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An Observation Chamber Technique for Evaluating Potential Biocontrol Agents of *Globodera rostochiensis*[1]

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Recent research has stimulated optimism that naturally occurring parasites may be useful biocontrol agents of nematodes (3,4,6–9). To evaluate their potential, collections of biocontrol agents must be screened against the target nematode. Screening isolates against one nematode generation in greenhouse pots is an often used standard test. Disadvantages of this test include the lack of controlled environmental conditions and the inability, without destructive sampling, to observe the effect of parasitism.

An observation chamber was developed (1) and used to continually observe parasitism of developing *Heterodera avenae* (Woll.) females by *Nematophthora gynophila* (5). This technique involved growing oat roots in clear petri dishes in soil infested with both the nematode and the fungal parasite under greenhouse conditions. The observation chamber allowed evaluation of parasitism at more than one point in the life cycle, but lacked controlled environmental conditions, which might increase sensitivity over greenhouse tests.

Foot (2) described a method to screen potatoes for resistance to the potato cyst nematode. Clear containers formed an enclosed sterile system which inhibited shoot growth and stabilized moisture without eliminating root growth, which was sustained by the seed tuber. We developed a modification of this procedure to evaluate fungal parasitism of *Globodera rostochiensis* (Woll.) Behrens. Results obtained with the canister method were compared with results obtained using greenhouse-grown potted plants.

Forty-one fungi isolated from *G. rostochiensis* in the Peruvian Andes and a parasite of *Meloidogyne incognita* (Kofoid & White) Chitwood, *Paecilomyces lilacinus* Thom. Samson (3) obtained from the International Potato Center were examined. Eight replications of each isolate were included. Potential fungal parasites were added to the system either on oat seeds or potato dextrose agar (PDA) strips. Oat seed medium consisted of 50 ml of oat seed and 25 ml of distilled water added to a 150-ml flask and autoclaved before inoculation. The infested oat seeds or PDA strips served both as an initial food source for the fungus

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and also as a means of distribution throughout the soil in the canister.

Ten infested oat seeds or ten infested 5-mm PDA strips were mixed with 250 ml of sterile sand in a 300-ml clear Nalgene plastic container. Single-eye seed pieces of certified 'Katahdin' seed potatoes were washed and planted in each canister. After root initiation, 5,000 second-stage juveniles of *G. rostochiensis* were added. Sterile water was added to adjust the contents to 10% moisture by weight.

After 3 weeks, 50–100 females developing on roots were located microscopically and their positions marked on the canister sides for three of the eight replications (Fig. 1). The marked canisters were checked weekly for abnormal or missing females. After 10 weeks at 20 C, the canister contents were removed, dried, and processed to recover cysts (10). Cysts were counted, hatched in potato root diffusate, and crushed to determine viability.

To evaluate isolates in greenhouse pots, single-eye seed pieces of 'Katahdin' potato were planted in 10-cm plastic pots containing autoclaved soil infested with 100–125 infested oat seeds or 100–125 infested PDA strips per pot. A 2.5-cm layer of sterile soil was added to the top of each pot to reduce contamination between treatments. When aerial shoots were 5–8 cm high, 5,000 eggs and juveniles of *G. rostochiensis* were added per pot. After 10–12 weeks, the shoots were cut off and the soil was dried and processed as before.

In canisters, the percentage of nonviable eggs, as determined by crushing 100 cysts/isolate, ranged from 7.6 to 46.5 (Table 1). The percentage of nonviable eggs from cysts subjected to fungal isolate 29 was higher ($P = 0.05$) than from the control and all other fungi. Four of the 42 fungal isolates had some pathogenic effect against potato and were not further tested.

Observation of developing white females with a dissecting microscope (10–50×) through the transparent canister revealed no parasitism or degeneration of females other than that associated with necrosis of the host root.

The number of cysts produced per greenhouse pot, an indication of the number of juveniles successfully completing the life cycle, was not different ($P = 0.05$) among fungal isolates. However, when 20 greenhouse-produced cysts from each of six replications were crushed, isolate 29 was found to have a greater ($P = 0.05$) incidence (27.4%) of nonviable eggs than did the control (14.3%). This result agrees with data obtained from the canisters, where isolate 29 had a higher ($P = 0.05$) incidence (46.5%) of nonviable eggs than did the control (21.4%).

