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SUSCEPTIBILITY OF *PIERIS RAPAE CRUCIVORA* (LEPIDOPTERA: PIERIDAE) TO THE IMPORTED ENTOMOGENOUS NEMATODE *STEINERNEMA FELTIAE*[1]

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Huei-Jung Wu and Yien-Shing Chow (1989) Susceptibility of *Pieris rapae crucivora* (Lepidoptera: Pieridae) to the imported entomogenous nematode *Steinernema feltiae*. *Bull. Inst. Zool., Academia Sinica* **28**(4): 237-244. Larvae, pupae, and adults of the cabbage worm, *Pieris rapae crucivora* (Boisduval), were exposed to four concentrations of *Steinernema feltiae* (=*Neoaplectana carpocapsae*) ranging from 5,000 to 40,000 per petri dish. Mean nematode-associated mortality 3 days after exposure ranged from 75 to 97.5% for host larvae and 32.5 to 62.5% for host adults. Host pupae were not susceptible to *S. feltiae*. Invasion tests revealed that the main routes of entry were through the mouth and anus of the host. The nematodes propagated within the host cadavers and released infective stage juveniles that were infective to new hosts.

Key words: Nematode, *Pieris rapae crucivora*, Susceptibility, Parasitism, Biocontrol.

The cabbage worm, *Pieris rapae crucivora* (Boisduval) has now widely distributed and become one of the major vegetable pests here in Taiwan as effective means of control are still being sought. Previous studies using the DD-136 strains of *Neoaplectana carpocapsae* Weiser (=*Steinernema feltiae*) against a wide variety of insects included over 250 species representing 75 familes (Poinar and Deschamps, 1981). Its wide host range is due to its associated bacterium, *Xenorhabdus nematophilus* (Poinar, 1975), which multiplies inside the host and provides a suitable habitat for nematode reproduction. The infective-stages of *S. feltiae* have long been thought to be able to enter the mouth, spiracles, and anus of their hosts during the last 3 decades. Yet there was still no direct evidence regarding the main routes of entry through which the nematodes invade the host body.

Some promising factors of using the infective-stage of *S. feltiae* and their symbiotic bacterium as potential agents against insect pests have been reported including: 1) ability to live in soil without hosts for more than 6 months, broad temperature tolerance (−10 to 35°C), and resistance to insecticides (Schmiege, 1963); 2) ability to enter the tunnels and kill most of the brood and

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adults of the host insects (Moore, 1970); 3) can be used against insect pests at where some benefitial insects, such as bees, occur by following certain normal spray precautions (Kaya *et al.*, 1982); and 4) might be used as self-sustaining biocontrol agents (Poinar and Deschamps, 1981). Recent advances on the mass production and storage methods, handling techniques, and application skills of the infective-stage of *S. feltiae* have made this nematode as control agent promising.

The absence of studies on the effect of *S. feltiae* on the cabbage worm and the difficulty in controlling this insect pest suggested the effect of this nematode on this insect should be investigated. The present paper describes the ability of this nematode to infect the cabbage worm and tests on the main routes of entry through which the nematodes invade the larvae and adults of the cabbage worms.

MATERIALS AND METHODS

The nematodes, *Steinernema feltiae*, used in this study were originally obtained from the USDA Stored Products Laboratory, Fresno, California, propagated in larvae of the onion orchid worm, *Brithys crini* (Fabricius), and stored in petri dishes as described by Hara and Kaya (1981). Counts were made by dilution. Host larvae of *Pieris rapae crucivora* were reared in plastic boxes drilled with 24 openings (5 mm in diam.) at the bottom and lined with moist soil to which kale plants were planted at all time. The boxes were placed in screen cages in the laboratory to prevent escape.

