THE INVASION, DEVELOPMENT AND REPRODUCTION OF *STEINERNEMA CARPOCAPSAE* (RHABDITIDA: STEINERNEMATIDAE) IN THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: YPONOMEUTIDAE)

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ABSTRACT

Ratnasinghe, G., and N. G. M. Hague. 1998. The invasion, development and reproduction of *Steinernema carpocapsae* (*Rhabditida: Steinernematidae*) in the diamondback moth, *Plutella xylostella* (*Lepidoptera: Yponomeutidae*). Nematropica 28:1-6.

The temperature range for invasion and mortality of diamondback moth (DBM) larvae by *Steinernema carpocapsae* was 20-30°C with an optimum at 25°C. There was a linear relationship between the dosage of infective juveniles of *S. carpocapsae* applied and the number of nematodes entering DBM larvae: the percentage invasion was in the range 10-13% over the dosage tested. Only one generation of *S. carpocapsae* occurred in DBM larvae and the number of juveniles produced was about 1/50 of the number produced in *Galleria mellonella* larvae: stunted female nematodes of *S. carpocapsae* were produced in DBM larvae.

Key words: biological control, diamondback moth, entomopathogenic nematodes.

RESUMEN

Ratnasinghe, G. y N. G. M. Hague. 1998. Invasión, desarrollo y reproducción de *Steinernema carpocapsae* (*Rhabditida:Steinernematidae*) en polilla de la col., *Plutella xylostella* (*Lepidoptera:Yponomeutidae*). Nematropica 28:1-6.

El rango de temperatura para la invasión y mortalidad de la larva polilla de la col. (DBM) por *Steinernema carpocapsae* fue de 20-30°C con óptimo a 25°C. Se observó una relación linear entre la dosis de juveniles infectivos de *S.carpocapsae* aplicados y el número de nematodos entrando en la larva. El porcentaje de invasión estuvo en el rango del 10-13% en relación a la dosis ensayada. Solamente una generación de *S. carpocapsae*, fue producida en la larva y el número de juveniles generados estuvo alrededor del 1/50, en comparación al número producido en la larva *Galleria mellonella*. Nematodos hembras de *S.carpocapsae* con malformaciones, fueron producidos en la larva DBM.

Palabras claves: control biológico, nematodos entomopatógenos, polilla de la col.

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* L., which attacks and damages cruciferous crops in both temperate and tropical countries, has been controlled reliably by chemical insecticides until recently when there has been a buildup of resistance both to conventional insecticides (Sun *et al.*, 1986) and to *Bacillus thuringiensis* Berliner (Tabashinik *et al.*, 1990). Alternative methods of controlling DBM have been proposed including entomopathogenic fungi (Wilding, 1985) and entomopathogenic nematodes (Morris, 1985).

Nematodes in the families Steinernematidae and Heterorhabditidae have been investigated mainly for the control of insects in the soil (Gaugler, 1981; Kaya, 1985). Attempts to use entomopathogenic

nematodes against Lepidoptera on the aerial parts of the plant have met with varied success because they can be inactivated by extremes in the physical environment, particularly on leaves where desiccation (Kamionek *et al.*, 1974), ultra violet radiation (Gaugler and Boush, 1978) and temperature (Molyneux, 1986) are the limiting factors.

Ratnasinghe and Hague (1995) investigated the efficacy of three Steinernematids and one Heterorhabditid against DBM larvae and reported that *Steinernema carpocapsae* Weiser was the most virulent. It was also observed that *S. carpocapsae* required at least 6 h exposure to DBM larvae to ensure 100% mortality. In the present experiments the effect of temperature on invasion of DBM larvae by *S. carpocapsae* was investigated, as well as development and reproduction of the nematode in the larvae of DBM.

MATERIALS AND METHODS

The nematode used in these experiments was *Steinernema carpocapsae* (All isolate) originally from Georgia, USA. It was cultured on late instar larvae of the greater wax moth, *Galleria mellonella* L., and the infective juveniles (IJs) extracted on a modified White trap (White, 1927). IJs were stored in distilled water at 6°C and were less than 2 weeks old when used in experiments.

Development of S. carpocapsae *in larvae of* P. xylostella—*Experiment 1:* Petri-dishes 4.5 cm in diameter were lined with filter paper and inoculated with 100 IJs of *S. carpocapsae* in 0.25 ml distilled water. Ten late instar DBM larvae were placed in each of 10 dishes, and they were covered with another moistened filter paper to prevent DBM larvae escaping or hanging on the lid of the Petri-dish. The space between the two layers of filter paper allowed free movement of the DBM larvae. After exposure for 3 h, DBM larvae were removed, washed in distilled water and placed in nematode-free Petri-dishes lined with moistened filter paper at 24°C. Every 3 h, up to 30 h, one Petri-dish was removed and the DBM larvae dissected to observe the development of the nematodes. The nematodes were mounted in TAF and the body length and greatest body width were measured. Only adult males were measured, because it was noted that many of the females were stunted.

