

**Strategies to Enhance Efficacy of Entomopathogenic Nematodes against Diamondback Moth
(*Plutella xylostella*) and Imported cabbageworm (*Pieris rapae*)**

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SABINA BUDHATHOKI

COMMITTEE MEMBERS

Koon Hui Wang (Chairperson), Ph.D.

Brent S. Sipes, Ph.D.

Ikkei Shikano, Ph.D.

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Abstract

Diamondback moth (DBM), *Plutella xylostella*, and imported cabbage worm (ICW), *Pieris rapae*, are the most destructive insect pests of cruciferous crops worldwide. Whereas various control measures against ICW are viable, DBM management in Hawaii is challenged by the development of insecticide resistant populations. This thesis focuses on exploring IPM strategies to enhance the efficacy of entomopathogenic nematodes (EPN) against DBM and ICW. Three approaches compatible with organic farming were examined to enhance the efficacy of foliar application of EPNs by integrating EPN sprays with 1) trap cropping, 2) intermittent sprinkler irrigation, and 3) using adjuvants comply with organic certification.

For the trap cropping approach, three 2×2 (trap crop \times EPN) factorial field trials were conducted by using kai choi (*Brassica juncea*) as trap crop and *Steinernema feltiae* for EPN sprays supplemented with 1.6 ml/L (or 20 fl oz/acre) on head cabbage (*Brassica oleraceae* var *capitata*) and kale (*Brassica oleraceae* var *acephala*). On head cabbage, trap cropping by kai choi reduced the abundance of DBM by 46%, ICW by 73%, DBM damage by 45% and ICW damage by 33%, respectively. On the other hand, EPN reduced DBM number in trap crop plots and ICW in no trap crop plots only. Effects of trap cropping were less on kale compared to that observed on cabbage. In the first kale trial, trap crop suppressed 50% of DBM abundance and 19% of leaves with DBM damage, whereas in the second trial, trap cropping only reduced 13% of leaves with ICW damage. EPNs was not effective against number and damage of both ICW and DBM in Kale Trial I, but it suppressed DBM numbers by 100% in Trial II ($P \leq 0.05$) soon after application. Overall, trap cropping did not improve the efficacy of EPN spray. EPN only suppressed DBM or ICW successfully when average pest pressure throughout a crop was below 0.5/plant.

For the intermittent sprinkler irrigation approach, two 3 × 2 (sprinkler irrigation regime × EPN +/-) factorial-split plot cabbage field trials were conducted. The three main plots of intermittent sprinkler irrigation regimes were: 1) 5-min sprinkler irrigation twice at dusk (6:00 and 8:00 pm) (S_d), 2) 5-min sprinkler irrigation from 8:00 am to 4:00 pm at 2-hour intervals and twice at dusk (6:00 pm and 8:00 pm) (S_{Dd}), and 3) no sprinkler irrigation (NS). Each main plot was split into subplots of foliar EPN (1.25 million IJs/ha) or no EPN (EPN-) applications. In Trial I, S_{Dd} and S_d decreased DBM damage on head cabbage by 19% compared to the NS but they had no effect in Trial II. S_{Dd} also reduced ICW damage by 88% in Trial I and by 45% in Trial II. In terms of insect abundance, S_{Dd} reduced ICW numbers on head cabbage by 86% compared to the NS control in Trial I and by 58% in Trial II. However, S_{Dd} and S_d did not affect DBM numbers. Unfortunately, no interaction occurred between sprinkler irrigation and EPN application, indicating that intermittent sprinkler irrigation also did not improve the performance of EPN on these pests.

To explore the third approach to improve EPN performance, two greenhouse experiments were conducted to test the efficacy of different adjuvants in enhancing the persistence of *Steinernema feltiae* MG-14 and its suppression against DBM. The adjuvant treatments tested were 3.9 ml/L (i.e. 50 fl oz/100 gal) of 1) Oroboost®, 2) Kinetic® and 3) Exit® compared to a 4) water control with EPN only where *S. feltiae* IJs were exposed to high heat in a greenhouse for 0, 30, 60, 120 and 180 min. Only Oroboost® extended the survival rates of *S. feltiae* for 2 hours. In fact, Kinetic® and Exit® showed a sign of toxicity against EPN. Oroboost® at 3.9 ml/L added to the EPN spray was more effective than Oroboost® at 1.6 ml/L (adjuvant rate used in the field trials as described above) in reducing the DBM population on cabbage plants 2 days after EPN application

in a greenhouse. A laboratory and a greenhouse experiment were followed up to test dosages of *S. feltiae* at 0, 0.625, 1.25 and 2.5 IJ/cm² mixed with Oroboost® at 3.9 ml/L against DBM larvae and pupae. Both experiments showed that 0.625 IJ/cm² was as effective as the commercial (1.25 IJ/cm² equivalent to 125 mil IJs/ha) and high (2.5 IJ/cm² equivalent to 250 mil IJs/ha) dosages of *S. feltiae* in killing and infecting DBM. Interestingly, both larva and pupa of DBM were equally susceptible to *S. feltiae* infection.

In summary, kai choi was more effective as a trap crop against DBM and ICW damage when intercropped with cabbage than with kale. Effect of kai choi as a trap crop in a kale cropping system was sporadic, mostly effective against DBM damage but not against ICW. The intermittent sprinkler irrigation (ISI) regimes tested in this thesis were only effective and rather significantly against ICW when ISI was in the daytime (> 80% in one trial and > 45% in another). ISI only reduced DBM damage by < 20% regardless of day or dusk ISI. More ISI regimes later at night need to be tested against DBM. The EPN efficacy tests conducted in the laboratory or greenhouse clearly showed that the high commercial EPN rate is not warranted but use of adjuvant is imperative. Future trap cropping field trials need to re-evaluate efficacy of *S. feltiae* with 62.5 mil IJs/ha supplemented with 3.9 ml Oroboost®/L. None-the-less, this project provided promising progress towards non-pesticide-based approaches against DBM and ICW that can help to mitigate insecticide resistance problem for organic farmers in Hawaii.

Keywords: adjuvant, cabbage, intermittent sprinkler irrigation, kai choi, *Steinernema feltiae*, trap crop.

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CHAPTER 1: Introduction

1.1 Head cabbage production

Head cabbage (*Brassica oleracea* var *capitata*) is an important vegetable crop in the US with an estimated production of 900 million kg in 2019 which is greater than 270 million kg for all other cole crops combined (USDA ERS 2019). This crop is known to have cancer-preventing properties and is rich in Vitamin C, β -carotene, lutein, DL- α -tocopherol, and phenolics (Singh et al., 2006). Cabbage production throughout the US is plagued by common specialist pests such as diamondback moth (DBM) (*Plutella xylostella*), imported cabbageworm (ICW) (*Pieris rapae*), cabbage webworm (*Hellula rogatalis*), and cabbage looper (*Trichoplusia ni* (Hübner)) (Mau et al., 2001). Among these pests, DBM and ICW are the most damaging (Talekar and Shelton, 1993; Furlong et al., 2013). DBM results in US\$4-5 billion yield loss worldwide and \$150-200 million in the USA alone (Zalucki et al., 2012). The overall loss caused by ICW can be about 71% on head cabbage (Maltais et al., 2012). Head cabbage, like all the cruciferous crops, contains glucosinolate or its metabolites and allyl isothiocyanates which serve as stimulants for oviposition and/or feeding of DBM and ICW (Reed et al., 1989). Both of these insect pests damage the leaf tissue making the produce unmarketable (Sarfraz et al., 2006).

In 2018, head cabbage was the top-volume vegetable crop in Hawaii with an estimated production of 3.60 million kg generating a farm gate value of \$3.93 million (NAAS, 2019). While the mild climate of Hawaii allows for the year-round production of cabbage, it also allows year-round insect pest reproduction. DBM and ICW have been the most recurring specialist insects in Hawaii threatening the yield and quality of major cruciferous crops including cabbage, cauliflower (*B. oleracea* var *botrytis*), broccoli (*B. oleracea* var *italica*), radish (*Raphanus sativus*), turnip (*B.*

rapa var *pekinensis*), Brussels sprouts (*B. oleracea* var *gemmifera*), kohlrabi (*B. oleracea* var *gongylodes*), mustard (*B. juncea*), rapeseed (*B. napus*), collard (*B. oleracea* var *acephala*), pak choi (*B. rapa* var *chinensis*), watercress (*Nasturtium officinale*), and kale (*B. oleracea* var *alboglabra*) (Nakhara et al., 1986). Farmers in Hawaii have experienced 20-40% and sometimes up to 100% of yield loss of crucifer crops attributed to DBM despite intensive attempts to manage the pest (Shimabukku et al., 1995).

1.2 Biology and Ecology of DBM and ICW

DBM takes about 41 days to complete its life cycle pending on the temperature (Liu et al., 2002). In the northern extremes of the DBM adaptable climate range, DBM has 3 to 4 generations per year, whereas in warmer areas, such as in Hawaii or Florida, DBM can breed continuously with as many as 15- 17 generations per year inflicting substantial pest pressure (Capinera, 2001). DBM are active at dusk and mating occurs the same day the adults emerge. Soon after mating, the female begins to lay eggs, and the eggs hatch in 4 days. Oviposition peaks between 7:00 PM and 8:00 PM with about 300 eggs laid by a female on either the upper or lower surfaces of a leaf (Talekar and Shelton, 1993). The larva molts through four instar stages. The larvae body tapers on both ends and a pair of pro-legs protrudes from the posterior end, forming a distinctive "V" shape. When disturbed, larvae will wriggle violently backward, eventually spinning down on a strand of silk (Capinera, 2001). The first-instar larvae mine in the spongy mesophyll tissue of the leaf, whereas older larvae feed on the lower leaf surface and usually consume leaf tissue leaving the wax layer on the upper surface creating a window screen on the leaf. Larval stages last for 14-21 days depending on the temperature. Pupation occurs in a loose silk cocoon, usually formed on the lower or outer leaves, and lasts for about 4-14 days. Therefore,

to manage DBM effectively, scientists have been targeting different stages of DBM. To disrupt oviposition and mating, Tabashnik et al. (1986) demonstrated overhead sprinkler irrigation as a vibrant strategy. Several other cultural and biological control measures have been practiced with varying degrees of success targeting the DBM larvae. However, due to the cryptic behavior of DBM hiding inside the spongy leaf tissue during its early instar stage, it is a challenge for the farmers to use contact pesticides to kill the larvae effectively.

Another important specialist pest of cruciferous crop is ICW. The complete life cycle of ICW requires 3–6 weeks, depending on weather. The number of generations reported annually is two to three in cool climates such as Canada, increasing to six to eight in southern areas (Capinera, 2008). In Hawaii, 15-17 generations/yr are found. The larvae proceed through five instars (Richards, 1940; Capinera, 2008). At the fifth instar, 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). In the temperate regions, the pupa remain in a quiescent stage (Richards, 1940; Capinera, 2008). The larvae feeds on leaves between the large veins and midribs and moves toward the center of the plant.

1.3 Immediate challenges of DBM and ICW Management in Hawaii

Different chemical and biological control measures have been deployed to manage ICW. Some of chemical pesticides such as diazinon, carbaryl, and permethrin are widely used as a measure to control ICW (Hutchison et al., 2009). Biological control using egg parasitoids (*Trichogramma* spp.), pupal parasitoids (*Pteromalus puparum* (L)), and brachonid wasps also offers variable control of the ICW. However, the 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). Other biopesticides such as the insect-pathogenic fungus *Beauveria bassiana*, the insect-pathogenic *Bacillus thuringiensis* (Capinera, 2008; Tompinks et al., 1986), and a Granulosis virus (Capinera, 2008; Tompinks et al., 1986) are found in the environment. However, many of these biopesticides are ineffective when applied alone. Use of Granulosis virus has limited success due UV radiation effects (Christian and Oakeshott, 1989). Hence, effective biological control of ICW is imperative to study to achieve an acceptable level of control.

Historically, management of DBM has been achieved using pesticides, but due to its propensity to develop insecticide resistance remains a serious pest (Saxena et al., 1989). Repeated use of insecticides with the same mechanism of action imposes insecticide selection pressure, yielding DBM populations that are highly resistant to several insecticides including organophosphate, carbamate, pyrethroid, organochlorine insecticides, and spinosad (Tabashnik et al., 1987; Mau and Gusukuma-Minuto, 2001). DBM is ranked second in the Arthropod Pesticide Resistance Database (APRD) for the highest number of insecticides with reported resistance in at least one population (Furlong et al., 2013).

Use of biopesticides such as fungi, nematodes, and bacteria has also been practiced. Gram-positive soil bacterium, *Bacillus thuringiensis* (*Bt*), once was a promising alternative to conventional insecticides against DBM but considerable resistance against *Bt* has been found in DBM worldwide. The first case of field resistance to *Bt* was reported from Hawaii in 1990 when field-collected DBM populations displayed 30-fold resistance to a *Bt* product, Dipel® (a.i. *B. thuringiensis* subsp. *kurstaki*) (Tabashnik et al. 1990). Besides *Bt* resistance, Mau and Gusukuma-

Minuto (2001) also reported DBM populations in Hawaii resistant to Pyganic (pyrethrin) and Entrust (a.i. spinosad). Therefore, researchers continue to search for alternative insecticides or biocontrol agents against DBM that can help to mitigate the insecticide resistant DBM population in Hawaii.

Cabbage growers employing conventional methods in Hawaii have at their disposal a handful of effective synthetic-based pesticides to employ in a 6-month pesticide rotational program (Mau and Gusukuma-Minuto, 2001), whereas organic growers only have a couple of pesticides that are effective, e.i. *Bt* and spinosad. Thus, it is imperative to identify more microbial biocontrol agents to add to the organic insecticide rotation program and identify cultural practices that can assist organic farmers in managing DBM effectively.

1.4 Biological Control

Natural parasitoids such as *Cotesia* (Braconidae), *Diadegma* (Ichneumonidae) and *Microplitis* (Braconidae) (Sarfraz et al., 2005) are present in Hawaii but are ineffective in suppression of DBM (Nofemela, 2013), but nonetheless do provide a low level of natural suppression in the field. Another group of natural enemies of DBM shown to have some potential. Often parasitoids do not provide effective suppression of insect pest populations due to unfavorable abiotic or biotic conditions (Gillespie et al., 2016)

Entomopathogenic nematodes (EPN) are obligate parasites of insects and are used as biological control agents of economically important insect pests (Shapiro-Ilan et al., 2012). EPNs can infect more than 200 species of insects under laboratory conditions (Hazir et al., 2003). While the fungal, bacterial, and arthropod biological control agents need days or weeks to kill the insect host, EPNs can kill the insect within 24-48 hours from infection. Application of EPNs does not

require special personal protective equipment and EPNs occur in a variety of soil types and environments (Georgis et al., 1991; Burnell and Stock, 2000). Species in two families (Heterorhabditidae and Steinernematidae) have been used as biological insecticides for pest management programs (Grewal et al., 2005). The third-stage juvenile (J3) known as Infective Juvenile (IJ), enters a dauer larvae state and is the only free-living stage of EPN. Both *Heterorhabditis* and *Steinernema* are mutualistically associated with bacteria of the genus *Xenorhabdus* and *Photorhabdus*, respectively. These are gram-negative bacteria in the family Enterobacteriaceae (Boemare et al., 1993). EPNs infect the host insect via the spiracles, mouth, anus, or in some species through intersegmental membranes of the cuticle, and then enter the hemocoel (Bedding and Molyneux, 1982). Once inside the host, bacteria kill the insect host via direct infection through the promotion of the secondary metabolites and toxins produced by the bacteria within 24-48 hours (Kaya and Gaugler, 1993). As the food depletes, the nematodes complete 1 to 3 generations, IJs leave the cadaver and disperse back into the soil in search of new target hosts (Ehlers, 1996).

EPNs have two foraging strategies to attack insects: ambush or cruise (Gaugler, 2002; Lacey and Kaya, 2000). Ambushers stand on their tails on the same spot for a long period and wait for opportunities to attack the prey. Chemical cues of the insect hosts are not important for ambushers (Lortkipanidze et al., 2016). Examples of ambusher EPN are *S. carpocapsae* and *S. scapterisci* (Gaugler, 2002; Lacey and Kaya, 2000). Cruisers move continuously in the environment in search of hosts. Cruisers largely use long-range cues (vibration, carbon dioxide, and other chemical cues) to locate the insect hosts. Examples of cruiser EPN include *S. glaseri*, and *H. bacteriophora* (Gaugler, 2002; Lacey and Kaya, 2000). However, some EPNs such as *S.*

riobrave and *S. feltiae* have intermediate foraging strategies, which means that they possess both ambusher and cruiser behaviors (Lacey and Kaya, 2000).

Application of EPN against insects has varying levels of success largely depending on the type of insects, life stages, habitat, species and doses of EPN, foraging strategies, application method, and environmental conditions. Shapiro et al. (2013) reported 95% (*S. riobrave*) and 77.5% (*S. feltiae*) control of Plum curculio weevil (*Conotrachelus nenuphar*), a soil-dwelling insect, when applied at 400 IJ/cm² in apple orchards. Similarly, *S. glaseri* has been found to control Japanese beetle, *Popillia japonica*, in turf grasses in the US (Gaugler et al., 1992). *S. scarabaei*, *H. bacteriophora* strain, *H. bacteriophora* strain TF, and *H. zealandica* strain X1 have provided field control of *P. japonica* by 100%, 34–97%, 65–92% and 73–98%, respectively (Grewal et al., 2005; Koppenhofer et al., 2006). For a foliar insect such as DBM, a high percentage of infectivity by EPN is found in laboratory assays. For example, Baur et al. (1995) reported 95% DBM control by *S. carpocapsae* and *S. riobrave* when applied at 2500 IJs/ml in a leaf disc assay. One study conducted by Morris et al. (1985) to compare the efficacy of *S. feltiae* and *H. indica* against DBM found *S. feltiae* to be more effective than *H. indica* when the same concentration was applied.

While EPNs showed a great degree of suppression of DBM and other Lepidopteran cabbage pests in the laboratory, EPN efficacy in the field was minimal. Belair et al. (2003) found only 35.5 % DBM control by *S. carpocapsae* in field application and 75.7% mortality of ICW in the laboratory. Similarly, Baur et al. (1995) achieved 41% control of DBM larvae with *S. carpocapsae* in a watercress wetland field, which is considered relatively promising. Somvanshi et al. (2006) reported a 40% mortality of DBM larvae when applying *S. thermophilum* at 2,000 IJs/ml in the field and could only increase mortality to 46 % when applying at 3,000 IJs/ml. It is uncertain what

concentration Somvanshi et al. (2006) applied in terms of the number of IJs/ha. If they applied at 945 liter/ha of spray coverage, this would be considered a very high concentration impractical to achieve in the field.

Failure to achieve > 50% of DBM control using EPN foliar application in the field could be due to various environmental factors such as desiccation, temperature, and ultraviolet radiation (UV) (Shapiro-Ilan et al., 2015). The high ambient temperatures, especially in tropical regions, adversely affect the efficiency of foliar applications of EPNs (Kamionek *et al.*, 1974). To increase the persistence and efficacy of the EPNs in the field, many approaches have been suggested. Some of them include adding adjuvants to protect EPN from desiccation and UV light. Adjuvants includes a variety of commercial products such as desiccation protectants, paraffin oil, alginate, arboxy-methylcellulose, guar gum, Arabic gum, and xanthan gum (Rhodes, 1993). The adjuvants either act as a wetting agent that can reduce the surface tension, increase the surface contact of the suspension to the leaves and reduce the runoff of the suspension. In addition, xanthan gum has proved to be the only adjuvant capable of delaying sedimentation of EPNs in suspension, thus improving the distribution of an EPN suspension during foliar application (Beck et al., 2013).

Chemicals such as PABA (para-amino benzoic acid), OMC (octyl methoxycinnamate) which are UV protectants, along with a fire protectant Barricade® gel were tested as adjuvants in an EPN suspension and were shown to increase the survival of *S. feltiae* exposed to UV light in the laboratory (Acar and Sipes, 2019). Other formulations such as the use of alginate beads are also suggested by Kapranas et al. (2020) in the early (during sowing of radish) and late (after sowing) before Cabbage fly (*Delia radicum*) infestation application of *S. feltiae* and *S. carpocapsae* which reduced the visible infestation and mining of cabbage fly on radish in laboratory and greenhouse

experiment. However, Jaffuel et al. (2019) found no difference in the efficacy of *H. bacteriophora* against banded cucumber beetle (*Diabrotica balteata*) between applying in alginate beads or aqueous suspension in laboratory conditions.

