

A Method for Obtaining Quantities of Clean *Meloidogyne* Eggs¹

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We have obtained gram quantities of clean, viable eggs of *Meloidogyne incognita* by a method which combined treatment with hypochlorite and differential centrifugation. Chili pepper plants (*Capsicum frutescens* L.) infected with *M. incognita* were dug at harvest from a naturally infested field. Egg sacs from roots of 50 plants were obtained by kneading the washed roots in water and recovering the egg sacs on a 60-mesh sieve (2). Egg sacs were placed in an electric blender in 100 ml of tap water, 500 ml of 1% sodium hypochlorite (20% commercial bleach) added, and the blender operated at maximum speed for 40 sec. The resulting suspension of eggs and root debris was poured quickly through nested 100- and 400-mesh sieves and eggs which were retained on the 400-mesh sieve were washed with several volumes of distilled water. Eggs which passed through the 400-mesh sieve (pore size 37- μ m) were recovered by repeated sieving and rinsing.

Eggs were eluted from the sieves, transferred in 40 ml of water to a 50 ml polycarbonate centrifuge tube and centrifuged at 1000 g for 5 min. The pellet, containing eggs and some debris, was resuspended in 40 ml of sucrose solution (454 g sucrose to 1 liter distilled water) and centrifuged at 1000 g for 40 sec. The supernatant was quickly decanted onto a 400-mesh sieve and the eggs retained on the sieve were washed three times with 100 ml portions of distilled water. Eggs that passed through the sieve were recovered by repeating the sieving technique.

During the above procedure, some second-stage larvae were released from the eggs. These larvae were removed by collecting them on a 200-mesh sieve. Eggs were concentrated by centrifugation at 1000 g.

By this method, 2 cc (approximately 10

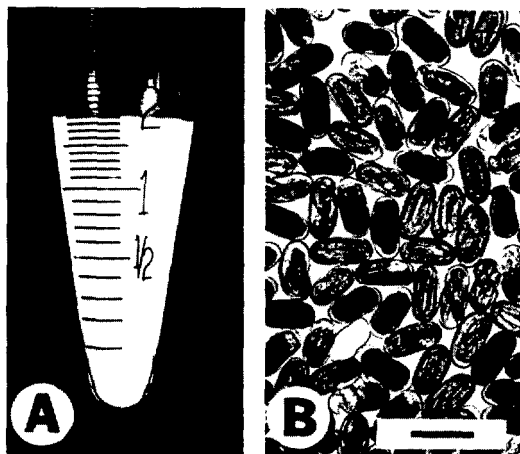


FIG. 1. Eggs of *Meloidogyne incognita*. A. Nearly 2 cc of eggs free of debris. B. Photomicrograph of a diluted suspension of debris-free eggs. Bar = 100 μ m.

million) of eggs free of debris were readily obtained from 50 highly infected chili pepper plants (Fig. 1). In another experiment, 2.1 cc of eggs were obtained from 11 chili pepper plants inoculated with 10,000 second-stage larvae and maintained in a greenhouse in 15-cm plastic pots for 3 months.

Although the molecular-level effects of hypochlorite on eggs are not known, Bird (1) indicated that a 2-3 min treatment in hypochlorite containing 0.5% available chlorine did not appear to influence either the hatching process or the fine structure of the egg shell. Eggs treated by our method (0.4% available chlorine) hatched normally and the hatched larvae readily penetrated and developed in roots of tomato seedlings.

LITERATURE CITED

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