

BIOCONTROL OF SQUASH BUG WITH *NEOAPLECTANA CARPOCAPSAE* (WEISER)¹

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Huei-Jung Wu (1988) Biocontrol of Squash Bug with *Neoaplectana Carpocapsae* (Weiser). *Bull. Inst. Zool., Academia Sinica* 27(3): 195-203. Some relationships likely to exist between the nematode, *Neoaplectana carpocapsae*, and the squash bug, *Anasa tristis*, have been examined under laboratory and field conditions at Fresno, California. Laboratory infection tests revealed that the infective larval stage of *N. carpocapsae* can penetrate into the squash bug via the anus. The nematode served as a carrier of the symbiotic bacterium, *Achromobacter nematophilus*, that were introduced into the body cavity and released in the hemocoel of the bug. The bacterium multiplied quickly and was responsible for the death of the bug which occurred within 25 to 72 hours. The nematodes that emerged from the eggs deposited by the second generation adult females in the dead bug formed infective juveniles that left the cadaver and entered the environment about 14 days after the infective larvae of the first generation penetrated the host. The application of *N. carpocapsae* and its associated bacterium as biological control agents for *A. tristis* is limited by their low host infection rate (24.1-70.8%) in the field trials. Circumstantial evidence suggested that the failure not be attributable to the nematode and its bacterium, but to the environment in which they were used. Nematodes required suitable moisture and temperature for survival, but their absence in Fresno preclude the use of the nematode against the squash bug in this area.

Key words: Nematode, Squash bug, Parasitism, Biocontrol.

The squash bug, *Anasa tristis* (de-Geer) (Hemiptera: Coreidae), has been reported as a serious pest of cucurbits and is one of the most difficult insects to control with insecticides (Borrer and DeLong, 1971; Metcalf and Flint, 1962). Although a tachinid fly, *Trichopogon pennipes* (Clausen, 1956), is known to parasitize the squash bug, there is not a successful biocontrol measure for this bug yet.

Nematodes of the genus *Neoaplectana* (Rhabditoidea: Steinernematidae) have been reported as the parasites of many insects (Niklas 1967, 1969). Natural host of neoap-

lectanids includes many species of the Coleoptera, Lepidoptera, Diptera, and Hymenoptera. Due to their special pathogenicity, the neoaplectanids are capable of killing, within 60 hours, most insects that ingest the infective juveniles (Poinar, 1975). This rapid death is due to the action of the symbiotic bacteria (Poinar, 1966; Poinar and Thomas, 1966a, 1966b; Poinar and others, 1971) rather than the direct injury of the nematode. Hence the nematode was designated as the carrier for bacterium. The hosts reported for the nematodes did not include species of the order Hemiptera.

The use of *Neoaplectana carpocapsae*

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(Weiser) in control of pests in the United States was limited to scientific research until recently. In February 1982, the U. S. Environmental Protection Agency cleared the product for commercial use in its action upon B. R. Supply Company's (Visalia, California) request for exemption from registration as a pesticide under FIFRA (Federal Insecticide Fungicide and Rodenticide Act).

The absence of studies on the effect of *N. carpocapsae* on the order Hemiptera and the difficulty in controlling squash bug suggested the effect of this nematode on the squash bug be investigated.

MATERIALS AND METHODS

Stock culture

A. tristis were collected from the field in Fresno, California. The bugs were sorted for noninfected specimens according to the methods of Poinar (1975). Members of the selected group were reared in screen cages (31×31 cm) with tissue paper and Zucchini squash present at all time.

Infective larvae of *N. carpocapsae* (Weiser) were obtained from the USDA Stored Product Pest Laboratory, Fresno, California. Three ml of concentrated larvae (about 3×10^5 /ml) were transferred with a disposable pipette to a plastic petri dish (150×15 mm) containing 27 ml of 0.1% formalin solution. The petri dish with the nematodes, now about 30,000/ml, was then stored at 6 °C until needed.

