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Influence of Environmental Factors on the Hatch and Survival of *Meloidogyne incognita*[1]

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Abstract: The influence of soil temperature and moisture on *Meloidogyne incognita* (Kofoid and White) Chitwood was examined in relation to hatching and survival of second-stage juveniles (J2). Nematodes were cultured on cotton (*Gossypium hirsutum* L. cv. Acala SJ2) under field conditions to provide populations similar to those found in the field in late autumn. Egg masses were placed in a temperature range (9–12 C and 21 C), and hatch was measured over a period equivalent to 20 degree days > 10 C (DD10). Hatch occurred below the reported 18 C activity threshold, was restricted below 12 C, and was inhibited below 10 C. Soil moisture influence on hatch was measured by placing egg masses in Hesperia sandy loam and subjecting them to suction pressures ranging from −1.1 bars to −4.5 bars. Suction potentials of less than −2 bars reduced hatch and less than −3 bars inhibited hatch. J2 were placed in sandy loam soil with soil moisture near field capacity, and their motility was measured over a period of 500 DD10. In the absence of a host, more than 90% of J2 became nonmotile over this period.

Key words: cotton, cultural management, *Gossypium hirsutum*, hatch, *Meloidogyne incognita*, soil moisture, soil temperature, survival.

Meloidogyne spp. are soil dwelling aquatic animals that are dependent on external sources of heat and water for their development (10,13,24,25,27,28), hatch (3,4, 10,23,27,29), and movement (19,20). In addition, since they are obligate plant parasites, the infective juveniles have limited stored reserves for survival in the absence of a host (25), and the survival duration is determined by temperature (22,25) and moisture (23).

While much is known about environmental influences on the life cycle of *Meloidogyne* spp., the influence of the specific temperature and moisture conditions of the San Joaquin Valley of California on *Meloidogyne incognita* (Kofoid and White) Chitwood have not been studied in detail. The purpose of this study was to investigate, for field populations of *M. incognita* in the San Joaquin Valley, the influence of temperature and moisture on hatch and on the length of survival of second-stage juveniles (J2) in the absence of a host.

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Materials and Methods

Experiment 1. Influence of temperature on hatch: Meloidogyne incognita was cultured on cotton (*Gossypium hirsutum* L. cv. Acala SJ2) at the USDA Cotton Research Station, Kern County, California, to provide populations of nematodes that had developed under environmental conditions similar to field populations. In this way, experimental animals which more closely resemble overwintering populations could be collected.

Individual clay pots (46 cm d × 31 cm deep) were buried to their rims in a cotton field and filled with methyl-bromide fumigated (2.2 kg/0.6 m^3) Hesperia sandy loam. Cotton was directly seeded into the pots on 2 May 1983 and inoculated on 17 July with 12,000 *M. incognita* J2 per pot. The culture plants were irrigated and fertilized in a similar manner to the field cotton. On 1 October the pots were removed from the ground and stored outdoors in a shaded location.

Cotton roots from the culture plants were soaked in phloxine B (ca. 3 g phloxine B per liter of water) for 10 minutes to highlight *M. incognita* egg masses. Ten egg masses of *M. incognita* attached to root fragments were placed on a 420-μm-pore sieve (40 mesh) and placed in a petri dish (90 × 20 mm). Water at the treatment temper-

ature was added to the level of the screen, and a small hypodermic needle was used to remove all trapped air between the screen and the water surface. Four petri dishes were placed in a plastic sandwich container and into incubators at the treatment temperature.

To examine the inherent variability in hatching capability of *M. incognita* near its developmental threshold of 10 C (24,27), the egg masses were subjected to a narrow range of temperatures: 9.0, 10.0, 10.5, 11.0, 11.5, and 12 C. As a comparison, egg masses were placed at 21 C to evaluate hatch under less extreme conditions. Thermographs were placed in each incubator during the experiment. In addition, the temperature of water-filled flasks was recorded at least twice daily in each of the incubators. The egg masses were left at the respective temperatures for 20 degree days > 10 C (DD10). Each treatment was replicated four times.

A physiological rather than calendar time scale was used to afford equivalent developmental time to the eggs at different temperatures. An equal proportion of hatch of viable eggs was expected to occur at each temperature until the point at which temperature became limiting.

