Improved Methods of Hatching Heterodera schachtii Larvae for Screening Chemicals

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Abstract: The rate of hatching of *Heterodera schachtii* larvae was greatly increased by placing cysts in sieves enclosed by small disposable cups. An apparatus that permitted rapid storage of second-stage larvae at 10 C prolonged the viability of the larvae. *Key Words:* Hatching apparatus, sugarbeet nematode, increased viability.

Syracuse watchglasses have been extensively used to evaluate hatching and emergence of larvae from cysts of *Heterodera schachtii* Schmidt (6). The use of watch glasses as hatching vessels is adequate for relatively small tests of about 50 samples. However, this method is not suitable for bioassay of large numbers of samples for hatching activity, especially when sample volumes available for bioassay are not greater than 5 ml/sample, and cysts must be transferred to fresh solutions during the bioassay. Reports have shown that greatly increased hatches can be obtained with methods employing sieves (2, 4, 5).

Hatched second-stage (L2) larvae of *H. schachtii* rapidly lose viability when stored at temperatures above 20 C. However, the rate of larval hatch may not peak until late in the second week when cysts are incubated at 24 C, the optimum hatching temperature for *H. schachtii* populations from the Salinas Valley of California (Steele, *unpublished*). As a result, the majority of hatched L2 that have accumulated may not be viable (1). Consequently, a sieve method that enabled rapid inactivation of larvae by storage at low temperatures (5-10 C) soon after hatching was developed, tested, and compared with the watchglass method. This method was used to obtain large numbers of hatched L2 which were then used to evaluate the nematicidal efficacies of oxime carbamates (8).

MATERIALS AND METHODS

The watchglass and sieve methods were evaluated for relative effectiveness in hatching larvae of *Heterodera schachtii*. Newly formed cysts were separated from sugarbeet roots and soil by floating and decanting suspended debris into screens. Cysts with viable eggs and larvae were selected and manually separated from soil and root debris as previously described (7) and stored 10 days at 8 C. Groups of 20 cysts were incubated in 15 ml of sugarbeet root diffusate (6) or tap water within Syracuse watchglasses or in small sieves (Fig. 1-C) (collection cups supplied by Hykro Pet Industries, Esbjerg, Denmark) enclosed by plastic portion cups (Thunderbird Container Corporation, El Paso, Texas 79912) (Fig. 1-A, B). The cups also contained 5 ml of treatment solution. Treatments were replicated four times. Cysts were transferred to fresh test solutions at 5-day intervals, and the emerged larvae were counted.

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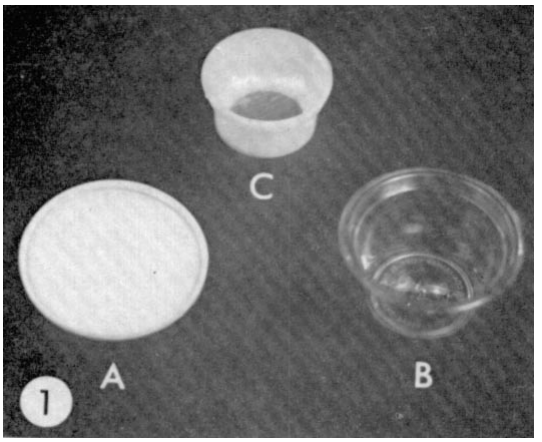

FIG. 1-(A to C). Plastic containers for hatching of *Heterodera schachtii* larvae. *A)* Cap. *B)* Cup. *C)* Sieve.

An apparatus (Fig. 2) was used to hatch and store L2 at 10 C until needed for inoculum or to evaluate the effects of aqueous chemical solutions. The temperature of hatch medium was regulated with an 11 x 2mm 35W flexible heating tape (Briscoe Mfg. Co., Columbus, Ohio 43216) and an electronic thermistor-activated heat control unit built to published specifications (3). The viability of larvae hatched with this apparatus was compared with the viability of larvae hatched with a similar apparatus that had no thermo-regulation of hatching and storage areas.