Nematode infectivity to constrained bugs

This study was done in a laboratory with a temperature of $26 \pm 1^\circ\text{C}$. Forty adult squash bugs were added to each one of seven sterile exposure petri dishes (100×15 mm, T1-T7) which contained four filter papers premoistened with 2 ml of 0.1% formalin solution. Each exposure petri dish was then placed in the middle of a sterile trapping petri dish (150×25 mm) containing 25 ml of 0.1% formalin solution. Two ml of the stored nematodes (about 30,000/ml) were

added to T1-T5 and T7 separately, but T6 which served as a control received only 2ml of 0.1% formalin solution without nematodes.

All the bug cadavers in T1 to T6 were removed when they died. They were washed with distilled water and placed in a 1% Ringer's saline solution, and dissected under a microscope (40 to 80X). The average cumulative infection rate was then calculated 15 days after the initial exposure to the nematode.

T7 was utilized for periodic observations over 15 days of the continuing destruction of the bug cadavers after death and the development of nematodes within the infected bug. Behavior of the nematodes and bugs were observed under a dissecting microscope (10 to 60X). As bugs died, the cadavers were observed and the level of destruction was recorded every 24 hrs. Each day one to three cadavers were dissected in Ringer's solution in small petri dishes and the development of the nematode was observed under a phase contrast microscope (400 to 1,000X).

An alternate method used for studying infection test was as follow: 1) 0.01 ml of distilled water mixed with 50-100 nematodes were force-injected into the hemocoel of the live insect with a glass tipped needle, 2) same amount of distilled water but without nematode (served as a control) was force-injected into the insect. After death, the bugs were dissected and observed as above.

Field behavior of the bugs and nematodes

1. The behavior of the squash bug on host plants: Fifty squash bugs were placed on two mature squash plants (Zucchini) in an open field. The bugs were observed for movement, resting, and feeding habits. Observations were made with visual and were recorded over one week.

2. The effect of moisture on nematode movement and mortality: Eight ml of the stored nematode suspension were diluted to 100 ml, and 50 ml was sprayed onto each of

the two squash plants using an electric sprayer. Tap water was periodically sprayed onto one plant at a rate to balance water loss: to the other plant no additional moisture was applied. The movement of the nematode was observed using a 10X hand lens and the mortality of the observed nematodes was daily estimated for 7 days.

3. The effect of temperature on nematode diurnal movement: 100 nematodes from the stored nematode solution were transferred into a petri dish (150×25 mm) containing a depth of no more than 1 cm of tap water to allow for oxygen exchange. Tap water was added to the petri dish periodically to replace that lost to evaporation. The bottom half of the petri dish was then placed in an open field in late July 1982. The environmental temperature (recorded by a maximum-minimum thermometer). The cumulative mortality (counted by removing the dead nematodes observed with a dissecting microscope at 40X), and the nematode activity were recorded every 4 hrs for 40 hrs. The same test was repeated in autumn (1982) and spring (1983).

Field tests on the nematode infectivity to the bug

Twenty squash plants (Zucchini) were planted every side around the greenhouse at California State University, Fresno, in the early September of 1982. The plants on the east side (group E) and those on the south side (group S) served as experimental groups and the plants on the west side (group W) and those on the north side (group N) were controls. When the plants were 2 months old, 6 adult squash bugs were released onto each of the 80 plants. Then 80 ml (about 2.4×10^8) of infective nematodes were sprayed onto each plant of the experimental groups. To the control groups, 80 ml of 0.1% formalin solution was sprayed onto each plant. Tap water was periodically sprinkled onto each plant of groups E and W to maintain a moist surface, but not on the plants of groups S and N. The dead bugs were colle-

cted and examined each day. The cumulative infection rate was calculated on the 8th day. The same procedure was repeated during June 7-15, 1983.

