Journal of Nematology 16(1):9–13. 1984.

Effect of the Entomogenous Nematode *Neoaplectana carpocapsae* on the Tachinid Parasite *Compsilura concinnata* (Diptera: Tachinidae)[1]

HARRY K. KAYA[2]

Abstract: The entomogenous nematode *Neoaplectana carpocapsae* and its associated bacterium, *Xenorhabdus nematophilus,* could not infect the pupal stage of the tachinid *Compsilura concinnata* through the puparium. *N. carpocapsae* had an adverse effect on 1-, 2- and 3-day-old *C. concinnata* larvae within the armyworm host in petri dish tests. All 1-day-old larvae treated with nematodes died in their hosts, whereas 61% and 69% of 2- and 3-day-old larvae treated with nematodes, respectively, died. However, the survivors developed to adults. Nine to thirty-seven percent of adult tachinids which emerged from nematode-treated soil (50 nematodes/cm^2) were infected with *N. carpocapsae.* The nematode adversely affects *C. concinnata* directly by the frank infection of the tachinid and indirectly by causing the premature death of the host which results in tachinid death.

Key words: interaction between parasite and nematode, biological control, insect–nematode interaction.

The tachinid *Compsilura concinnata* parasitizes a large number of lepidopterous insects (1). Its biology has been studied by Culver (3) and Fusco et al. (5). The adult is larviparous, and more than one larva may emerge from a host. The parasitized host becomes moribund in 3–4 days, but the tachinid larva does not emerge until the 7th day. After emergence, the larva forms a puparium in the soil or on any convenient surface such as silken webs or bark crevices. Adult flies emerge 9–12 days after pupation.

The entomogenous nematode *Neoaplectana carpocapsae* (= *Steinernema feltiae*) (see 13), along with its associated bacterium, *Xenorhabdus nematophilus,* infects a wide variety of insects, including many pest species (11,12). The impact of this nematode-bacterium complex on beneficial parasitic insects has been examined only recently by Kaya (6,7) and Kaya and Hotchkin (10). They showed that *N. carpocapsae* infects and kills the lepidopterous host, resulting in the death of the larval stages of certain hymenopterous parasites. The pupal stages of the hymenopterous parasites *Glyptapanteles militaris* (= *Apanteles militaris*), *Cotesia medicaginis* (= *Apanteles medicaginis*), *Hyposoter exiguae,* and *Chelonus* spp., however, are resistant to nematode infection because the nematode cannot penetrate the nonporous, inner silken layer of the cocoon (10). Laumond et al. (11) found that adults of the tachinid *Metagonistylum mi-*

Received for publication 24 January 1982.

[1] I thank Dr. Ronald M. Weseloh, Connecticut Agricultural Experiment Station, New Haven, and Mr. Robert Chianese, New Jersey Department of Agriculture, Trenton, for providing *Compsilura concinnata*; Mr. Hugh Lothrop, University of California, Davis, for technical assistance; Dr. R. A. Bedding, CSIRO, Tasmania, Australia, for providing an advance copy of his manuscript, and Dr. R. C. Reardon, U.S. Forest Service, Davis, California, and Dr. Randy Gaugler, Rutgers University, New Brunswick, New Jersey, for critically reading the manuscript. Supported in part by USDA Grant 5901-0410-0268 and CDFA grant 8557.

[2] Division of Nematology, University of California, Davis, CA 95616.

nense are highly susceptible to the nematode-bacterium complex in the laboratory. The effect of this nematode-bacterium complex on immature tachinid parasites has not been previously examined. I report herein the interactions between the immature stages of *C. concinnata* and *N. carpocapsae* and between emerging adult tachinids in soil and *N. carpocapsae.*

Materials and Methods

N. carpocapsae, originally isolated by Dr. John All, University of Georgia, Athens, was propagated in *Galleria mellonella* larvae and harvested as described by Dutky et al. (4). Nematodes were held at 10 C at a concentration of 1,000 nematodes/ml and dilutions made as needed.