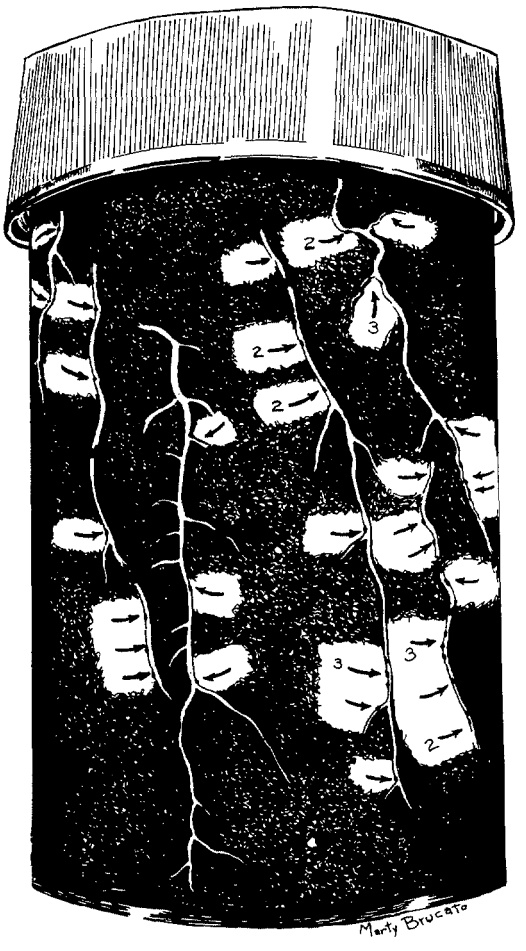

FIG. 1. Canister used for evaluating fungal parasitism of *Globodera rostochiensis* showing number and distribution of females on potato roots.

To evaluate parasitism of second-stage juveniles in eggs, surface-sterilized cysts containing juveniles were exposed to each fungal isolate on the surface of water agar plates for 20 days at 20 C. Each isolate was replicated five times, and the experiment was performed twice. No isolate was pathogenic to eggs, and no differences between isolates were apparent.

TABLE 1. Percent nonviable canister produced encysted juveniles of *Globodera rostochiensis.*

Isolate no.	% nonviable*	Isolate no.	% nonviable
20	7.6 a†	4	16.6 abcde
21	8.5 ab	2	17.8 abcde
17	11.7 abc	37	18.5 abcde
22	12.2 abcd	38	19.5 abcdef
23	12.2 abcd	33	20.0 abcdef
35	12.3 abcd	42	20.2 abcdef
11	12.3 abcd	16	20.2 abcdef
36	12.9 abcd	41	20.9 abcdef
15	13.3 abcd	Control	21.4 abcdef
24	13.7 abcd	31	21.7 abcdef
30	13.8 abcd	1	22.9 bcdef
8	13.9 abcd	6	23.2 bcdef
28	14.3 abcd	25J‡	25.8 cdef
12	14.4 abcd	5	26.6 def
32	14.6 abcde	10	29.2 ef
25	15.2 abcde	40	33.4 f
9	15.3 abcde	18	33.5 f
34	15.8 abcde	19	33.9 f
26	16.3 abcde	29	46.5 g
7	16.5 abcde		

* Percent kill determined by crushing 100 cysts.
† Numbers followed by the same letter not significantly different ($P = 0.05$) according to Duncan's multiple-range test.
‡ *Paecilomyces lilacinus.*

It is our belief that the effect of parasitic organisms on nematodes is a reflection of the stage of the life cycle parasitized, agent specificity, and the relative aggressiveness of the parasitic organism. There is a need for standardized techniques that evaluate these parameters and reduce the probability of rejecting a potentially effective agent. The canister screening technique appears to be effective for the potato cyst nematode system. As with Crump and Kerry's observation chamber technique (1), parasitism can be evaluated at more than one point in the nematode life cycle without destructive sampling. Development of the host plant and the female nematode can be directly observed through the transparent canister. The number of visible females may be directly proportional to the total number (2). Individual females can be extracted without drying to facilitate fungal recovery from parasitized females.

Results indicated that physical conditions in the canisters led to a greater mortality than in greenhouse pots, even though fungal inoculum in the greenhouse was 10 times that of the canisters. Controlled conditions of moisture, temperature, and photoperiod increased the sensitivity of the screening technique. The covered canister reduces transpiration and water loss, stabilizing moisture conditions. Also, limited shoot growth allows for placement of the canister in a constant temperature incubator in complete darkness.

Isolate 29, as yet unidentified, substantially decreased *G. rostochiensis* viability in both the greenhouse and canisters. The fungus parasitized eggs of developing females, but not those of mature cysts. It is postulated that this fungus invades immature or undeveloped eggs before development to the second-stage juvenile.

Paecilomyces lilacinus retained its parasitism of *Meloidogyne incognita* but caused no reduction in viability of any stage of *G. rostochiensis.*

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