

SUPPRESSION OF FULLER ROSE BEETLE (COLEOPTERA:
CURCULIONIDAE) ON CITRUS WITH *STEINERNEMA*
CARPOCAPSAE (RHABDITIDA: STEINERNEMATIDAE)

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ABSTRACT

A laboratory bioassay with larvae and adults of the Fuller rose beetle, *Asynonychus godmani* Crotch, and the Kapow strain of *Steinernema carpocapsae* (Weiser), resulted in 12, 44, and 67% mortality with rates of 50, 150, and 500 infective juveniles per 3-wk old larva, respectively; 100% mortality with 150 infective juveniles per 3-mo old larva; and 24, 48, and 83% mortality with adults. A field trial was conducted on Valencia orange trees harboring high levels of Fuller rose beetle late instar larvae. A single application of either the Kapow or All strain of *S. carpocapsae* each applied at 3 rates (50, 150, and 500 infective juveniles per cm²) reduced the number of emerging adult Fuller rose beetles a combined 55 and 38% compared with the water control the year following treatment and 79 and 82%, respectively, the 2nd year. Because of high variability between treatments, however, it was difficult to choose between the two nematode strains or the 3 rates of each strain. Infested fruit was reduced by a combined mean level of 62% one year after treatment. Based on nematode recovery at 6 months and the further reduction of Fuller rose beetle emergence in the second year

after application, we suspect that nematodes persisted and recycled in the soil and provided added control in the second year of the trial.

In order to conduct the laboratory bioassay, a method of rearing Fuller rose beetle from egg to adult was developed. A corn rootworm artificial diet was used but resulted in high larval mortality. After 6 months on the diet under laboratory conditions, 12 parthenogenetic females resulted from approximately 1,000 eggs and 8 of these females produced viable eggs masses.

Key Words: *Asynonychus godmani*, parasitic nematodes, Kapow strain, All strain, Biovector™, biological control

RESUMEN

Un bioensayo con larvas y adultos de *Asynonychus godmani* Crotch, y la cepa Kapow de *Steinernema carpocapsae* (Weiser), dió como resultado 12, 44, y 67% de mortalidad con dosis de 50, 150 y 500 juveniles infectivos por larva de 3 días de edad, respectivamente; 100% de mortalidad con 150 juveniles infectivos por larvas de 3 meses de edad, y 24, 48 y 83% de mortalidad con adultos. Un ensayo de campo fue conducido en árboles de nanranjo Valencia, con altos niveles de instares tardíos de *A. godmani*. Una sola aplicación de la cepas Kapow o All de *S. carpocapsae* aplicadas cada una a 3 dosis (50, 150, y 500 juveniles infectivos por cm²) redujo el número de *A. godmani* emergidos en un 55 y 38%, comparado con un testigo con agua al año siguiente al tratamiento, y 79 y 82%, respectivamente, en el segundo año. Sin embargo, debido a la alta variabilidad entre los tratamientos, fue difícil de escoger entre las dos cepas del nemátodo o las tres dosis de cada cepa. Los frutos infestados fueron reducidos por un nivel de media combinada del 62% un año después del tratamiento. Basados en la recuperación de nemátodos en el segundo año después de la aplicación, sospechamos que los nemátodos persistieron, se reciclaron en el suelo, y aportaron control adicional en el segundo año del ensayo.

Para conducir los bioensayos de laboratorio, fue desarrollado un método de cría para *A. godmani* desde el huevo hasta el adulto. Fue usada una dieta artificial para gusanos de la raíz del maíz pero produjo una alta mortalidad larval. Luego de 6 meses en la dieta bajo condiciones de laboratorio, 12 hembras partenogenéticas se obtuvieron de aproximadamente 1,000 huevos y 8 de esas hembras produjeron masas viables de huevos.

The Fuller rose beetle (FRB), *Asynonychus godmani* Crotch (*Pantomorus cervinus* (Boheman)), is a flightless, parthenogenetic beetle which was reported in California as early as 1879 (Chadwick 1965). Until recently, however, it was considered to be a relatively unimportant pest of citrus in the state. In 1985, viable egg masses were found under the calyx of fruit shipped to Japan. Japan lists FRB as a quarantine pest and loads of fruit found to contain a single fruit infested with a viable egg mass are fumigated with methyl bromide. Fumigation damages the fruit and is costly to the grower.