Host exposure to nematode

For infection experiments, 20 hosts (larvae, pupae or adults) were placed in sterile petri dishes (150×25 mm; d1-d36, Table 1) lined with filter paper. Into each of the experimental dishes was added 2 ml of nematode suspension. The four treatments consisted of 2.5×10^3, 5×10^3, 1×10^4 and 2×10^4 infective stage nematodes per ml. Control dishes contained 20 hosts with 2 ml of distilled water. After the addition of the nematodes or water, the dishes were covered with a modified lid with 24 openings (.7 mm in diam.) and incubated at room temperature (21-26°C) and 71-94% RH. Three days after the initial host exposure to *S. feltiae*, the hosts were removed from each dish, rinsed with water, and transferred into new sterile petri dishes (d1′-d36′, 100×15 mm) lined with 50 ml of fine-finish plaster to which 28 ml of distilled water had been added 16-24 h earlier. Each of the 100×15 mm dishes (without lid) with the hosts placed inside was then placed into a larger petri dish (150×25 mm) designed to collect the emerging infective stage nematodes in water at the bottom. A modified lid was covered to each of these 150×25 mm petri dishes and the whole unit incubated at room temperature. During the next 21 days, the water containing the nematodes (in dish units d31′-36′) was removed daily, the nematodes counted, and then new distilled water was added to the containers. The emerging nematodes were tested for infectivity against healthy hosts. Six days after the initial exposure, the hosts in each of d1′-d30′ were collected and dissected under a dissecting microscope to verify nematode parasitism. Four additional petri dishes (150×25 mm) with the nematodes (4×10^4/dish), 20 host larvae, and 20 kale sprouts (as a food source for the host larvae) were set up for monitoring behavior and development of the nematodes.

Determination of invasion routes

Host larvae, pupae and adults were tested to determine the route(s) of entry

through which the nematodes invade the host body, Twenty hosts were injured, by cutting a small portion (5×2 mm) of cuticle or exoskeleton at the body surface, and/or carefully blocked by immobilizing them in cold, gluing the mouth parts and/or anus with melt wax (Crown candle, Huang Kuan Co. Ltd., R. O. C.), and cooling in water. After the revival, the blocked insects were placed in a petri dish for an hour and their blocked part(s) checked under a dissecting microscope before exposed to the infective-stage of *S. feltiae*. Most of the blocked insects were able to keep alive for 14-18 h without breaking the wax film glued on their mouth parts and/or anus. The injured insects were exposed to the nematodes soon after wounded. Nematode invasion and infectivity to the manipulated insects in the petri dishes (150×15 mm) were monitored under the conditions of different treatments as shown in Table 2.

RESULTS

Host infections

A total of 120 young (1st and 2nd larval stages) and 120 mature (3rd and 4th larval stages) larvae of *Pieris rapae crucivora* were exposed to the infective-stage of *Steinernema feltiae*. All larval stages were very susceptible to the nematodes. The mean percentage of verified nematode infection 3 days after exposure was dose-related ranging from 75% at the lowest nematode dosage level to 97.5% at the highest dose (Table 1). Host larvae began to die 16 h after

Table 1
Mean percentage of verified nematode infection of *P. rapae crucivora* 3 days after exposure as larvae, pupae or adults to the infective-stage of *S. feltiae* (n=20 per dish)

Dose	Larvae*				Adults**			Pupae***			
	Dish No.	IL (%)	IPL (%)	UL (%)	Dish No.	IA (%)	UA (%)	Dish No.	IP (%)	IEA (%)	UPA (%)
5,000	1,2	75.0[a]	0.0	25.0	3, 4	32.5[a]	67.5	5, 6	0.0[a]	0.0	100.0
10,000	7,8	85.0[b]	2.5	12.5	9,10	40.0[b]	60.0	11,12	0.0[b]	2.5	100.0
20,000	13,14	92.5[c]	2.5	5.0	15,16	47.5[c]	52.5	17,18	2.5[c]	0.0	97.5
40,000	19,20	97.5[d]	0.0	2.5	21,22	62.5[d]	37.5	23,24	2.5[d]	0.0	97.5
0	25,26	0.0	0.0	100.0	27,28	0.0	100.0	29,30	0.0	0.0	100.0

