

INTEGRATED STRATEGIES FOR MANAGING DIAMONDBACK MOTH,
PLUTELLA XYLOSTELLA L. IN CABBAGE USING COMPANION PLANTING AND
REDUCED-RISK INSECTICIDES

By

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To my mom and dad for being my first teacher in life
To my husband and children for continuous support

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Abstract of Thesis Presented to the Graduate School
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Cabbage is an important crop in Florida. Current management strategies to control major pests of cabbage including diamondback moth (DBM), *Plutella xylostella* (L), rely heavily on insecticides. There are concerns that overuse of insecticides will lead to the development of resistance and negative effects on non-target organisms. The purpose of this study was to evaluate alternatives to chemical control and to develop a more sustainable approach to manage insect pests of cole crops. The colonization of cabbage pests and their natural enemies were investigated for 2 years in cabbage intercropped with marigolds, roselle and collards. Populations of natural enemies increased in cabbage intercropped with marigolds and roselle in both years. Diamondback moth populations were reduced in cabbage treated by Entrust[®], followed by cabbage intercropped with roselle and marigold. Laboratory studies evaluating roselle fruit extracts demonstrated that oviposition by DBM adults were reduced and larvae avoided treated cabbage discs. In a semi-field based study, we evaluated several insecticides that are labelled for organic use including Entrust[®], Azera[®], Aza-Direct[®] and Grandevo[®]. Entrust[®] effectively reduced DBM larvae within 12 h and 100% mortality was recorded at 24 h after exposure; the other insecticides resulted in significant

mortality after 48 h. In the second year, a field efficacy study evaluated the effectiveness of insecticide combinations with Entrust[®] including Azera[®], Aza-Direct[®] and Grandevo[®]. Entrust[®] + Azera[®] showed similar efficacy with Entrust[®] alone in reducing DBM populations and maintaining marketable cabbage yields. Entrust[®] + Aza-Direct[®] was found to significantly reduce aphid populations compared with Entrust[®] alone treatment. Findings from these studies will be useful for providing information on alternative strategies that can be incorporated into an IPM program to manage cabbage pests in organic or conventional cole crop production.

CHAPTER 1 INTRODUCTION

The Cabbage Industry in the United States and Florida

Cabbage, *Brassica oleracea* L. var. *Capitata* (family Brassicaceae) belongs to a diverse group of plants with 350 genera and more than 3,500 species, which include cauliflower, Brussels sprouts, sprouting broccoli, kohlrabi, and curly kale. The brassicas are listed in the top ten of economically important plant families (Warwick et al. 2013) and are grown worldwide on more than 2.2 million hectares annually (Vickers et al. 2004). In the United States (US), cabbage is produced mainly for the fresh market, contributing 5% of total fresh market production in the US in 2015.

Florida's cabbage production contributes 13% of the total cabbage production nationally, which makes Florida the third largest cabbage producer in the US after California and New York (Wells 2016). There are several regions listed as being the principal cabbage-production in the state which include Hastings, Sanford, Oviedo, Zellwood, Plant City, Palmetto, Ruskin, Sarasota, Martin county and Homestead (USDA/NASS 2014). In 2012, southeast and northeast Florida contributed a total of 54% of the cabbage acreage for Florida. Southeast Florida (Palm Beach and Okeechobee counties) contributed about 29% of cabbage acreage while 25% of cabbage acreage was contributed from the Hastings area in northeast Florida (Flagler and St Johns counties) (USDA 2014).

Cabbage Production and Nutrient Content

Cabbage is categorized as a cool-weather crop that grows at minimum temperature of 0 to 25 °C. The optimum temperature for this crop ranges from 15 to 20 °C (Criddle et al. 1997). In Florida, cabbage planting seasons are between August

through March and may vary between regions; September and December (Northeast Florida), September to February (Central Florida), and September to January (South Florida) (Olson et al. 2012). Cabbage is grown by transplanting 4 to 6 weeks old seedlings that are previously sown in a greenhouse. Transplants are usually either seedlings with bare-root or container-grown plugs (Elwakil and Mossler 2010). Cabbage seedlings are transplanted on raised-beds covered with plastic mulch, combined with drip irrigation installed below the mulch. Plastic mulch helps to maintain soil moisture, reduce nutrient leaching (Romic et al. 2003), and reduce weed growth.

In United States, cabbage is ranked as the 10th most consumed fresh vegetable, with an average consumption of 7.1 pounds per person in 2014 (USDA/ERS 2015). According to data collected in 2013 by Economic Research Service at the U.S. Department of Agriculture (USDA), cabbage was reported to be the third most economical vegetable in terms of per edible cup. In terms of nutritional value, while being a good source of vitamin A and C, every 100 g of edible portion of cabbage also contains 1.8 g protein, 0.1 g fat, 4.6 g carbohydrate, 0.6 g mineral, 29 mg calcium, 0.8 mg iron, 14.1 mg sodium (Tiwari et al. 2003). Furthermore, cabbage also contains glucosinolates, secondary metabolites that are widely distributed across the Brassicaceae family. The hydrolyzed glucosinolate such as isothiocyanates play an important role by having protective properties against cancer (Dekker et al. 2000, Keck and Finley 2004). In agriculture, isothiocyanates is widely used as an active ingredient for soil fumigants (Gamliel et al. 2000).

Cabbage Pests

Although cabbage is shown to have high economic importance and nutritional values, like other vegetable crops, cabbage is also susceptible to pest infestations.

These infestations could cause serious damage and reduce yield. Insects that are considered as pests of cabbage can be divided into 3 sub-groups; 1) the main lepidopteran pests include the diamondback moth (DBM), *Plutella xylostella* (Linnaeus), the cabbage looper (CL), *Trichoplusia ni* (Hübner), and the imported cabbage worm (CW), *Pieris rapae* (Linnaeus); 2) secondary lepidopteran pests include the beet armyworm, *Spodoptera exigua* (Hübner), the cabbage webworm, *Hellula rogatalis* (Hulst), black cutworm, *Agrotis ipsilon* (Hufnagel), and the granulate cutworm, *Feltia subterranea* (Fabricius); and 3) other pest families include the aphid species group; cabbage aphid (CA), *Brevicoryne brassicae* (Linnaeus), turnip aphid, *Lipaphis erysimi* (Kaltenbach), and the green peach aphid, *Myzus persicae* (Sulzer). The sweet potato whitefly, *Bemisia tabaci* biotype B is an occasional pest. Other insect pests only occur occasionally and are less problematic (Webb 2010).

The major pest problem faced by Florida's grower is damage caused by the diamondback moth. Among the cruciferous pests, the DBM has gained notoriety for being resistant to many classes of insecticides (Furlong et al. 2013). In 2016, Arthropod Pesticide Resistance Database (APRD) reported that the DBM had shown resistance to 95 active ingredients. According to Sarfraz and Keddie (2005) several factors including intensive application of pesticides in cruciferous crops that facilitated the exclusion of beneficial predators and parasitoids, DBM migratory ability, and the magnitude of damage on cruciferous crops which allowed it to achieve pest status especially in new areas that lack its natural enemies. The situation becomes even more complicated because frequent pesticide applications contribute to the increase in the cost of cabbage production. Based on cost and profitability analysis a report that was published

in 2013 for cabbage production in Ventura County, California, the cost for three pesticide treatment applications in cabbage was about 5% of the total production cost (Takele and Daugovish 2013).

Justification

Conventional cabbage growers in some regions such as the Mariana Islands usually apply a rotation of reduced-risk and synthetic pesticides to manage cabbage pests. In addition to *Bacillus thuringiensis* (Bt) insecticides, conventional insecticides such as malathion (55%) and carbaryl (30%) were being applied to manage pests in cabbage fields and applications were as frequent as 8 to 10 times per cropping season (Reddy 2011). This practice reduced the damage inflicted by cabbage pests and increased marketable yields. However, the reliance on synthetic pesticides alone is not sufficient to effectively manage DBM and other cabbage pests in the field. Furthermore, frequent applications of conventional pesticides will increase the production cost, increase potential for resistance and augment the detrimental effects of non-target organisms and the environment.

Although, reduced-risk pesticides have been labelled as safe to humans and the environment, there were several studies indicating that some reduced-risk pesticides including abamectin and spinosad can cause side effects and mortality to biological control agents including the minute pirate bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Gradish et al. 2011, Biondi et al. 2012), and Swirski mites, *Amblyseius swirskii* (Athias-Henriot) (Arachnida: Mesostigmata: Phytoseiidae) (Gradish et al. 2011). Additionally, the neonicotinoid insecticide (imidacloprid) may impair *Bombus terrestris* (Latreille) (Hymenoptera: Apidae) foraging behaviors (Mommaerts et al. 2010). Hence, it is crucial to reduce the amount of pesticides irrespective of its status (reduced-risk or

conventional) and promote more sustainable management of cabbage pests through an integrated approach.

Alternative control strategies that are cost effective and ecologically sound are needed in a more integrated approach. The primary goal of this study was to develop tactics to integrate companion plants with the main crop (cabbage) that will facilitate reducing the application of pesticides for management of cabbage pests specifically diamondback moth. This can be partially achieved by measuring the colonization of key pests in cabbage in presence of companion plants, and conserving natural enemy populations in the field through habitat modification (introducing companion plants into the agro-ecosystem).

Hypothesis

Ho. It is possible to suppress key cabbage pests including *Plutella xylostella* using companion plants and reduced-risk pesticides (biorationals) without suffering major yield loss.

Ha. The alternative hypothesis is that companion plants and reduced-risk pesticides (biorationals) cannot suppress key cabbage pests and will result in major economic loss in yield.

Objectives

The specific objectives of this study were: 1) to evaluate the colonization of cabbage by key pests, DBM, cabbage worm (CM), cabbage looper (CL), and cabbage aphids (CA) in the presence companion plants; 2) to determine the potential of using extracts from selected companion plants as bio-pesticides. 3) to assess the effect of selected insecticides that are labelled for organic use on diamondback moth

The outcome of this study will be beneficial to agricultural industry groups and growers in the state especially organic growers that are introducing companion plants into the agro-ecosystem. This study will identify alternative plants that can be planted with cabbage in a companion planting system.

CHAPTER 2 LITERATURE REVIEW

Diamondback Moth

The diamondback moth (DBM), *Plutella xylostella* (L), is a key pest of cabbage and one of the most destructive pest of cruciferous crops. The pest was accidentally introduced into the United States in 1854 in Illinois and it spread rapidly and reached Florida and the Rocky Mountains by 1883 (Capinera 2001). The wide distribution of DBM is believed to be associated with the expansion of cruciferous cultivation worldwide and the migration ability of adult DBM (Chu 1986). This moth was described as “diamondback” due to the formation of three or four yellow diamond shapes that are observed from the dorsal view of adult wings when the insect is at rest (Ankersmit 1951).

Biology

The life cycle of DBM includes an egg, four larval instars, pupa and an adult. The DBM takes approximately 25-30 d (depending on temperature) to complete its life cycle (Talekar and Shelton 1993, Capinera 2001). Adult DBM can be distinguished from other plutellid moth by the unique coloration of the wings and are described in detailed by (Moriuti 1973). The upper 2/3 of the forewing range from light dusky brownish to partially ochre tinged, with mixed of whitish scales and small blackish dots, while 1/3 of the lower side has a pale yellowish-brown to white coloration. Females have lighter wing color compared to males (Marsh et al. 1917). Hindwings usually have longer fringe on the inner margin compared to the forewings. The body length is 9 mm long and the wingspan of the adult varies from 7 mm to 55 mm (Heppner 2004). Adult moths are weak flyers and the distance of dispersal were limited and influenced by the availability

of suitable host for oviposition (Mo et al. 2003). During the daytime, adult DBMs are inactive and are observed resting on the lower leaf surface of the leaf (Harcourt 1957). Mating occurred during dusk, on the same day of adult emergence. If a suitable host is available, females will oviposit several patches of small clusters of eggs soon after mating. Oviposition often last up to 4 d with an average of 150 eggs laid per generation (Harcourt 1957, Talekar and Shelton 1993, Capinera 2001). Females show preference to deposit eggs on the concave surface of foliage usually near leaf veins (Talekar and Shelton 1993).

Eggs are minute in size ~ 0.44 mm long x 0.26 mm wide, oval shaped, and yellow to pale green color. Under favorable conditions, first instar larvae hatched within 24 hours to 6 d after eggs are deposited (Harcourt 1957, Talekar and Shelton 1993, Capinera 2001). The incubation period may last up to 55 d in extremely low temperature of 6 to 4 °C; however, larvae that hatched at these temperatures are unable to survive to adult stages (Liu et al. 2002).

Once hatch, neonate larvae approximately 1.7 mm in size will start mining on spongy mesophyll of the leaf epidermis. The first and second instar (\approx 3.5 mm) larvae mining activities are less visible. First instar larvae are colorless but as it grows, the color changes to pale brown or pale green. In later instars, the head capsule becomes darker and black short hairs on the abdomen become more visible. Fully grown neonate larvae sized may reach approximately 10 mm long (Capinera 2001, Phillips et al. 2014). The larva can be distinguished from other lepidopteran larvae by the unique behavior when it being disturb. Diamondback moth larvae will wriggle backward and if it fell off from the leaves, it will also produce fine silken threads, which allow it to be hanged from

the foliage for some time (Harcourt 1957). Larval developmental period may last for 3 to 14 d depending on the climate (Harcourt 1957, Ooi 1986).

Larvae will stop defoliating leaves during the pre-pupal stage and after 1 to 3 d they will begin to spin silky cocoon, forming a white and loose oval shape cocoon (Golizadeh et al. 2007). Cocoons can be found sticking on the midrib of the foliage and most of the times larvae will pupate on the lower surface of the leaves. Pupal stage may last from 5 to 15 d depending on environmental conditions (Harcourt 1957, Talekar and Shelton 1993).

Temperature plays an important role in affecting the duration of each life stage, development time, and survival period of DBM (Golizadeh et al. 2007). Several studies proved that DBM have the ability to tolerate a wide range of temperatures, as low as 6.1°C and as high as 32.5 °C (Liu et al. 2002, Marchioro and Foerster 2011, Bahar et al. 2014). Development of DBM is rapid in warmer climates compared to cooler climates. In tropical regions, there can be as many as 20 generations per year whereas this can be restricted to 3 - 5 generations per year in cooler regions such as in the Northern United States (Capinera 2001).

Plant Injury

Diamondback moth larvae are known to be the damaging stage of this pest. Although the size of the larva is several times smaller than the cabbage looper and cabbage worm larva, the DBM larva causes more injury on cabbage leaves than the aforementioned cabbage pests. Injury on cabbage leaves is more significant as the second generation of the pest begins to emerge (Knodel et al. 2008). Another factor that affects the severity of injuries is the plant growth stage. The population of DBM may be more abundant when the cabbage plant begins to form folded leaves and in the early

pre-heading stage (Ayalew 2006, Knodel et al. 2008). Multiple defoliation during early formation of folded leaves (cupping stages) and pre-heading stages significantly reduced the cabbage head-weight, stem diameter, and root weight (Alishah 1987, Baidoo et al. 2012).

The feeding behavior of larvae differs for early and late instars. The neonate larvae mining on leaf surface cause irregular patches of white marks that look like window pane (Figure 2-1). Feeding by older larvae (3rd and 4th instar) cause more injury as they voraciously feed on cabbage leaves except the leaf veins (Ivey 2015). Heavy infestation of this pest can cause complete skeletonization of foliar tissues and disturb the formation of the cabbage head (Capinera 2001, Webb 2010).

Insecticide Resistance

In the 1940s control measures for DBM focused almost exclusively on insecticides; with a goal of eradication (Talekar and Shelton 1993). Diamondback moth population in Indonesia was first reported to develop resistance against DDT in 1953 (Johnson 1953). In early 1990s, the biological pesticide *Bacillus thuringiensis* (Bt) was reported to be less effective against DBM (Talekar and Shelton 1993, Tabashnik 1994, Ferrè and Van Rie 2002). Currently, DBM is reported to be resistant against > 95 compounds of insecticide (ARDC). It is among the top 20 of resistant arthropods; DBM was listed as the second most resistant arthropod after *Tetranychus urticae* Koch (Acari: Tetranychidae) (Whalon 2008).

In Hasting, Florida, where more than 50% of the cabbage acreage of the state were grown, Yu and Nguyen (1992) found that the DBM strain that were collected in the area exhibited resistance to six pesticides, pyrethroids (permethrin, cypermethrin,

fenvalerate, esfenvalerate, cyhalothrin, and fluvalinate) from 2132 to 82,475 fold. This pest was also shown to be resistant to other synthetic pesticides including to two carbamates (methomyl and carbofuran), five organophosphates (OPs) (chlorpyrifos, methyl parathion, malathion, methamidophos, and diazinon) and a cyclodiene (endosulfan) with resistance of 409-504 fold, 20-73 fold and 25 fold, respectively (Yu and Nguyen 1992).

There are several factors that contribute to the rapid development of pesticide resistance among DBM population worldwide. These includes high reproductive rate and rapid generation times, continuous growing season of host plants, and monoculture agricultural practices (Talekar and Shelton 1993). Additionally, overuse of synthetic insecticides from few classes as the main control tactic has increased the potential for insecticide resistance. In developing countries, such as Malaysia, cabbage is grown on a small scale mostly in the highland areas. Whereas, in develop countries such as United States, cabbages are planted on a commercial scale. Both develop and developing countries used insecticides as primary control strategies to prevent crop damage by insect pests (Talekar and Shelton 1993). A survey on insecticide usage among cabbage growers in the Cameron highlands, Malaysia indicated that 96% of growers were highly dependent on insecticide applications as the major control tactics to manage cabbage pests (Mazlan and Mumford 2005). Pesticides are used at least on average of 2 times per week during the production season (Mazlan and Mumford 2005).

The intensive and repeated use of broad-spectrum insecticides can also lead to negative effects on non-target organisms including reducing the potential of natural enemies to regulate pests population (Bommarco et al. 2011), harmful to pollinators,

and contamination of the environment by contaminating ground water (Talekar and Shelton 1993, Orr 2009, Furlong et al. 2013).

Integrated pest management to control DBM on crucifers includes routine monitoring of crop injury and pest populations, conservation of natural enemies, cultural control, and application of selective insecticides (Orr 2009, Furlong et al. 2013, Philips et al. 2014).

Monitoring

Efficient and effective monitoring of crop injuries and DBM population in the field is an essential step in IPM. This step is crucial to determine the presence of DBM in the field and to establish guidelines for proper management (Orr 2009). Monitoring can be conducted using various sampling techniques such as visual examination on plant injuries due to larval feeding, *In-situ* counts of DBM immature stages (Philips et al. 2014), deployment of sticky cards, pheromone-baited traps (Miluch et al. 2013), and sweep netting (Dosdall et al. 2011).

In situ counts and visual observations give an estimation of larval density while other techniques as mentioned above yield an estimation of adult DBM density (Dosdall et al. 2011, Philips et al. 2014). Although information obtain from both methods can be useful to predict pest populations, *In-situ* counts provide a more accurate estimation of pest populations in the field (Dosdall et al. 2011). This information is crucial to establish guidelines for proper decision making. Therefore, *In-situ* counts will be used as the main tool for monitoring pest populations. Additionally, deployment of yellow sticky cards and pitfall traps will be installed to observe the population of natural enemies especially parasitoids and ground crawling predators.

Routine monitoring is important in predicting the severity of the infestation and as an indicator for management action. Once the number of the pest reached the economic threshold level (ET), management action should be taken to avoid crop damage. The economic threshold level varies with the type of cash crop grown (Philips et al. 2014). Threshold in warmer climates are usually lower compared with cooler climates. For example, a lower threshold is used in southeastern states such as Georgia and Florida as compared to other more northern states (New York and Virginia) in US. A ET of one or more larvae feeding on the leaf is used as a threshold for DBM in Florida and Georgia (Capinera 2001). Effective monitoring is important to determine when and where control action is warranted.

Reduced-Risk Pesticides

Application of reduced-risk pesticides as a last resort is considered as a major component of an IPM program for many pests including DBM (Sarfranz and Keddie 2005). The term of “reduced risk” introduced by Environmental Protection Agency (EPA), to reflect insecticide with less risk to the operator, human health and environment (Fishel 2013). Reduced-risk pesticides are usually compatible to be used with other management tactics in IPM such as the release of a biocontrol agent (Fishel 2013). Examples of reduced-risk pesticides include botanical based pesticides that are derived from plant materials or extracts of essential oils. These include neem *Azadirachta indica* (Meliaceae), and pyrethrum *Chrysanthemum cinerariaefolium* (Asteraceae). Azadirachtin is commonly used in IPM program as an antifeedant tool for many insects. Several formulations with different trade names containing azadirachtin as the active ingredient can be found on the market such as Neemix (Certis USA, L.L.C, Columbia, MD), Azatrol EC (Gordon corp., Kansas City, MO), and Aza-Direct® (Gowan

company, Yuma, AZ). Azadirachtin is known to have various effects on insect pests, which include as antifeedant (Liang et al. 2003), insect growth regulator, and an oviposition deterrent (Isman 2006). Azadirachtin can be applied either as a stand-alone application or in combination with other biological pesticide such as *Bacillus thuringiensis* (Bt) or Entrust® (Spinosad). Several studies show an increase in marketable yield of cabbage when the field was treated with combination of botanical and biological pesticides. This includes application of Aza-direct® and Bt. (DiPel DF, Valent BioSciences Corporation, Libertyville, IL) on a rotational basis (Reddy 2011).

Azera® is the newest formulation that contains a combination of 1.2% azadirachtin and 1.4% pyrethrins (extracts of *Chrysanthemum cinerariaefolium* Vis.). At present, only few studies (Pezzini and Koch 2015, Morehead 2016) have shown the efficacy of this Azera® against key arthropod pests. However, to date only one paper by (Seaman et al. 2015) that was published show a significant difference of Azera® treatment plot compared to other treatments (untreated, Venerate, Grandevo, Veratran D, Nu Film P and Surround) against DBM.

Other formulations of reduced-risk pesticides that are available are microbial-based insecticide such as *Bacillus thuringiensis* (Bt), *Saccharopolyspora spinosa*, Entrust®/ Spinosad, and *Chromobacterium subtsugae*, Grandevo®. Application of Bt in the early 1980s significantly reduced the amount chemical insecticide treatments in cabbage fields to about 50% (Biever 1996). However, as new tools become available, Entrust® (Spinosad, Dow AgroScience LLC, Indianapolis, IN) was shown to provide consistent control of DBM and others lepidopteran pests compared with Bt. (Dipel and

XenTari) and Novaluron (insect growth regulator) in Cole crops (Maxwell and Fadamiro 2006).

A three-year study (1998-2001) by Burkness and Hutchison (2008) showed that implementation of reduced-risk pesticides together with effective monitoring reduced pesticide residues and improve application timing for fresh-market cabbage. This study also showed an increase in marketable-yields, which resulted in an increase of the net profit for cabbage production.

