Journal of Nematology 24(1):156–159. 1992.

Response of *Steinernema carpocapsae* Infective Juveniles to the Plasma of Three Insect Species[1]

WIROTE KHLIBSUWAN, NOBUYOSHI ISHIBASHI, AND EIZO KONDO[2]

Abstract: Exsheathed infective juveniles of *Steinernema carpocapsae* All strain were attracted to the plasma of three species of insects in agar plate bioassays. Plasma of *Pieris rapae crucivora*, *Spodoptera litura*, and *Agrotis segetum* attracted 88.6%, 80.4%, and 64.4%, respectively, of *Steinernema carpocapsae* juveniles added to plates. Autoclaved plasma of *S. litura* larvae attracted more juveniles than saline controls, but less than nonautoclaved plasma. The active agent passed through a 14,000 MW dialysis membrane.

Key words: attraction, nematode, plasma, *Steinernema carpocapsae*.

Nematode attraction to hosts, food sources, and chemical stimuli has been observed for nematodes from different habitats. The entomopathogenic nematode, *Steinernema carpocapsae,* responds positively to CO_2 (5), insect body temperature (1,2), the mutualistic bacterium *Xenorhabdus nematophilus* (7,9), insect fecal components (8,11), and the insect host (6,12). This investigation reports on the attraction of *S. carpocapsae* to plasma from three species of host insects.

MATERIALS AND METHODS

Steinernema carpocapsae All strain was continuously cultured in *Galleria mellonella* larvae (4). Two weeks after the death of insects by infection with nematodes, test tubes containing the insect cadavers on a filter paper strip wetted with 0.01% formalin were stored at 6–10 C for 2 months or less. Most of the infective juveniles exsheathed during this storage. In preliminary bioassays, this procedure appeared to enhance juvenile response to insect plasma; the response was 20% at most for ensheathed juveniles, compared to more than 80% for exsheathed ones. Before applying juvenile nematodes to agar plates, they were washed with distilled water and allowed to move through a nylon mesh screen (32 μm) to recover active worms. The juveniles were then washed three times in phosphate buffered saline (PBS) (20 mM potassium phosphate, 150 mM NaCl, pH 7.2) (3).

Full-grown last-instar larvae of three host insects were used: the cabbage worm *Pieris rapae crucivora,* the cutworm *Spodoptera litura,* and the turnip moth *Agrotis segetum.* Insect hemolymph, obtained by puncturing the second prolegs, was centrifuged at 5,000*g* for 5 minutes (4 C) to remove hemocytes. A few crystals of phenylthiourea were added to the supernatant to prevent melanization; the supernatant was stored at −70 C until use.

Attraction test: A 4-mm-thick layer of 0.6% agar in PBS was prepared in each petri dish (45 mm d). Two wells (5 mm d, 2 mm deep) were made in the agar on opposite sides and 1 cm from the central inoculation well (2 mm d, 2 mm deep). Fifty microliters of insect plasma were applied in one side well and the same volume of PBS in the opposite well (control). To make comparisons between plasma of different insect species, the same volume of plasma was applied in opposite side wells. One microliter of PBS containing 30 infective juveniles was applied in the inoculation well 2 hours after addition of solutions to the side wells. After the nematodes were allowed to move for 2 hours at 25 C in the dark, the plates were placed in a freezer at −20 C for 5–10 minutes to stop nematode movement. The number of nematodes in

Received for publication 29 January 1991.

[1] Supported by Grant-in-Aid for Developmental Scientific Research, Ministry of Education, Culture and Sciences, Japan, Grant No. 02506001.

[2] Graduate student, Professor, and Associate Professor, Department of Applied Biological Sciences, Saga University, Saga 840, Japan.

The authors thank Dr. S. Tojo of Saga University for technical advice.

the plasma well, the PBS well, the inoculation well, and outside of the wells in the petri dish were counted and expressed as percentages. The number of nematodes in the plasma well was recorded as the attractive response of nematodes. Each treatment was conducted with four replications. The experiment was performed three times.

Properties of active agents: To test the thermal stability of plasma attractants, *S. litura* plasma was autoclaved (120 C) for 20 minutes at 3.3 lb/in^2 and examined for its attractiveness using PBS or nonautoclaved intact plasma in opposite wells. Four replicates were done for each treatment, and the experiment was performed three times.

To estimate the molecular size of active components, *S. litura* plasma was dialyzed through a cellulose membrane (cut-off substances with MW less than 14,000) against distilled water at 4–6 C for 48 hours. After dialysis, the outer dialysate fraction was evaporated and condensed under reduced pressure to the same volume as the inner dialysate fraction. The activity of inner dialysate fraction was assayed against the outer dialysate fraction or the nondialysed plasma. Each treatment was conducted with four replications. The experiment was performed three times.