RESULTS AND DISCUSSION

Nematode infectivity to constrained squash bugs

The results of the infection test in T1 to T6 are summarized in Table 1. About 52.0% (within 72 hrs), 71.5% (within 192 hrs), and 77.5% (within 288 hrs) of the bugs, that were exposed to the nematode in T1 to T5, were found parasitized by *N.*

TABLE 1
The effect of *N. carpocapsae* infective stage larvae on constrained *A. tristis* adults

Hours after initial exposure	Experimental					Control	
	T1	T2	T3	T4	T5	T6	
	ID	ID	ID	ID	ID	D	ID
24	0	0	0	0	0	0	0
48	0	1	1	2	0	0	0
72	14	21	24	25	16	0	0
96	2	0	0	2	5	0	0
120	2	0	2	0	1	0	0
144	2	3	2	0	0	1	0
168	1	2	3	7	1	0	0
192	1	1	0	1	1	0	0
216	1	2	1	0	0	0	0
240	0	0	1	0	1	1	0
264	1	1	1	2	0	0	0
288	0	0	0	0	1	0	0
312	0	0	0	0	0	1	0
336	0	0	0	0	0	1	0
360	0	0	0	0	0	2	0
ACIR (%)	72			52.0			0
at hr	192			71.5			0
	288			77.5			0
ACR (%) of dead but non-infected bugs at hr 360				16.0			15.0
AR (%) of survived bugs at hr 360				6.5			85.0

D: non-nematode-infected bug; ID: infected dead bug; ACIR: average cumulative infection rate; ACR: average cumulative rate; AR: average rate.

carpocapsae. None of the dead bugs that were exposed to the 0.1% formalin solution in T6 was found parasitized by *N. carpocapsae*. The average cumulative rate of dead, but non-nematode-infected bugs, was 16.0% in T1 to T5 and 15.0% in T6 at hour 360. The cause of death on the non-nematode-infected bugs may have been due to the hunger, age, or unsatisfactory environment of the petri dishes. The average rate of survived bugs was 6.5% in T1 to T5 and 85% in T6 at hour 360. No additional dead bugs were found in T1 to T5 between hour 312 and hour 360. This apparent resistance of the surviving bugs to the nematode is unexplained.

During the first 4 hrs in the exposure petri dish T7, the nematodes moved over the surface of the squash bug. Around the spiracle, a few nematodes were found to remain by the peritreme for about 5 minutes before they moved into the spiracle. Others attacked the anus, whereas some were found penetrating into the rectum within 20 to 30 minutes. The nematodes which were swarming at the membranes between the leg segments did not penetrate into the body until the bug was dead and parts of these membranes were transformed from a more solid to a less solid state within 120 hrs. The above results indicate that nematodes penetrate into the bug's body via spiracles and anus at the early infection, and is hard to penetrate through intact membranous areas.

The dead bugs in T7 were dissected and examined for nematodes. The number of nematodes found in each bug ranged from 1 to 9×10^4 with about 78% of the bugs parasitized within 12 days.

The infective nematode at 3rd stage of larvae molted twice. Testis (with sperm) and ovaries (with eggs) became visible under a phase contrast microscope (400-1,000X). These nematodes were matured within 120 hrs after penetrating the host bug. The size at maturation was increased about 10X

comparing to the third stage larva (50u) to an adult male and about 40X to an adult female.

The infective *N. carpocapsae* at 3rd larval stage showed a mass of rod-shaped bacteria in the anterior part of the intestine. These bacteria, *Achromobacter nematophilus* (Poinar, 1966; Poinar and Thomas, 1966a), were released from the broken nematode by pressing the coverglass. The same bacteria were found in increasing number in the hemocoel of the infected bugs beginning at about 25 hrs after the initial exposure. They were not found in the hemocoel of the control bugs. Apparently, the bacteria were carried into the host's hemocoel by the infective nematode.

Mating was not seen, but ovoviviparous eggs were seen in adult females from the 49th to 136th hour. The first stage larvae from the hatched eggs remained inside the first generation female adults for about 24 to 36 hrs before the skin of the mother was seen to break and the larvae escaped into the host hemocoel. The new generation was seen to develop and mature in the hemocoel. In average, it took about 140 hrs from first generation larva to second generation larva, and the same time occurred between the second generation and third generation.

