

**BIOLOGICAL CONTROL OF IMPORTED
CABBAGEWORM (*Pieris rapae*,
Lepidoptera: Pieridae) WITH *Steinernema
feltiae***

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Abstract

Imported cabbageworm (*Pieris rapae* L., Peiridae) is an economically important pest of cabbage throughout temperate and subtropical zones. Damage from *P. rapae* feeding causes irregular holes on older cabbage leaves. Under severe infestations, larvae skeletonize leaves. Over all, losses can be up to 71% in cabbage. Concerns about effects of pesticides on human health and non-target organisms have led to a reduction in the use of these chemicals. *Pieris rapae* has many natural enemies, including predators, parasitoids, and pathogens. Entomopathogenic nematodes (EPNs) represent another potential biological agent group for *P. rapae*. *Steinernema carpocapsae* and *S. feltiae* can play a role in the control of *P. rapae* but face several obstacles to their successful deployment. Insects have an immune response to EPNs and their symbiotic bacteria. However, EPNs react and can be non-recognized (evasion) by the insect host, tolerate insect encapsulation (tolerance), or suppress insect encapsulation (suppression). EPNs are adversely affected by temperature, sunlight, lack of moisture, and UV radiation. Many approaches can be utilized to enhance the application of EPN to control foliage-feeding insects. This thesis research is aimed to determine whether low population densities of EPN infective juveniles (IJs) can work as well as high population densities, whether the efficacy of low EPN population densities on mortality of *P. rapae* is sufficient, whether *S. feltiae* infects *P. rapae* before dying due to lethal environmental conditions, and whether mixing *S. feltiae* and *S. carpocapsae* can increase the mortality of *P. rapae*. Just 1.6 IJs/cm² were able to defend an area and give 92% mortality of *P. rapae* 4th instar larvae. A 3-hour exposure was sufficient for *S. feltiae* to infect *P. rapae* larvae and give 90% mortality. A minimum number of IJs should penetrate the larvae to achieve

successful infection and produce a new generation. Not all the cadavers from a 2-hour exposure produced EPNs. Some cadavers apparently were invaded by IJs sufficient to kill the insect but not by enough IJs to escape the insect immunity, survive, and reproduce. Combining EPN species further increases the level of insect mortality over that of a single EPN species. Exposure time is also important to achieve a successful invasion of the target insect, especially for low population densities of EPNs. The basic knowledge for making a decision to use EPNs in the field is to apply during the day avoiding high temperatures and UV radiation. Mixing species of EPNs can enhance EPN efficacy.

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Chapter One

Introduction

Imported cabbageworm (*Pieris rapae* L., Peiridae) is an economically important pest of cabbage throughout temperate and subtropical zones (Capinera, 2008; Vincent, 2007; Cameron et al., 2002). This insect completes 2-3 generations/year in temperate regions and more in tropical regions (Richards, 1940; Capinera, 2008). Females lay individual eggs on the lower surface of the outer leaves of Cruciferae (Capinera, 2008), which hatch after 3-7 days (Richards, 1940). The larvae proceed through five instars (Richards, 1940; Capinera, 2008). At the 5th instars, larvae grow to 24.5-30.1 mm in length. The larval stage is completed in 15 days, when the larvae pupate (Richards, 1940; Capinera, 2008; Hutchison et al., 2009). Adults emerge from the pupae in 2-3 weeks (Richards, 1940; Capinera, 2008; Hutchison et al., 2009). *Pieris rapae* remains a quiescent pupa during the winter (Richards, 1940; Capinera, 2008) in temperate regions but remains active year round in tropical regions. Adults are 16 mm long, white in color with black spots on the front wings: two in the center of the front wings in the females and one on the front wings in the males. One black spot is found on the forewings of both genders (Capinera, 2008). The body is covered with white bristles on the females and darker bristles on the males (Capinera, 2008). Each female lays between 300-400 eggs (Capinera, 2008; Jogar et al., 2008).

Imported cabbage worm is highly sensitive to glucosinolate, a compound that is released by cabbage plants. This butterfly can be stimulated by glucosinolate and have preferences selecting different cabbage cultivars that vary in glucosinolate levels (Jugar,

et al., 2008). In addition, the color, shape, quality, and leaf age of the cabbage plant also affect the egg laying preference of this butterfly (Jogar et al., 2008).

Damage from *P. rapae* feeding causes irregular holes on older cabbage leaves (Capinera, 2008; Hutchison et al., 2009). Under severe infestations, larvae skeletonize leaves (Capinera, 2008). The larvae usually progress from the outer leaves, eventually reaching the cabbage head (Capinera, 2008; Jogar et al., 2008; Hutchison et al., 2009). Larvae may enter the head of the cabbage, specifically in the 4th and the 5th instars (Capinera, 2008; Jogar et al., 2008). Feeding on the cabbage head causes economic yield loss. Feeding on the outer leaves also causes economical loss due the need for application of pesticides to control the larvae. Larval feeding results in the production of large amount of frass between the leaves (Capinera, 2008; Hutchison et al., 2009) that may contribute to secondary infection by fungal pathogens of cabbage plants. Over all, losses can be up to 71% in cabbage in southern New Brunswick (Maltais et al., 1998).

Chemical pesticides including diazinon, carbaryl, and permethrin, have been used to control *P. rapae* (Hutchison et al., 2009). Although application of chemical pesticides may be the easiest approach to control, many insect pests develop resistance to chemical pesticides (Hutchison et al., 2009). Concerns about effects of pesticides on human health and non-target organisms have led to a reduction in the use of these chemicals (Barbercheck, 1992; Lacey and Kaya, 2000). Resistance to a chemical may alter the cabbageworm's response to natural enemies. Based on a fitness cost trade-off concept, developing cellular immunity against toxic chemicals may make the larvae more susceptible to their enemies (Foster et al., 2006). Thus, using natural enemies of an insect

pest as a biological control-agent is a viable alternative to control these pests in many cases (Gaugler and Kaya, 1990).