The colony of *C. concinnata* was maintained as follows: Tachinid adults were held in screen cages, cubes of 30 cm on each side, and fed sugar and water and undiluted honey. Ten 6th-stage larvae of the armyworm, *Pseudaletia unipuncta* (ratio ca. one host to two female flies), were introduced into the cage and removed 24 hours later. Armyworm hosts were introduced into the cages for 7 consecutive days. Hosts were held in petri dishes (100 × 15 mm) and fed artificial diet. Dead hosts were transferred onto sterilized sand for larval emergence and pupation.

The Dutky method of exposing insects to nematodes, as modified by Kaya and Hara (9), with a nematode concentration of 200 nematodes per tachinid, was used in all petri dish tests. One-day-old puparia were exposed individually to the nematodes for 48 hours, washed in distilled water, and held in 39-ml vials containing moist vermiculite until adults emerged. Puparia from which flies did not emerge were dissected and examined for nematodes. Control puparia were treated with distilled water. There were two trials with 22 and 30 puparia in the nematode treatments in trials 1 and 2, respectively, and 11 puparia in the control treatments for both trials. Newly-emerged tachinid larvae from the host were also exposed to the nematodes as described above. There were three trials with 10 larvae in each trial. Finally, armyworm hosts parasitized by tachinids for 1–3 days were exposed individually to nematodes in petri dishes. After 48 hours the armyworm hosts were rinsed in distilled water and placed into moist vermiculite. The number of dead and live tachinid larvae within the armyworm host, dead tachinid larvae and pupae with or without nematodes, and tachinid adults were recorded. *P. unipuncta* hosts were examined for the presence of developing nematodes 7 days after exposure to the infective nematodes. Eight tachinid-parasitized *P. unipuncta* were exposed to nematodes for the 1-day-old group, 30 for the 2-day-old group, and 21 for the 3-day-old group. Tachinid-parasitized armyworms treated with distilled water served as controls. Chi-square analysis was used to show differences between treatments.

Infectivity of *N. carpocapsae* to larvae, pupae within puparia, and emerging adults of *C. concinnata* in soil was tested in plexiglass tubes as described by Kaya and Grieve (8). Briefly, each tube (5 cm height × 4.4 cm inner diameter) was filled with moist sterilized soil (76.8% sand, 15.2% silt, 8% clay). A second tube was placed over the first and joined with masking tape; the top was covered with a petri dish half (55 × 13 mm). Two ml of nematode suspension was added to the soil to provide a concentration of 50 nematodes/cm^2 of surface. Soil to which distilled water was added served as controls.

In the first test, a moribund parasitized armyworm host was placed on the soil. Nematodes were added to the soil 7 days after the first tachinid larva emerged from the host. Emergence of adult tachinids was monitored for 5 days after the first flies emerged. Adult flies were held individually in test tubes (16 × 150 mm) and fed undiluted honey. Each test tube was plugged with moist cotton which served as a source of water for the fly. Flies dead within 48 hours after emergence were held an additional 5 days, dissected, and examined for infecting nematodes. The soil was examined for emerged and nonemerged puparia 8 days after the first fly had emerged, and the depth at which pupation occurred was recorded. Nonemerged puparia were dissected and pupae examined for nematodes. There were two trials with seven armyworms per trial per treatment.

In the second test, five 5-day-old puparia were placed 1 cm below the soil surface and nematodes were added to the soil 2

TABLE 1. Effect of exposure of *Pseudaletia unipuncta* larvae parasitized for 1, 2, or 3 days by the tachinid *Compsilura concinnata* to 200 infective *Neoaplectana carpocapsae*.

	No. *P. unipuncta* larvae	Tachinids/host $\bar{x} \pm SD$	Total no. tachinids	% tachinids dead with nematode		% tachinids dead without nematode		% adult tachinids
				Larvae	Pupae	Larvae	Pupae	
1-day-old tachinid								
Control	10	2.8 ± 1.8	28			14.3	10.7	75.0
Nematode	8	2.6 ± 1.7	21	28.6	0	71.4	0	0
2-day-old tachinid								
Control	38	3.3 ± 2.2	127			11.0	3.9	85.1
Nematode	30	3.2 ± 2.1	97	18.6	4.1	35.0	3.1	39.2
3-day-old tachinid								
Control	15	5.6 ± 3.7	84			3.6	11.9	84.5
Nematode	21	4.8 ± 3.8	101	5.9	2.0	48.5	12.9	30.7

