Technique for Obtaining Eggs and Juveniles of Heterodera glycines[1]

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We describe here our method of obtaining clean *Heterodera glycines* eggs and hatched infective juveniles in quantity.

Similar methods of obtaining eggs of other nematode genera have been published (1,2,4,7). Minigawa (5) compared the efficiency of a double layer centrifugal method (DCF) with extraction in Baermann funnels and direct examination for recovery of nematodes from soil. He concluded that DCF is unsuitable for quantitative extraction of nematode eggs from soil. He did not examine *Heterodera* spp.

Collection of eggs: We collect cysts from soil on a 60-mesh screen (250-μm apertures) by elutriation or by settling and decanting. Screenings are washed onto a 100-mesh sieve (150 μm) and macerated by gentle rubbing with a rubber stopper to release eggs. Eggs and debris are collected in a bucket with the aid of a gentle stream of water, concentrated by centrifugation at 800 g for 5 min in 50-ml conical tubes in a swinging horizontal head rotor. The supernatant is siphoned off until about 2 ml remain above the pellet. The pellet is then

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stirred and the slurry is layered over a sucrose gradient in a 50-ml conical centrifuge tube.

We prepare the sucrose gradient by successive addition of the following into 50-ml conical tubes: (i) 10 ml of 50% sucrose solution (614.8 g sucrose + 1 liter water). Specific gravity 1.23. (ii) 10 ml of 40% sucrose solution (470.6 g sucrose + 1 liter water). Specific gravity 1.18. (iii) 10 ml of 20% sucrose solution (216.2 g sucrose + 1 liter water). Specific gravity 1.08. Before use, tubes are held for 10 min or overnight in the refrigerator.

During centrifugation the clean embryonated eggs concentrate in a band in the 40% layer (Fig. 1). In addition, the band may contain hatched juveniles and a few fungus spores. The band is collected by siphon, caught on a 31-μm aperture nylon sieve, and rinsed gently with water. Other components of the sample (mycorrhizal spores, fungus mycelia, or parasitized eggs) accumulate in other parts of the gradient. Empty egg shells and other debris collect in the pellet. One-celled eggs usually accumulate in the 20% layer. The distribution of eggs and larvae collected from 900 cm^3 of soil and separated in a sucrose gradient is shown in Figure 2.

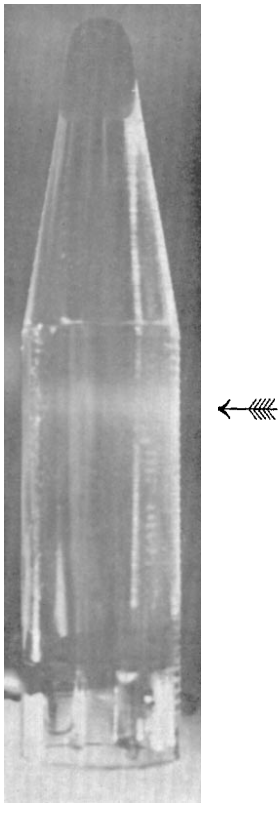

Fig. 1. A band (arrow) containing > 200,000 eggs of *Heterodera glycines* in the 40% layer of a sucrose gradient (10–40–50%) after centrifugation for 5 min at 800 g.

Hatching: Second-stage juveniles and spores of mycorrhizal fungi are removed by passing the suspension through a 63-μm nylon sieve. Eggs caught in the 31-μm sieve are transferred to a 41-μm nylon sieve for hatching. The hatching apparatus consists of upper halves of two 50-ml plastic beakers. A nylon mesh cloth is supported by the two beakers inserted into each other. The apparatus fits into a 60 × 20 mm plastic petri dish or a syracuse dish. Sufficient water to cover the eggs is added to the dish. The unit is held in a desiccator with the lid in place, water in the bottom, and the pore to the atmosphere open.

We routinely incubate the eggs at 30 C for 3–5 days. Then the water is replaced with 0.01 M $ZnSO_4$ or other hatching factor and incubated at 25 C (6). Problems with bacterial and fungal growth during incuba-

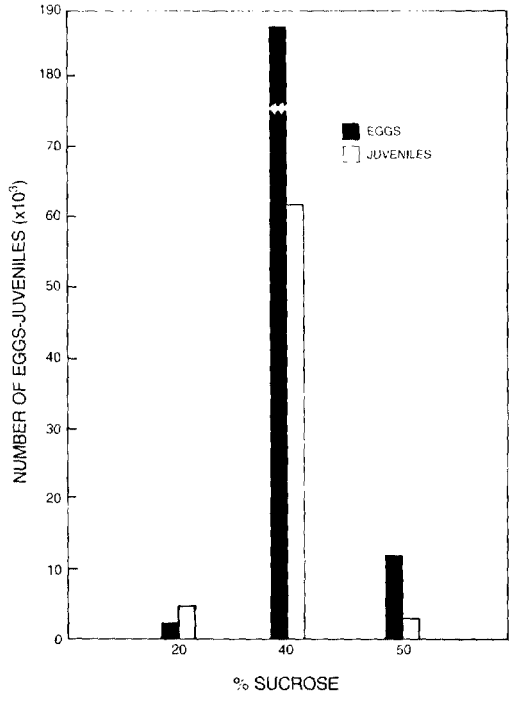

Fig. 2. Distribution in a sucrose gradient of *Heterodera glycines* eggs and juveniles from 900 cm^3 of infested soil.

tion are avoided by immersing eggs in 0.5% chlorhexidine diacetate (Hibitane) for 15 min, followed by a water rinse preceding incubation at 30 C.

We have not determined the quantitative recovery of eggs from soil or the hatching of infective juveniles in this procedure. For each experiment we hatch between 100,000 and 500,000 eggs. Under our conditions, more than 50% of the eggs collected from greenhouse populations hatch over a period of 12 days, with the peak hatching 5–8 days after transfer to $ZnSO_4$. Clean, viable eggs of *Meloidogyne incognita* were also collected by our method from egg masses separated with 1% NaOCl (3).

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