Pieris rapae has many natural enemies, including predators, parasitoids, and pathogens (Capinera, 2008). Some of these natural enemies have been developed as effective biological control agents (Garcia-Gutiérrez et al., 2010). *Cotesia rubecula* (Marshall) (Braconidae), *Cotesia glomerata* (L.) (Braconidae), *Pteromalus puparum* (Petromalidae), and *Trichogramma evanescence* (Chacidoidae) are parasitoids that have been used against *P. rapae* (Vincent et al., 2007; Van Dreische, 2008). *Cotesia rubecula*, *C. glomerata*, and *T. evanescence* are not particularly good biological control agents against *P. rapae* (Vincent et al., 2007). *T. evanescence* attacked non-target butterfly eggs whereas *C. rubecula* and *C. glomerata* were hyperparasitized upon introduction to the USA and Canada (Vincent et al., 2007). Pimentel (1961) has shown that the predators *Coleomegilla maculata fuscilabris* (Coccinellidae) De Geer, *Coccinella transversoguttata* (Coccinellidae) Fald., *Hippodamia convergens* (Coccinellidae) Guer., *Sphaerophoria cylindrica* (Syrphidae) Say, *S. nata* (Syrphidae) Say, and *Chrysopa* spp. (Chrysopidae) preyed upon *P. rapae* 1st and 2nd instar larvae. However, these predators were not as effective in controlling *P. rapae* populations as parasitoids and pathogens (Pimentel, 1961). Other natural enemies of *P. rapae* include the insect-pathogenic fungus *Beauveria bassiana* (Garcia-Gutiérrez et al., 2010), the insect-pathogenic *Bacillus thuringiensis*, (Capinera, 2008; Tompinks et al., 1986), and a Granulosis virus (Capinera, 2008; Tompinks et al., 1986). However, UV radiation limits the viral control of *P. rapae* (Christian and Oakeshott, 1989).

Entomopathogenic nematodes (EPNs) represent another potential biological control agent for *P. rapae*. EPNs attack insects as infective juveniles (IJ) (Lacey and Kaya, 2000). These nematodes have several characteristics that make them desirable biological agents. They kill the insect host quickly, have a high reproductive rate, are easily mass-reared in vitro, and are easily applied. Thus, EPNs are among the best natural enemies to control edaphic insect pests but can also play a role in the control of foliar insect pests (Fenton, et al., 2000). EPNs employ two foraging strategies in their attack on insects: ambush or cruise (Gaugler, 2002; Lacey and Kaya, 2000). An ambusher EPN stands on its tail and waits for an insect to cross in its path, e.g. *S. carpocapsae* and *S. scapterisci* (Gaugler, 2002; Lacey and Kaya, 2000). Cruiser EPNs move about searching for a host, e.g. *S. glaseri* (Gaugler, 2002; Lacey and Kaya, 2000). Cruiser EPNs tend to be better in controlling insect pests than ambusher EPNs (Gaugler, et al., 1996). However, some EPNs such as *S. riobrave* and *S. feltiae* employ both foraging strategies (Lacey and Kaya, 2000). Most current uses of EPNs focus on application of a single nematode species. Greater efficacy might be achieved by exploiting the cruising and ambushing behaviors of a mixture of EPN species.

EPNs enter their insect host through openings in the insect exoskeleton. Spiracles provide entry points for EPN into the insect hemocoel (Gaugler and Kaya, 1990). After the IJ enters the insect hemocoel, the EPN releases symbiotic bacteria, *Xenorhabdus sp.* in *Steinernema* (Gaugler and Kaya, 1990; Fenton et al., 2000; Selvan et al, 1993). These bacteria feed on the hemocoel fluids, multiply, and secrete a toxin that kills the insect within 24-48 hours after infection (Fenton et al., 2000; Selvan et al., 1993). The IJs in the hemocoel feed on the bacteria and atrophying insect host tissue (Fenton et al., 2000;

Selvan et al., 1993). Within 1-2 weeks, these nematodes grow, develop, mature, and produce new generations (Fenton et al., 2000; Selvan et al., 1993). A new generation of IJs is formed when food becomes limited. The new IJs leave the insect cadaver and search for a new host (Selvan et al., 1993).

Since *P. rapae* larva are foliar feeders usually living on cabbage leaves, using EPNs which are soil-dwelling organisms to control a foliage insect like *P. rapae* is a challenge. However, *S. carpocapsae* and *S. feltiae* infect and kill *P. rapae* (Salem et al., 2007; Arthurs, 2004). This thesis has three objectives designed to develop a strategy for using EPNs to control *P. rapae*.

Objectives:

The overall goal of this work is to investigate whether low population densities of EPNs can effectively defend cabbage plants from the imported cabbageworm. Three specific objectives will aid in reaching this goal:

- 1- Determine the number of *S. feltiae* necessary to defend an area against *P. rapae* 4th instar larvae;
- 2- Determine the time required for *S. feltiae* to infect and kill *P. rapae* 4th instar larvae; and
- 3- Determine the effect of *S. feltiae* and *S. carpocapsae* alone and in combination on the control of *P. rapae* 4th instar larvae.

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Chapter Two

Testing the efficacy of *Steinernema feltiae* MG 14 against *Pieris rapae* (Lepidoptera: Peiridae) at low population densities

Introduction

EPNs have shown a wide range in their ability to control different insect pests. Under ideal circumstances, a single EPN is capable of killing an individual insect pest. However, ideal conditions depend upon the interaction between the insect species and the nematode species (Campos-Herrera and Gutiérrez, 2009; Belair et al., 2003), host immunity to the EPN (Gaugler, 2002), the habitat in which the pest lives (Gaugler and Kaya, 1990), and the time of EPN application (Haukeland and Lola-Luz, 2010).

Belair et al. (2003) tested the response of three different crucifer insect pests exposed to three different species of EPNs. The three insect species responded with different susceptibility to the three species of EPNs. Campos-Herrera and Gutiérrez (2009) have shown that *S. feltiae* has a different Lc_{50} , ranging from 0.27 to 99.61 IJ/cm², against three different insect targets. Clearly, one EPN species is not universally effective against all insect pests.

Insects have an immune response to EPNs and their symbiotic bacteria. This immunity is often sufficient to protect the insect but has limitations. Insects respond to

EPN infection as a non-self recognition followed by encapsulation of the EPN and phagocytosis or nodulation of the symbiotic bacteria (Gaugler, 2002). This is not a static interaction and EPN IJs may take actions against encapsulation. EPNs may evade recognition, tolerate encapsulation, or suppress encapsulation (Gaugler, 2002). *Steinernema carpocapsae* IJs have lipid components covering their body which make *Galleria mellonella* (Lapsus) (Pyralidae) larvae hemolymph unable to recognize the nematode. When the IJs are treated with lipase, *G. mellonella* larvae recognize the IJ and capsule the EPN (Gaugler, 2002).

Tolerance occurs when the EPNs invade the host in large number and the insect is unable to encapsulate all the IJs (Gaugler, 2002). *Leptinotarsa decemlineata* (Say) (Chrysomelidae) is able to encapsulate a maximum of 21 *S. carpocapsae*, any additional EPNs will remain free in the hemolymph (Gaugler, 2002).

Steinernema carpocapsae and *S. glaseri* have can suppress encapsulation when they invade the turnip moth, *Scotia segetum* (Schiff.) (Noctuidae) (Gaugler, 2002). Two proteins are involved in the suppression process. One protein secreted by the IJ decreases the number of haemocytes in the insect and the other protein limits production of melanin by the insect (Gaugler, 2002). This complex and dynamic interaction between the insect host and infective EPN plays a key role in the successful killing of the insect.