Biological Control

Natural enemies (predators and parasitoids) play an important role in regulating and maintaining pest density in agroecosystem at tolerable levels (Talekar and Shelton 1993, Philips et al. 2014). Beneficial arthropods can occur naturally in agroecosystems and the population can also be enhanced by providing habitats for egg laying and adequate food sources (van Lenteren 2012). In agroecosystem that have low population of natural enemies, augmentive or inundative releases of control agents might be appropriate tactics that can be adopted (Collier and Van Steenwyk 2004).

Diamondback moth is known to have numerous natural enemies that attacked all life stages of this pest (Philips et al. 2014). Adult DBM often attacked by birds and spiders (Talekar and Shelton 1993). Meanwhile, other predators including carabid beetles (Suenaga and Hamamura 1998), ants, lacewings, big-eyed bugs, and staphylinids beetles were reported to cause mortality on DBM larvae (Harcourt 1957). Spiders were also known to attack 3rd and 4th instars of DBM larvae (Nemoto 1986). Delvare (2004) reported that DBM were known to be parasitized by more than 135 parasitoids world-wide. However, only few species are commonly used as biological control agents in IPM (Sarfranz et al. 2005), and are effective at controlling this pest (Lim

1986). These include parasitoids from family Ichneumonidae (genus; *Diadegma* and *Diadromus*), Braconidae (genus; *Microplitis* and *Cotesia*), and Eulophidae (genus; *Oomyzus*) (Lim 1986, Sarfraz et al. 2005). Although numerous beneficials were reported to attack DBM, most research has focused on larval parasitoids. These are considered as more effective control agents and species/strain specific, thus providing more targeted control against DBM (Furlong et al. 2013). However, to ensure that the biological control tactics will be successful, it is important to accurately identify which DBM life stages is being targeted, species and strain of both parasitoids and pest before any release program is initiated (Sarfraz et al. 2005).

Sarfraz et al. (2005) suggested that since parasitoids and DBM were shown to be strain specific, obtaining parasitoids from the pests' native area would lead to more promising and effective control agent. Additionally, native natural enemies (predators and parasitoids) also play an important role in regulating secondary pest of cabbage such as aphids and whitefly (Razze et al. 2016). Therefore, conserving beneficial insects from native population can provide a more sustainable control of pest complex in the field. Furthermore, a study by Kfir (2005) indicated that the integration of naturally occurring and introduced control agents in conjunction with application of bio-pesticides will help to reduce DBM population.

Cultural Control

Cultural control is another major component of IPM in managing DBM. Cultural tactics involve manipulating the agroecosystem so that it becomes less favorable for the pests while maintaining the high productivity of the cash crop. Since cultural control does not directly cause pest mortality, this tactic is useful in conserving naturally occurring predators and parasitoids in agroecosystem (Dhawan and Peshin 2009).

Integration of cultural control tactics with other IPM component such as a biorational pesticide can help to reduce DBM population (Philips et al. 2014). Examples of cultural control tactics include the use of companion plants, intercropping, trap cropping, crop rotation, and field sanitation of plant materials (Talekar and Shelton 1993).

Companion planting is the planting of two or more crops concurrently within the same field (Smith and Liburd 2012). The companion planting can either be different species or non-host crops (Talekar and Shelton 1993). This practice is normally adopted by small scale farmers where they intensively used available crop land to maximize the profit returns (Talekar and Shelton 1993). Companion planting with non-host floral plants into cabbage fields will serve to enhance the diversity of parasitoids and predators in the field by providing oviposition sites, food source and shelter for natural enemies. This strategy will increase the biological control activity which will eventually suppress the pest population overtime (Wäckers 2004, Lu et al. 2014). Several floral plants have been used as companion plants in cabbage fields including *Centaurea cyanus* (cornflower) (Ditner et al. 2013, Généau et al. 2013, Balmer et al. 2014), *Tagetes patula nana* (french marigold) and *Calendula officinalis* (pot marigold) (Jankowska et al. 2009). These studies showed a significant increase in arthropods species richness when using floral plants as companion plants.

In addition to floral plants, other crops can be used in companion planting systems including *Allium sativum* (garlic) (Cai et al. 2010, Karavina et al. 2014), Celery (Bavec et al. 2012), and onion (Asare-Bediako et al. 2010). Garlic and onion belongs to the plant family Amaryllidaceae and are known repellents against certain types of insects (Boulogne et al. 2012, Mobki et al. 2014) including those that commonly attack

cabbage (Mousa et al. 2013). Other plants that have been documented to repel pests of Brassicaceae and can be used as companion plants include sage (*Salvia officinalis* L.), rosemary (*Rosemarinus officinalis* L.), thyme (*Thymus vulgaris* L.), dill (*Anethum graveolens* L.), and mint (*Menta* L. spp.) (Parker et al. 2013), yellow rocket (Badenes-Perez et al. 2004, 2005), non-glossy collards (Mitchell et al. 1997, Shelton and Nault 2004, Musser et al. 2005), indian mustard, and wild mustard (Shelton and Badenes-Perez 2006) has been used as trap crops instead of companion plants because these plants attract DBM to oviposit on them.

Besides cabbages, there are other crops where companion planting has been known to reduce pest complex in other vegetable crops such as squash (Razze et al. 2016), bell pepper (Bickerton and Hamilton 2012), and tomatoes (Mutisya et al. 2016). For example, buckwheat that was planted within squash crop has showed a reduction of aphids' densities and reduces the transmission of aphid-borne pathogens while promoting natural enemies population (Razze et al. 2016). Similarly, study by Bickerton and Hamilton (2012) where they planted three flowering plants including Dill, *Anethum graveolens* L., coriander, *Coriandrum sativum* L., and buckwheat, *Fagopyrum esculentum* Moench on the edge of bell peppers proved to increase natural enemies densities and reduced the population of aphids in the companion planting system.

Red sorrel/Roselle (hereafter referred as roselle), *Hibiscus sabdariffa* L. (family Malvaceae) is a common crop in many tropical and sub-tropical regions of the world, including Africa and the Caribbean. This plant is native to Asia or Tropical Africa (Morton 1987). Its calyx is edible and is used for drinks, jams, and jellies. The calyxes produced a cranberry like flavor (Julia 1987) and the foliage gives off a pungent smell

and are sometimes used as greens in salads and stews. In Florida, the plant was initially grown as an ornamental and commonly planted as home garden crop. Despite the edible part of this plant, it is also known to be resistant to root-knot nematode (Wilson and Menzel 1964) and have potential to be used as alternative method in controlling leaf cutting ants (Boulogne et al. 2012). The fruits and leaves of this plant contain phenolic compounds (anisaldehyde) that were identified to show insecticidal activities (Mahadevan et al. 2009, Boulogne et al. 2012). Our goal was to: a) intercrop Roselle into a primary cash crop (cabbage) to determine if there are any reductions in pest populations and, 2) evaluate fruit dip solutions of Roselle to determine if there is any activity against key insect pests in cabbage (DBM).



Figure 2-1. "Window pane" cause by mining activities of diamondback moth larvae.
Photo courtesy of Z. Mazlan.

CHAPTER 3 COLONIZATION OF ORGANIC CABBAGE BY KEY PESTS AND BENEFICIAL INSECTS IN THE PRESENCE OF COMPANION PLANTS

In the United States (US), cabbage is produced mainly for the fresh market, contributing 5% of total fresh market production in 2015 (USDA/NASS 2014). Although cabbage is traditionally grown using conventional practices, there is an increasing demand for green agriculture (chemical-free food products) and a rapidly emerging organic industry (Rigby and Cáceres 2001, Balusu et al. 2015). In organic agriculture, management of cabbage pests includes the integration of several approaches such as routine monitoring of plant injury, pest, and beneficial insect populations, cultural practices, enhancement of natural enemies and application of reduced-risk insecticides that are labelled for organic use (Orr 2009, Furlong et al. 2013, Philips et al. 2014).

Companion planting is one of the cultural practices used in managing cabbage pests. Parolin et al. (2012) defined companion planting as secondary plants that are grown close to the main crop that influence the 1st trophic level by nutrition enhancement and/or chemical defense of the main crop. This tactic also aims to encourage the population and enhance of locally existing natural enemies (Simpson et al. 2011, Parolin et al. 2012). Therefore, companion planting may provide sustainable management of cabbage pests which complements other IPM approaches in organic agriculture.

In previous studies, cabbage pest populations were reduced when cabbage was intercropped with onion, tomato (Asare-Bediako et al. 2010), garlic or lettuce (Cai et al. 2010) compared to a monoculture system. Floral plants including French Marigold (*Tagetes patula* nana 'Kolombina') and Pot Marigold (*Calendula officinalis* 'Promyk') were intercropped with cabbage and shown to reduce cabbage pest populations

including the cabbage aphid *Brevicoryne brassicae* L., flea beetles *Phyllotreta* spp., imported cabbageworm *Pieris rapae* L., large white butterfly *P. brassicae* L., cabbage moth *Mamestra brassicae* L. and DBM (Jankowska et al. 2009). Floral plants improved the agroecosystem by providing food sources and suitable habitats (Pickett and Bugg 1998) that enhanced the establishment of natural enemies (Landis et al. 2000). Cai et al. (2010) suggested that integration of an intercropping system allowed sustainable (long term) management of cabbage pests, specifically diamondback moth (DBM). Additionally, this integration approach improved the timing of insecticide applications (Philips et al. 2014).

Collards (*Brassica oleracea*) and marigolds (genus *Tagetes*) have been commonly planted with cabbage. A study conducted by Badenes-Perez et al. (2004) showed that DBM preference to oviposit on collards was 300 times greater than on cabbage. DBM preferred host (collards) can be intercropped with the main crop cabbage in organic systems where pest management tools are limited. Previous studies demonstrated that marigold attracts generalist predators including ladybug beetles *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) (Adedipe and Park 2010) and minute pirate bugs *Orius insidiosus* Say (Hemiptera: Anthocoridae) (Gredler 2001). Marigold was also found to attract different species of parasitoids wasps (Gredler 2001).

Another plant that has potential to be integrated in a companion planting system is roselle (*Hibiscus sabdariffa* Herb). This plant was also shown to be attracting generalist predators including different species of ladybird beetles *Coccinella undecimpunctata* L. and *Scymnus interruptus* Mulsant and minute pirate bugs (Abdel-Moniem and Abd El-Wahab 2006). In cabbage system, generalist predators are known

to feed on lepidopteran larvae, and other cabbage pests, consequently regulating pest populations. Roselle had been previously intercropped in tomato where it increased parasitoid diversity (Smith et al. 2001).

Implementation of companion planting into traditional monocrop systems shows potential for sustainable management of cabbage pests, reducing the frequency and amount of insecticides and enhancing natural enemies. The objectives of this study were to 1) evaluate the colonization of key pests on organic cabbage including DBM, imported cabbageworm (CW), cabbage looper *Trichoplusia ni* (Hübner) (CL), and aphids in the presence of companion plants, 2) record how selective companion plants and an insecticide (Entrust®) labelled for organic use affect natural enemy populations and marketable yields in cabbage system.

Materials and methods

Study Site

The study was conducted for two growing seasons from Feb to May in spring 2016 and spring 2017. Both studies were located at the University of Florida Plant Science Research and Education Unit (PSREU) in Citra, (location: 29.410868N, 82.141572W) Marion County, Florida (Figure 3-1).

Plant Material

Bravo Cabbage *Brassica oleracea* var. *capitata* (Urban Farmers Seed, Westfield, IN) was the main crop for these studies. Three companion plants were evaluated which includes roselle *Hibiscus sabdariffa* Herb. (SeedArea, Hong Kong, China), lemon star marigolds *Tagetes tenuifolia* Cav. (Johnny's Selected Seeds, Winslow, ME) and collards Champion *Brassica oleracea* var. *acephala* (Johnny's Selected Seeds, Winslow, ME).

In 2016, cabbage and collard seeds were first grown in the Small Fruit and Vegetable IPM (SFVIPM) greenhouse using standard production techniques (Zotarelli et al. 2017). Seeds were planted in organic garden soil potting mix (Miracle-Gro, Marysville, OH) in seedling trays. Seedlings were irrigated manually three to four times per week. After six weeks on Feb 17, 2016, cabbage and collard seedlings were transplanted to the field. On the same day, marigold and roselle seeds were also hand seeded onto the respective bed.

In 2017, cabbage and collard seeds were planted in seedling trays and were grown in the SFVIPM greenhouse for 7 weeks, while marigolds and roselle were grown for 4 weeks. The sowing of marigold and roselle during 2017 was to synchronize the blooming periods for marigolds and roselle with the growth of the cabbage plants. All seedlings were transplanted to the field on Feb 22, 2017.

Crop Management and Experimental Design

Bravo cabbage seedlings were planted 30 cm apart on raised beds covered with black plastic mulch (TriEst Ag Group Inc., Greenville, NC). Each treatment was established on plots containing two beds with double irrigation lines installed for each bed. Each bed consisted of two rows of cabbage (one row on each side of the bed) and one row of the respective companion plant treatment in the middle. In 2016, each bed was measured 3.0 m x 0.9 m while in 2017 wider beds measuring 3.0 m x 1.2 m were prepared. This modification allowed a wider space for companion plants to grow.

Five treatments with four replications were arranged in a randomized complete block design (RCBD). Treatments include cabbage planted with 1) roselle, 2) marigolds, 3) collards, 4) no companion plant and treated with Entrust[®] (Dow AgroScience LLC, Indianapolis, IN) plot (positive control), and 5) no companion plant and an untreated plot

(negative control). In the positive control plots, Entrust® was applied biweekly at the recommended rate of 0.29 L per ha using a backpack sprayer (model 425, SOLO, Newport News, VA) fitted with XR Teejet nozzle (11004 VK). Total of five applications of Entrust® were applied throughout the growing season. Liquid fertilizer (N-P-K: 6-0-8, Mayo Fertilizer, Lafayette, FL) was applied weekly at 236 liter per ha.

In 2016, a cover spray of *Bacillus thuringiensis* (Bt) (DiPel DF, Valent BioSciences Corporation, Libertyville, IL) was applied two weeks before harvesting at the rate of 1.12kg per ha on all treatment plots to suppress the high population of DBM. In 2017, cutworms *Agrotis ipsilon* Hufnager, CW and CL became a significant problem, therefore four cover sprays with Bt were applied throughout the 3-month cropping periods. Two applications to reduce infestation of cut worms early in the growing season, and then two applications to reduce CW and CL later in the growing season. Cover sprays were applied to all treatments therefore there were no effects of treatment differences.

Sampling

Diamondback moth, other cabbage pests and natural enemies were sampled using visual counting (*In-situ*), yellow sticky Pherocon AM unbaited traps (Great Lakes IPM, Vestaburg, MI, USA), and pitfall traps. In 2016, weekly sampling was conducted from Mar 18 to May 6. In 2017, weekly sampling was conducted from Mar 15 to May 13.

Estimating Pest Population

Cabbage pests and other insects were visually counted (*In-situ*) every week. Five randomly selected cabbage plants and four companion plants from each plot were observed and pest and natural enemy populations were recorded. Visual counting was

conducted starting from the second week after transplanting and ended a week before harvest.

Predators and Parasitoids Densities among Companion Plants and Entrust® Treated Plots

In 2016, two yellow sticky traps were deployed on each bed throughout an 8 week period. In 2017 yellow sticky traps were reduced to one trap per bed and sampling was conducted for a 10 week period. Yellow sticky traps were collected and replaced weekly after 5 d throughout the growing season. In 2016, sampling was conducted from Mar 18 through May 6 and in 2017 data was collected from Mar 15 through May 17. Yellow sticky traps measuring 14 cm x 11.5 cm (Great Lakes IPM, Vestaburg Michigan) were used to observe the presence of natural enemies (predators and parasitoids). Additionally, these cards also gave relative density information on other cabbage pests including adult DBM, alate aphids, adult whiteflies, thrips and plant hoppers.

Traps were brought back to SFVIPM and assessed under a 10X dissecting microscope. The number of cabbage pests and natural enemies caught on the traps were recorded and natural enemies were identified to the family level.

To observe the parasitism activities on each treatment plot, a total of 20 DBM pupae per treatment were hand collected and were labelled according to the treatment. However, no pupae were found on the Entrust® treated plots. All pupae collected were kept for 14 d in the environmental chamber at a temperature of 26 °C with 63% RH, and a light: dark cycle of 16: 8 respectively. After 14 d, the number of parasitoids or DBM adults that emerged was recorded. Parasitoids that emerged were identified to the species level. After 3 week, inactive cocoons were dissected to observe the presence of dead parasitoids or adult DBM.

Ground predators were observed using one pitfall trap per treatment which was installed in either one of the two beds. A hole measured about 14.5 cm deep was dug in the middle of the bed and a pitfall container sized (14 cm height, 11 cm in diam.) was placed inside the hole. The container was filled with one quarter of water with few drops of dish liquid (Ajax®, Colgate-Palmolive, NY) to break the surface tension. Pitfall traps were left in the field for 7 d and sampling was conducted biweekly from Mar 18 to Apr 29 in 2016 and from Mar 22 to May 3 in 2017. The contents were brought back to the laboratory and assessed under a 10X dissecting microscope. The number of natural enemies were recorded and identified to the family level.

Marketable Cabbage

In both seasons, twenty cabbage heads were harvested randomly from each treatment. Cabbage heads with no observable insect damage to minor damage (no damage after removing 4 folded leaves) were rated as marketable, while cabbage heads with obvious to severe damage were rated as non-marketable (Figure 3-2). Both marketable and non-marketable heads were counted and weighed separately.

Data Analysis

The assumption of normality of the data was first examined and data that did not meet these assumptions were square-root transformed to fit the model. The data collected was analyzed using repeated measures analysis of variance procedures (ANOVA; PROC GLM, SAS Institute 2013) with treatment, time and treatment × time as the fixed effects to determine if there were any differences between insect counts and time. The data were then pooled together and an analysis of variance (ANOVA) was used to determine if treatment means were significantly different (SAS Institute Inc.

2013). Means were compared using a least significant difference (LSD) test. For all statistical tests, $\alpha = 0.05$.

Results

Main Cabbage Pests

***In-situ* counts in 2016.** Overall, with the exception of marigold, all treatments reduced populations of DBM compared with the control ($F = 27.74$; $df = 4,760$; $P < 0.001$). Plots treated with Entrust® had the lowest number of DBM and were significantly lower than all the other treatments. Roselle as a companion plant was the second-best treatment after Entrust® in reducing the population of DBM; however, it was not significantly different from the collard treatment. Marigold as a companion plant was not significantly different from the control and the collard treatment (Figure 3-3).

Weekly *In-situ* counts for DBM in 2016 showed that significant differences varied throughout the 8 weeks of sampling (Table 3-1). There were no differences among all the treatments for week 1 ($F = 1.63$; $df = 4,95$; $P = 0.17$), week 2 ($F = 1.31$; $df = 4,95$; $P = 0.27$), and week 3 ($F = 2.13$; $df = 4,95$; $P = 0.08$). However, during the fourth week, only plots treated with Entrust® had significantly fewer DBM than the control ($F = 5.01$; $df = 4,95$; $P = 0.001$). During the fifth week, plots treated with Entrust®, and plots that had collard planted as a companion plant had significantly fewer DBM than the control ($F = 11.41$; $df = 4,95$; $P < 0.0001$). None of the other treatments were significantly different from the control. During the sixth week, all treatment plots had significantly fewer DBM than the control ($F = 12.44$; $df = 4,95$; $P < 0.0001$). Plots treated with Entrust® had significantly fewer DBM than the other treatments. During the seventh week, only plots treated with Entrust® had significantly fewer DBM than the control ($F = 9.83$; $df = 4,95$; $P < 0.0001$). During the final week, only plots treated with Entrust® and

plots with treated with roselle planted as a companion plant had significantly fewer DBM than the control ($F = 4.63$; $df = 4,95$; $P = 0.002$) (Table 3.1).

***In-situ* counts in 2017.** Three major lepidopteran pests including DBM, CW and CL were recorded (Table 3-2).

There were significantly fewer DBM larvae observed in cabbage treated with Entrust® compared with the other treatments. All other treatments were not significantly different from each other (Table 3-2). Throughout the sampling period, plots treated with Entrust® had significantly fewer DBM at week 5 (50 d: $F = 2.88$; $df = 4,95$; $P = 0.03$), week 7 (64 d: $F = 3.09$; $df = 4,95$; $P = 0.02$), and week 8 (70 d: $F = 4.30$; $df = 4,95$; $P = 0.003$) after planting compared with the control. There were no significant differences among other treatments for DBM population (Table 3-3).

Significantly fewer CW larvae were recorded in plots treated with Entrust® compared with other treatments (Table 3-2). Among the companion plants significantly fewer CW larvae were recorded in cabbage interplanted with collards compared with control, but this treatment was not significantly different from marigold and roselle treatments (Table 3-2). Both of these treatments (marigold and roselle) had numerically lower numbers of CW compared with control. Over the 9 weeks of sampling, significant differences in CW populations were recorded at weeks 5, 6, and 7 (43, 50 and 58 d) after planting. (Table 3-4). During the 5th week of sampling cabbage plots interplant with roselle and marigold, and treated with Entrust® had significantly fewer CW than the control ($F = 3.2$; $df = 4, 95$; $P = 0.02$). During the 6th week of sampling all treatments had fewer CW compared with the control ($F = 3.82$; $df = 4,95$; $P = 0.006$). During the 7th

week all treatments had fewer CW than cabbage interplanted with roselle ($F = 4.26$; $df = 4,95$; $P = 0.003$).

There was no significant difference among the treatments for CL overtime and total counts (Table 3-2).

Yellow sticky traps observations. In 2016, fewer adult DBM were captured in Entrust® plots compared with all the other treatments ($F = 5.58$; $df = 4,600$; $P = 0.0002$). Plots that were intercropped with roselle had significantly fewer DBM than the collard treatment; however, these counts were not significantly different to the control and marigold treatment (Table 3-5).

In 2017 there was no significant difference in the number of DBM captured on yellow sticky traps (Table 3-6).

Secondary Pests

***In-situ* counts in 2016.** Aphids species include the green peach aphid *Myzus persicae* (Sulzer) and the cabbage aphid *Brevicoryne brassicae* (Linnaeus). The whitefly species recorded was sweet-potato whitefly biotype B *Bemisia tabaci* (Gennadius). The principle thrips species recorded was Florida flower thrips *Frankliniella bispinosa* (Morgan). There were no significant differences among secondary pests including aphids, whitefly, and thrips for *In-situ* counts in 2016 (Table 3-7).

Yellow sticky traps in 2016. The species of secondary pests recorded were similar to those observed in *In-situ* counts. There were no differences among treatments for aphids and adult whiteflies. (Table 3-5).

***In-situ* counts in 2017.** Secondary pests that were recorded include armyworms (*Spodoptera* spp.), aphids, whiteflies, and blister beetles (Meloidae) but populations of the secondary pests were not significantly different between treatments (Table 3-8).