The FRB is thought to have one generation per year in California with peak emergence of adults from the soil occurring August to October although a few adults emerge each month of the year (Morse et al. 1987). After emerging from a pupal chamber in the soil, adults feed on the leaves of citrus for several wks prior to laying egg masses in cracks and crevices in the tree and under the calyx of fruit. Eggs hatch after 3-4 wks, larvae drop to the ground, enter the soil, and feed on citrus roots for most of a year before building a pupation chamber several centimeters below the soil surface.

Methods of reducing FRB infestation of citrus shipped to Japan include: (1) monitoring citrus groves or fruit lots in the packinghouse and prioritizing fruit with low levels of viable FRB egg masses for shipment to Japan, (2) skirt-pruning and trunk treatments to exclude adult beetles from ovipositing on fruit in the tree, (3) foliar chemical treatments to kill adults in the tree, and (4) holding lemons in cold storage long enough to allow eggs to hatch (Morse et al. 1987, 1988, Haney & Morse 1988, Lakin & Morse 1989). None of these options has yet proven to be totally satisfactory due to a combination of logistic and economic constraints consistent with the need for almost 100% control required by quarantine protocols (Morse et al. 1987). This research investigates an alternative control strategy—use of the entomopathogenic nematode, *Steinernema carpocapsae* (Weiser) (= *S. feltiae* Filipjev), to reduce FRB larval populations in the soil thereby reducing subsequent adult emergence and oviposition on the fruit.

MATERIALS AND METHODS

FRB Laboratory Rearing

Adult FRB for larval rearing were collected from the Fairview citrus grove in Hemet, CA, and approximately 25 were isolated in each of 4 ventilated plastic sweater boxes (11 cm × 26 cm × 36 cm) with Valencia orange leaves, *Citrus sinensis* (L.) Osbeck, provided as a food source, and 10 tightly folded pieces of wax paper as an oviposition substrate. Eggs were collected, allowed to hatch, and larvae were reared for 3 wks or 3 mo at room temperature ($24.4 \pm 1.7^\circ\text{C}$) on a corn rootworm artificial diet (Diet Premix No. 1675, Bio-Serv. Inc., Frenchtown, NJ) as suggested by Dr. W. J. Schroeder, USDA-ARS, Orlando, FL. The diet mixture was prepared by mixing 25 liters cold water, 750 g agar, 60 ml formaldehyde, and 10 kg diet premix. The mixture was heated to 93°C in a mixing machine, poured while still hot into small 25-ml plastic diet cups, dried for 5 d, and refrigerated prior to use.

Laboratory Nematode Bioassay

Preliminary laboratory bioassays with the Kapow selection of *S. carpocapsae* were conducted on five dates with adult FRB, and on a single date each with 3-week old (1-2 mm in length) FRB larvae, and a small number of 3-month old larvae (about 5 mm in length). Adult FRB bioassays were conducted using beetles collected from a commercial citrus grove in Hemet, CA. Larvae used in the bioassays were reared from eggs collected in the laboratory.

Kapow selection infective juveniles (IJ), selected since 1984 from the Mexican strain for increased production and pathogenicity (Agudelo-Silva et al. 1987, Lindegren 1990), were produced at the USDA-ARS, Horticultural Crops Research Laboratory, Fresno, California using the *in vivo* method described by Lindegren et al. (1993). The laboratory bioassays were conducted with recently harvested Kapow infective juveniles (IJ) counted by pipetting 0.02-ml drops of IJ-water suspension onto the bottom of a 10-cm diam, 1.5-cm high, plastic petri dish. The drops were overlaid with 9-cm diam filter paper, and deionized water was added to give a total volume of 1 ml per dish. The dish was covered with a lid and allowed to equilibrate for 1 h, then 10 FRB adults or larvae were added to each petri dish. Each test consisted of 10 replications of 0, 5, 15, and 50 nematodes per FRB (i.e., 0, 50, 150, and 500 IJ per dish). Adult FRB were bioassayed on 5 dates, 3-wk old larvae were bioassayed once, and because only twenty 3-mo old larvae were available, a single replicate was bioassayed using a wa-

ter control and 150 IJ/ larva. The dishes were incubated at 25°C and 45% RH. Mortality was evaluated after 72 h, and data were corrected for control mortality using Abbott's formula (1925). All larval and adult mortalities were verified by dissection.