Other cultural and biological methods are also being integrated with EPN in the field to increase its efficacy. For example, Baur et al. (1998) increased the performance of *S. carpocapsae* against DBM in a watercress field in Hawaii from 41% to 58% when integrated with Bt foliar spray. On the other hand, Sáenz-Aponte et al. (2020) combined the application of *H. indica* at 1200 IJ/cm² with the entomopathogenic fungus *Metarhizium anisoplae* at 1×10^5 conidia/cm² and reduced DBM damage from 60% with EPN alone to 40% when applied in combination and increase the productivity of broccoli in the field. Mohammed (2012) found that combining the EPN species *S. feltiae* and *S. carpocapsae* further increased the level of ICW mortality over that of a single EPN species tested. Gumus et al. (2015) developed another strategy that utilized living goat moth (*Cossus cossus*) pre-inoculated with EPNs as 'EPN bombs' controlled 86% of goat moth compared to the untreated control. However, this 'EPN bomb' strategy only worked against insect pests with cryptic behavior such as goat moth that hides inside chestnut logs. This approach was found not effective against non-cryptic insect pests such as lawn caterpillar (*Spodoptera cilia*) in the turf arena (Gumus et al., 2015). The success of foliar application of EPNs is still a concern. Hence, appropriate techniques of integration of EPNs with other cultural practices might be beneficial to manage foliar pests like the DBM in the field.

The literature indicates that EPNs are not providing sufficient control of DBM in cruciferous crop fields, but we know that EPNs are highly effective against DBM in the laboratory. One approach to overcome this challenge is to apply EPN at a very high dosage such as that

recommended by most commercial EPN products. The recommended rate of Millenium (a.i. *S. carpocapsae*, BASF, Research Triangle, NC) is 2.5 billion IJ/ha. NemaSeek (a.i. *Heterorhabditis bacteriophora*) and NemAttack (a.i. *S. feltiae*, Arbio Organics, Oro Valley, AZ) are recommended to be applied at 125 million IJ/ha. Studies showed that a very high concentration of EPNs is crucial to achieving effective larval mortality. Farmers cannot afford to apply EPNs at the high rates if they can purchase commercial EPN products. Unfortunately, due to restrictions on the importation of alien organisms into the State of Hawaii, commercial formulations of EPNs are not permitted to be brought in by growers, making the use of EPN as a biocontrol agent against agricultural pests difficult in Hawaii.

Fortunately, indigenous EPNs both in the genera of *Heterorhabditis* and *Steinernema* has been found in Hawaii (Hara *et al.*, 1991; Myers *et al.*, 2015). A newly described species, *Heterorhabditis hawaiiensis*, was found in Hanalei, Kauai and described by Gardner *et al.*, 1994). Myers *et al.* (2015) found two *Heterorhabditis* species to be abundant and widespread in Hawaii, among which, *H. indica* OM160 was shown to be highly virulent on several important insect pests. *Steinernema feltiae* MG-14 isolated from Waikapu, Maui had also been found to be a virulent strain against imported cabbage worm 4th instar (Mohammed, 2012).

1.4 Integrated Pest Management with EPN

One strategy to make the use of EPN effective against DBM is to integrate multiple management options mentioned above including trap cropping, intermittent sprinkler irrigation, and adding adjuvants to increase the persistence of indigenous EPN. This thesis will focus on integrating EPN foliar application using the locally isolated EPN, *S. feltiae* MG-14, with 1) trap crops and 2) intermittent sprinkler irrigation to manage DBM and ICW. Since the most vulnerable

cabbage and kale farmers that suffer from DBM damage are the organic farmers, this research focuses on developing IPM strategies that are compatible with organic farming. The effective and feasible rates of *S. feltiae* MG-14 that are prepared *in vivo* (not available commercially) need to be determined. It is anticipated that multiple focus of this thesis would contribute to better decision making for organic cruciferous crop growers in Hawaii.

1.5 Objectives and Hypothesis

The overarching objective of this study is to integrate cultural and biological tactics of pest management against DBM suitable for organic cruciferous crop farmers in the tropics. This thesis emphasizes enhancing the efficacy of a locally isolated EPN species (*S. feltiae* MG-14) in Hawaii under field conditions which have shown promising results in the laboratory but need improvement. Specific objectives of this project are to:

1. Evaluate the benefits of integrating EPNs with trap cropping to manage DBM

H₀ 1: Kai choi as a trap crop would suppress the abundance and damage of DBM and ICW

H₀ 2: EPN foliar spray would suppress the abundance and damage of DBM and ICW

H₀ 3: Combination of trap crop and EPN would produce an additive effect than each of these treatments alone

2. Examine the potential of integrating intermittent sprinkler irrigation and trap cropping in enhancing EPN efficacy against DBM.

H₀1. Daytime intermittent sprinkler irrigation not only would disrupt the flying and feeding of ICW but would also reduce DBM feeding on the wet leaf surface

H₀2. Dusk intermittent sprinkler irrigation would disrupt the mating and oviposition of DBM

H₀3. Overhead sprinkler irrigation could increase leaf moisture and enhance the longevity and survival of EPNs

3. Determine EPN foliar spray concentration and of appropriate adjuvants most effective for DBM management,

H₀1. Adjuvants will increase the persistence and efficacy of EPNs when exposed to high heat and desiccation which normally cause high EPN mortality.

H₀2. Lab reared EPNs isolated in Hawaii will allow lower EPN dosages to be effective for reducing DBM in cabbage plants if coupled with appropriate adjuvants.

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CHAPTER 2: Efficacy of Integrating Trap Cropping and EPN Foliar Spray on Abundance and Damage of DBM and ICW on Head Cabbage and Kale Agroecosystems

2.1 Introduction

Cabbage (*Brassica oleracea* var *capitata*) and kale (*Brassica oleracea* var *acephala*) production throughout the US is plagued by several common pests including diamondback moth (DBM), imported cabbageworm (ICW) (*Pieris rapae*), cabbage webworm (*Hellula rogatalis*), and cabbage looper (*Trichoplusia ni* (Hübner) (Mau et al., 2001). Among these pests, DBM and ICW are considered the most damaging pests (Talekar and Shelton, 1993; Furlong et al., 2013). DBM causes up to 90% (Talekar and Shelton, 1993; Kfir, 1998; Amoabeng et al., 2013) and ICW about 71% (Maltais et al., 1998) crop losses on cruciferous crops. DBM early instars mine the leaf tissue, while the later instars consume leaf tissue from the underside of the leaf creating a window-screen appearance. ICW causes irregular holes on the leaves (Capinera, 2008; Hutchison et al., 2009), and under severe infestations, larvae skeletonize leaves. In Hawaii, DBM and ICW have been the most recurring pests threatening the yield and quality of major cruciferous crops. While ICW damage is also economically damaging, it can be managed by the application of commercially available insecticides, but DBM management is challenging due to its propensity to develop resistance against commonly used insecticides such as organophosphates, organochlorine, carbamates, and pyrethroid compounds (Tabashnik et al., 1987). Additionally, DBM resistance to *Bacillus thuringiensis* (*Bt*) in Hawaii was discovered in 1986 (B E Tabashnik et al., 1990), and to spinosad in 2000 (Mau and Gusukuma-Minuto, 2001). The insecticide resistance management (IRM) program in Hawai'i recommended a complete 6-month insecticide rotation of different mechanism of action groups every month (Chou et al., 2018; Mau and Gusukuma-

Minuto, 2001) to battle Bt and spinosad-resistant DBM. While conventional growers can mitigate resistance by doing this, organic growers have fewer pesticide options and are primarily limited to the rotation of only Bt, spinosad, and *Bueveria bassiana*. Thus, it is imperative to identify other biocontrol agents and cultural practices to assist organic farmers in managing DBM infestations.

Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are obligate parasites that kill insects with the help of mutualistic bacteria that inhabit the intestine of the infective juveniles (IJs) (Poinar, 1990; Boemare, 2002). EPNs have been used with variable success against insects occupying different habitats. Most of the EPN success cases have targeted soil-dwelling insects or insects residing in cryptic habitats (Begley, 1990; Klein, 1990; Williams and Walters, 2000). For foliar pests such as DBM and ICW, even though laboratory assays have shown successful control, field results are variable (Begley, 1990). For example, although Ganguly and Gavas (2004) found 100% mortality of DBM larvae caused by *Steinernema thermophilum* within 48 h after inoculation in laboratory conditions, the same EPN applied at 1,000 to 3,000 IJs/ml along with an adjuvant (0.033% APSA80) as a foliar spray caused only 35-46% mortality of DBM on head cabbage (Somvanshi et al., 2006). Similarly, although Zolfagharian et al. (2016) reported that *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* caused 84 % and 100 % DBM mortality, respectively in the laboratory, Baur et al. (1995) only achieved 41% control of DBM larvae with *S. carpocapsae* in a watercress wetland field.

Failure of EPNs to achieve more than 50% of DBM control using EPN application in the field could be due to various environmental factors that affect the survival of EPN such as desiccation, heat, and ultraviolet (UV) radiation (Shapiro-Ilan et al., 2015). Some scientists

suggested increasing the persistence and efficacy of the EPNs in the field by adding spray adjuvants. Schroer et al. (2004) found a single application of 0.5 million *S. carpocapsae*/m² with 0.3% xanthan gum and 0.3% Rimulgan achieved a significant reduction of DBM with >50% control after 7 days. However, Baur et al. (1997) reported that the efficacy of EPN against DBM was similar to with and without the use of adjuvants.

Cultural control such as crop rotations, planting resistant varieties, and trap cropping have been suggested as effective strategies against DBM and ICW in the field (Furlong et al., 2013). Among these, the trap cropping approach was studied the most. Trap crops are plants grown close to a cash crop that can attract, retain, or intercept target pests to reduce or eliminate the pest damage on the cash crop (Shelton and Badenes-Perez, 2006). The most tested trap crops against DBM are collard green (*Brassica oleracea* var *acephala*), mustard (*Brassica juncea*), kale (*B. oleraceae* var *acephala*), and yellow rocket (*Barbarea vulgaris*) (Pinero and Manandhar, 2015; Sherbrooke et al., 2020). However, many contradictory findings are found among these literatures on the ability of trap crops to reduce the pest pressure of DBM on the cash crops. For example, Mitchell et al. (1997) reported that the numbers of DBM never exceeded the action threshold 0.3 DBM/plant in eight of the nine fields that were completely surrounded by collards but did exceed the action threshold in three of the fields without collards. In another study where Indian mustard was planted as a trap crop in every 15 to 20 rows of cabbage, a significant reduction of DBM population densities was observed in India (Srinivasan and Moorthy, 1992) and in Sweden (Asman, 2002). However, Luther et al. (1996) and Bender et al. (1999) both found that Indian mustard planted as a trap crop did not reduce DBM population on head cabbage in Hawaii and Texas.

A preliminary experiment conducted in Hawaii that compared 5 brassica crops including ‘Joy choi’ and ‘Mei Ching’ pak choi (*Brassica rapa* subsp. *Chinensis*), ‘Hirayama’ kai choi (*Brassica juncea*), ‘KK’ head cabbage, and ‘Starbor’ kale for their attractiveness to and feeding damage of DBM showed that DBM caused most damage on kai choi or mustard green when interplanted with head cabbage (Budhathoki et al., 2020). Further research to confirm trap cropping effect of ‘Hirayama’ kai choi on DBM is needed. In addition, Somvanshi et al. (2007) suggested to include EPNs as one of the components in an integrated pest management (IPM) program against DBM. Thus, the specific objectives of this research were to integrate the use of an indigenous EPN from Hawaii, *Steinernema feltiae* MG-14, as a biological control agent with kai choi as a trap crop to reduce DBM and ICW damage on head cabbage and kale in field settings. We hypothesized that 1) kai choi as a trap crop would suppress the abundance and damage of DBM and ICW, 2) EPN foliar spray would suppress the abundance and damage of DBM and ICW, and 3) combination of trap crop and EPN would produce an additive effect than each of these treatments alone.

2.2 Materials and Methods

EPN inoculum: *S. feltiae* MG 14 was cultured using mealworm larvae (*Tenebrio molitor*). Ten mealworm larvae were placed in 100-mm-d petri dishes lined with Whatman #1 filter paper. Each mealworm larvae were inoculated with 100 IJs of *S. feltiae*. The paper was moistened with 1 ml of water. The larvae were monitored 24 and 48 h after inoculation and dead larvae were placed on White traps (White, 1927). IJs were collected and stored at 15°C for inoculum. No cultures used in this experiment were over 30-day-old.

Head cabbage experiment: A head cabbage field trial was conducted from March to May 2020 at Go Farm Hawaii, Waialua, HI (21°3339.5 N, 158°0740.1 W). This farm had been practicing

organic farming for the last 4 years. Six-week-old 'KY' cross head cabbage was transplanted at 30-cm spacing between plants in a row on a 90-cm wide planting bed. A 2 × 2 (trap crop × EPN) factorial designed experiment was installed. For plots designated as 'Trap Crop', all cabbage plants were surrounded by 'Hirayama' kai choi as a border crop at 30-cm between plants (Fig. 2-1A) and planted 15-cm away from the head cabbage. No trap crop plots only had head cabbage planted. Each plot had 5 cabbage plants. The entire field trial was surrounded by bare fallow areas during the trial. For treatment receiving EPN (EPN+), plants were sprayed with *S. feltiae* MG-14 at 1,000 IJs per plant (equivalent to 125 million IJs/ha) delivered through 8 ml of water suspension per plant the 4th week after transplanting. The spray solution contained Barricade® gel (Barricade International, Inc., Hobe Sound, FL) as an anti-desiccant and PABA (Sigma Chemical, Steinheim, Germany) as a UV protectant. EPN- plots did not receive the foliar spray. Each treatment had 4 replications. Crops were irrigated, fertilized and occasionally sprayed with *Bt* as needed following the commercial farming practice.

Percent of leaves with DBM or ICW damage on each plant were recorded from 4 plants per plot weekly from transplanting to harvesting, a 7-week interval. The abundance of insect eggs, larvae, pupae, and adult stages of DBM and ICW as well as parasitoid pupae per plant were also recorded from 4 plants per plot weekly. Head cabbage weight from each plant was recorded at harvest.

Kale experiment: Two field trials were conducted at Kahumana Organic Farm (21°27'13.2 N 158°09'03.8 W) in Waianae, HI. The first trial (Trial I) was conducted on May 13 to June 24, 2020, to evaluate the effect of trap crop and EPN spray-on curly kale against DBM and ICW. The experiment was arranged in a 2 × 2 (trap crop × EPN) factorial design with 4 replicated plots.

Seven-week-old kale seedlings were transplanted in two rows per plot at the 30-cm spacing between plants and between rows. Each plot was $1.2 \times 1.8 \text{ m}^2$ surrounded by 2 rows of kai choi seedlings transplanted at the same time. A total of 16 plots with 12 kale plants per plot were established. For 'Trap Crop', 'Hirayama' kai Choi were planted at the edge of the plot with 30 cm between plants and 30 cm from the kale plants (Fig. 2-1B). For the plots designated as EPN+, a water suspension containing 1.6 ml/L of Oroboost® (Oro Agri, Inc. 2788 S. Maple Ave., Fresno, CA 93725) and *S. feltiae* MG-14 was applied at 1,000 IJs/plant (125million IJs/ha) once on the 3rd week after initiation of the trial. Crops were irrigated, fertilized, and occasionally sprayed with *Bt* as needed following the commercial farming practice.

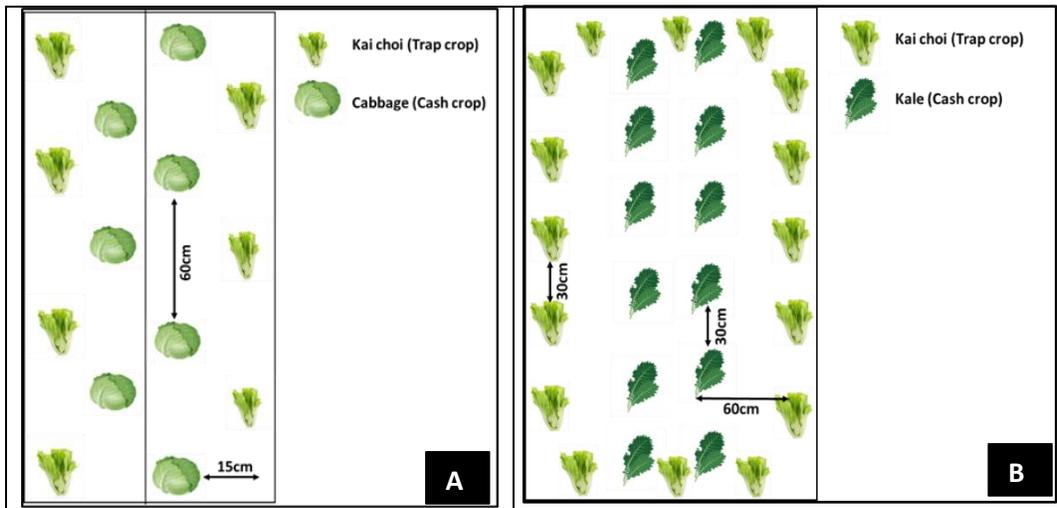


Fig.2-1. Kai choi as a trap crop around A) cabbage and B) kale planted at Waialua and Waianae.

Four randomly selected plants were recorded from each plot for 1) percent of leaves with DBM or ICW damage, and 2) abundance of eggs, larvae, pupae, and adult stages of DBM and ICW per plant weekly for 5 weeks. Kale leaves were harvested weekly starting from the third week after transplanting till 5 weeks after planting.

A second field trial (Trial II) was conducted from June 22 to July 16, 2020, in a kale field next to Trial I. Slight modification from Trial I was performed whereby plot size was reduced to $1.2 \times 0.9 \text{ m}^2$ with 8 kale plants per plot. Kai choi was planted 66 cm away from the kale planting rows. EPN+ plots received a foliar spray of *S. feltiae* at 125 million IJ/ha using Oroboost® as an adjuvant like Trial 1 except that it was applied on the first week of data collection. Crops were irrigated, fertilized, and occasionally sprayed with *Bt* as needed following the commercial farming practice. Insect counts and damage data were collected over 5 weeks from 4 plants per plot. Kale was harvested from the 3rd to 5th week after initiation of Trial II.

Statistical analysis: Data from each field trial were checked for normality using Proc Univariate in Statistical Analytical Software (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA). Insect counts were normalized using $\log_{10}(x + 1)$ whenever needed before analysis of variance (ANOVA). The insect count and damage data from cash crop in each trial were subjected to repeated measures ANOVA using Proc GLM in SAS with sampling dates blocked within treatments. If no significant interaction between treatment and sampling date, data across sampling dates were pooled for ANOVA, whereas if significant interaction occurred, data were analyzed by date. All data were subjected to 2×2 (Trap crop \times EPN) factorial analysis of variance (ANOVA) in a randomized complete block design using SAS (SAS Institute, Cary, NC). To examine the short-term effect of EPN, reduction in DBM and ICW abundance 1 week after EPN application

were compared among treatments. To further understand the feeding preference of DBM and ICW, abundance and damage of DBM and ICW on cabbage and kai choi within the trap crop plots were compared using one-way analysis of variance. Means were separated by the Waller-Duncan k -ratio ($k=100$) t -test wherever appropriate, but only the true means were presented. Similarly, yield data were also subjected to 2×2 factorial ANOVA and Waller-Duncan k -ratio ($k=100$) t -test wherever appropriate.

2.3 Results

Head cabbage experiment: Based on the 2×2 ANOVA with sampling dates blocked within treatments, interaction between trap crop and EPN effects were observed for number of DBM ($F_{1, 333} = 6.5$, $P = 0.01$) but not DBM damage, ICW number and damage ($P > 0.05$). However, interaction between treatment (Trap \times EPN) and date was not significant for number of DBM but were significant for DBM damage, ICW number and damage. Thus, the means of DBM were presented by Trap Crop \times EPN on each sampling date (Fig. 2-2A), whereas the means of DBM damage, ICW number and damage were presented for trap crop by date (Fig. 2-3). Since EPN was only expected to be effective after EPN spray, comparison between EPN treatments was only conducted on the changes before and after EPN spray on May 23, 2020 (Fig. 2-4). Interaction between Trap crop \times EPN was significant for changes in DBM abundance after EPN application, but no significant difference between EPN treatments was observed regardless of T or NT (Fig. 2-4A). Significant interaction between Trap crop and EPN also occurred in changes of ICW number after EPN application ($F_{1, 56} = 8.93$, $P < 0.01$) where EPN only reduced ICW abundance in the NT plots ($F_{1, 56} = 8.93$, $P < 0.01$).