Five-hundred L2 obtained with each apparatus were inoculated on each of 10 sugarbeet plants. After they had grown 35 days in a greenhouse, roots of inoculated plants were examined for female *H. schachtii*.

RESULTS AND DISCUSSION

Figure 3 illustrates that more rapid hatching takes place in sieves than in watchglasses. At 10 days, more than 90 percent of L2 hatching in sieves had emerged, and this amount was greater than the total hatch from watchglasses in 25 days.

In addition to increasing hatch rate, the sieve method offers other advantages. Transfer of cysts with sieves (as through a series of solutions) minimizes handling and prevents damage to cyst walls which may occur when cysts are transferred with brushes, forceps, or pipets. Capped containers also prevent evaporation of test solutions and

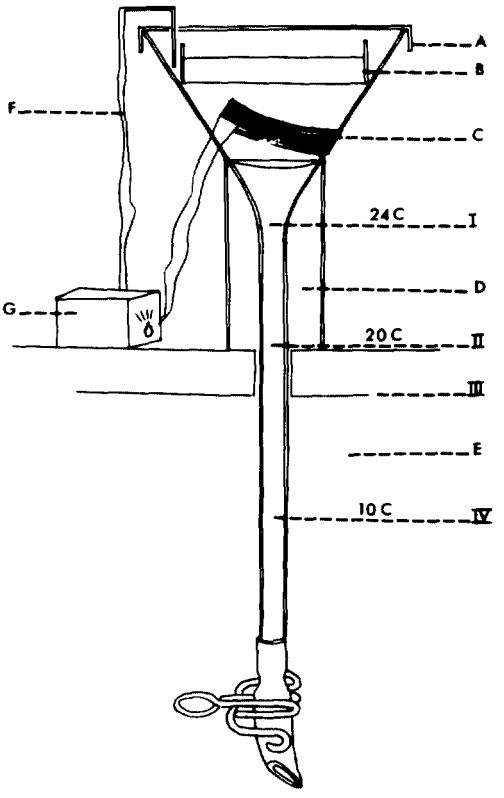

FIG. 2. Apparatus for hatching and cold storage of *Heterodera schachtii* larvae. (A) Plastic cover. (B) Sieve of plastic tubing and nylon mesh of 40/cm. (C) Flexible heating tape. (D) Funnel support (plastic tubing). (E) Interior of refrigerator cabinet kept at 8 C. (F) Thermistor probe. (G) Electronic thermoregulator. Lengths of funnel stem: from neck of funnel (I) to top of refrigerator (II), 85 mm; from top inside wall of refrigerator (III) to (IV), 50 mm.

only 2 ml of test solution are required for evaluation. The unit cost of materials for the sieve method (includes sieves, disposable cups, and caps) amounts to less than 15% of the cost of a single watchglass. This method has been used successfully to evaluate the effects of nonvolatile nematicides on hatching and to bioassay more than 5,000 samples for hatching activity during a period of 6 months.

Examination of sugarbeet inoculated with L2 revealed that storage of L2 at 10 C soon after emergence more than doubled the number of viable L2. Plants inoculated with L2 hatch with the refrigerated apparatus had a mean of 193 adult females per plant compared with 85 females per plant for the nonrefrigerated apparatus.

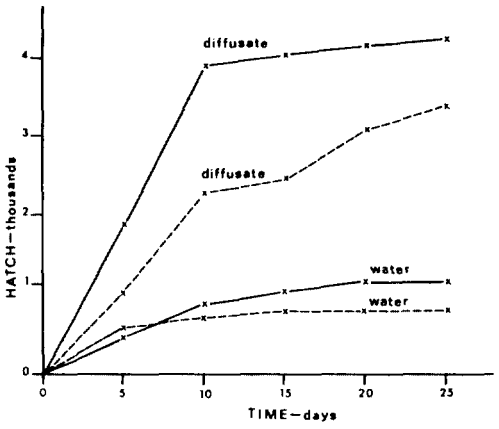

FIG. 3. Accumulated hatch of *Heterodera schachtii* larvae from cysts treated with sugarbeet root diffusate or water in sieves (solid line) or Syracuse watchglasses (broken line) at 5-day intervals.

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