Field Trial

A mature Valencia orange grove (Block 8, Fairview Ranch) in Hemet, CA was chosen for the field trial based on high levels of FRB adults observed the previous year. Pre-treatment counts of fruit calyx infestation with FRB egg masses (both hatched and unhatched eggs) were taken 7 June 1989 by removing and examining the calyx of 20 fruit randomly picked from the exterior canopy of each of 160 trees. Seventy trees with the highest infestation levels were chosen and seven single tree replicates were assigned to each of 10 blocks based on the percent of fruit infested (there were 10 replicates per treatment). One tree in each block was assigned randomly to each of the following seven treatments: water control, Kapow strain at 50, 150, and 500 nematodes per cm², and All strain (Biovector™, Biosys, Columbia, MD) at 50, 150, and 500 per cm² applied to the soil surface beneath the drip line of the tree. Data trees were separated by a minimum of one untreated buffer tree in each direction down the row and were spread uniformly over an area of 13 rows by 47 trees (611 trees total). Tree spacing was 6.1 m both down and across the row, trees were approximately 5.5 m high, and the tree canopy extended approximately 5.5 m in diam. Tree skirts were pruned to 1 m above ground level and, if necessary, between data trees and adjacent trees in a row, so that beetles could access data trees only via the trunk. Treatments were applied 26 June 1989 using a 189-liter diaphragm pump sprayer (Lindgren et al. 1987) to apply the nematodes in 1.9 liters of water, uniformly under the drip line of each tree. The grove was irrigated for 9 h before and for 15 h after nematode applications were made. The irrigation system consisted of fanjet J2 minisprinklers (Teejet, Wheaton, IL) positioned mid-way between each trunk down the row emitting 60.0 liters of water per h.

FRB adult emergence from the soil was monitored every two wks July to December for two years following treatment using three 58.4-cm square emergence boxes placed under each tree. Emergence box frames were constructed of wood, covered with screen, and were pushed one cm into the soil to prevent beetle escape. Counted adults were released under the sampled data tree. Fruit FRB egg mass contamination was determined again 21 June 1990 by examining 100 fruit selected randomly from the exterior of each data tree.

Persistence of nematodes in the soil was bioassayed on 0-, 0+ (several h before and after treatment, respectively), 9, 15, 23, and 193 d post-treatment using soil cores (5.0-cm diam, 7.0-cm deep) collected from 5 of the 10 replicates from each treatment using a circular aluminum cylinder with a volume of 137 cm³. On each date, 3 soil cores were collected 1.5 m from the trunk of each of the same 5 replicate trees (initially chosen randomly) and were shipped by 2-d mail to Fresno where bioassays were conducted one d later using wax moth larvae, *Galleria mellonella* (L.). The three samples from each tree were mixed and an aliquot of 88.4 cm³ of soil was spread evenly in individual 15-cm diam by 2.5-cm high, plastic petri dishes with 10 larvae. Mortality was evaluated after 72 h incubation at 25°C. Soil samples from +15 and +23 d were quite dry and 10 ml of water was mixed with the soil prior to bioassays being conducted.

Statistical analysis was conducted using PROC GLM in SAS (SAS Institute 1985) and mean separation was determined using the REGWQ option.

RESULTS

FRB Laboratory Rearing

The corn rootworm diet appears suitable for rearing FRB larvae although mortality rates were quite high especially during early larval instars. Diet moisture content may be important and some manipulation of this might lead to increased survivorship. Under laboratory conditions, 12 adults were obtained from approximately 1,000 neonate larvae placed on the diet with the larval and pupal stages lasting five and one month, respectively. Of the 12 adults obtained, 8 laid egg masses within 4 wks of pupal emergence. Egg hatch was comparable to that observed with field collected adults and neonate larvae appeared to be normal.