Statistical analyses: Data expressed in percentages were transformed to arc sine to ensure normality. The single-classification ANOVA test and Duncan's multiple-range test were employed for the statistical analyses.

Results

Attraction to plasma: Exsheathed infective juveniles of *S. carpocapsae* were attracted to the insect plasma, although some remained in the central inoculated zone. All plasma tested were significantly more attractive than PBS; attraction rates to the plasma of *P. rapae crucivora, Spodoptera litura,* and *A. segetum* were 88.6%, 80.4%, and 64.4%, respectively (Table 1). The difference between the *A. segetum* plasma and plasma of the other two species was significant ($P < 0.01$). The proportion of nematodes wandering outside the two wells were 9.6%, 14.8%, and 19.0% with the plasma of *P. rapae crucivora, S. litura,* and *A. segetum,* respectively. These differences were significant ($P < 0.05$). When two kinds of insect plasma were placed in opposite wells, the nematodes were attracted ($P < 0.01$) to plasma of *P. rapae crucivora* > *S. litura* > *A. segetum* (Table 2).

Table 1. Migration of *Steinernema carpocapsae* All strain infective juveniles toward plasma of *Pieris rapae crucivora, Spodoptera litura,* and *Agrotis segetum* larvae on agar plates

Test plasma	Average ± SE
P. rapae crucivora	88.6 ± 1.1 a
Inoculation zone	1.8 ± 0.7 b
PBS	0.0 ± 0.0 c
Outside above zones	9.6 ± 0.7 d
S. litura	80.4 ± 2.2 a
Inoculation zone	4.3 ± 0.4 bf
PBS	0.5 ± 0.1 c
Outside above zones	14.8 ± 2.0 d
A. segetum	64.4 ± 1.6 e
Inoculation zone	15.2 ± 1.8 f
PBS	1.4 ± 0.6 c
Outside above zones	19.0 ± 1.9 d

Values followed by the same letter are not different ($P = 0.01$) according to Duncan's multiple-range test. Averages and standard errors are mean percentages of three trials with four replications each.

Table 2. Migration of *Steinernema carpocapsae* infective juveniles to insect plasma of *Pieris rapae crucivora, Spodoptera litura,* and *Agrotis segetum* placed on opposing sides of agar plates.

Test plasma	Average ± SE
P. rapae crucivora	76.5 ± 2.4 a
S. litura	11.7 ± 1.6 b
Inoculation zone	0.0 ± 0.0 c
Outside above zones	11.8 ± 1.5 b
P. rapae crucivora	80.2 ± 2.8 a
A. segetum	11.5 ± 1.5 b
Inoculation zone	0.0 ± 0.0 c
Outside above zones	8.3 ± 1.5 b
S. litura	50.0 ± 1.9 a
A. segetum	21.5 ± 2.0 b
Inoculation zone	8.1 ± 0.8 c
Outside above zones	20.4 ± 2.0 b

Values followed by the same letter in each set are not different ($P = 0.01$) according to Duncan's multiple-range test. Averages and standard errors are mean percentages of three trials with four replications each.

Properties of attractive agents: Heat treatment of *S. litura* plasma by autoclaving significantly decreased its attractiveness, but relatively high activity still remained (Table 3); 59.3% of the nematodes were attracted to autoclaved plasma when assayed against PBS.

The active agents in the insect plasma passed through a cellulose membrane (cutoff MW < 14,000) after dialysis for 48 hours against distilled water. Attraction rates to the outer dialysate fraction and to the inner dialysate fraction present on the same plate were 77.3% and 6.7%, respectively (Table 4). No nematodes were attracted to the inner dialysate fraction in the tube after dialysis when assayed against the nondialysed plasma of *S. litura.*

Discussion

The present experiments demonstrated that *S. carpocapsae* infective juveniles were attracted to the host insect plasma, although the degree of the attraction was different among insect species. Of the three species examined, the cabbage worm plasma was significantly more attractive than plasma of cutworm and turnip moth larvae. This finding agrees with laboratory and field tests, showing that the cabbage worm is more susceptible than the other two insects to *S. carpocapsae* (12).

Table 3. Migration of *Steinernema carpocapsae* infective juveniles toward autoclaved plasma of *Spodoptera litura* larvae.