Planting of kai choi as a trap crop suppressed the total abundance of DBM ($F_{(1, 333)} = 13.13$, $P \leq 0.01$; Fig. 2-2A) regardless of EPN treatment. While no significant effects of EPN application on DBM abundance was observed in the repeated measure analysis, slight reduction in DBM number was observed 1 week after the application of EPN on April 23, 2020 ($P > 0.05$; Fig. 2-2B) but only in the trap crop treatment. On the other hand, EPN treatment slightly increased DBM numbers when no trap crop was planted ($P > 0.05$; Fig. 2-2B).

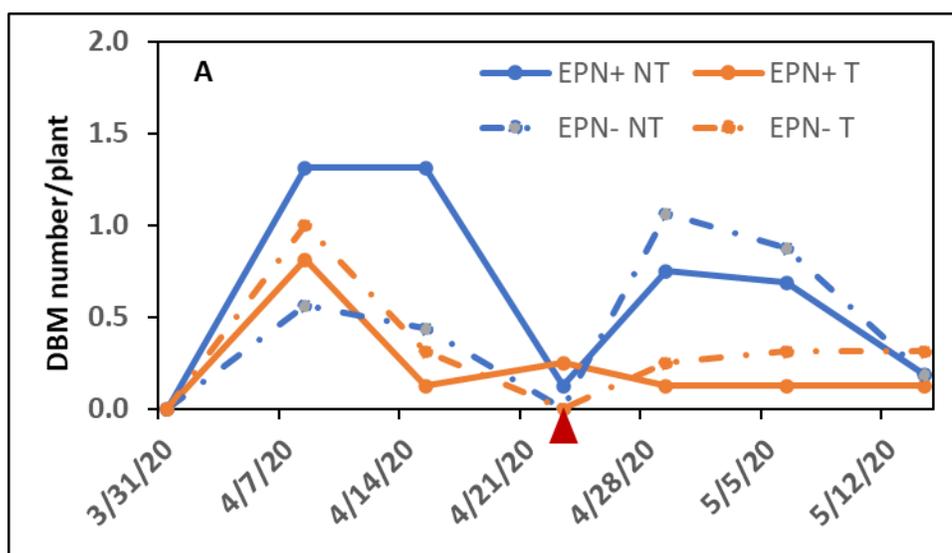


Fig.2-2. Effect of kai-choi as trap crop and entomopathogenic nematode (EPN) on reduction of abundance of diamondback moth (DBM) on head cabbage in the Waialua Trial after EPN application. T= trap crop, NT = no trap crop. ▲ Indicates EPN application. @ Indicates difference between EPN+ and EPN- at $P \leq 0.10$ level.

Planting of kai choi trap crop reduced percent of leaves with DBM damage ($F_{(1, 333)} = 13.6$, $P \leq 0.01$) by 45% compared to no trap crop on head cabbage (Fig. 2-3A). Trap crop also reduced the abundance ($F_{(1, 333)} = 140.54$, $P \leq 0.01$) and leaf damage of ICW ($F_{(1, 333)} = 30.8$, $P \leq 0.01$) by 69% and 33%, respectively (Fig. 2-3B, C). Soon after EPN application, though not significant, EPN+ was showing a trend in reducing DBM only in the T ($P > 0.05$) (Fig. 2-4 A). In contrary, EPN only reduced ICW number in the NT but not in the T plot ($F_{(1, 333)} = 8.64$, $P \leq 0.05$; Fig. 2-4B).

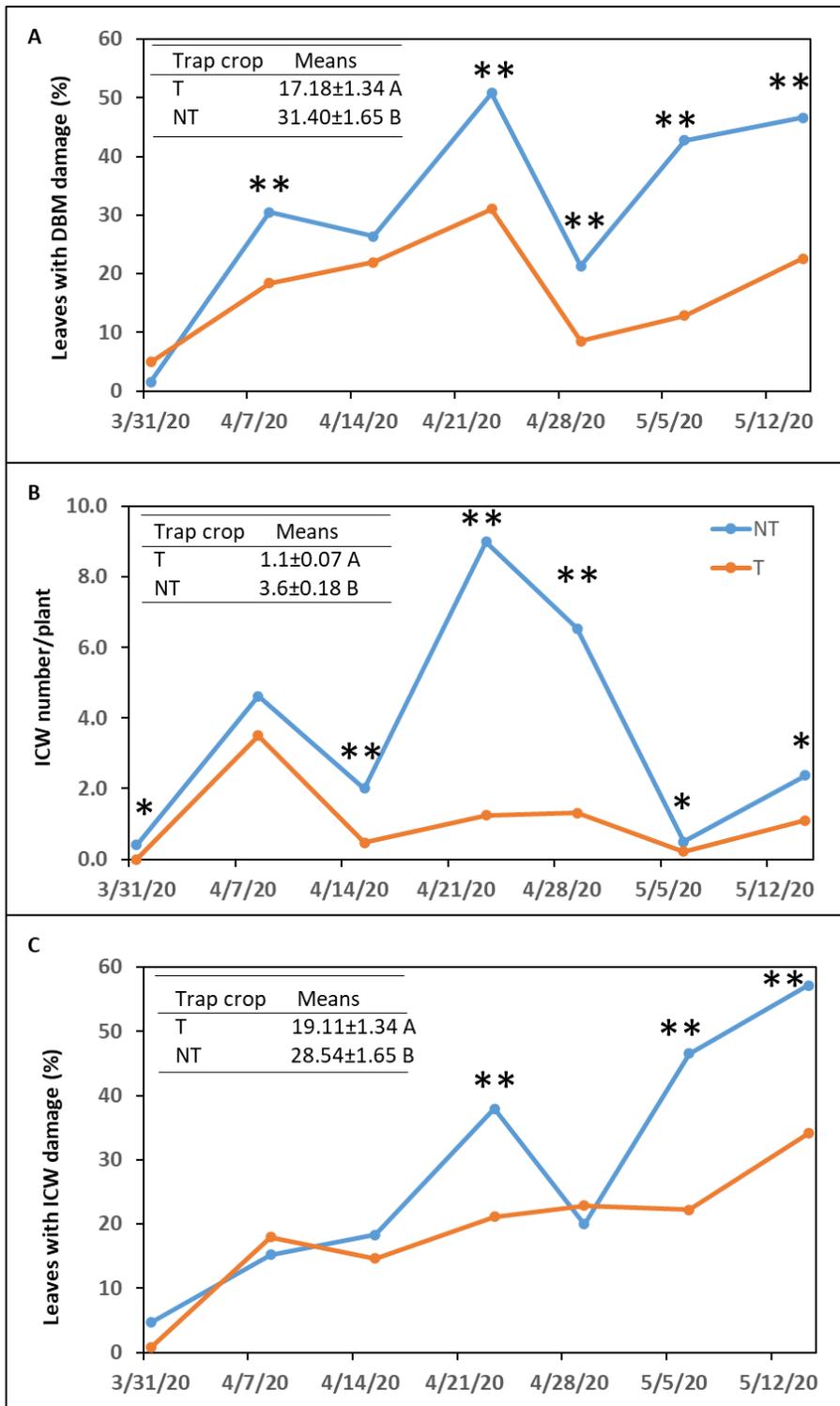


Fig.2-3. Effect of kai choi as a trap crop on A) percentage of leaves with diamondback moth (DBM) damage B) abundance of imported cabbageworm (ICW) and C) percentage of leaves with imported cabbageworm damage on head cabbage. * and ** indicate significant difference between trap crop (T) and no trap crop (NT) at $P \leq 0.05$ and 0.01 , respectively.

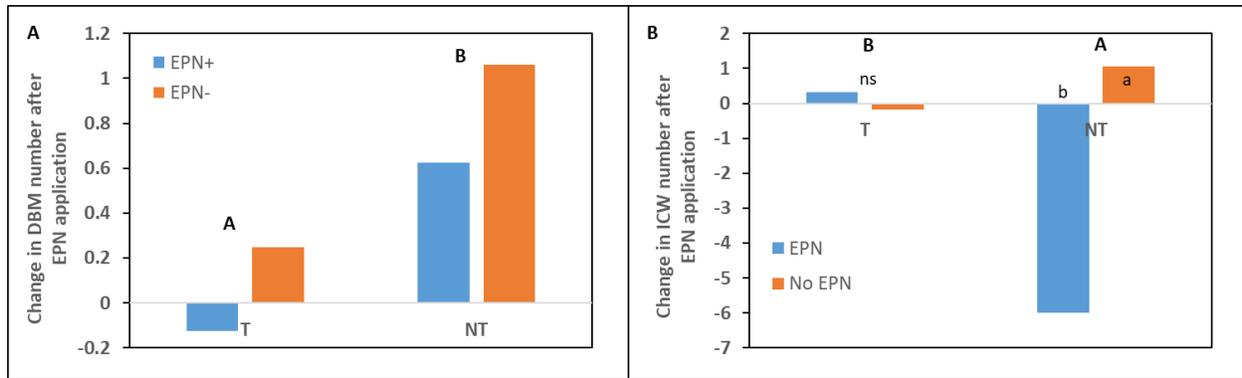


Fig.2-4. Effect of entomopathogenic nematodes (EPN) on reduction in number of A) diamondback moth (DBM) and B) imported cabbageworm (ICW) before and one week after EPN application in trap crop (T) and no trap crop (NT) plots. Means (n=16) of trap crop treatments or EPN treatments followed by the same letter were not different according to analysis of variance.

When comparing kai choi and cabbage within the trap crop plots, the abundance of ICW ($F_{(1, 420)} = 88.43, P \leq 0.01$) and DBM ($F_{(1, 420)} = 41.02, P \leq 0.01$) were higher on the head cabbage than the kai choi (Fig. 2-5 A, B), but the feeding damage of ICW ($F_{(1, 420)} = 65.27, P \leq 0.01$) and DBM ($F_{(1, 484)} = 145.74, P \leq 0.01$) were more severe on kai choi compared to the head cabbage (Fig. 2-5 C, D). No interaction between crop type and date was observed throughout the trial, thus only means of the main factor was presented.

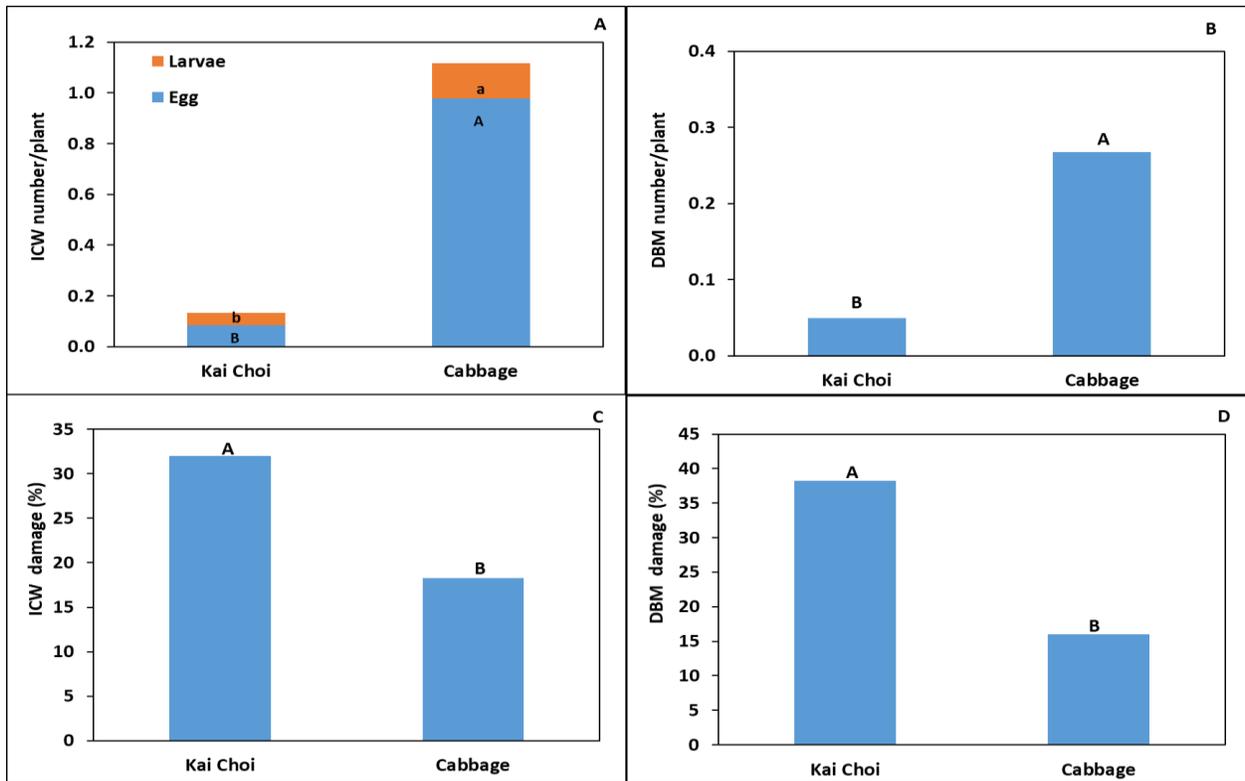


Fig. 2-5. Abundance of A) imported cabbage worm (ICW) larvae and eggs, and B) diamondback moth (DBM larvae) percentage of C) ICW and D) DBM leaf damage on kai choi and head cabbage in the Waialua Trial. Columns (n=224) followed by the same letter in a graph are not different based on analysis of variance.

No interaction between trap cropping and EPN effect was observed. Thus, the data for main effect on cabbage yield were pooled together. ANOVA results showed that EPN application was not significant. Therefore, only means of trap cropping were presented. Planting of kai choi as a trap crop 15-cm away from the cabbage planting row reduced the head cabbage weight ($F_{(1, 60)} = 77.11, P \leq 0.01$) by 41% compared to cabbage in no Trap Crop (Fig.2-6).

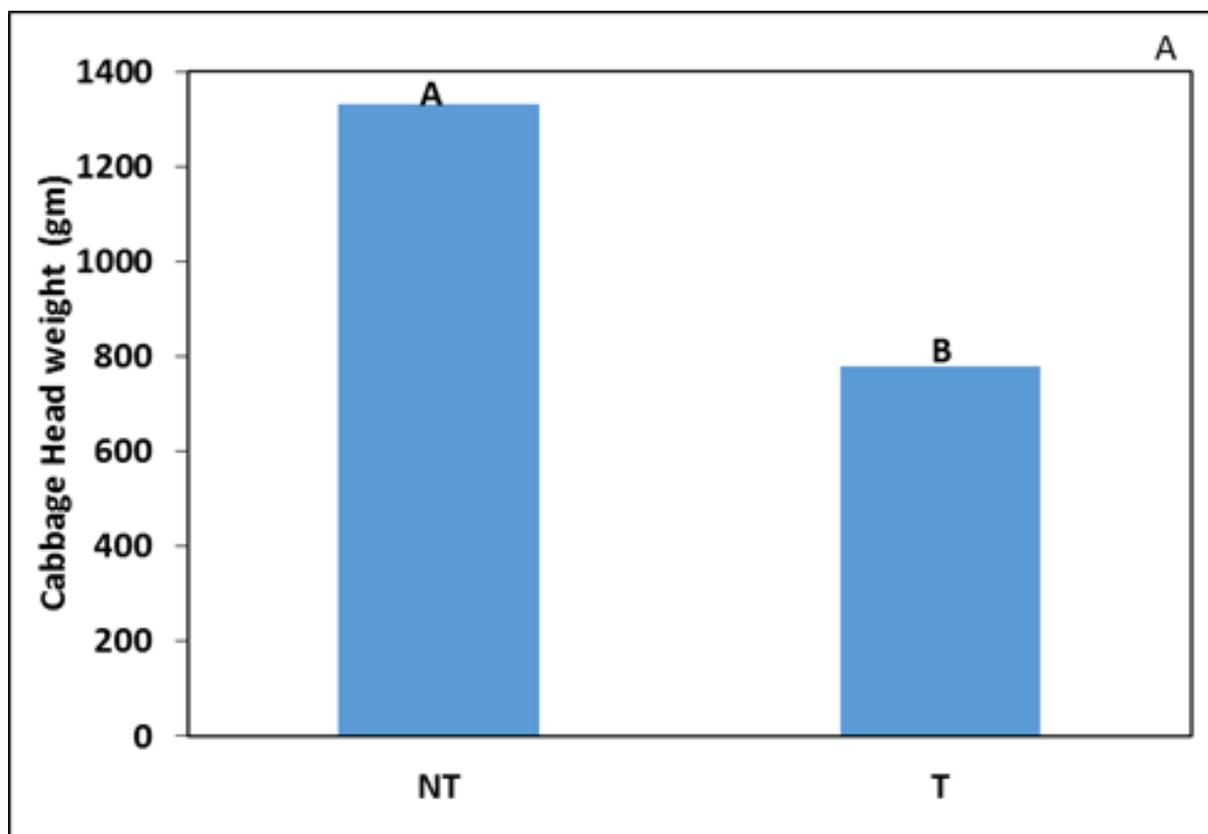


Fig.2-6. Effect of Trap crop on the productivity of head cabbage. Means followed by the same letters are not different based on analysis of variance.

Kale experiment: In Kale Trial I, based on the 2×2 ANOVA, no interaction between trap crop and EPN effects was observed for any of the parameters measured, thus only means of the main treatments were presented. It is encouraging that planting of kai choi as a trap crop suppressed 50% of DBM abundance ($F_{(1, 237)} = 8.68, P \leq 0.05$), mostly dominated by larvae and pupae stages, and 19% of leaves with DBM damage ($F_{(1, 237)} = 11.54, P \leq 0.01$) on kale (Fig. 2-7 A, B). However, the trap cropping effect was not significant ($P > 0.05$) for ICW number and percent of leaves with DBM damage ($P > 0.05$) in Trial I of kale (Fig. 2-7 C, D). On the other hand, EPNs did not affect the number and damage of both ICW and DBM ($P > 0.05$), thus data were not shown.

When comparing the abundance and damage of ICW between kale and kai choi within the trap crop treatment, ICW numbers (larvae and eggs) ($F_{(1, 420)} = 38.42, P \leq 0.01$) and ICW damage ($F_{(1, 420)} = 4.61, P \leq 0.01$) were both higher on kale than on kai choi. No interaction between crop type and date was observed, thus only means of the crop type were presented (Fig. 2-8). Similarly, DBM abundance (larvae and pupae), was also higher on kale than on kai choi ($F_{(1, 420)} = 12.12, P \leq 0.01$) but DBM damage ($F_{(1, 420)} = 44.61, P \leq 0.01$) was higher on kai choi than on kale.

In terms of kale yield, ANOVA showed no interaction between trap cropping and EPN, and no significant effect from EPN. Thus, only means of trap cropping was shown. Trap cropping reduced kale weight in Trial I by 24% (Fig. 2-9A) even though the leaf numbers were not different (Fig. 2-9B).