Laboratory Nematode Bioassay

The laboratory bioassay with 3-week old FRB larvae and the Kapow strain of *S. carpocapsae* resulted in 7% control mortality and 12, 44, and 67% corrected mortality with rates of 50, 150, and 500 IJ per larva, respectively (Table 1). A limited number of large FRB larvae (3 mo old, about 5 mm in length) were available and in the single bioassay conducted, 10% control mortality was observed and 100% mortality with 150 IJ per larva.

Five laboratory bioassays were conducted with adult FRB and results were variable (Table 1). On average, 7.6% control mortality was observed and 24.1, 47.6, and 83.3% corrected mortality with 50, 150, and 500 IJ per larva, respectively. Based on the above data, a field trial was determined to be worthwhile.

Field Trial

The Valencia orange grove in Hemet chosen for the field trial had as high a level of FRB infestation for the two years prior to the trial as we have seen in California since 1985. As a result of choosing the most heavily infested trees for the trial and blocking treatments based on fruit egg mass infestation levels, pre-trial levels were similar among treatments and varied from 24.0 to 25.0% of the outside fruit infested just prior to harvest in June, 1989 (Table 2). Petal-fall for these fruit was May, 1988 and thus, they were exposed to FRB oviposition for 13 mo prior to harvest with the highest levels of oviposition expected approximately August to December, 1988 (Morse et al. 1987).

Nematodes were applied 26 June 1989. Morse et al. (1987) monitored FRB soil emergence in four groves in Riverside and San Bernardino counties. One of these groves was the same Block 8 used in this *S. carpocapsae* trial and a second was a nearby grapefruit grove. Based on data from all four groves, monthly adult emergence was 2.7, 6.8, 32.0, 34.9, 16.0, 6.0, and 1.5% of the yearly emergence during the months of June through December, respectively, and only 0.1% of the FRB adults emerged in the months of January through May. Thus, at the time of nematode application, we assume that the majority of the FRB were in the late larval or pupal instars.

Soil laboratory wax moth bioassays indicated that nematodes initially persisted in the soil for at least 23 d and the All strain was recovered in the persistence bioassay 193 d later (Table 2). Soil samples from the +9 d sample overheated in transport and low bioassay mortalities for this date were suspect. Although not statistically different than laboratory wax moth mortality seen with soil from the water control, numerically higher mortality was observed with soil from the 150 and 500 nematodes per

TABLE 1. IMPACT OF THE KAPOW SELECTION OF *NEOAPLECTANA CARPOCAPSAE* ON FRB ADULTS AND LARVAE IN LABORATORY TRIALS.

Nematode Rate (Ij/FRB)	Mean Corrected Percent Mortality with 3-wk Old Larvae	Mean Corrected Percent Mortality with 3-mo Old Larvae	Corrected Percent Adult FRB Mortality in Tests on 5 Dates					Mean Corrected Percent Adult Mortality ¹
			27-Nov-88	10-Jan-89	15-Jan-89	31-Jan-89	7-Feb-89	
Control	7	10	2	4	14	10	8	7.6 c
50	12	— ²	4	22	30	44	48	24.1 bc
150	44	100	20	40	78	60	56	47.6 b
500	67	—	58	84	98	96	84	83.3 a

¹Means followed by the same letter within a column are not significantly different ($p = 0.05$, REGWQ; $F_{3,18} = 19.69$). Percentage data were transformed using arcsine square-root fractional mortality prior to analysis; untransformed means are listed.

²Due to an insufficient number of 3-mo old larvae, only a single replicate was conducted with the control and 150 Ij/larva.