Experiment 2: Ten, fourth instar DBM larvae were placed in Petri-dishes, 4.5 cm in diameter, lined with filter paper and inoculated with 100 IJs of *S. carpocapsae* at 24°C, replication being five-fold. After exposure for 48 h, five larvae from each Petri-dish were placed on White traps and the number of IJs emerging over 10 days counted. The remaining five DBM larvae in each Petri-dish were dissected at 24 h intervals to observe how many females developed in the larvae and to observe how many generations of *S. carpocapsae* occurred in larvae of *P. xylostella.*

Dosage of infective juveniles of S. carpocapsae *and invasion into larvae of* P. xylostella: Petri-dishes (4.5 cm in diameter) were lined with filter paper and inoculated with dosages of 10, 20, 40, 80, and 160 infective juveniles in 0.25 ml distilled water. Five, fourth instar DBM larvae were placed in each dish which was sealed with Parafilm to prevent desiccation and placed in an incubator at 24°C for 48 h, replication being 12-fold. All larvae were dissected to confirm invasion and the number of nematodes invading each larva counted. The data was analyzed using PROC GLM (Procedure General Linear Model), and plotted with the graphic package, Cricket Graph.

Effect of temperature on the establishment of S. carpocapsae *in larvae of* P. xylostella:

Petri-dishes (3.5 cm in diameter) were lined with filter paper and inoculated with 100 IJs of *S. carpocapsae* in 0.15 ml distilled water, and five late instar DBM larvae were placed in each dish which was sealed with Parafilm, replication being six-fold. The dishes were placed on a temperature gradient plate with an overall dimension of 711 × 711 mm (Murdoch *et al.*, 1989). The area of copper plate was sub-divided into 169 (13 × 13) small squares by polystyrene matrix plates, so that each cell contained a single Petri-dish (3.5 cm) which was placed directly on the copper plate. The whole of the polystyrene matrix divisions were covered with a triple-glazed perspex lid to prevent heat loss to the surrounding environment. The temperature range investigated was 15-35°C. After 48 h exposure, the number of dead DBM larvae was recorded, all the larvae were dissected and the number of IJs invading the larvae counted. The data obtained were analyzed using the SAS package.

RESULTS

IJs developed into 4th stage juveniles about 3 h after infecting last instar larvae of *P. xylostella*, and pre-adults started to form after 12 h, the pre-adult stage being the longest stage in the developmental life cycle (Fig. 1). There was a gradual increase in body width of the developing nematodes, with a marked increase after 21 h. The measurements of 24 h were similar to those observed elsewhere for males of *S. carpocapsae*. Only first generation adults of *S. carpocapsae* occurred in DBM larvae.

Among the females of *S. carpocapsae* that developed, stunted females of various sizes were observed in the cadavers and these females produced comparatively smaller IJs when infected DBM larvae were placed on White traps. The new generation of IJs started emerging after 5 days

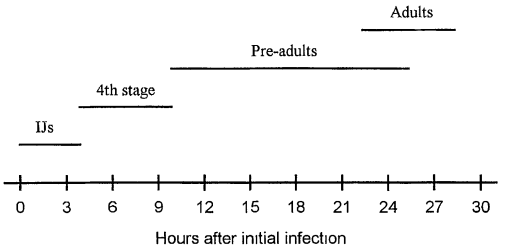

Fig. 1. Schematic diagram for the development of *S. carpocapsae* in last instar larvae of *P. xylostella* after an initial exposure of the DBM larvae to IJs for 3 hours.

and continued to emerge for 5 more days. The mean number of female nematodes observed per DBM larvae was 2.4 and the number of IJs per DBM larvae recovered from the White trap was approximately 4000.

The mean number of IJs invading *P. xylostella* larvae after 48 h exposure was linearly related to the dosage of IJs applied (Fig. 2). The percentage invasion was in the range of 10-13% over the range of dosages tested. The temperature range for infection of DBM larvae by IJs of *S. carpocapsae* was 20-30°C (Fig. 3), the optimum temperature for both mortality and invasion being about 25°C.