EPNs are soil inhabiting nematodes. Consequently, EPNs are most effective against insect pests that have an edaphic life stage. EPNs are intolerant to desiccation (Gaugler and Kaya, 1990). Shelter et al. (1988) have shown that EPNs, belonging to the genera *Steinernema* and *Heterorhabditis*, require at least 0.64 cm of pretreatment

irrigation water and 0.64 cm post-treatment irrigation for successful infection. EPNs are also very susceptible to UV radiation (Gaugler and Boush, 1978). Gaugler and Boush (1978) found that irradiating *H. bacteriophora* and *S. caprocapsae* decreased their reproductive capacity and efficacy in control. Shairo-Ilan et al. (2006) demonstrated that using polymeric materials as an application formulation enhanced the foliage survival of EPN. Advances in formulation may help to overcome these limitations.

The time of EPN application also affects efficacy. Haukeland and Lola-Luz (2010) applied EPNs in the field to control black vein weevil, *Otiorrhynchus sulcatus* F. (Curculionidae). They found that applying a particular EPN population in the early autumn was more effective in controlling the pest than applying twice the nematode population and/or two applications in late autumn. Haukeland and Lola-Luz proposed that decreasing the temperature below 10°C limited the control attributable to EPNs. The season of application can play a significant role in determining success in the control of black vein weevil.

Given these limiting factors, laboratory studies and field applications have employed massive EPN population densities to achieve successful control of a variety of insects. EPN population densities used in field applications to control Lepidoptera pests typically reach as high as 3000 IJ/ml (Gaugler and Kaya, 1990). Producing EPN infective juveniles in high population densities is costly which limits the use of EPNs in the field (Lacey and Kaya, 2000). It is possible that low EPN population densities may achieve results similar to high population densities in the control of an insect, if appropriate measures are taken to reduce the impacts of deleterious environmental factors.

If the correct selection of EPN species has been made for a particular insect, an appropriate timing has been chosen for EPN application, and good technique has been followed in EPN application, EPNs might be used at lower population densities while still providing effective control, similar to that achieved with high IJ population densities. To determine if low population densities of IJs can work as well as high population densities, the efficacy of low EPN population densities on the mortality of *P. rapae* (Linnaeus) (Peiridae) was investigated. The objective of this series of experiments was to determine the minimum number of *S. feltiae* necessary to defend an area against *P. rapae* 4th instar larvae.

Material and Methods

Cabbage plants. Cabbage, *Brassica oleraceae*, was grown in the field to supply *P. rapae* eggs and larvae. Cabbage seeds were germinated in 10-cm-diameter clay pots at the rate of 15 seeds/pot. After 4 weeks, the seedlings were transplanted into field plots. Plots for the cabbage were established at the Magoon Research Facility located in Manoa valley, Honolulu, Hawaii. The soil was tilled and drip tubes were laid on 1-m centered rows. Eight 7-m rows were planted with 14 cabbage seedlings each. Seedlings were observed and protected from insects and diseases. Cabbage transplants that died or grew poorly were replaced. The plots were irrigated regularly and maintained weed free.

Pieris rapae. Eggs and larvae of the cabbageworm were collected from the cabbage plants at 1-week intervals and transferred to the laboratory. Pieces of cabbage leaf upon which eggs and/or larvae were found were removed from the plant and placed in a plastic container (29 cm long × 27 cm wide × 13cm deep), covered with cheese cloth and held in the laboratory at 25°C. Paper towels were placed under the cabbage leaves to absorb

excess moisture. The cabbage leaves were changed and the container cleaned daily. All 4th instars were transferred to a 750 ml disposable plastic container and stored at 4°C to slow their development, thus allowing collection of sufficient number of larvae for each run of an experiment.

Steinernema feltiae. The nematodes used in all experiments were from cultures less than 1 month old. EPN cultures were renewed by placing 2 filter paper disks in a 90 mm×10 mm Petri dish. One thousand nematodes carried in 1000 µl water were taken from an existing nematode culture. The volume was adjusted to 2000 µl and pipetted onto the double layer filter paper. Seven mealworm (*Tenebrio molitor* L.) larvae were placed in the Petri dish and the dish covered. The culture was placed on the laboratory bench and held at 25°C for 48 hours. Dead mealworms were transferred to white traps (Kaya and Stock, 1997) and EPNs were collected 14-20 days later.

Experiment 1. The ability of *S. feltiae* MG 14 to defend an area was evaluated at five different population densities. The experiment consisted of five replications (runs). Each treatment was represented by a single Petri dish. The treatments were 0.8, 1.6, 2.4, 3.2, and 4 IJ/cm². Each EPN density was delivered in 2000 µl water. Two layers of filter paper were placed in the bottom of each Petri dish and the EPNs pipetted over the entire area. Cabbageworm larvae were removed from storage, returned to 25°C, and placed on cabbage leaves and allowed to feed for 6 hours before exposure to EPNs. Ten larvae were selected arbitrarily from the container and placed on the filter paper in each dish. The larvae were covered by another disk of filter paper to prevent larvae from crawling to the lid of the Petri dish and escaping from the EPN. The dish was covered and labeled. The five treatments were transferred to a 25°C incubator. After 36 hours, dead larvae were

collected, their numbers recorded, and cadavers placed in white traps (Kaya and Stock, 1997). The surviving larvae were transferred to EPN-free Petri dishes with cabbage leaves for an additional 48 hours. Any additional dead larvae observed after the 48 hours were transferred to the white trap. After 5-7 days, dead larvae were examined with the aid of a dissecting microscope for the presence of *S. feltiae*.

Experiment 2. *Steinernema feltiae* MG 14 defense of an area was evaluated with 0, 0.25, 0.5, 0.75, and 1 IJ/cm². The experiment consisted of ten replications. Each experimental unit was a single Petri dish. EPNs were delivered in 2500 μ l water for each treatment. As in the first experiment, two layers of filter paper were placed in the bottom of the Petri dish. EPNs were spread across the dish. Cabbageworm larvae were fed as before and 5 arbitrarily selected larvae were set in each dish. The larvae were covered with a disk of filter paper. The dish was covered and labeled. Dishes were transferred to a 25°C incubator. After 36 hours, dead larvae were collected, their number recorded, and cadavers placed in white traps (Kaya and Stock, 1997). The surviving larvae were transferred to EPN-free Petri dishes with cabbage leaves for 48 hours. Any additional dead larvae were transferred to the white trap. After 5-7 days, dead larvae were examined with the aid of a dissecting microscope for the presence of *S. feltiae*.

