Susceptibility of Various Species of Lepidopterous Pupae to the Entomogenous Nematode *Neoaplectana carpocapsae*[1]

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Abstract: The susceptibility of certain species of lepidopterous pupae occurring in different ecological situations to the entomogenous nematode, *Neoaplectana carpocapsae,* was tested. Soil- or litter-pupating lepidopterous insects were not highly susceptible to *N. carpocapsae.* The most susceptible insect pupating in the soil was *Spodoptera exigua* with 63% pupal mortality, while *Harrisinia brillians,* which pupates in litter, had 55% mortality. Other soil- and litter-pupating insects had mortalities of less than 25%. Some insect species that pupate above ground were highly susceptible (> 84% mortality) to *N. carpocapsae* infection. *Key words:* biological control, nematode infection, pupal mortality.

Differential susceptibility of lepidopterous pupae to infection by the nematode, *Neoaplectana carpocapsae,* and its associated bacterium, *Xenorhabdus nematophilus,* was demonstrated with the greater wax moth, *Galleria mellonella,* the beet armyworm, *Spodoptera exigua,* and the armyworm, *Pseudaletia unipuncta* (2). Pupae of *G. mellonella* were most susceptible to the nematode with 100% mortality, followed by *S. exigua* with ca. 75% mortality and *P. unipuncta* with ca. 50% mortality. Sandner and Stanuszek (7) reported 100% infection with pupae of *G. mellonella.* Moyle and Kaya (5) found that pupae of the silkworm, *Bombyx mori,* and of *G. mellonella* that were in silken cocoons were highly susceptible (100% mortality) to *N. carpocapsae.* Pupae of the spruce budworm, *Choristoneura fumiferana,* the jack pine budworm, *C. pinus,* (8) and the sphingid, *Herse convoluli* (3) were moderately susceptible to this nematode. Lewis and Raun (4) reported that 30% of the pupae of the European corn borer, *Ostrinia nubilalis,* exposed to *N. carpocapsae* became infected. Bedding and Akhurst (1) suggested that soil-inhabiting insects may have evolved some protection from parasitic rhabditid nematodes. We conducted a survey to determine the susceptibility of soil-, litter- and above-ground-pupating lepidopterous insects to *N. carpocapsae.* This information is important in determining whether *N. carpocapsae* can be used as a biological control agent against the pupal stage.

MATERIALS AND METHODS

Test insects: Pupae of various insect species were obtained by rearing field-collected larvae or from laboratory-reared insects (see Table 1 for common names). Insects obtained from the field and fed on their plant hosts in the laboratory until pupation were *Hyphantria cunea* (walnut), *Papilio zelicaon* (parsley), *Pieris rapae* (cabbage), *Precis coenia* (snapdragons), *Sabulodes aegrotata* (ivy), and *Schizura concinna* (walnut). Field-collected insects from other laboratories were *Platyptilia carduidactyla* (artichoke—University of California, Davis) and *Harrisina brillians* (grape—California Department of Food and Agriculture, Sacramento). The following insects were reared in California laboratories and provided for testing: *Phthorimaea operculella* and *Trichoplusia ni* (University of California, Riverside), *Platynota stultana* (University of California, Davis), *Choristoneura occidentalis* (U. S. Forest Service, Berkeley), *Manduca sexta* (Stauffer Chemical, Palo Alto), *Laspeyresia pomonella* and *Anagasta kuehniella* (University of California, Berkeley), and *Amyelois transitella, Cadra figulilela, Ephestia cautella,* and *Plodia interpunctella* (USDA, Stored Product Insects Research Laboratory, Fresno). The following insects were reared to pupation on artificial diets in our laboratory: *Pseudaletia unipuncta, Spodoptera exigua, G. mellonella, Estigmene acrea,* and *Heliothis zea.*

Infectivity tests: Pupae were exposed to *N. carpocopsae* (All strain) using Dutky's

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method as described by Kaya and Hara (2), except where noted. Pupae of known age were placed individually in 60- × 15-mm plastic petri dishes in which 200 infective juveniles in 0.5 ml of water had been pipetted onto a piece of 5.5-cm d filter paper (Whatman No. 1). Petri dishes were placed in a plastic bag to prevent desiccation. After 48 h each pupa was rinsed in three washes of sterile distilled water and placed into a 39-ml plastic vial containing ca. 0.6 g of moist vermiculite. Pupae were dissected 8 to 12 d after treatment and examined for presence of nematodes. Adult insects emerging before the 8–12-d period were recorded as healthy.