Yellow sticky traps observations in 2017. Overall, cabbage plots interplanted with marigolds and roselle had significantly fewer pests (DBM, aphids, whiteflies, and thrips) than collard and Entrust® (Table 3-6). The number of aphids captured was not significantly different between treatments for overall and weekly captures. However, there were significantly fewer adult whiteflies collected in cabbage plots treated with marigolds compared to the other treatments (Table 3 -6). Significantly fewer adult whiteflies were also collected in cabbage plots treated with roselle compared to plots treated with Entrust® and interplanted with collards. Finally, plots interplanted with marigolds had significantly higher thrips population compared with the other treatments (Table 3-6).

Beneficial Insects

Predators count in 2016. Overall, significantly higher number of predators were recorded on cabbage plots interplanted with marigolds compared with Entrust®, roselle and collards (Figure 3-4). The predators observed on yellow sticky traps include ants (Formicidae), green lacewings (Chrysopidae), brown lacewings (Hemerobiidae), minute pirate bugs (Anthocoridae), ladybird beetles (Coccinellidae), and spiders (Araneae). There were no differences in predator numbers on yellow sticky traps between all treatments (Table 3-9). The predators that were captured in pitfall trap include ground beetles (Carabidae), spiders, ants, ladybird beetles, and dragonflies (Coenagrionidae). Among the predators captured, only ground beetles showed significant differences between treatments. Cabbage plots interplanted with roselle had significantly higher

populations of ground beetles compared to the other treatments except the control plots. Also cabbage plots interplant with collard had fewer ground beetles than the control (Table 3-10).

Predators count in 2017. Predators observed on yellow sticky traps included ants (Formicidae), green lacewings (Chrysopidae), minute pirate bugs (Anthocoridae), ladybird beetles (Coccinellidae), spiders (Araneae), predatory thrips (Aeolothripidae), ground beetles (Carabidae), rove beetles (Staphylinidae), big-eyed bugs (Geocoridae), and tachinid flies (Tachinidae) (Table 3-11). Among these families, only minute pirate bugs showed significant difference between treatments. There were significantly more predators (Figure 3-5), specifically minute pirate bugs recorded in plots interplanted with marigolds compared to the other treatments (Table 3-12). Among the ground crawling predators captured in pitfall traps (ants, spiders, and ground beetles), there were significantly more carabids (ground beetles) recorded in the marigold treatment compared to the other treatments (Table 3-12).

Parasitoids count in 2016. There were more parasitoids recorded in the control plots compared with all the other treatments. I recorded no difference in parasitoid numbers when cabbage was intercropped with collards, roselle and marigolds. Numerically, plots treated with Entrust[®] had the lowest number of parasitoids but these were not significantly different to plots intercropped with roselle, collard and marigold (Figure 3-6).

There were significant differences among selected parasitoid families (Table 3-13). In all, 20 families of parasitoids were recorded including Aphelinidae, Chalcididae, Encyrtidae, Eulophidae, Eupelmidae, Eurytomidae, Mymaridae, Pteromalidae,

Signiphoridae, Trichogrammatidae, Ceraphronidae, Megaspilidae, Bethyridae, Figitidae, Evaniidae, Brachonidae, Ichneumonidae, Mymarommatidae, Platygasteridae and Diapriidae. Significant differences were recorded within the family Pteromalidae. Yellow sticky traps in plots interplanted with roselle had significantly fewer pteromalids than the other treatments ($F = 2.74$; $df = 4,600$, $P = 0.03$). In the family Trichogrammatidae, plots treated with Entrust® had significantly lower counts than all the other treatments (Table 3-5: $F = 10.52$; $df = 4,600$; $P = 0.0001$). There were no differences in trichogrammatid populations between plots interplanted with marigold, roselle, and collards; however, these counts were significantly lower than the control. There were more ceraphronids in the control, collard, and Entrust® treatments compared with plots interplanted with marigolds ($F = 3.32$; $df = 4,600$; $P = 0.01$). The population of brachonids in treatments of marigold, roselle and collard were significantly lower than Entrust® ($F = 3.22$; $df = 4,600$; $P = 0.01$). Among the platygasterids, plots interplanted with marigold and roselle had significantly lower populations than the control, Entrust®, and collard (Table 3-5: $F = 3.96$; $df = 4,600$; $P = 0.004$).

Parasitoids count in 2017. Significantly more parasitoids were recorded in plots interplanted with marigolds compared to the other treatments. Significantly more parasitoids were also collected from plots interplanted with roselle compared to the control and Entrust® treatment. Cabbage plots interplanted with collards, treated with Entrust® and the control had fewer beneficial insects and were not significantly different from each other (Figure 3-7). Throughout the season, the number of parasitoids increased in selected treatments and significant differences were observed among treatments from 43 d after planting until the end of the season. Parasitoid populations

peaked at 64 d after planting in all treatments except for roselle, which peaked later at 78 d (Table 3-14).

Similar to the previous year's observations, there were 20 families of parasitoids recorded in 2017. Significant differences were observed on selected parasitoid families including Aphelinidae, Encyrtidae, Ceraphronidae, and Platygastriidae (Table 3-15). Significantly more aphelinids were recorded in cabbage plots interplanted with roselle and collards compared with the other treatments. Significantly fewer aphelinids were recorded in the plots treated with Entrust[®] compared to the control, but were not significantly different from the marigold treatment. There were significantly more encyrtids recorded in the marigold treatment compared to the Entrust[®] treatment and the control, but this was not significantly different from the roselle and collard treatments. There were significantly more mymarid parasitoids recorded in plots interplanted with roselle compared to plots treated with Entrust[®] and the control, but numbers were not significantly different from marigold and collard treatments. There were significantly more parasitoids in the family Ceraphronidae in the marigold treatment compared to the other treatments. There were also significantly more ichneumonids recorded in plots interplanted with roselle, collards, and the control compared to the marigold treatment. In contrast, there were significantly more platygastriids recorded in the marigold treatment compared with other treatments. Plots interplanted with roselle also had significantly more platygastriids compared to the Entrust[®] treatment (Table 3-15).

From DBM larvae that were parasitized and collected from the field, three species of parasitoids were reared from the larvae including *Diadegma insulare*

Cresson (Ichneumonidae) (Figure 3-8), *Oomyzus* sp. (Eulophidae) (Figure 3-9, 3-10), and *Conura* sp. (Chalcididae) [Figure 3-11, 3-12].

Marketable Yield

In both years, the plots treated with Entrust® had significantly greater yields compared to the other treatments for 2016 ($F = 13.44$; $df = 4,15$; $P < 0.0001$) and 2017 ($F = 4.07$; $df = 4,15$; $P = 0.02$) (Figure 3-13). Plots treated with Entrust® had an average of 30X more cabbage heads than the other treatments during 2016 and 6X as many cabbage heads compared to the other treatments in 2017. There were no significant differences between the control and the companion planting treatments. Injuries from DBM were so high in cabbage interplanted with roselle and collards that no marketable yield could be assessed.

Discussion

Major Cabbage Pests

One of the objectives of this study was to investigate the colonization of key cabbage pests on cabbage interplanted with selected plants or treated with the reduced-risk insecticide Entrust®. As expected our findings indicate that Entrust® provided the most consistent and effective control of DBM over the two-year study. This was not surprising since other researchers including Maxwell and Fadamiro (2006) reported that Entrust® provided the most consistent and lowest mean damage ratings against lepidopteran pests in cole crops. Entrust® belong to the spinosad group in the class Naturalytes. It is derived from the fermentation of the bacterium *Saccharopolyspora spinosa* Mertz and Yao (Sparks et al. 1998) and is effective against lepidopteran and thrips (Maxwell and Fadamiro 2006, Liburd et al. 2017). Regardless of the effectiveness of Entrust®, there are restrictions (for resistance management) on the

number of applications or the amount of Entrust® that can be used in one growing season. Organic growers have the option of rotating Entrust® with *Bacillus thuringiensis* (Bt) for management of lepidopteran complex in cole crops. However, field populations of DBM have been shown to develop resistance to commercial formulations of Bt (Tabashnik 1994).

Interplanting insect deterring plants such as roselle or marigold are alternatives to relying exclusively on insecticides that are labelled for organic use. In my study, fewer DBM were found in cabbage plots interplanted with roselle and collards in 2016. It is unclear why a reduction in DBM was found in the roselle; however, Al-Mamun et al. (2011) recorded 100% mortality against *Tribolium castaneum* Herbst (Tenebrionidae) using fruit extracts from roselle (*Hibiscus sabdariffa*). They concluded that the mortality of *T. castaneum* was related to the insecticidal properties of roselle but failed to discuss the exact nature of these insecticidal properties. Similarly, in my laboratory bioassay with roselle (detailed in Chapter 4), I found evidence of oviposition deterrence or repellency effects where DBM adults oviposit fewer eggs on cabbage leaf discs treated with roselle extracts compared with untreated discs. Additional studies indicated that DBM larvae avoided cabbage discs treated with roselle extracts in favor of untreated discs. It is hypothesized that glycosides, alkaloids (Faizi et al. 2003), saponins, flavonoids, and steroids that are associated with roselle extracts (Tolulope 2007) may have contributed to a reduction in DBM population.

Besides roselle, other plants in the same family Malvaceae and or genus are known to have insect deterring effects. These include rose of Sharon, *Hibiscus syriacus* L. (Bird et al. 1987), and globemallow *Sphaeralcea emoryi* Torrey (Honda and Bowers

1996), which exhibited a deterrent effect on feeding and oviposition by the boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). Bird et al. (1987) also reported that fatty acids and methyl ester produced in the calyx of *H. syriacus* contain active insect deterrent elements. Similarly, secondary chemicals produced in *S. emoryi* flowers were reported to serve primarily as feeding deterrent (Honda and Bowers 1996).

Collard was the second best treatment interplanted with cabbage that reduced DBM population on cabbage. The hypothesis is that collard is a more attractive host and when given a choice between cabbage and collard DBM will choose to alight and lay eggs on collard. Subsequently fewer DBM larvae were found on cabbage than in the control during 5, 6, and 7 week sampling periods in 2016. These results support the findings of Badenes-Perez et al. (2004) who demonstrated that DBM oviposited on glossy collard (*B. oleracea* L. var. *acephalla*) about 300 times more than cabbage. Collards have also been used as a trap crop in cabbage systems and was found to be effective in reducing DBM population (Mitchell et al. 1997, 2000, Badenes-Perez et al. 2004, Musser et al. 2005).

In 2017, lowest number of cabbage pests was recorded on cabbage plot interplanted with marigold. Volatiles emitting from marigold have been reported to be toxic to insects. Jankowska et al. (2009) and Jankowska (2010) found the lowest number of DBM and CW eggs on cabbage intercropped with marigold. Essential oil volatiles extracted from marigold in the genus *Tagetes* was reported to reduce aphid reproduction (Tomova et al. 2005). Other related studies found that marigold in the genus *Tagetes* contains volatiles that were highly toxic to the yellow fever mosquito *Aedes aegypti* L. and *Anopheles stephensi* Liston (Wells et al. 1992, 1993, Vasudevan

et al. 1997). Finally, the flowers produced by marigold were found to attract predators and parasitoids into research plots (Silveira et al. 2009). Moreover marigolds also provides suitable refugia for beneficial insects therefore, enhancing their population.

The absence of cabbage yield in 2016 caused me to modify my production techniques in 2017 and apply cover sprays using *Bacillus thuringiensis* (Bt) when the DBM threshold of one or more larvae per plant was reached. The cover spray was applied to all treatments and should not have affected the results of the study. Unfortunately, this may have contributed to the low population of DBM recorded in 2017 making it difficult to assess the differences in DBM population within treatments. The insecticide (Bt) that was used for the cover spray is one of the few tools that organic cole crop growers have at their disposal. It is used as an alternative to Entrust[®], a grower standard.

Fewer numbers of DBM's were observed in the first 30 days after planting and the population peaked approximately 70 days after planting. Vanlaldiki et al. 2013 found a similar trend while working on DBM in cabbage system. This information is important as a guideline in order to more accurately time the application of insecticide sprays because the residual activity of these compounds are relatively short lived (López et al. 2005). Knowing the pest population dynamics and the susceptible crop stages allow growers to effectively timing their control strategies such as monitoring, biological control, and pesticide treatments (Herms 2004, Marchioro and Foerster 2011). As a result, subsequent pesticide treatment could be reduced and therefore maximizing profitability and promoting sustainability in the environment (D'Auria et al. 2016).

Only a few imported cabbage worm (CW) larvae were recorded during *in situ* counts in the 2016 growing season; however, CW were common in 2017. We hypothesize that the difference in CW populations may have been influenced by mustard plants, *Brassica rapa* L. that were grown in a research plot less than 50 m from our experimental site (Figure 3-14). During the early cabbage season, mustards on the adjacent research plot were flowering (Figure 3-15), promoting high densities of CW in our field plots. Early study indicated that CW have strong preference to bright yellow color flower and mustard flowers scent, which stimulate the frequent visitation of this pests on mustard (Ômura et al. 1999).

Flowering companion plants were also found to attract CW into the field. More CW were recorded on cabbage planted with marigolds and roselle. This finding was comparable with Zhao et al. (1992) who reported that higher CW were found on broccoli interplanted with nectar-producing plants than the monoculture system.

Secondary Pests

Among secondary pests, fewer adult whiteflies were found in cabbage plots planted with marigold and roselle. As previously stated this may be related to the repellency or the insect deterring effects of these plants (Bird et al. 1987, Faizi et al. 2003). Smith et al. (2001) evaluate the potential to reduce whitefly population in common bean, *Phaseolus vulgaris* L., by intercropping the poor and non-host plants that include velvet bean, roselle, cilantro, cabbage, corn, and tomato in row and a mixed field design. They found that less whitefly immatures were recorded on roselle, which was a poor hosts for this pests. In contrast, more adult whiteflies were recorded in collards weekly, suggesting that collards were an attractive and suitable host for whiteflies where they can reproduce quite efficiently. In a study to compare whitefly

infestation in brussels sprouts, collard, kale, broccoli, and cabbage demonstrated that whiteflies had higher preference on infesting collards, brussels sprouts, and kale than cabbage and broccoli (Farnham and Elsey 1994).

Aphid population remained relatively low throughout the growing seasons, especially in plots that were intercropped with roselle and marigolds. Lower populations of aphids may suggest that the population were successfully regulated by the natural occurring predators and parasitoids. The lower population seen in 2017 may be related to the increase in natural enemy populations that were recorded in 2017. The early establishment of flowering companion plants in 2017 may have positively influenced the natural enemy population by providing more resources for oviposition and reproduction. Similarly, Razzi et al. (2016) also reported that aphid densities were reduced in squash production system when intercropped with buckwheat, *Fagopyrum esculentum* Moench.

Flower thrips belonging to genus *Frankliniella* were recorded in cabbage plots with marigold as companion plant. In this study, the marigold that were planted produced yellow flowers with a dark red in the centers. Flower thrips were known to be associated with flowering plants and were reported to be attracted to bright yellow flowers (Reitz 2005). Therefore, the marigold flower may be attracting more thrips than in other companion plots.

Natural Enemies Population

The interaction between plants, pests, and natural enemies were complex throughout the study. The highest number of predators were found in cabbage interplanted with marigold. Among the predators that were recorded in the marigold-cabbage treatment, carabid beetle population were found to have the highest count. This result could be attributed to the habitat modification which made it more favorable

for the development of carabid populations. One of the hypothesis is that marigolds provided more ground covered areas that may have influenced predation activities for these beetles. Previous studies using similar system showed that companion plants including clover (Armstrong and McKinlay 1997, Björkman et al. 2010), and cornflower (Ditner et al. 2013) provided additional groundcover that encouraged carabid beetles activities. Therefore, increasing the possibilities of prey to be encountered by this predator. In 2017, minute pirate bug population were also in the marigold. As previously stated thrips *Frankliniella* spp. population in the marigold was very high and their population is usually regulated by minute pirate bugs (Funderburk et al. 2000). Also, previous study by Peshin (2014) indicated that marigolds, particularly *T. tenuifolia*, positively influence minute pirate bugs and parasitoid wasps population.

Parasitoid abundance was positively correlated with the flowering phase of marigold and roselle in both growing seasons. This was shown by the higher parasitoid densities recorded in 2017 specifically in cabbage plots with marigold followed by roselle in treatment plots. In 2016 parasitoid families varied across treatments. The highest population of Trichogrammatidae was seen on untreated cabbage (control plot). Trichogrammatids are the major egg parasitoids for agricultural pests specifically lepidopteran pests (Smith 1996). The abundance of this wasps recorded on untreated plot may be associated with the high DBM pressure on this plot. In 2017, the parasitoid in the family platygasteridae was 3-fold higher than other treatment plots. Parasitoid abundance may be influenced by the nectar source provided by marigold flowers. Marigold is known to support parasitoid activities in agricultural systems (Rahat et al. 2005). It is well known that nectar-producing plants could enhanced biological control

activities of parasitoids (Cortesero et al. 2000, Silveira et al. 2009). Supplemental nectar was found to be vital for longevity and fecundity of most parasitoid species.

Marketable Yield

The absence of marketable yields in 2016 was due to significant apparent injuries on cabbage heads caused by a high population of DBM. Very little injuries were caused by imported cabbage worm, cabbage looper, aphids and whiteflies. In 2017, although a total of four cover sprays were applied throughout the season and primarily when DBM reached the threshold limit, injuries caused by CW during early heading stage greatly affected the marketable yield for that season. This finding suggest that frequent monitoring of all cabbage pests during the early growing season is crucial to ensure good marketable yield.

The current study shows that marketable yields of cabbage interplanted with companion plants and the untreated control were similar suggesting that interplanting cabbage with roselle, marigold or collard does not provide sufficient protection to prevent cabbage pest from reducing marketable yields. However, these three plants did provide some reduction in major and secondary cabbage pests. If these tools are integrated with effective reduced risk insecticides that are labelled for organic use they have the potential of providing sufficient protection for cabbage pest and reducing economic damage.

In summary, from these studies we found that integrating flowering companion plants could enhance natural enemy population by providing additional groundcover and extra floral nectar to predators and parasitoids. These plants also positively influence the parasitism and predation activities on cabbage pests (Balmer et al. 2013). Roselle were found to have deterrent effect on DBM and this was further studied in Chapter 4.

As the conclusion, adopting flowering companion plants would be an important tool to promoting biological and cultural control strategies in agricultural systems and this strategy may provide an economically viable alternative or complementary tactics to the current insecticide-based pest control practice specifically in organic production system (Bommarco et al. 2011).



Figure 3-1. Cabbage planted on a raised bed covered with black plastic mulch for the companion planting study. Photo courtesy of Z. Mazlan.



Figure 3-2. Non-marketable (left) and marketable (right) cabbage heads harvested. Photo courtesy of Z. Mazlan.

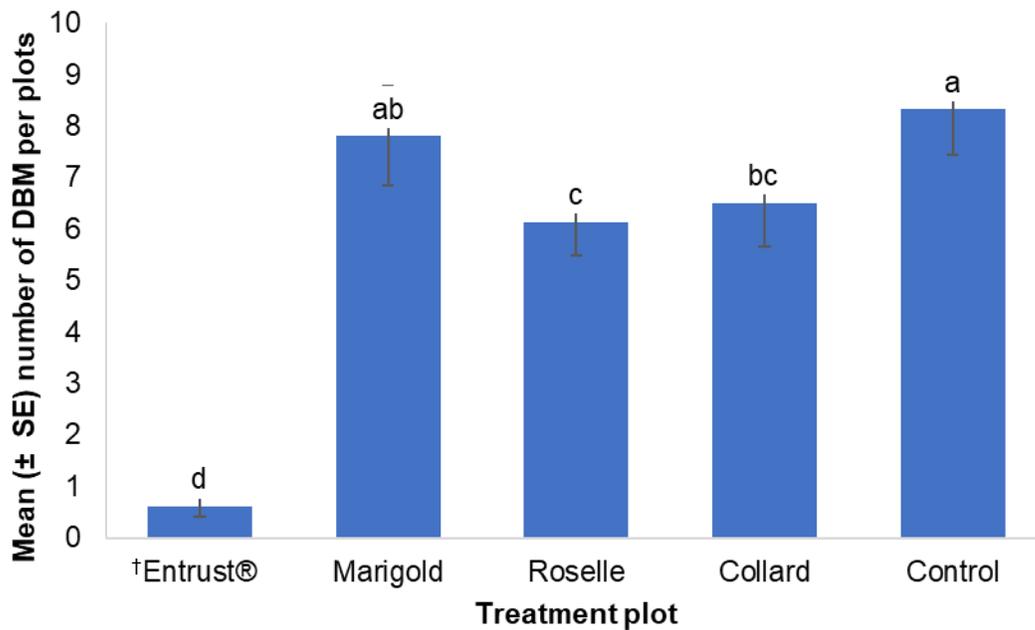


Figure 3-3. Overall mean \pm SE number of diamondback moth (DBM) recorded in *In-situ* counts over eight-week period for the companion planting study from Mar to May 2016. Treatments included cabbage plot treated with Entrust®, cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control). Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD).
 †Entrust® application rate (0.29 L per ha)

Table 3-1. Mean \pm SE number of diamondback moth (DBM) population observed weekly during *In-situ* counts over eight-week period for the companion planting study from Mar to May 2016. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Plant age days (week)	Observation date	Treatment				
		†Entrust [®]	Marigold	Roselle	Collard	Control
31 (w 1)	18 Mar	0.05 \pm 0.05 a	0.10 \pm 0.07 a	0.10 \pm 0.07 a	0.45 \pm 0.21 a	0.25 \pm 0.16 a
38 (w 2)	25 Mar	0.15 \pm 0.08 a	0.70 \pm 0.28 a	0.45 \pm 0.23 a	0.70 \pm 0.23 a	0.30 \pm 0.18 a
45 (w 3)	1 Apr	0.65 \pm 0.18 a	0.80 \pm 0.22 a	1.05 \pm 0.26 a	1.75 \pm 0.49 a	1.45 \pm 0.31 a
51 (w 4)	7 Apr	0.10 \pm 0.07 b	2.55 \pm 0.51 a	2.30 \pm 0.56 a	2.70 \pm 0.65 a	2.85 \pm 0.53 a
58 (w 5)	14 Apr	0.45 \pm 0.21 c	5.85 \pm 0.90 ab	6.35 \pm 1.02 ab	4.40 \pm 0.73b	8.15 \pm 1.12 a
65 (w 6)	21 Apr	1.15 \pm 0.27 c	5.85 \pm 0.90 b	6.35 \pm 1.02 b	4.40 \pm 0.73 b	20.20 \pm 3.40 a
72 (w 7)	28 Apr	0.75 \pm 0.26 c	18.75 \pm 2.50 ab	15.80 \pm 2.26 b	15.45 \pm 3.35 b	19.75 \pm 2.72 a
78 (w 8)	5 May	1.60 \pm 0.78 c	21.20 \pm 4.92 a	11.70 \pm 2.77 b	15.25 \pm 4.15 ab	13.65 \pm 2.33 ab

Means for all variable are untransformed values. Means in row followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

Transplanting date was on 17 Feb 2016.

