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Recovery and Longevity of Egg Masses of *Meloidogyne incognita* during Simulated Winter Survival[1]

J. L. STARR[2]

Abstract: Effects of soil matrix potential on longevity of egg masses of *Meloidogyne incognita* were determined during simulated winter conditions. Egg masses were recovered from isolated root fragments incubated in field soil at matrix potentials of −0.1, −0.3, −1.0, and −4.0 bars throughout winter survival periods of 10 weeks for tomato roots and 12 weeks for cotton roots. Egg masses were more superficial on cotton roots than on tomato roots and were more easily dislodged from cotton roots during recovery of root fragments by elutriation. The rate of decline in numbers of eggs and J2 per egg mass was greater in wet as compared to dry soils ($P = 0.01$), with the relationship between numbers of eggs and J2 per egg mass and time being best described by quadratic models. Percentage hatch of recovered eggs declines linearly with time at soil matrix potentials of −0.1 and −0.3 bars, but at −1.0 and −4.0 bars the percentage hatch of recovered eggs increased before declining. Effects of soil matrix potential on numbers of eggs per egg mass and percentage hatch of recovered eggs were consistent with previous reports that low soil moisture inhibits egg hatch before affecting egg development. Estimations of egg population densities during winter survival periods will be affected by ability to recover infected root fragments from the soil without dislodging associated egg masses. There is a need for procedures for extraction of egg masses not attached to roots from the soil.

Key words: egg mass, matrix potential, *Meloidogyne incognita,* nematode, root knot, soil moisture, winter survival.

Reliable estimates of nematode population densities are critical to the selection of appropriate management strategies if losses due to nematodes are to be minimized and crop productivity maximized (4). This frequently requires that nematode populations be sampled several months prior to actual initiation of the appropriate management tactics (1). In many climates, it is necessary to sample nematode populations at the end of one cropping season or during the early winter months and to estimate winter survival of the population to determine the hazard level for the succeeding crop. Eggs represent a substantial portion of the total population for *Meloidogyne* species from the end of a cropping season through the early winter months (1,14) and play an important role in winter survival (3,14). Ability to estimate population densities of eggs in soil samples is dependent on the recovery of root fragments with adhering egg masses, yet there are few data available that document the longevity of egg masses during the winter months. On several occasions, attempts to monitor winter survival dynamics were hampered by erratic recovery of eggs from infested soil (9). Because temperature and moisture level are known to have major effects on survival of *Meloidogyne* spp. (6,7,18–20), the objectives of this study were to determine the effects of soil matrix potential on egg mass longevity and egg viability of *M. incognita* during in vitro, simulated winter conditions common to Texas. During the simulated winter survival period, soil temperatures were not held constant but were decreased in a systematic manner.

Materials and Methods

Egg masses for these experiments were obtained from greenhouse cultures of a population of *Meloidogyne incognita* (Kofoid & White) Chitwood race 3 originally isolated from cotton in 1982. The nematodes were reared on *Lycopersicon esculentum* Mill. cv. Rutgers or on *Gossypium hirsutum* L. cv. Rowden in a sand-peat soil mix (6:1; v/v). The soil used for simulated winter survival experiments was a loamy sand (91% sand, 2% silt, 7% clay; <1% organic matter; pH

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[2] Department of Plant Pathology and Microbiology, Texas Agricultural Experiment Station, College Station, TX 77843.

7.9) obtained from a field planted to cotton in the previous 2 years. The moisture content of 12-kg lots of air-dried field soil was adjusted gravimetrically, based on its moisture release curve, to −0.1, −0.3, −1.0, and −4.0 bars matrix potential. Immediately after adjusting the matrix potential, the soil was thoroughly mixed by hand and then placed in sealed containers for 1 week at room temperature to equilibrate.

For each experiment, *Meloidogyne*-infected roots of cotton or tomato were collected from 8-week-old greenhouse cultures and washed free of soil. The roots were blotted dry with paper towels and cut into 2- to 4-cm long segments, with each segment containing one or two well-developed light brown egg masses. Ten to 12 infected root fragments were placed in each of several plastic bags containing ca. 500 g soil adjusted to one of the four different matrix potentials. There were 12 replicate bags of soil for each matrix potential treatment. The bags of soil were then placed in an incubator at 25 ± 0.5 C. To simulate fall and winter soil temperatures typical of Texas, the temperature of the incubator was decreased by 5 C every 2 weeks until the minimum temperature of 5 C was reached; this temperature was then maintained for the final 2 weeks in two experiments with infected tomato roots and for the final 4 weeks in a single experiment using cotton as the host.