Dose	Larvae*			Adults**			Pupae***		
	Dish No.	IL (%)	M-np/H	Dish No.	IA (%)	M-np/H	Dish No.	IP (%)	M-np/H
40,000	31,32	95.0	Approx. 9,500	33,34	57.5	Approx. 3,700	35,36	2.5	Approx. 7,000

* IL=% infected larvae; IPL=% infected pupated larvae; UL=% uninfected larvae; M-np/H=mean nematode production per host larva.

** IA=% infected adults; UA=% uninfected adults; M-np/H=mean nematode production per host adult.

*** IP=% infected pupae; IEA=% infected emerging adults; UPA=% uninfected pupae and emerging adults; M-np/H=mean nematode production per host pupa.

Superscripts *a* vs *c* and *a* vs *d* in IL column and superscripts *a* vs *d* in IA column are significantly different, but superscripts *a*, *b*, *c*, *d* in IP column are not significantly different ($p<0.05$; Duncan's multiple range test).

exposure and at 72 h, 196 (98%) out of 200 experimental larvae were dead. Among these dead larvae 90.8% (178) was, as verified 6 days after the initial exposure, nematode-associated mortality. Tissues of parasitized larvae were yellowish brown and contained developing *S. feltiae* adult or larval stages. Sixteen (40%) out of 40 control larvae also died in this period but they were free of nematodes. All experimental and control larvae were dead 6 days after the initial exposure, and 2 of the parasitized mature larvae died as young pupae.

A total of 240 host adults were tested for nematode infectivity. Adults of *P. rapae crucivora* were moderately susceptible to *S. feltiae*. The mean percentage of verified nematode infection of experimental adults was also dose-related ranging from 32.5 to 62.5% (Table 1). Host adults began to die 20 h after exposure and at 72 h, 134 (67%) out of 200 experimental adults were dead. Among these dead adults 71.6% (96) was later verified to be nematode-associated mortality. Fifteen (37.5%) out of 40 control adults also died in this period and they did not contain nematodes. All experimental and control adults died 5 days after the initial exposure.

A total of 240 host pupae were tested for nematode infectivity. Pupae of *P. rapae crucivora* were resistant to *S. feltiae* under conditions used in the experiments. A total of only 3 (1.5%) out of 200 experimental pupae were infected and killed by the nematodes after 3 days exposure (Table 1). Yet the 4 emerging adults in the experimental dishes were marginally susceptible at 25% (1) verified infection in this period.

Infective-stage of *S. feltiae* that emerged from diseased hosts (in dish units d31′-d36′) were collected and counted over a period of 15-17 days. Nematode production began 7-9 days after the initial exposure at room temperature (21-26°C). At the end of the emergence period (days 19-24) an average of approximately 9,500, 3,700, and 7,000 infective stage nematodes emerged from each of the parasitized 38 host larvae, 23 adults and 1 pupae respectively (Table 1). These nematodes were tested for infectivity against new healthy hosts of *P. rapae crucivora* at a dose of 4×10^4 nematodes per dish ($n=20$); 38 (95%) of the new host larvae, 26 (65%) of the adults and 0 (0%) of the pupae tested were killed by the nematode infection 72 h after exposure.