After removal from the incubators, the egg masses in two of the temperature treatments (12 and 9 C) were treated with 0.4 M NaCl to prevent their immediate hatch (10) during the phloxine B soak. This procedure was discontinued after concurrent moisture experiments indicated that after transfer to optimal temperature and moisture conditions, hatch was generally delayed 24 hours.

After 20 DD10, the screens were flushed with water (at the same temperature as the treatment) to remove the J2 that had hatched but not moved through the screen. The J2 in the petri dish were counted with a dissecting microscope. The egg mass matrices were dissociated in a 10% solution of commercial bleach (6), the suspension was passed through a 25-μm-pore sieve (500 mesh) and backwashed into a beaker, and the eggs were counted. Data were expressed as a percentage of hatched J2 plus remaining eggs.

A previous report (26) and exploratory experiments (Goodell, unpubl.) indicated that the hatch rate per DD10 was unchanged over the temperature range from 12 to 21 C. Muscular activity is probably required in the hatch process (9,10,30), however, and Roberts (20) indicated that below 18 C *M. incognita* motility was prevented. To examine whether the ability to hatch at temperatures less than 18 C implied a lower activity temperature (19,20), the following experiment was conducted. Nematodes from the field culture were reared on Acala SJ2 cotton in the greenhouse. Freshly hatched J2 were collected by placing roots into aerated water after discarding those J2 collected in the first 24 hours. An average of 215 J2 were placed in modified Moje vials (18,22) containing water at 26 or 15 C. The Moje apparatus consisted of vials (25-ml capacity) with a 2-cm hole in the cap (18). The holes had a 100-mesh plastic screen and Whatman #2 filter paper cut to fit the top of the vial. The vials were placed in a rack, inverted, and suspended over petri dishes (60 × 15 mm) which had enough water to make contact with the inverted vial (18,22). The reason for using the Moje apparatus instead of a standard Baermann funnel was to increase the resistance to nematode motility. The tissue paper used in a Baermann funnel allows passage of even weakened individuals, whereas the Whatman filters provided a controllable path of resistance.

The entire apparatus was placed in a plastic box. The nematodes were left for 24 hours at 15 ± 0.5 C or 26 ± 1.15 C. The J2 that moved through the screen and filter paper were counted. Each temperature treatment was replicated three times.

Experiment 2. Influence of moisture on hatch: The field-reared population of *M. incognita* was used as in the previous experiment. Roots were collected from cotton plants and stained with phloxine B, and the egg masses were collected. A 5-bar ceramic plate was placed in a 15-bar pressure chamber with enough water to cover it. After 1

hour, approximately 40 g steam-sterilized Hesperia sandy loam soil was placed in polyethylene rings (50 × 10 mm) (8) and excess water was removed. Five egg masses were placed in the soil of each ring, and the pressure equivalent to −1.1 bars was imposed for 3 hours. The pressure was increased to the experimental pressures for 24 hours. Experimental treatments included pressures equivalent to −1.1, −2.0, −3.0, −3.5, −4.0, and −5.0 bars.

After removal from the pressure plate, the soil rings were weighed, placed in a sealed plastic bag, and stored at 20 C for 24 hours (10 DD10). They were weighed again to measure any moisture loss that might have occurred during this period. The soil was removed from the ring, suspended in water, and poured through a 420-μm-pore sieve (40 mesh) to remove the egg masses; the J2 were extracted from the soil by modified sugar flotation (7). The sugar suspension and soil was triple sieved through a 25-μm-pore sieve (500 mesh). The eggs were dissociated from the egg mass using a 10% solution of commercial bleach (6) and collected on a 25-μm-pore sieve (500 mesh). Both eggs and J2 were counted; juvenile counts were expressed as a percentage of hatched J2 plus eggs.

Two controls were maintained throughout this experiment. The first assessed background hatch by placing five egg masses onto a hatching screen suspended over water as described in Experiment 1 and replicating two times. The screens were set up when egg masses were being placed in the soil rings. The hatched J2 were counted after 24 hours and at the conclusion of the experiment. The second control consisted of five egg masses placed in a soil ring as described. It measured any hatch which might have occurred in the soil rings between the time when the pressure was first applied and when the rings were removed for storage. The J2 in these controls were extracted as described.

