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The Pathogenicity of *Neoaplectana carpocapsae* to Blackfly Larvae[1]

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Laboratory assays indicated that infective-stage juveniles of *Neoaplectana carpocapsae* are highly pathogenic to *Simulium* spp. larvae. Instar susceptibility increased with larval size, with early instars being nonsusceptible. High rates of mortality (75–100%) were achieved in assays against late instars. These results indicate that *N. carpocapsae* may have potential value as a blackfly biocontrol agent.

KEY WORDS: *Neoaplectana carpocapsae; Simulium* spp.; biological control of blackflies.

INTRODUCTION

Progress toward the biological control of blackflies has been slow, due in part to an inability to obtain quantities of inoculum sufficient to permit comprehensive investigations. At present, none of the naturally occurring parasites of blackflies which are seriously being considered for use can be inexpensively mass produced, and workers are often dependent on field collections from infected host populations. This has led to consideration of insect parasites not normally found in blackflies for which mass production technology is currently available. One such parasite is the entomogenous nematode *Neoaplectana carpocapsae*.

N. carpocapsae possesses several characteristics which make it an attractive candidate for control of insect pests. Mass rearing is accomplished easily and inexpensively, with in vitro production costs of two cents per million juveniles (Bedding, 1976). The nematode is highly virulent, capable of causing death in less than 24 hr, due primarily to the action of its associated bacterium. Moreover, it is resistant to many agricultural chemicals (Dutky, 1969), thus making it suitable for use in integrated control programs.

Despite extensive testing against terrestrial insects, few studies have evaluated the potential of this nematode against aquatic insect pests. The rate of nematode ingestion by *Culex pipiens* larvae was reported by Dadd (1971) to be directly proportional to larval size, with first and second instars not becoming infected because of exclusion. Webster (1973), investigating the effect of temperature on ingestion, found the greatest number of nematodes within larvae of the blackfly *Simulium vittatum* exposed at 20°C and within larvae of the mosquito, *Culiseta inornata*, exposed at 25°C. Detailed accounts of the infection process and host defense reaction in mosquitoes have been presented (Bronskill, 1962; Andreadis and Hall, 1976). In the only field trial against aquatic insects, Welch (1962) reported that applications of *N. carpocapsae* in *Aedes* spp. habitats reduced mosquito larval populations and adult emergence, but did not result in nematode establishment.

Strains of *N. carpocapsae* have been isolated only from terrestrial insect populations. The major limitation to its use as a control agent of terrestrial insects has been the nematode's inability to tolerate unfavorable environmental conditions. When applied in terrestrial environments, infective-stage juveniles of *N. carpocapsae*

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are inactivated by desiccation (Moore, 1973; Simons and Poinar, 1973), high temperature (Schmiege, 1963), and ultraviolet (Gaugler and Boush, 1978), and thus are often ineffective as control agents. In contrast, applications for control of blackfly larvae, which are restricted to flowing water, would occur under the more favorable survival conditions of free water, low temperature, high oxygen, and absence of ultraviolet light. Moreover, the indiscriminant filter-feeding behavior of blackfly larvae make them particularly susceptible to nematode ingestion. The assays reported here were designed to provide a preliminary assessment of the pathogenicity of this nematode to larval blackflies and its potential for biological control.

MATERIALS AND METHODS

The DD-136 strain of *N. carpocapsae* was reared in *Galleria mellonella* as outlined by Dutky et al. (1964), and stored in a 0.1% formalin solution at 5–7°C in oxygenated jars. All assays had parallel controls in which blackflies were exposed to concentrations of formalin equal to that added with the inoculum. Nematode viability in assays ranged between 90 and 100%.

Relative instar susceptibility. Early-, mid-, and late-instar *S. verecundum* larvae, laboratory reared from field collected eggs, were placed in aerated glass bowls holding 250 ml of stream water at 11±1°C, incubated for 4 hr, and then exposed to nematodes for 24 hr. Following exposure, larvae were transferred to bowls of fresh aerated stream water, held for another 24 hr, and then dissected and examined microscopically for infection.

Pathogenicity to late instars. Laboratory reared late instars of *S. verecundum* and *S. vittatum* were placed in glass battery jars containing 1500 ml of aerated stream water. After 4 hr of acclimation, the larvae were exposed to infective-stage nematodes for 30 min at 11 ± 0.5°C. The mean concentration of live nematodes per milliliter in the test jars was determined by sampling at 5-min intervals. Following exposure, the larvae were removed from the jars, rinsed with stream water on an organdy cloth filter for 1 min, and then transferred to running water troughs (Jamnback and Frempong-Boadu, 1966) for incubation at 10–12°C for 6 days. These flow-through troughs simulate natural stream conditions as they are continuously supplied with stream water flowing out of the troughs and over effluent lips to which the larvae are attached. During this post-treatment period, dead and moribund larvae were removed and dissected daily. All surviving blackflies were also examined for nematode infection at the termination of the incubation period.

RESULTS AND DISCUSSION

Relative Instar Susceptibility

Susceptibility rose as larval size increased, with infection rates of 80–100% achieved in late instars (Table 1). Early instars remained uninfected. Subsequent larval examination established that infective-stage juveniles had been readily ingested by late-instar larvae, but not by early instars. Supplementary observations with a stereomicroscope confirmed that early instars cannot easily capture and ingest nematodes, presumably because of the size disparity between the nematode and the blackfly (Fig. 1). These instar susceptibility results parallel those of Dadd (1971) with mosquito larvae.

