Effect of Soil Water Potential on Survival of *Meloidogyne javanica* in Fallow Soil[1]

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Abstract: A natural infestation of *Meloidogyne javanica* in an aggregated Oxisol declined at an exponential rate when aliquots of the soil were stored for 72 days in polyethylene bags at various soil water potentials (ψ). Time periods required for reduction in soil infestations by 50% were 2.7, 4.9, 110, 10, and 2.6 days at ψ of −0.16, −0.30, −1.1, −15, and −92 bars, respectively. In the wetter soils, at ψ of −0.16, −0.30, and −1.1 bars, the predominant stage recovered was the second-stage larva. In the drier soils, at ψ of −15 and −92 bars, both eggs and larvae were recovered with neither stage predominating. Incidence of coiled larvae was inversely related to the ψ value of the soil, a greater incidence occurring in the drier soils. After 15–32 days, percentages of coiled larvae were 13, 27, 55, 65, and 88% in soil at ψ of −0.17, −0.60, −1.9, −15, and −82 bars, respectively. *Key words:* nematode extraction, quiescence, coiling.

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Root-knot nematodes (*Meloidogyne* Goeldi spp.) can survive in fallow field soil for years (8,12,14,16). Studies have shown that survival diminishes more rapidly if the soil is either wetted (15) or dried (2). The reporting of soil moisture content in field studies has been limited to gravimetric percentage, percentage of saturation, or percentages of the moisture equivalent and wilting point. Soil moisture content in terms of water potential (Ψ) has not been reported. To determine the effect of Ψ on survival of *M. javanica* (Treub) Chitwood in soil is the object of this study. The effect of Ψ on the incidence of anhydrobiotic coiling of larvae (5,6,7) will also be described.

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MATERIALS AND METHODS

A naturally infested soil of the Wahiawa series, an aggregated Oxisol, was taken from fields of the former experiment station of the Pineapple Research Institute at Waipio on Oahu. The fields had been under continuous pineapple cultivation for more than 20 years and were heavily infested with *M. javanica*. Other plant parasitic nematodes—

Paratylenchus sp. *Tylenchus* sp., and *Pratylenchus* sp.—were also present.

Soil moisture release characteristics were obtained by using a pressure chamber (Ψ values of −0.1 to −15 bars) and by holding thin layers of soil over sulfuric acid solutions for 1 month (Ψ values less than −15 bars) and calculating Ψ values from the relative humidity (17,18).

Survival in a naturally infested soil: Infested soil was passed through a screen (32-mm openings) to remove root galls and thoroughly mixed. One 20-liter aliquot was sealed in double polyethylene bags; another aliquot was wetted, mixed, and sealed. The remainder of the soil was dried in a layer about 15 cm deep with daily mixing under conditions of low light, a relative humidity of 70%, and a temperature of 23–24 C. Twenty-liter aliquots were removed after 1, 2, and 4 days and sealed in polyethylene bags. Three 50-g aliquots were periodically removed from each bag and evaluated for the presence of *M. javanica*. Data were recorded as mean percentage of the initial level of infestation.

To evaluate the overall level of infestation, a 50-g aliquot was added to 250 g greenhouse soil in which a cucumber seedling was planted, and the root galls counted 25 days later. A log-log transformation of the data converted the time-survival curves to straight lines, from which were calculated the time periods necessary to reduce population densities by 50%.

Survival of larvae was evaluated by passing a 50-g aliquot in a roiled suspension through a screen (246-μm openings) to remove egg masses. Larvae were concentrated on a screen (25-μm openings) and added to soil for a cucumber bioassay. From a second 50-g aliquot, larvae were collected with a centrifugal-flotation technique (13) and those not stained by 0.1% potassium permanganate (11) counted.

Survival of eggs was evaluated by passing a 50-g aliquot in a roiled suspension through a screen (246-μm openings) to collect egg masses. Screenings were added to soil for a cucumber bioassay. From a second 50-g aliquot, eggs were dispersed with 0.5% NaOCl (3), collected by centrifugal-flotation (13) with zinc sulfate (sp gr 1.4) substituted for sucrose (10), and counted.