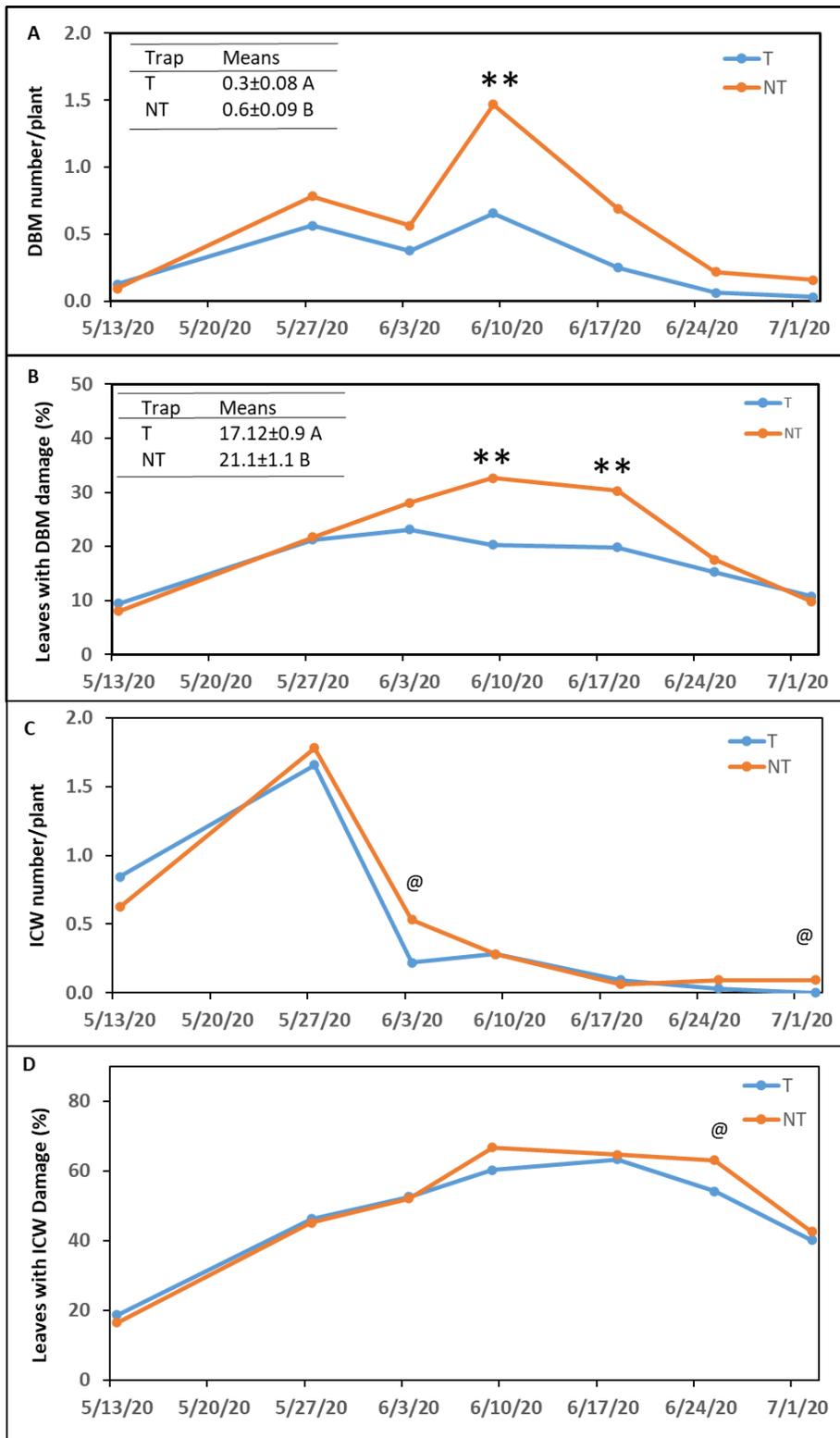


Fig.2-7. Effects of kai choi as a trap crop on A) abundance of diamondback moth (DBM) B) percent leaves with DBM damage, C) abundance of imported cabbageworm (ICW), and D) percent leaves with ICW damage in kale Trial I.

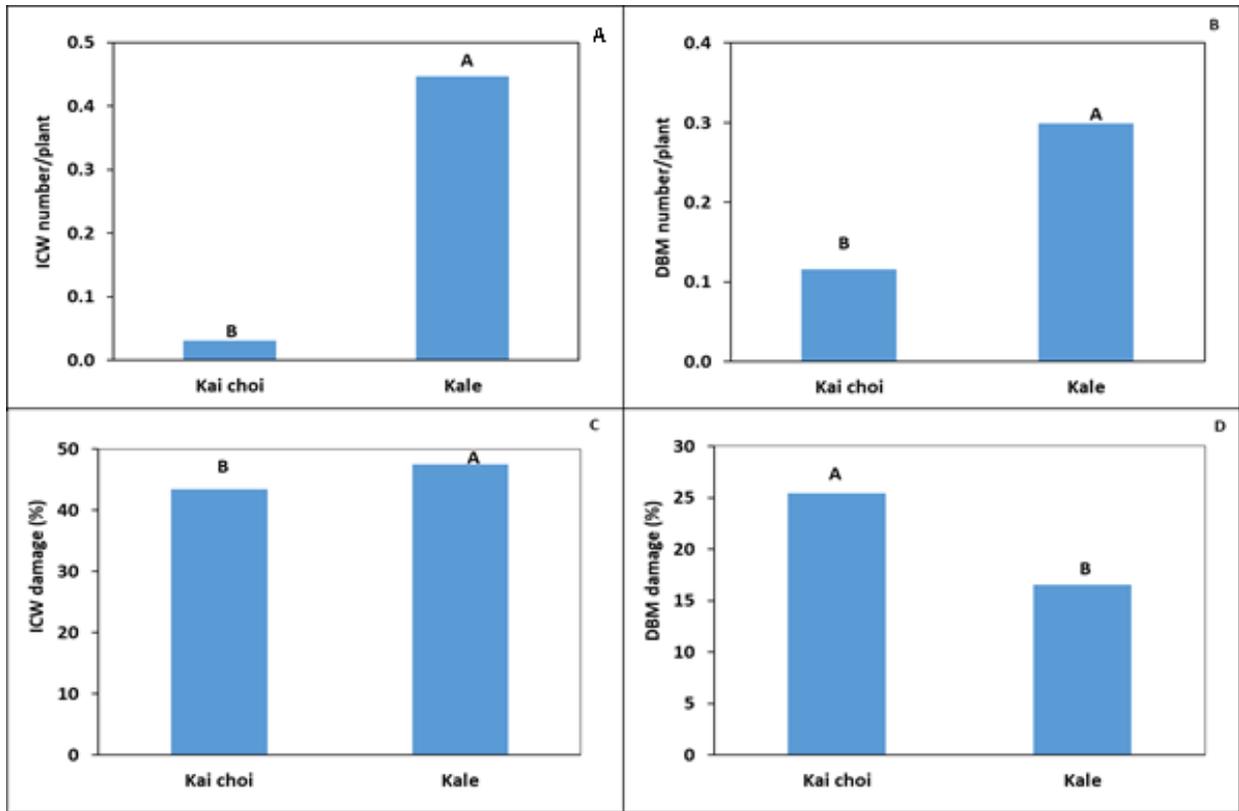


Fig.2-8. The abundance of A) imported cabbageworm (ICW) larvae and eggs, and B) diamondback moth (DBM) (larvae and pupae), and percentage of C) ICW and D) DBM leaf damage on kai choi and kale in the trial I at Waianae. Means followed by the same letters in a graph are not different based on analysis of variance.

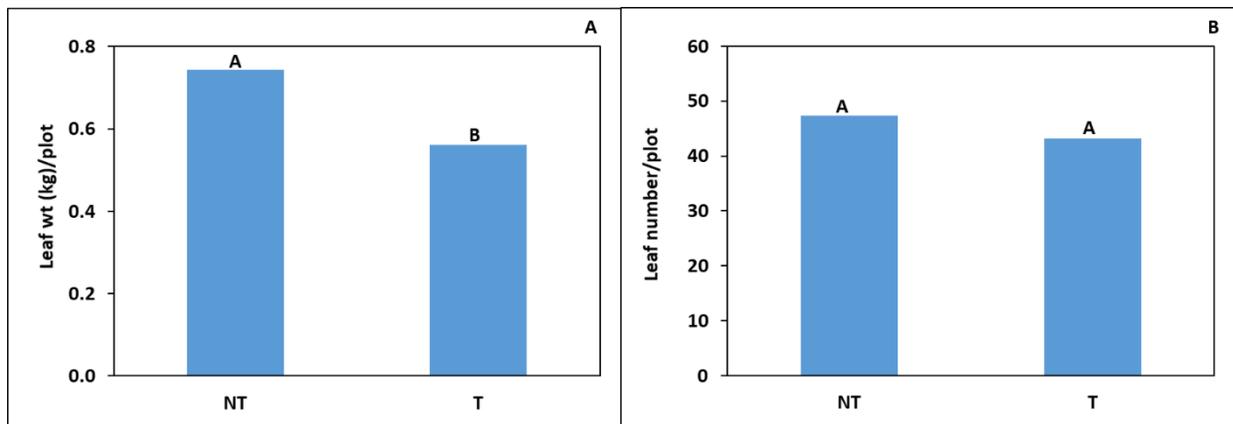


Fig.2.9. Effect of Trap crop on kale A) leaf weight and B) leaf number in kale Trial I. Means followed by the same letters are not different based on analysis of variance.

In Trial II of kale, no interaction between trap crop and EPN was observed for all parameters. However, an opposite trend from Trial I occurred. Encouragingly, one week after EPN application, EPN suppressed DBM numbers by 100% ($F_{(1, 237)} = 19.14, P \leq 0.05$) regardless of trap crop treatment (Fig. 2-10), but no effect was observed on ICW in Trial II. Trap cropping did not affect DBM abundance, DBM damage and ICW abundance. However, trap cropping reduced ICW damage ($F_{(1, 237)} = 7.83, P \leq 0.05$) by 13% (Fig. 2-11D).

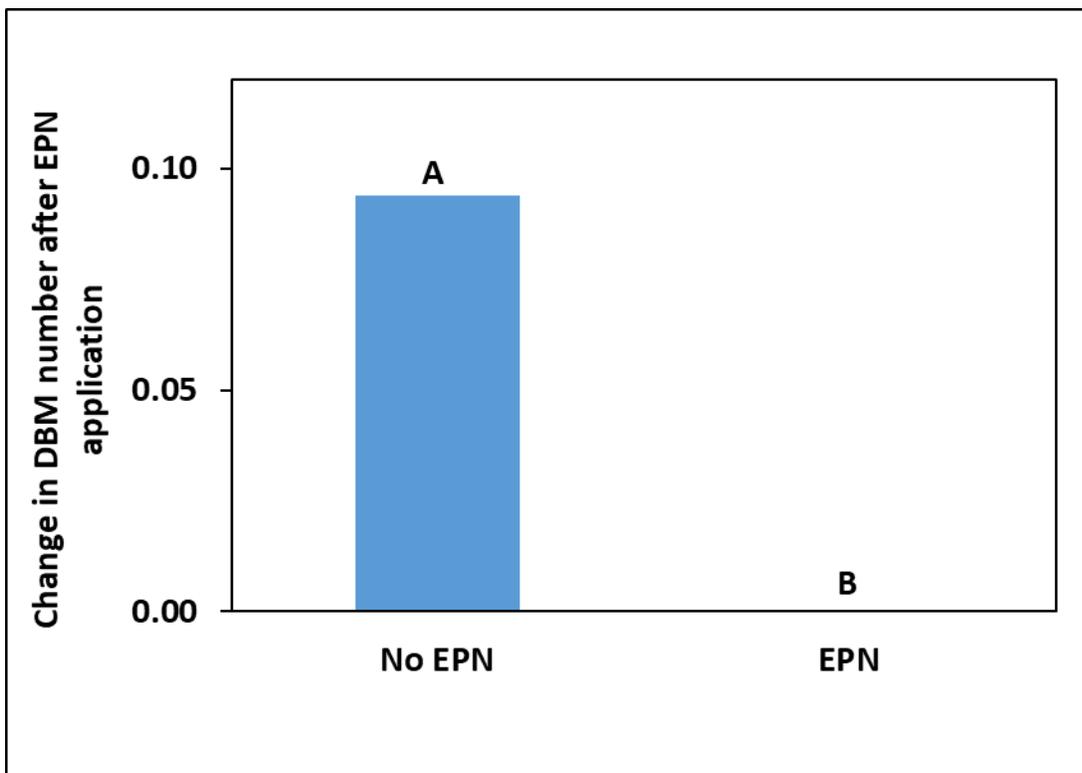


Fig.2-10. Effects of EPN on abundance of diamondback moth (DBM) in kale Trial II. Means followed by the same letters in a graph are not different based on analysis of variance.

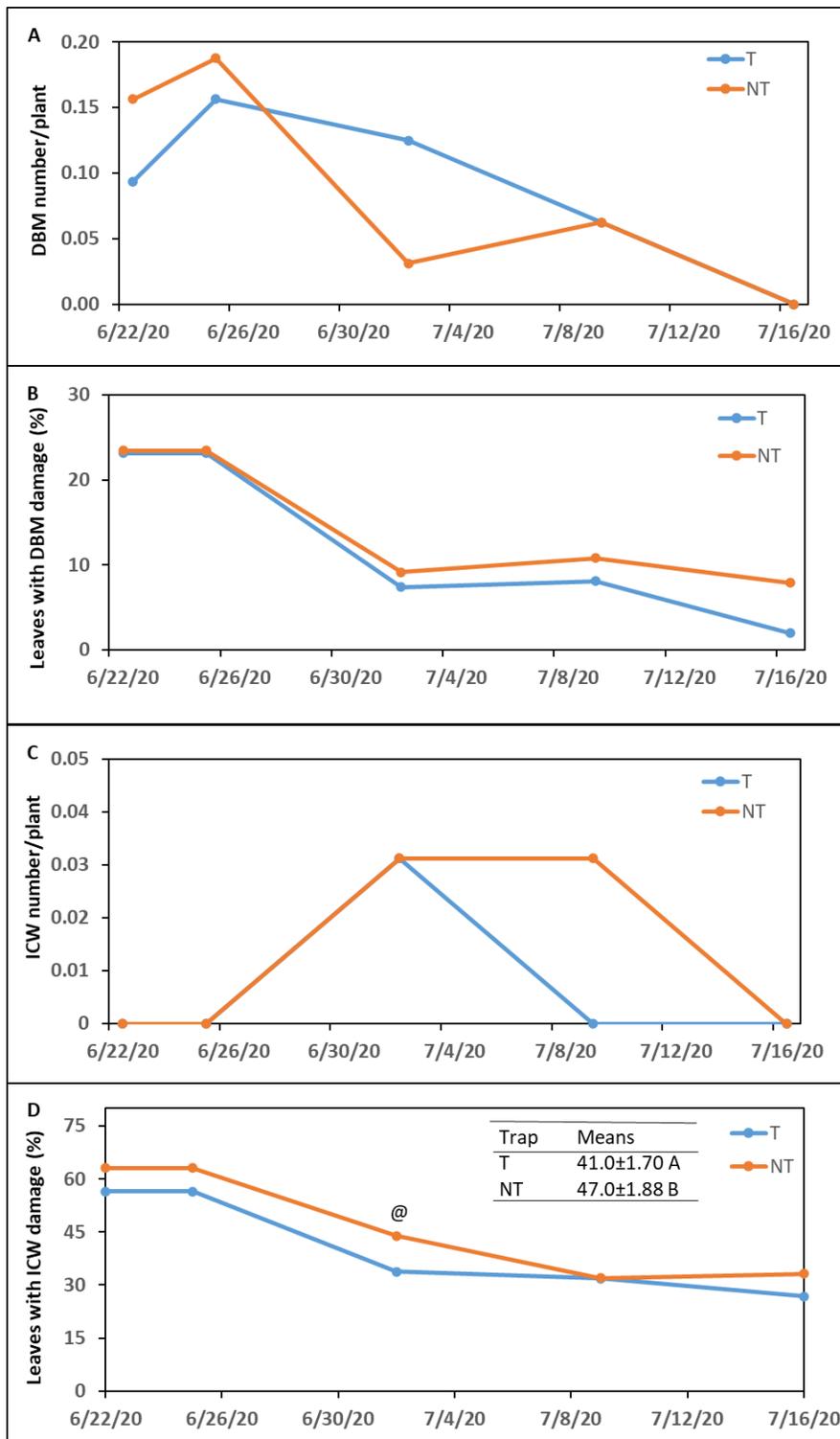


Fig.2-11. Effects of kai choi as trap crop on A) abundance of DBM B) percent leaf with DBM damage, C) abundance of ICW, and D) percent leaf with ICW damage on kale in Trial II. T = Trap crop, NT = No trap crop. Means followed by the same letter are not different based on analysis of variance. * indicates significant difference between EPN+ and EPN- on a particular date.

When comparing kale and kai choi within the trap crop plots, ICW numbers ($F_{(1, 300)} = 37.42, P \leq 0.01$) and damage ($F_{(1, 300)} = 17.10, P \leq 0.01$) were both higher on kai choi than kale (Fig. 2-12 A, C). Similarly, DBM numbers ($F_{(1, 300)} = 20.55, P \leq 0.01$) DBM damage ($F_{(1, 300)} = 11.23, P \leq 0.01$) were also higher on kai choi compared to kale (Fig. 2-12 B, D). No interaction between crop type and date was observed, thus only means of the crop type were presented.

In terms of kale yield, ANOVA showed no interaction between trap cropping and EPN, and no significant effect from EPN. Both kale weight (Fig. 2-13A) and leaf numbers (Fig. 2-13B) were not affected by trap cropping in Trial II.

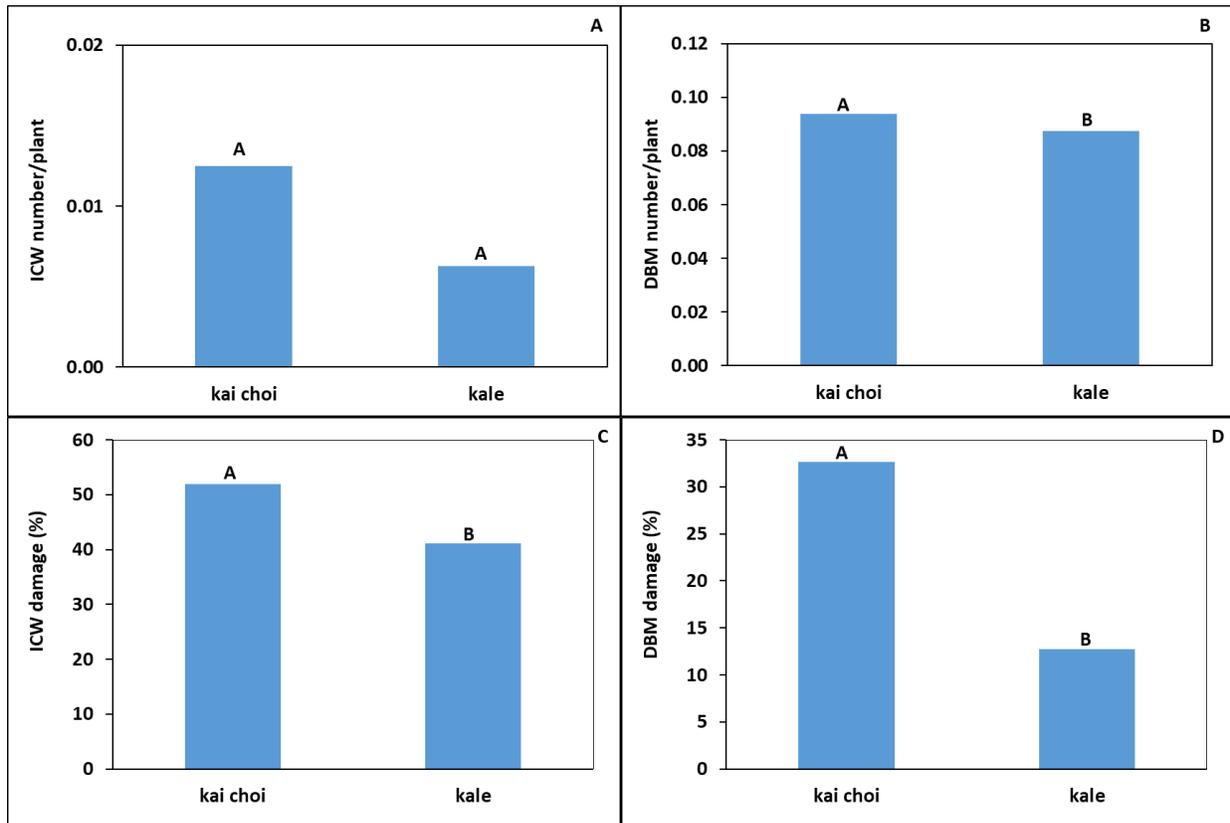


Fig.2-12. Abundance of A) ICW larvae B) DBM larvae C) ICW leaf damage D) DBM leaf damage on kai choi and kale in trial II at Waianae. Means followed by the same letters in a graph are not different based on analysis of variance.

