FIELD EVALUATION OF THE ENTOMOGENOUS NEMATODE, *NEOAPLECTANA CARPOCAPSAE*, AS A BIOLOGICAL CONTROL AGENT OF BLACK FLIES (DIPTERA: SIMULIIDAE)[1]

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ABSTRACT. The DD-136 strain of *Neoaplectana carpocapsae* Weiser was tested in a small stream against black fly larvae at a dosage of 34.5 nematodes/ml over a 15 min exposure period. Mortality averaged 50% among late instar larvae treated in the stream and subsequently held in the laboratory under simulated stream conditions. Death was rapid: larval mortality commenced within 2–4 hr, and 64% of all deaths occurred within 24 hr of treatment. The intensity of infection in black fly cadavers was related to the day of larval death, with the heaviest infections observed in larvae dying within 1–2 days after treatment. Larvae dying toward the end of the 6 day observation period were lightly infected or uninfected. Treatment did not result in significant mortality among early and mid instars. Low stream temperature (9–12 °C) was probably the main factor limiting nematode effectiveness. No evidence of nematode establishment or recycling was detected.

INTRODUCTION

The broad host range of the entomogenous nematode *Neoaplectana carpocapsae* Weiser has prompted considerable research into its potential for insect control. Although numerous investigators have established the effectiveness of this parasite in causing host mortality under laboratory conditions, field trials against terrestrial insects have met with variable and often disappointing results (Poinar 1979). Failures have usually been attributed to the vulnerability of the infective-stage juveniles to environmental extremes, especially their sensitivity to desiccation (Poinar 1971). Several workers have experimented with evaporation retardants in efforts to extend nematode survival time following field applications (Nash and Fox 1969, Webster and Bronskill 1968). An alternative approach is the use of the nematode against insect pests inhabiting areas possessing a microenvironment favorable for nematode survival.

Laboratory studies have demonstrated that *N. carpocapsae* shows promise for the control of black flies (Gaugler and Molloy 1981, Molloy et al. 1980). High rates of mortality were achieved in these tests against late instar *Simulium* spp. without evidence of a host defense reaction. This nematode is the only potential biological control agent of black flies, other than the entomopathogen *Bacillus thuringiensis* var. *israelensis* de Barjac (Undeen and Colbo 1980), for which mass production procedures are currently available. Because black fly larvae inhabit swiftly flowing streams, nematode survival would not be a problem in control attempts, since near optimal conditions are provided by the aquatic environment. Moreover, since released nematodes are carried rapidly downstream, there is a high potential for host-parasite contact soon after application. Despite these advantages, virtually all field tests have been conducted against terrestrial invertebrates. *Neoaplectana carpocapsae* has not been evaluated in the field against a stream insect, and there is only a single report of field application against any aquatic invertebrate, i.e., mosquito larvae (Welch 1962).

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Reported here is a determination of the field efficacy of *N. carpocapsae* against black flies.

MATERIALS AND METHODS

The test was conducted during October 1979 in a small stream located on property of the New York State Science Service near Cambridge, N.Y. This stream was hand dug 9 weeks before treatment and natural stream water diverted into the bed. The stream averaged 30 cm in width, 8 cm in depth, and had a flow rate of 187 liters/min. No pools were present along its 40 m length. Sod removed when the channel was cut was replaced in the streambed, where it served as a substrate for the black flies and nontargets that quickly colonized the stream. These natural populations were augmented by stocking 1–3 weeks before testing. Species composition of late larval instars was 90.4% *Simulium vittatum* Zetterstedt, 7.4% *S. verecundum* Stone and Jamnback, and 2.2% *S. aureum* Fries. The incidence of patent infections (i.e., microsporidia, *Coelomycidium simulii* Debaisieux, and mermithids) among *S. vittatum* larvae was 9.1%. No other larvae were so infected.

The DD-136 strain of *N. carpocapsae* was produced in larvae of the greater wax moth, *Galleria mellonella* (Linnaeus). An aqueous suspension of infective-stage nematodes (93.2% viability) was introduced into the stream from a 20 liter carboy, using the technique of Fredeen (1970) to deliver a dosage of 34.5 viable nematodes/ml over a 15 min exposure period. The carboy was agitated during treatment to ensure uniform nematode suspension. Water temperature was 11.4°C at the time of treatment (1230 hr October 5) and ranged from 9.0–11.8°C during the 5 day test. Rain fell almost daily, with a particularly strong storm occurring the evening following treatment, when 3.3 cm of precipitation was recorded.

