A Technique for the Collection of Larvae of *Meloidogyne* spp. and a Comparison of Eggs and Larvae as Inocula

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The infectivity of second-stage larvae of *Meloidogyne* spp. is a function of soil temperature, aeration, moisture, texture, and density, as well as a function of the distance the second-stage larvae have to travel prior to root penetration (3, 4). Because eggs may require up to 2 weeks to hatch under greenhouse conditions, infection periods with larvae are normally shorter than those with eggs. Larvae inoculum is easily obtained by placing galled roots with egg masses in a container over a funnel in a mist chamber. However, a large percentage of larvae become noninfective or they die within 12 to 24 h when numbers accumulating in the funnel stem exceed a few thousand.

The object of this investigation was to compare the yield of larvae of *Meloidogyne incognita* (Kofoid and White) Chitwood obtained from egg masses in a mist chamber, and that obtained with a new technique that allows for longer periods of accumulation of larvae without loss of infectivity. The infectivity of the larvae hatched on the sieve was compared to that of larvae hatched from intact egg masses and from eggs released from egg masses by a sodium hypochlorite (NaOCl) solution (2).

Method for collection of infective larvae: Heavily galled roots from three tomato plants (*Lycopersicon esculentum*) 'Manapal' infected with *M. incognita* were washed clean of soil, cut in 2- to 5-cm segments, mixed, and weighed. Half of the root segments were divided into fifths and placed on top of five funnels in a mist chamber at 23 C. The remaining roots were treated to release the eggs from the egg masses by means of a procedure modified from Hussey and Barker (2). The root pieces were stirred for 3 min in 4 liters of a 0.53% NaOCl solution at 23 C. The egg suspension was poured through four nested metallic sieves (20-cm diam) with decreasing pore size from top to bottom: 420 μm (40 mesh), 149 μm (100 mesh), 53 μm (270 mesh), and 26 μm (500 mesh). The eggs were quickly rinsed with tap water and washed down onto the bottom sieve. The number of eggs collected was determined before they were placed on top of a 28-μm plastic sieve in a closed chamber with sufficient water to cover the eggs. The numbers of larvae obtained with both techniques were determined every 24 h and transformed to percentage of the number of eggs placed on the sieve.

Comparison of infectivity of various inocula: Three types of inocula were evaluated: *(i)* intact egg masses, *(ii)* eggs released from egg masses with NaOCl, and *(iii)* larvae hatched from NaOCl-treated eggs on the sieve. Ten egg masses containing 100 to 400 eggs each, or 1,000 eggs released from egg masses by dissolution of the matrix in 0.53% NaOCl solution, or 2,000 second-stage larvae hatched on the sieve in 24 h were mixed with 100 cm^3 of a sterilized loamy sand (texture: 82% sand, 14% silt, 4% clay) in plastic cups. A 2-week-old tomato seedling was transplanted into each cup. A complete range of treatments was placed in an air-conditioned greenhouse at 24 ± 2 C. In addition, tomato seedlings in cups inoculated in the same manner with

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2,000 larvae each were placed in another greenhouse at 30 ± 5 C. All treatments were replicated 3 to 5 times. After 20 days, the roots were washed, stained with acid-fuchsin lactophenol, and examined microscopically for larval penetration. Eggs released from egg masses with NaOCl and placed on the sieve hatched at a low rate for the first 3 days and at an increased rate for the next 4 days (Fig. 1). Unhatched second-stage larvae were very sensitive to NaOCl after the lipoid membrane had been enzymatically digested (1). This fact apparently was responsible for the observed delay in hatching. Maximum larval emergence from egg masses on the roots in the mist chamber occurred after 24 h (Fig. 1). The second-stage larvae accumulated at the bottom of the funnels in high numbers, and 60-80% of them were immobile. The numbers of larvae collected on succeeding days decreased, but the percentage of immobile larvae remained high. Inoculation with these larvae usually resulted in poor infections (2).

Oxygen (O_2) saturation measurements (Oxygen monitor 53, Yellow Spring Instruments, Yellow Spring, Ohio) of water in the funnel stem showed almost complete depletion of O_2 in 3-10 h when 10,000 to 50,000 larvae were placed in a funnel. With fewer larvae/funnel (5,000), the percentage of immobile larvae was relatively low but increased within 24 h, even though O_2 saturation decreased only to 12-6%. Since *Meloidogyne javanica* (Treub) Chitwood

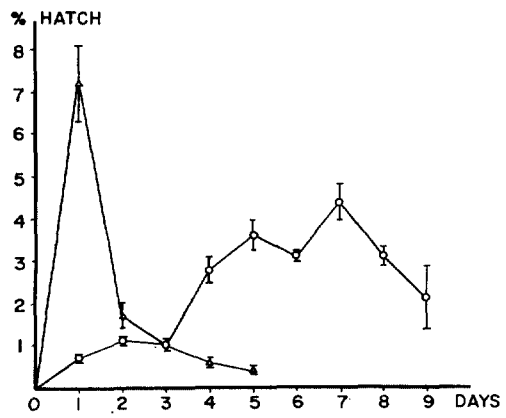

FIG. 1. Daily hatch of *Meloidogyne incognita* larvae from NaOCl-treated eggs on a 28-μm sieve (o) and from roots with egg masses in a mist chamber (△).

second-stage larvae have been shown to retain infectivity for 24 days in soil with an atmosphere containing 2-3% O_2 (3), and to withstand complete lack of O_2 in water for 4 days (5), the high density of larvae in the stem of the funnels and the accumulation of their own excretions may be major causes for loss of infectivity in this collection technique.

Infectivity was also affected by temperature in the greenhouse, as significantly fewer larvae penetrated roots at 30 ± 5 C than at 24 ± 2 C (Table 1). At 24 C, root penetration by larvae hatched from eggs in egg masses was similar to that by larvae hatched from NaOCl-treated eggs on the sieve. The amount of root penetration by larvae hatched from NaOCl-treated eggs used as inoculum was lower than that of the other two inocula sources.

TABLE 1. Influence of inoculum type and temperature on infectivity of *Meloidogyne incognita*.

Type of inoculum	Greenhouse temperature (C)	Inoculum level	Infection[a] %
Larvae[b]	24	2,000	46.5
Larvae[b]	30	2,000	17.8
Egg masses	24	2,640[c]	51.4
Eggs (NaOCl)	24	1,000	19.8
		LSD: $P=0.5$	7.6
		$P=0.01$	10.5

[a]Percentage of numbers of larvae (or eggs inoculated that yielded infective larvae) inside tomato roots after 20 days.
[b]Larvae hatched from NaOCl-treated eggs on 28-μm sieve.
[c]Average number of eggs from 10 egg masses.

The data presented herein show some of the problems encountered when inocula of *Meloidogyne* species are used. The limitations of egg masses (2), the low infectivity resulting from treatment of eggs with NaOCl, and the sensitivity of larvae to relatively high temperatures should be considered in selecting the type of inoculum for experiments involving *Meloidogyne* spp.

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