At the start of each experiment, 10 egg masses from the same population used for that experiment were digested with 1% NaOCl (8) to determine the initial number of eggs per egg mass. Every 2 weeks after the initiation of the experiment, one bag of soil from each matrix potential treatment was removed from the incubator, and the root fragments were extracted on a 250-µm-pore sieve by elutriation (2). Egg mass condition was observed by microscopic examination at each sample time. Eight egg masses per matrix potential treatment were then hand-picked from extracted root fragments and placed individually in 50-ml beakers. At the 12-week sample time for the experiment with cotton, only six egg masses were collected due to reduced recovery of egg masses. Each egg mass was treated with 1% NaOCl (8) to dissolve the matrix, and the number of eggs and second stage juveniles (J2) per egg mass was enumerated. Viability of recovered eggs was measured on termination of the second tomato experiment (10 weeks) and after 4, 8, and 12 weeks in the cotton experiment. To estimate egg viability, eggs from each matrix potential treatment were pooled, and four replicated samples of 100 to 200 eggs each from each treatment were placed in hatching chambers constructed from PVC tubing (13). Hatched J2 were counted every 3 to 4 days for 14 days. Significant treatment effects were identified by analysis of variance using the SAS general linear models procedure (12). Regression models were obtained from the curve fitting function of Cricket Graph version 1.3 (Cricket Software, Malvern, PA).

To determine fecundity of mature nematode females on isolated root fragments, 10 2-cm long root fragments, each bearing a single gall with a uniform size and light brown colored egg mass, were collected as described above and placed separately in 1.8-cm diameter dishes with 2 ml of water. The egg matrix and all visible eggs were carefully removed with forceps, and the samples were incubated at room temperature (24 ± 2 C). Additional eggs produced by these females during the next 14 days were then determined.

Results

Egg masses associated with tomato or cotton root fragments were extracted from soil throughout the length of these experiments, 10 weeks for tomato and 12 weeks for cotton. Greater than 90% of the root fragments in each sample were recovered by elutriation. The egg masses became a darker brown in color with time at all soil matrix potentials. In all cases, the roots deteriorated substantially during the simulated winter survival period. With tomato, the egg masses were partially embedded in

the root tissue and, based on the number of galls that had pores where an egg mass appeared to have been attached, less than 20% of the egg masses were lost from the roots during elutriation. Egg masses were more superficial on cotton roots, and 60% were lost during elutriation after 12 weeks in the soil.

At the beginning of the two experiments with tomato roots, there were means of 494 ± 60 and 635 ± 145 eggs and J2 per egg mass, respectively. The initial mean numbers of eggs and J2 per egg mass in the cotton experiment was 770 ± 190. Juveniles were generally less than 10% of the number of eggs in the egg masses and were not enumerated separately. Soil matrix potential affected the rate of decline of numbers of eggs and J2 per egg mass (P = 0.01) in all three experiments. In general, the rate of decline was greater for wetter soils (−0.1 and −0.3 bars) than for dryer soils (−1.0 and −4.0 bars) (Fig. 1). In each instance, the relation between mean numbers of eggs and J2 per egg mass and time during simulated winter survival was best described by a quadratic model (Table 1). No difference in decline in numbers of eggs and J2 per egg masses between the two hosts was noted, other than percentage recovery of egg masses as described above.

Soil matrix potential affected viability of eggs present in egg masses (P = 0.01). Eggs recovered from tomato roots exhibited a 41% mean hatch in the second experiment after 10 weeks in soil at −4.0 bars as compared to 27% and 32% mean hatch of eggs recovered from soil with matrix potentials of −0.1 and −1.0 bars, respectively. Insufficient eggs were recovered from the −0.3-bar treatment after 10 weeks to test egg hatch. Both time and matrix potential affected (P = 0.01) percentage hatch of eggs recovered from cotton roots. At soil matrix potentials of −0.1 and −0.3 bars, the decrease in percentage hatch was linear with time (Fig. 2). In the drier soils at −1.0 and −4.0 bars, percentage egg hatch increased before declining (Fig. 2), and was best described by quadratic models.