Sampling to assess treatment effectiveness was conducted at 2.5 m upstream (control site), and at 2.5 and 26 m downstream (treatment sites) from the point of application. Larvae were collected from these sites 2 hr after exposure and transferred to the laboratory incubation system, where host response could be closely monitored without loss due to to drift, predation, or sampling error. Each sample site was represented in the laboratory by 5 replicates, each with ca. 35 mixed instar larvae. These larvae were held under simulated stream conditions with a continuous supply of fresh flowing stream water (Gaugler et al. 1980). The stream water used in the incubation system was diverted from the same source used in the test stream and was therefore equivalent in all respects (i.e., temperature, pH, suspended material). Previous tests have demonstrated that the mortality rates obtained with this procedure correspond closely to those obtained in the field (Molloy and Jamnback 1981). Mortality was recorded daily over a 6 day posttreatment period. All black flies were categorized as to instar, infection intensity, and species; pupae were examined at the termination of the test. Instar was determined by recording larval postgenal length. Since younger larvae (< 380 μm postgenal length) show limited susceptibility to *N. carpocapsae* infection (Gaugler and Molloy 1980), these black flies were excluded from all computations except those concerning instar susceptibility. Infection intensity was determined according to the number of nematodes invading the host hemocoel: larvae penetrated by $\geq$ 50 nematodes were classified as heavily infected, 4–49 as moderately infected, and 1–3 as lightly infected.

Tests to determine if the nematode could become established in the aquatic environment were also conducted. Since the stream emptied into a 6 m diameter concrete holding pond located 30 m downstream from the treatment point, most of the more than 100 million released nematodes settled in this area. Black flies in the stream and nontargets in the stream and pond were collected with a D-net at 3 and 6 months after treatment and examined for evidence of *N. car-*

pocapsae infection. Using the technique of Bedding and Akhurst (1975), soil samples were collected from the pond bottom 6 months after treatment and placed into 8 petri dishes (10 cc of soil/dish) with 10 *G. mellonella* larvae/dish. These larvae were observed over a 6 day period for evidence of *N. carpocapsae* induced mortality.

Replicates were analyzed by chi-square and all data were transformed by arcsin before calculating confidence limits.

RESULTS AND DISCUSSION

Treatment of late instar black flies with *N. carpocapsae* resulted in the death of half of the exposed larvae. In contrast, early and mid-instars were not affected (Table 1). This result had been anticipated from earlier laboratory studies which have shown susceptibility to sharply increase with increasing larval size (Molloy et al. 1980). Early instars are resistant because they physically exclude ingestion of the relatively large juvenile nematodes (Gaugler and Molloy 1981). Because mortality did not decline with distance, it was concluded that downstream carry over the 26 m distance was excellent and that negligible settling of the inoculum had occurred. All subsequent data for the 2 treatment sites were consequently pooled.

Larval mortality following treatment was generally rapid, with 64% of all deaths occurring within 24 hr of treatment (Table 2), and some deaths within 2–4 hr. Mortality leveled off quickly thereafter, without evidence of a trend. This type of skewed mortality progression was expected, based on laboratory exposures conducted at approximately the same temperature (Molloy et al. 1980).

The proportion of black fly cadavers infected by nematodes was strongly correlated ($r = +0.99$) to day of larval death (Table 2). Thus, cadaver infection rate decreased steadily over the 6 day post-treatment period, from 96 to 20%. These results, obtained from larvae treated in the field and then transferred to the laboratory for observation, correspond well with data obtained from cadavers collected from the stream 2 and 5 days after treatment, when the infection rates were 100 (20/20) and 23.5% (4/17), respectively. The data support our earlier contention that larval death may occur in the absence of nematode infection. Such deaths are theorized to result from gut damage inflicted by nematodes unsuccessfully attempting to penetrate into the larval hemocoel (Molloy et al. 1980).

Infection intensity was also related to the day of larval death (Table 3). Generally, the more nematodes infecting a larva, the quicker death occurred. Larvae dying within a few hours of treatment had invariably been penetrated by several

Table 1. Mortality of early to mid-instar and late instar *Simulium* spp. larvae following exposure to *Neoaplectana carpocapsae*[a].

