numbers of *M. chitwoodi* eggs hatching between 25 and 35 days than at 10 C, so both temperatures are relatively favorable for egg hatch of this species (Figs. 1A–D, 2). Hatch of *M. hapla* eggs at 7 C was less (P = 0.05) that at 10 C, so temperatures above 10 C are more favorable for egg hatch of this parasite (Figs. 1E–H, 2). At 10 C the duration of egg hatch of both species was shorter than that of embryogenic development indicating that at the beginning of the experiment the eggs were in an advanced stage of development. Temperatures of 15, 20, and 25 C had similar effects on the hatch of *M. chitwoodi* and *M. hapla* eggs; 25 C was the most favorable temperature for both species.

Under field conditions, infections of *M. chitwoodi* are expected to occur earlier than those of *M. hapla,* since 7 and 10 C are relatively more favorable to egg hatch of *M. chitwoodi* than to *M. hapla* egg hatch. However, because of the increased length of embryogenic development at these low temperatures, *M. chitwoodi* initial population densities will be limited and lower than those that occur at higher temperatures. At temperatures above 10 C, however, root infections of both nematode species are expected to be similar.

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