Data describing survival of eggs and larvae were log-log transformed so that time-survival curves approximated straight lines, the slopes of which were compared by t-test.

Incidence of coiled larvae: A separate lot of infested soil was wetted or dried by the same method described above, from which 20-liter aliquots were removed and sealed in polyethylene bags. From each bag three 50-g samples were periodically removed and sampled for the presence of coiled larvae. A coiled larva is one with its head recurving on itself at least 1.25 times (Fig. 1). Larvae in the soil were fixed by immersing each 50-g aliquot in 400 ml 4% formalin at 40 C (9). The 4% formalin solutions had been shown not to induce

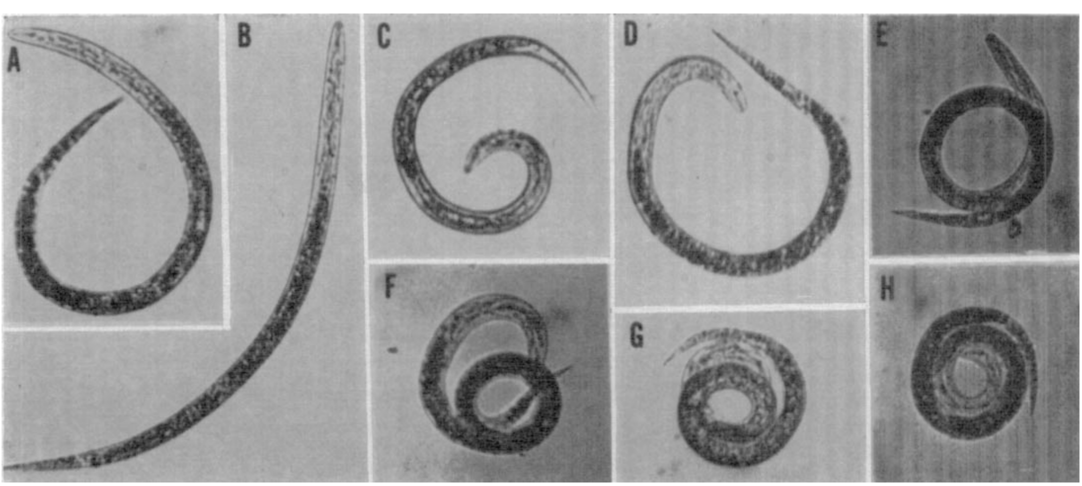

Fig. 1. *Meloidogyne javanica* larvae fixed in 4% formalin before recovery from soil. A, B, D are not coiled. C. E, F, G, H are coiled.

coiling. After 24 h, larvae were extracted by centrifugal-flotation (13) modified to include 4% formalin. At least 35 specimens were observed in each sample, and mean percentages of coiled root-knot nematode larvae in each sample were recorded. After 32 days, the coefficient of linear correlation between Ψ and percentage of coiled larvae was calculated.

RESULTS

Survival in a naturally infested soil: Infestation of soil by *M. javanica* diminished at an exponential rate from an initial level of 370 galls/cucumber seedling (Fig. 2). Log-log transformation of the data resulted in significantly linear relationships between time period and survival ($P \leq 0.05$). Durations in storage necessary for a 50% reduction in the level of infestation were 2.7, 4.9, 110, 10, and 2.6 days at Ψ of −0.16, −0.30, −1.1, −15, and −92 bars, respectively.

Means of 360 eggs and 680 larvae/50 g soil were counted in soil before it was dried or wetted. Inoculation of cucumber bioassays with extracted eggs and larvae produced means of 120 and 41 galls/cucumber plant, respectively. In soil at Ψ of −0.16 bar, survival of eggs descended to a barely detectable level within 45 days; survival of larvae was reduced by about 90% during the same time period (Fig. 3). Survival declined significantly less among larvae than eggs in soils at Ψ of −0.30 ($P \leq 0.05$) and −1.1 bars ($P \leq 0.10$), according to count data (Figs. 4, 5). In the drier soils, at Ψ of −15 and −92 bars, survival of eggs and larvae declined at comparable rates (Figs. 6, 7).