Distance from treatment point (m)	Early to mid-instars[b] No. tested	Percent mortality ±SE	95% confidence limits	Late instars[c] No. tested	Percent mortality ±SE	95% confidence limits
2.5 upstream (control)	15	0	—	125	5.5 ±3.10	0–11.58
2.5 downstream	44	2.3 ±2.03	0–6.28	152	47.6 ±4.56	38.66–56.54
26 downstream	51	9.8 ±4.89	0.22–19.38	128	52.7 ±1.31	50.13–55.27
2.5+ 26 downstream	95	5.6 ±2.39	0.92–10.28	280	49.9 ±2.52	44.46–54.84

[a] Larvae collected from field 2 hours after treatment and transferred to simulated stream units in the laboratory.

[b] Larvae with <380 μm postgenal lengths.

[c] Larvae with >380 μm postgenal lengths.

Table 2. Daily mortality and infection rates of late instar *Simulium* spp. larvae following exposure to *Neoaplectana carpocapsae*[a].

Days postexposure	Mortality[b]			Infection		
	No. dead	% mortality ±SE	95% confidence limits	No. of dead infected	% of dead infected ±SE	95% confidence limits
1	82	64.1 ±3.07	58.08–70.12	79	96.3 ±2.89	89.60–99.41
2	13	10.2 ±2.64	5.03–15.37	12	92.3 ±5.61	75.25–99.82
3	6	4.7 ±2.90	0–10.38	5	83.3 ±17.95	—[c]
4	8	6.3 ±3.84	0–13.82	4	50.0 ±16.07	—
5	14	10.9 ±3.29	4.45–17.35	4	28.6 ±10.5	—
6	5	3.9 ±3.09	0–9.96	1	20.0 ±15.18	—

[a] Larvae collected from the field 2 hours after treatment and transferred to simulated stream units in the laboratory.
[b] Ten pupae deleted because of inability to determine day of death.
[c] Low sample size did not permit meaningful computation.

hundred infective-stage juvenile nematodes. Conversely, larvae dying after the initial 48 hr were never heavily infected. This apparent relationship between rapid mortality and heavy infection suggests that larval death during the first 24 hr after treatment is a result of severe physiological disruption resulting from massive invasion of the hemocoel. It is doubtful the nematode's associated bacterium, *Xenorhabdus nematophilus* (Poinar and Thomas), is capable of contributing significant lethal action in such a short period at the low temperatures (9–12°C) our tests were conducted, because the bacterium shows little activity at temperatures near 10 °C (Kaya 1977).

The wide host range attributed to *N. carpocapsae* against terrestrial insects (Poinar 1979) generates some concern regarding its potential for causing adverse effects among nontarget stream insects. However, dissections of nontarget invertebrates (including filter-feeding hydropsychids and philopotamids) collected from stream vegetation 2 and 5 days after treatment did not reveal the presence of *N. carpocapsae*. Examination of black flies at these intervals established that ingestion and infection had occurred.

Table 3. Infection intensity in late instar *Simulium* spp. larvae following exposure to *Neoaplectana carpocapsae*.[a]

Days postexposure	No. of larval cadavers infected:			
	Heavily[b]	Moderately[c]	Lightly[d]	Uninfected
1	61	16	2	3
2	3	9	0	1
3	0	5	0	1
4	0	4	0	4
5	0	0	4	10
6	0	0	1	4
Total(%)	64 (50.0)	34 (26.6)	7 (5.5)	23 (18.0)

[a] Larvae collected from the field 2 hours after treatment and transferred to simulated stream units in the laboratory.
[b] ≥50 nematodes/cadaver.
[c] 4–49 nematodes/cadaver.
[d] 1–3 nematodes/cadaver.

Nematodes were not detected in dissections of over 300 black flies and nontarget insects collected from the stream and holding pond 3 and 6 months after treatment. Viable juveniles were also absent in microscopic examinations of soil samples from the pond and stream bed, although dead nematodes, corresponding to the dimensions of *N. carpocapsae* juveniles, were sometimes found in the pond samples. Large numbers of juveniles had been found in the pond and stream bed immediately after treatment. Attempts to recover viable infective-stage juveniles by exposing *Galleria* larvae to soil samples collected from the pond 6 months after treatment were unsuccessful. It may be speculated that the nematode's surprising inability to persist in the aquatic environment was due to unknown biotic mortality factors, since the physical environment seemed optimal for *N. carpocapsae* survival (i.e., moisture, high oxygen level, low temperature), and since many organisms are known to prey upon nematodes (Mankau 1980). The possibility of the terrestrially adapted *N. carpocapsae* establishing and recycling in a stream, therefore, appears to be remote, particularly since in this test we observed that black fly cadavers degenerated before the nematode could complete reproduction. Welch (1962) similarly noted that *N. carpocapsae* did not become established in an aquatic environment (mosquito pools) and concluded that the nematode could be used only as an insecticide for short-term mosquito control. Clearly, its use against black flies is also limited to the inundative control method.