About 290 hrs after the infection, tremendous clumps of larvae were present in the hind gut of the host and swarms emerged via the anus. Around 360 hrs after infection, about 1 million infective stage larvae that had left the host bugs were found in the trapping petri dish (T7).

The cadavers of the infected bugs were observed to undergo color (lighter) and odor (obnoxious) change, body swelling, and membrane, trachea, and tissue decay.

All bugs which were force-injected with nematodes died within 80 hrs. The infection rate was 92.5%. The development of the nematode inside the cadavers was similar to that of those infected in the petri dish (T7). In contrast, none of the bugs which were

force-injected with 0.1% formalin solution died within 120 hrs.

Field behavior of squash bugs and nematodes

Flying and dispersal movements were not detected on the winged squash bugs during the study. About 69.6% of the squash bugs were found hidden around the base of the plants under the lower leaves or amongst the clods and 30.4% on or under the upper leaves or vines (Fig. 1). And 88.4% were found feeding during the day time and 11.6% during the night. But the squash bug could be found feeding at any time, day or night, throughout the summer in Fresno.

The effect of temperature on nematode diurnal movement and relative mortality are listed in Table 2. In summer, the average cumulative mortality was 42% during the day time (0600-2000h), with temperature from 21.0 to 40.5°C; and 8% during the night time (2000-0600h), while temperature ranged from 21.0 to 29.0 °C. The difference between the average cumulative mortalities during the day time and the night time in autumn and spring were negligible in Fresno. The nematodes were more active and evenly

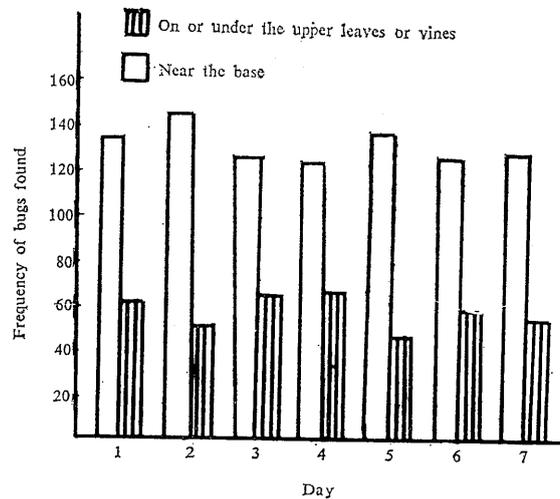


Fig. 1. Frequency and place of squash bugs found within 7 days after being released onto two mature Zucchini squash plants.

distributed between 2200h and 1000h in summer, and between 1800h and 1000h in both autumn and spring (Table 2).

The occurrence and behavior of *N. carpocapsae* on the surface of two mature squash plants were observed in early September of 1982 in Fresno. High mortality (nearly 90%)

TABLE 2
The effect of temperature on *N. carpocapsae* diurnal movement and relative mortality

Hour intervals	Summer (1982)			Autumn (1982)			Spring (1983)		
	T(°C)	CM(%)	A	T(°C)	CM(%)	A	T(°C)	CM(%)	A
1000-1400h	25.4-35.3	5	ad	23.3-26.7	3	ad	21.7-26.1	4	ad
1400-1800h	35.3-40.5	39	ad	26.7-30.0	6	ad	26.1-28.3	7	ad
1800-2200h	40.5-29.0	46	ad	30.0-22.2	7	bc	28.3-21.1	9	bc
2200-0200h	29.0-24.1	48	bc	22.2-16.1	8	bc	21.1-14.4	9	bc
0200-0600h	24.1-21.0	52	bc	16.1-13.3	8	bc	14.4-11.1	9	bc
0600-1000h	21.0-31.4	55	bc	13.3-22.2	8	bc	11.1-19.4	9	bc
1000-1400h	31.4-39.8	78	ad	22.2-28.9	10	ad	19.4-25.6	10	ac
1400-1800h	39.8-40.4	85	ad	28.9-30.6	12	ad	25.6-26.7	12	ac
1800-2200h	40.4-27.5	88	ad	30.6-21.1	15	bc	26.7-19.4	13	bc
2200-0200h	27.5-25.2	89	bc	21.1-17.2	15	bc	19.4-12.8	13	bc