†Entrust[®] application rate (0.29 L per ha)

Table 3-2. Mean \pm SE number of main lepidopteran pests observed during *In-situ* counts over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Treatment	DBM	Cabbage worm	Cabbage looper	Total
†Entrust [®]	0.07 \pm 0.03 b	0.03 \pm 0.01 c	0.01 \pm 0.01 a	0.10 \pm 0.03 b
Marigold	0.34 \pm 0.05 a	0.16 \pm 0.04 ab	0.06 \pm 0.02 a	0.55 \pm 0.08 a
Roselle	0.38 \pm 0.06 a	0.16 \pm 0.03 ab	0.07 \pm 0.03 a	0.61 \pm 0.09 a
Collard	0.49 \pm 0.07 a	0.12 \pm 0.03 b	0.04 \pm 0.01 a	0.64 \pm 0.09 a
Control	0.45 \pm 0.08 a	0.21 \pm 0.04 a	0.05 \pm 0.02 a	0.71 \pm 0.09 a
Trt (df=4,950)	$F=9.50; P<0.0001$	$F=5.28; P=0.0003$	$F=2.22; P=0.07$	$F=13.36; P<0.0001$
Trt*obs (df=36,950)	$F=2.44; P<0.0001$	$F=2.88; P<0.0001$	$F=1.28 P=0.1262$	$F=2.47; P=0.02$

Means for all variables are untransformed values. Means in column followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

†Entrust[®] application rate (0.29 L per ha)

Table 3-3. Mean \pm SE number of diamondback moth (DBM) population observed during *In-situ* counts over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Plant age days (week)	Observation date	Treatment				
		†Entrust [®]	Marigold	Roselle	Collard	Control
22 (w 1)	15 Mar	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.05 \pm 0.05 a	0.00 \pm 0.00 a
29 (w 2)	22 Mar	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.05 \pm 0.05 a
36 (w 3)	29 Mar	0.15 \pm 0.08 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.05 \pm 0.05 a	0.10 \pm 0.07 a
43 (w 4)	05 Apr	0.00 \pm 0.00 a	0.15 \pm 0.08 a	0.20 \pm 0.16 a	0.25 \pm 0.16 a	0.05 \pm 0.05 a
50 (w 5)	12 Apr	0.10 \pm 0.10 b	0.80 \pm 0.24 a	0.95 \pm 0.22 a	0.75 \pm 0.20 a	0.60 \pm 0.17 a
58 (w 6)	20 Apr	0.40 \pm 0.20 a	0.30 \pm 0.15 a	0.35 \pm 0.13 a	0.50 \pm 0.17 a	0.25 \pm 0.12 a
64 (w 7)	26 Apr	0.00 \pm 0.00 b	0.45 \pm 0.14 a	0.20 \pm 0.09 ab	0.50 \pm 0.17 a	0.45 \pm 0.14 a
70 (w 8)	02 May	0.05 \pm 0.05 b	1.25 \pm 0.31 a	1.70 \pm 0.38 a	1.95 \pm 0.48 a	1.85 \pm 0.48 a
77 (w 9)	09 May	0.00 \pm 0.00 a	0.35 \pm 0.13 a	0.20 \pm 0.12 a	0.50 \pm 0.18 a	0.65 \pm 0.28 a
84 (w 10)	16 May	0.00 \pm 0.00 a	0.10 \pm 0.07 a	0.15 \pm 0.11 a	0.30 \pm 0.13 a	0.50 \pm 0.26 a

Means for all variable are untransformed values. Means in row followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

Transplanting date was on 22 Feb 2017.

†Entrust[®] application rate (0.29 L per ha)

Table 3-4. Mean \pm SE number of imported cabbage worm (CW), *Pieris rapae* population observed during *In-situ* counts over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Plant age days (week)	Observation date	Treatment				
		†Entrust [®]	Marigold	Roselle	Collard	Control
22 (w 1)	15 Mar	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
29 (w 2)	22 Mar	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
36 (w 3)	29 Mar	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.10 \pm 0.10 a	0.00 \pm 0.60 a
43 (w 4) ^a	05 Apr	0.00 \pm 0.00 c	0.10 \pm 0.07 bc	0.00 \pm 0.00 c	0.45 \pm 0.15 ab	0.60 \pm 0.22 a
50 (w 5) ^b	12 Apr	0.05 \pm 0.05 b	0.40 \pm 0.18 b	0.20 \pm 0.09 b	0.35 \pm 0.15 b	0.90 \pm 0.26 a
58 (w 6) ^c	20 Apr	0.05 \pm 0.05 b	0.10 \pm 0.07 b	0.45 \pm 0.15 a	0.05 \pm 0.05 b	0.05 \pm 0.05 b
64 (w 7)	26 Apr	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
70 (w 8)	02 May	0.15 \pm 0.11 a	0.80 \pm 0.22 a	0.55 \pm 0.17 a	0.20 \pm 0.16 a	0.50 \pm 0.11 a
77 (w 9)	09 May	0.00 \pm 0.00 a	0.10 \pm 0.07 a	0.05 \pm 0.05 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
84 (w 10)	16 May	0.00 \pm 0.00 a	0.05 \pm 0.05 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.05 \pm 0.05 a

Means for all variable are untransformed values. Means in row followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

†Entrust[®] application rate (0.29 L per ha)

^a $F = 3.22$; $df = 4,95$; $P = 0.02$

^b $F = 3.82$; $df = 4,95$; $P = 0.006$

^c $F = 4.26$; $df = 4,95$; $P = 0.003$

Table 3-5. Mean \pm SE number of cabbage pests collected from yellow sticky traps over eight-week period for the companion planting study from Mar to May 2016. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Treatment	DBM	Aphids	whitefly	thrips	Total
†Entrust [®]	1.13 \pm 0.15 c	7.86 \pm 0.59 a	6.56 \pm 0.56 a	45.99 \pm 4.95 a	62.12 \pm 5.31 a
Marigold	1.77 \pm 0.25 ab	7.20 \pm 0.58 a	5.77 \pm 0.48 a	54.43 \pm 6.61 a	69.80 \pm 7.00 a
Roselle	1.71 \pm 0.24 b	7.47 \pm 0.66 a	6.35 \pm 0.60 a	42.16 \pm 3.91 a	58.28 \pm 4.19 a
Collard	2.29 \pm 0.27 a	7.27 \pm 0.55 a	6.43 \pm 0.66 a	51.17 \pm 4.60 a	67.59 \pm 4.99 a
Control	2.14 \pm 0.26 ab	7.83 \pm 0.54 a	6.22 \pm 0.51 a	46.78 \pm 3.99 a	63.59 \pm 4.39 a
Trt (df=4,600)	$F=5.58$; $P=0.0002$	$F=0.41$; $P=0.80$	$F=0.47$; $P=0.76$	$F=1.27$; $P=0.28$	$F=1.04$; $P=0.39$
Trt * obs (df=28,600)	$F=1.86$; $P=0.005$	$F=0.95$; $P=0.54$	$F=1.73$; $P=0.01$	$F=0.69$; $P=0.89$	$F=0.72$; $P=<0.0001$

Means for all variables are untransformed values. Means in column followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

†Entrust[®] application rate (0.29 L per ha)

Table 3-6. Mean \pm SE number of cabbage pests collected from yellow sticky traps over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Treatment	DBM	Aphids	Whitefly	Thrips	Total pests
†Entrust [®]	0.23 \pm 0.07 a	2.55 \pm 0.35 a	50.25 \pm 9.70 a	10.55 \pm 1.76 b	63.58 \pm 9.24 ab
Marigold	0.35 \pm 0.08 a	2.38 \pm 0.32 a	15.73 \pm 3.38 c	23.22 \pm 5.10 a	41.67 \pm 5.39 d
Roselle	0.38 \pm 0.15 a	2.69 \pm 0.45 a	35.73 \pm 7.03 b	10.63 \pm 1.49 b	49.42 \pm 6.70 dc
Collard	0.36 \pm 0.11 a	2.56 \pm 0.41 a	56.63 \pm 12.23 a	11.14 \pm 1.77 b	70.69 \pm 11.80 a
Control	0.33 \pm 0.08 a	2.17 \pm 0.29 a	44.41 \pm 9.89 ab	9.77 \pm 1.29 b	56.67 \pm 9.55 bc
Trt (df=4,280)	$F=0.35$; $P=0.84$	$F=0.61$; $P=0.66$	$F=11.88$; $P<0.0001$	$F=11.03$; $P<0.0001$	$F=5.20$; $P=0.0005$
Trt * obs (df=28,280)	$F=1.86$; $P=0.006$	$F=1.87$; $P=0.006$	$F=6.44$; $P<0.0001$	$F=5.24$; $P<0.0001$	$F=6.78$; $P<0.0001$

Means for all variable are untransformed values. Means in column followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

†Entrust[®] application rate (0.29 L per ha)

Table 3-7. Mean \pm SE number of secondary pests observed during *In-situ* counts over eight-week period for the companion planting study from Mar to May 2016. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Treatment	Aphids	Whitefly	Thrips	Total
†Entrust [®]	2.34 \pm 0.51	0.21 \pm 0.07	0.03 \pm 0.02	2.58 \pm 0.51
Marigold	2.03 \pm 0.60	0.22 \pm 0.08	0.03 \pm 0.02	2.28 \pm 0.61
Roselle	1.64 \pm 0.27	0.23 \pm 0.05	0.07 \pm 0.03	1.94 \pm 0.28
Collard	1.51 \pm 0.29	0.27 \pm 0.07	0.06 \pm 0.02	1.84 \pm 0.31
Control	1.76 \pm 0.37	0.21 \pm 0.06	0.04 \pm 0.02	2.00 \pm 0.37
trt (df=4,760)	$F=0.65; P=0.63$	$F=0.17; P=0.96$	$F=0.80; P=0.52$	$F=0.52; P=0.72$
Trt*obs (df=28,760)	$F=1.52; P=0.04$	$F=1.82; P=0.006$	$F=0.27; P=0.999$	$F=1.51; P=0.04$

Means for all variable are untransformed values.

†Entrust[®] application rate (0.29 L per ha)

Table 3-8. Mean \pm SE number of secondary pests observed during *In-situ* counts over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Treatment	Secondary leps	Aphids	Whitefly	Blister beetle	Total
†Entrust [®]	0.00 \pm 0.00	0.20 \pm 0.09	1.40 \pm 0.24	0.00 \pm 0.00	1.59 \pm 0.25
Marigold	0.07 \pm 0.05	0.20 \pm 0.05	1.10 \pm 0.16	0.00 \pm 0.00	1.30 \pm 0.17
Roselle	0.03 \pm 0.02	0.26 \pm 0.09	1.10 \pm 0.12	0.00 \pm 0.00	1.36 \pm 0.14
Collard	0.01 \pm 0.01	0.08 \pm 0.02	1.58 \pm 0.21	0.26 \pm 0.26	2.17 \pm 0.55
Control	0.01 \pm 0.01	0.36 \pm 0.15	1.43 \pm 0.15	0.00 \pm 0.00	1.78 \pm 0.22
Trt (df=4,950)	$F=1.03$; $P=0.34$	$F=1.26$; $P=0.28$	$F=1.80$; $P=0.13$	$F=1.00$; $P=0.41$	$F=1.44$; $P=0.22$
Trt*obs (df=36,950)	$F=0.98$; $P=0.50$	$F=1.02$; $P=0.43$	$F=1.42$; $P=0.05$	$F=1.00$; $P=0.47$	$F=0.99$; $P=0.49$

Means for all variables are untransformed values.

†Entrust[®] application rate (0.29 L per ha)

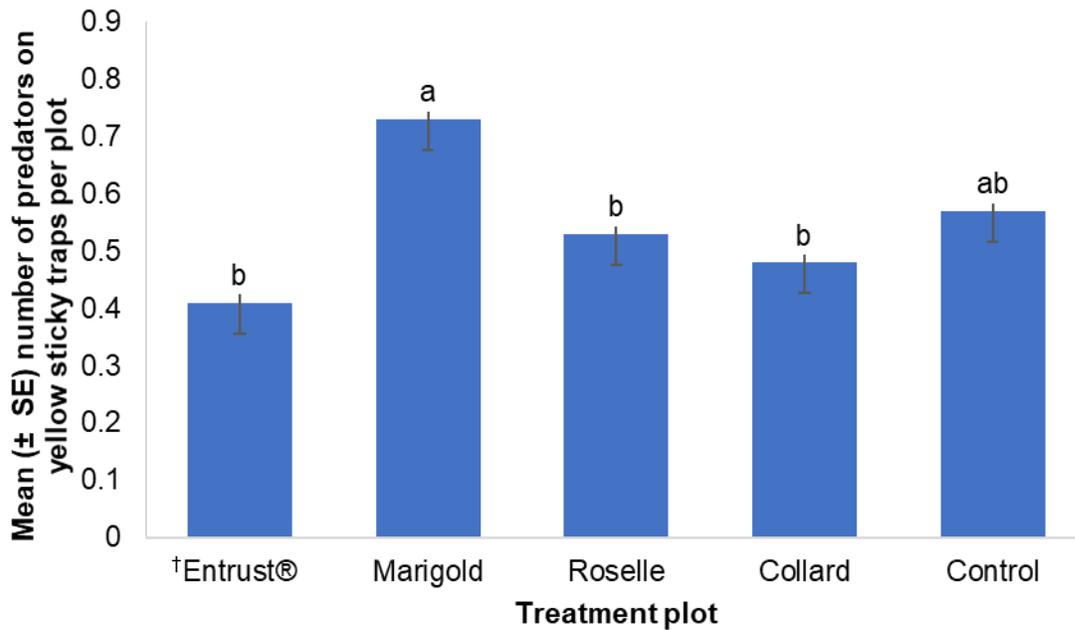


Figure 3-4. Overall mean \pm SE number of predators collected from yellow sticky traps over eight-week period for the companion planting study from Mar to May 2016. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot. Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD).

†Entrust[®] application rate (0.29 L per ha)

Trt $F = 3.21$; $df = 4,600$; $P = 0.013$

Trt * obs $F = 0.97$; $df = 28,600$; $P < 0.0001$

Table 3-9. Mean \pm SE number of predators collected from yellow sticky traps over eight-week period for the companion planting study from Mar to May 2016. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Predators	†Entrust [®]	Marigold	Roselle	Collard	Control	Trt (df= 4, 600)	Trt*week (df=28, 600)
Ant (Formicidae)	0.76 \pm 0.11	0.83 \pm 0.08	0.77 \pm 0.09	0.89 \pm 0.11	0.98 \pm 0.11	<i>F</i> =0.96; <i>P</i> =0.43	<i>F</i> =0.59; <i>P</i> =0.95
Lacewing; green (Chrysopidae) and Brown (Hemerobiidae)	0.17 \pm 0.03	0.27 \pm 0.05	0.32 \pm 0.06	0.27 \pm 0.05	0.26 \pm 0.05	<i>F</i> =1.47; <i>P</i> =0.21	<i>F</i> =0.52; <i>P</i> =0.98
Minute pirate bug (Anthocoridae)	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.02 \pm 0.01	0.03 \pm 0.02	<i>F</i> =0.31; <i>P</i> =0.87	<i>F</i> =0.79; <i>P</i> =0.82
Ladybug beetle (Coccinellidae)	0.01 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	<i>F</i> =1.00; <i>P</i> =0.41	<i>F</i> =1.00; <i>P</i> =0.43
Spider (Araneae)	0.12 \pm 0.05	0.03 \pm 0.02	0.07 \pm 0.09	0.06 \pm 0.02	0.09 \pm 0.03	<i>F</i> =1.19; <i>P</i> =0.31	<i>F</i> =0.82; <i>P</i> =0.73

Means for all variables are untransformed values.

†Entrust[®] application rate (0.29 L per ha)

Table 3-10. Mean \pm SE number of predators collected from pitfall traps over eight-week period for the companion planting study from Mar to May 2016. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Predators	†Entrust [®]	Marigold	Roselle	Collard	Control	Trt (df=4,20)	Trt*week (df=12,20)
Ant (formicidae)	16.56 \pm 5.18 a	17.25 \pm 4.78 a	8.38 \pm 1.44 a	11.00 \pm 3.00 a	13.44 \pm 2.60 a	$F=1.30$; $P=0.28$	$F=1.30$; $P=0.28$
Dragonfly (Coenagrionidae)	0.00 \pm 0.00 a	0.19 \pm 0.14 a	0.13 \pm 0.09 a	0.00 \pm 0.00 a	0.13 \pm 0.09 a	$F=1.42$; $P=0.24$	$F=1.42$; $P=0.18$
Ground beetle (Carabidae)	0.19 \pm 0.10 bc	0.19 \pm 0.14 bc	0.75 \pm 0.27 a	0.13 \pm 0.09 c	0.63 \pm 0.26 ab	$F=2.93$; $P=0.03$	$F=1.35$; $P=0.22$
Ladybug beetle (Coccinellidae)	0.00 \pm 0.00 a	0.06 \pm 0.06 a	0.00 \pm 0.00 a	0.06 \pm 0.06 a	0.00 \pm 0.00 a	$F=0.75$; $P=0.56$	$F=0.75$; $P=0.70$
Spider (Araneae)	0.56 \pm 0.22 a	0.63 \pm 0.30 a	0.50 \pm 0.16 a	0.50 \pm 0.24 a	0.31 \pm 0.12 a	$F=0.32$; $P=0.87$	$F=0.38$; $P=0.97$
Total mean	8.88 \pm 2.83	19.38 \pm 9.14	8.50 \pm 1.90	8.63 \pm 3.69	16.88 \pm 4.59	$F=1.12$; $P=0.37$	$F=0.79$; $P=0.66$

Means for all variables are untransformed values. Means within row followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

†Entrust[®] application rate (0.29 L per ha)

Table 3-11. Mean \pm SE number of predators collected from yellow sticky traps over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Predator	†Entrust [®]	Marigold	Roselle	Collard	Control	Trt (df=4,280)	Trt*obs (df=28,280)
Labybird beetle (Coccinellidae)	0.02 \pm 0.02 a	0.05 \pm 0.03 a	0.03 \pm 0.02 a	0.03 \pm 0.02 a	0.02 \pm 0.02 a	$F=0.39$; $P=0.13$	$F=0.64$; $P=0.92$
Spider (Araneae)	0.42 \pm 0.09 a	0.19 \pm 0.06 a	0.33 \pm 0.08 a	0.25 \pm 0.06 a	0.19 \pm 0.06 a	$F=1.95$; $P=0.10$	$F=0.68$; $P=0.89$
Ground beetle (Carabidae)	0.08 \pm 0.03 a	0.06 \pm 0.04 a	0.02 \pm 0.02 a	0.00 \pm 0.00 a	0.02 \pm 0.02 a	$F=1.94$; $P=0.10$	$F=1.28$; $P=0.17$
Ant (Formicidae)	0.08 \pm 0.03 a	0.03 \pm 0.03 a	0.09 \pm 0.04 a	0.06 \pm 0.04 a	0.05 \pm 0.03 a	$F=0.51$; $P=0.73$	$F=1.08$; $P=0.36$
Six-Spotted Thrips (Thripidae)	0.17 \pm 0.06 a	0.36 \pm 0.12 a	0.13 \pm 0.05 a	0.23 \pm 0.07 a	0.42 \pm 0.14 a	$F=2.28$; $P=0.06$	$F=1.30$; $P=0.15$
Green lacewing (Chrysopidae)	0.00 \pm 0.00 a	0.03 \pm 0.02 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	$F=2.00$; $P=0.09$	$F=0.86$; $P=0.68$
Rove beetles (Staphilinidae)	0.23 \pm 0.05 a	0.20 \pm 0.05 a	0.19 \pm 0.06 a	0.28 \pm 0.07 a	0.16 \pm 0.06 a	$F=0.65$; $P=0.63$	$F=0.63$; $P=0.93$
Minute pirate bug (Anthochoridae)	0.11 \pm 0.05 b	1.03 \pm 0.22 a	0.14 \pm 0.09 b	0.06 \pm 0.04 b	0.06 \pm 0.03 b	$F=29.62$; $P<0.0001$	$F=7.60$; $P<0.0001$
Big eyed bug (Geocoridae)	0.02 \pm 0.02 a	0.00 \pm 0.00 a	0.02 \pm 0.02 a	0.00 \pm 0.00 a	0.03 \pm 0.02 a	$F=0.94$; $P=0.44$	$F=1.40$; $P=0.09$
Tachina flies (Tachinidae)	0.16 \pm 0.06 a	0.11 \pm 0.04 a	0.17 \pm 0.07 a	0.17 \pm 0.17 a	0.13 \pm 0.05 a	$F=0.27$; $P=0.90$	$F=0.57$; $P=0.96$

Means for all variables are untransformed values. Means in row followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

†Entrust[®] application rate (0.29 L per ha)

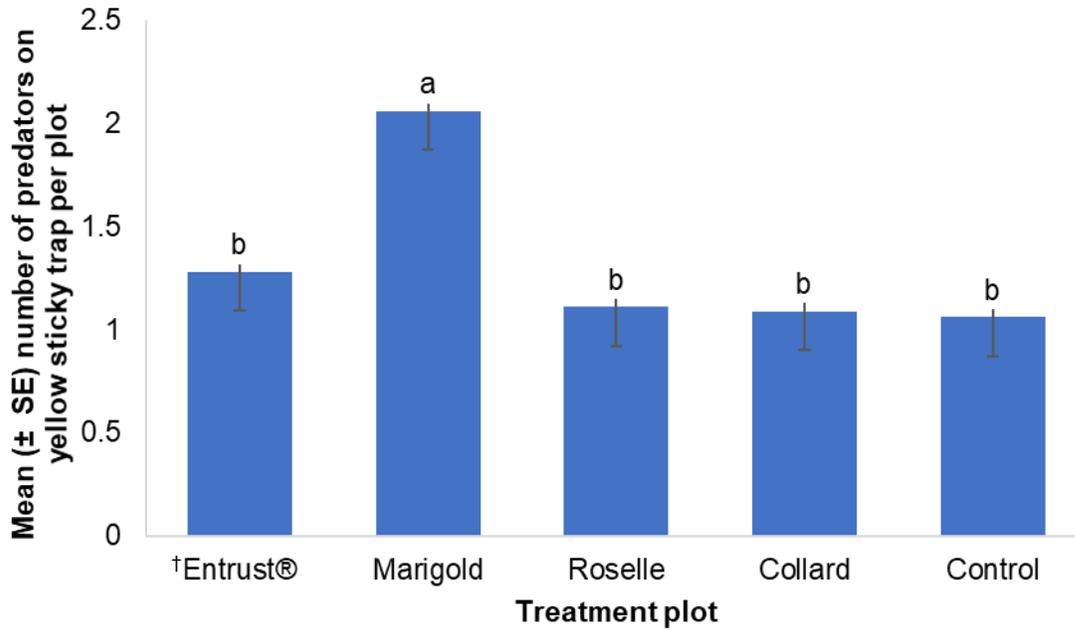


Figure 3-5. Overall mean \pm SE number of predators collected from yellow sticky traps over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust®, cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control). Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD).