Experiment 3. The susceptibility of *P. rapae* over a wide range of 8 population densities of *S. feltiae* MG 14 was evaluated. The experiment consisted of eight replications of each treatment. The EPN population densities evaluated were 0, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, and 3 IJ/cm². EPN application, larval exposure, and larval mortality were conducted as described for the first two experiments.

Analysis. Each dead larva was evaluated for EPN infection. A darkening or color change in body color was considered indicative of EPN infection (Gaugler, 2002). A turgid cadaver body was also considered a sign of EPN infection. Larvae infected by non-toxic bacteria or lethal virus were indicated by changing the body color to brown and softening of the cadaver. Cadavers with visible mycelia were considered killed by fungi. The non-EPN infected dead larvae were discarded and not included in the data analysis. Larval mortality was calculated for each EPN population density by totaling the number of dead larvae at 36 hours exposure and additional dead larvae 48 hours after transfer from EPN defended areas. Total dead larvae were divided by the number of larvae initially exposed to EPNs. Dead larvae in which no EPNs were observed under the microscope or larvae that did not exhibit typical EPN infection upon death were not included in the data analysis.

Data for the three experiments were analyzed by regression using Sigma plot 10.0 to determine the relationship between EPN population density and mortality of cabbageworm larvae. Experiments were analyzed separately and then combined.

Results and Discussion

In general, early mortality of *P. rapae* larvae at 36 hours EPN exposure increased as the IJ population densities increased (Tables 1, 2, and 3). However, the late mortality, 48 hours after exposure, was higher in the lowest EPN population densities (Tables 1, 2, and 3). The late mortality appears to result from infection with a small number of IJs. The speed of death is positively correlated to the amount of the toxic protein secreted by symbiotic bacteria (Forst and Neilson, 1996; Gaugler, 2002). When the symbiotic

bacteria have been released from large number of IJs, the insect dies faster whereas the insect will die slowly if the bacterial population is small because of an initial low number of IJs. Pest immunity may play a role in extending the killing time. When a small number of IJs infect the larvae, the IJs will be challenged to survive and many times the IJ will be encapsulated in the hemolymph with their symbiotic bacteria sequestered in nodules (Gaugler, 2002). However, nodulated bacteria continue to multiply and can ultimately erupt from nodules and proceed to kill the larvae (Gaugler, 2002). Low initial bacterial inoculum levels and insect immunity probably account for the late mortality observed in the three experiments.

Not all larvae with typical EPN infection symptoms yielded *S. feltiae* in the white traps. The lack of observed IJ can be expected at low EPN population density exposure. Insect immunity is likely to be active and able to encapsulate all IJs that penetrate the body when the IJs are present in small numbers. The larvae, in this case, die because of the bacteria released by the IJs release prior to encapsulation. The IJs did not survive. Also, *S. feltiae* is amphimictic. The probability of both genders infecting the same larva is only 50%, so IJs of the same gender will survive and grow but without reproduction. If only two IJ infect, successfully avoid insect immunity, and survive, these IJ may be weakened and unable to produce a normal number of eggs and/or produce low quality eggs because of exposure to the resistance response of the insect. However, producing a new generation of EPN is not important in augmentative field application for control of foliage insects because the new generation of IJs will die soon after leaving the dead larvae due to exposing to lethal UV radiation and high temperature.

Table 1. The percent mortality of *Pieris rapae* 4th instar at 36 hours exposure to *Steinernema feltiae* infective juveniles (IJ) and 48 hours after exposure.

Time	IJ/cm ²				
	0.8	1.6	2.4	3.2	4
36 hours	66	84	80	94	100
48 hours	12	8	14	2	-
Total (84 hours)	78	92	94	96	100

Table 2. The percent mortality of *Pieris rapae* 4th instar at 36 hours exposure to *Steinernema feltiae* infective juveniles (IJ) and 48 hours after exposure.

Time	IJ/cm ²				
	0	0.125	0.25	0.5	1
36 hours	0	32	42	74	84
48 hours	0	12	14	12	4
Total (84 hours)	0	44	56	86	88

Table 3. The percent mortality of *Pieris rapae* 4th instar at 36 hours exposure to *Steinernema feltiae* infective juveniles (IJ) and 48 hours after exposure.

EPN population density (IJ/cm ²)	Mean of dead larvae at 36 hours	Mean of dead larvae at 48 hours	Over all death mean
0	0	3	3
0.1	25	10	35
0.2	45	20	65
0.3	63	5	68
0.4	63	18	81
0.5	68	13	81
1	82	13	95
2	85	10	95
3	97	3	100

Data from experiments 1, 2, and 3 were combined to reflect the strength of *S. feltiae* MG 14 against *P. rapae* 4th instar larvae (Fig.1). The mean of the 0.5 and 1 IJ/cm² treatments from experiment 2 and 3 were calculated and used for analysis. Data followed a sigmoid regression model (Sigma Plot 10.0). The model was

$$y = \frac{92.53}{\left(1 + e\left(-\frac{x - 0.16}{0.11}\right)\right)}$$

($P < 0.01$, $R^2 = 0.93$), where y is mortality and x is EPN population. The 1 IJs/cm² was the best defending population (Fig. 1), being the lowest density that gave the highest mortality of *P. rapae* 4th (100%). The mortality of *P. rapae* larvae between 0 and 1 IJ/cm² decreased rapidly which reflects that efficacy is related to EPN population densities. The three experiments demonstrated that 1 IJ/cm² was lowest population density appropriate for controlling *P. rapae*. Population densities < 1 IJ/cm² were not sufficient for control.

Batalla-Careera et al. (2010) applied three different EPN species at 25 and 50 IJs/cm², relatively high population densities, to control the larval and pupal stages of *Tota absoluta* (Meyrick) (Gelechiidae). Insect mortality was 78.6-100 % with 25 IJ/cm² and 86.6-100 % with 50 IJ/cm². The present experiments have shown *S. feltiae*'s ability to infect and kill *P. rapae* at much lower population densities, 0.1-3 IJ/cm². Campos-Herrera and Gutiérrez (2009) have shown that *S. feltiae* controlled 70% and 80% of *Sodoptera littoralis* Biosduaval (Noctuidae) and *Trichoplusia ni* Hubner (Noctuidae) respectively at 2 IJ/cm². The current results demonstrate that low population densities of IJs can kill insects as effectively as the high population densities *in vitro*. It may therefore be possible to reduce the EPN populations used in field applications. There are limits to