Incidence of coiled larvae: Coiled larvae were recovered from soils at all Ψ values tested. Percentages of coiled larvae increased in all soils during the first 5–10 days, then remained approximately constant. Between 15 and 32 days percentages of coiled larvae were 13, 27, 55, 65, and 88% in soil at Ψ of −0.15, −0.60, −1.9, −15, and −82 bars, respectively (Fig. 8). The coefficient of linear correlation between Ψ and percentage of coiled larvae was r = −0.82, which was not significant ($P \leq 0.05$).

DISCUSSION

Overall survival of *M. javanica* was greatest at Ψ of −1.1 bars, which was slightly

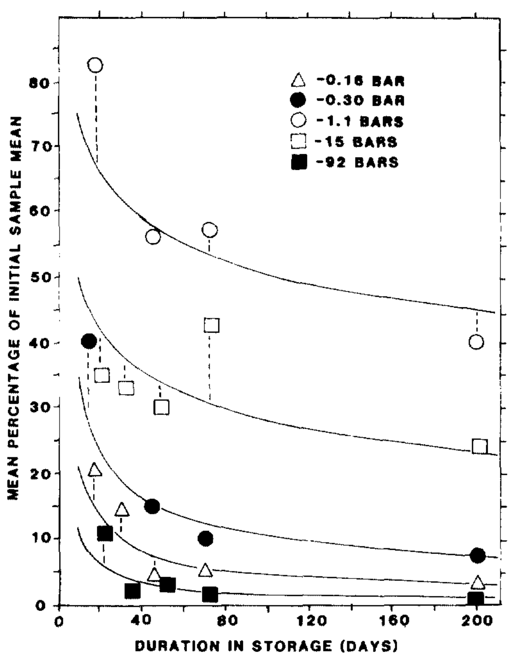

Fig. 2. Survival of *Meloidogyne javanica* in soil stored for various time periods at various ψ values, according to bioassay.

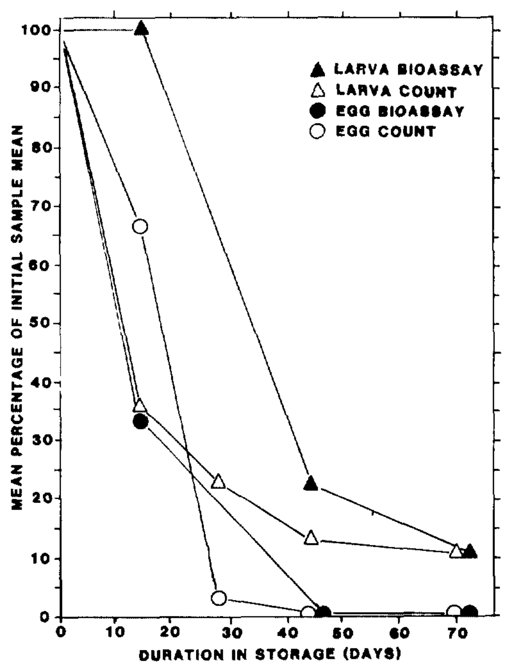

Fig. 3. Survival of eggs and larvae of *Meloidogyne javanica*, evaluated by counting recovered specimens and by bioassay, in soil stored for various time periods at ψ of −0.16 bar.