In field trials against *Aedes* mosquitoes, Galloway and Brust (1976) reported that low water temperatures severely limited infections by the mermithid nematode *Romanomermis culicivorax* Ross and Smith. We have similarly noted that the pathogenicity of *N. carpocapsae* against black flies is reduced at low temperatures (unpublished data). Thus, significantly enhanced mortality probably would have been obtained had our test been conducted at summer (16–22°C) rather than fall (9–12°C) water temperatures.

Considerable success has attended recent trials of *N. carpocapsae* against terrestrial pests occupying moist, cryptic habitats (Lindegren et al. 1978, Bedding and Miller 1981). Our results, however, suggest that the nematode shows less promise as a control agent of black fly larvae, as its use appears restricted to short-term reductions of late instar populations inhabiting warm water streams.

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References Cited

Bedding, R. A. and R. J. Akhurst. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21:109–116.

Bedding, R. A. and L. A. Miller. 1981. Disinfesting blackcurrant cuttings of *Synanthedon tipuliformis* using the insect parasitic nematode, *Neoaplectana bibionis*. Environ. Entomol. (in press).

Fredeen, F. J. H. 1970. A constant-rate liquid dispenser for use in blackfly larviciding. Mosq. News 30:402–405.

Galloway, T. D. and R. A. Brust. 1976. Field application of the mermithid nematode, *Romanomermis culicivorax* Ross and Smith, for control of mosquitoes, *Aedes* spp., in spring in Manitoba. Manit. Entomol. 10:18–25.

Gaugler, R. and D. Molloy. 1981. Instar susceptibility of *Simulium vittatum* (Diptera: Simuliidae) to the entomogenous nematode, *Neoaplectana carpocapsae*. J. Nematol. 13:1–5.

Gaugler, R., D. Molloy, T. Haskins and G. Rider. 1980. A bioassay system for the evaluation of black fly (Diptera: Simuliidae) control agents under simulated stream conditions. Can. Entomol. 112:1271–1276.

Kaya, H. K. 1977. Development ot the DD-136 strain of *Neoaplectana carpocapsae* at constant temperatures. J. Nematol. 9:346–349.

Lindegren, J. E., C. E. Curtis, and G. O. Poinar, Jr. 1978. Parasitic nematode seeks out navel orangeworm in almond orchards. Calif. Agric. 32:10–11.

Mankau, R. 1980. Biological control of nematode pests by natural enemies. Ann. Rev. Phytopathol. 18:415–440.

Molloy, D. and H. Jamnback. 1981. Field evaluation of *Bacillus thuringiensis* var. *israelensis* as a black fly biocontrol agent and its effect on nontarget stream insects. J. Econ. Entomol. 74:314–318.

Molloy, D., R. Gaugler and H. Jamnback. 1980. The pathogenicity of *Neoaplectana carpocapsae* to black fly larvae. J. Invertebr. Pathol. 36:302–306.

Nash, R. F. and R. C. Fox. 1969. Field control of the Nantucket pine tip moth by the nematode DD-136. J. Econ. Entomol. 62:660–663.

Poinar, G. O., Jr. 1971. Use of nematodes for microbial control of insects. Pp. 181–203 *in* H. D. Burges and N. W. Hussey, eds. Microbial control of insects and mites. Academic Press, London.

Poinar, G. O., Jr. 1979. Nematodes for biological control of insects. CRC Press, Boca Raton, FL. 277 pp.

Undeen, A. H. and M. H. Colbo. 1980. The efficacy of *Bacillus thuringiensis* var. *israelensis* against blackfly larvae (Diptera: Simuliidae) in their natural habitat. Mosq. News 40:181–184.

Webster, J. M. and J. F. Bronskill. 1968. Use of Gelgard M and an evaporation retardant to facilitate control of larch sawfly by a nematode-bacterium complex. J. Econ. Entomol. 61:1370–1373.

Welch, H. E. 1962. Nematodes as agents for insect control. Proc. Entomol. Soc. Ont. 92:11–19.