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Effect of Temperature on Development and Survival of the Mermithid *Filipjevimermis leipsandra*[1]

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Abstract: The development and survival of egg, juvenile (parasitic), preovipositing, and ovipositing stages of the parasitic mermithid *Filipjevimermis leipsandra* were determined at eight temperatures. Infection of the host, *Diabrotica balteata*, peaked at 30 C and fell off sharply at warmer or cooler temperatures. No development, oviposition, or infection occurred at < 20 C. Fecundity was highest at 25 C, and 50% of the eggs were laid in the first 4–7 days of oviposition.
Key words: *Diabrotica balteata, Filipjevimermis leipsandra,* mermithid, oviposition, survival.

The mermithid nematode *Filipjevimermis leipsandra* Poinar and Welch parasitizes several species of economically important chrysomelid beetles (3) and may have potential for biological control of these pests. Briefly, the life cycle of *F. leipsandra* is as follows (4): After overwintering in the adult postparasitic stage, the nematode lays its eggs in the soil. After hatching, the preparasitic stage (juvenile) initiates active searching, and individuals successful in finding young host larvae parasitize them by directly penetrating the cuticle. The early parasitic stage migrates into a host ganglion, and, after considerable growth, the nematode eventually enters the host hemocoel where it grows further and kills the full-grown larva when it exits. Usually one or two postparasitic molts occur before oviposition. Males are rare and not necessary for propagation of the species.

Application of eggs and preparasitic stages of *F. leipsandra* appeared promising in suppressing artificial infestations of the banded cucumber beetle, *Diabrotica balteata* LeConte (2). Temperature was important in these fields tests (2) because synchronization of nematode egg hatch with susceptible larvae was a critical factor in obtaining successful control. In addition, knowledge of the role of temperature in development and survival is essential for optimum rearing and for storing and accumulating eggs or gravid females. Thus, our objective was to test the effect of various temperatures on development, survival, fecundity, and infectivity of this nematode.

MATERIALS AND METHODS

The colony of *F. leipsandra* used in this study was reared at the U.S. Vegetable Laboratory, Charleston, South Carolina, according to the methods of Creighton and Fassuliotis (1). It was established in 1987 from local material using *D. balteata* as the host insect. All tests were conducted at various temperatures in growth chambers with an accuracy of ± 1 C and at 14 hours light : 10 hours dark.

Egg development: For each temperature test, newly laid eggs were placed in 10 wells (50–60 per well) of plastic tissue culture plates containing distilled water. Egg hatch was recorded daily. Means and standard deviations were calculated on the basis of variation among wells.

Parasitic stage development: Infectivity tests were carried out at each temperature by exposing first-instar *D. balteata*, previously allowed to feed on newly sprouted wheat seed, to preparasitic nematodes in clay pots (5 cm d) filled with moist soil. A small hole was made in the soil of each pot into which ca. 1,000 preparasites, 100 beetle larvae, and 50 wheat seeds were added. The pots were sealed with Parafilm, enclosed in a plastic crisper, and placed in a growth chamber. After 4–5 days the beetle larvae were dumped from the pots into the

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TABLE 1. Effect of temperature on the rate of development, fecundity, egg hatch, and infectivity of *Filipjevimermis leipsandra*.

	Duration of development (days)						Infection of *Diabrotica* larvae
	Egg stage ± SD	Parasitic stage ± SD	Preoviposition stage ± SD	Total	Eggs laid ± SD ($\bar{x}$)	Egg hatch ± SD (%)	(%)
37 C	10.7 ± 1.1				0.0	7.9 ± 3.9	0.0
35 C	12.6 ± 0.5	12.0 ± 0.0			0.0	64.1 ± 9.7	15.6
32 C	11.9 ± 0.4	12.0 ± 0.0	7.9 ± 1.0	31.8	1,778.3 ± 1,227.3	86.8 ± 5.8	35.7
30 C	12.6 ± 0.3	13.1 ± 0.4	6.6 ± 1.6	32.4	2,520.1 ± 1,192.9	87.8 ± 7.9	78.0
25 C	16.8 ± 0.5	18.1 ± 0.7	11.2 ± 3.5	46.1	3,459.2 ± 837.8	92.9 ± 3.3	14.0
20 C	25.5 ± 0.7	26.8 ± 0.4	15.1 ± 1.2	67.4	2,461.1 ± 403.8	59.0 ± 23.7	9.0
18 C	No hatch				0.0	0.0	0.0
15 C	No hatch				0.0	0.0	0.0

crisper and allowed to feed on the wheat until they were fully grown. The larvae were then transferred to individual plastic jelly cups containing moistened Starblast (a commercial blasting abrasive) and several grains of wheat. Cups were checked daily for pupation or, if infected, emergence of the postparasitic nematode. Means and standard deviations were calculated on the basis of variation among individual larvae.