†Entrust® application rate (0.29 L per ha)

Trt $F = 6.20$; $df = 4,600$; $P < 0.0001$

Trt * obs $F = 1.88$; $df = 28,600$; $P = 0.006$

Table 3-12. Mean \pm SE number of predators collected from pitfall traps over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Predators	†Entrust [®]	Marigold	Roselle	Collard	Control	Trt (df=4,60)	Trt*week (df=12,60)
Ant (formicidae)	4.31 \pm 1.76 a	5.81 \pm 2.36 a	7.56 \pm 4.21 a	6.94 \pm 3.26 a	6.19 \pm 2.48 a	$F=0.22$; $P=0.93$	$F=0.25$; $P=0.99$
Ground beetle (Carabidae)	0.13 \pm 0.13 b	3.94 \pm 2.11 a	0.44 \pm 0.20 b	0.44 \pm 0.18 b	0.75 \pm 2.86 b	$F=4.70$; $P=0.002$	$F=4.02$; $P=0.0001$
Spider (Araneae)	0.56 \pm 0.20 a	1.06 \pm 0.19 a	0.88 \pm 0.36 a	1.06 \pm 0.39 a	0.56 \pm 0.16 a	$F=1.25$; $P=0.30$	$F=3.63$; $P=0.0004$
Total mean	8.88 \pm 2.83	19.38 \pm 9.14	8.50 \pm 1.90	8.63 \pm 3.69	16.88 \pm 4.59	$F=1.12$; $P=0.37$	$F=0.79$; $P=0.66$

Means for all variables are untransformed values. Means within row followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

†Entrust[®] application rate (0.29 L per ha)

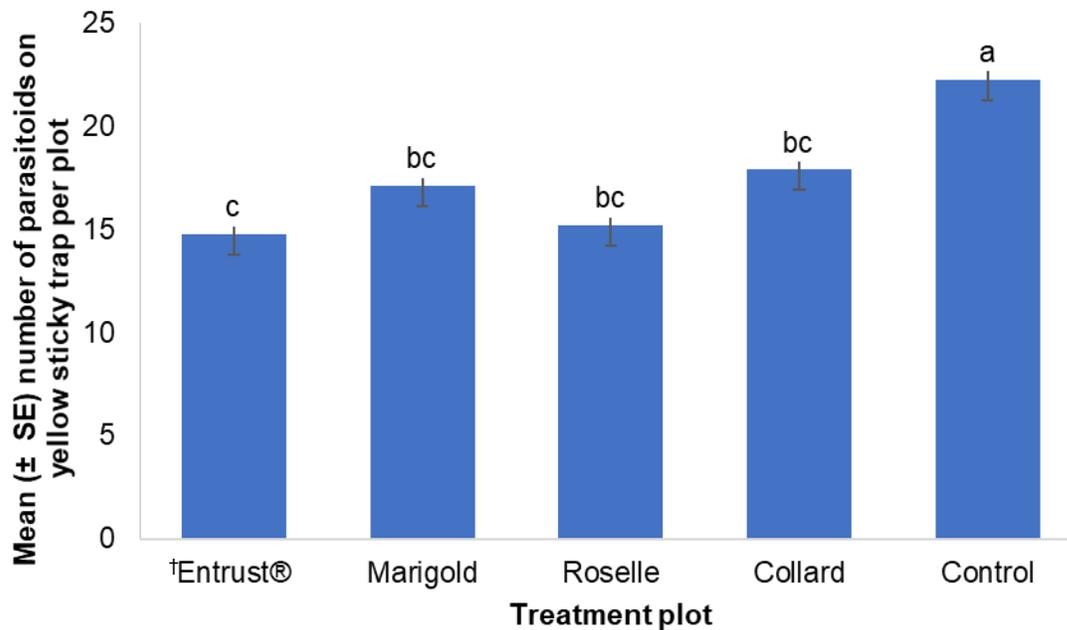


Figure 3-6. Overall mean \pm SE number of parasitoids collected from yellow sticky traps over eight-week period for the companion planting study from Mar to May 2016. Treatments included cabbage plot treated with Entrust®, cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control). Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD).

†Entrust® application rate (0.29 L per ha)

Trt $F = 9.33$; $df = 4,600$; $P < 0.0001$
 Trt * obs $F = 4.24$; $df = 28,600$; $P < 0.0001$

Table 3-13. Mean \pm SE number of parasitoid families collected from yellow sticky traps over eight-week period for the companion planting study from Mar to May 2016. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Superfamily	Family	†Entrust [®]	Marigold	Roselle	Collard	Control
Chalcidoidea	Aphelinidae	1.34 \pm 0.15 a	1.11 \pm 0.13 a	1.02 \pm 0.12 a	1.24 \pm 0.13 a	1.36 \pm 0.14 a
	Chalcididae	0.01 \pm 0.01 a	0.01 \pm 0.01 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Encyrtidae	0.76 \pm 0.11 a	0.83 \pm 0.08 a	0.77 \pm 0.09 a	0.89 \pm 0.11 a	0.98 \pm 0.11 a
	Eulophidae	1.27 \pm 0.18 a	1.09 \pm 0.12 a	1.02 \pm 0.15 a	1.24 \pm 0.14 a	1.35 \pm 0.14 a
	Eupelmidae	0.12 \pm 0.05 a	0.03 \pm 0.02 a	0.07 \pm 0.02 a	0.06 \pm 0.02 a	0.09 \pm 0.03 a
	Eurytomidae	0.00 \pm 0.00 a	0.02 \pm 0.01 a	0.01 \pm 0.01 a	0.00 \pm 0.00 a	0.01 \pm 0.01 a
	Mymaridae	1.42 \pm 0.15 a	1.60 \pm 0.14 a	1.25 \pm 0.11 a	1.55 \pm 0.13 a	1.74 \pm 0.14 a
	Petromalidae ^a	0.20 \pm 0.05 a	0.19 \pm 0.04 a	0.05 \pm 0.02 b	0.16 \pm 0.04 a	0.18 \pm 0.04 a
	Signiphoridae	0.09 \pm 0.03 a	0.05 \pm 0.02 a	0.09 \pm 0.03 a	0.09 \pm 0.02 a	0.07 \pm 0.03 a
	Trichogrammatidae ^b	5.49 \pm 0.80 c	9.11 \pm 1.38 b	7.78 \pm 1.17 b	8.75 \pm 1.28 b	12.39 \pm 1.99 a
Ceraphronoidea	Ceraphronidae ^c	0.32 \pm 0.05 a	0.16 \pm 0.03 b	0.30 \pm 0.05 ab	0.42 \pm 0.06 a	0.34 \pm 0.06 a
	Megaspilidae	0.01 \pm 0.01 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a

Table 3-13. Continued

Superfamily	Family	†Entrust®	Marigold	Roselle	Collard	Control
Chrysoidea	Bethylidae	0.04 ± 0.02 a	0.01 ± 0.01 a	0.05 ± 0.02 a	0.03 ± 0.02 a	0.05 ± 0.02 a
Cynipoidea	Figitidae	0.07 ± 0.02 a	0.07 ± 0.02 a	0.13 ± 0.03 a	0.09 ± 0.03 a	0.09 ± 0.03 a
Evanoidea	Evaniidae	0.00 ± 0.00 a	0.00 ± 0.00 a	0.01 ± 0.01 a	0.00 ± 0.00 a	0.00 ± 0.00 a
Ichneumonoidea	Brachonidae ^d	1.30 ± 0.12 a	0.94 ± 0.09 b	0.84 ± 0.08 b	0.92 ± 0.10 b	1.04 ± 0.11 ab
	Ichneumonidae	0.04 ± 0.02 a	0.12 ± 0.04 a	0.09 ± 0.05 a	0.14 ± 0.04 a	0.09 ± 0.04 a
Mymarommatoidea	Mymarommatidae	0.01 ± 0.01 a	0.01 ± 0.01 a	0.00 ± 0.00 a	0.02 ± 0.01 a	0.00 ± 0.00 a
Platygastroidea	Platygastridae ^e	2.21 ± 0.17 a	1.73 ± 0.16 b	1.72 ± 0.13 b	2.23 ± 0.16 a	2.41 ± 0.19 a
Proctotrupeoidea	Diapriidae	0.01 ± 0.01 a	0.01 ± 0.01 a	0.01 ± 0.01 a	0.02 ± 0.01 a	0.00 ± 0.00 a
	Other	0.07 ± 0.02 a	0.05 ± 0.02 a	0.02 ± 0.01 a	0.05 ± 0.02 a	0.08 ± 0.02 a

Means for all variables are untransformed values. Means within row followed by the same letters are not significantly different $P \leq 0.05$ (LSD).
†Entrust® application rate (0.29 L per ha)

^a $F = 2.74$; $df = 4, 600$; $P = 0.03$

^b $F = 10.52$; $df = 4, 600$; $P < 0.0001$

^c $F = 3.32$; $df = 4, 600$; $P = 0.01$

^d $F = 3.22$; $df = 4, 600$; $P = 0.01$

^e $F = 3.96$; $df = 4, 600$; $P = 0.004$

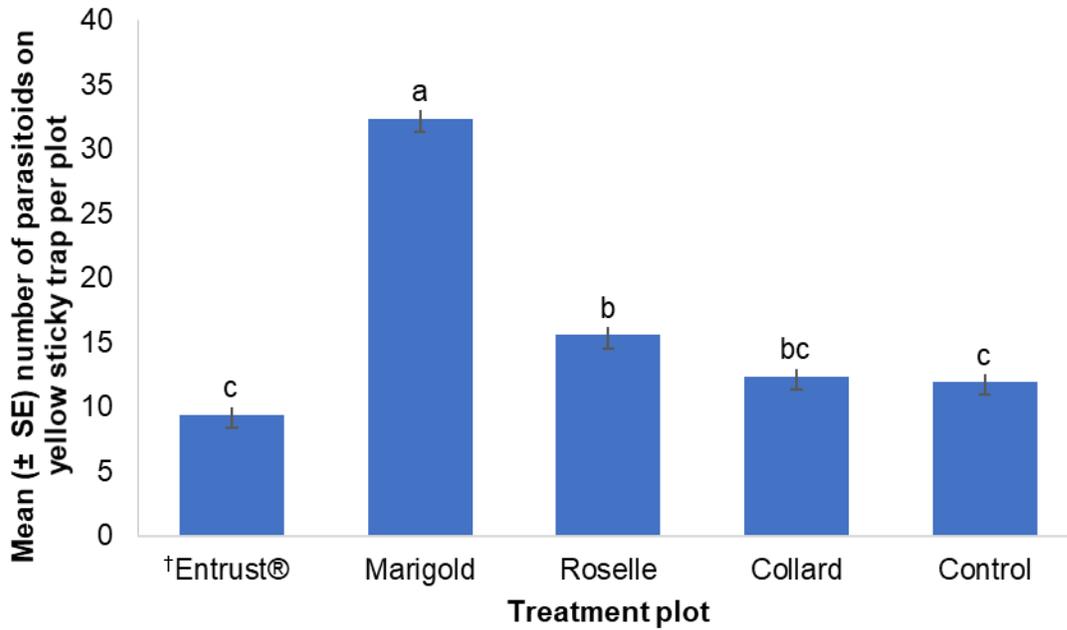


Figure 3-7. Overall mean \pm SE number of parasitoid families collected from yellow sticky traps over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust®, cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot. Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD).

†Entrust® application rate (0.29 L per ha)

Trt $F = 58.16$; $df = 4,280$; $P < 0.0001$
 Trt * obs $F = 7.85$; $df = 28,280$; $P < 0.0001$

Table 3-14. Mean \pm SE number of parasitoids collected from yellow sticky traps over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Plant age days (week)	Observation date	†Entrust [®]	Marigold	Roselle	Collard	Control	Trt (df=4,35)
29 (w 2)	22 Mar	5.25 \pm 0.84 a	5.25 \pm 1.29 a	7.88 \pm 2.19 a	6.50 \pm 1.63 a	6.25 \pm 1.18 a	<i>F</i> =0.52; <i>P</i> =0.72
36 (w 3)	29 Mar	4.50 \pm 0.94 a	7.75 \pm 1.91 a	6.88 \pm 1.01 a	7.13 \pm 1.16 a	4.88 \pm 0.77 a	<i>F</i> =1.40; <i>P</i> =0.25
43 (w 4)	05 Apr	3.25 \pm 1.08 c	8.50 \pm 1.55 a	7.38 \pm 1.59 ab	6.63 \pm 1.28 abc	4.00 \pm 0.96 bc	<i>F</i> =2.90; <i>P</i> =0.04
50 (w 5)	12 Apr	8.38 \pm 1.75 b	21.38 \pm 4.11 a	12.13 \pm 1.84 b	14.25 \pm 2.40 b	11.00 \pm 0.87 b	<i>F</i> =4.07; <i>P</i> =0.008
57 (w 6)	20 Apr	11.38 \pm 2.63 b	32.38 \pm 4.10 a	17.63 \pm 2.33 b	14.50 \pm 1.16 b	17.00 \pm 1.25 b	<i>F</i> =10.22; <i>P</i> <0.0001
64 (w 7)	26 Apr	19.63 \pm 7.48 b	73.63 \pm 10.96 a	20.88 \pm 6.94 b	22.63 \pm 4.81 b	18.63 \pm 2.67 b	<i>F</i> =11.16; <i>P</i> <0.0001
71 (w 8)	03 May	n/a	n/a	n/a	n/a	n/a	n/a
78 (w 9)	10 May	12.13 \pm 2.38 c	53.00 \pm 3.19 a	32.13 \pm 4.24 b	14.13 \pm 3.07 c	17.13 \pm 2.85 c	<i>F</i> =28.67; <i>P</i> <0.0001
85 (w 10)	17 May	10.50 \pm 1.49 b	57.25 \pm 7.89 a	19.63 \pm 2.46 b	12.88 \pm 1.74 b	16.38 \pm 1.52 b	<i>F</i> =24.47; <i>P</i> <0.0001

Means for all variable are untransformed values. Means in row followed by the same letters are not significantly different $P \leq 0.05$. (LSD).

Transplanting date was on 22 Feb 2017.

†Entrust[®] application rate (0.29 L per ha)

Table 3-15. Mean \pm SE number of each parasitoid family collected from yellow sticky traps over ten-week period for the companion planting study from Mar to May 2017. Treatments included cabbage plot treated with Entrust[®], cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control).

Superfamily	Family	†Entrust [®]	Marigold	Roselle	Collard	Control
Chalcidoidea	Aphelinidae ^a	0.27 \pm 0.07 c	0.53 \pm 0.11 bc	1.17 \pm 0.21 a	1.19 \pm 0.25 a	0.77 \pm 0.14 b
	Chalcididae	0.02 \pm 0.02 a	0.05 \pm 0.03 a	0.05 \pm 0.03 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Encyrtidae ^b	0.28 \pm 0.07 c	0.94 \pm 0.15 a	0.78 \pm 0.12 ab	0.67 \pm 0.13 ab	0.56 \pm 0.10 bc
	Eulophidae	0.52 \pm 0.09 a	0.72 \pm 0.13 a	0.66 \pm 0.12 a	0.72 \pm 0.11 a	0.47 \pm 0.09 a
	Eupelmidae	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.03 \pm 0.02 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Eurytomidae	0.00 \pm 0.00 a	0.03 \pm 0.02 a	0.00 \pm 0.00 a	0.02 \pm 0.02 a	0.00 \pm 0.00 a
	Mymaridae ^c	0.66 \pm 0.10 b	0.98 \pm 0.15 ab	1.06 \pm 0.15 a	0.88 \pm 0.13 ab	0.64 \pm 0.11 b
	Petromalidae	0.03 \pm 0.02 a	0.14 \pm 0.05 a	0.19 \pm 0.06 a	0.09 \pm 0.04 a	0.09 \pm 0.04 a
	Signiphoridae	0.08 \pm 0.04 a	0.06 \pm 0.03 a	0.13 \pm 0.04 a	0.03 \pm 0.02 a	0.13 \pm 0.04 a
	Trichogrammatidae	0.83 \pm 0.15 a	1.31 \pm 0.20 a	1.14 \pm 0.12 a	1.06 \pm 0.18 a	0.81 \pm 0.12 a
Ceraphronoidea	Ceraphronidae ^d	0.13 \pm 0.05 b	0.41 \pm 0.09 a	0.17 \pm 0.05 b	0.09 \pm 0.04 b	0.13 \pm 0.04 b
	Megaspilidae	0.02 \pm 0.02 a	0.03 \pm 0.02 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.02 \pm 0.02 a

Table 3-15. Continued

Superfamily	Family	†Entrust®	Marigold	Roselle	Collard	Control
Chrysoidea	Bethylidae ^e	0.13 ± 0.05 b	0.41 ± 0.09 a	0.17 ± 0.05 b	0.09 ± 0.04 b	0.13 ± 0.04 b
Cynipoidea	Figitidae	0.02 ± 0.02 a	0.03 ± 0.02 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.02 ± 0.02 a
Evanoidea	Evaniidae	0.02 ± 0.02 a	0.06 ± 0.03 a	0.05 ± 0.03 a	0.02 ± 0.02 a	0.02 ± 0.02 a
Ichneumonoidea	Brachonidae	0.00 ± 0.00 a	0.05 ± 0.03 a	0.02 ± 0.02 a	0.02 ± 0.02 a	0.05 ± 0.03 a
	Ichneumonidae	0.05 ± 0.03 a	0.03 ± 0.02 a	0.03 ± 0.02 a	0.03 ± 0.02 a	0.06 ± 0.03 a
Mymarommatoidea	Mymarommatidae	0.36 ± 0.09 a	0.38 ± 0.08 a	0.47 ± 0.10 a	0.48 ± 0.12 a	0.63 ± 0.11 a
Platygastroidea	Platygastridae ^f	0.59 ± 0.17 ab	0.31 ± 0.09 b	0.84 ± 0.23 a	0.83 ± 0.20 a	0.81 ± 0.16 a
Proctotrupoidea	Diapriidae	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.02 ± 0.02 a	0.02 ± 0.02 a

Means for all variables are untransformed values. Means within row followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

†Entrust® application rate (0.29 L per ha)

^a $F = 8.96$; $df = 4, 280$; $P < 0.0001$

^b $F = 4.90$; $df = 4, 280$; $P = 0.0008$

^c $F = 2.34$; $df = 4, 280$; $P = 0.05$

^d $F = 5.21$; $df = 4, 280$; $P = 0.0005$

^e $F = 2.26$; $df = 4, 280$; $P = 0.06$

^f $F = 75.40$; $df = 4, 280$; $P < 0.0001$



Figure 3-8. Ichneumonidae, *Diadegma insulare* (Cresson) emerged from a diamondback moth pupa collected in the companion planting study. Photo courtesy of Z. Mazlan.



Figure 3-9. Eulophidae (*Oomyzus* sp.) emerged from a diamondback moth pupa collected in the companion planting study. Photo courtesy of Z. Mazlan.



Figure 3-10. Eulophidae (*Oomyzus* sp.) dissected from a diamondback moth pupa collected in the companion planting study. Photo courtesy of Z. Mazlan.



Figure 3-11. Chalcididae (*Conura* sp.) emerged from a diamondback moth pupa collected in the companion planting study. Photo courtesy of Z. Mazlan.



Figure 3-12. Chalcididae (*Conura* sp.) inside a diamondback moth pupa collected in the companion planting study. Photo courtesy of Z. Mazlan.

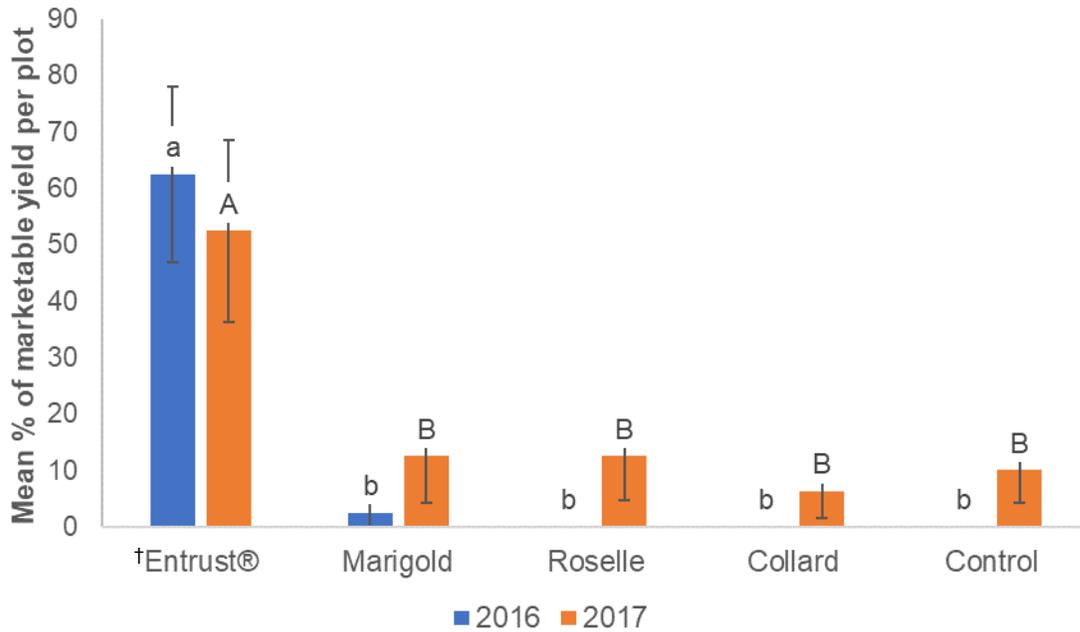


Figure 3-13. Overall mean \pm SE percentage of marketable yield for the companion planting study in 2016 and 2017. Treatments included cabbage plot treated with Entrust®, cabbage intercropped with marigold, cabbage intercropped with roselle, cabbage intercropped with collard, and untreated cabbage plot (control). Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD).

†Entrust® application rate (0.29 L per ha)



Figure 3-14. Strawberry research plot adjacent to the companion planting study in 2017. Photo courtesy of Z. Mazlan.



Figure 3-15. Imported cabbage worm, *Pieris rapae* adult on Mustard *Brassica rapa* flowers. Photo courtesy of Z. Mazlan

CHAPTER 4 THE EFFECTS OF ROSELLE FRUIT EXTRACTS ON DIAMONDBACK MOTH

The use of botanical-based products was one of the earliest approach to managing insect pests before being replaced by chemical insecticides in the 1940s (Pedigo and Rice 2014). Subsequently, organophosphate, carbamate, organochlorine, and pyrethroid chemical insecticides became more widely used in agriculture because of the ability to provide more effective and persistent pest control (Khater 2012). However, the overuse of chemical insecticides led to numerous problems including the persistence of insecticide residues in the environment, contamination of ground water, negative impact on beneficial insects, emergence of secondary pests, and insecticide resistance (Dubey et al. 2011). These negative impacts increased the need to develop alternatives to chemical insecticides that are more environmentally friendly and ecologically sound, such as plant-based insecticides (Prakash and Rao 1997, Isman et al. 2011).