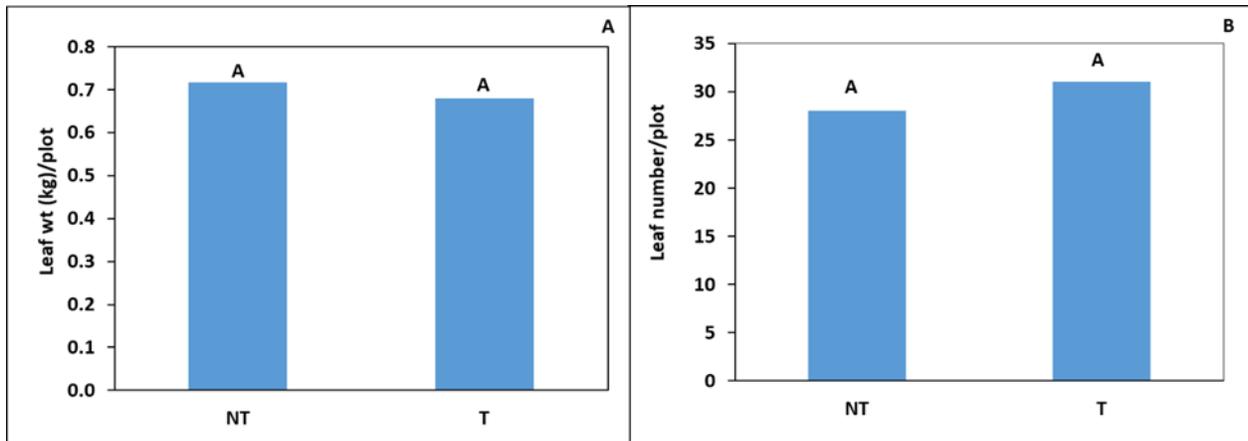


Fig.2-13. Effect of trap cropping on kale yield in Trial I and Trial II at Waianae. Means followed by the same letters in a graph are not different based on analysis of variance.

2.4 Discussion

Trap cropping: All three field trials partially supported the hypothesis that planting kai choi as a trap crop next to the head cabbage or kale would reduce the population densities and damage of DBM and ICW on both cash crops. This result is similar to that reported by Srinivasan and Moorthy (1991) where planting 15 rows of head cabbage with two border rows of mustard (*B. juncea*) reduced DBM population densities on cabbage in India. Asman et al. (2002) also reported increased DBM oviposition on cabbage surrounded by mustard compared to cabbage monoculture in the field. Similar results were also reported by Hasheela et al. (2010) where the Indian mustard planted 60 cm away from the cabbage as a border crop had lower abundance and damage of DBM on cabbage compared to cabbage planted with other non-host border crops and no border control. However, cabbage field trials in Texas (Bender et al., 1999) and Hawaii (Luther et al., 1996) showed no effect of trap crop on larval densities between monoculture cabbage and cabbage with Indian mustard as a trap crop when the distance between the mustard border and the cabbage was spanning from 180 cm and 60-120 cm in the trials conducted by Bender et al.

(1999) and Luther et al. (1996), respectively. These ambiguous results of trap cropping could be due to differences in the distance between trap crop and cash crop, or the population densities of DBM or ICW on the cash crop. There is a trend that trap crops are more effective if the population densities of DBM or ICW was ≥ 0.5 /plant, which is also the economic threshold for DBM in Hawaii (Baur et al., 1998). For example, kai choi reduced the abundance and damage of DBM and ICW on cabbage in the Waialua cabbage trial where there were 4 ICW /plant and about 0.5 DBM/plant. However, kai choi as a trap crop did not reduce the ICW population in Trial I of kale because the ICW abundance was < 0.5 /plant but did reduce DBM abundance and damage where DBM was > 0.5 /plant. In Trial II of kale, the trap crop did not suppress DBM because the average abundance of DBM was < 0.5 /plant. Even though trap crop slightly reduced ICW damage in kale Trial II where the ICW abundance was only < 0.01 /plant, the reduction was only 12.7% which was minimal. Banks and Ekbohm (1991) also concluded that the success of the trap cropping system relies on abundance of target pests in the field.

Current results also suggested that the distance between the trap crop and cash crop could affect the efficacy of the trap crop. Though the field trials conducted were not designed to determine the optimal distance between trap crop and cash crop for kai choi to be effectively trapping DBM, it can be concluded that the distance should be at least 60 cm to avoid competition with cash crop growth. At 60 cm between kai choi and kale, DBM abundance can still be reduced by kai choi trap crop. Future study is needed to determine how far kai choi trap crop can be intercropped with kale or head cabbage beyond 60 cm and can still be effective.

Although Bender et al. (1999) and Luther et al. (1996) concluded that the use of Indian mustard as a trap crop for cabbage failed to reduce DBM abundance when planted 100-200 cm

or 60-180 cm away from cabbage, host feeding preference of DBM and ICW could also affect the efficacy of trap cropping. Charleston and Kfir (2000) found that the number of DBM larvae was the same or lower on the Indian mustard plants compared to other cruciferous crops such as cabbage, cauliflower, broccoli, and Chinese cabbage in a study conducted in South Africa. Results of the current field trials suggested that kai choi is a better trap crop in a cabbage field (reducing 45% of DBM damage and 33% of ICW damage) than a kale field (only reducing DBM damage by 19% in Trial I or ICW damage by 13% in Trial II). Differential host preferences of DBM and ICW may be a consequence of leaf wax content which could influence oviposition of these insects (Spencer, 1996). In general, glossy leaves such as kai choi have a reduced wax load (Andrahennadi and Gillott, 1998; Stoner, 1992; Eigenbrode and Shelton, 1992), which improves the adhesiveness of eggs (Uematsu and Sakanoshita, 1989), but reduces larval survival (Eigenbrode and Shelton, 1992) due to increased predation on DBM. This is because predators are known to move easily on glossy leaf surface (Eigenbrode et al., 1995). This explained why kai choi works well as a trap crop with cabbage with low glossiness. Another reason for kai choi as trap crop was not so effective in kale trials is because Manandhar et al. (2015) reported that DBM and ICW were more attractive to kale compared to the other cruciferous crop including mustard and cabbage for oviposition. However, current kale trials showed that DBM preferred to feed on kai choi than kale. Glucosinolate sinigrin concentration is another factor that increases the feeding preference and behavior of DBM (Robin et al., 2017). Therefore, more DBM damage was observed to be more on kai choi (with higher sinigrin) than head cabbage and kale.

As the DBM larvae are more prone to predation on leaves with high glossiness (e.g. kai choi) suggested by Eigenbrode et al. (1995), it explained why less DBM abundance was observed

on kai choi (higher glossiness) compared to kale and cabbage. Among these three crops, head cabbage has the least glossiness, thus kai choi works better as a trap crop in cabbage field than in a kale field.

Despite a positive result of using kai choi as a trap crop to reduce DBM and ICW damage on the cash crop when population densities of these pests are higher than 0.5/plant, planting of trap crop could exhibit a resource competition effect on cash crop yield depending on the planting distance between the trap crop and the cash crop. Up to 41% yield loss of head cabbage was found when seedlings were planted 15 cm away from the kai choi, and 24% of kale yield loss was observed in Trial I of kale when seedlings were planted 30 cm from the kai choi. However, no yield loss was found in Trial II of kale when seedlings were planted 66 cm from the kai choi in addition to the reduction of ICW damage on kale. Hasheela et al. (2010) reported the highest marketable yield of cabbage when it was planted 60 cm away from the mustard row. Hence, more research is needed to determine the fine line between planting distance to avoid competition while functioning effectively as a trap crop.

Effect of EPN: All three field trials partially supported the hypothesis that foliar application of EPNs can suppress ICW and DBM. In the current project, *S. feltiae* applied once at 1000 IJs/plant, which is equivalent to the commercial recommended rate of 125 million IJ/ha, suppressed abundance of ICW in no trap crop plots whereas it only reduced DBM abundance in the trap crop plots for the cabbage. Amazingly, EPN suppressed 100% of DBM in kale Trial II at 1 week after EPN application. Baur et al. (1995) reported effective control of DBM in a watercress field when they applied high dosage of *S. carpocapsae* at 5 billion IJs/ha as foliar spray and achieved 41% control of DBM larvae. Suppression of *S. feltiae* in kale Trial II was surprisingly

impressive, achieving 100% reduction of DBM larvae. One of the reasons for high efficacy of EPN in this trial could be due to low abundance of DBM in kale Trial II (0.15 DBM/plant). On the other hand, lack of effect of *S. feltiae* against ICW in kale Trial II is simply due to lack of ICW visits (0.01/plant) to the kale.

Current application rate of *S. feltiae* in the field trials here is 125 million IJs/ha which is a commonly recommended rate in commercial EPN product. Failure of *S. feltiae* to suppress DBM when DBM population was higher in the Waialua, and Kale Trial I could be resolved by better protection of EPN using different adjuvants. Current research used Oroboost® (alcohol ethoxylate), a registered material for use in organic agriculture. Acar and Sipes (2019) reported the importance of using UV protectant and anti-desiccant to improve the efficacy of *S. feltiae*. Future research should examine other formulation of adjuvant compatible for organic food crop production to improve the EPN performance against DBM.

Overall, this study demonstrated that the EPN foliar application did not provide consistent suppression against DBM on cabbage or kale. Nonetheless, EPN could still be a viable tool to be added into the pesticide rotation program for organic farmers because it is less likely for DBM to develop resistance against EPN. EPN foliar application at 125 million IJs/ha is effective against ICW when population pressure is below 0.5/plant. It is vital to maintain DBM population below 0.5/plant for cabbage and kale to be economically profitable. Organic farmers could achieve this by rotating *Bt*, spinosad, *B. bassiana* if insecticide resistant DBM population was not present in their field. Having EPN in the pesticide rotation program would reduce the frequency of *Bt* and spinosad application which will improve the DBM management program. It is disappointing that integration of trap crop with EPN did not improve the suppression against DBM and ICW.

However, planting kai choi as a trap crop provide a consistent reduction of damage from both DBM and ICW when the seedlings are planted at least 60 cm away from the cash crop to avoid plant growth competition. This study showed that kai choi as a trap crop works better for cabbage than for kale. Future research should explore more effective trap crops for kale against DBM and ICW.

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**CHAPTER 3: Integration of Intermittent Sprinkler Irrigation with Foliar Spray of
Entomopathogenic Nematodes to manage Diamondback moth and Imported Cabbageworm
in a Head cabbage Agroecosystem**

3.1 Introduction

Head cabbage (*Brassica oleracea* var. *capitata*) is an important vegetable crop in the US with an estimated production of 900 million kg in 2019 (USDA ERS, 2019). Cabbage production throughout the US is plagued by several common pests including diamondback moth (DBM), imported cabbageworm (ICW) (*Pieris rapae*), cabbage webworm (*Hellula rogatalis*), and cabbage looper (*Trichoplusia ni* (Hübner) (Mau et al., 2001). Among these pests, DBM and ICW are considered the most damaging pest on Brassicaceous crops (Wallingford, et al., 2012). DBM causes up to 90% loss (Talekar and Shelton, 1993; Kfir, 1998; Amoabeng *et al.*, 2013) and ICW feeding results in about 71% crop loss (Maltais et al., 1998) . DBM affects cabbage yield by directly feeding on the leaves, reducing cabbage marketability, and contaminating the produce (Bopape, 2013). ICW causes irregular holes on the leaves (Capinera, 2008; Hutchison et al., 2009), and under severe infestations, skeletonizes the leaves. DBM is distributed worldwide where cruciferous crops are planted. DBM has a fast-colonizing capacity as a result of its short life cycle where the first to fourth instar can develop in 19 days at 23 °C (Guilloux et al., 2013). Additionally, it can reach up to 10 generations per year in temperate regions (Bahar et al., 2013) and 15-17 in tropical regions like Hawaii (Mau and Gusukuma-Minto, 2001). The sparse rainfall during summer in Hawaii is more conducive to DBM and ICW infestation. Although some natural enemies such as *Cotesia glomerata* and *Pteromalus puparum* for ICW, and *Diadegma insularis* and *Microptilis plutellae* for DBM are present (Capinera.,2008), insecticides are still needed to manage ICW and

DBM populations below the economic threshold. However, the overuse of insecticides has caused the development of insecticides resistant DBM (Shelton et al., 1993; Tabashnik et al., 1990; Tabashnik, 1994) against a broad spectrum of insecticides including *Bacillus thuringiensis* (*Bt*) in different parts of the world including Hawaii. Hence, alternative pest control strategies including application of biocontrol agents are being investigated (Mahar et al., 2004).

Another strategy to manage DBM and ICW being suggested is the use of overhead sprinkler irrigation. The mode of action of using overhead sprinklers to reduce insect numbers is like a simulation rainfall on a crop (Davis et al., 1995; Gameel, 1977; Kaakeh and Dutcher, 1993). Intermittent overhead irrigation has been shown to reduce thrips infestation on cotton (Leigh, 1995), lettuce (Palumbo et al., 2004), and onions (Hoffman et al., 1996). In addition, whiteflies on melons (Castle et al., 1996) and *Drosophilla* on blueberry (Rendon and Walton, 2019) have also been suppressed by intermittent overhead irrigation. However, in some cases, excess water or additional irrigation can be detrimental to insects. For example, irrigation can delay development and affect the survival of cotton bollworm, *Helicoverpa*, pupae in the soil (Yu et al., 2008). Sprinkler irrigation has also been reported to suppress the DBM population on multiple cruciferous crops. For example, Tabashnik et al. (1986) reported that overhead sprinkler irrigation for 5-min in 30-min intervals throughout the day and night reduced DBM oviposition rates by 7-fold on watercress compared to intermittent sprinkler irrigation only in the daytime in a cage experiment. Nakahara et al. (1985) further found that an overhead sprinkler irrigation in a watercress field reduced DBM population from 156 DBM larvae and 23 adults to no DBM when the irrigation was discharged for 5-min at 30-min intervals from 8:00 am - 10:00 pm. Unlike watercress that is cultivated in natural spring water or wet land system, it might not be practical

for cabbage farmers to use intermittent sprinkler irrigation in dry land all day long because of the high irrigation cost and probable increase of diseases such as black rot and downy mildew. It is encouraging that Talekar et al. (1986) reported a reduction in DBM infestation and an increase in head cabbage yield when sprinkler irrigation was only applied for 5 min once at dusk every 2 days for the first 3-4 weeks after planting and every day thereafter. Intermittent sprinkler irrigation might also contribute other benefits such as washing DBM larvae off the foliage and creating a wet leaf surface that can reduce the feeding intensities of DBM (Talekar et al., 1986).

Besides, as a component of integrated pest management, intermittent sprinkler irrigation can also provide an additive effect in controlling DBM when combining with the use of biocontrol agents. Entomopathogenic nematodes (EPNs) have been widely studied as biocontrol agents against DBM but with variable efficacy in the field (Nyasani et al., 2008). However, the success of EPN foliar application in controlling insects in the field is limited (Georgis et al., 2006). Arthurs et al. (2004) reported that the efficacy of EPNs is dependent on the habitats which are more effective when insects are residing in cryptic foliage than in exposed foliage. EPNs are also more effective in the laboratory than in the field. The exposure of EPN on foliage to extreme temperature (Kaya, 1977; Molyneux, 1985; Grewal et al., 1994), ultraviolet radiation (Gaugler and Boush, 1978; Gaugler et al., 1992), and rapid fluctuation in moisture (Simons and Poinar, 1973; Baur et al., 1995) causes low survival rates of EPNs. Kaya et al. (1984) reported highest infection rates of codling moth larvae with the application of *S. carpocapsae* when temperatures were just below optimal, and rain was common. Retention of moisture on the leaf surface is important to facilitate EPN movement, infection and to prevent abiotic stress on the nematodes. Knight (1998) demonstrated that overhead sprinkler irrigation reduced 60-90% of the injury

caused by codling moths on apple trees as well as providing a conducive environment for the EPNs to infect the larvae on the foliage.

Therefore, this project aimed to explore the potential benefits of day and dusk intermittent sprinkler irrigation in combination with the use of EPN as a biocontrol agent against DBM and ICW on the head cabbage. It is hypothesized that daytime intermittent sprinkler irrigation not only would disrupt the flying and feeding of ICW but would also reduce DBM feeding on the wet leaf surface. It was also hypothesized that overhead sprinkler irrigation could increase leaf moisture and enhance the longevity and survival of EPNs. Specific objectives of this study were to 1) compare day and dusk intermittent sprinkler irrigation vs dusk only intermittent sprinkler irrigation, and 2) evaluate the potential of intermittent sprinkler irrigation to enhance the infectivity of EPNs against DBM and ICW.

3.2 Materials and Methods

Two field trials were conducted at Poamoho Experiment Station (21°32'42.2" N 158°05'18.6" W), University of Hawaii at Manoa, Waialua, Oahu. The first trial (Trial I) was conducted from September 3 to Oct 15, 2020, to evaluate the effect of intermittent sprinkler irrigation and EPN foliar spray on head cabbage against DBM and ICW. The experiment was arranged in a 3 × 2 (sprinkler irrigation regime × EPN +/-) factorial split plot design with 4 replications. The three main plots of intermittent sprinkler irrigation regimes were: 1) 5-min sprinkler irrigation twice at dusk (6:00 and 8:00 pm) (S_d), 2) 5-min sprinkler irrigation from 8:00 am to 4:00 pm at 2-hour intervals and twice at dusk (6:00 pm and 8:00 pm) (S_{Dd}), and 3) no sprinkler irrigation (NS). Each main plot was split into two subplots of foliar EPN application (EPN+) or no EPN application (EPN-). All plants were drip irrigated as the main form of irrigation. For treatments receiving

intermittent sprinkler irrigation, micro-sprinkler heads assembled with 60-cm tall micro tubing with a barb connector to a 1.27-cm-d poly tubing system were installed. Six-week-old 'KK cross' head cabbage seedlings were transplanted into the field at 60-cm spacing between the plants within a row. 'Caliente 199' brown mustard (*Brassica juncea*) was transplanted 45 cm from the head cabbage row with 60 cm between plants at the border of the head cabbage to serve as a trap crop for DBM and ICW. Brown mustard was planted 1 week before head cabbage planting and was replaced with 4-week-old brown mustard seedlings 3 weeks after cabbage planting to avoid growth competition with the head cabbage. Each plot was 3.6×0.9 m². Twenty-four plots, each containing 6 cabbage plants and 12 brown mustard plants, were established.

Steinernema feltiae MG-14 isolated from Waikapu Maui and reared on *Tenebrio molitor* larvae in the laboratory were used in the EPN+ plots. Water suspension containing *S. feltiae* MG-14 was mixed with 1.6 ml of Oroboost® /L water (Oro Agri, Inc., Fresno, CA) and was foliar sprayed at 125 million IJs/ha at 2, 4, and 6 weeks after cabbage planting.

Every week, 3 cabbage plants were randomly selected and 1) DBM damage, 2) ICW damage, 3) number of eggs, larvae, pupae, and adult stages of DBM and ICW were counted and recorded for each plant. Data were collected over 7 weeks. The level of DBM or ICW damage was based on a scale of 0-4 where 0 = no damage, 1 = 1–10% damage, 2 = 11–25% damage, 3 = 26–50% damage, and 4 = 51–100% damage (Fig. 3- 1). Cabbage was harvested over 3 weeks as a solid head was formed.

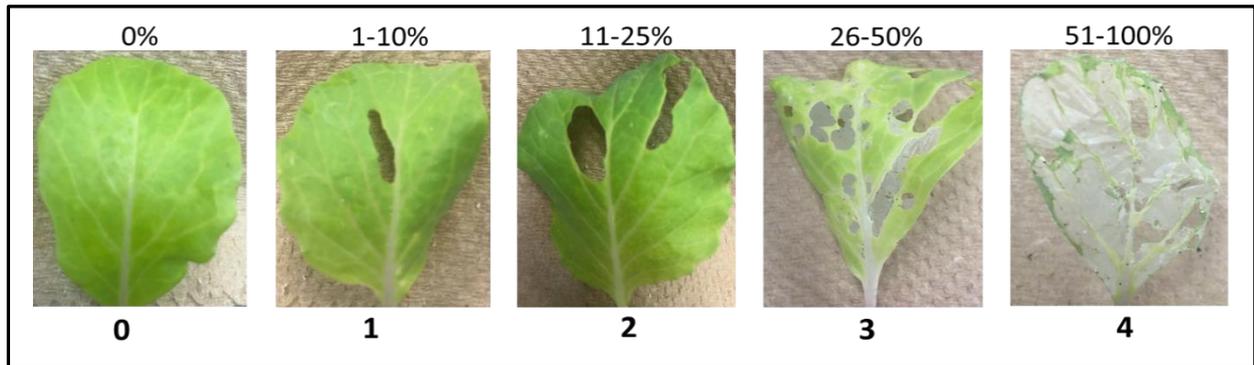


Fig.3-1. Damage index in a scale of 0-4 cause by diamondback moth or imported cabbageworm damage on a cabbage leaf.

