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Overwintering and Cold Tolerance of the Mermithid *Filipjevimermis leipsandra*[1]

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Abstract: Overwintering survival of *Filipjevimermis leipsandra* postparasitic adults ranged from 12 to 15% over 2 years. Nematodes recovered from biweekly samples oviposited within a week when exposed to warm temperatures. More nematodes were recovered from the lower portions of soil cores than from the upper. In laboratory tests of cold tolerance, the LD_{50} at −5 C was 5.5 hours, whereas exposure to −2 C for as long as 96 hours resulted in no mortality.
Key words: cold tolerance, cucumber beetle, *Diabrotica balteata, Filipjevimermis leipsandra,* mermithid, overwintering.

The mermithid nematode *Filipjevimermis leipsandra* Poinar & Welch is a parasite of the cucumber beetle *Diabrotica balteata* in the southeastern United States with potential as a biological control agent (2). This parthenogenic nematode overwinters in the adult postparasitic stage in the soil and begins to oviposit when soil temperatures reach about 20 C (4). An important aspect of this nematode's fitness to its habitat is its ability to survive cold winter soil temperatures with sufficiently fecund individuals to increase the population with the onset of warm weather. Elsey (4) found *F. leipsandra* to be sensitive to cool temperatures during its developmental stages, which may restrict its economic value in cooler regions. This work was undertaken to ascertain the ability of the mermithid to overwinter and tolerate subfreezing temperatures.

METHODS AND MATERIALS

Filipjevimermis leipsandra used in this study were obtained from a culture maintained at the U.S. Vegetable Laboratory in Charleston, South Carolina, and reared by the methods of Creighton and Fassuliotis (1). A 2-year study of overwintering survival was conducted from late autumn to early spring of 1988–89 and 1989–90 on a plot of ground inside a large screenhouse. One hundred cylindrical cages (7.6 cm × 30.5 cm) constructed of 5-mm-thick PVC were buried vertically to a depth of 25.0 cm and filled with soil (Lynchburg loamy fine sand) to that level. A circular sheet of fine nylon screen (25 mesh/cm) covered the bottom of the cylinder. Soil in these cages and the surrounding area had been fumigated with methyl bromide 1 week before the start of the experiment. The cages were arranged in a 10 × 10 pattern with each cage positioned 20–30 cm from the others. Twenty postparasitic nematodes, 1–5 days after emergence from the host larvae of *Diabrotica balteata* LeConte, were placed in a hole (2.5 cm deep) in each cage on 4 November 1988 and 2 November 1989 and covered with soil. Temperatures were monitored with a soil thermograph at a depth of 15 cm. Moisture was monitored by placing two Irrometer Moisture Indicator tensiometers (Irrometer Co., Riverside, CA) at 15-cm depths in the study area. When readings above 10 centibars were recorded, the area was sprinkled with water in order to maintain moist soil conditions. This was necessary only in February 1989. Starting on 12 December 1988 and 14 November 1989, 10 cages were unearthed and cut into five 5-cm sections with a band saw. The soil from each section was searched carefully for nematodes, and those found alive were placed in individual plastic dishes (60 × 15 mm) containing 10 ml water. After 7 days the dishes were examined for eggs.

To investigate the effect of subfreezing temperatures on survival, 20 nematodes were placed into each of four snap-tight

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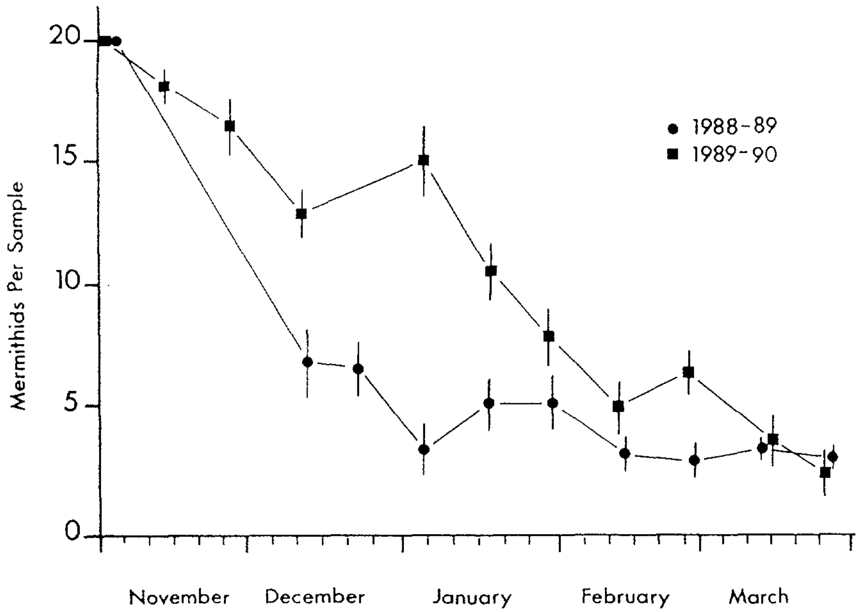