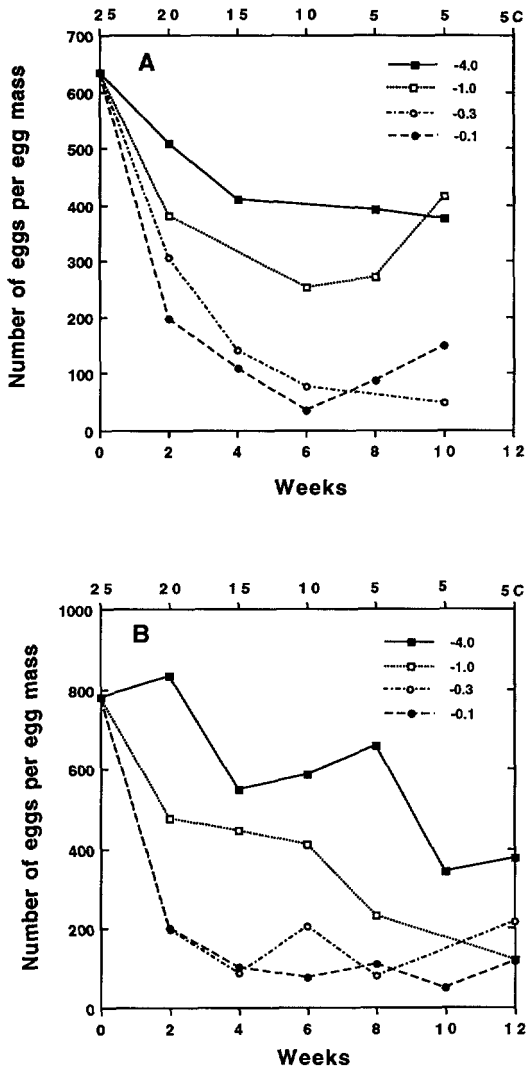

FIG. 1. Effect of soil matrix potential on numbers of eggs and J2 per egg mass for *Meloidogyne incognita* during simulated winter survival. Values are means of 6 to 8 egg masses for each soil matrix potential at each sample time. Soil temperature was initially 25 C and was decreased by 5 C every 2 weeks until the minimum temperature of 5 C was reached. A) Egg masses on tomato roots. B) Egg masses on cotton roots.

During the experiment with cotton (Fig. 1) and in the first experiment with tomato (data not shown) it was observed that the mean numbers of eggs and J2 per egg mass increased between weeks 0 and 2 in the −4.0-bar soil matrix potential treatment. In all other treatments, numbers of eggs and J2 declined during this time. In

TABLE 1. Relationship between mean number of eggs and J2 per egg mass and time for *Meloidogyne incognita* during simulated winter survival at different soil matrix potentials.

Soil matrix potential	Model	R^2
	Tomato	
−0.1 bars	$Y = 585 - 176X + 14X^2$	0.94**
−0.3 bars	$Y = 613 - 155X + 10X^2$	0.98**
−1.0 bars	$Y = 623 - 132X + 11X^2$	0.98**
−4.0 bars	$Y = 628 - 68X + 4X^2$	0.97**
	Cotton	
−0.1 bars	$Y = 661 - 167X + 11X^2$	0.84*
−0.3 bars	$Y = 658 - 164X + 11X^2$	0.78*
−1.0 bars	$Y = 724 - 79X + 2X^2$	0.92**
−4.0 bars	$Y = 802 - 31X + X^2$	0.74*

Data are from the second of two experiments with tomato. Results of the first experiment gave similar trends but differed in numbers of total eggs per egg mass and in absolute values of the quadratic models.

*,** Indicate significance at the $P = 0.05$ and 0.01 levels, respectively.