T: temperature; CM, cumulative mortality; A: activity; a: swarming at the edge on the bottom of the petri dish; b: evenly distributed on the bottom of the petri dish; c: tend to be active; d: tend to be quiescent.

of the observed nematodes was found within 24 hrs on the first plant in which no additional moisture was added after the nematodes were sprayed; while a lower mortality (about 60%) was observed on the second plant in which tap water was sprayed periodically to retain a moist surface. Most of the live nematodes were found near the base of the second plant during the first 24 hrs after being sprayed. They then moved down, clumping and swarming on the wet soil near the base of the plant during the following 72 hrs, and finally migrated into the soil. There were no live nematodes in 144 hrs after the plants were sprayed.

The above results suggest that evening (1900h to 2000h) be an optimum time for spraying the nematode for field tests in summer and autumn in Fresno provided that tap water is applied periodically to maintain a moist surface on the plants.

Field test on the nematode infectivity to bugs

As shown in Table 3 and 4, the cumulative infection rate at 8th day was higher in groups E' and E (63.4% and 70.8% respectively), while tap water was sprinkled periodically to retain a moist surface on the squash plants. It is lower in groups S' and S (24.1% and 47.6% respectively), while no

TABLE 3
Cumulative infection rate of 480 *A. tristis* (120 bugs in each group)
followed for 8 days after the spray on November 11, 1982

EXPERIMENTAL GROUPS: Nematodes applied						
Time after initial exposure	Group E (water sprinkled)			Group S (no water sprinkled)		
	Db	Ib	CIR (%)	Db	Ib	CIR (%)
24h	5	4	3.3	3	2	1.7
48h	19	19	19.1	16	14	13.4
72h	42	40	52.4	27	25	34.2
96h	12	11	61.6	9	8	40.9
120h	7	6	66.6	5	5	45.1
144h	4	3	69.1	3	3	47.6
168h	2	0	69.1	0	0	47.6
192h	3	2	70.8	2	0	47.6
Total at hour 192	94	85	70.8	65	57	47.6
CONTROL GROUPS: No nematodes applied						
Time after initial exposure	Group W (water sprinkled)			Group N (no water sprinkled)		
	Db	Ib	CIR (%)	Db	Ib	CIR (%)
24h	1	0	0.0	1	0	0.0
48h	2	0	0.0	2	0	0.0
72h	0	0	0.0	1	0	0.0
96h	2	0	0.0	0	0	0.0
120h	0	0	0.0	0	0	0.0
144h	1	0	0.0	0	0	0.0
168h	1	0	0.0	1	0	0.0
192h	0	0	0.0	0	0	0.0
Total at hour 192	7	0	0.0	5	0	0.0

CIR: cumulative infection rate; Db: dead bug; Ib: infected dead bug.

Place of spray: California State University, Fresno.

TABLE 4
Cumulative infection rate of 480 *A. tristis* (120 bugs in each group)
followed for 8 days after the spray on June 7, 1983

EXPERIMENTAL GROUPS: Nematodes applied						
Time after initial exposure	Group E' (water sprinkled)			Group S' (no water sprinkled)		
	Db	Ib	CIR (%)	Db	Ib	CIR (%)
24h	4	2	1.7	2	1	0.8
48h	18	17	15.9	5	4	4.1
72h	38	36	45.9	18	17	18.3
96h	11	11	55.1	7	5	22.5
120h	6	5	59.3	1	1	23.3
144h	5	3	61.8	2	0	23.3
168h	2	1	62.6	0	1	24.1
192h	0	1	63.4	1	0	24.1
Total at hour 192	84	76	63.4	36	29	24.1
CONTROL GROUPS: No nematodes applied						
Time after initial exposure	Group W' (water sprinkled)			Group N' (no water sprinkled)		
	Db	Ib	CIR (%)	Db	Ib	CIR (%)
24h	2	0	0.0	1	0	0.0
48h	0	0	0.0	1	0	0.0
72h	2	0	0.0	0	0	0.0
96h	1	0	0.0	3	0	0.0
120h	0	0	0.0	0	0	0.0
144h	0	0	0.0	1	0	0.0
168h	1	0	0.0	0	0	0.0
192h	0	0	0.0	1	0	0.0
Total at hour 192	6	0	0.0	7	0	0.0

CIR: cumulative infection rate; Db: dead bug; Ib: infected dead bug.