A second field trial (Trial II) was conducted from December 3, 2020, to January 13, 2021, in the same field as Trial I with a slight modification. Sprinkler irrigation timing was reduced to 1 min at 2-hour intervals for all the irrigation treatments instead of 5 min. Instead of ‘Calientee 199’ brown mustard used in Trial I, ‘Hirayama’ kaichoi (*B. juncea*) was planted as the border trap crop surrounding the head cabbage in Trial II. Kai choi and cabbage were planted at the same time. While row spacing between head cabbage and the trap crop remained the same, the number of head cabbage plants within each plot was increased to 12 plants/plot. Same data were collected as described for Trial I except that data were collected over 6 weeks.

Statistical analysis: Data from each field trial were checked for normality using Proc Univariate in Statistical Analytical Software (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA). Insect counts were normalized using $\log_{10}(x + 1)$ whenever needed before analysis of variance (ANOVA). The insect count and damage data in each trial were subjected to repeated measures ANOVA using Proc GLM in SAS with sampling dates blocked within treatments. If no significant interaction between treatment and sampling date across sampling dates was found, data were

pooled for ANOVA. If significant interaction occurred, data were analyzed by date. In both cases, a 3×2 (Sprinkler irrigation × EPN) factorial ANOVA was performed. Means were separated using the Waller-Duncan k -ratio ($k = 100$) t -test whenever appropriate, but only the true means were presented. Similarly, cabbage yield data was subjected to 3×2 factorial ANOVA and Waller-Duncan k -ratio ($k=100$) t -test wherever appropriate.

3.3 Results

Intermittent sprinkler irrigation (ISI): Based on the 3×2 ANOVA of the repeated measure of DBM and ICW abundance and damage, no interaction between sprinkler irrigation and EPN, and between treatment (sprinkler irrigation and EPN combination) and sampling date were observed in both trials (Appendix A). Thus, only means of each main factor (sprinkler irrigation or EPN) were presented. In Trial I, S_{Dd} and S_d decreased DBM damage ($F_{(2, 459)} = 3.32, P \leq 0.05$) on head cabbage by 19% compared to the no sprinkler irrigation control (Fig 3-2A). However, in Trial II, both ISI treatment and EPN did not reduce DBM damage (Fig 3-2B). In terms of ICW, S_{Dd} reduced ICW damage ($F_{(2, 459)} = 3.97, P \leq 0.05$) by 88% in Trial I (Fig. 3-2C) and 45% in Trial II ($F_{(2, 390)} = 4.21, P \leq 0.05$) on the head cabbage (Fig. 3-2D).

In terms of insect abundance, S_{Dd} reduced ICW abundance ($F_{(2, 459)} = 7.27, P \leq 0.05$) on head cabbage by 86% compared to the NS control in Trial I (Fig. 3-3A) and by 58% ($F_{(2, 390)} = 8.80, P \leq 0.05$) in Trial II (Fig. 3-3B). S_{Dd} also reduced DBM population on head cabbage by 22% in Trial I and 52% in Trial II, but the effect was not significant ($P > 0.05$, data not shown). On the other hand, S_d did not suppress ICW and DBM population densities in both trials ($P > 0.05$).

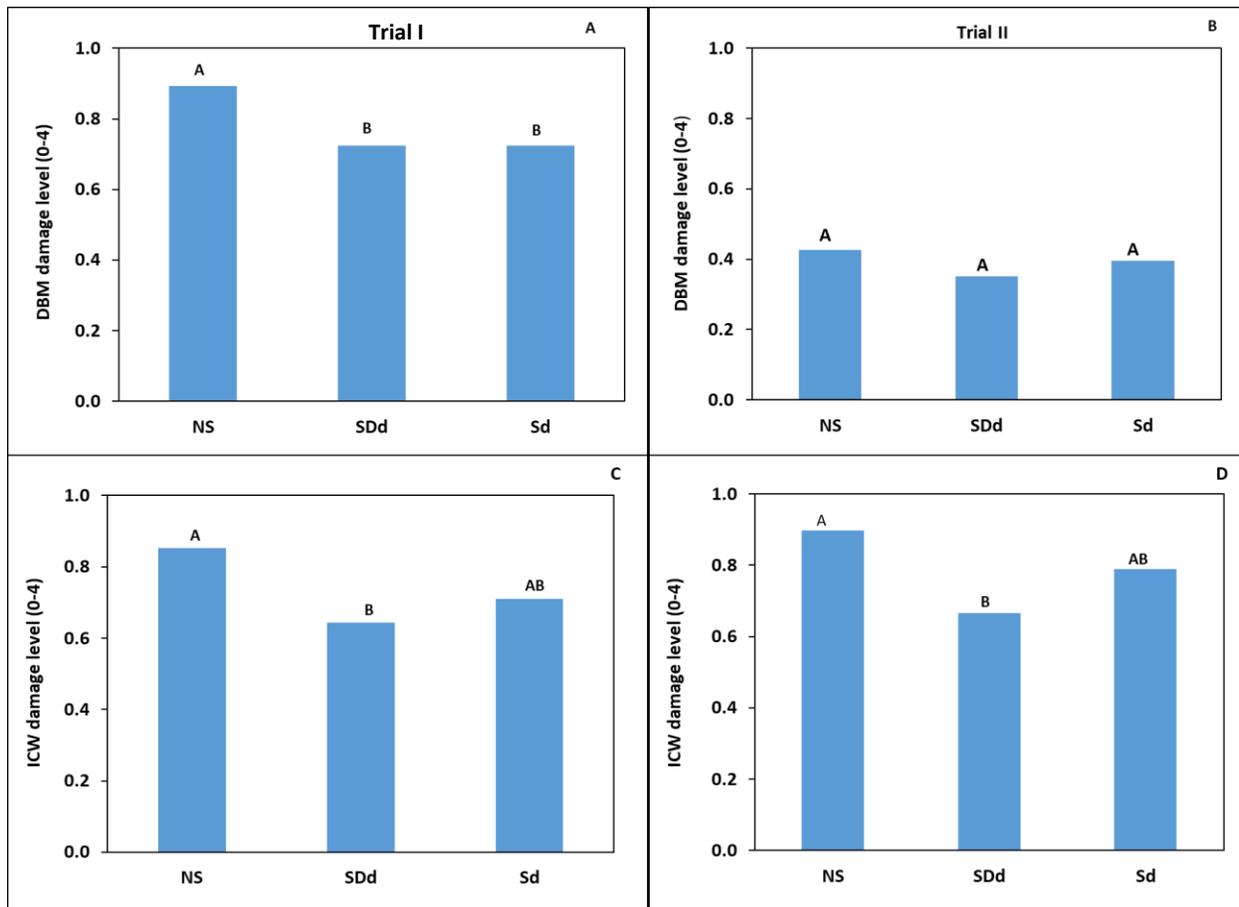


Fig.3-2. Effects of sprinkler irrigation at dusk (S_d) and day and dusk (S_{Dd}) compared to no sprinkle irrigation (NS) on diamondback moth (DBM) damage and imported cabbageworm (ICW) damage on head cabbage in Trial I (A, B) and Trial II (C, D). Means followed by the same letter(s) were not different according to the Waller-Duncan k -ratio ($k=100$) t -test. Damage level 1 = 1-10%, 2 = 11-25%, 3 = 26-50% and 4 = 51-100% leaf damage.

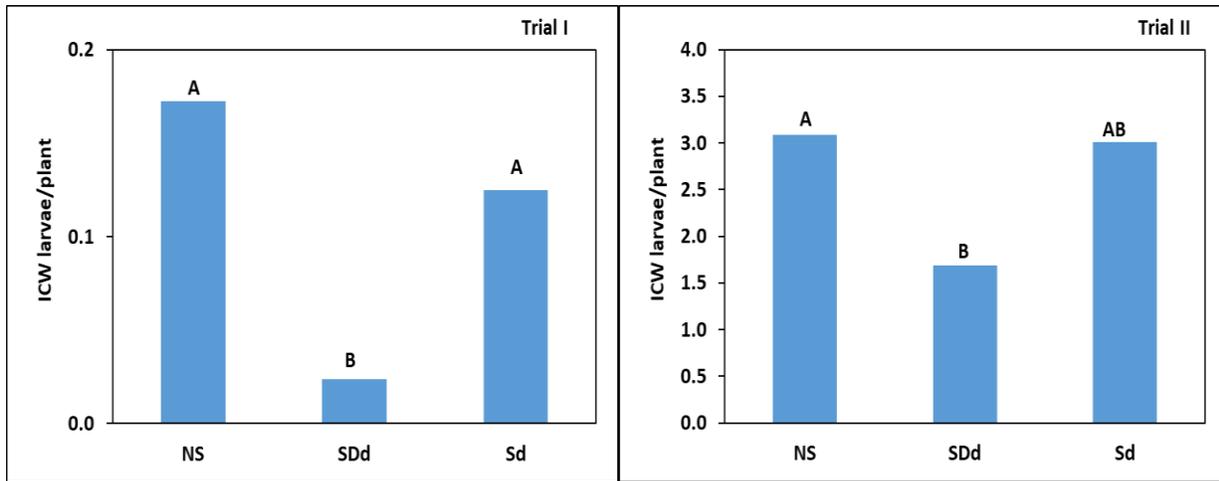


Fig.3-3. Effects of sprinkler irrigation at dusk (S_d), day and dusk (S_{Dd}) compared to no sprinkle irrigation (NS) on abundance of Imported cabbageworm in Trial I (A) and Trial II (B). Means followed by the same letter(s) were not different according to the Waller-Duncan k -ratio ($k=100$) t -test.

Overall, in Trial I, S_{Dd} increased the marketable head cabbage weight by 44% ($F_{(1, 121)} = 3.17, P \leq 0.05$) compared to NS and S_d increased cabbage weight by 25% though not significantly different from NS (Fig. 3-4A). In Trial II, head cabbage weight was not different among the irrigation treatments ($P > 0.05$) (Fig. 3-4B). In both trials, EPN foliar application did not suppress DBM or ICW larvae nor their damage to head cabbage (data not shown). No interaction between sprinkler irrigation and EPN was observed for the head cabbage yield.

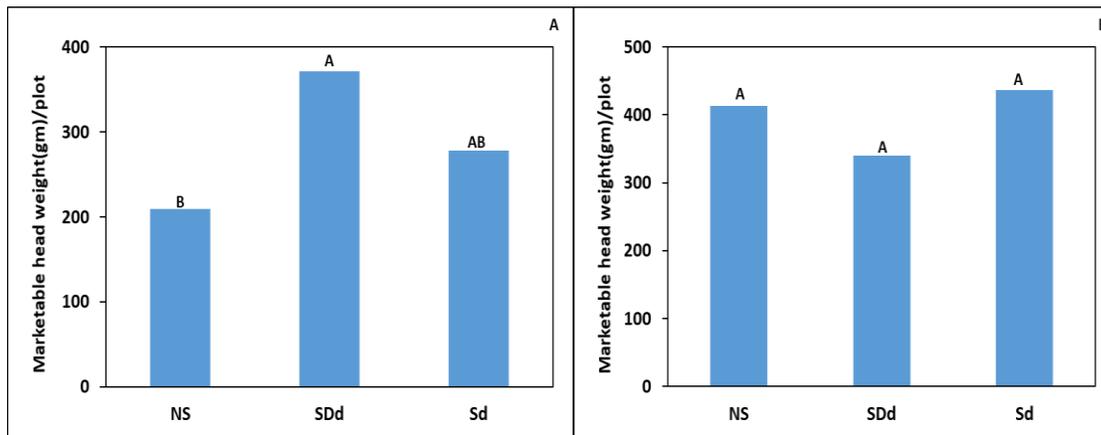


Fig.3-4. Effects of sprinkler irrigation at dusk (S_d), day and dusk (S_{Dd}) compared to no sprinkle irrigation (NS) on head weight of cabbage per plant in Trial I and Trial II. Means followed by same letter(s) were not different according to Waller-Duncan k -ratio ($k=100$) t -test.

3.4 Discussion

Intermittent sprinkler irrigation effects: Results from both field trials supported the hypothesis that intermittent sprinkler irrigation at dusk and during the day can reduce DBM and ICW damage on head cabbage compared to no sprinkler irrigation. Intermittent sprinkler irrigation only at dusk reduced DBM damage in Trial I but not in Trial II. Failure of sprinkler irrigation at day and dusk to reduce damage from DBM in Trial II could be due to the reduction of intermittent sprinkler irrigation duration from 5 min to 1 min. The reason for cutting back intermittent sprinkler irrigation from 5 min to 1 min in Trial II was due to massive algae growth on the alleyways. The purpose of using intermittent sprinkler irrigation for DBM damage was mainly to disrupt DBM oviposition which was supposed to occur at dusk (Harcourt, 1957). One explanation of intermittent sprinkler irrigation during the day and at dusk to be effective against DBM damage in Trial I could be that intermittent sprinkler irrigation can wash DBM larvae off the plants, thus disrupting DBM feeding during the day and at dusk. Talekar et al. (1986) reported that a wet leaf surface is unfavorable for DBM larvae feeding. In addition, dusk irrigation would not be sufficient because ICW is more active in the daytime, and DBM and ICW often coexist in the same field. Further research is needed to determine if intermittent sprinkler irrigation all night long would further reduce DBM damage, but night irrigation might have bad implications for farmers due to the extensive irrigation inputs and the repercussions of creating an environment conducive to plant diseases.

While previous studies in intermittent sprinkler irrigation have focused on insect count data, this is the first investigation on the use of intermittent sprinkler irrigation against ICW and DBM damage on cabbage. The result supported the hypothesis that S_{Dd} would interfere with the

damage of ICW on cabbage. ICWs are more active during the daytime, hence daytime irrigation plays an important role in reducing the damage caused by ICW. Maurico et al. (1990) reported that ICW larvae are mostly feeding during the day, therefore maintenance of wet leaf moisture all day long deterred the feeding of ICW larvae. The results here also showed that intermittent sprinkler at dusk could not further decrease ICW feeding damage in both trials as ICW is not actively feeding at dusk.

The results supported the hypothesis that S_{Dd} could suppress ICW population effectively on head cabbage, but it did not reduce DBM abundance. Intermittent sprinkler irrigation during the daytime (S_{Dd}) might have disrupted the oviposition and mating of ICW on head cabbage which cannot be achieved by S_d . Although Talekar et al. (1986) reported that 5-min intermittent sprinkler irrigation during the dusk once in every 2 days for the second and third week after transplanting and every day thereafter significantly reduced DBM population on the head cabbage, results from the current study showed minimal to no effect of S_{Dd} and S_d in reducing the abundance of DBM on the cabbage. Interestingly, S_{Dd} performed slightly better against DBM abundance than S_d although not significantly different. This could be because S_{Dd} disrupted the feeding environment of DBM which might have increased time to maturity, thus resulting in slightly lower DBM abundance. McHugh and Foster (1995) also did not find significant intermittent sprinkler irrigation effects on DBM abundance when examining intermittent sprinkler irrigation (at 30-min durations and 30-min intervals) at different times at night on head cabbage. They found 85.9% reduction of DBM infestation when applying intermittent sprinkler irrigation from 8:00 pm to 11:30 pm compared to a 53.7% reduction when applying intermittent sprinkler irrigation from 3:00 to 5:00 pm. Tabasnik and Mau (1986) also reported that DBM

oviposition was most active between 5:00 pm to 11: 00 pm reaching a peak between 8:00 -11:00 pm and decline thereafter. More research can be conducted to fine-tune the duration, intervals, and timing of intermittent sprinkler irrigation for best DBM suppression on head cabbage. Intermittent sprinkler irrigation all day long in watercress wetland system had been widely adopted for various insect pest management (Nakahara et al., 1985). Fine-tuning intermittent sprinkler irrigation on cabbage could possibly be economical if DBM could be managed effectively.

EPN effects: EPN application did not reduce ICW or DBM numbers and damage in both trials. This is inconsistent with Unruh and Lacey (2001) who suggested the wet leaf surface before and after application of *S. carpocapsae* could result in 100% mortality of codling moths. In addition, there was no interaction between intermittent sprinkler irrigation and EPN treatments on all the parameters measured.

The dose of EPN used in this trial (125 million IJ/ha) is 8-fold lower than the 2.5 billion IJs/ha rate recommended by Shapiro et al. (2006) for using commercial EPN. However, studies done by Mohammad et al. (2012) reported that 16 IJs/10 cm² were effective in killing ICW larvae in a petri dish test, which is equivalent to 160 mil IJs/ha. The concentration used in the current study at 125 mil IJs/ha was equivalent to 10 IJ/10 cm². Thus, we can conclude that intermittent sprinkler irrigation did not improve EPN foliar spray efficacy in this experiment. Future research could explore different sprinkling pressure from sprinkler nozzles on EPN spray.

Effect on head cabbage yield: Parallel to the reduction of ICW and DBM damage by S_{Dd} on head cabbage, an increase in the head cabbage yield was observed for S_{Dd} in Trial I but not in Trial II. Differential effects between Trial I and Trial II could be due to the amount of sprinkler irrigation

outputs from 5 min to 1 min. Much higher population densities of ICW with 3/plant in the NS control in Trial II compared to ICW < 1/plant in Trial I could be another reason for the lack of yield improvement by intermittent sprinkler irrigation in Trial II.

This study revealed some promising results in using intermittent sprinkler irrigation against ICW that led to cabbage yield increase by 41% in one of the two trials. Further research is still needed to fine-tune the best intermittent sprinkler irrigation practice that is more economical for head cabbage production. An ideal situation would be finding the best timing, sprinkling duration, and frequency during the nighttime beyond dusk for DBM control much like the work done by McHugh and Foster (1995) but with monitoring of DBM damage. Effective intermittent sprinkler irrigation against ICW demonstrated in this project would reduce the need of intensive insecticide spray while mitigating pesticide resistance problem imposed by the need to manage ICW chemically.

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CHAPTER 4: Determine Factors Influencing Foliar Spray Efficacy of *Steinernema feltiae* against Diamondback Moth

4.1. Introduction

Diamondback moth (DBM) (*Plutella xylostella* L., Lepidoptera: Plutellidae) is one of the most serious pests of cruciferous crops and causes economic losses of \$4-5 billion annually worldwide (Zaluki et al., 2012). Glucosinolates found in cruciferous crops are egg-laying stimulants and feeding attractants of DBM and ICW (Nayar and Thorsteinson, 1963; Hillyer and Thorsteinson, 1971). Although other caterpillar pests occur on cruciferous crops in Hawaii, DBM is the main pest of concern due to its propensity to develop resistance to several insecticides including organophosphate, carbamate, pyrethroid, organochlorine (Tabashnik et al., 1987), *Bacillus thuringiensis* (Bt) and, spinosad (Mau and Gusukuma-Minuto, 2001). Hence, alternative pest control tactics including improvement of the efficacy of biocontrol agents are urgently needed (Mahar et al., 2004).

Entomopathogenic nematodes (EPNs) have been used as biological control agents for a great variety of insect pests (Georgis et al., 2006; Lacey and Georgis, 2012). However, the application of EPN against insects has varying levels of success largely depending on the target insect's life stages and habitat, the EPN species used, the EPN dose, and the foraging strategies of EPN. The application method and environmental conditions at the time of application also affect the efficacy of insect control (Kaya, 1990; Shapiro-Ilan et al., 2000). *Steinernema* spp. has been reported to be more effective than *Heterorhabditis* spp. against DBM (Belair et al., 2003; Maher et al., 2004; Nyasani et al., 2008; Ratansinghe and Hague, 1995; Somvanshi et al., 2006). Baur et al. (1995) reported more than 95% mortality of 4th stage DBM larvae with *Steinernema*

carpocapsae (Weiser), whereas *Heterorhabditis* spp caused below 77% mortality when applied at 100 IJs/DBM larvae in both greenhouse and laboratory condition. *Steinernema carpocapsae* occurs naturally near the soil surface (Kaya, 1990; Campbell and Gaugler, 1993) and has been reported to be able to tolerate desiccation better than other species (Simons and Poinar, 1973; Glazer and Navon, 1990; Kung et al., 1991; Koppenhofer et al., 1995). However, Shinde and Singh (2000) reported the lowest LD₅₀ with *H. bacteriophora* compared to other *Steinernema* spp. tested against DBM.