TABLE 2. PERSISTENCE OF NEMATODES IN SOIL AND IMPACT OF NEMATODES ON ADULT FULLER ROSE BEETLE EMERGENCE AND FRUIT INFESTATION.¹

Percent Mortality in Laboratory Soil Bioassays with Wax Moth Larvae													
Nematode Rate (nem/sq cm)	Nematode Strain	Date											
		Days Post-treatment					FRB Adult Emergence per Tree, July-Dec.						
		26-Jun-89	26-Jun-89	5-Jul-89	11-Jul-89	19-Jul-89	5-Jan-90	[% Reduction Compared to Control]				Percent Infested Fruit Post-trial 21-Jun-90	
		0-	0+	9	15	23	193	1989	1990	Both years			
—	Control	4 a	4 c	0 a	0 a	0 a	0 a	24.1—a	19.0—a	43.1—a	10.7—a		
50	Kapow	0 a	96 ab	2 a	0 a	0 a	0 a	13.4[44]a	4.5[76]b	17.9[59]b	3.9[64]b		
50	All	0 a	82 b	0 a	0 a	0 a	12 a	15.2[37]a	3.0[84]b	18.2[58]b	3.6[66]b		
150	Kapow	2 a	94 ab	2 a	4 a	0 a	0 a	10.6[56]a	4.4[77]b	15.0[65]b	3.3[69]b		
150	All	4 a	98 a	24 a	20 a	6 a	9 a	12.2[49]a	2.0[90]b	14.2[67]b	4.4[59]b		
500	Kapow	0 a	100 a	2 a	18 a	4 a	0 a	8.2[66]a	3.1[84]b	11.3[74]b	4.3[60]b		
500	All	0 a	100 a	16 a	32 a	10 a	0 a	17.6[27]a	5.4[72]b	23.0[47]b	5.2[51]b		
								Mean reduction [46.6]		[80.4]	[61.5]		
F _{6,68}		1.58	46.51	1.74	1.48	1.31	1.35	1.89	10.34	5.21	3.73		

¹Means followed by the same letter within a column are not significantly different (p = 0.05, REGWQ). Percentage data were transformed using arcsine square-root fractional infestation or mortality prior to analysis; untransformed means are listed.

cm² treatments of the All strain at 9, 15, and 23 d and with the 500 per cm² treatment of the Kapow strain at 15 d. Because the life cycle of *S. carpocapsae* is approximately 2 to 3 wks, we assume that this indicates persistence and recycling of the nematodes for at least one generation on FRB or other insects present in the soil and the 193 d data suggest persistence for 6 months.

Soil emergence of adult FRB followed the general pattern reported by Morse et al. (1987), with the highest levels of emergence occurring in August through October, independent of treatments. In the water control plots, total emergence over the six months July - December was 24.1 and 19.0 adults collected from the three emergence boxes per tree for 1989 and 1990, respectively. In 1989, treatments reduced emergence 27% (All 500) to 66% (Kapow 500) (mean of 47% over the 6 treatments) compared with the water control although data were not statistically significant due to high variability among replicates. In 1990, however, all nematode treatments resulted in statistically reduced emergence, 72% to 90% compared with the control, with an average reduction for all 6 treatments of 80%. Based on this further reduction of FRB emergence in 1990 over that seen in 1989, and the 193 d *Galleria* bioassay, we suspect that nematode populations persisted in the soil beyond 1989 and gave added control of FRB larvae and pupae in the second year of the trial.

Fruit infestation by FRB egg masses one year after the trial began were significantly reduced 51 (All 500) to 69% (Kapow 150) with an average of 62% compared with levels in the water control. Note that these mature Valencia oranges were set May, 1989 and thus, the majority of egg masses observed resulted from adults emerging July to December 1989. We have no clear explanation for the decline in egg mass infestation in the water control trees from June, 1989 (25.0% infested) to June, 1990 (10.7%). Data trees were separated by at least one buffer tree down the row and a total of 60 of 611 trees in this part of the grove were treated with nematodes in this trial. It is possible that nematode applications in June, 1989, to selected heavily infested trees distributed throughout the grove, suppressed FRB levels in the entire block resulting in reduced oviposition in the control trees. Persistence and dispersal of entomopathogenic nematodes has been observed in other tests (Rick Miller, Biosys, Inc., personal communication). This possibility, however, remains unproven.