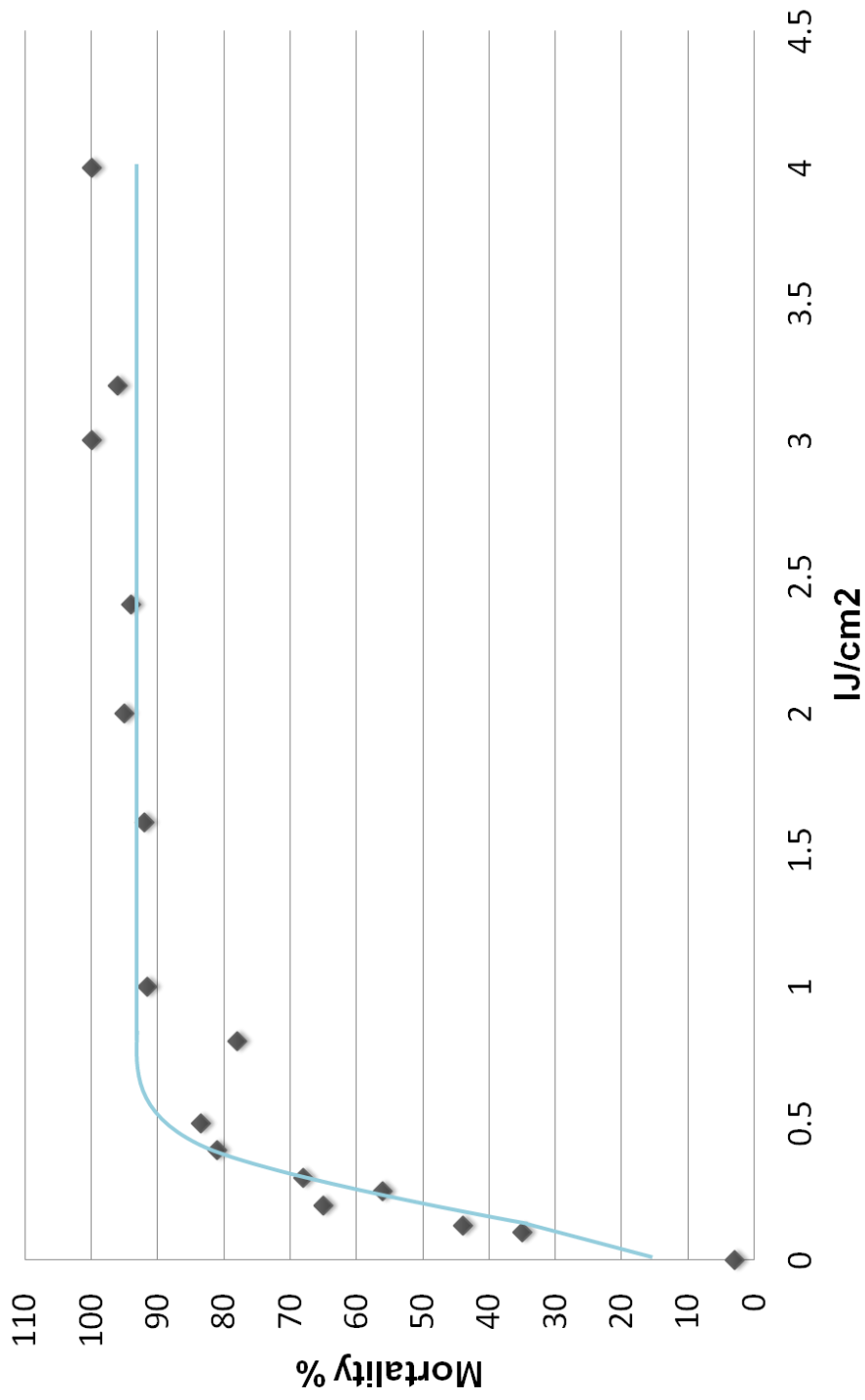


Figure 1: Mortality of *Pieris rapae* 4th instar larvae for combined three experiments showed the efficacy of *Steinernema feltiae* in defending an area against *Pieris rapae* at low population densities. The model data points are presented as \blacklozenge . The model was $y = \frac{92.53}{(1 + e^{(-\frac{x - 0.16}{0.11})})}$, ($P=0.01, R^2=0.93$)

EPNs' efficacy and ability to defend an area at very low population densities. EPNs are highly effective in killing their host even though the number of the individual EPN is small.

This work has demonstrated the potential for low population densities of *S. feltiae* to effectively locate, infect, and kill *P. rapae* larvae under laboratory conditions. To provide a more general approach to select EPNs to control *P. rapae*, we suggest similar experiments with other EPN species like *S. caprocapsae*, *S. glaseri*, or *H. bacteriophora*.

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Chapter Three

Employing *Steinernema feltiae* characteristics to enhance field applications utilizing low population densities

Introduction

Soil is the natural habitat for EPNs. This environment provides the nematodes with essential protection from UV radiation and desiccation. Consequently, EPNs suffer from lethal environmental effects when used in augmentative biological control applications to control foliar insects. EPNs are adversely affected by temperature, sunlight, lack of moisture, and UV radiation (Gaugler, 2002; Gaugler and Kaya, 1990). Gaugler and Boush (1978) found that long wave length UV radiation of 800 $\mu\text{W}/\text{cm}^2$ did not affect EPN efficacy or survival, but did find that when EPNs were exposed to sunlight without shielding, EPN survival and infection of insects decreased dramatically. Temperature is also very important for EPN activity. Moderate temperatures (7-30°C) are favored by EPNs (Chen et al., 2003). Elevated temperatures (>32°C) adversely affect EPN efficacy, their development inside the host, and their reproduction (Gaugler, 2002; Gaugler and Kaya, 1990). Moisture is another important factor affecting EPN activity and survival rate all of which will affecting its ability to control foliage-feeding insects (Gaugler and Kaya, 1990). Developing and choosing appropriate application techniques can help EPNs to survive on foliage and effectively kill the target insect. Current research

focuses on testing adjuvants to protect EPNs from lethal conditions in the field until the EPNs have penetrated the insect body. Even though these adjuvants increase the mortality of the target insect, adjuvants do not completely protect the EPNs. EPN characteristics may provide basic information to enhance application approaches improving EPN survival until penetration of the target insect.

EPN characteristics that might be exploited to achieve better control include their foraging behavior, speed of infection, response to insect immunity and behavior towards previously infected insects. EPN infection speed may be used to maximize efficacy against foliar insects. Some EPNs infect target insects rapidly whereas other species require several hours to infect an insect (Gaugler, 2002). EPNs may seek out their insect prey, or lie in waiting and ambush their prey (Gaugler, 2002; Gaugler and Kaya, 1990). Field applications can take advantage of these different behaviors to maximize insect control. Some EPNs have lipid components on their cuticles that allow the nematode to enter insect prey and go unrecognized and therefore not be encapsulated in the insect haemolymph (Gaugler, 2002). EPNs, such as *S. carpocapsae*, do not prefer insects already infected with another EPN species (Gaugler, 2002). *Steinernema carpocapsae* will avoid infecting these insects. This characteristic may be employed in field applications if two EPN species are mixed and applied simultaneously. More insects may be infected because IJs of *S. carpocapsae* will seek out other uninfected insects, thus increasing mortality of target insect.