Plant and insect interactions are mediated by secondary plant chemicals. Plant chemical compounds are mainly used in defense mechanisms against insect herbivory. These secondary metabolites affect insects in a number of ways including repellency, oviposition deterrent, feeding inhibitor, toxins, and growth regulator (Maia and Moore 2011). Plant compounds have been studied extensively and are used directly on crops for arthropod pest management or indirectly in companion planting (Parker et al. 2013, Balmer 2014, Mutisya et al. 2016), trap cropping (Mitchell 2000, Badenes-Perez et al. 2004, 2005, Musser 2005, Shelton and Badenes-Perez 2006), and dead-end trap crops (Shelton and Nault 2004) to manage pest populations. Plant compounds have also

been extracted and used as active ingredients in many botanical insecticides (Schmutterer 1992, Liang et al. 2003, Ahmad et al. 2012).

In an early study, neem seed extracts were reported to control cabbage pests through feeding deterrent, and growth regulator (Schmutterer 1990, 1992). In 1992 and 1993, a neem-based insecticide product (Azadirachtin) was tested on cabbage to manage diamondback moth (DBM) and cabbage looper (CL) in Texas and was found to effectively managing these pests (Leskovar and Boales 1996). Another plant extract that had been studied on cabbage pests was a wild crucifer *Erysimum cheiranthoides* L. extract which was reported to deter oviposition by cabbage worm (CW) (Dimock and Renwick 1991). Ethanol extracts from harmel seeds *Peganum harmala* L. (Nitrariaceae) treated on cabbage leaf discs also exhibited insecticidal effects, feeding and oviposition deterrent, and sublethal effects on DBM (Abbasipour et al. 2010).

Plants in the family Malvaceae have also been reported to have a deterrent effects on selected arthropod pests. Some of these plants include *Hibiscus syriacus* L. (Bird et al. 1987), *Sphaeralcea emoryi* Torrey (Honda and Bowers 1996) and portia tree, *Thespesia populnea* Cav. (Dongre and Rahalkar 1992). Previous studies by Bird et al. (1987), and Honda and Bowers (1996) found that the boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) only fed on *H. syriacus* and *S. emoryi* flower buds after calyxes were removed. Detailed studies found that the calyxes of these plant contain secondary chemicals which responsible for feeding and also oviposition deterrents. Other plant species in the Malvaceae family include the portia tree, *Thespesia populnea* Cav., which can act as an oviposition deterrent and have antifeedant and antibiosis effect on spotted bollworm *Earis vittella* Fabricious

(Lepidoptera: Nolidae) when rearing diet (okra) were treated with *T. populnea* extract (Dongre and Rahalkar 1992).

Although several studies on plant-based insecticides have been reported and published in the past 15 years (Berry et al. 2008, Isman and Grieneisen 2014), only two types of new plant-based products have been commercialized, which are neem-based (Schmutterer 2002) and essential oil-based (Isman 2000). The recent rapid growth in the number of organic growers to accommodate the increased demand for organic foods make it is necessary to have more options for natural insecticide products for managing pests specifically in organic agroecosystems.

Roselle *Hibiscus sabdariffa* L. (Malvaceae) is a common crop in many tropical and sub-tropical regions of the world, including Africa and the Caribbean. This plant is native to Asia or Tropical Africa (Julia 2017). In many parts of the world, the calyces are commonly used for beverages, jams, jellies, and as greens in salads and stews. This plant is rich in anthocyanins, a property that creates the red color (Duangmal et al. 2008) and provides a rich source of antioxidants (Ali et al. 2005). Despite the edible part of this plant, it is also known to be resistant to root-knot nematode (Wilson and Menzel 1964) and have potential to be used in controlling leaf cutting ants (Boulogne et al. 2012). The fruits and leaves of this plant contain phenolic compounds (anisaldehyde) that have insecticidal properties (Mahadevan et al. 2009, Boulogne et al. 2012). Previous studies reported that roselle extracts have the potential to be used in management of stored product pests which include *Tribolium castaneum* Herbst (Hajera Khatun et al. 2011) and *Trogoderma granarium* Everts (Coleoptera; Dermestidae)

(Musa et al. 2007). However, there is a lack of research that has evaluate the effect of this extract on lepidopteran pests.

Therefore, laboratory experiments were conducted to evaluate the effect of roselle fruit extracts (RFE) on the activity of diamondback moth (DBM) larvae and adult oviposition activity. Plant extract solution was prepared and tested on adult DBM in an oviposition study and 3rd and 4th instar larvae in a larval orientation and settlement choice assay. The goal for this study was to evaluate the existence of any deterrent effect from roselle fruit extracts that can be utilized in integrated pest management for DBM.

Materials and Methods

Study Site

Experiments were conducted at the Fruit and Vegetable IPM laboratory, Entomology and Nematology Department, University of Florida.

Diamondback Moth Colony

Larvae used in the laboratory assay were taken from a DBM colony established in 2015. Initially, the colony was started from 100 DBM pupae that were obtained from an untreated cabbage field (from experiment 1) and reared in plastic containers measuring 15 cm x 10 cm x 6 cm. Plastic containers were kept inside an environmental chamber at 26 °C, 63% RH, and 16:8 h L: D photoperiod until adult emergence. Adult moths were kept separately inside a 1 mm mesh cage measuring 30 cm x 30 cm x 30 cm and supplied with 10% honey solution for a food source. Fresh cabbage leaves were placed inside the rearing cage overnight for collecting eggs. Larvae were fed with organic cabbage leaves purchased from a local store. These leaves were washed in distilled water and air-dried before being placed into the rearing container.

Preparation of Roselle Fruit Extracts (RFE)

Roselle fruits were collected from companion planting study (Chapter 3). The calyces were separated from the seed capsules, cleaned with distilled water and air-dried inside the fume hood. Dried calyces were weighed into 100 g groups and each group was mixed with 200 ml (70% grade) ethanol in a Farberware® 4-speed digital blender (Model 103742, Farberware Licensing Company, LLC.). The extraction was filtered using fine mesh (1 mm) to separate the RFE from the calyces. The RFE solution was concentrated by evaporation inside the fume hood up to 100 ml. This solution was kept in an air-tight bottle stored inside the refrigerator as the main stock and labelled as 100% solution. The calyces were discarded and only RFE diluted with distilled water in 1:1 ratio was used in the laboratory assays.

Roselle as an oviposition deterrent against diamondback moth

Pupae collected from the laboratory colony were separated in vials and reared until adults emerged. The sexes of emerging adults were identified by observing the last abdominal segment. Adult males have a pair of claspers while the female has an ovipositor inside the last abdominal segment (Figure 4-1). After adults were allowed 24 h to emerge, a pair of adults (male and female) were then introduced into the oviposition chamber measuring 16 cm x 16 cm x 16 cm with 3 side openings covered with fine mesh (1mm) to allow for air flow, and a 10% honey solution was provided inside each of the oviposition chambers (Figure 4-2).

Two treatments and a total of 30 replicates were assessed and arranged in a completely randomized design (CRD). Each set of replicates consisted of two Petri dishes measuring 6 cm diam. with wet cotton and filter paper (Whatman Q5, 5.5 cm diam.) at the base of each Petri dish to avoid early drying of tested leaf. One Petri dish

contained a cabbage disc (measuring 6 cm diam.) dipped in 50% RFE for 30 sec and the other was an untreated cabbage disc (treated with distilled water) as the control treatment. Both Petri dishes (50% RFE and control) were put inside the oviposition cage and kept in an environmental chamber (26 °C, 63% RH, and 16:8 h L: D) overnight. After 24 h, both Petri dishes were taken out from the oviposition chamber and the number of eggs laid on each leaf disc were recorded. This procedure was repeated for each replicate using new set of leaf disc (RFE treated and untreated) replaced every day until the female died, up to 7 days of adult life.

Orientation and Settlement of DBM Larva

Choice assays evaluating larval orientation and settlement on treated cabbage discs were conducted using methods as described by Midega et al. (2011). Third instar DBM larvae (aged 5 - 7 d, 3 - 5 mm long) and fourth instar DBM larvae (aged 8 - 10 d, 6 - 9 mm long) were used and each instar were studied separately. The experimental arena included an inverted Petri dish cover measuring 15 cm diam. with filter paper (Whatman Q5, 15 cm diam.) placed at the base of the cover. The inverted bottom of the Petri dish was used as the cover of the Petri dish to prevent the larvae from escaping (Figure 4-3).

The experiment was designed as a choice assay with each Petri dish (replicate) receiving untreated (control) and treated (50% RFE) cabbage leaf discs, each one measuring 2 cm diam. and placed equidistant from the larval release point. Cabbage discs with the same treatment were placed opposite to each other. Each leaf disc was about 3 mm away from the edge of the Petri dish. A total of 15 replicates were prepared. Five third instar larvae were released in the middle of the Petri dish for each replicate and were kept inside a dark room. The number of larvae on the control leaf

discs and on the treated leaf discs were recorded after 15 min, 30 min, and 1 h of exposure to observe the orientation and after 24 h for larval settlement. This study were repeated for fourth instar larvae.

Data Analysis

The assumption of normality of the data was first examined. Data that did not meet the assumption were square root transformed to fit the model.

The data recorded in the oviposition study, and the mean percentage per replicate at 1 h for orientation and at 24 h for settlement study were analyzed using Student's t-test (SAS Institute Inc. 2013). For all statistical tests, $\alpha = 0.05$.

Results

Overall significantly more eggs were laid on untreated cabbage leaf discs (control) than cabbage leaf discs treated with RFE ($t = 11.10$; $df = 418$; $P < 0.0001$). Almost 4X as many DBM eggs were laid on untreated cabbage leaf discs compared with discs treated with RFE (Table 4-1). This trend was seen when observations were recorded over a 7 d period (Table 4-2)

Within the first hour of introduction into the bioassay chamber, the mean percentage of DBM larvae were significantly more oriented toward untreated cabbage compared with discs treated with RFE for third instar larvae ($t = 12.61$; $df = 28$; $P < 0.0001$), and fourth instar larvae ($t = 13.10$; $df = 28$; $P < 0.0001$) (Table 4-3). Similarly, both larval instars had higher settlement rates on untreated cabbage compared with cabbage treated with RFE for third instar larvae: $t = 8.50$; $df = 28$; $P < 0.0001$, and fourth instar larvae: $F = 11.34$; $df = 28$; $P < 0.0001$ (Table 4-4).

Discussion

This study investigated the effect of roselle fruit extracts (RFE) on DBM larval and adult activity. We found that DBM females preferred to lay eggs on untreated cabbage and that cabbage leaf discs that were coated with roselle residues were 4X less like to have DBM eggs on them. This is a new finding since this species, *Hibiscus sabdariffa* has not been evaluated against DBM. Previously, several crude extracts derived from plants were reported to have a deterrent effect on oviposition by DBM. These included the extract from neem *Azadirachta indica* A. Juss. (Meliaceae) (Qiu et al. 1998), siam weed *Chromolaena odorata* L. (Asteraceae) (Ling et al. 2003), syringa trees *Melia azedarach* L. (Meliaceae) (Chen et al. 1996, Charleston et al. 2005), *Peganum harmala* L. (Nitrariaceae) (Abbasipour et al. 2010), yeheb *Cordeauxia edulis* Hemsl. (Fabaceae) (Egigu et al. 2010), and yam bean *Pachyrhizus erosus* L. (Fabaceae) (Basukriadi and Wilkins 2014). Plant extracts were also reported to have a deterrent effect on oviposition by other agricultural pests. For example, oviposition by adult two-spotted spider mites *Tetranychus urticae* Koch (Acari: Tetranychidae) were reduced on bean leaves treated with thorn apple *Datura stramonium* L. (Solanaceae) extract mixed with ethanol (Kumral et al. 2010). Cabbage worm oviposition was reported to be reduced on cabbage treated with wild mustard *Erysimum cheiranthoides* L. (Brassicaceae) (Dimock and Renwick 1991).

In the present study, DBM moth was found to oviposit significantly less on RFE treated cabbage leaf discs compared with untreated cabbage leaf discs. This suggests that RFE may contain an oviposition deterrent compound or volatiles that are responsible for preventing oviposition on treated cabbage discs. Studies have reported on non-host secondary plant compounds including rutin and coumarin were found to

have oviposition deterrent effect on DBM (Tabashnik 1985). These compound occur in non-crucifers plants at relatively high concentration (Leung 1980). For example, rutin was found in tomato (Tabashnik 1985) while coumarin was found in yellow sweet clover *Melilotus officinalis* L. (Fabaceae) (Gupta and Thorsteinson 1960). Therefore, future studies on plant chemicals and volatiles derived from roselle fruit will be useful to determine these deterrent compounds in this plant.

Findings from the larval choice bioassay suggested that both third and fourth instar larvae were highly oriented and more likely to settle on untreated cabbage compared with cabbage treated with RFE. This suggests that treating cabbage leaves with RFE makes the host less attractive to DBM larvae and influences their dispersal behavior and host acceptance. The RFE may also contain plant chemicals that act as a feeding deterrent. Diamondback moth larval feeding is highly influenced by secondary plant metabolites including glucosides, sinigrin, sinalbin, and glucocheirolin which act as specific feeding stimulants (Talekar and Shelton 1993, Shelton 2004). Cabbage treated with crude plant extracts including extracts from *Melia volkensii* Guerke (Meliaceae) (Akhtar and Isman 2004) and sweet flag *Acorus calamus* L. (Acoraceae) (Reddy et al. 2016) have been reported to have an antifeedant effects on DBM larvae. Other plants that may deter oviposition and larval feeding include *M. azedarach* (Charleston et al. 2005) and *C. edulis* (Egigu et al. 2010). Akhtar and Isman (2004) also reported that *M. volkensii* have the potential to act as feeding deterrents on other insect pests including armyworm, *Pseudaletia unipuncta* Haworth and mexican bean beetle, *Epilachna varivestis* Mulsant when exposed with corn (cv. Hybrid sweet corn) and broad bean (cv. Mirado) plants treated *M. volkensii* extracts (Akhtar and Isman 2004).

Our findings suggest that cabbage treated with roselle extracts deterred feeding and oviposition by DBM. This finding is supported by previous studies by Musa et al. (2007) and Hajera Khatun et al. (2011), which indicated that roselle fruit extract has the potential to be used in managing insect pests other than DBM in cabbage or other cropping systems. In addition, this plant has the potential to be integrated as a companion plant in cabbage production. In conclusion, roselle has strong potential to be utilized in a crop protection program against DBM, and also could aid in the development of new botanical insecticides which can be utilized in IPM program for cabbage pests. Future studies should investigate oviposition and feeding deterrent using different concentration of RFE and as well as the mechanisms behind these effects. In addition, the effect of RFE on other cabbage pest especially on lepidopterous pests should also be considered.

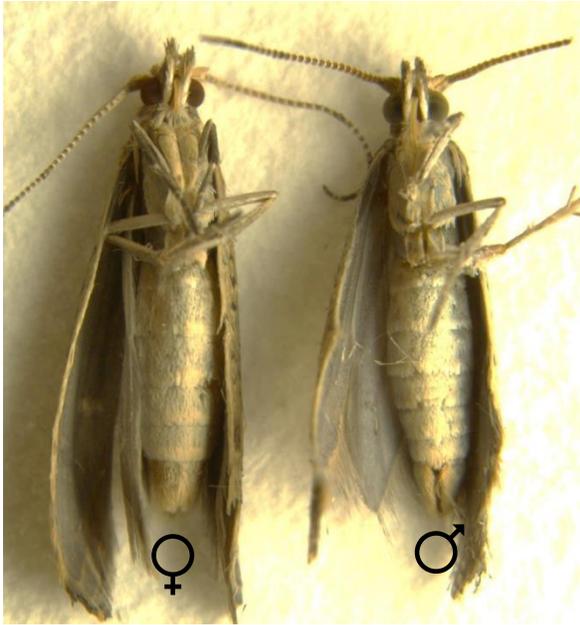


Figure 4-1. Diamondback moth; female (Left) and male (right). Photo courtesy of Z. Mazlan.



Figure 4-2. Oviposition study experimental arena. Treatments included cabbage leaf disc treated with roselle fruit extracts and untreated cabbage leaf disc. Photo courtesy of Z. Mazlan.

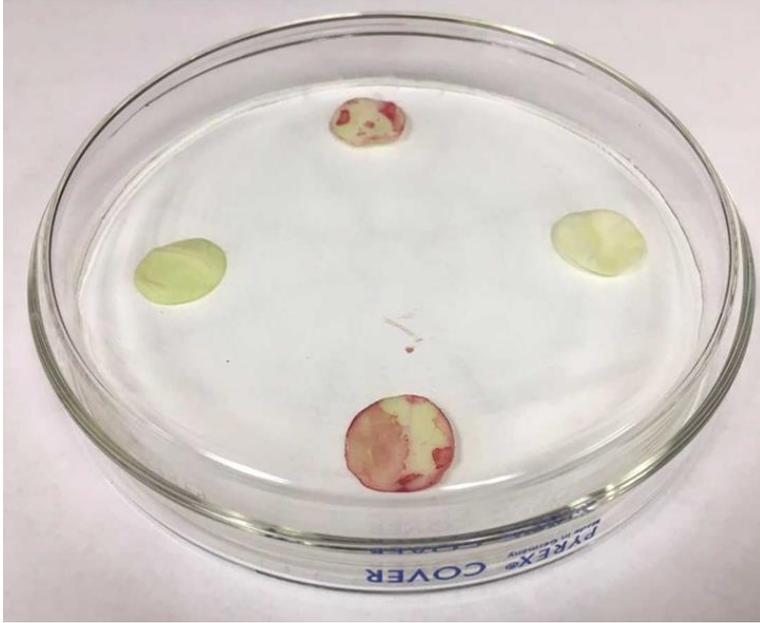


Figure 4-3. Choice assay experimental arena. Treatments included cabbage leaf discs treated with roselle fruit extracts and untreated cabbage leaf discs. Photo courtesy of Z. Mazlan.

Table 4-1. Overall mean \pm SE number of diamondback moth eggs deposited on cabbage leaf discs over seven-day period for the oviposition study. Treatments included cabbage leaf disc treated with roselle fruit extracts (RFE) and untreated cabbage leaf disc.

Trt	Mean \pm SE
Cabbage treated with RFE	3.08 \pm 0.40
Cabbage	12.02 \pm 0.70

$t = 11.10; df = 418; P < 0.0001$

Mean in the same column marked by different letter are significantly different by student T-Test ($P < 0.05$)

Table 4-2. Mean \pm SE number of diamondback moth eggs deposited on cabbage leaf discs over seven-day period for the oviposition study. Treatments included cabbage leaf disc treated with roselle fruit extracts (RFE) and untreated cabbage leaf disc.

Days	Cabbage treated with RFE	Cabbage	t Value (df=58)	P Value
1	5.27 \pm 1.84 a	12.73 \pm 2.69 b	2.29	0.03
2	5.30 \pm 1.31 a	12.77 \pm 2.07 b	3.05	0.003
3	3.90 \pm 1.19 a	13.30 \pm 1.93 b	4.13	0.0001
4	2.47 \pm 0.53 a	17.03 \pm 1.45 b	9.42	<0.0001
5	2.00 \pm 0.50 a	12.83 \pm 1.56 b	6.60	<0.0001
6	1.33 \pm 0.34 a	9.23 \pm 1.30 b	5.87	<0.0001
7	1.30 \pm 0.99 a	6.23 \pm 0.94 b	4.99	<0.0001

Mean in the same row marked by different letter are significantly different by student T-Test ($P < 0.05$)

Table 4-3. Overall mean \pm SE percentage of orientation and settling for third instar larvae of diamondback moth. Treatments included cabbage leaf discs treated with roselle fruit extracts (RFE) and untreated leaf discs.

Treatment	% orientation	% settling
Cabbage treated with RFE	6.67 \pm 2.52 b	10.67 \pm 3.84 b
Cabbage (Control)	84.00 \pm 5.59 a	61.33 \pm 4.56 a
$t=12.61$; $df=28$; $P<0.0001$		$t=8.50$; $df=28$; $P<0.0001$

Mean in the same column marked by different letter are significantly different by student T-Test ($P<0.05$)

Table 4-4. Mean \pm SE percentage of orientation and settling for fourth instar larvae of diamondback moth. Treatments included cabbage leaf discs treated with roselle fruit extracts (RFE) and untreated leaf discs.

Treatment	% orientation	% settling
Cabbage treated with RFE	1.33 \pm 1.33 b	9.33 \pm 2.67 b
Cabbage (Control)	80.00 \pm 5.86 a	76.00 \pm 5.24 a
$t=13.10$; $df=28$; $P<0.0001$		$t=11.34$; $df=28$; $P<0.0001$

Mean in the same column marked by different letter are significantly different by student T-Test ($P<0.05$)

CHAPTER 5 EFFECT OF SELECTED INSECTICIDES THAT ARE LABELLED FOR ORGANIC USE ON DIAMONDBACK MOTH

Applications of insecticides are considered as the main approach in managing diamondback moth (DBM) and other cabbage pests including cabbage looper (CL), imported cabbage worm (CW), aphids, whiteflies, and thrips in cole crops. Insecticides are applied as frequent as 8 to 10 times throughout the cropping season in cabbage to manage DBM (Reddy 2011). In organic production, only insecticides approved by USDA Organic Standards and certified by the Organic Materials Review Institute (OMRI) board are allowed, therefore limiting the options for crop protection for organic cabbage growers.

Reduced-risk insecticides include bacterial derived insecticides, *Bacillus thuringiensis* (Bt), *Chromobacterium subtsugae* (Grandevo®), Marrone Bio Innovations, Davis, CA), and spinosad (Entrust®, Dow AgroSciences LLC, Indianapolis, IN).

Botanical-based insecticides include azadirachtin (Aza-direct®, Gowan Company LLC, Yuma, AZ) which is derived from seeds of the neem tree (*Azadirachta indica*). Aza-direct® is one formulation of a pesticide that is OMRI approved and can be used to manage pests in cole crops (Olson et al. 2012). It causes cessation of feeding in insects and is known to affect growth, development, and reproduction in insects (Koul 2004).

Another insecticide that is approved for organic use and formulated as mixture of pyrethrins and azadirachtin is Azera® (Valent BioSciences, Walnut Creek, CA). Azera® is labelled for organic use in cole crops and it is hypothesized that the pesticide displays some synergy since it possess the combine attributes of pyrethrum and azadirachtin. All

aforementioned insecticides have been registered for managing DBM and other cabbage pests.

Among registered insecticides, Entrust® had been used most extensively in organic production because of its faster knockdown action compared with other insecticides (Dayan et al. 2009), and its effectiveness against a range of lepidopterans especially with regards to cole crops (O.E. Liburd personal comm). Spinosad disrupts the nicotinic acetylcholine receptor (nAChRs) by targeting binding sites of the insect nervous system (Salgado et al. 1998, Millar and Denholm 2007) which causes immediate effect after the insect begins feeding. Although reduced-risk insecticides are relatively safe to humans and the environment, several studies indicated that some reduced-risk insecticides including abamectin and spinosad can negatively affect biological control agents (BCA) (Bommarco et al. 2011).