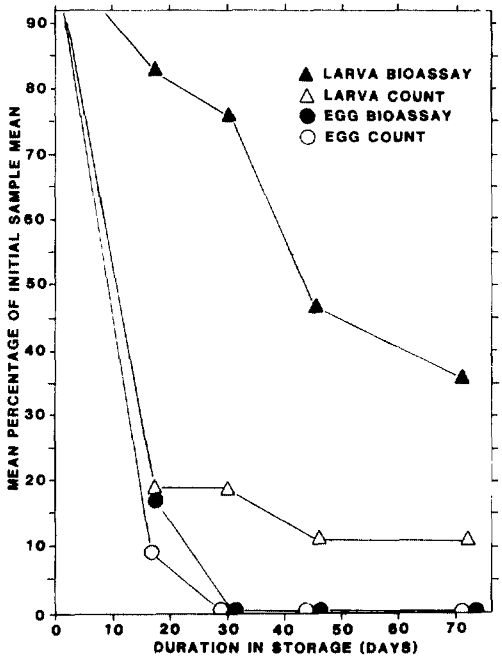

Fig. 4. Survival of eggs and larvae of *Meloidogyne javanica,* evaluated by counting recovered specimens and by bioassay, in soil stored for various time periods at ψ of —0.30 bar.

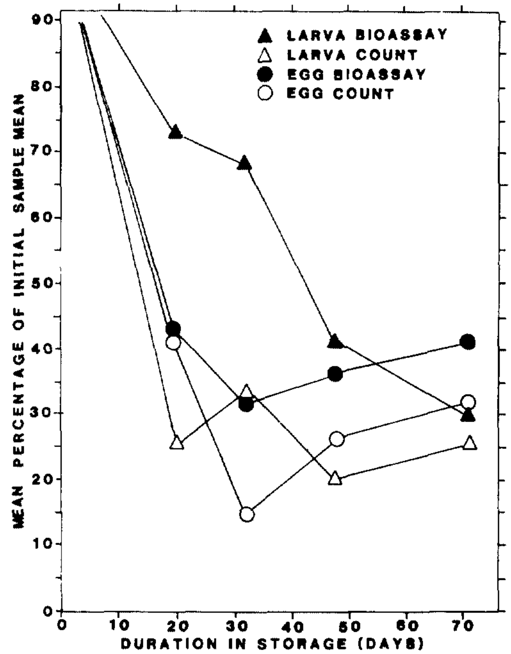

Fig. 6. Survival of eggs and larvae of *Meloidogyne javanica,* evaluated by counting recovered specimens and by bioassay, in soil stored for various time periods at ψ of —15 bars.

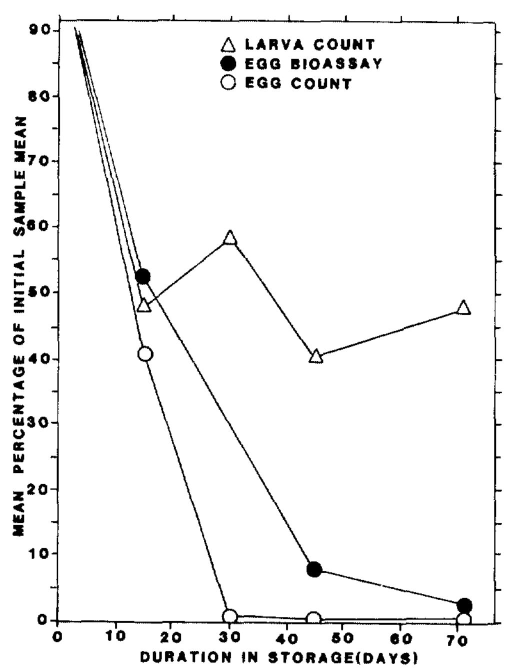

Fig. 5. Survival of eggs and larvae of *Meloidogyne javanica,* evaluated by counting recovered specimens and by bioassay, in soil stored for various time periods at ψ of —1.1 bars.