Environmental conditions also affect the efficacy of EPNs. Arthurs et al. (2004) reported the efficacy of EPNs in targeted habitats to be more effective in insect bored holes and cryptic foliage but less effective on exposed foliage. Foliar applications fail because EPNs are more adapted to the soil environment and are susceptible to UV radiation (Gaugler et al., 1992), temperature extremes (Molyneux, 1985; Grewal et al., 1994), and desiccation (Womersley, 1990). Gumus et al. (2015) reported 86% control of goat moth (*Cossus cossus*, Lepidoptera: Cossidae) with EPN when EPN were inoculated by releasing live EPN-preinoculated greater wax moth, *Galleria mellonella*, larvae in chestnut logs compared to foliar spray on the logs using the same dose of EPN. Adjuvants are also found to enhance the efficacy of EPNs. Schröder et al. (2005b) screened several adjuvants such as alginate, carboxymethylcellulose, guar gum, arabic gum, xanthan gum and found that xanthan gum added to EPN increased DBM mortality. Chemicals such as PABA (para-amino benzoic acid), OMC (octyl methoxycinnamate) which are UV protectants, along with a fire protectant Barricade® gel were tested as adjuvants in an EPN suspension and were shown to increase the survival of *S. feltiae* exposed to UV light in a laboratory trial (Acar and Sipes, 2019). However, adjuvants such as Agral, Triton X100, Triton

X155, or Tween 60 mixed with *S. carpocapsae* did not significantly increase DBM control in water cress (Baur et al., 1997). EPN strains vary in susceptibility to different adjuvants, so all adjuvants may not be equally good for each nematode strain. Therefore, it is essential to test the toxicity of the adjuvants and to assess their optimal dosages for the nematode to be used in biocontrol programs.

Generally, a high dose of EPNs is reported to lead to high mortality of DBM larvae in the laboratory (Baur et al., 1995; Tolera et al., 2016). Field application of *S. carpocapsae* resulted in more than 50% control of DBM at 0.5 million IJs/m² in a formulation containing 0.3% Xanthan gum and 0.3% Rimulgan as surfactants in a cabbage field trial (Schroer et al., 2005a). Similarly, Somvanshi et al. (2006) found 35-46% mortality of DBM on cabbage with foliar spray of 1,000 - 3,000 IJs/ml where the EPN were in a solution with 0.033% APSA80. All these studies were testing extremely high dosage of EPNs against DBM. Commercial production of EPNs since the 1980s has made the availability of mass amounts of EPNs feasible. *Steinernema feltiae* (Filipjev), *S. carpocapsae* (Weiser), and *Heterorhabditis bacteriophora* (Poinar) are some of the commonly used commercial EPNs for IPM programs (Wraight et al., 2017). However, in a recent review on the successful use of EPNs in sustainable food crop production in the U.S., DBM control by commercial EPNs was not listed (Koppenhöfer et al., 2020). This suggested more research on how to improve the efficacy of EPN against DBM is needed.

Commercial EPNs remain restricted in Hawaii, making the use of EPNs in Hawaii limited to *in vivo* rearing of indigenous EPNs isolated from Hawaii. Different isolates of EPNs both in the genera of *Heterorhabditis* and *Steinernema* have been isolated in Hawaii (Hara et al., 1991; Myers et al., 2015) and their efficacy has been tested against various economically important insect

pests such as imported cabbageworm (Mohammed, 2012) and coffee berry borer (Martiney et al., 2018).

In this project, we used laboratory *S. feltiae* MG-14 reared on *Tenebrio molitor* as this EPN possess intermediate foraging strategies, i.e. both ambusher and cruiser behavior. We combined them with adjuvants to test the efficacy of *S. feltiae* MG-14. The strain MG-14 was isolated from Waikapu, Maui, thus is maybe adapted to the climate of Hawaii. The critical research needed on the use of EPN in Hawaii is to determine lower dose of EPN foliar spray to achieve effective control of DBM in the field. Adjuvants tested were Oroboost® (Oro Agri, Inc., Fresno, CA), Kinetic® (Helena Chemical Company, Collierville, TN) and Exit® (Miller Chemical and Fertilizer, LLC, Hanover, PE). Oroboost® and Kinetic® are OMRI certified adjuvants whereas Exit® is not. It is hypothesized that laboratory reared (or in vivo produced) indigenous EPNs might allow for lower EPN population densities to be effective if coupled with appropriate food-grade adjuvants.

Specific objectives of this study were to determine 1) OMRI certified adjuvants that can improve persistence of EPN, 2) efficacy of EPN against DBM affected by adjuvant on cabbage leaves; and 3) effective dosage of *S. feltiae* MG-14 and its infection rates on DBM larvae and pupae.

4.2 Materials and Methods

EPN persistence affected by adjuvants: Two greenhouse trials were conducted to test the persistence of *S. feltiae* MG-14 affected by different adjuvants. Three adjuvants tested were Oroboost®, Kinetic® and Exit®. Four treatments consisted of 3.9 ml/L (i.e. 50 fl oz/100 gal) of 1) Oroboost®, 2) Kinetic® and 3) Exit® as recommended on the label, and a 4) control with

nematodes only. *S. feltiae* MG-14 IJs were harvested from meal worm cadavers and used for the two trials within 1 week after harvest.

In Trial I, a total of 60 petri dishes (60-mm-d) each contained an excised leaf of head cabbage were placed on a greenhouse bench without petri dish lids. Each petri plate was sprayed with a 2 ml suspension of *S. feltiae* MG-14 containing 500 IJs mixed with one of the 4 adjuvant treatments. Three replications of each treatment were removed from the greenhouse bench at 0, 30, 60, 120 and 180 minutes after exposing to the heat in the greenhouse starting at 3:00 and ending at 6:00 pm. Thus, this was a 4 × 5 (adjuvant × time of exposure) factorial experiment with 3 replications, arranged in a randomized complete block design. The experiment was replicated again in Trial II with a slight modification where only 250 IJs was sprayed per dish, and experiment was initiated at 2:30 pm and terminated at 5:30 pm. Trial I and Trial II were conducted at two days apart. During the 3 hours of exposure time in the greenhouse, the average light hours was 178 $\mu\text{mol}^{-2}\text{s}^{-2}$, temperature was 40°C (38- 48°C), dew point was 23.7°C (13-21°C) and relative humidity was 24% (22-27%) in Trial I; whereas the average light hours was 177 $\mu\text{mol}^{-2}\text{s}^{-2}$, average temperature was 49°C (44-53°C), dew point was 19.1°C (21-26°C), relative humidity was 18% (22-53%) in Trial II.

After each exposure, nematodes on each cabbage leaf were washed with 20-25ml of tap water into a petri dish. IJs collected were observed under an inverted microscope and quantified for number of actively moving vs straight and immobile IJs (Fig. 1A, B). Percentage of IJ persistence was calculated for each plate.

Data were subjected to 4×5 (adjuvants× time of exposure) factorial analysis of variance (ANOVA) in a randomized complete block design using SAS (SAS institute, Cary, NC). Means were separated by Waller-Duncan *k*-ratio (*k*=100) *t*-test wherever appropriate.

Efficacy of EPN on DBM affected by adjuvant: A greenhouse experiment was conducted to test the efficacy of adjuvants added to *S. feltiae* MG-14 foliar spray against DBM larvae. Six-week-old 'KK cross' head cabbage seedlings in 10-cm diameter pots were grown in a greenhouse to be sprayed with one of the 5 treatments: 1) Oroboost® at 3.9 ml/L (i.e. 50 fl oz/100 gal), 2) Oroboost® at 1.6 ml/L (i.e. 20 fl oz/100 gal), 3) Kinetic® (3.9 ml/L), 4) Exit® (3.9 ml/L), and 5) no adjuvant control. EPN were applied at 1,235 IJs /plant equivalent to 250 million IJs/ha. An additional water control without IJs and adjuvant was included as the negative control. The experiment was arranged in a RCBD with 4 replications. Prior to the foliar spray, laboratory reared DBM were introduced at 5 eggs per plant, the eggs were allowed to hatch, and develop into 3-4th stage larvae for a week. One week after DBM eggs were introduced, *S. feltiae* MG-14 were sprayed at 250 million IJs/ha by mixing with the designated adjuvants using a 50-ml mister bottles at dusk. The number of DBM alive, lost, and dead on each plant were recorded at 24 and 48hr after EPN application. The number of DBM larvae lost and dead were considered as the total number of DBM reduced after 2 days of EPN application.

Data were subjected to one way analysis of variance (ANOVA) in a randomized complete block design using SAS (SAS institute, Cary, NC). Means were separated by Waller-Duncan *k*-ratio (*k*=100) *t*-test wherever appropriate.

Effects of EPN dosages in petri dish: A laboratory trial was conducted to determine effective foliar spray dosages of *S. feltiae* MG-14 against DBM larvae and pupae in petri dishes.

Less than 1-month old *S. feltiae* MG-14 harvested from mealworms were used as inoculum. Four 3rd-4th instar DBM larvae or 4 pupae were placed on a 90-mm-d petri dish lined with Whatman #1 filter paper, individually. Four different dosages of EPNs: 0, 40, 80 and 160 IJs/plate in 1.5 ml water equivalent to 62.5, 125 and 250 million IJs/ha (0.625, 1.25 and 2.5 IJs/cm²), respectively was dispersed in each petri dish using a pipette. Each dosage was replicated 4 times. Mortality of DBM was observed at 24 and 48 hours after inoculation. Dead DBM cadavers were placed in individual White traps and checked for EPNs emergence every day over 2 weeks (Kaya and Stock, 1990). Percentage of DBM infection was calculated by the number of larvae/pupae with IJs emergence divided by initial DBM larvae/pupae per plate. Data were subjected to a 2×4 factorial (DBM Stage × EPN dosage) ANOVA using PROC GLM in SAS 9.4 (SAS Inc., Cary, NC). Means were separated by Waller-Duncan *k*-ratio (*k*=100) *t*-test wherever appropriate.

Effects of EPN spray dosage in the greenhouse: A greenhouse experiment was conducted to determine effects of dosage of *S. feltiae* on efficacy against DBM larvae. Six-week-old 'KK cross' head cabbage seedlings grown in 15-cm diameter pots were sprayed with: 0, 40, 80 and 160 IJs/plant in 2 ml water equivalent to 62.5, 125 and 250 million IJs/ha, mixed with Oroboost® at 3.9 ml/L (i.e. 50 fl oz/100 gal). Each treatment was replicated in 4 pots of plants. Five DBM eggs/plant were introduced on cabbage plants and allowed to develop to 3-4th stage larvae. After a week, EPNs were sprayed on the cabbage seedlings during the dusk. The survival or mortality of DBM were recorded at 24 and 48 hours after EPN inoculation. Dead DBM cadavers were placed in individual White traps to check for their reproduction i.e. cadavers with EPNs emergence every day over 2 weeks on White traps (Kaya and Stock, 1990). Data were subjected to one-way ANOVA

using PROC GLM in SAS 9.4 (SAS Inc., Cary, NC). Means were separated by Waller-Duncan k -ratio ($k=100$) t -test wherever appropriate.

4.3 Results

EPN persistence: A significant interactions between the adjuvants and time of exposure of cabbage leaves after EPN spray under high temperature in the greenhouse was observed ($P \leq 0.05$) in both trials. Thus, persistence of EPNs over time of exposure were presented by adjuvant treatments in a line graph (Fig. 4-2 A). Overall, in Trial I survival rate of IJs decreased over exposure time except for a slight increase by Oroboost® treatment. While no significant difference in persistence of EPNs among adjuvant treatments were observed from 0 to 60 min in the greenhouse, Oroboost® provided the highest persistence of IJs at 120 min. Exit® showed significantly lower EPN survival at 120 min of exposure ($P \leq 0.05$, Fig. 4-2 A). At 180 min after exposure, EPN survived at around 42.8% regardless of Oroboost®, Exit® or water treatments, but it only had 13% survived in the Kinetic® solution.

In Trial II, where the experiment was conducted at a warmer time than Trial I, a steady decline of IJs survival rate over time of exposure was observed for all treatments and only 2.9% of EPN survived at 180 min after exposure. At 0 and 30 min, Kinetic® showed lower EPN survival compared to other adjuvants including control ($P \leq 0.05$; Fig. 4-2b). It was promising to see that Oroboost® maintained a higher survival of EPN at 60 and 120 min after heat exposure compared to the water control ($P \leq 0.05$). However, Oroboost® failed to protect EPNs at 180 min after heat exposure.



Fig.4-1. *Steinernema feltiae* infective juveniles (IJs) A) actively moving and B) immobile observed under an inverted microscope.

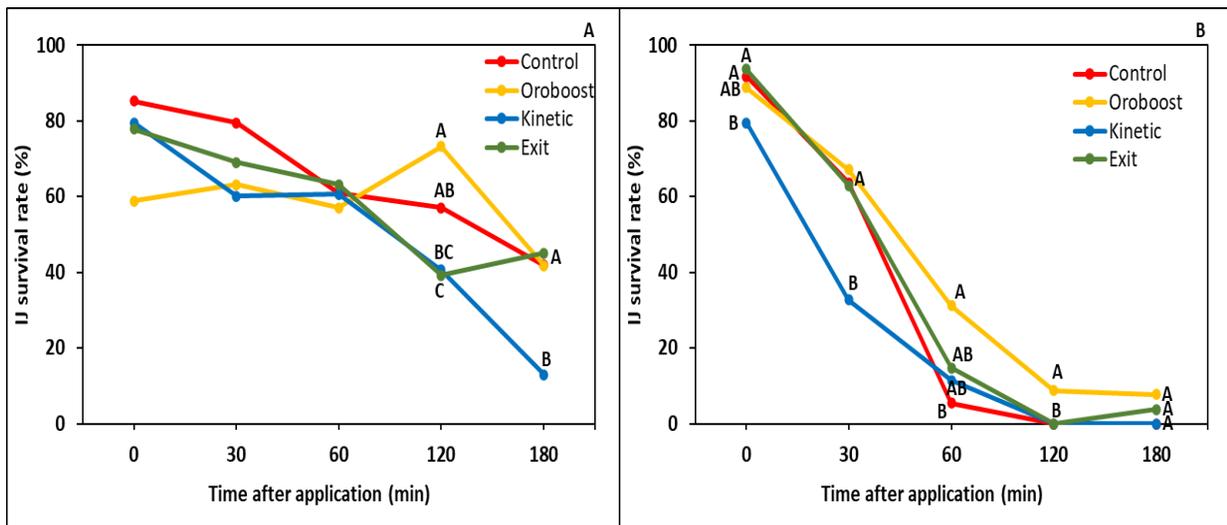


Fig.4- 2. Effect of adjuvants on the survival rate of *Steinernema feltiae* MG-14 infective juveniles (IJs) at different exposure time after foliar application in greenhouse Trial I (A) and Trial II (B). Means followed by the same letters within each exposure time are not different based on Waller-Duncan k -ratio ($k=100$) t -test.

Efficacy of EPN on DBM affected by adjuvant: The greenhouse pot trial showed that O-50 (3.9 ml/L) and K-50 (3.9 ml/L) were able to increase the efficacy of *S. feltiae* spray against DBM larvae on cabbage plant as compared to EPN alone without adjuvant and the water control ($P \leq 0.05$; Fig. 4-3). These results also clearly showed that *S. feltiae* alone without adjuvant was not able to reduce DBM population on the cabbage plants ($P > 0.05$). Whereas using adjuvant O-20 (1.6 ml/L) and E-50 (3.9 ml/L) were not able to improve the efficacy of EPN against DBM larvae.

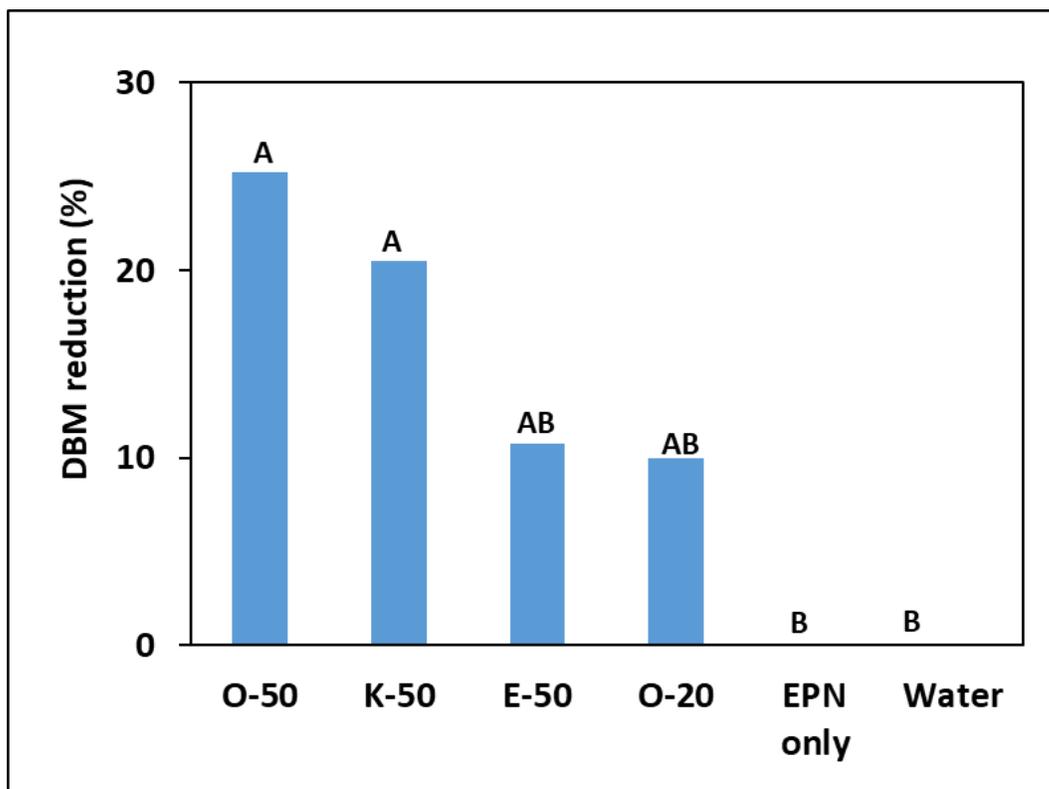


Fig.4-3. Effect of adjuvants O-50 (Oroboost® at 3.9 ml/L, equivalent to 50 fl oz/100 gal), K-50 (Kinetic® at 3.9 ml/L), E-50 (Exit® at 3.9 ml/L), and O-20 (Oroboost® at 1.6 ml/L equivalent to 20 fl oz/100 gal) on efficacy of *Steinernema feltiae* MG-14 (EPN) against diamondback moth (DBM) on cabbage seedlings in a greenhouse pot trial. Percent reduction of DBM = (number of DBM dead and lost 2 days after EPN application/5 DBM×100). Means followed by the same letters are not different based on Waller-Duncan k -ratio ($k=100$) t -test.

EPN dosage in petri dish: All DBM larvae and pupae died within 1 hour after EPN inoculation, thus mortality rates were 100% for all EPN inoculated treatments regardless of dosage or DBM stages tested. Based on the 2×4 ANOVA, infection rate of DBM represented by the emergence of IJs from dead DBM cadaver, were not different among different EPN dosage, but were all higher than the uninoculated control ($P \leq 0.05$; Table 1) which also did not have DBM mortality. No interaction between EPN dosage and stages of DBM was detected for percent of EPN infection.

Table 4-1. Effect of *Steinernema feltiae* dosage on infection rates of diamondback moth larvae and pupae.

	% Infection
Dosage (IJs/cm ²)	
0	0 b ²
0.625	33.33±11.18 a
1.25	37.50±12.49 a
2.5	50.00±11.18 a
Stages	
Larvae	31.25 A
Pupae	29.16 A

² Means (n=6) followed by the same letter(s) are not different according to Waller-Duncan *k*-ratio (*k*=100) t-test.

EPN dosage in greenhouse: Although no significant difference in the mortality rates of DBM was observed among the EPN dosages tested and the untreated control ($P > 0.05$, Fig 2-4), there was an increasing trend of mortality with the increase in EPN dosages. Whereas the untreated control had no DBM mortality, the highest dosage of 2.5 IJs/cm² resulted in 9% mortality.