Many approaches can be utilized to enhance the application of EPN to control foliage-feeding insects. The two objectives of this experiment are to determine the infection speed of *S. feltiae* in controlling *P. rapae* (Lepidoptera: Peridae) and to

determine whether mixed applications of *S. feltiae* and *S. carpocapsae* can increase the mortality of *P. rapae*.

Material and Methods

***Pieris rapae*.** Larvae and eggs of *P. rapae* were collected from cabbage plants at the Magoon Research Facility located near the University of Hawaii at Manoa. Larvae and eggs were brought to the laboratory and fed on cabbage leaves until reaching the 4th instar. Larvae were then placed in separate containers and stored at 4°C until used in an experiment (Mohammed, 2012).

***Steinernema feltiae* and *S. carpocapsae*.** IJs were cultured on mealworm (*Tenebrio molitor* L.). IJs emerging from dead mealworm larvae were collected, washed by 3 times in water, and adjusted to 1000 IJ/ml. These IJ were stored 15°C, for no more than 30 days, until used in the experiments (Mohammed, 2012).

Experiment 1. Six time intervals (0, 8, 10, 12, 14, and 36 hours) were compared to determine the speed of EPN infection. Petri dishes were filled with a double layer of filter paper. A 2500 µl water aliquot that contained 200 IJs of *S. feltiae* was pipetted over the filter paper. Five of the stored larvae that had been warmed to room temperature and fed on cabbage leaves for 6 hours were placed in each dish. These larvae were covered with a single filter paper disk and the plate covered with its top. The dishes were incubated at 25°C. At each time interval, the larvae were observed for mortality. Dead larvae were removed and placed in white traps (Kaya and Stock, 1997). At the 36 hours observation, all remaining live larvae were transferred to clean Petri dishes with cabbage leaves for a 48-hour feeding period. Any additional dead larvae were removed to a white trap. After

7-10 days, the larvae in the white traps were observed for the presence of EPN. This experiment was repeated four times, with two replications per time. A complete randomized block design was used. The data were analyzed by regression to detect the relationship between the exposure time to *S. feltiae* and the mortality of *P. rapae* 4th instar larvae.

Experiment 2. The interaction between *S. feltiae* and *S. carpocapsae* was tested in five combinations with one non-EPN treatment as a control. The experiment was replicated 4 times. EPN population density was maintained at 8 IJs in all the treatments. The combinations tested were *S. feltiae* to *S. carpocapsae* in ratios of 8:0; 6:2; 4:4; 2:6; and 0:8. Five 4th instar larvae were brought to room temperature and set in each dish. The larvae were covered by a piece of filter paper, then the Petri dish was covered. The plates were maintained in a 25°C incubator. After 36 hours, the dishes were removed from the incubator, dead larvae were collected and transferred to white traps. The surviving larvae were transferred to clean dishes with cabbage leaves and held for an additional 48 hours. After 48 hours, additional dead larvae were placed in white traps. After 7-10 days in the white traps, the larvae were observed for EPNs. EPNs were counted according to their species. The experiment was a complete randomized design.

Analysis: In the first experiment, data were collected by recording the number of dead *P. rapae* 4th instar larvae for each treatment in the end of the interval and 48 hours after exposure. The two records for each treatment were combined for final mortality for all replications. The data were analyzed by a regression using Sigma plot 10.0. Mortality data from the second experiment were collected similarly. Using SAS Enterprise 4.3, data

were analyzed for variance and contrast conducted between the number of *S. feltiae* IJs and *S. carpocapsae* IJs recorded in the white traps in the combination treatments.

Results and Discussion

Mortality of *P. rapae* was related to the length of exposure to the EPNs. A sigmoid model represented the EPN infection speed ($P < 0.01$, $R^2 = 0.97$),

$$y = \frac{98.20}{\left(1 + e\left(-\frac{x - 1.34}{0.64}\right)\right)}$$

where y is the mortality of *P. rapae* 4th instar and x is the time. Nearly 100% mortality was achieved by 4 hours (Fig. 2). No difference was detected between the 3-hour exposure and the longer exposure times. Only the 1-hour exposure had lower mortality. The 3-hour exposure was sufficient for *S. feltiae* to infect *P. rapae* larvae and give a high rate of mortality (Fig. 2).

The population density of emerged IJs depended on the exposure period that allowed IJs to search, find, and infect the larvae in a reliable number. The 3-, 4-, and 36-hour exposures resulted in very high EPN population densities, 11535-13844 IJ/cadaver. The IJs emerged earlier from the 3-, 4-, and 36-hour exposure periods than from the 1- and 2-hour exposures. The mean IJ population was 7,025 IJ/cadaver for the 2-hour exposure and 0 IJ/cadaver for the 1-hour exposure (Fig. 3). The number of available IJs is important because the first IJs to infect the insect usually die due to the defense mechanism in the insect haemolymph (Gaugler, 2002). The life cycle of the EPNs in the