Behavior and development

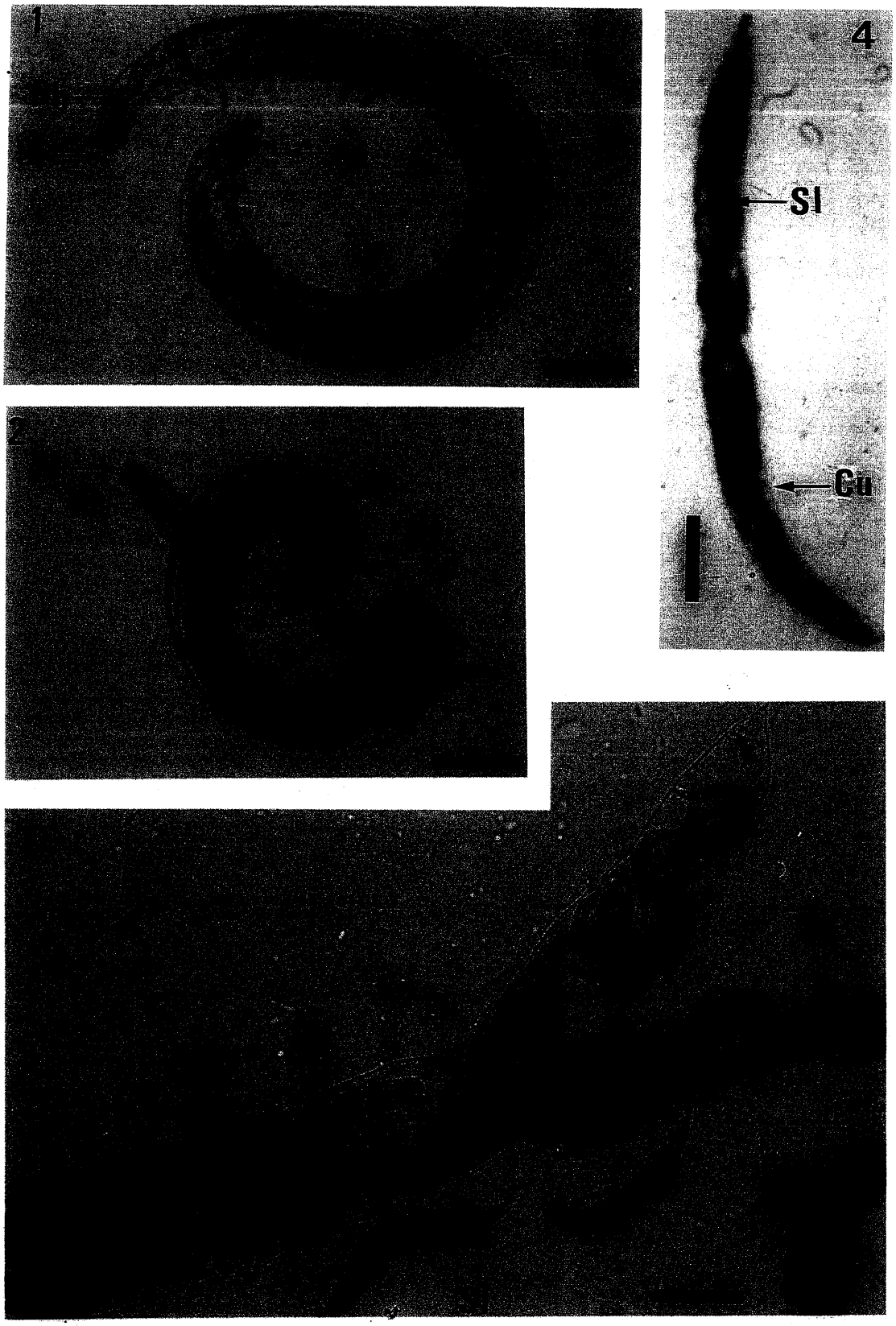
Hosts and nematodes were seen moving very actively soon after the exposure. Nematode locomotion was made either by swimming in the water at the bottom of the petri dish or looping and bridging between two substrates on the moist surface of the host. No clear-cut evidence was observed to show the

Fig. 1. An adult male of *Steinernema feltiae* isolated from an infected larva of *Pieris rapae crucivora*. Bar: 80 μm; Ac: alimentary canal; Cu: body cuticle; Ph: pharynx; Sp: spicule; St: stroma; Te: testis.