Experiment 3. Influence of temperature on the survival of J2 in the absence of a host: Meloidogyne incognita were reared on Acala SJ2 cotton plants as in experiments 1 and 2. Roots from the host plant were placed in aerated water for 72 hours. The J2 were collected every 24 hours, but the J2 collected in the first 24 hours were discarded. Approximately 170 J2 were placed in 50 g steam-sterilized Hesperia sandy loam in a sealed plastic bag. These bags were placed in a soil drying can (8 cm d × 5.2 cm deep) in a 29-C incubator. All bags of soil were weighed after infestation, and extra bags were prepared to measure any soil moisture loss (14). Soil moisture was approximately equal to field capacity. Each treatment was replicated eight times. Five additional bags were prepared and infested to establish baseline mortality caused by handling.

Cans were removed at intervals of 100 DD10 up to 500 DD10. Individual bags were weighed, the soil was placed on Baermann funnels for 24 hours, and the J2 recovered were placed on a modified Moje motility test (18,22), replicated four times. The Moje method previously described was modified by using a Whatman #5 filter paper, and the apparatus was placed into a 29-C controlled environment for 24 hours.

Nematode counts were expressed as percentage of baseline recovery (0 DD10). The moisture check was weighed wet and dry, and the moisture percentage was calculated (14). The experiment was repeated three times.

Results

Experiment 1. Influence of temperature on hatch: Hatch rate per DD10 increased exponentially between 9 and 11 C and reached its maximum at 12 C (Fig. 1). The curve took the form of $y = ae^{bx}$ and was linearized by ln y transformation (expressed as cumulative hatch at 12 C). The resulting equation, $\ln y = \ln a + bx$, yielded the coefficients $b = 1.79$ and $a = -21.18$, and provided the equation,

$$y = 6.33 * 10^{-10} * e^{1.79x},$$
$$\text{for } 9.0 \geq x > 12.0,$$
$$(r^2 = 0.94)$$

where y is a proportion of the hatch at 12 C and x is temperature (Fig. 2). There were

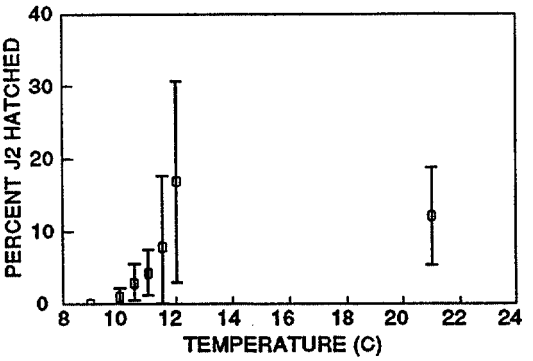

FIG. 1. Percentage of hatch of *Meloidogyne incognita* as affected by temperature. Data represent mean of four replications and a confidence interval of one standard deviation.

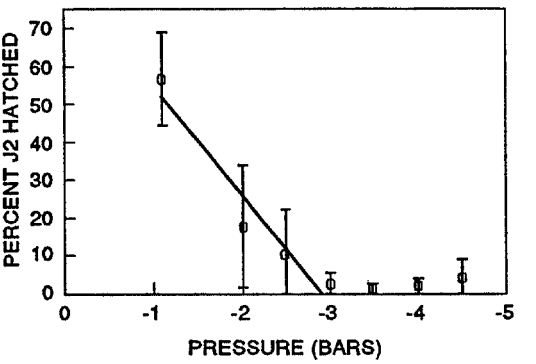

FIG. 3. Percentage of hatch of *Meloidogyne incognita* as influenced by moisture. Data represent mean of five replications with a confidence interval of one standard deviation. See text for regression equation.

no significant differences between 12 C and 21 C treatments.

Motility of J2 at 15 C represented less than 1% of the original population and was significantly different ($P < 0.01$) from the motility of the 26 C treatment.

Experiment 2. The influence of moisture on hatch: Hatch decreased with decreasing moisture (Fig. 3). At −1.1 bars, 57% of the eggs hatched compared to 4.0% at the driest treatment, −4.5 bars. The relationship between hatch percentage and soil moisture was linear between −1.1 and −3.0 bars and was described by the equation,

$$y = 83.89 + 28.94x,$$
$$\text{for } -2.90 \leq x \leq -1.1,$$
$$(r^2 = 0.88)$$

where y is hatch percentage and x is soil suction in bars.

There was no measurable moisture loss from soil samples during the course of the experiment. Results from the hatching controls indicated that no measurable hatch occurred during the first 24 hours when the egg masses were in saturated conditions.