days later. Adult flies and nonemerged puparia were monitored as in the first test. There were two trials with 15 puparia per treatment. In the third test, nematodes were added to the soil 3 days before the first tachinid larva was expected to emerge. A 4-day-old parasitized moribund host was suspended ca. 1.5 cm above the soil by a piece of 1.2-mm² mesh hardware cloth (3 × 3 cm) to prevent nematodes from invading the host. Adult flies and nonemerged puparia were handled as in the first test. There were two trials with seven armyworms per trial per treatment.

RESULTS

The combined results of two trials of the petri dish test showed that tachinids in 1-day-old puparia were not susceptible to nematode infection at the concentration of nematodes tested. In the control 73.9% and in the nematode treatment 80.9% of the puparia produced adults. None of the tachinids that died in the pupal stage were infected with nematodes. One of the 30 emerged tachinid larvae was nematode-infected, one died of unknown causes, and the remainder (93.3%) pupated and emerged as adults. The one infected as a larva formed a puparium containing an adult female nematode. The majority (90%) of the 30 control tachinids pupated and emerged as adults, while the other 10% died as pupae within the puparia from unknown causes.

The development of 1–3-day-old *C. concinnata* larvae in armyworm hosts in the petri dish test was adversely affected by nematodes (Table 1). All 1-day-old nematode-treated tachinid larvae died within their hosts, with 29% of the tachinid larvae in these hosts infected with the nematode at the time of host dissection. Of the 2-day-old nematode-treated tachinids, 61% died as larvae or pupae, with 22.7% of them infected with *N. carpocapsae*. Forty-five of 97 tachinids (46%) were dead in the nematode-treated hosts compared to 5 of 127 (4%) in the controls. Forty-six of 54 tachinids (85.2%) from nematode-infected hosts died as larvae, while eight (14.8%) formed puparia, six of them producing adults. Of the 3-day-old nematode-treated tachinids, 69% died as larvae and pupae with 8% of the tachinid larvae and pupae infected with *N. carpocapsae*. Fifty-five of 101 tachinids (54.4%) were dead inside the nematode-treated hosts. In contrast, 1 of 84 (1.2%) died inside control hosts. There was no significant difference in adult emergence between 2- and 3-day-old nematode-treated tachinids (χ^2 test, $P > 0.10$).

N. carpocapsae could utilize some of the tachinid-parasitized lepidopterous insects as hosts. *P. unipuncta* larvae containing 1-day-old or 2-day-old tachinids at the time of exposure to *N. carpocapsae* showed that 75% (n = 8) and 50% (n = 30) of the *P. unipuncta* larvae, respectively, supported nematode reproduction. None of the *P. unipuncta* larvae containing 3-day-old tachinids supported nematode reproduction when exposed to *N. carpocapsae*.

Nematodes placed on soil infected tach-

TABLE 2. Degree of nematode infection of *Compsilura concinnata* adults emerging from soil inoculated with 50 *Neoaplectana carpocapsae*/cm² of surface area.

	No. hosts treated*	No. puparia	No. (%) adults emerged	% adults dead†		% adults alive
				With nematodes	Without nematodes	
Test 1‡						
Control	14	44	43 (97.7)		41.9	58.1
Nematode	14	52	51 (98.1)	37.3	25.5	39.2
Test 2§						
Control		29	25 (86.2)		44.0	56.0
Nematode		28	22 (78.6)	13.6	36.4	50.0
Test 3‖						
Control	14	77	72 (93.5)		54.2	45.8
Nematode	14	80	57 (71.3)	8.8	54.4	36.8

* Combined data of two separate trials.
† Percentage calculated on number of adults emerged.
‡ Nematodes added 7 days after the first tachinid larva emerged from host.
§ Five-day-old puparia buried 1 cm below soil surface and nematodes added to the soil 2 days later. Total of 30 puparia placed into soil but all were not recovered.
‖ Nematodes added to soil 3 days before the first tachinid larva was expected to emerge.

inid larvae. In the test (test 3) where the tachinid larvae emerged into nematode-infested soil, 8 of 80 (10%) pupae became infected with *N. carpocapsae* and in five of them the nematodes reproduced. Another 15 (18.7%) pupae were dead, and of these, four showed signs of nematode infection in that the internal tissues were gummy and ocher in color. The remainder of the pupae in the puparia appeared to have died from other causes. In tests 1 and 2, and in the control of test 3, there was mortality of pupae, but none appeared to be nematode related.

