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An Efficient New Device to Release Eggs From *Heterodera glycines*

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Abstract: A new apparatus to release eggs from cysts of soybean cyst nematode (*Heterodera glycines*) is described and its efficiency evaluated. A rubber stopper was mounted on a bolt, and cysts were ground against a 60-mesh screen. Eggs and second-stage juveniles were washed into a series of screens nested underneath the apparatus. This method was fast and efficient, and had no ill effect on prepared inoculum.

Key words: apparatus, chemical, eggs, extraction, *Heterodera glycines,* inoculation, mechanical, method, nematode, soybean, soybean cyst nematode.

Soybean cyst nematode eggs and juveniles can be released from cysts in the laboratory by chemical or physical methods (Acedo and Dropkin, 1982; Faghihi et al. 1986; Niblack et al. 1993). Acedo and Dropkin (1982) described a method of releasing eggs and juveniles by macerating cysts with a rubber stopper. We have enhanced this concept by constructing an apparatus that maximizes the number of eggs released and have compared results with those obtained using a chemical method of egg release.

Materials and Methods

Components: The cyst-crushing apparatus consists of three components: chamber, plunger, and electric motor.

The chamber consists of two pieces of PVC pipe each with a 31-mm i.d. One piece is 65 mm long, and the other is 20 mm long. The chamber is constructed with epoxy glue applied to the adjoining sides of each section to hold in place a piece of 42-mm-diam. 60-mesh screen (250-µm pore size) that is glued between the two pipe sections (Fig. 1a).

The plunger consists of a no. 6 rubber stopper (32 × 26 × 25 mm) and a metal bolt (130 mm long, 7 mm in diam.). A 15-mm-long hole (5-mm-diam.) is drilled in the center of the 25-mm-diam. end of the rubber stopper. A second hole (2-mm-diam.) is drilled, at a right angle to the first hole, from the side 10 mm from the top of the rubber stopper. A similar-size hole is drilled, 7 mm from the end, into the long bolt. A nut with washer is screwed into the end of the long bolt. The bolt is inserted into the rubber stopper. A 2-mm- × -20-mm-long finish nail is inserted into the side hole of the rubber stopper and passed through the hole of the long bolt. This nail prevents the rubber stopper from turning during operation. The washer and nut at the top of the rubber stopper are tightened to prevent any movement. All of the open holes in the rubber stopper are filled with silicon glue (Figure 1b).

Specifications for a suitable electric motor are 1.3 amp, 7,500 rpm, with a 7-mm shaft. The crushing plunger is attached to the electric motor with a piece of Tygon tubing, 40 mm long. The motor speed can be controlled with a variable-speed dial and timed with an electric clock.

Egg collection: Cysts are collected with a sieving-and-decanting method (Faghihi et al., 1986) into a 50-ml beaker. The cysts are poured into the crushing chamber, the plunger is inserted into the chamber, and the motor is turned on for 1 minute. The cysts are ruptured when they become trapped between the rotating rubber stopper and the 250-µm screen. A gentle stream of water is poured into the chamber throughout this operation to wash eggs through the apparatus and prevent them from being damaged. A nested set of 150- and 38-µm sieves is stacked underneath the chamber to collect the released eggs.

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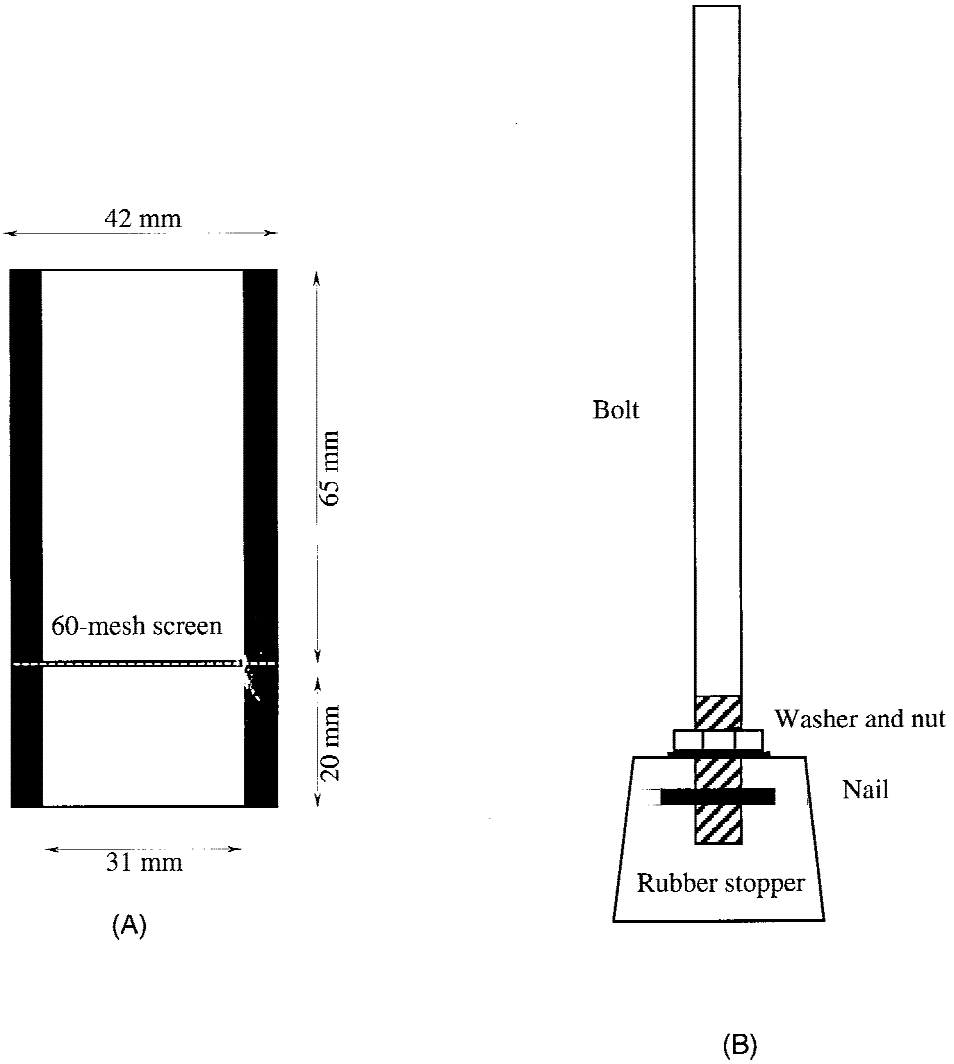