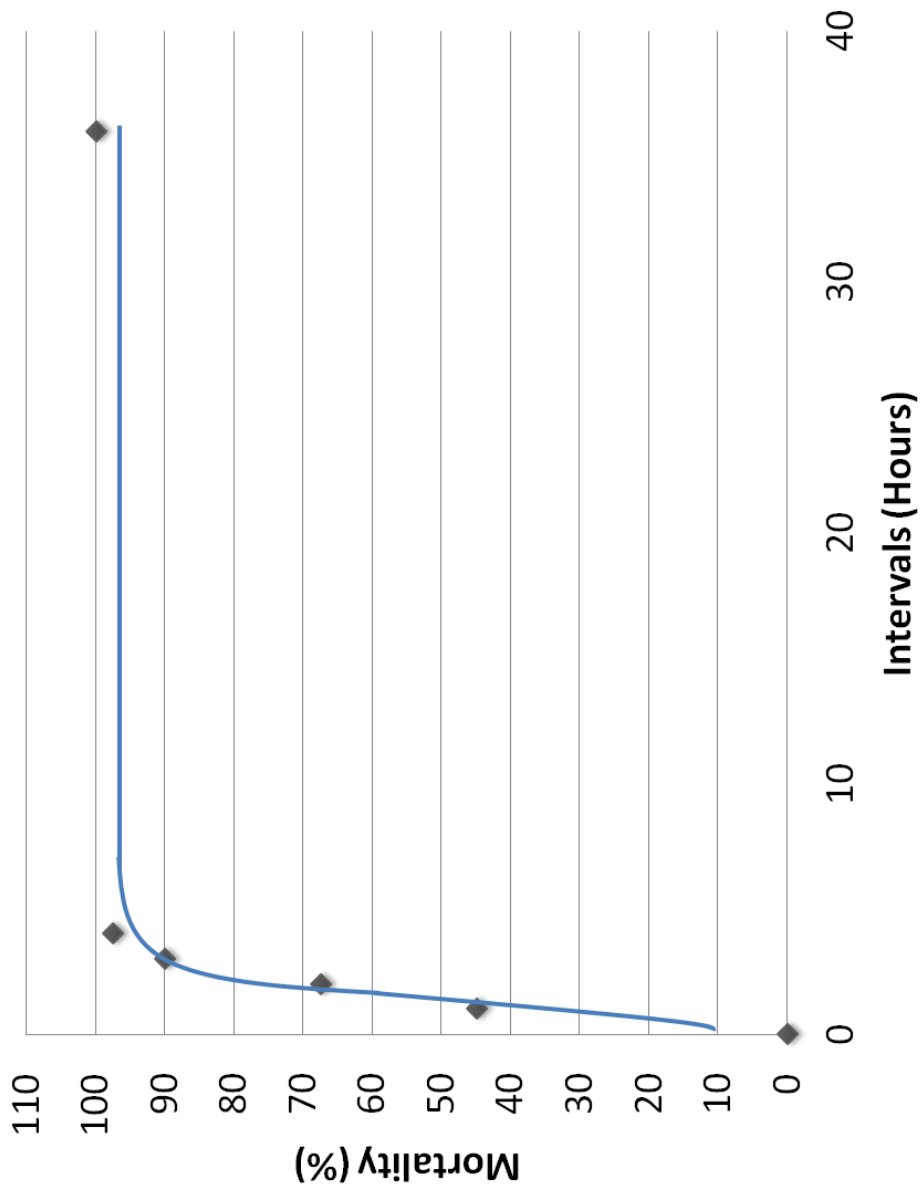


Figure 2: regression curve between the exposure time and percentage of mortality for *Pieris rapae*. The model logarithmic was followed. The model was $y = \frac{98.20}{(1 + e^{(-\frac{x - 1.34}{0.64})})}$, ($P < 0.01$, $R^2 = 0.97$). The data points are shown by \blacklozenge .

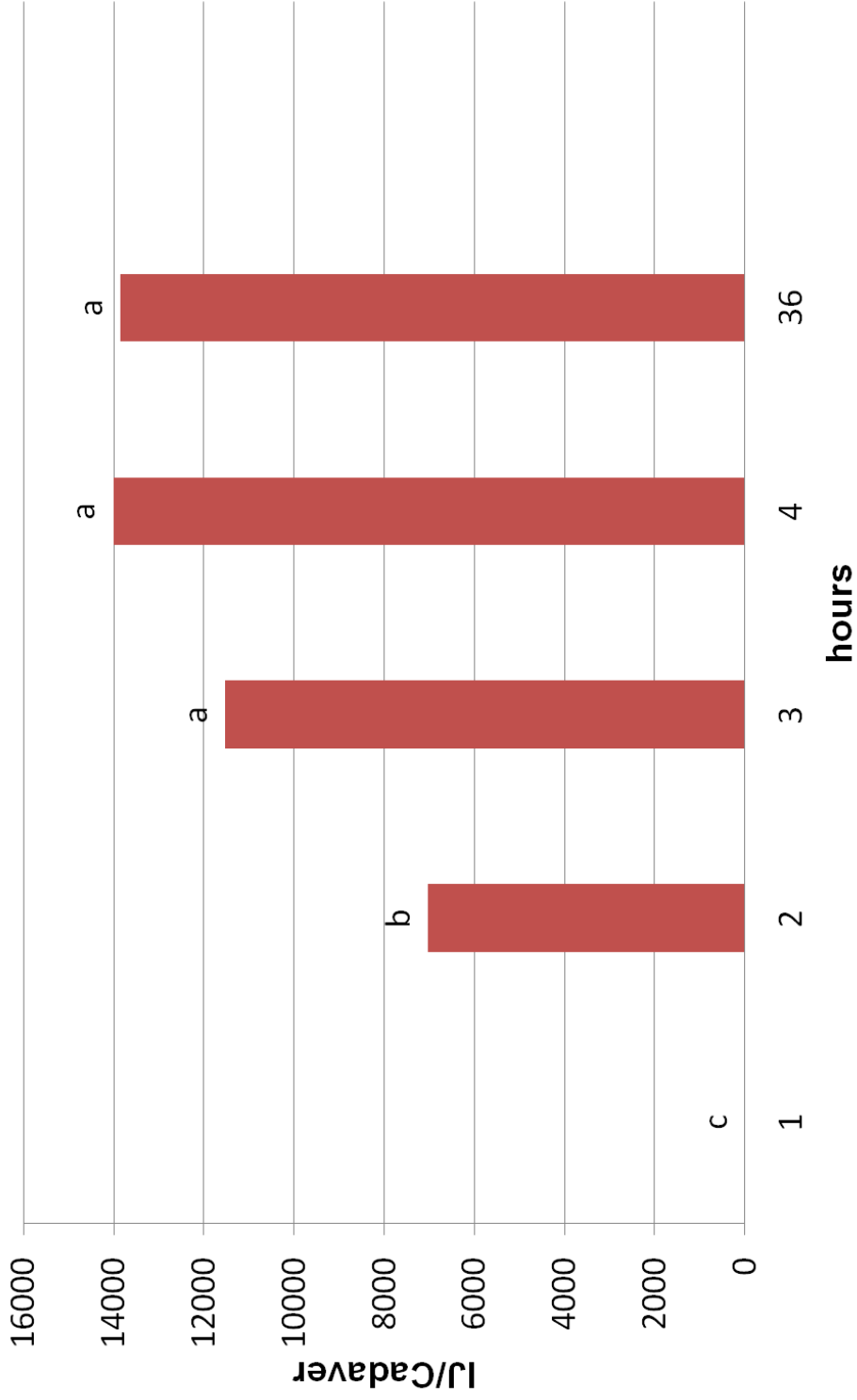


Figure 3: The number of *Steinernema feltiae* I/cadaver *Pieris rapae* after exposure for increasing period time. Bars with the same letters are not different according to W. D. K-ratio t test (k= 100).

dead larva depends on the number of surviving IJs. An initially large number of surviving IJs mean earlier depletion of the food and faster emergence of the next generation of IJs from the insect. A minimum number of IJs should penetrate the larvae to achieve successful infection and produce a new generation (Gaugler, 2002). The lack of IJ in the 1-hour exposure period suggested that this amount of time was not sufficient for the IJs to locate and infect the larvae. Not all the cadavers from the 2-hour exposure produced EPNs. Some cadavers apparently were invaded by sufficient IJs to kill the insect but not enough IJs to escape the insect immunity, survive, and reproduce. Thus the total number of emerged IJ was lower.