Experiment 3. Influence of temperature on survival of J2 in the absence of a host: In all three experiments, the reduction of motile *M. incognita* J2 was greater than 90% of the original population over the 500 DD10 exposure. Averaging the three experiments provided a linear relationship (Fig. 4) and the equation,

$$y = 100.0 - 0.171x,$$
$$\text{for } 0 \geq x \geq 500,$$
$$(r^2 = 0.94)$$

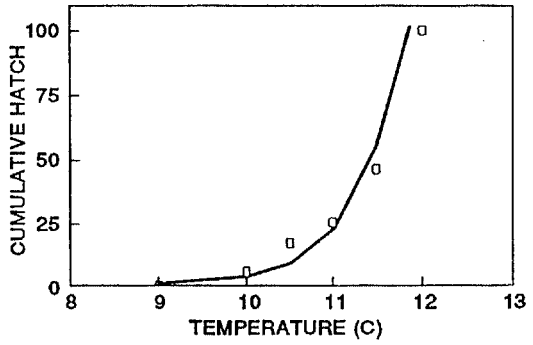

FIG. 2. Cumulative hatch of *Meloidogyne incognita* with increasing temperature. See text for regression equation.

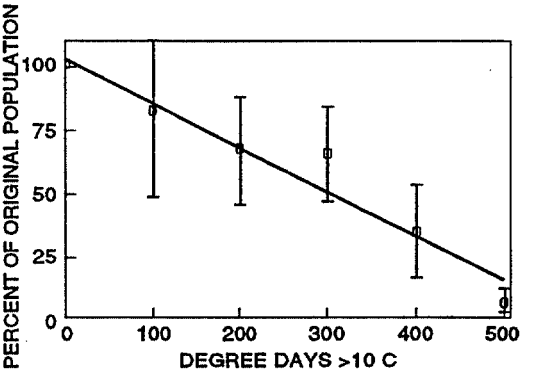

FIG. 4. Motility of *Meloidogyne incognita* juveniles after storage in soil in the absence of a host. Data represent the mean of three experiments with four replications each. Bars represent one standard deviation. See text for regression equation.

where y is the proportion of juveniles that remained motile and x is DD10.

Discussion

The experiments were designed to clarify the role of environmental influence on hatch and survival of *M. incognita*. The hatch studies were conducted within a normal range of temperature and moisture for the development of *Melodiogyne* spp. (13,24,27,28). They were conducted on a physiological time scale to allow equivalent development to occur at all temperatures above the basal threshold of development.

Nematode movement and penetration of host tissue requires that the environmental temperature be above some threshold below which nematode muscular activity is greatly reduced (19,20). The motility response to temperature of this field-cultured population was similar to that in other reports (19,20). The hatching process is a combination of activities including chemical (5), behavioral (9,10,30), and physical (30). Changes in egg shell permeability caused by the hydrolysis of the inner lipid layer (5) take place before hatch (2,28). These changes occur after the first molt, when the juvenile has a fully developed cuticle (5), to increase the flexibility of the egg shell (5,30) and to accommodate the increased juvenile activity before hatch (9,10,30). As the activity of the juvenile increases, the resistance between the nematode and the egg shell also increases, until a point is reached when the friction is greater than the ability of the nematode to overcome it (30). When the nematode comes to rest, the head is pressed against the egg shell. The stylet is actively thrust against the shell creating perforations (9,10,30). The internal force produced by the nematode causes the shell to tear and the nematode to slip through the slit (30).

While movement by the J2 appears to be a requirement for hatch (5,30), these results indicate that hatching does not require a specific temperature threshold (> 9 C). If motility is reduced or prevented below 18 C (20), then a passive component may be involved in the hatch process, which is in contradiction to Wallace (30). No hypothesis is presented to explain this contradiction, but several factors could be involved. First, the internal pressure developed by the J2 before being subjected to experimental temperatures may be sufficient to tear through a weakened egg shell. Also, stylet activity may occur at a temperature below that required for locomotion, resulting in a weakened egg shell and hatch if sufficient internal pressure develops. Finally, genetic diversity within a population may allow some individuals to be active at these lower temperatures.

