# RESEARCH NOTES

## A Laboratory Technique for Culturing Filipjevimermis leipsandra, a Nematode Parasite of Diabrotica balteata Larvae (Insecta: Coleoptera)[1]

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The mermithid nematode *Filipjevimermis leipsandra* Poinar and Welch is a potential biological agent for suppressing the population of the banded cucumber beetle, *Diabrotica balteata* LeConte. The life cycle of *F. leipsandra* has been described (1,2,4). Also, the seasonal fluctuation of field populations of the nematode and its parasitism of the beetle have been reported (1). Our results showed that laboratory infections can be achieved readily by exposing the insect host to naturally infested field soil or to sterilized potting soil inoculated with *F. leipsandra* eggs or preparasitic juveniles. However, for a large-scale field test of control of *Diabrotica* by the mermithid nematode, the organism must be mass reared. Techniques for mass rearing the insect host are available (3); this paper reports a laboratory technique for rearing the nematode.

Postparasitic mermithids emerging from naturally infested beetle larvae were washed several times in distilled water and individually placed in the cells of multicell tissue culture plate (127 mm × 75 mm) in distilled water and allowed to complete development. After adults oviposited, the eggs were removed from the cells and transferred into a Baermann funnel containing a bolting cloth sieve (71 $\mu^2$ openings). Hatched juvenile nematodes were collected periodically and stored at 5 C until used as inoculum.

Each experimental unit consisted of a clay pot (7.6 cm d) filled with methyl bromide fumigated soil (ca. 17% moisture). A hole 2.5 × 2.5 cm was made in the soil into which the desired number (1, 2, 4, or 6 × $10^3$) of nematodes, 100 first instar *D. balteata* larvae, and 10 sprouted corn seeds (for insect food) were added. The tops of the pots were sealed with Parafilm "M" and placed in a plastic crisper (47 × 25 × 15 cm) containing a damp sponge. The crisper was also sealed with Parafilm "M." After 5 or 8 d the soil in each pot was combed for insect larvae to determine percentage insect survival. The percentage of insect parasitism was determined by individually caging 10 of the surviving larvae in 7 dm plastic vials containing a sprouted corn seed in moist soil. The remaining larvae were incubated in a crisper to which fresh sprouted corn was added. The crisper lids were punctured with small holes to allow for air exchange. The number of parasitic nematodes emerging from the insect larvae were recorded about 2 wk later to determine the yield of mermithids per pot.

All experiments were conducted in a controlled environment room maintained at 25.3 ± 1.4 C and 57.5 ± 7.5% RH with a 12 h photoperiod.

Experiment 1 was designed to determine optimum parasite:host ratio for maximum yield of parasitized *Diabrotica* larvae and postparasitic nematodes. Each experimental unit was inoculated with 1,000, 2,000, 4,000, or 6,000 juvenile nematodes and replicated four times in a completely randomized block. Survival counts of *D. balteata* larvae were made 8 d after inoculation. Table 1 summarizes the percentage of the insect larvae that survived inoculation at the various nematode population densities, the percentage of insect parasitism, and the average number of postparasitic emerging from each host. No significant differences in host survival and parasitism or in the number of postparasites were obtained between soil infested with 1,000 to 6,000 juveniles. It

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Table 1. Survival of infected *Diabrotica balteata* larvae and yield of postparasitic *Filipjevimermis leipsandra* resulting from single and double inoculations.

| Density of preparasites/ pot | % insect survival* | % insect parasitism | Yield postparasitic nematodes |
|---|---|---|---|
| | Experiment 1 | | |
| 0 | 65.2a | 0.0a | 0.0a |
| 1,000† | 61.7a | 22.5b | 26.5b (16-41) |
| 2,000 | 60.7a | 10.0b | 25.7b (18-32) |
| 4,000 | 51.2a | 12.5b | 34.7b (20-45) |
| 6,000 | 38.2a | 35.0b | 13.2b (12-17) |
| | Experiment 2 | | |
| 0 | 59.5a | 0.0a | 0.0a |
| 1,000‡ | 58.2a | 40.0b | 40.2b (15-40) |
| 1,000‡ + 1,000 3 d later | 65.0a | 47.5b | 57.7b (21-61) |
| 1,000‡ + 1,000 6 d later | 68.0a | 37.5b | 63.0b (35-75) |

*100 first-instar *D. balteata* larvae added to each of four pots (replicate) on 8 May in experiment 1 and on 25 May in experiment 2. Means followed by the same letters were not significantly different ($P = .05$) by Duncan's multiple-range test.

†Each density of preparasites used in experiment 1 introduced on 8 May 1979.

‡Preparasites introduced on 25 May 1979.

appeared that a ratio of 1,000 nematodes per 100 *Diabrotica* larvae (i.e., 10:1) was sufficient for maximum recovery of post parasitic nematodes.

Experiment 2 was an attempt to increase the efficiency of the inoculum. When the nematode inoculum was applied as a split application of 1,000 preparasites each at 3 or 6 d intervals, no significant increase over the nonsplit application was obtained in the number of postparasites (Table 1).

Inoculum density of preparasitic juveniles greater than 1,000 preparasites per 100 insect larvae did not significantly increase the yields of postparasites. Since only the number of nematode juveniles was varied in these experiments, it appears that the maximum number of postparasites were produced in cultures inoculated at the 10:1 (parasite:host) ratio. The reduced yield of postparasites with higher inoculum density could be due to a high mortality rate of insect larvae burdened by massive parasitism.

Since each adult mermithid can produce between 2,000 and 7,000 eggs (1), the system reported here has the potential for producing between 80,000 and 240,000 eggs per experimental unit.

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