Pupae of *M. sexta* were too large to be treated in the above manner. Consequently, each pupa was placed in a glass petri dish bottom (90 × 20 mm) containing a filter paper (9.0 cm) treated with 200 nematodes in 1 ml of water. Another petri dish bottom was placed over this and the two dishes sealed with a strip of Parafilm. For the following insects—*A. kuehniella, C. figulilella, E. cautella, P. operculella,* and *P. interpunctella*—five pupae were placed into a petri dish (60 × 15 mm) with 200 nematodes. For the majority of insect species, there were at least three separate trials with a minimum of 8–10 insects per trial. Pupae of *S. aegrotata, P. zelicaon,* and *P. coenia* were treated as pupation occurred because of the asynchronous pupation of these field-collected insects. Also, only a limited number of *E. acrea, C. occidentalis, P. stultana, S. aegrotata, A. transitella,* and *P. coenia* were available for testing.

RESULTS AND DISCUSSION

The survey showed that soil- or litter-pupating lepidopterous insects generally are not highly susceptible to the entomogenous nematode, *N. carpocapsae.* The most susceptible soil-pupating insect was *S. exigua* with 63% mortality, followed by the litter-pupating insect, *H. brillians,* with 55% mortality. Other soil- and litter-pupating insects had mortality rates of less than 27%. In contrast, *S. aegrotata, G. mellonella, A. transitella, P. carduidactyla, A. kuehniella,* and *P. zelicaon,* which pupate above ground, were far more susceptible (> 84% mortality) to *N. carpocapsae.* However, pupation above ground per se did not result in high pupal mortality by *N. carpocapsae.* Pupae of *P. coenia, T. ni, P. stultana, P. rapae,* and *E. cautella* appeared to be less susceptible to the nematode than *S. exigua. L. pomonella,* which pupates above ground, and *S. exigua* pupae appeared to be equally susceptible to *N. carpocapsae.* The comparative resistance of soil- and litter-pupating lepidopterous pupae to *N. carpocapsae* appears to be true, but not all lepidopterous insects which pupate above ground are highly susceptible to this nematode.

Differences in infectivity of *N. carpocapsae* to pupae due to age were noted with *H. zea* (Table 1). When pupal age ranged from 1 to 3 days, the mortality was 26%, but when pupal age ranged from 3 to 6 days, the mortality rate was 6%. A t-test showed significant differences in the mortality rate according to age ($t = 4.71$, df 4, $P < 0.05$). On the other hand, Kaya and Hara (2) reported that *N. carpocapsae* caused a pupal mortality of 25–50% for *P. unipuncta* and 50–75% for *S. exigua* with pupal age not an important factor. Pupal age may be an important factor with certain insect species, and more studies are needed before generalizations can be made.

With some species, pupal mortality without nematodes exceeded that with nematodes (see Table 1), although most of these pupae showed typical signs of a neoaplectanid infection. The reasons for the absence of nematodes in these pupae are unknown. No nematode development may be related to the physiological state of the host or the nematode or both. The nematodes may have been weakened upon entering the host and may not have been able to develop. From a biological control standpoint, the important aspect is the death of the target insect. However, if establishment of the nematode in the host's environment is desired, nematode reproduction in its target insect must occur. In all insect species except *M. sexta* tested in the present study, nematode reproduction occurred in the host insect to some degree. Thus, establishment and recycling could occur in the soil where conditions are favorable for nematode survival.

In general, the utilization of *N. carpocapsae* against species which pupate above

Table 1. Susceptibility of various lepidopterous pupae to the entomogenous nematode, *Neoaplectana carpocapsae*.