FIG. 1. A) Longitudinal section of a cyst-crushing chamber built with two pieces of PVC pipe with 60-mesh (250-µm pore size) screen between them. B) Plunger constructed with a no. 6 rubber stopper and a 130-mm-long bolt.

Comparisons of eggs recovered with a chemical method of egg release: In the first experiment, a 50% concentration of household bleach (5.25% sodium hypochlorite) was used to dissolve the cyst wall to release the eggs (Faghihi et al., 1986). This test was repeated twice—each time with four replications—and the eggs and uncrushed cysts were counted.

In the second experiment, identical numbers of eggs, extracted with either the chemical or the new mechanical method, were placed on Baermann funnels. A 10-ml sample was drawn from each funnel every day during a 2-week period, and the number of juveniles and their movement under a microscope was observed and recorded. Nematodes were considered mobile if they made any movement while being observed.

Comparison of quality of inoculum from the two methods: In the third experiment, Williams 82 susceptible soybean plants were grown in seedling trays (2.5 × 2.5-cm cells) for 2 weeks before being inoculated with two levels of 3,600 and 7,300 eggs/juveniles from each of the two release methods. The

TABLE 1. Mean numbers of cysts that remained intact and the numbers of eggs recovered after the chemical and mechanical procedure.

Variable	Chemical	Mechanical	*P*
Intact cysts	16	1	0.005[a]
Number of eggs	75,150	32,100	0.034[b]

[a] Significance at $P = 0.01$.
[b] Significance at $P = 0.05$.

plants were grown in a greenhouse under identical conditions for 1 month. Cysts that developed on the roots were separated from the soil with a sieving-and-decanting method and were counted under a microscope. Data were subjected to ANOVA to determine their significance.

RESULTS

Thirty-one percent of cysts treated with bleach remained intact, whereas, with the new mechanical method, 2% of the cysts from the crushing chamber were left unbroken. The number of cysts that remained intact was higher ($P = 0.01$) when they were treated with bleach as compared to those ruptured mechanically (Table 1). More eggs were extracted with the mechanical method ($P = 0.05$) than with the chemical method (Table 1).

The total number of juveniles hatched following the chemical method and their mobility were not different ($P = 0.48$) from the number of juveniles released with the mechanical method (Table 2). Differences ($P = 0.882$ and $P = 0.604$) between the methods were not found for the number of cysts recovered following inoculation with the same number of eggs and juveniles (Table 3).

TABLE 2. Mean numbers of second-stage juveniles that hatched in 2 weeks using chemical and mechanical procedures.

Number of juveniles	Chemical	Mechanical	*P*
Mobile	390	574	0.480 NS
Immobile	1,206	639	0.276 NS

NS = not significant.

TABLE 3. Mean numbers of cysts recovered from susceptible soybean roots 1 month after inoculation with inoculum prepared with chemical and mechanical methods.

Inoculum level[a]	Chemical	Mechanical	*P*
Low	236	248	0.882 NS
High	323	366	0.604 NS

[a] Low inoculum level was 3,600 eggs and second-stage juveniles; high level was 7,300.
NS = not significant.

DISCUSSION

This mechanical method of crushing the cyst wall provides a quick, reliable, and effective way to release eggs from cysts for enumeration or inoculation. Almost all of the cysts in the samples can be crushed using this method.

When soil organic matter was high, the time required to crush all of the cysts had to be increased. The organic matter prevented a full contact between rubber stopper and the screen. However, some of the debris can be removed by a modified sugar flotation method using a 50:50 (g sugar: ml water) solution before crushing the cysts. Though not addressed in this study, we anticipate the new crushing apparatus can be adapted for use with other cyst nematode species or even root knot nematodes.

LITERATURE CITED

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