Test plasma	Average ± SE
Test 1	
Autoclaved	59.3 ± 2.6 a
Inoculation zone	16.2 ± 1.6 b
PBS	1.0 ± 0.5 c
Outside above zones	23.5 ± 1.8 b
Test 2	
Autoclaved	20.3 ± 3.1 a
Nonautoclaved	52.2 ± 3.3 b
Inoculation zone	10.0 ± 0.7 a
Outside above zones	17.5 ± 2.0 a

Values followed by the same letter in each set are not different ($P = 0.01$) according to Duncan's multiple-range test. Averages and standard errors are mean percentages of three trials with four replications each.

Table 4. Migration of *Steinernema carpocapsae* infective juveniles toward dialysed plasma of *Spodoptera litura* larvae.

Test plasma	Average ± SE
Inner dialysate	0.0 ± 0.0 a
Nondialyzed	76.6 ± 2.0 b
Inoculation zone	5.3 ± 1.4 a
Outside above zones	18.1 ± 1.6 c
Inner dialysate	6.7 ± 1.1 a
Outer dialysate	77.3 ± 2.4 b
Inoculation zone	0.0 ± 0.0 c
Outside above zones	16.0 ± 2.1 d

Values followed by the same letter in each set are not different ($P = 0.01$) according to Duncan's multiple-range test. Averages and standard errors are mean percentages of three trials with four replications each.

The active agent(s) in the host insect plasma is(are) not thermally stable, because attraction to autoclaved *S. litura* plasma was significantly lower than to nonautoclaved plasma. The molecular weight of the attractant(s) was less than 14,000, because it was small enough to pass through a cellulose membrane during dialysis against distilled water. Purification and characterization of the attractant(s) in the plasma should help to explain the differential response of the nematode to plasma of different insect species.

Infective juveniles of *S. carpocapsae* are attracted to an aqueous surface wash of larvae of the greater wax moth, *Galleria mellonella* (10), suggesting that attraction is due to chemical gradients excreted or emanated from the host insect body. Further studies are needed to determine how the attractive agent(s) in the plasma of the host reach its body surface.

Literature Cited

1. Burman, M., and A. E. Pye. 1980. *Neoaplectana carpocapsae:* Movements of nematode populations on a thermal gradient. Experimental Parasitology 49:258–265.

2. Byers, J. A., and G. O. Poinar, Jr. 1982. Location of insect hosts by the nematode, *Neoaplectana carpocapsae,* in response to temperature. Behavior 79:1–10.

3. Dunphy, G. B., and J. M. Webster. 1987. Partially characterized components of the epicuticle of dauer juvenile *Steinernema feltiae* and their influence on hemocyte activity in *Galleria mellonella.* Journal of Parasitology 73:584–588.

4. Dutky, S. R., J. V. Thompson, and G. E. Cantwell. 1964. A technique for the mass propagation of the DD-136 nematode. Journal of Insect Pathology 6:417–422.

5. Gaugler, C. D., L. Lebeck, B. Nakagaki, and G. M. Boush. 1980. Orientation of the entomogenous nematode *Neoaplectana carpocapsae* to carbon dioxide. Environmental Entomology 9:649–652.

6. Gaugler, R., J. F. Campbell, and T. R. McGuire. 1989. Selection for host-finding in *Steinernema feltiae.* Journal of Invertebrate Pathology 54:363–372.

7. Ishibashi, N., and E. Kondo. 1990. Behavior of infective juveniles. Pp. 139–150 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

8. Kondo, E. 1989. Studies on the infectivity and propagation of entomogenous nematodes, *Steinernema spp.,* (Rhabditida: Steinernematidae), in the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). Bulletin of Faculty of Agriculture, Saga University, Saga 840, Japan 67;1–88.

9. Pye, A. E., and M. Burman. 1981. *Neoaplectana carpocapsae:* Nematode accumulations on chemical and bacterial gradients. Experimental Parasitology 51:13–20.

10. Schmidt, J., and J. N. All. 1978. Chemical attraction of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) to insect larvae. Environmental Entomology 7:605–607.

11. Schmidt, J., and J. N. All. 1987. Attraction of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae) to common excretory products of insects. Environmental Entomology 8:55–61.

12. Tojo, S., Y. Nishimoto, M. Noda, and N. Ishibashi. 1987. Physiological analysis of different infectivities of *Steinernema feltiae* (str. DD-136) to the larvae of two lepidopteran insects. Pp. 133–138 *in* N. Ishibashi, ed. Recent advances in biological control of insect pests by entomogenous nematodes in Japan. Research Project on Biological Control, Ministry of Education, Japan. Saga University, Saga 840, Japan. (In Japanese, with English summary.)