Fig. 2. An adult female of *Steinernema feltiae* isolated from an infected larva of *Pieris rapae crucivora*. Bar: 240 μm; Ac: alimentary canal; Cu: body cuticle; Gp: genital pore; Ov: ovaries; Ph: pharynx; St: stroma.

Fig. 3. A mother nematode of *Steinernema feltiae* isolated from an infected larva of *Pieris rapae crucivora*. Bar: 160 μm; Cu: body cuticle; Dr: decomposing remnant of the mother's digestion system; Eu: envelope of uterus; Hl: newly hatched nematode; Oe: an ovoviviparous egg; Sl: ensheathing second stage larva.

Fig. 4. Young nematodes broke out from a depleted mother nematode of *Steinernema feltiae*. Bar: 640 μm; Cu: mother's body cuticle; Sl: ensheathing second stage larvae.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nematodes could direct its movements in responce to the host larvae. But many of them were able to locate the host larvae soon after the releasing indicating a possible means of detection other than encountered through random movements. The attacking behavior was not obvious. None of the nematodes which moving over the general surface of the host was seen successfully piercing the host cuticle or penetrating through the spiracles. Yet many of them were seen entering the host body orally, either passively when the insect was feeding or actively at other time.

Dissections showed that 24 h after exposure, infective-stage of *S. feltiae* remained in the lumen of the alimentary canal and many of them entered, apparently by piercing through the decomposing gut wall, into the hemocoel of the host larvae. Other nematodes were also found inside the tissues in head, thorax and/or abdomen of the host. But none of the intruded nematodes was found in the tracheal system in any dissected host larva. A large part of the host tissues were decomposed and most of the parasitized hosts were killed in this period.

After molting, the nematodes grew rapidly into adult males and females (Figs. 1-2). Sexual maturity of the first generation occurred in about 72-120 h after the initial exposure. Mating was not seen. The ovoviviparous eggs, ranging from about 60-140 (mean 96, $n=10$), hatched within the uterus of the female adult (Fig. 3). These young nematodes fed on the contents of the uterus and later broke into the body cavity where they fed within the mother's cuticle and grew into the second stage of development. At about 121-168 h after exposure, the young nematodes broke out of the mother's cuticle (Fig. 4). Some of them molted and retained the old cuticle, becoming infective-stage juveniles, while others continued to mature and formed the third generation in the decomposed tissues of the host larvae. This process continued until the host larvae was depleted. The nematodes then escaped from the host cuticle and migrated into the water in the trapping dish designed to collect them.

Nematode invasion

A total of 20 healthy pupae (in d41) and 20 injured pupae (in d42) of *P. rapae crucivora* were exposed to infective-stage of *S. feltiae* (40,000/dish). As shown in Table 2, none of the intact pupae in d41 was nematode-infected while 65% of the wounded pupae in d42 were. This result, as supported by the observations made under a dissecting microscope, strongly suggested that the infective-stage of *S. feltiae* could not enter the intact pupae by piercing the hard pupae case and penetrating through it.

A total of 220 host larvae (d43-d53, Table 2) were exposed to the nematodes (5,000/dish). A mean of 67.5% of the healthy larvae in d45-d46 (without kale sprouts) was nematode-infected while 90% of those in d43-d44 (with kale sprouts as a food source) was nematode-infected. One of the reasons to this result, as observed under a dissecting microscope, was that ingestion of the kale sprouts by the host larvae in d43-d44 increased the chance of passive invasion of the nematodes moving amongst the sprouts. About 52.5% of the anus-blocked larvae, 27.5% of the mouth-blocked larvae and 2.5% of the mouth- and anus-blocked larvae in d47-d48, d49-d50 and d51-d52 were nematode-infected respectively (Table 2). This result strongly suggested that the main routes of entry to the host larvae were through the mouth and anus, of which the mouth was the major entrance and the anus was the minor one. There was a 40% infection rate for

Table 2
Mean percentage of verified nematode infection of manipulated *P. rapae crucivora* 3 days after exposure as healthy or injured and/or partially blocked larvae, pupae or adults to the infective-stage of *S. feltiae* (n=20 per dish)