In previous studies, applications of spinosad caused 100% mortality of *Diadegma insulare* (a major DBM parasitoid) (Harcourt 1960, Ooi 1992, Xu et al. 2010), minute pirate bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Gradish et al. 2011, Biondi et al. 2012) and swirski mites, *Amblyseius swirskii* (Athias-Henriot) (Arachnida: Mesostigmata: Phytoseiidae) (Gradish et al. 2011). Elimination of BCA often leads to more serious attacks by insect pests. Hence, it is critical to reduce the amount of insecticides in the agricultural ecosystem and to promote sustainable management of cabbage pests through an integrated approach.

Similar to the benefits derived from using Azera® it may be possible to exploit other chemistries that are labelled for organic use by mixing them from various classes. This may extend the residual activity of selected insecticides and more protection of

cole crops from key pests. Furthermore, this tactic could decrease the labor cost by reducing the number of insecticide applications required (Cabello and Canero 1994, Blackshaw et al. 1995). Insecticide mixtures may cause synergistic interaction which can enhance the effectiveness against target pests (Warnock and Cloyd 2005, Cloyd et al. 2007). Previous studies of Brownbridge et al. (2000) found increased efficacy when insecticide mixtures were used against whiteflies and western flower thrips (*Frankliniella occidentalis* Pergande) (Cloyd 2003). Mixing two insecticides with different modes of action have also been reported to delay the development of insecticide resistance (Ahmad 2004); although there is evidence that this strategy (insecticide mixtures) can increase the potential of pests for developing insecticide resistance.

A semi-field bioassay and a field efficacy study were conducted to evaluate four reduced-risk (biorational) insecticides labelled for use in organic cole crop production. Insecticides included Entrust[®], Azera[®], Aza-Direct[®], and Grandevo[®]. The objectives of the study were; 1) to identify tools (insecticides) that growers can use to manage key pests of cole crops including cabbage 2) to determine if there were any synergy in combining two insecticides from various classes and 3) to investigate the effects of reduced-risk insecticides on BCA populations or key natural enemies.

Materials and methods

Study Site

The semi-field bioassay and field efficacy studies were conducted at the University of Florida Plant Science Research and Education Unit (PSREU) in Citra (location: 29.410868N, 82.141572W), Marion County, Florida in spring 2017. Soil type of the experimental plot is sandy loom and the pH is 7. The field was prepared by using standard commercial practices. In the semi-field bioassay, insecticide treatments were

applied in the field and DBM larvae were exposed to the treated cabbage leaf discs in the Laboratory, Department of Entomology and Nematology, University of Florida, Gainesville, FL.

Growing Seedlings

Cabbage seedlings (*Brassica oleracea var. capitata*) were sown from seeds (Urban Farmer Seeds, Westfield, IN) in organic garden soil potting mix (Miracle-Gro, Marysville, OH) in styrofoam seedling trays (Speeding Inc., PO Box 7220, Suncity FL 33586). Irrigation was done manually, three to four times per week to maintain soil moisture at 5 – 8%. Cabbage seedlings were grown according to standard production practices (Zotarelli et al. 2017) inside the greenhouse located at the Department of Entomology and Nematology, University of Florida in Gainesville, FL. Seedlings were allowed to grow in the greenhouse for six weeks before they were planted in the field.

Field Preparation and Maintenance of Crops

The field was prepared by following standard commercial practices using moldboard plow (Case IH, Hinsdale, IL) and disking (Athens Disc Machine). Afterward, raised beds each 0.9 m wide and 6 inches were prepared by machine (KENNCO Manufacturing Inc.). Granular fertilizer (N-P-K: 10-10-10) was applied at 448 kg per ha in a furrow 20 cm from and parallel to the both sides of seed row and was incorporated within 15 cm of the soil surface. Halo sulfuron methyl (37 ml per ha, Sandea®, Gowan Company LLC., Yuma, AZ) was used as a pre-emergence herbicide to control weeds. For performing irrigation, two drip tapes (Ro-Drip, USA) with 30 cm emitter spacing were placed 15 cm apart on each side parallel to the center of a bed. Each bed was then covered with black plastic mulch (TriEst Ag Group Inc., Greenville, NC).

Cabbage seedlings were planted 30 cm apart in two rows on a raised beds measuring 3 m x 0.9 m. Seedlings were spaced 30 cm within the same row and 42 cm in between rows (Figure 5-1). Liquid fertilizer (N-P-K: 6-0-8, Mayo Fertilizer, Lafayette, FI) was applied weekly at 236 liter per ha. No additional insecticides, except experimental insecticides were used to maintain crop.

Experimental Insecticides

Insecticides used in both studies include Entrust® (22.5% Spinosad, Dow AgroSciences LLC, Indianapolis, IN), Aza-Direct® (1.2% Azadirachtin, Gowan Company LLC, Yuma, AZ), Azera® (1.2% Azadirachtin and 1.4% Pyrethrins, Valent BioSciences, Walnut Creek, CA), and Grandevo® (30% *Chromobacterium subtsugae* strain PRAA4-1^T, Marrone Bio Innovations, Davis, CA). These insecticides met the USDA organic standards and were listed on the Organic Material Review Institute (OMRI).

Diamondback Moth Colony

Diamondback moth (DBM) larvae were obtained from a laboratory colony reared inside an environmental chamber at 26°C, 63% RH, and 16:8 h L:D photoperiod as described in the Chapter 4. The 3rd and 4th instar larvae from F₁₅ colony aged 10 to 15 d with length approximately 4 – 10 mm were used for this study.

Semi-field Bioassay

Field. Cabbage seedlings were transplanted into raised beds measuring 3 m x 0.9 m. Each treatment plot consisted of one bed of 3 m long and was separated with a buffer row of cabbage and at least 60 cm between treatments (Figure 5-2). Five treatments were assessed and arranged in a completely randomized design (CRD) with 4 replicates for each treatment. Treatments include: 1) Entrust® (0.29 L per ha) , 2) Aza-Direct® (4.09 L per ha), 3) Azera® (2.34 L per ha), 4) Grandevo® (3.36 kg per ha), and 5)

Control (untreated). Insecticides were applied in the field using a backpack sprayer (model 425, SOLO, Newport News, VA) fitted with XR Teejet nozzle (11004 VK). Leaves were allowed to air-dry for approximately 30 min after application, a leaf was extracted from each treatment plot. The leaves were placed in a zip lock bag which was marked with date, treatment, and plot. All samples were transported to the laboratory to be used in the laboratory assay.

Laboratory. Treated leaves were cut into discs measuring 6.5 cm diam. Each leaf disc was placed onto Whatman No. 5 filter paper (9 cm diam.) in a 10 cm diam. Petri dish. Wet cotton was placed around the inner wall of the Petri dish to avoid desiccation. Four fourth instar larvae were introduced into each of Petri dish. Five treatments were assessed and arranged in CRD with 8 replicates. Larvae were visually observed at 2, 6, 12, 24, 48 and 72 h after introduction to assess their activity. Larval activity observed was rated by the following categories; 0 (dead), 3 (reduced activity), and 5 (normal activity) (Liburd et al. 2003).

Field-efficacy Study for Tank Mixing of Reduced-Risk Insecticides Against DBM

In the field efficacy trial, cabbage seedlings were initially grown in the greenhouse as described above and then transplanted into the field. Cabbage was planted 30 cm apart and each treatment plot consisted of two beds (with 2 rows per bed) and each bed measuring 3 m x 0.9 m and was 0.9 m apart. Each treatment was separated with at least 3 m buffer zone of uncultivated area. Five treatments were assessed and arranged in a completely randomized design (CRD) with 4 replicates for each treatment. Treatments include 1) Entrust[®] (0.29 L per ha) alone, 2) a tank mix of Entrust[®] (0.15 L per ha) + Aza-Direct[®] (2.05 L per ha), 3) a tank mix of Entrust[®] (0.15 L per ha) + Azera[®] (1.17 L per ha), 4) a tank mix of Entrust[®] (0.15 L per ha) + Grandevo[®]

(1.68 kg per ha), and 5) an untreated plot (control). Insecticides were applied in the field using a backpack sprayer (model 425, SOLO, Newport News, VA) fitted with XR Teejet nozzle (11004 VK). Insecticide applications were done on a weekly basis from the fourth week through ninth week after planting with a total of 6 applications.

Sampling was conducted weekly by randomly selecting five cabbage plants per plot to be observed. *In situ* counts of insect pests and beneficial insects were done prior to the application of each treatment and two days after each treatment was applied. Cabbage pests and beneficial insects were also monitored using yellow sticky Pherocon AM unbaited traps (Great Lakes IPM, Vestaburg, MI, USA) that were mounted on wooden stakes and placed just above the plant canopy. One yellow sticky trap per plot was replaced weekly from 4 weeks after transplanting cabbage seedlings for 6 weeks of insecticide treatment application. Yellow sticky traps were brought to the laboratory and observed under a 10X dissecting microscope for adult DBM, alate aphids, whiteflies, thrips, and BCA (parasitoids and predators). All BCA that were caught on the trap were identified to the family level.

At harvest, all cabbage heads in the inner rows (non-sampling rows) for each treatment plot were harvested and weighed. The number of marketable and non-marketable heads was counted and weighed. Cabbage heads sized more than 1 kg with no insect damage or minor damage (i.e., no damage after removing 2 folded leaves) were rated as marketable, while cabbage heads with apparent or severe damage was rated as non-marketable.

Data Analysis

The assumption of normality of the data was first examined. Data that did not meet the assumption were square root transformed to fit the model.

In the semi-field bioassay, the mean rating per replicate was calculated and analyzed by repeated measures analysis of variance using the SAS GLM procedure (SAS Institute Inc. 2013). Treatment means were compared using the least significant difference (LSD) test to determine if there were any significant differences. For all statistical tests, $\alpha = 0.05$.

The data collected in the field efficacy study were analyzed using repeated measures analysis of variance procedures with treatment, time and treatment \times time as the fixed effects to determine if there were any differences between larval/insect counts over time. The data were then pooled together and analysis of variance (ANOVA) was used to determine if treatment means were significantly different. Means were compared using the least significant difference (LSD) test. For all statistical tests, $\alpha = 0.05$.

Results

Semi-field Bioassay

Based on mean larval activity ratings, the Entrust[®] treatment killed significantly more DBM larvae compared with all treatments at 2, 4, 6, 12, 24, 48 and 72 h (Table 5-1). At 48 h, there was greater mortality of DBM larvae introduced to cabbage leaf discs and treated with Aza-Direct[®] (mean rating of 4.47 ± 0.22) and Grandevo[®] (mean rating of 4.53 ± 0.23) compared with the control (mean rating of 5.00 ± 0.00). At 72 h, there was greater mortality of DBM larvae in the Aza-Direct[®] treatment (mean rating of 3.94 ± 0.34) compared with the Azera[®] treatment (mean rating of 4.69 ± 0.20) and the control (mean rating of 5.00 ± 0.00). The Grandevo[®] treatment also had fewer larvae alive compared with the control; however it was not significantly different to Aza-Direct[®] (Table 5-1). Overall, there were significant interactions within treatments $F = 472.66$; df

= 4,245; $P < 0.0001$ with the control having the highest number of DBM larvae alive per replicate, but this was not significantly different from the number recorded for larvae in the Azera® treatment. The Entrust® treatment had the fewest larvae alive which was significantly different from the other treatments. Grandevo® was the second best treatment with an overall larval activity rating significantly lower than Azera® and the control but not different to Aza-Direct® (Figure 5-3).

Field-efficacy Study for Tank Mixing of Reduced-Risk Insecticides Against DBM

There was a significant reduction of total pest populations including DBM, aphids, and whiteflies observed on cabbage in all insecticide treatments compared with the control (Table 5-2). Fewer DBM larvae were observed in cabbage plots treated with Entrust® and plots treated with the tank mix of Entrust® + Azera®, but this was not significantly different to tank mix of Entrust® + Grandevo®. The control had significantly greater numbers of DBM compared with the other plots (Table 5-2). Throughout the six-week sampling period for pests on cabbage treated with Entrust®, Entrust® + Azera®, and Entrust® + Grandevo®, we observed a reduction in DBM populations on cabbage after each treatment (Table 5-3).

The aphid species recorded was green peach aphid, *Myzus persicae* (Sulzer) and the cabbage aphid, *Brevicoryne brassicae* (Linnaeus). Fewer aphids were recorded on cabbage treated with the tank mix of Entrust® + Aza-Direct® compared with plots treated with Entrust® and the control. Aphid populations in cabbage treated with Entrust® + Aza-Direct® were not significantly different to aphid populations in Entrust® + Azera® and Entrust® + Grandevo® treatments (Table 5-2).

The whitefly species recorded was sweet-potato whitefly biotype B *Bemisia tabaci* (Gennadius). Fewer whiteflies were observed on cabbage in all insecticide

treatment plots compared with the control (Table 5-2). Whitefly population in the control was at least 2.5 times higher in the control than any other treatment.

The principle thrips species recorded was Florida flower thrips *Frankliniella bispinosa* (Morgan). There were no significant differences among thrips species based on *In-situ* counts.

Pest populations recorded on yellow sticky traps were not significantly different between treatments; however, there were numerically fewer DBM and whitefly populations in plots treated with insecticides compared with control plots (Table 5-4).

A total of 15 parasitoid families were captured on yellow sticky traps. These include Aphelinidae, Encyrtidae, Eulophidae, Eupelmidae, Mymaridae, Pteromalidae, Signiphoridae, Trichogrammatidae, Ceraphronidae, Bethylidae, Figitidae, Brachonidae, Ichneumonidae, Mymarommatidae, and Platygasteridae. Among these families, only ichneumonids showed significant differences between treatments. All insecticide treatments plots had significantly fewer ichneumonids ($F = 4.51$; $df = 4, 150$; $P = 0.002$) than the control with no significant differences between insecticide treatments (Table 5-5).

For predators, five families were recorded on yellow sticky traps including Tachinidae, Formicidae, Chrysopidae, Coenagrionidae, Araneae, and Coccinellidae (Table 5-6). However, none of the treatments had a significant effect on the families.

The highest marketable yields were harvested from plots treated with Entrust[®] and Entrust[®] + Azera[®] compared with plots treated with Entrust[®] + Grandevo[®] and the control. The control had 1.6 and 1.5 fewer cabbage heads than Entrust[®] and Entrust[®]

+Azera[®] respectively. Marketable yields were not significantly different between Entrust[®], Entrust[®] + Azera[®], and Entrust[®] + Aza-Direct[®] (Table 5-7).

Discussion

The major objective of this study was to identify effective tools (insecticides) for use in organic cole crop pest management. The results of the semi-field bioassay indicated that Entrust[®], Aza-Direct[®] and Grandevo[®] are tools that can be integrated into the cole crop production system because larval activity counts were less than the control. However, Entrust[®] was 5 times as effective in reducing larval activity counts as Grandevo[®] the second overall best tool identified in the study. The mortality of DBM larvae occurred almost immediately when exposed to Entrust[®] treated leaf discs. The quick knockdown of DBM may be explained by the different mode of action for Entrust[®] compared with the other insecticides. Entrust[®] cause the disruption of nicotinic acetylcholine receptors (Millar and Denholm 2007) which has immediate effect once the insect begins feeding. In addition, the residual activity of Entrust[®] has been reported to persist for more than a week after application (Balusu and Fadamiro 2012). This may increase its efficacy against DBM and other cabbage pests.

Other insecticides showed mortality only after 48 h of exposure. In contrast, Aza-Direct[®] was one of the effective insecticides identified in our semi-field bioassay. It took almost 48 h before a reduction in larval activity was observed. Aza-Direct[®] act as a feeding deterrent or as an insect growth regulator and requires adequate consumption before the symptoms are expressed (Nisbet et al. 1996). The disruption of insect development by an insect growth regulator product could lead to the reduction of subsequent generations of the pests. Effect of neem-based insecticides on DBM including feeding deterrent (Liang et al. 2003), increase larval developmental time,

failure to complete molting process, and reduced in adult fecundity and longevity (Ahmad et al. 2012). These effects decrease the potential of new adult emerging and therefore reducing the population of subsequent generation. This was also suggested by Razze et al. (2016) which indicate that growth regulator product could exhibit higher efficacy through multiple generation.

Similarly, *Chromobacterium subtsugae*, the active ingredient for Grandevo® was reported to exhibit antifeedant activity against lepidopteran pests which include the rice-cotton cutworm *Spodoptera litura* Fabricius (Baskar and Ignacimuthu 2012) and the cotton bollworm *Helicoverpa armigera* Hubner (Hirata et al. 2003). It is not clear whether the lepidopteran that is no longer feeding will eventually die but the activity within the cropping system is significantly reduced. This will allow them to be more exposed to predators and other natural biotic factors. Grandevo® was the second most effective insecticide after Entrust® and can be a good tool to be integrated in the organic cropping system. Martin et al. (2007) found that *C. subtsugae* showed insecticidal activity against DBM larvae, colorado potato beetle *Leptinotarsa decemlineata* Say larvae, corn rootworm *Diabrotica* spp. larvae, and gypsy moth *Lymantria dispar* L. larvae. Grandevo® was also previously tested against pecan weevil *Curculio caryae* Horn (Curculionidae) in the field efficacy study resulting in a reduction in plant injuries (Shapiro-Ilan et al. 2013).

Azera® did not perform well in the field-bioassay trial the overall mean larval activity rating was equal to the control. Azera® is formulated from a mixture of pyrethrin and azadirachtin. It is not clear why Azera® did not perform well in the field bioassay and warrants further research.

The second objective of the study was to evaluate synergy from tank mix based on direct field studies. In the field efficacy study, all insecticides that were tested effectively reduced DBM and other cabbage pest populations in the field. A tank mixture of Entrust® and Azera® showed similar efficacy against DBM larvae as the single application of Entrust® alone. Alternatively, the tank-mixture of Entrust® and Aza-Direct® showed lower overall efficacy against DBM compare to Entrust® alone. This is an interesting finding because Aza-Direct® was more effective than Azera® when used singly. The reason for this inverse finding is unclear and needs research.

Entrust® in combination with Aza-Direct® proved to be an effective tool in reducing aphid populations. In fact it was more effective than using Entrust® alone. Entrust® has been used as a standard tool by organic growers for management of aphids in cabbages and other vegetables (O.E. Liburd per com) but the finding from this research indicates that other tools (insecticides) can be more effective.

Several lepidopteran species have been reported to develop resistance against spinosad which include DBM (Shelton et al. 2000, Zhao et al. 2006), the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Reyes et al. 2012), and the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Ahmad et al. 2003, Wang et al. 2009) as result of extensive applications of this insecticide in conventional and organic systems (Zhao et al. 2006). Furthermore, Entrust® is among the most expensive insecticide (\$ 481 per L) that is available for organic production, hence it is crucial to find other alternative insecticides with different mode of action that can either be applied in a rotational program or tank mixed with Entrust®.

Conserving BCAs population in cropping system is crucial especially to ensure the compatibility of the integration with cultural practices such as companion planting therefore improving the effectiveness of the IPM program for cabbage pests. Most of the natural enemies in the various families were not affected by the reduced-risk (biorationals) insecticides used in this study. However, parasitoids in the family Ichneumonidae were greatly reduced in all insecticide treated plots. The greatest reduction in ichneumonids was recorded in plots treated with Entrust® alone. This finding was supported by previous studies which reported that spinosad, the active compound of Entrust®, was harmful to parasitoids (Williams et al. 2003), predators (Gradish et al. 2011, Biondi et al. 2012), and also demonstrated a potential threat to pollinators, especially honey bees (Morandin et al. 2005). Biocontrol agents or natural enemies are important in regulating secondary pest populations and attacking pests that may have escaped from insecticide treatment (Hardin et al. 1995). Therefore, destroying BCA populations could lead to resurgence of the secondary pests and could potentially cause more serious damage on cash crops.

Overall, insecticide treatments effectively managing cabbage pests in the field as I recorded increased marketable yields from all insecticide treatment plots compared with the control (untreated plots). Among insecticide treatment plots, plot treated with Entrust® alone, and plots tank mixed with Entrust® and Azera® had numerically the highest yields. Yields from Entrust® in combination with Aza-Direct® were not statistically different although numerically lower than Entrust® alone and tank mix of Entrust® and Azera®.

In summary, this study is useful in identifying effective tools for organic production of cole crops and for providing different options for insecticide mixture which could provide adequate control for cabbage pests. An important finding from this study is that Aza-Direct[®] and Grandevo[®] can be used singly in rotation programs with Entrust[®] in cole crop management for key pests and that the efficacy of Azera[®] can be increased in tank mixed with Entrust[®]. Finally, with the exception of Entrust[®] none of these compounds negatively affect BCA or Natural enemies. These reduced-risk insecticides can be used to increase marketable yields in cole crop systems. Further evaluation are needed to determine the compatibility of these new tools (Azadirect[®], Grandevo[®]) and insecticide mixture (Entrust[®] + Azera[®]) with other IPM practices such as companion planting.



Figure 5-1. Cabbage planted on a raised bed covered with black plastic mulch for the field efficacy study. Photo courtesy of Z. Mazlan.

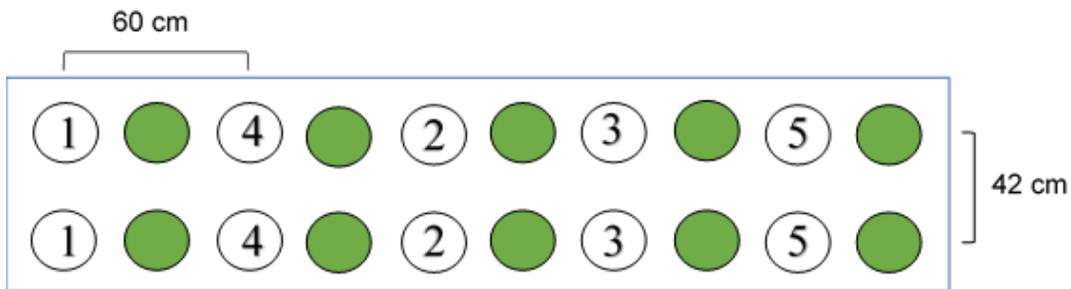


Figure 5-2. Experimental design for the semi field-based insecticide study. Treatments included application of Entrust[®], Aza-Direct[®], Azera[®], Grandevo[®], and untreated cabbage plot.

Table 5-1. Mean \pm SE rating of diamondback moth larval activities on cabbage leaf discs treated with different insecticides over a 72-hour of observation period for the semi-field efficacy study in Apr 2016. Treatments included application of Entrust[®], Aza-Direct[®], Azera[®], Grandevo[®], and untreated cabbage plot (control).