Place of spray: California State University, Fresno.

additional moisture was applied. In contrast, dead bugs were not found due to nematode infection in all control groups (N', W', N, W) at summer and autumn tests. The higher infection rates in groups E and S than those in groups E' and S' might due to relatively higher temperature in summer (21.6-41.1°C during June 7-14, 1983) which caused the mortality of nematodes increase as indicated from the previous tests (Tabel 2). The nematodes concentrated near the base of the plant. This appeared to be fortuitous as the quash bugs also spent most of their time at the same place. The percentages (6.7-7.5% in November and 5.8-6.7% in June) of the

dead bugs, which were not nematode infected, in experimental groups, were close to those (4.2-5.8% in November and 5.0-5.8% in June) in the control groups. Cause of death of non-infected bugs is unknown. As in laboratory studies, most of the bugs died on the third day after the nematodes were sprayed.

CONCLUSIONS

The potential relationship between *N. carpocapsae* and *A. tristis* is a case of parasitism. Infection of the squash bug is initiated when the infective larvae of *N. carpocapsae*

penetrate into the bug via the anus. As soon as they reach the bug hemocoel, the larvae begin to develop, and the bacterial cells, *A. nematophilus*, which were carried in the lumen of the intestine are released, via the anus (Poinar, 1966), to the bug hemocoel. The death of the squash bug occurs within 25 to 72 hours. The infective nematode larvae develop into first generation adults that mate and oviposit in the now dead squash bug. The eggs hatch inside the adult females and the nematodes mature to smaller second generation adults which in turn mate and oviposit. In general, the nematodes emerging from the eggs deposited by second generation females form infective larvae that leave the cadaver and enter the environment about 14 days after the infective larvae of the first generation penetrated into the host bug.

The application of *N. carpocapsae* and its associated bacterium, as the biological control agents, for *A. tristis* is limited by their low host infection rate (24.1-70.8%) in the field trials (Table 3 and 4). The actual causes of their failure cannot be positively determined but circumstantial evidence suggested that the failure not be attributable to the nematode and its bacterium, but to the environment in which they were used. Nematodes were sprayed in water droplets, and, as nematodes require moisture for survival, the rate of droplet evaporation and drying of leaves is critical. More reliable data, including ideal conditions of moisture (or water sprinkling), air movement, temperature, and nematode dosage need to be obtained before additional efficacy field tests can be made.

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顯微線蟲 (*Neoaplectana carpocapsae*) 對菜瓜蟲 (*Anasa tristis*) 防治潛能之研究

吳 輝 榮

室內感染實驗，發現卡波線蟲第三齡幼蟲可由肛門侵入菜瓜蟲體，並在菜瓜蟲體腔內放出與線蟲共生之螢光菌 (*Achromobacter nematophilus*)，導致菜瓜蟲在 25-72 小時內死亡。入侵線蟲在菜瓜蟲體內成熟及繁殖，歷經三代，約於第十四天，當菜瓜蟲體被消耗後，其第三子代始離開寄主。進一步在美國 Fresno 城所做的田間試驗，指出該線蟲對菜瓜蟲感染殺傷率，視氣溫和濕度之不同，由 24.1~70.8% 不等。研究和觀察並顯示出 Fresno 城炎熱及乾燥的氣候導致線蟲的高死亡率，因而限制了線蟲在該地的實用潛能。將來田間施用法之改進以及線蟲噴灑量的研究，將有助於以該線蟲做為生物防治的發展。

