Infection of the Cat Flea, Ctenocephalides felis (Bouche) by Neoaplectana carpocapsae Weiser

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Abstract: Infection of cat flea, *Ctenocephalides felis,* larvae by the entomophilic nematode *Neoaplectana carpocapsae* was accomplished in the laboratory. The Breton strain of *N. carpocapsae* provided higher larval mortality at lower dosages than did the DD-136 strain. Adult nematodes were evident in the insect hemocoel after 48 h; however, no infective third-stage larvae were produced. Larval flea infection increased with an increase in the moisture content of sand from 2% to 7% and of sandy clay from 7% to 12%. Larval flea infection was also obtained on turf containing dauer larvae. Nematode penetration of cocoons with invasion of prepupal and pupal fleas was apparent. *Key words:* entomogenous nematodes, biological control.

Journal of Nematology 14(3):394-397. 1982.

The cat flea, *Ctenocephalides felis* (Bouchè), is a cosmopolitan pest of man and domestic pets but also attacks many feral mammals. In addition to being annoying, flea bites may cause localized allergic reaction in hosts (2). Of greater medical importance, *C. felis* is capable of transmitting plague, murine typhus, and dog tapeworm, *Dipylidium caninum,* to man and animals (10). Control of *C.* felis currently involves treatment of pets and their indoor and outdoor habitats with chemical insecticides.

The entomophilic nematode, *Neoaplectana carpocapsae* Weiser, was first isolated and described independently by Weiser (11) and Dutky and Hough (1) from codling moth larvae. Poinar (7) lists 250 insect species comprising 10 orders which have been infected by *N. carpocapsae.* The order Siphonaptera was not included in this host listing.

The present laboratory study was undertaken to determine the susceptibility of *C. felis* to infection with *N. carpocapsae* and to assess the potential of *N. carpocapsae* as a biological control agent for larval fleas on natural substrates.

MATERIALS AND METHODS

Fleas in all experiments were obtained from a stock culture maintained as described in Silverman et al. (9). The DD-136 and Breton strains of *N. carpocapsae* were obtained from Dr. H. Kaya at the University of California, Davis, and reared in late-instar larvae of the cabbage looper, *Trichoplusia ni* (Hüber). Dauer larvae recovered 8–14 days postinfection were stored at 5 C in 50-ml flasks containing 5 ml of distilled water. Since dauer larval mortality exceeded 50% after 4 wk of storage, individuals were used for experiments prior to this time.

A bioassay was developed to provide moisture adequate for nematode survival but not deleterious to flea larvae, since flea larvae quickly succumb when exposed to free water. Moist filter paper (Whatman No. 42, 7-cm-d) was placed on a sintered glass funnel attached to a suction flask. Dauer larvae suspended in 2 ml distilled water were added to each of four plexiglass cylinders (2.5-cm-d × 7.5 cm) pressed against the filter paper. Suction was applied until the water in each tube was no longer visible. A layer of sand one grain thick was placed at the bottom of each cylinder and 10 third-instar flea larvae were added. The sand prevented the larvae from making continuous direct contact with the moist paper, thus providing 100% survival on uninoculated substrates. The cylinders were held against the filter paper by the weight of a rubber stopper to prevent larvae from escaping. The cylinders and inoculated paper were then placed in water-saturated air at 22 ± 1 C. After a 24-h exposure period, mortality among the flea larvae was assessed and the larvae were dissected to determine the level of infection.

The Breton strain of *N. carpocapsae* was tested against larval fleas on soil and turf. Ten grams of sand or sandy clay (47% sand, 16% silt, and 37% clay) at 0, 5, 10, or 20% moisture content were placed in individual glass vials (2.5-cm-d × 7 cm) to a depth of

Received for publication 10 August 1981.