C. concinnata pupated within 1 cm of the soil surface. Puparia were found 9 ± 4 mm (test 1) and 7.5 ± 5 mm (test 3) below the soil surface.

Nematodes infected tachinid adults as they emerged from the soil (Table 2). Based on adult emergence, the highest adult mortality was 37% in test 1 where *N. carpocapsae* were added 7 days after the first tachinid larvae emerged. Test 2 was similar to test 1 except that the puparia were manually placed into the soil. Adult mortality due to nematode infection was 14%. The lowest mortality due to nematode infection was 9% in test 3. However, when both pupal and adult mortalities due to nematode infection were taken into account, the percentage was 21.3% (17/80—including four pupae with signs of nematode infection). Adult mortality, apparently not caused by nematodes, was quite high in all three tests. This mortality was probably the result of the conditions under which the tachinids were held.

DISCUSSION

The *N. carpocapsae*-bacterium complex has indirect and direct deleterious effects on the tachinid, *C. concinnata*. The indirect effect is due to death of the host before the tachinid can complete its development. The direct effect occurs when the nematode infects the larval, pupal, or adult stages of the tachinid. The puparium, an exceptional protective structure, appears to be a physical barrier to the nematode. Infection of the pupa occurs when the nematodes infect the tachinid larva prior to pupation. These deleterious effects of *N. carpocapsae* on tachinids are similar to those obtained for hymenopterous parasites (6, 7, 10). However, differences were also observed.

Tachinid larvae are less affected by nematode infection in their lepidopterous host than hymenopterous parasites. Some 2-day-old and 3-day-old tachinid larvae in nematode-treated hosts can continue to develop and some eventually become adults, but 8-day-old or 9-day-old larvae of the hy-

menopterous parasite, *G. militaris*, cannot. In the case of *C. concinnata*, an additional 4–5 days in the nematode-treated host are required before development is complete. In the case of *G. militaris*, larval development requires 11–12 days and only 10-day-old and 11-day-old parasites can pupate normally when the host is exposed to infective nematodes (6). Another difference is that the nematode cannot utilize the tachinid-parasitized armyworm host after the third day of parasitism, but it can develop and reproduce in *G. militaris* armyworm host even after the parasite has emerged from the armyworm. In the former case, the armyworm host is dead in 3–4 days after tachinid parasitization (3) which probably affects the growth of the nematode-bacterium complex. In the latter case, the host is still alive after parasite emergence but dies 1–2 days later.

At the nematode concentration (50 nematodes/cm^2) used against the tachinids in the soil tests, 84–93% of adults of the lepidopterous pest *Spodoptera exigua* became infected as they emerged from the soil (8). In contrast, 9–37% of tachinid adults were infected under similar conditions. Moreover, at this nematode concentration, only a small percentage of tachinid larvae were infected as they entered the soil, whereas 100% of *S. exigua* prepupae were infected. Even at concentrations of five nematodes/cm^2 of soil surface, 89–96% of the *S. exigua* prepupae became infected with *N. carpocapsae*. At 50 nematodes/cm^2 or less of soil surface, *C. concinnata* larvae, pupae, and adults are less susceptible to *N. carpocapsae* than are certain lepidopterous pests. However, Bedding et al. (2) have shown that the degree of infectivity of *Neoaplectana* species/strain for different hosts varied considerably. Accordingly, more research in the laboratory and field with this tachinid and other *N. carpocapsae* strains is needed to adequately demonstrate their compatibility. If insect parasites are important mortality components of a pest species and entomogenous nematodes show potential as additional biological control agents, the compatibility of these biological control agents should be further evaluated.

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