Although results were not statistically separable among the six nematode treatments, there were numerical trends. With the Kapow strain, there was a consistent trend towards reduced emergence and reduced post-trial fruit infestation with higher rates of nematodes. With the All strain, however, despite the numerically higher levels of nematode soil persistence as indicated by the *G. mellonella* laboratory bioassays, FRB emergence and fruit infestation was numerically higher at the highest rate of 500 nematodes per cm². Because of this unusual result, it is difficult to choose between the Kapow and All strains although the Kapow strain at 500 nematodes per cm² gave the best results numerically.

DISCUSSION

Parasitic rhabditid nematodes have been shown to be effective against a number of insect pests (Poinar 1971, 1979, Kaya 1985, Morris 1985, Gaugler & Kaya 1990, Parkman et al. 1994), have been evaluated in a number of trials against root weevils (Diaz & Hernandez 1978, Bedding & Miller 1981, Montes et al. 1981, Simons 1981, Beavers 1984, Belair & Boivin 1985, Roman & Figueroa 1985, Rutherford et al. 1987, Schroeder 1987, 1990a, b, 1992, 1994, Boivin & Belair 1989, Downing et al. 1991, Mannion & Jansson 1992, Burlando et al. 1993, Mracek et al. 1993, Smith 1994, Duncan & McCoy 1996) and are currently applied commercially for control of *Diaprepes*

abbreviatus L. in Florida citrus. Results in different trials have been variable, however, and as might be expected, appear to be influenced by: application method; air, water suspension, and soil temperatures; soil type; soil salinity; soil moisture level as influenced by irrigation or natural rainfall; host species; vertical distribution of the host species; the level of alternate hosts available for nematode parasitization and persistence; and nematode species and quality.

In this study, we evaluated soil persistence of nematodes using the following three parameters: (1) laboratory bioassays of field-collected soil using wax moth larvae as substitute hosts, (2) reduction in adult FRB emergence for two years after treatment, and (3) reduction in FRB egg masses deposited on fruit one year after treatment. With one nematode application (June 25, 1989), and three rates of two strains of *S. carpocapsae*, a combined average of 95% of the wax moth larvae were killed in soil collected 1 day after treatment. In addition, emergent adult and egg mass reductions of 46.6 (year 1), 80.4 (year 2), and 61.5% were observed, respectively.

An exceedingly high level of FRB control is required to reduce egg mass contamination of fruit to the degree required to pass quarantine inspection in Japan (Morse et al. 1987). In citrus groves with moderate to high levels of FRB, it appears that repeated nematode applications, perhaps multiple applications per year over 2-3 successive years, might be required to approach this level of control. Cost is a factor, although treatment with 2 billion nematodes per acre (50 IJ/ cm²) is considered economically feasible (Smith 1994). Because large prepupal larvae are most susceptible, nematode applications should be optimally timed to impact this stage. Nevertheless, with a single treatment applied in June, 1989, the additional reduction of FRB emergence observed in 1990 beyond that seen in 1989 is encouraging. The obvious implication is that nematodes persisted in the soil, providing additional control of FRB larvae in 1990.

Application costs may be reduced by the use of more efficacious entomopathogenic nematodes. FRB susceptibility should be evaluated for *S. riobravus* Cabanillas, Poinar & Raulston, a more temperature tolerant nematode, indigenous to the Rio Grande Valley of Texas (Cabanillas et al. 1994). This commercially available nematode was the most efficacious of six species and strains tested with a substitute host - *G. melonella* (Lindegren et al. 1993), persisted for 3 months in spring field tests in Phoenix, AZ (Lindegren et al. 1995), and was significantly more effective than *S. carpocapsae* in mid-summer small-scale field tests on pink bollworm, *Pectinophora gossypiella* (Saunders) in Phoenix (Lindegren et al. 1994).

This study indicates nematode establishment and reduction of FRB populations into the second year after application. Because FRB infested trees can be readily identified by notched leaves, costs might also be reduced by applying nematodes to single previously identified and marked infested trees throughout the orchard, similar to the method used in this study.

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