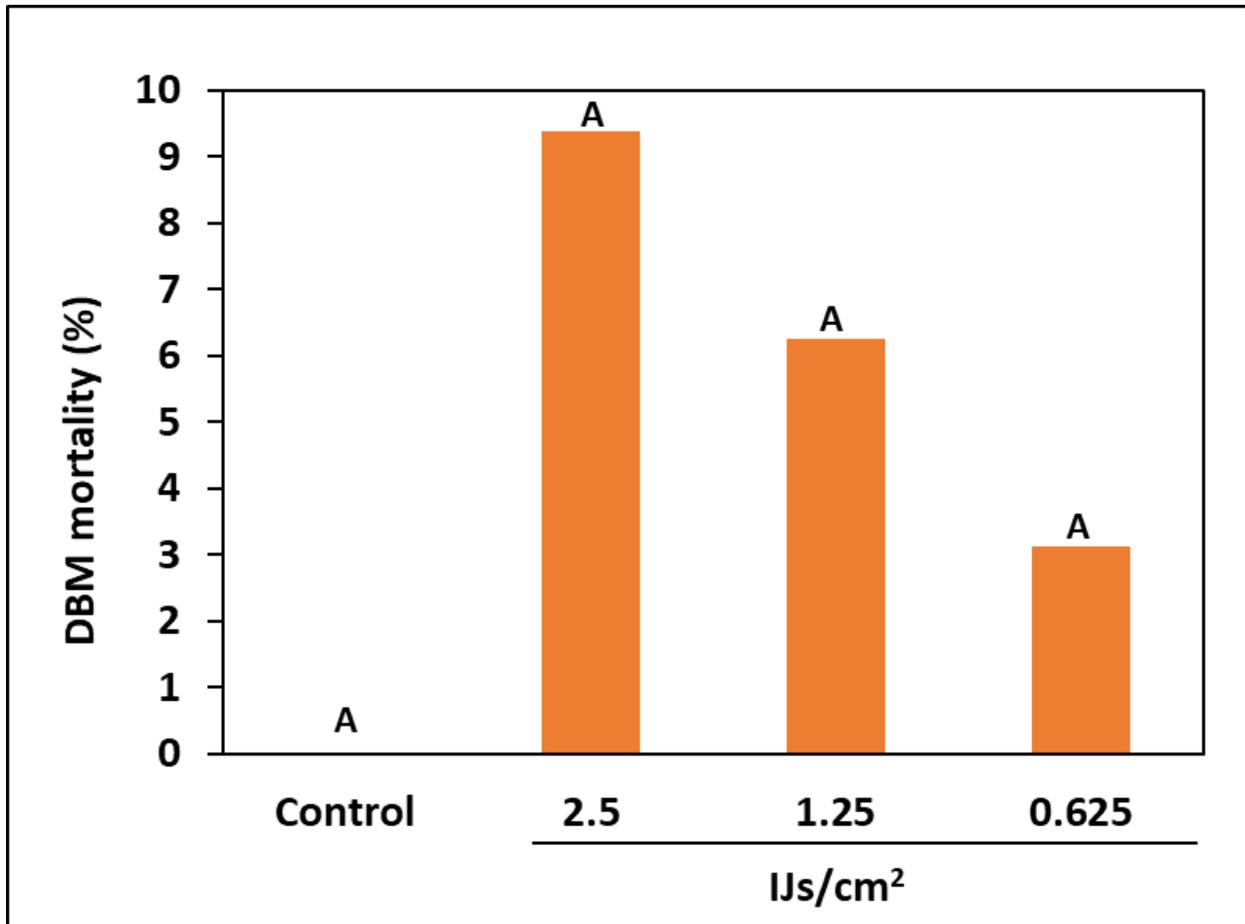


Fig.4- 4. Effect of *Steinernema feltiae* MG-14 dosage on mortality of diamondback moth larvae in greenhouse conditions. Means followed by the same letters are not different based on Waller-Duncan k -ratio ($k=100$) t -test.

4.4 Discussion

EPN persistent affected by adjuvants in petri dish: Results in the greenhouse EPN persistence experiment verified the challenge of using EPN as foliar spray against insect pests especially when adjuvants were not used. Survival of EPNs were 42 % and 0 % in Trial I and Trial II, respectively 3 hours after application. Higher heat and lower relative humidity (RH) in Trial II than Trial I resulted in lower to no EPN survival in Trial II. Jin et al. (2004) reported zero survival of *S. carpocapsae* 160 min after inoculation at 35°C. Among the adjuvants tested, Oroboost® showed some promising EPN protection, but the effect was short-lived where it only extended the survival rates of EPNs for 160 min compared to no adjuvant control. Higher survival rates of EPN might occur if the temperature were to be reduced to that similar to a field conditions in Hawaii. None-the-less, this study provided consistent results on ability of Oroboost® to extend the survival rates of EPN in foliar spray. This experiment also verified that Oroboost® was not toxic to EPN and can achieve 25 % DBM reduction in the greenhouse trial. EPNs + Oroboost® at a lower rate, 1.6 ml/L (O-20), can still achieve 10% reduction of DBM numbers on cabbage compared to the use of EPNs alone which failed to suppress DBM. Kinetic® another adjuvant registered for organic farming, was determined to be not compatible for EPN foliar spray based on the less survival of EPNs in the persistency test than control from the beginning of exposure of cabbage leaves. However, Kinetic® + EPN achieved similar reduction of DBM to the Oroboost® (O-50), suggesting that Kinetic® might have toxicity to DBM larvae. Baur et al. (1997) also found that not all adjuvants tested can improve DBM control by *S. carpocapsae* on water cress.

EPN dosage: Although many researchers used very high EPN dosage to test efficacy of EPN on DBM such as 0.5 million IJs/m² in cabbage field (Schroer et al., 2005a), the laboratory trial conducted here showed that 0.625 IJs/cm² (6,250 IJs/m²) could result in 100 % mortality, and 33% DBM infection rate which was not significantly different from the highest rate (2.5 IJs/cm²) with 50% infection rate. The greenhouse experiment did show a trend in increasing of DBM mortality as the EPN dosage increase. However, the high EPN dosage, 2.5 IJs/cm² only achieved 9% of DBM mortality which is very low compared to 35-50% DBM mortality achieved by other EPNs at much higher concentrations demonstrated by Baur et al. (1995) and Schroer et al. (2005). Extreme high temperature and low relative humidity in the first greenhouse trial may not represent the conditions in the field, thus the data from the second greenhouse trial would be more representative of what could happen to EPN foliar spray in the field. Nyugen et al. (2014) reported extreme and longer durations contributes to higher mortality of DBM.

While increasing EPN dosage to 2.5 IJs/cm² was not warranted based on the laboratory and first greenhouse trials, it is very important to maintain the survival rates of EPN applied to the foliage. Considering the short time frame for selected adjuvants to protect EPN, it is more important to reduce desiccation and provide good coverage of EPN during the foliar spray. Thus, using spreader as adjuvant would help to improve the uniform spreading, distribution and reduce the desiccation of the spray droplets (Schröer et al., 2005b) and eventually increase the likelihood of DBM infection. Knowing that low rates of *S. feltiae* MG-14 can provide as effective control as commercial rates is encouraging. Due to the stringent restriction on the import of commercial EPNs into Hawaii, *in vivo* rearing of EPNs is a must. A lower effective rate of EPN would make *in vivo* rearing of EPN more feasible in Hawaii. This finding showed that laboratory reared (or *in vivo*

produced) indigenous EPNs can be effective at lower EPN population densities if coupled with appropriate adjuvants such as Oroboost®.

Unfortunately, EPN foliar application for DBM management must currently rely on inundative release of EPN due to the low survival rate in the field environment. Thus, repeated application of EPN is needed. Interestingly, both DBM larvae and pupae were equally susceptible to *S. feltiae* MG-14 infection. Kary et al. (2019) also found that DBM pre-pupae were more susceptible to *S. feltiae* infection than DBM larvae. If one life cycle of DBM is 21-24 days in Hawaii, spraying EPNs at two-week intervals would increase the chances of EPNs to encounter DBM larvae and pupae.

In conclusion, this study identified an OMRI certified adjuvant, Oroboost® at 3.9 ml/L with promising effects in extending the survival of *S. feltiae*. Oroboost® also increased the efficacy of *S. feltiae* by reducing the number of DBM surviving on cabbage leaves within 2 days after EPN application. This study also demonstrated that without environmental stress, EPNs dosage as low as 1 IJ/cm² was as effective as the commercial rate as well as the high dosage of application rate in infecting and killing DBM.

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Chapter 5: Conclusion

Among the three integrated approaches examined to enhance the efficacy of entomopathogenic nematode (EPN) sprays against diamondback moth (DBM), *Plutella xylostella*, and imported cabbage worm (ICW), *Pieris rapae*, trap cropping with 'Hirayama' kai choi (*Brassica juncea*) was most effective in reducing abundance of DBM (46%) and ICW (73%), and in reducing damage caused by DBM (45%) and ICW (33%) on cabbage. EPN spray using an indigenous species, *Steinernema feltiae* MG-14, reared on mealworm (*Tenebrio molitor*) alone also reduced ICW number in the cabbage trial only in no trap crop plots. Surprisingly EPN suppressed DBM population by 100% in kale Trial II though it had no effect in kale Trial I. This finding coincides with many results found in the literature that suggest inconsistent effects of EPN in the field. There was a trend from all trials that EPN was only effective when DBM and ICW numbers were < 0.5/plant. Unlike the hypothesis, integrating a trap cropping strategy with EPN foliar spray did not increase the performance of EPN against these two pests. The pitfall of the trap cropping design used in this study was the close distance (15 cm) between the trap crop and the cash crop which compromised cabbage and kale yield at harvest. However, when the distance was increased to 66 cm between the trap crop and cash crop, yield of kale was not compromised. Future research should examine appropriate distance necessary to avoid growth competition while achieving effective trap cropping effect against DBM and ICW. Performance of the kai choi trap crop was less impressive on kale than on cabbage, especially against ICW.

When using the intermittent sprinkler irrigation (ISI) approach, 5-min sprinkler irrigation from 8:00 am to 4:00 pm at 2-hour intervals and twice at dusk (6:00 pm and 8:00 pm) (S_{Dd}) was most effective in reducing ICW (86% and 58% in Trial I and Trial II, respectively), and ICW damage

(88% and 45% in Trial I and Trial II, respectively). Unlike what was hypothesized, Sd did not perform well in interfering DBM oviposition that could lead to reduction of DBM number or damage. More research should be conducted to fine-tune the duration, intervals, and timing of ISI for best DBM suppression. Nonetheless, ISI against ICW determined in Chapter 3 is still beneficial as organic farmers would have another tool to manage DBM and ICW without strongly relying on pesticide spray that can lead to the vicious cycle of pesticide treadmill. It is evident that integration of ISI and EPN spray is not warranted as ISI did not improve the performance of EPN in this cabbage experiment despite spraying EPN for three times throughout the crop. This is in part due to EPN possibly being washed off the foliage by the water from ISI.

The last approach being tested to enhance EPN efficacy showed Oroboost® at 3.9 ml/L (i.e. 50 fl oz/100 gal) extended the survival rates of *S. feltiae* for 2 hours in greenhouse trials and was more effective than Oroboost® at 1.6 ml/L when added to *S. feltiae* in suppressing DBM on cabbage. Additional laboratory and greenhouse experiments showed that high dosage of *S. feltiae* at 125 mil IJs/ha recommended for commercial EPN application was not necessary as the lower concentration of 62.5 mil IJs/ha was as effective as 125 mil IJs/ha and 250 mil IJs/ha dosages in killing and infecting DBM.

Overall, this thesis provides some insights on how to improve efficacy of EPN performance against DBM and ICW in the field and should be research further to help organic brassica crop farmers to mitigate the use of pesticides or biopesticides that are challenged by pesticide resistant DBM populations.

Appendix A

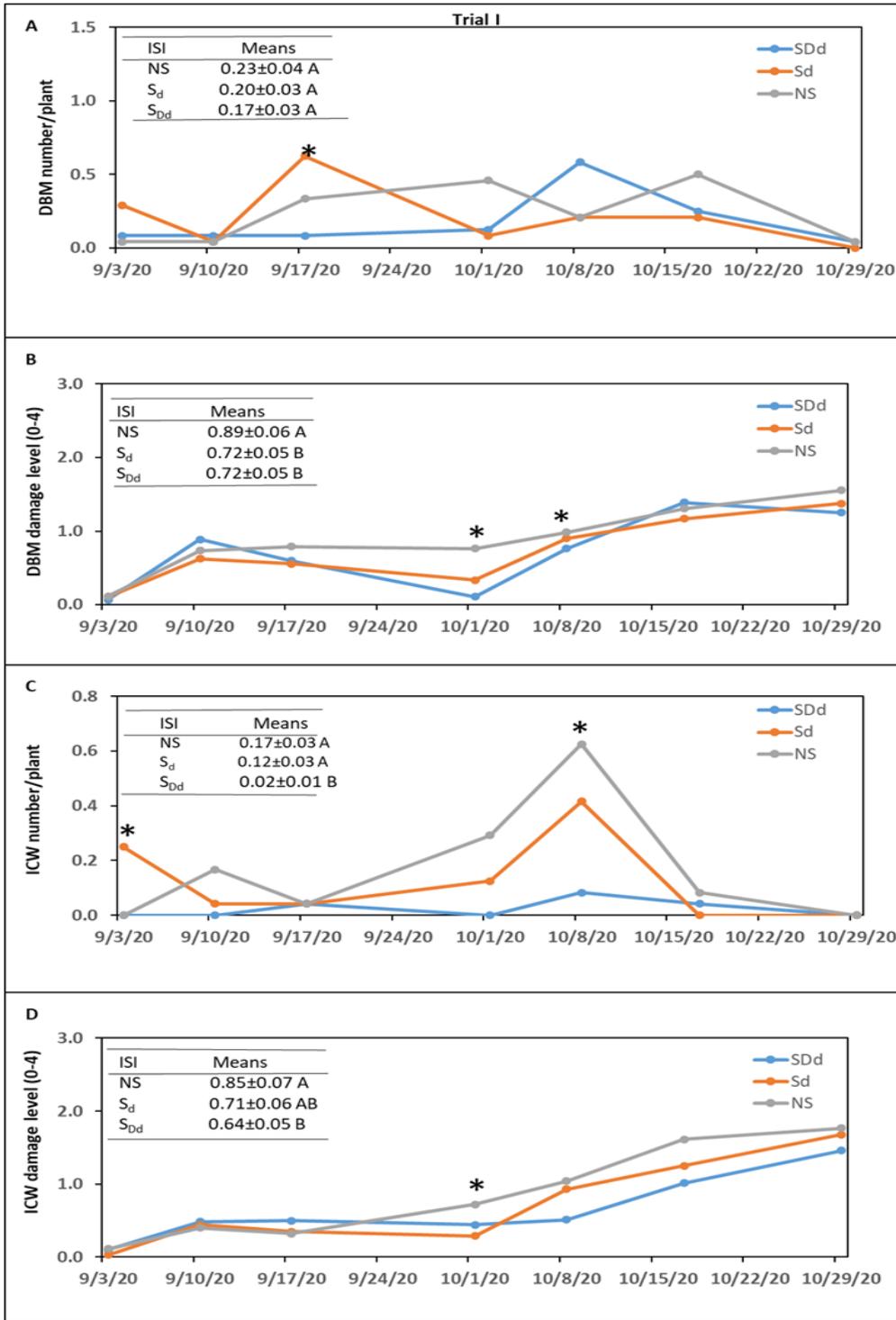


Fig. A-1. Effects of intermittent sprinkler irrigation (ISI) at dusk (S_d), and day and dusk (S_{Dd}) compared to no sprinkle irrigation (NS) on A) abundance of diamondback moth (DBM), B) percent leaf with DBM damage, C) abundance of imported cabbageworm (ICW), and D) percent leaf with ICW damage on head cabbage in Trial I (Chapter 3). Damage level 1 = 1-10%, 2 = 11-25%, 3 = 26-50% and 4 = 51-100% leaf damage. Means followed by the same letter on each date are not different based on analysis of variance. * indicates significant difference among treatments.

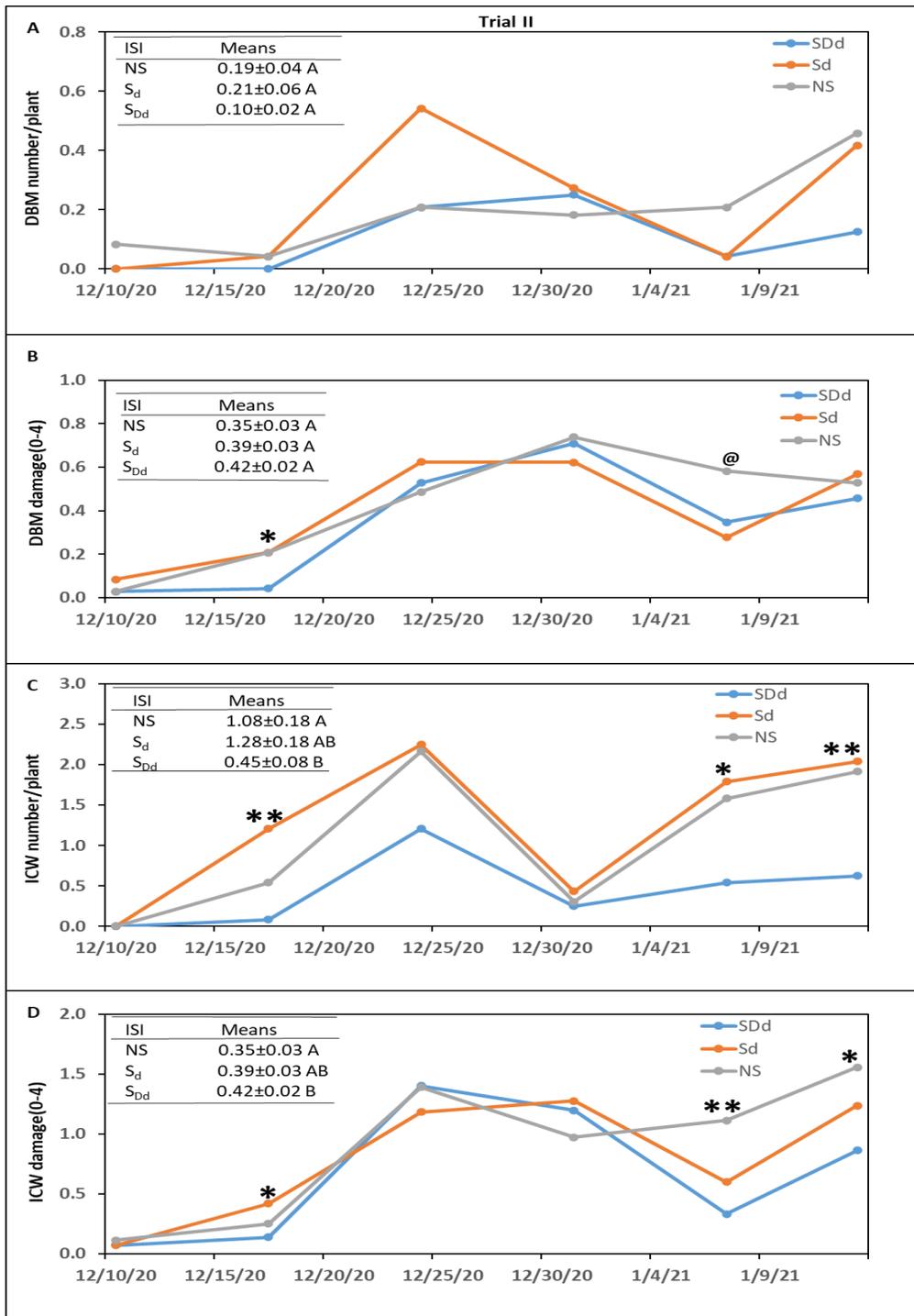


Fig. A-2. Effects of intermittent sprinkler irrigation (ISI) at dusk (S_d), and day and dusk (S_{Dd}) compared to no sprinkle irrigation (NS) on A) abundance of diamondback moth (DBM), B) percent leaf with DBM damage, C) abundance of imported cabbageworm (ICW), and D) percent leaf with ICW damage on head cabbage in Trial II (Chapter 3). Damage level 1 = 1-10%, 2 = 11-25%, 3 = 26-50% and 4 = 51-100% leaf damage. Means followed by the same letter on each date are not different based on analysis of variance. * indicates significant difference among treatments.