Preoviposition stage and fecundity: Newly emerged individual postparasitic nematodes were put in each of 20 plastic dishes (9 cm^2) containing 25 ml distilled water and placed at each temperature. Eggs were counted daily until oviposition ceased or the nematode died. Means and standard deviations were calculated on the basis of variation among adults actually laying eggs.

RESULTS AND DISCUSSION

Developmental and survival statistics for the stages of *F. leipsandra* are in Table 1. Warm temperatures, in the 20–32 C range, favored the nematode's rate of development, egg survival, and fecundity. Although unreplicated, the high rate of infection obtained at 30 C, in contrast to higher or lower temperatures, was particularly striking. The thermal maximum of development appeared to be ca. 37 C. Some

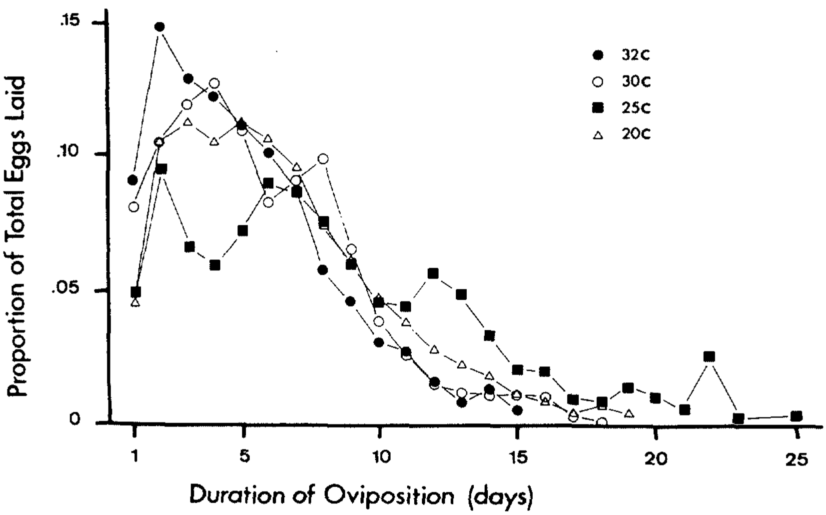

FIG. 1. Proportion of total eggs laid daily by *Filipjevimermis leipsandra* at four temperatures.

TABLE 2. Regression equations for rate of development of stages of *Filipjevimermis leipsandra* in relation to temperature.

	Temperature (C)	Regression equations	*R*
Egg	20–37	−0.0160 + 0.0037X	0.960
Juvenile (parasitic)	20–35	−0.0280 + 0.0032X	0.983
Preoviposition period	20–32	−0.0624 + 0.0064X	0.904
Total development	20–32	−0.0134 + 0.0014X	0.988

Regression equation is $y = A + bX$ where y is reciprocal of the number of days and X is temperature.

eggs hatched at this temperature, but no oviposition or larval infection was observed.

Regression equations of the percentage of development per day are in Table 2. Using these equations to extrapolate minimum thresholds of development was unsatisfactory. Experimentally, no development, oviposition, or infection occurred under 20 C; however, threshold values of 10.2 C, for egg development, 10.5 C for juvenile development, and 13.0 C for the preoviposition period were predicted from these equations. The oviposition curves (Fig. 1) are similar in shape regardless of temperature and show that most eggs were laid during the early part of the oviposition period. While length of the oviposition period varied from 15 days at 32 C to 24 days at 25 C, the number of days by which 50% of the eggs were laid ranged only from 4.5 to 7.

Our results indicate that *F. leipsandra* can be reared successfully at temperatures between 20 and 30 C and also that high infection rates are probable only at warmer temperatures. It is apparent that experiments using *F. leipsandra* as a control agent should be conducted when the soil is warm enough to obtain high infection rates. Long-term storage of eggs or adults at low temperatures should be done with caution, considering the abrupt decline in viability of all stages at temperatures below 20 C. Further tests are necessary to determine the influence of cool storage on stages that are returned subsequently to rearing conditions.

Wallace (5) suggested that nematodes have optimum temperature requirements in the range of 20–30 C and a minimum requirement of 10–15 C. If so, *F. leipsandra* is on the high side of these ranges with an optimum of 25–30 C and a minimum of 20 C. While the geographic range of *F. leipsandra* is not known, the high temperature required for infection and its sensitivity to cool temperatures suggest a tropical or subtropical origin. Thus, it would not be surprising to find that Charleston, South Carolina, is near the northern limit of its range. This should be taken into account if attempts at permanent establishment in new areas are contemplated.

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