Treatment	2	4	6	12	24	48	72
Entrust [®]	3.44 \pm 0.64 b	2.59 \pm 0.76 b	0.63 \pm 0.31 b	0.16 \pm 0.16 b	0.00 \pm 0.00 b	0.00 \pm 0.00 c	0.00 \pm 0.00 d
Aza-Direct [®]	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	4.47 \pm 0.22 b	3.94 \pm 0.34 c
Azera [®]	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	4.69 \pm 0.20 ab
Grandevo [®]	5.00 \pm 0.00 a	5.00 \pm 0.00 a	4.84 \pm 0.16 a	4.84 \pm 0.16 a	4.84 \pm 0.16 a	4.53 \pm 0.23 b	4.10 \pm 0.38 bc
Control	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a	5.00 \pm 0.00 a
Trt (df=4,35)	<i>F</i> =5.98; <i>P</i> =0.0009	<i>F</i> =10.06; <i>P</i> <0.0001	<i>F</i> =156.34; <i>P</i> <0.0001	<i>F</i> =473.25; <i>P</i> <0.0001	<i>F</i> =1009; <i>P</i> <0.0001	<i>F</i> =228.39; <i>P</i> <0.0001	<i>F</i> =68.58; <i>P</i> <0.0001

Means for all variables are untransformed values. Means in columns followed by the same letters are not significantly different $P \leq 0.05$ (LSD). Larval activity observed was rated by the following categories; 0 (dead), 3 (reduced in activity), and 5 (normal activity) (Liburd et al. 2003).

Application rate:

Entrust[®] 0.29 L per ha
Aza-Direct[®] 4.09 L per ha
Azera[®] 2.34 L per ha
Grandevo[®] 3.36 kg per ha

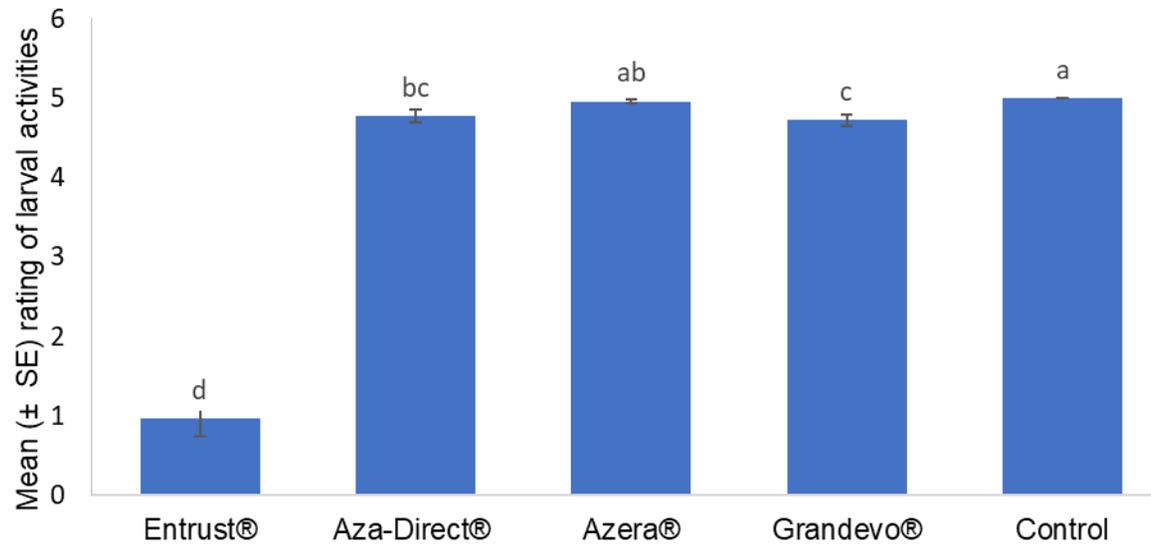


Figure 5-3. Overall mean \pm SE rating of larval activities for the semi-field efficacy study in Apr 2016. Treatments included application of Entrust®, Aza-Direct®, Azera®, Grandevo®, and untreated cabbage plot (control). Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD).

Application rate:

Entrust®	0.29 L per ha
Aza-Direct®	4.09 L per ha
Azera®	2.34 L per ha
Grandevo®	3.36 kg per ha

Table 5-2. Mean \pm SE number of cabbage pests observed during *In-situ* counts for the field efficacy study from Mar to Apr 2016. Treatments included cabbage plot treated with Entrust[®], cabbage plot treated with tank-mix of Entrust[®] and Aza-Direct[®], Entrust[®] and Azera[®], Entrust[®] and Grandevo[®], and untreated cabbage plot (control).

Treatment	DBM	Cabbage looper	Cabbage worm	Aphids	Whitefly	Thrips	Total pests
Entrust [®]	0.08 \pm 0.03 c	0.20 \pm 0.19 a	0.01 \pm 0.01 a	1.64 \pm 0.26 b	0.06 \pm 0.02 b	0.04 \pm 0.02 a	2.03 \pm 0.32 b
Entrust [®] + Aza-Direct [®]	0.23 \pm 0.06 b	0.01 \pm 0.01 a	0.02 \pm 0.01 a	0.89 \pm 0.12 c	0.08 \pm 0.03 b	0.13 \pm 0.07 a	1.37 \pm 0.15 b
Entrust [®] + Azera [®]	0.08 \pm 0.02 c	0.01 \pm 0.01 a	0.01 \pm 0.01 a	1.34 \pm 0.21 bc	0.04 \pm 0.02 b	0.07 \pm 0.03 a	1.54 \pm 0.21 b
Entrust [®] + Grandevo [®]	0.09 \pm 0.02 bc	0.00 \pm 0.00 a	0.00 \pm 0.00 a	1.35 \pm 0.23 bc	0.08 \pm 0.03 b	0.08 \pm 0.03 a	1.61 \pm 0.23 b
Control	0.92 \pm 0.11 a	0.00 \pm 0.00 a	0.03 \pm 0.01 a	2.41 \pm 0.32 a	0.20 \pm 0.05 a	0.05 \pm 0.02 a	3.61 \pm 0.33 a
Trt (df=4,840)	F=47.18; P<0.0001	F=1.08; P=0.36	F=1.79; P=0.13	F=6.49; P<0.0001	F=4.41; P=0.002	F=0.96; P=0.43	F=14.07; P<0.0001

Means for all variable are untransformed values. Means in column followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

Application rate:

Entrust[®] (0.29 L per ha)

Entrust[®] (0.15 L per ha) + Aza-Direct[®] (2.05 L per ha)

Entrust[®] (0.15 L per ha) + Azera[®] (1.17 L per ha)

Entrust[®] (0.15 L per ha) + Grandevo[®] (1.68 kg per ha)

Table 5-3. Mean \pm SE number of diamondback moth (DBM) population observed in *In-situ* counts before and two days after insecticide application for the field efficacy study from Mar to Apr 2016. Treatments included cabbage plot treated with Entrust[®], cabbage plot treated with tank-mix of Entrust[®] and Aza-Direct[®], Entrust[®] and Azera[®], Entrust[®] and Grandevo[®], and untreated cabbage plot (control).

Observation Date	Entrust [®]	Entrust [®] + Aza-Direct [®]	Entrust [®] + Azera [®]	Entrust [®] + Grandevo [®]	Control	Trt (df=4,70)
16 Mar	0.33 \pm 0.16 a	0.33 \pm 0.19 a	0.33 \pm 0.19 a	0.20 \pm 0.11 a	0.40 \pm 0.13 a	F=0.22 P=0.93
18 Mar†	0.07 \pm 0.07 a	0.20 \pm 0.14 a	0.00 \pm 0.00 a	0.07 \pm 0.07 a	0.20 \pm 0.11 a	F=0.97 P=0.43
23 Mar	0.13 \pm 0.09 b	0.33 \pm 0.13 ab	0.13 \pm 0.13 b	0.13 \pm 0.09 b	0.67 \pm 0.19 a	F=3.19 P=0.02
25 Mar†	0.00 \pm 0.00 a	0.07 \pm 0.07 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.13 \pm 0.09 a	F=1.40 P=0.24
30 Mar	0.07 \pm 0.07 b	0.07 \pm 0.08 b	0.07 \pm 0.07 b	0.00 \pm 0.00 b	0.47 \pm 0.17 a	F=4.38 P=0.003
01 Apr†	0.00 \pm 0.00 b	0.13 \pm 0.09 b	0.00 \pm 0.00 b	0.00 \pm 0.00 b	1.07 \pm 0.37 a	F=7.43 P<0.0001
05 Apr	0.00 \pm 0.00 b	1.33 \pm 0.51 b	0.00 \pm 0.00 b	0.40 \pm 0.16 b	0.53 \pm 0.22 a	F=4.44 P=0.003
08 Apr†	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.07 \pm 0.07 a	0.33 \pm 0.21 a	F=2.14 P=0.09
12 Apr	0.07 \pm 0.07 b	0.07 \pm 0.07 b	0.07 \pm 0.07 b	0.07 \pm 0.07 b	0.93 \pm 0.37 a	F=4.83 P=0.002
15 Apr†	0.00 \pm 0.00 b	0.00 \pm 0.00 b	0.13 \pm 0.09 b	0.00 \pm 0.00 b	1.20 \pm 0.33 a	F=11.99 P<0.0001
19 Apr	0.00 \pm 0.00 b	0.07 \pm 0.07 b	0.07 \pm 0.07 b	0.07 \pm 0.07 b	1.93 \pm 0.50 a	F=13.38 P<0.0001
22 Apr†	0.33 \pm 0.33 b	0.20 \pm 0.20 b	0.13 \pm 0.09 b	0.13 \pm 0.09 b	3.20 \pm 0.69 a	F=13.99 P<0.0001

Means for all variables are untransformed values. Means in column followed by the same letters are not significantly different $P \leq 0.05$ (LSD).

†Observation after insecticide treatment

Application rate:

Entrust[®] (0.29 L per ha)

Entrust[®] (0.15 L per ha) + Aza-Direct[®] (2.05 L per ha)

Entrust[®] (0.15 L per ha) + Azera[®] (1.17 L per ha)

Entrust[®] (0.15 L per ha) + Grandevo[®] (1.68 kg per ha)

Table 5-4. Mean \pm SE number of cabbage pests collected from yellow sticky traps over six-week period for the field efficacy study from Mar to Apr 2016. Treatments included cabbage plot treated with Entrust[®], cabbage plot treated with tank-mix of Entrust[®] and Aza-Direct[®], cabbage plot treated with tank-mix of Entrust[®] and Azera[®], cabbage plot treated with tank-mix of Entrust[®] and Grandevo[®], and untreated cabbage plot (control).

Treatment	DBM	whitefly	Aphids	thrips	Total
Entrust [®]	0.94 \pm 0.24	4.11 \pm 1.46	7.83 \pm 1.04	131.53 \pm 27.79	143.47 \pm 28.08
Entrust [®] + Aza-Direct [®]	0.64 \pm 0.17	2.72 \pm 0.79	8.86 \pm 1.22	195.81 \pm 38.66	207.39 \pm 38.81
Entrust [®] + Azera [®]	0.53 \pm 0.14	2.47 \pm 0.68	8.25 \pm 0.79	165.06 \pm 30.24	175.78 \pm 30.40
Entrust [®] + Grandevo [®]	0.81 \pm 0.19	3.53 \pm 0.94	9.64 \pm 0.97	138.75 \pm 29.82	151.92 \pm 30.38
Control	1.17 \pm 0.28	4.25 \pm 1.22	8.69 \pm 1.12	123.72 \pm 22.12	136.67 \pm 22.32
Trt (df=4,150)	<i>F</i> =1.75; <i>P</i> =0.14	<i>F</i> =1.86; <i>P</i> =0.12	<i>F</i> =0.70; <i>P</i> =0.60	<i>F</i> =2.21; <i>P</i> =0.07	<i>F</i> =2.07; <i>P</i> =0.09
Trt*obs (df=20,150)	<i>F</i> =0.76; <i>P</i> =0.76	<i>F</i> =1.56; <i>P</i> =0.07	<i>F</i> =0.32; <i>P</i> =0.998	<i>F</i> =1.10; <i>P</i> =0.35	<i>F</i> =1.09; <i>P</i> =0.37

Means for all variables are untransformed values.

Application rate:

Entrust[®] (0.29 L per ha)

Entrust[®] (0.15 L per ha) + Aza-Direct[®] (2.05 L per ha)

Entrust[®] (0.15 L per ha) + Azera[®] (1.17 L per ha)

Entrust[®] (0.15 L per ha) + Grandevo[®] (1.68 kg per ha)

Table 5-5. Mean \pm SE number of each parasitoid family collected from yellow sticky traps over six-week period for the field efficacy study from Mar to Apr 2016. Treatments included cabbage plot treated with Entrust[®], cabbage plot treated with tank-mix of Entrust[®] and Aza-Direct[®], cabbage plot treated with tank-mix of Entrust[®] and Azera[®], cabbage plot treated with tank-mix of Entrust[®] and Grandevo[®], and untreated cabbage plot (control).

Superfamily	Family	Entrust [®]	Entrust [®] + Aza-Direct [®]	Entrust [®] + Azera [®]	Entrust [®] + Grandevo [®]	Control
Chalcidoidea	Aphelinidae	0.69 \pm 0.13 a	0.42 \pm 0.13 a	0.64 \pm 0.18 a	0.58 \pm 0.14 a	0.94 \pm 0.19 a
	Encyrtidae	0.58 \pm 0.15 a	0.50 \pm 0.12 a	0.58 \pm 0.12 a	0.42 \pm 0.10 a	0.61 \pm 0.14 a
	Eulophidae	0.47 \pm 0.15 a	0.53 \pm 0.16 a	0.47 \pm 0.13 a	0.44 \pm 0.13 a	0.47 \pm 0.14 a
	Eupelmidae	0.00 \pm 0.00 a	0.03 \pm 0.03 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
	Mymaridae	1.00 \pm 0.18 a	1.08 \pm 0.18 a	1.28 \pm 0.21 a	1.17 \pm 0.22 a	1.31 \pm 0.24 a
	Petromalidae	0.22 \pm 0.08 a	0.17 \pm 0.06 a	0.33 \pm 0.14 a	0.17 \pm 0.09 a	0.33 \pm 0.10 a
	Signiphoridae	0.14 \pm 0.08 a	0.08 \pm 0.05 a	0.11 \pm 0.07 a	0.08 \pm 0.05 a	0.14 \pm 0.07 a
	Trichogrammatidae	2.14 \pm 0.38 a	1.92 \pm 0.42 a	1.50 \pm 0.33 a	1.67 \pm 0.34 a	2.03 \pm 0.43 a
Ceraphronoidea	Ceraphronidae	0.28 \pm 0.09 a	0.31 \pm 0.10 a	0.36 \pm 0.11 a	0.25 \pm 0.09 a	0.28 \pm 0.09 a
Chrysoidea	Bethylidae	0.06 \pm 0.04 a	0.03 \pm 0.03 a	0.11 \pm 0.05 a	0.03 \pm 0.03 a	0.00 \pm 0.00 a
Cynipoidea	Figitidae	0.11 \pm 0.05 a	0.06 \pm 0.04 a	0.03 \pm 0.03 a	0.08 \pm 0.05 a	0.06 \pm 0.04 a
Ichneumonoidea	Brachonidae	0.75 \pm 0.16 a	0.61 \pm 0.14 a	1.03 \pm 0.18 a	1.03 \pm 0.16 a	1.17 \pm 0.20 a
	Ichneumonidae	0.08 \pm 0.05 b	0.11 \pm 0.05 b	0.14 \pm 0.06 b	0.11 \pm 0.05 b	0.42 \pm 0.11 a
Mymarommatoidea	Mymaromatidae	0.00 \pm 0.00 a	0.03 \pm 0.03 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a	0.00 \pm 0.00 a
Platygastroidea	Platygastridae	1.11 \pm 0.21 a	1.36 \pm 0.23 a	1.36 \pm 0.28 a	1.56 \pm 0.28 a	1.64 \pm 0.28 a

Means for all variables are untransformed values. Means within row followed by the same letters are not significantly different $P \leq 0.05$ (LSD)

Application rate:

Entrust[®] (0.29 L per ha)

Entrust[®] (0.15 L per ha) + Aza-Direct[®] (2.05 L per ha)

Entrust[®] (0.15 L per ha) + Azera[®] (1.17 L per ha)

Entrust[®] (0.15 L per ha) + Grandevo[®] (1.68 kg per ha)

Table 5-6. Mean \pm SE number of each predator family collected from yellow sticky traps over six-week period for the field efficacy study from Mar to Apr 2016. Treatments included cabbage plot treated with Entrust[®], cabbage plot treated with tank-mix of Entrust[®] and Aza-Direct[®], cabbage plot treated with tank-mix of Entrust[®] and Azera[®], cabbage plot treated with tank-mix of Entrust[®] and Grandevo[®], and untreated cabbage plot (control).

Predators	Entrust [®]	Entrust [®] + Aza-Direct [®]	Entrust [®] + Azera [®]	Entrust [®] + Grandevo [®]	Control
Tachinids flies (Tachinidae)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.03 \pm 0.03	0.00 \pm 0.00
Ant (Formicidae)	0.08 \pm 0.05	0.03 \pm 0.03	0.08 \pm 0.05	0.03 \pm 0.03	0.03 \pm 0.03
Green lacewing (Chrysopidae)	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.03 \pm 0.03	0.00 \pm 0.00
Dragonfly (Coenagrionidae)	0.00 \pm 0.00	0.03 \pm 0.03	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Spider (Araneae)	0.08 \pm 0.06	0.25 \pm 0.10	0.00 \pm 0.00	0.19 \pm 0.08	0.11 \pm 0.05
Ladybug beetle (Coccinellidae)	0.06 \pm 0.04	0.03 \pm 0.03	0.00 \pm 0.00	0.11 \pm 0.07	0.03 \pm 0.03

Means for all variables are untransformed values

Application rate:

Entrust[®] (0.29 L per ha)

Entrust[®] (0.15 L per ha) + Aza-Direct[®] (2.05 L per ha)

Entrust[®] (0.15 L per ha) + Azera[®] (1.17 L per ha)

Entrust[®] (0.15 L per ha) + Grandevo[®] (1.68 kg per ha)

Table 5-7. Mean \pm SE weight of marketable heads harvested from plots for the field efficacy study from Mar to Apr 2016. Treatments included cabbage plot treated with Entrust[®], cabbage plot treated with tank-mix of Entrust[®] and Aza-Direct[®], cabbage plot treated with tank-mix of Entrust[®] and Azera[®], cabbage plot treated with tank-mix of Entrust[®] and Grandevo[®], and untreated cabbage plot (control).

Treatment	Application rate	Marketable yield per plot (kg)
Entrust [®]	0.29 L per ha	20.22 \pm 1.52 a
Entrust [®] + Aza-Direct [®]	0.15 L per ha and 2.05 L per ha	18.20 \pm 1.11 ab
Entrust [®] + Azera [®]	0.15 L per ha and 1.17 L per ha	19.28 \pm 0.39 a
Entrust [®] + Grandevo [®]	0.15 L per ha and 1.68 kg per ha	14.26 \pm 1.91 bc
Control	-	12.32 \pm 1.11 c
Interaction (df=4,10)		<i>F</i> =6.73; <i>P</i> =0.007

Means for all variables are untransformed values. Means in column followed by the same letters are not significantly different; *P* \leq 0.05 (LSD)

CHAPTER 6 CONCLUSION

In the field study evaluating the colonization of key pests and natural enemies on cabbage interplanted with selected plants compared with cabbage treated with reduced-risk insecticides, we demonstrated that Entrust[®] provided consistent and effective management of DBM over the two-year study. Diamondback moth populations were also reduced in cabbage intercropped with roselle, followed by collard and marigold. Companion plants were suggested to provide additional groundcover and extra floral nectar that contributed to an increase in natural enemy populations. Adopting companion planting in cabbage production could be an important tool in promoting higher densities of natural enemies in an agricultural system. By enhancing the natural regulation of pests by natural enemies, growers could ultimately reduce their reliance on insecticides and delay the development of resistance in major economic pests.

In the laboratory study evaluating the effect of plant extracts on DBM, adult females were found to oviposit fewer eggs on cabbage discs treated with roselle fruit extracts (RFE) compared with untreated cabbage discs. Similarly, DBM larvae avoided RFE treated cabbage in the orientation and settlement study. These findings suggest that RFE deterred oviposition and feeding by DBM on cabbage. Roselle may contain plant volatiles which act to deter DBM activity on a host plant. Therefore, future studies to determine the chemical compounds could serve as the foundation for developing a new botanical insecticide derived from roselle. Furthermore, this study also supported the finding that roselle is a potential candidate for companion plants that could be utilized in an IPM program for organic cabbage production.

In the semi-field efficacy study that evaluated four biorational insecticides, Entrust® had greater efficacy compared with Azera®, Aza-Direct® and Grandevo®. Larval mortality when introduced to Entrust® treated leaf discs was recorded at 24 h, while larval mortality was recorded after 48 h when cabbage was treated with Azera®, Aza-Direct®, and Grandevo®. In the field efficacy study evaluating Entrust® alone and the reduced rate of Entrust® mixed with Azera®, Aza-Direct®, and Grandevo®; Entrust® alone and Entrust® + Azera® showed similar efficacy in reducing DBM populations overtime. Additionally, cabbage treated with Entrust® + Azera® had similar yields to the Entrust® alone treatment. This study also found that Entrust® mixed with other insecticides provided better management of aphid populations. Fewer aphids were recorded on cabbage treated with Entrust® + Aza-Direct®, followed by other mixtures compared with the Entrust® alone treatment. Overall, reducing the rate of Entrust® by half and mixing Entrust® with other insecticides did not affect the overall performance of Entrust®. In addition, reducing the application rate of Entrust® could positively influence natural enemy populations.

This project evaluated several IPM strategies to manage DBM and other pests in organic cabbage production. The main goal of this study was to provide information for organic cabbage growers on alternatives to chemical control, such as the use of companion plants that have the potential to be integrated in IPM for cabbage. However, adopting a companion planting system alone is not sufficient for achieving effective control of cabbage pests. Effective management of cabbage pests requires the integration of cultural practices with other pest management strategies including biological control and the application of OMRI certified insecticides. Although chemical

control is still considered as the major component of an IPM program for cabbage pests, the findings from this study suggest that Entrust[®] could be applied as tank mix with biorational insecticides without affecting the effectiveness in managing cabbage pests. Future research should investigate the compatibility of companion planting with reduced-risk insecticide mixtures to manage cabbage pest populations while conserving natural enemy populations. In addition, research should explore the potential of using roselle extracts as new botanical insecticide which can be integrated with other IPM tactics including biological control for crop protection program in cabbage and other cole crop systems.

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BIOGRAPHICAL SKETCH

Zulaikha was born in Johor, Malaysia. She received her Bachelor of Science with honor in applied biology from the Universiti Sains Malaysia in 2007. In November 2007, she joined Malaysian Agricultural Research and Development Institute (MARDI) as research officer. After 7 years of service, she was granted with full scholarship to pursue her Master at the University of Florida. She began her master's program with Oscar Liburd in August 2015 at the Small Fruit and Vegetable IPM Laboratory. Her research focused on the management of cabbage pests through the integration of companion planting and application of reduced-risk insecticides for in organic cabbage production.