Stage tested (Nematode dose/dish)	Dish No.	Treatment			Verified infection (%)
		Body part(s) blocked*	Body part injured	Other	
Pupae	41	—	—	—	0.0
(40,000)	42	—	Pupal case	—	65.0
Larvae (5,000)	43,44	—	—	Food**	90.0
	45,46	—	—	—	67.5
	47,48	Anus	—	—	52.5
	49,50	Mpts	—	—	27.5
	51,52	Mpts, Anus	—	—	2.5
	53	Mpts, Anus	Cuticle	—	40.0
Adults (40,000)	54,55	—	—	Food***	65.0
	56,57	—	—	—	67.5
	58,59	Anus	—	—	5.0
	60,61	Prob	—	—	65.0
	62,63	Prob, Anus	—	—	0.0
	64	Prob, Anus	Cuticle	—	35.0

* Mpts=mouth parts; Prob=proboscis.

** Thirty kale sprouts (5 cm tall) were provided to the host larvae in the petri dish as a food source.

*** Honey water (0.1 ml) was mixed with nematode suspension in the petri dish as a food source for the adult hosts.

the larvae (in d53) with mouth- and anus-blocked and surface cuticle injured.

A total of 220 host adults (d54-d64, Table 2) were exposed to the nematodes (40,000/dish). The results, as shown in Table 2, suggested that the anus of the host adults was the main route that the nematodes penetrated through. The mouth (or the proboscis) of the hosts was, however, very slender and was probably too narrow to allow either passive or active invasion of the infective-stage of *S. feltiae*. There was a 35% infection rate for the adults (in d64) with anus- and proboscis-blocked and surface exoskeleton injured.

DISCUSSION

Infection tests showed that both larval and adult stages of *Pieris rapae crucivora* were susceptible to the infective-stage of *Steinernema feltiae*. The mean percentage of nematode-associated mortality was dose-related. Microscopic observations and host dissections suggested that the nematodes might not be able to enter the host larvae by penetrating the cuticle and tracheal system. Invasion tests revealed that the nematodes could not pierce the pupal case and adult exoskeleton of *P. rapae crucivora* and that the main routes of entry were through the mouth, anus and/or surface cuticle when wounded, the injured body parts of the host larvae and adults. The nematodes matured and propagated inside all host stages of *P. rapae crucivora* and released infective stage juveniles that were infective to new hosts. Thus the nematodes could be used as self-sustaining biological control agents. The yield

of nematodes per host, the propagation generations in the host, and the nematode emergence period varies with the size and condition of the insect. Further studies are needed to determine the practical application of the nematode as a biological control agent of *P. rapae crucivora*.

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顯微線蟲 (*Steinernema feltiae*) 對紋白蝶 (*Pieris rapae crucivora*) 防治潛能之初步研究

吳 輝 榮　　周 延 鑫

室內感染實驗，將紋白蝶幼蟲、成蟲及蛹分別暴露於費爾地線蟲 (*Steinernema feltiae*) 之第三齡幼蟲，發現寄主紋白蝶各期幼蟲和成蟲都會在暴露後短時間內被該線蟲寄生，其在 72 小時內被感染致死率 (32.5-97.5%) 與線蟲劑量之高低成正比；但於蛹期則因線蟲不能穿過堅硬的蛹殼，而不容易被該線蟲感染。進一步的觀察和實驗，顯示出該線蟲入侵寄主的主要孔道是經由寄主幼蟲的口器和肛門或寄主成蟲的肛門。入侵線蟲在寄主體內快速成熟及繁殖，歷經 2-3 代 (16-24天)，產生爲數衆多的子代，該子代離開原寄主後，經實驗證明，其對新寄主的感染能力，和其親代並無差別，因此該線蟲可做爲一種應用於蟲害防治的自我生產的生物防治劑。