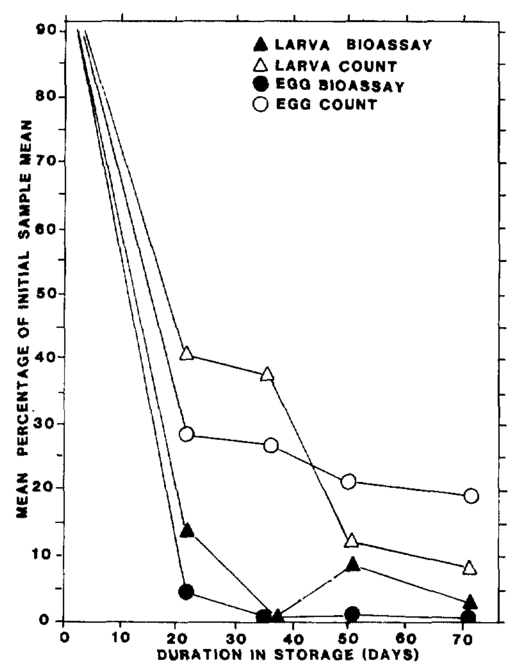

Fig. 7. Survival of eggs and larvae of *Meloidogyne javanica,* evaluated by counting recovered specimens and by bioassay, in soil stored for various time periods at ψ of —92 bars.

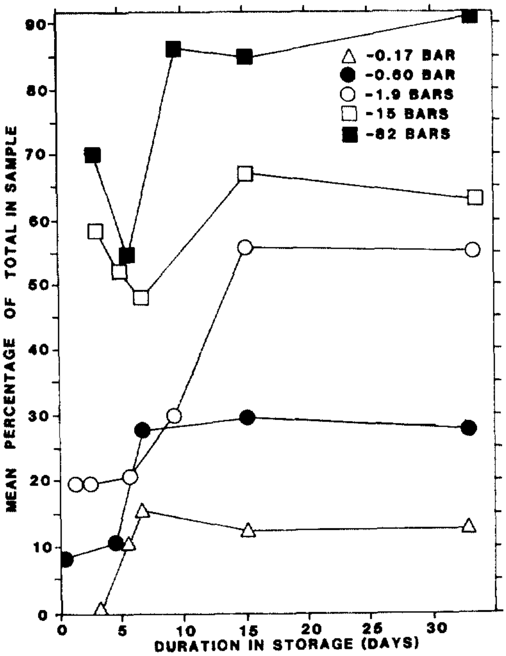

Fig. 8. Percentage of coiled larvae of *Meloidogyne javanica* recovered from soil stored for various time periods at various ψ values.

drier than the soil water potential when the soil mass was first removed from the field, at Ψ of −0.3 bars. Both wetting and drying the soil reduced the level of infestation of *M. javanica*. This and similar research (19) show promise of having practical application in the pineapple industry where it has been shown that by pre-irrigating weekly 1 month before planting, populations of the reniform and the root-knot nematode are significantly reduced. Furthermore, pre-irrigation induces egg hatching of both species. By eliminating the egg stage, the nematodes are more susceptible to nematicidal treatment.

In the drier soils, at Ψ of −15 and −92 bars, desiccation effectively reduced numbers of eggs and larvae. Survival of some eggs was due to inhibition of hatching (1); survival of larvae was due to anhydrobiosis (4). In the wetter soils, at Ψ of −0.16 and −0.30 bars, numbers of eggs declined rapidly because hatching took place; numbers of larvae declined because there was enough soil moisture for motility and depletion of stored energy. In soil at Ψ of −1.1 bars, where overall survival was greatest, the predominant form of survival was the larva. More than 90% of these larvae were found to be coiled at the termination of the experiment (6 months after removal from the field). The desiccating effect of soil at Ψ of −1.1 bars had a negligible influence on the survival of larvae. The number of eggs surviving the same conditions was very low because of hatching.

Percentages of coiled larvae recovered from soil during a period of 1 month after removal from the field were inversely correlated with the Ψ value (Fig. 8). This supports earlier data indicating that coiling is a response to desiccation (5,6,7). It was further observed that percentage recovery of coiled *M. javanica* larvae greatly increased over a period of 1 to 6 months from fallow soil held at Ψ of −0.16 to −15 bars (unpublished). This could be due either to differential survival of coiled nematodes or to subsequent coiling of surviving nematodes. Coiling has been shown necessary for subsequent survival of *Aphelenchus avenae* Bastian in dry air (5,6), but its role in aiding survival of other nematodes has not been established.

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