In the second experiment, no significant difference in mortality among the combinations was detected (Fig. 4). Mortality with the combination of the two EPN species, however, tended to be better than mortality associated with a single EPN species (Fig. 4). The emerged IJs varied in the number of IJ/ cadaver and the EPN species population (Table 4). Eighteen out of fifty six cadavers did not produce IJs. The initial population was very low and may not have provided enough IJs of the same species to kill and produce a new generation. The population density of emerged IJs for the 2 *S. feltiae* vs 6 *S. caprocapsae* treatment was relatively high in comparison to the other treatments (Table 4) *Steinernema caprocapsae* was not apparently affected by the secretions from the symbiotic bacteria of *S. feltiae* while *S. feltiae* may have been affected by the symbiotic bacteria of *S. caprocapsae*.

The two EPN species combinations showed a slightly higher control of the insect than one EPN species alone. There are a number of potential explanations for this. Two different species may adapt to different foraging strategies to search, find, and infect the

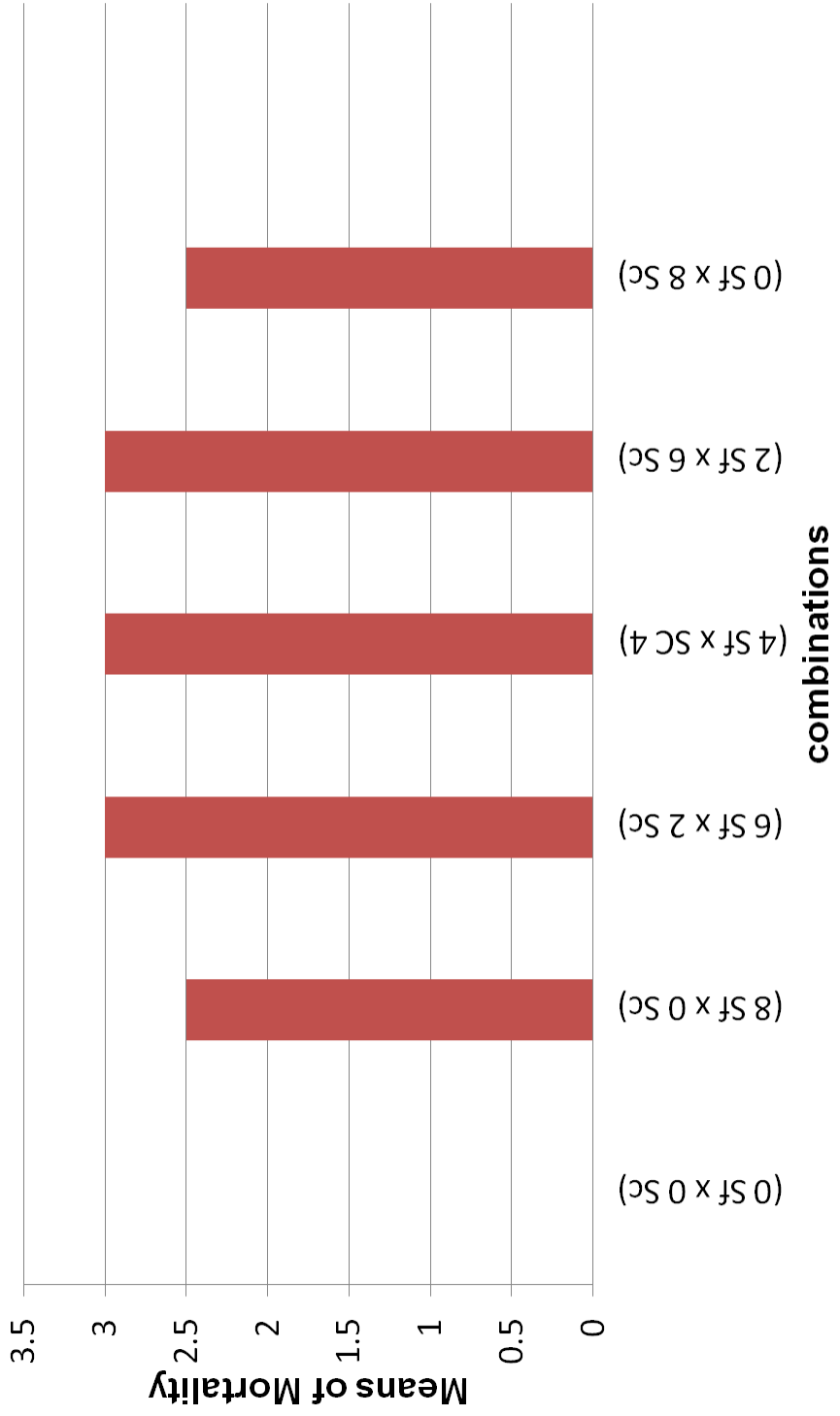


Figure 4: Comparison of mortality of *Pieris rapae* 4th instar larvae killed by different combinations of *Steinernema feltiae* and *S. carpocapsae* in population density of 0.125 IJ/cm².

Table 4: The effect of mixed entomopathogenic nematode (EPN) species on the mortality of *Pieris rapae* and the combination effects on the EPNs themselves (S. f. = *Steinernema feltiae*; S. c. = *Steinernema carpocapsae*).

Combinations <i>S. f.</i> x <i>S. c.</i>	Cadaver No. out of 20	Cadavers producing EPN (%)	EPN/cadaver	<i>S. feltiae</i> (%)
8 x 0	10	70	3750	100
6 x 2	12	50	502	95
4 x 4	12	67	816	43
2 x 6	12	58	2946	23
0 x 8	10	100	7996	-

host, which increase chances of successful infection. With two different symbiotic bacteria, the insect hemolymph may be under greater stress, which may offer a greater likelihood to kill the host within a shorter time. Mixing two species of EPN may stress and weaken the insect immunity such that while the first species may be capsulated, the hemolymph capacity to capsule the second species is reduced. The low population density of IJs that were used in the experiment may be the reason for the lack of differences in larvae mortality. The mix of two EPN species may add to insect control in general. The assumption that the combination of two different EPN species adapted to two different foraging strategies and employing different characteristics in their search for a host gives a greater chance to infect the target insect may be true but requires additional data.

Exposure time is important to achieve a successful invasion of the target insect, especially for low population densities of EPNs. The basic knowledge needed to make a decision to use EPNs in the field is to apply during the day avoiding the high temperatures and UV radiation. Mixing species of EPNs can enhance EPN efficacy. More laboratory and field work is needed to demonstrate clear results conclusively. Enhancing EPNs for foliage application can be through developing the application approach besides choosing the right time and selecting the best species of EPNs.

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