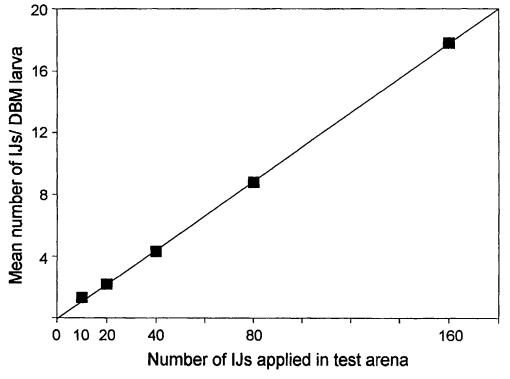

Fig. 2. The relationship between the mean number of IJs of *S. carpocapsae* recovered from last instar larvae of *P. xylostella* and the dosage of IJs applied.
$Y = 0 + 0.1111X$ (no intercept in the model)
$R^2 = 0.9388$

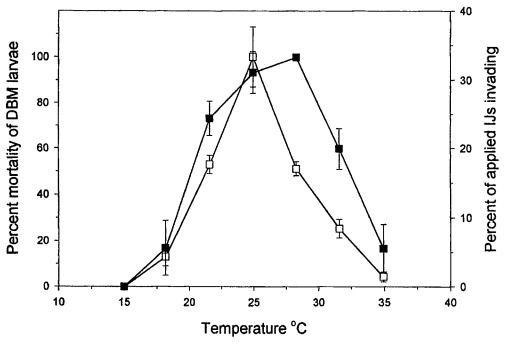

Fig. 3. The relationship between temperature, and the mortality and invasion by IJs of *S. carpocapsae* in last instar of *P. xylostella*: ■% mortality; □% of applied IJs invading, both estimates with SEs.

DISCUSSION

Host size clearly influences the size of the developing Steinernematid. In neonate larvae of the black vine weevil, *Otiorhynchus sulcatus*, stunted female *S. carpocapsae* were observed (Kakouli, 1995), and Gouge and Hague (1995) also reported stunted females of *S. feltiae* in fungus gnats (*Bradysia* spp.). Stunted female nematodes produce smaller IJs, but there is no effect on their pathogenicity to hosts (Gouge and Hague, 1995). In small hosts, only one generation of Steinernematid nematodes occur (Gouge and Hague, 1995; Kakouli, 1995) compared to 2-3 generations in larger hosts such as *G. mellonella* (Otto, 1996). In DBM larvae, only approximately 4000 IJs were produced per host compared to 250 000 in *G. mellonella* (Nyachuru, 1994). However, this is dependent on the size of the host.

The invasion and establishment of an entomopathogenic nematode in a host using a dosage-response assay is useful for comparing the virulence of nematode isolates (Hominick and Reid, 1990). Such assays evaluate all IJs capable of invading and establishing in the host under the conditions of the experiment. In the present experiments, only 10-13% of the *S. carpocapsae* IJs applied actually invaded compared to 35-40% in *G. mellonella* (Otto, 1996).

Table 1. The body width and length of developing *S. carpocapsae* in last instar larvae of *P. xylostella* for 30 h after initial exposure to IJs.

Time (h after exposure)	Body width ± (SE)	Body length ± (SE)
3	33.4 g[z] ± 1.4	634.4 d ± 21.6
6	36.8 g ± 1.2	711.8 c ± 18.9
9	43.8 fg ± 1.8	704.2 c ± 7.4
12	70.2 de ± 3.1	717.0 c ± 19.1
15	63.6 ef ± 4.7	761.4 c ± 15.7
18	90.0 cd ± 3.1	778.6 c ± 36.7
21	112.2 c ± 6.5	775.4 c ± 20.9
24	*254.8 b ± 15.7	*1144.0 b ± 17.9
27	*247.0 ± 4.1	*1349.4 a ± 21.5
30	*322.8 a ± 15.7	*1203.8 b ± 36.9

[z]Means within a column followed by the same letter are not significantly different ($P = 0.05$; DMRT.SAS).
*The measurements recorded are for male nematodes.

Steinernema carpocapsae is a very effective nematode for controlling DBM larvae (Ratnasinghe and Hague, 1995). In the present study, the effective temperature range for invasion and mortality has been shown to be 22-30°C, which is the temperature range at which others have shown *S. carpocapsae* to be most effective (Grewal *et al.*, 1994).

Under field conditions, DBM is difficult to control because the larvae feed firstly as leaf miners and then subsequently on the underside of the leaf surface where they are not easy to control either with conventional insecticides or biological products. Enhanced control of insect larvae on leaves can be obtained by the use of anti-desiccants (Glazer *et al.*, 1992) and optical brighteners (Ratnasinghe, 1996). The results of the present experiments suggest that DBM larvae can be controlled by *S. carpocapsae* at temperatures between 22 and 30°C, which is the temperature range experienced in Sri Lanka where the senior author has done trials with anti-desiccants and optical brighteners. Ratnasinghe (1996) achieved 40-65% control of DBM when nematodes were applied with anti-desiccants on potted cabbage plants. To obtain satisfactory control of lepidopterous pests on leaves, nematodes must be kept moist to retain their effectiveness; therefore, applications should be made immediately after rain and preferably after sundown when the ambient temperature falls, and the relative humidity increases, particularly in the tropics.