In contrast, hatch was inhibited over the range of negative pressures used in this study (Fig. 3, Table 2). The negative pressure range was weighted too heavily in the dry region to allow a complete examination of the relationship of moisture to hatch. Results are similar to those for *M. javanica* (Treub) Chitwood (23) in which there was no decrease in egg hatch at low negative pressures (−0.16, −0.3, and −1.1 bars) but hatch was inhibited at extreme pressures (−15 and −92 bars). Baxter and Blake (2) studied free eggs of *M. javanica* to determine the role of suction (both matric and osmotic) on hatch. They found no reduction in the rate of hatch until ca. −4 bars, but hatch decreased asymptotically beyond −4 bars and was inhibited beyond −10 bars.

Wallace (29) hypothesized that egg hatch of *M. javanica* should be greatest near the point on a moisture release curve where the slope is greatest. This is associated with open, but not completely drained, soil pores. The current studies were conducted in a range of moisture levels just beyond the inflection point of the soil moisture curve (Fig. 5), ca. −0.5 bars. These results support Wallace's (29) hypothesis by demonstrating that hatch rapidly decreases beyond the point of inflection on the soil moisture curve.

The actual mechanism for inhibition of hatch in low soil moisture was debated in the literature during the late 1960s without complete resolution (2,28,29). The egg shell was known to be semipermeable after

the first molt (2,4,28), and it was suggested (2) that the egg shell became compressed as a result of free moisture being withdrawn, restricting nematode movement (28). With the constriction of the shell against the J2, perhaps space is inadequate for the J2 to develop sufficient internal force for expulsion.

The restriction of hatch at negative pressures greater than −1.1 bars (and the inhibition of hatch beyond −3.0 bars) has adaptive value, by preventing the juvenile from hatching into an environment not conducive to host germination and development (9,10,30). Under moderately dry conditions, this survival mechanism allows the nematodes to develop but not hatch, thereby preserving the infective units until hosts are present.

Field populations of root-knot nematodes decrease with time in the absence of a host (12,15,17,21). Bergeson (3) found that *M. incognita* infectivity was reduced after in vitro storage at temperatures above 15.68 C for approximately 1 month. Thomason et al. (22) found that the motility of *M. javanica* J2 decreased significantly after 8 days of in vitro storage at 27 C but did not change even after 16 days storage at 15 C. Van Gundy (25) investigated the physiological and biochemical factors involved in the starvation process for *M. incognita* and found rapid decrease in motility, infection, and lipid contents after 8 days of in vitro storage at 27 C.

The results of this study agree with previous reports that under soil moisture conditions adequate for movement, a *Meloidogyne* J2 will gradually lose its ability to find and (or) penetrate a host. The average rate of decline was 0.2% of the original population per degree day above 10 C.

The assumption that energy reserves are depleted at a constant rate relative to physiological time can be questioned when the temperature is above the developmental threshold but below the activity threshold. Is 100 DD10 at 29 C, where muscular activity requires greater energy utilization, the same as 100 DD10 at 15 C, where only maintenance respiration is required? Future studies should be designed to separate the depletion of energy reserves during muscular activity from that of a resting state.

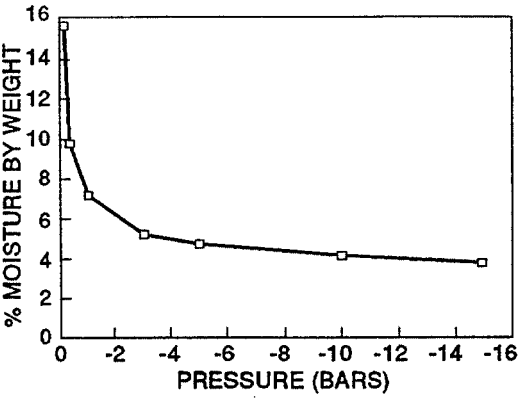

FIG. 5. Soil moisture release curve for Hesperia sandy loam at USDA Cotton Research Station, Kern County, California, averaged for the 0–30-cm depth. Adapted from D. W. Grimes (pers. comm.).

This study has provided quantitative information about the influence of temperature and moisture on the hatch and survival of *M. incognita* in the absence of a host. The results support the concept of "wet fallow" where application of water to cropland in the absence of the host (1,30) could be used to increase *M. incognita* mortality. The data provided the basic parameters for the development of a computer simulation model. Although several *M. incognita* models have been proposed (12,16,17), there are data gaps in describing the direct influence of the environment on the biology of the nematode. Development of a simulation based on environmental factors would provide a more flexible and generalized model. Such a simulation could be used as part of a larger multi-year, multicrop nematode management optimization model (11) or used independently as a tool for further research.

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