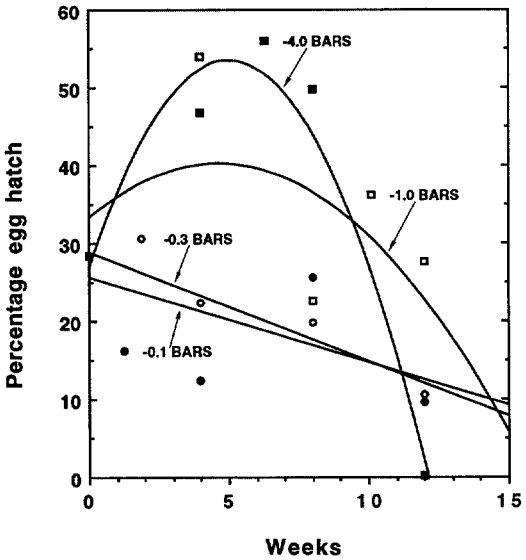

FIG. 2. Effect of time and soil matrix potential on hatch of eggs of *Meloidogyne incognita* recovered from infected cotton roots during simulated winter survival. Values are means of four replicated samples of 100 to 200 eggs each. Soil temperature was initially 25 C and was decreased by 5 C every 2 weeks until the minimum temperature of 5 C was reached. Regression equations where Y equals percentage hatch are: −0.1 bars, $Y = 25.6 - 1.1X$, $R^2 = 0.36$; −0.3 bars, $Y = 28.8 - 1.4X$, $R^2 = 0.96$; −0.1 bars, $Y = 33.1 + -0.3X^2$, $R^2 = 0.28$; −4.0 bars, $Y = 26.6 + 10.7X - 1.1X^2$, $R^2 = 0.95$.

the separate in vitro test, mature females produced a mean of 148 eggs on excised tomato root fragments and a mean of 125 eggs on excised cotton root fragments incubated in water at 24 ± 2 C for 2 weeks.

DISCUSSION

These data support previous work on the role of eggs of *Meloidogyne* species in winter survival (14). That egg masses did not deteriorate rapidly in field soil is further evidence that they are important winter survival structures. Although numerous fungal and bacterial species have been isolated from egg masses (5,15,16) and can probably utilize the egg matrix and eggs as a source of nutrients, egg masses are proposed to have antibiotic activity (10) important for the longevity of the matrix.

Nearly all of the egg masses recovered were attached to root fragments; only rarely were egg masses not associated with root tissue extracted from soil with a 250-μm pore sieve. The physical connection between egg masses and roots and the factors that influence root decay affect the accuracy of estimating densities of eggs in the soil by methods that measure only the egg masses attached to root fragments. Estimates of population densities of eggs are likely to be much lower than actual values when egg masses are easily dislodged from host roots. In cases where population densities of eggs declined rapidly following soil tillage in the fall (Starr, unpubl. data), poor recovery of infected root fragments and increased dislodging of egg masses from roots may have played as major a role in the observed decline as did actual decline in population densities. New methods for extraction of egg masses from soil when not attached to roots are needed to improve estimates of egg population densities.

The effects of soil matrix potential on rate of decline of numbers of eggs per egg mass and on subsequent egg hatch are consistent with previous reports (6,7,19,20) that low soil moisture levels inhibit egg hatch. The more rapid decline in egg numbers in the wetter soils was probably due to egg development and eclosion dur-

ing the first 4 weeks of the simulated winter survival period when soil temperatures were 20 to 25 C. Decline in numbers of eggs per egg mass is slower in drier soil because eggs developed but did not hatch (6,7,19,20). Additionally, even though the egg matrix may have antimicrobial activity (10), it is likely that increased microbial activity in the wetter soils may have contributed also to a more rapid decline in numbers of eggs per egg mass as compared to the drier soils.

The increase in eggs per egg mass in soils at −4.0 bars matrix potential observed at 2 weeks was probably due to additional production of eggs after the root fragments were placed in the soil as well as to inhibition of hatch by dry soil conditions. In the wetter soils, the higher rates of egg hatch relative to the rate of egg production would account for the observed decline in egg per egg mass.

The excellent fit of the rate of decline in mean numbers of eggs and J2 per egg mass over time to the quadratic models, as opposed to simple linear or exponential models, was somewhat surprising and most likely due to random variation in initial numbers of eggs and J2 per egg mass rather than to an actual increase in egg numbers at that time. The soil temperatures during the last 2 or 4 weeks of these experiments were 5 C and were too low for additional egg production by *M. incognita* (5,11,17).

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