Pathogenicity to Late Instars

High rates of mortality (>75%) were achieved in late instars of both *Simulium* spp. (Table 2). Nematode ingestion did not, however, insure simuliid mortality, since some larvae survived the gut passage of dozens of active nematodes. Dissection of cadavers revealed that nematode penetration into the hemocoel invariably resulted in host death. Nematode penetration, however, was not a prerequisite for larval death, since corrected mortality rates at times exceeded infection rates. Approximately one-third of all cadavers did not contain nematodes within their hemocoels.

TABLE 1
INFECTION RATES OF *Simulium verecundum* EARLY, MID-, AND LATE INSTARS EXPOSED TO INFECTIVE-STAGE JUVENILES OF *Neoaplectana carpocapsae* FOR 24 HR

Instar	Initial nematode concentration (juveniles/ml)	Percentage infection[a]
Early	21.6	0.0 (0/18)
	28.8	0.0 (0/24)
Mid	24.0	5.0 (1/20)
	33.6	21.4 (6/28)
Late	3.5	82.8 (24/29)
	3.6	80.0 (24/30)
	26.4	100 (22/22)
	30.0	84.0 (21/25)

[a] Infection rates in early, mid-, and late instars in parallel control trials = 0% (respectively, 0/24, 0/22, and 0/25).

Poinar and Ennik (1972) in a study of the effect of *N. carpocarpsae* on adult yellowjackets also noted cadavers in which nematodes could not be found. It is suggested that, in the present tests, ingested nematodes, which were observed to be highly active during their passage through the blackfly gut, may have caused sufficient damage by their probing to allow leakage of gut contents.

The high mortalities recorded in these assays are encouraging, especially since they were achieved at temperatures near the threshold of infection for *N. carpocapsae* in *G. mellonella* (≈10°C (Dutky et al., 1964)). The nematode's pathogenicity to blackflies at higher temperatures now needs to be determined. Virulence at high temperatures, e.g., 20–30°C, would make it suitable for use under tropical conditions.

Nematode virulence was demonstrated not only by the high rates of mortality

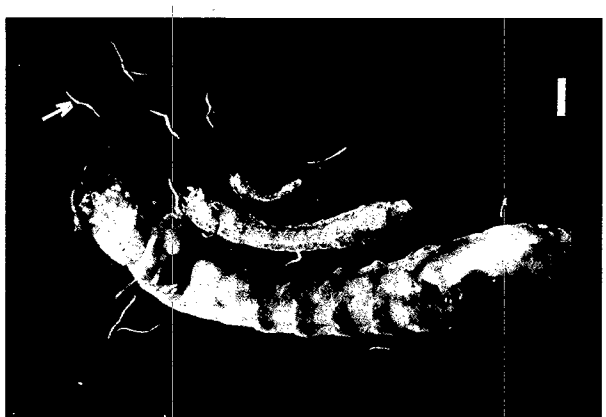

FIG. 1. Size relationship between late-, mid-, and early-instar blackfly larvae and infective stages of *Neoaplectana carpocapsae* (arrow). Bar = 0.5 mm.

TABLE 2
INFECTION AND MORTALITY RATES OF LATE INSTAR *Simulium* spp. EXPOSED TO INFECTIVE-STAGE JUVENILES OF *Neoaplectana carpocapsae* FOR 30 MIN[a]

Mean exposure rate (average No. juveniles/ml/30 min)	No. larvae treated	Mortality		Infection[c]	
		No.	%[b]	No.	%
S. verecundum					
0.1	20	7	33.3	1	5.0
0.2	21	4	17.0	0	0.0
3.1	21	16	75.6	13	61.9
3.4	21	14	65.8	6	23.8
50.4	21	17	80.5	14	66.7
87.2	18	18	100.0	10	55.6
100.6	20	15	73.4	12	60.0
S. vittatum					
0.1	21	1	4.8	0	0.0
0.2	18	1	5.6	1	5.6
3.1	19	10	52.6	10	52.6
3.4	18	5	27.8	2	11.1
50.4	19	12	63.2	10	52.6
87.2	19	9	47.4	5	26.3
100.6	18	14	77.8	12	66.7

[a] Test ended 6 days post-treatment.
[b] Corrected by Abbott's formula with control mortality = *S. verecundum*, 2.5% (2/81) and *S. vittatum*, 0.0% (0/77).
[c] All infected larvae were dead.

achieved, but also by the relatively rapid rate at which these deaths occurred. Thus, 54% (77/143) of all blackfly mortality occurred within 24 hr post-treatment, with generally high numbers of nematodes within the hemocoels of these cadavers. At 3.4 nematodes/ml, for example, an average of 129 (range, 93–189) nematodes were found in the five larvae which succumbed within 24 hr post-treatment. Daily mortality rates dropped quickly after the first day, with 4.9, 6.9, 16.0, 9.7, and 9.0% mortality recorded for days 2–6, respectively.

Encapsulated nematodes were not found in any blackflies. This is in sharp contrast to studies with mosquito larvae in which encapsulation of *N. carpocapsae* was commonly observed (Welch and Bronskill, 1962; Andreadis and Hall, 1976).

These preliminary assays demonstrate the pathogenicity of *N. carpocapsae* to blackfly larvae. The heterogeneity of the data, however, suggests a more detailed examination of experimental parameters. Since *N. carpocapsae* is virulent to a wide variety of insects (Laumond et al., 1979), its effects on aquatic nontargets and its potential for stream establishment also need to be examined. A comprehensive study of these problems is now underway.

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