Family	Species	Common name	Pupae Age (days)	Pupae No.*	% dead with nematodes	% dead without nematodes	% alive
		Pupation in soil					
Arctiidae	*Estigmene acrea*	saltmarsh caterpillar	3-5	25	8.0	0	92.0
	Hyphantria cunea	fall webworm	1-4	72	2.5	0	97.5†
Noctuidae	*Heliothis zea*	bollworm	1-3	79	24.0	2.5	73.5
	Heliothis zea	bollworm	3-6	49	2.0	4.1	93.9
	Pseudaletia unipuncta	armyworm	1-4	30	16.7	10.0	73.3
	Spodoptera exigua	beet armyworm	1-4	30	46.7	16.6	36.7
Notodontidae	*Schizura concinna*	redhumped caterpillar	1-4	55	10.9	1.8	87.3
Sphingidae	*Manduca sexta*	tobacco hornworm	12	30	0	0	100
		Pupation in litter					
Gelechiidae	*Phthorimaea operculella*	potato tuber moth	1-3	70	2.9	15.7	81.4
Zygaenidae	*Harrisinia brillians*‡	western grapeleaf skeletonizer	1-4	33	7.8	47.7	44.5†
		Pupation above ground					
Geometridae	*Sabulodes aegrotata*§	none	1-4	20	100	0	0
Noctuidae	*Trichoplusia ni*§	cabbage looper	3-4	39	17.9	17.9	64.1
Nymphalidae	*Precis coenia*§	buckeye butterfly	1-4	16	12.5	0	87.5
Olethreutidae	*Laspeyresia pomonella*§	codling moth	2-3	28	60.7	7.1	32.2
Papilionidae	*Papilio zelicaon*§	anise butterfly	1-4	25	84.0	16.0	0
Pieridae	*Pieris rapae*§	cabbage butterfly	1-3	45	46.7	4.4	48.9
Pterophoridae	*Platyptilia carduidactyla*§	artichoke plume moth	2-4	48	87.5	2.1	10.4
Pyralidae	*Amyelois transitella*§	navel orangeworm	1-4	25	84.0	8.0	8.0
	Anagasta kuehniella‖	Mediterranean flour moth	1-4	30	63.3	23.3	13.4
	Cadra figulilella‖	raisin moth	2-5	70	18.6	54.3	27.1
	Ephestia cautella‖	almond moth	2-5	50	26.0	28.0	46.0
	Galleria mellonella‖	greater wax moth	3-4	30	100	0	0
	Plodia interpunctella‖	Indian meal moth	1-2	70	71.4	2.9	25.7
Tortricidae	*Choristoneura occidentalis*§	western spruce budworm	1-4	19	47.4	10.5	42.1
	Platynota stultana§	omnivorous leafroller	0-3	24	25.0	0	75.0

*For the majority of insect species, the number of pupae represents the total of three separate trials. (See text for details.)
†Corrected mortality by Abbott's Formula.
‡Pupation in litter or grape vine.
§Pupation on plant or leaf.
‖Pupation above ground (stored product insects—*G. mellonella* feeds on wax of honey bees).

ground (including stored product insects) is not feasible. (For obvious reasons, the use of *N. carpocapsae* against stored product insects is not feasible.) The nematode's high moisture requirement precludes its use in foliar applications until better formulations are developed. Under laboratory conditions, high pupal mortality occurs with some species which pupate on foliage; under field conditions, these pupae are less likely to become infected because of the nematode's inability to survive desiccation. However, Dutky reported that this nematode was effective against larvae and pupae of the codling moth, *L. pomonella,* which occurred under bands placed around trunks of apple trees (see 6). Environmental modification that favors the nematode and traps the target insect offers another control method for this important pest of fruit.

Overall, the use of *N. carpocapsae* only against the pupal stage does not appear practical. However, Kaya and Grieve (unpublished data) have shown that *N. carpocapsae* in soil will kill prepupae, pupae, and emerging adults of *S. exigua.* The combined mortalities can be significant; thus utilization of this nematode against these stages that occur in the soil is feasible. Other soil-pupating insects may be killed in a similar manner by the nematode. Certain insect pest species should be evaluated as to the feasibility of this control tactic.

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