LITERATURE CITED

GAUGLER, R., and G. M. BOUSH. 1978. Effects of ultraviolet radiation and sunlight on the entomogenous nematode, *Neoaplectana carpocapsae*. Journal of Invertebrate Pathology 32:291-296.

GLAZER, I., M. KLEIN, A. NAVON, and Y. NAKACHE. 1992. Comparison of efficacy of entomopathogenic nematodes combined with antidesiccants applied by canopy sprays against three cotton pests (Lepidoptera: Noctuidae). Journal of Economic Entomology 85:1636-1641.

GOUGE, D. H., and N. G. M. HAGUE. 1995. The development of *Steinernema feltiae* (Nematoda: Steinernematidae) in the sciarid fly *Bradysia paupera* (Diptera: Sciaridae). Annals of Applied Biology 126:395-401.

GREWAL, P. S., S. SELVAN, and R. GAUGLER. 1994. Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment and reproduction. Journal of Thermal Biology 19:245-253.

HOMINICK, W. R., and A. P. REID. 1990. Prospectives on entomopathogenic nematology. Pp. 327-343 *in* R. Gaugler, and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL, U.S.A.

KAKOULI, T. 1995. Biological control of the black vine weevil, *Otiorhynchus sulcatus* (F) (Coleoptera: Curculionidae) with entomopathogenic nematodes. Ph.D. Thesis. The University of Reading, Reading, U.K.

KAMIONEK, M., I. MASLANA, and H. SANDNER. 1974. The survival of invasive larvae of *Neoaplectana carpocapsae* Weiser in a waterless environment under various conditions of temperature and humidity. Zesz. Probl. Post. Nank Rohn. 154:409-412.

KAYA, H. K. 1985. Entomogenous nematodes for insect control in IPM systems. Pp. 283-302 *in* M. Hoy, and D. C. Herzog, eds. Biological Control in Agricultural IPM Systems. Academic Press, New York, NY, U.S.A.

MOLYNEUX, A. S. 1986. *Heterorhabditis* spp. and *Steinernema* (=*Neoaplectana*) spp.: temperature, and aspects of behavior and activity. Experimental Parasitology 62:169-180.

MORRIS, O. N. 1985. Susceptibility of 31 species of agricultural insect pests to the entomogenous nematodes *Steinernema feltiae* and *Heterorhabditis bacteriophora*. Canadian Entomologist 117:401-407.

MURDOCH, A. J., E. H. ROBERTS, and C. O. GOEDERT. 1989. A model for germination responses to alternating temperatures. Annals of Botany 63:97-111.

MYACHURU, G. 1994. Production of entomopathogenic nematodes in *Spodoptera littoralis* compared with *Galleria mellonella*. M.Sc. Thesis. The University of Reading, Reading, U.K.

OTTO, A. A. 1996. Invasion, establishment and activity of entomopathogenic nematodes at different temperatures. PhD Thesis. The University of Reading, Reading, U.K.

RATNASINGHE, G. 1996. Biological control of diamondback moth, *Plutella xylostella* (Lepidoptera:

Yponomeutidae) with entomopathogenic nematodes (Nematoda: Rhabditida). Ph.D. Thesis. The University of Reading, Reading, U.K.

RATNASINGHE, G., and N. G. M. HAGUE. 1995. The susceptibility of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) with entomopathogenic nematodes. Afro-Asian Journal of Nematology 5:20-23.

SUN, C.N., T. K. WU, J. S. CHEN, and W. T. LEE. 1986. Insecticide resistance in diamondback moth. Pp. 359-371 *in* N. S. Talekar, and T. D. Griggs, eds. Diamondback Moth Management: Proceedings of 1st International Workshop, AVRDC, Shanhuwa, Taiwan.

TABASHINIK, B. E., N. L. CUSHING, N. FINSON, and M. W. JOHNSON. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). Journal of Economic Entomology 83:1671-1676.

WHITE, G. F. 1927. A method for obtaining infective nematode larvae from cultures. *Science* 66:302-303.

WILDING, N. 1985. The pathogens of diamondback moth and their potential for its control. Pp. 329-345 *in* N. S. Talekar, and T. D. Griggs, eds. Diamondback Moth Management: Proceedings of 1st International Workshop, AVRDC, Shanhuwa, Taiwan.

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