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The authors thank D. A. Reierson and M. S. Mulla for their critical review of this mansuscript.

about 3 cm. Ten third-instar flea larvae were placed in each vial 30 min prior to nematode exposure. Three replicates were performed for each soil type and soil moisture. Dauer larvae were then added to each vial in 50-μl droplets of distilled water increasing the moisture in each vial by 2%. Turf was also provided as a substrate for flea larvae and *N. carpocapsae*. Plugs of hybrid Bermuda var. Tilgreen turf (ca. 3 cm tall) were pressed into individual plastic vials (3-cm-d × 8 cm) containing a 2-cm layer of sand. Ten flea larvae were added to each vial and 1 h later dauer larvae were applied on the turf with a DeVilbiss (No. 152) fine mist sprayer. Each inoculum level (0, 60, 125, or 500 nematodes per container; three replications) was tested on separate days; as a consequence the turf moisture varied for each inoculum. Soil moisture of single uninoculated plugs of turf was determined from the wet and dry weights of soil removed from the roots of the turf. The effect of increased moisture content was tested by adding 2 ml water to turf plugs in three additional experimental groups (0, 60, and 125 nematodes). Excess water was allowed to drain before flea larvae were introduced. Each vial was covered with a piece of paper towel held in place with a rubber band to prevent larval fleas from escaping. Following a 24-h exposure period, all living flea larvae were placed on dry uninoculated sand and held an additional 24 h at 22 ± 1 C and 75% RH to determine latent mortality.

RESULTS AND DISCUSSION

The Breton strain of *N. carpocapsae* was significantly more pathogenic to flea larvae than the DD-136 strain (Fig. 1). The median lethal densities for the Breton and DD-136 strains were 6.5 ± 1 and 88 ± 1.6 dauer larvae/cm^2, respectively. The number of fourth-stage nematodes in a dead host 24 h after exposure ranged from 1 to 180. Although first-generation adult nematodes were evident within 2 days, no further development was observed in host cadavers observed for up to 8 days, presumably as a result of nutrient depletion and loss of host integrity.

Oviposition by *C. felis* occurs while on the host animal. The eggs have a non-sticky

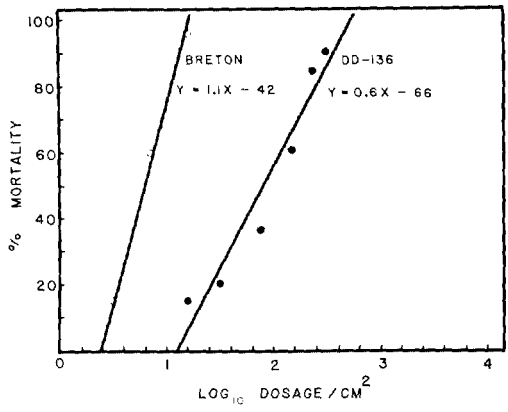

Fig. 1. Mortality of *Ctenocephalides felis* larvae following a 24-h exposure to the Breton and DD-136 strains of *Neoaplectana carpocapsae*.

smooth chorion which facilitates easy removal from the host. Consequently, the substrate upon which larvae develop depends on the location of the host when the eggs fall from the host. Cat flea development may occur both in and around human dwellings. The high moisture requirements of *N. carpocapsae* dauer larvae would preclude its use indoors, but soil and turf may provide the necessary moisture requirements for dauer larvae and cat flea larvae. Larvae of *C. felis* survived in dry soil when ambient RH was 50% or greater (9). Cat flea larvae survived at 12% RH when soil moisture was 1–20%, but high mortality increased considerably with higher soil moisture (Silverman & Rust, unpublished). Moore (4) observed that DD-136 dauer larvae survived at least 24 days in moist soil and 20 days when the soil was dried slowly at 70% RH.

By increasing the moisture of sand from 2 to 7%, flea larval mortality increased significantly at inoculum levels of 250 and 60 nematodes (Table 1). There was considerable variation at all inoculum levels contributing to the insignificant difference between larval mortality of 2% and 7% sand moisture when exposed to 125 nematodes. At 2% moisture most flea larvae and dauer larvae were isolated, fleas being 0.5–2.0 cm below the soil surface while the nematodes were on the surface probably unable to disperse within this dry environment. After 24 h no living nematodes were observed. When the moisture content was increased to 7%, water filled the interstitial soil spaces, forcing flea larvae to the surface.

Table 1. Mortality of *Ctenocephalides felis* larvae infected by the Breton strain of *Neoaplectana carpocapsae* on sand and sandy clay substrates with varying moisture contents.