FIG. 1. Average number of mermithids (± SE) recovered from cages sampled during winters of 1988–89 and 1989–90, Charleston, South Carolina.

plastic dishes (60 × 10 mm) filled with moistened blasting abrasive (50 ml water/ 200 ml abrasive). After 1 hour the dishes were placed in a refrigerated water bath at a constant temperature for controlled exposure periods. At the end of each exposure period, dishes were removed from the bath and nematodes were put in plastic dishes (100 × 15 mm) containing 15 ml water and put in a growth chamber at 23 C. After 24 hours nematodes showing movement were counted as alive. Some surviving nematodes were held 2 weeks at 23 C in individual plastic dishes (60 × 15 mm), and egg laying was noted.

RESULTS AND DISCUSSION

Biweekly examination of core samples indicated that overwintering survival of *F. leipsandra* was about 12–15% in both years of the study. The shapes of the survival curves (Fig. 1), however, were quite different. In 1988–89 there was an abrupt drop in survival of nematodes placed in the soil cages during the first 6 weeks, after which the number of nematodes recovered changed little for the rest of the winter. In 1989–90 the number of nematodes recovered dropped at about the same rate from November to late March. Both winters were warmer than average, with the exception of a long cold spell in late December 1989, with 85 consecutive hours of subfreezing ($\bar{x}$ = −4.7 C) temperatures. During this extended period of cold weather, the soil temperature at 15 cm deep reached a minimum of 3.0 C.

Most of the nematodes recovered at each sampling date began to oviposit soon after being introduced to warm temperatures. An average of 87.7% (SE = 1.5) in 1988–89 and 91.4% (SE = 1.0) in 1989–90 laid eggs 1 week after recovery. Thus, there appeared to be no persistent state of reproductive diapause.

More nematodes were recovered from the lower 10 cm of the soil cores than from the upper 15 cm, particularly the top 5 cm (Table 1). These results differ markedly from a similar study conducted during spring and summer in which no mermithid adults were found at 20–25 cm and 63% were found at or above 15 cm deep (G. Fassuliotis, pers. comm.).

Results of the cold tolerance tests showed that *F. leipsandra* is more resistant to cold

TABLE 1. Surviving mermithids (% total ± SE) found at indicated soil depth, Charleston, South Carolina.

Depth (cm)	1988–89	1989–90
1–5	6.5 ± 1.7 a	11.1 ± 1.5 a
5–10	12.5 ± 2.3 a	21.4 ± 2.3 ab
10–15	18.3 ± 2.1 a	17.6 ± 2.2 ab
15–20	32.7 ± 4.8 b	19.8 ± 2.2 ab
20–25	30.0 ± 4.6 b	30.1 ± 3.7 b

Means with the same letters are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

than its temperature thresholds for development and infection (4) might indicate. The LD_{50} at −5 C was 5.5 hours (0.95 fiducial limits 4.1–12.2). Exposure of the nematodes to −2 C for 96 hours (the maximum time) resulted in no mortality and 2 weeks later 87% of them had laid eggs. Since the coldest soil temperature during this study was above freezing, even during a period of much colder than average air temperature in 1989, mortality from acute cold probably is not a factor in the Charleston area. From the standpoint of surviving subfreezing temperatures, *F. leipsandra* may be able to overwinter in areas north of Charleston. However, low temperatures (< 20 C) were found to lower viability of *F. leipsandra* (4), so the observed mortality (with the exception of the unexplained sharp drop in November and December of 1988) may be gradual attrition due to chronic effects of cold temperatures (4).

With a soil temperature of 20 C as a threshold for the initiation of oviposition (4), it is possible that egg laying in 1989 may have commenced in early February when the maximum temperature at 15 cm reached or exceeded 20 C on several days during a warm spell. Hatching of these eggs would have been unlikely, however, since warm temperatures did not resume until mid-March when soil temperatures exceeded 20 C regularly. In 1990 soil temperatures reached 20 C by 5 March. Since eggs of *F. leipsandra* take 25 days to hatch at 20 C (4), preparasitic nematodes would not be available to parasitize host beetle larvae until April in most years. Therefore, the first-generation larvae of banded and spotted cucumber beetles (*D. undecimpunctata howardi* Barber), which are active in February and March in the Charleston area (3), would escape parasitism. *Filipjevimermis leipsandra* may be better synchronized with the striped cucumber beetle, *Acalymma vitatum* (F.), which first appears in early April.

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