Substrate	Inoculum	% Mortality at various moisture levels*			
		2%	7%	12%	22%
Sand	250	13 ± 12 b	83 ± 12 a	90 ± 17 a	90 ± 10 a
	125	13 ± 15 b	66 ± 12 ab	76 ± 21 a	60 ± 10 ab
	60	0 b	70 ± 10 a	63 ± 6 a	43 ± 31 a
	0	0	0	0	15 ± b
Sandy clay	250	0 c	30 ± 27 b	93 ± 6 a	100 a
	125	7 ± 6 b	40 ± 20 b	93 ± 12 a	100 a
	60	3 ± 6 b	7 ± 6 b	66 ± 32 a	76 ± 15 a
	0	0	0	0	7 ± 6

*For each inoculum (rows), means ± S.D. followed by different letters are significantly different ($P \leq 0.05$) with Duncan's multiple-range test after arc sin transformation.

The added moisture also facilitated nematode movement resulting in greater host-parasite contact. Soil moisture increases to 22% did not significantly change the mortality. Soil moistures above 22%, however, caused greater than 20% mortality of control larval fleas.

Flea larval mortality increased significantly when the soil moisture of sandy clay was increased from 7% to 12%. Although nematodes were viable on 7% moist sandy clay after 24 h, flea larval infection was low. Unlike sand, silt and clay particles have a high affinity for water molecules. When water adheres to these particles at low soil moisture levels, the interstitial soil spaces are left unfilled. Consequently, flea larvae were not forced to the surface where they would encounter nematodes. Infection of 30–40% of the flea larvae at 7% soil moisture indicates that some flea larvae migrated upwards and/or nematodes dispersed beneath the soil surface, thereby coming into contact. However, DD-136 shows little tendency for downward migration when placed on the surface of sand or soil (5,8). At 12% or 22% moisture, the soil became compacted impeding flea larval penetration below the soil surface and thereby rendering them susceptible to nematode attack. The only significant mortality differences between the highest and the lowest inocula on either soil type occurred on substrates containing 22% moisture.

When nematodes were sprayed on turf in large numbers (500 or 250 dauer larvae), most hosts died after 24 h (Table 2). Soil moisture varied considerably in these tests and flea larval mortality was directly related to numbers of nematodes. Mortality of control flea larvae was increased by the addition of water. Although much greater host mortality occurred with as few as 60 dauer larvae on bare soil (Table 1), the reduced effectiveness of the dauer larvae in this experiment (Table 2) was probably a consequence of the increased surface area of the turf. We observed that in the experimental group

Table 2. Percent mortality of *Ctenocephalides felis* larvae infected with the Breton strain of *Neoaplectana carpocapsae* on Bermuda turf, with and without added water.

Inoculum	No water added		2 ml water added	
	% moisture	% mortality*	% moisture	% mortality*
500	6	100	...	...
250	10	97 ± 5	...	...
125	4	53 ± 21	14	73 ± 12
60	12	17 ± 21	15	20 ± 6
0	12	0	17	10 ± 10

*Average of three replications ± S.D. Mortalities at same inoculum ± water addition were compared statistically by means of Student's *t*-test by means were not significantly different at $P \leq 0.05$.

with added water, flea larvae moved on the soil surface and onto the blades of grass. But this did not alter the effectiveness of the parasite-host contact since there were no significant differences between equivalent inocula.

After completing its development, the larva of *C. felis* voids its gut then spins a silken cocoon adorned with bits of substrata. Within the cocoon the larva assumes a U-shaped posture termed the prepupa. In 4 days the exarate pupa is evident. Since larva and cocoons of *C. felis* are found in similar environments, the potential for prepupal and pupal infection with *N. carpocapsae* was also considered. When removed from cocoons and placed on moist sand containing 1,000 Breton strain dauer larvae, all the prepupae (n = 30) and 73% of the pupae (n = 30) became infected within 24 h. All prepupae and 60% of prepupae in cocoons were infected also showing that dauer larvae were able to penetrate this "protective" structure. Recent studies by Kaya's group (3,6) showed that silken cocoons of many lepidopterous species were ineffective barriers to invasion by *N. carpocapsae*.

In conclusion, *C. felis* larvae and pupae are susceptible to infection by *N. carpocapsae*. If applied to a moist substrate nematode, dauer larvae may survive to infect more than one generation of fleas. However, since fleas do not support complete nematode development and *N. carpocapsae* is an obligate parasite, continuous control would probably require repeated applications of dauer larvae.

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