PROCEEDINGS

VIII International Conference on Management of the Diamondback Moth and Other Crucifer Insect Pests

R. Srinivasan, P. Sotelo-Cardona, M.P. Zalucki, A.M. Shelton

Editors

4-8 March 2019
World Vegetable Center
Shanhua, Tainan, Taiwan

World Vegetable Center

Cornell University

PROCEEDINGS

VIII International Conference on Management of the Diamondback Moth and other Crucifer Insect Pests

R. Srinivasan, P. Sotelo-Cardona,

M.P. Zalucki, A.M. Shelton

Editors

4-8 March 2019

World Vegetable Center

Shanhua, Tainan, Taiwan

World Vegetable Center • Cornell University

World Vegetable Center

The World Vegetable Center is an international nonprofit research and development institute committed to healthier lives and more resilient livelihoods through the increased production and consumption of nutritious, diverse, and health-promoting vegetables.

World Vegetable Center
P.O. Box 42
Shanhua, Tainan 74199
TAIWAN

Tel: +886 6 583 7801
Fax: +886 6 583 0009

Email: info@worldveg.org
Web: worldveg.org

WorldVeg Publication No. 21-1041
ISBN: 92-9058-234-0

Cover design: Amy Chen

Suggested citation

Srinivasan R, Sotelo-Cardona P, Zalucki MP, Shelton AM (eds.) 2021. Proceedings, VIII International Conference on Management of the Diamondback Moth and other Crucifer Insect Pests. World Vegetable Center, Taiwan. 195 p.

PROCEEDINGS

VIII International Conference on Management of the Diamondback Moth and Other Crucifer Insect Pests

SPONSORS

Gold Level sponsor (US$ 5,000)

CropLife Taiwan R.O.C.

Travel grant sponsor

International Organisation for Biological Control (IOBC)

Table of Contents

Foreword

Brassica vegetables are important crops globally, cultivated in an area of 3.88 million ha, with annual production of about 97 million t. They are an important source of vitamins, minerals, folic acid, and dietary fiber. In addition, brassica vegetables are found to reduce the incidence of cancer diseases due to their relatively high glucosinolate content. They are also high value, repeat-cycle crops that could lift smallholder farmers out of poverty. For instance, Chinese kale cultivation in Cambodia can generate an income of about US$ 7,000/ha. However, biotic constraints, especially insect pests, severely limit the productivity of vegetable brassicas and stimulate the overuse of pesticides. Besides diamondback moth (DBM, *Plutella xylostella*) – the cosmopolitan pest of brassicas – secondary lepidopterans such as head caterpillar, web worm, cabbage butterflies, common armyworm, flea beetles, and aphids cause severe yield losses worldwide. As a prophylactic measure, farmers predominantly rely on the use of chemical pesticides. Damage and management costs for DBM alone amount to an astonishing US$ 4-5 billion annually, worldwide. Besides escalating production costs, pesticide misuse and overuse pose serious threats to human and environmental health.

The World Vegetable Center (WorldVeg) pioneered scaling out DBM biological control using a guild of introduced parasitoids such as *Diadegma semiclausum*, *Cotesia plutellae*, and *Diadromus collaris* in South- and Southeast Asia from 1985-2005. This practice was extended to East Africa from 2004-2008 in collaboration with *icipe*. The International Working Group on DBM and Other Crucifer Insects, an informal group of researchers worldwide who are actively engaged in research and development in brassica pest management, is coordinated by WorldVeg. This working group has successfully organized eight international conferences since 1985. After three decades, WorldVeg in collaboration with Cornell University has hosted the conference once again at its headquarters in Tainan, Taiwan from March 4-8, 2019. About 70 participants from 14 countries participated in the conference. The *Proceedings* of this conference, which summarizes recent advances in the management of DBM as well as other brassica pests, will be a useful resource for researchers and plant protection practitioners.

Marco Wopereis
Director General
World Vegetable Center

Acknowledgements

The VIII International Conference on Management of the Diamondback Moth and other Crucifer Insect Pests was organized by the World Vegetable Center in collaboration with Cornell University (USA). The workshop was held in World Vegetable Center's headquarters, Tainan, Taiwan during March 4-8, 2019. Thirty oral presentations and six poster presentations were made in seven scientific sessions, *viz.,* Diamondback moth and other crucifer pest: the global challenge in a changing climate, Biology, Ecology and Behavior of Diamondback Moth and Other Crucifer Pests, Biological and non-chemical methods of management of crucifer pests (including organic agriculture), Insect Plant Interactions, Host Plant Resistance, and Chemical Ecology of Crucifer Pests, Insecticide Resistance and Management in Crucifer Pests, Genetic approaches to manage crucifer pests, and At the Farm and Landscape Level: Barriers to and Innovations for Management of Crucifer Pests, during the workshop. I thank all the 70 delegates from 14 countries whose participation made this workshop a great success.

I would like to acknowledge the support from Scientific Committee members, especially Dr. Paola Sotelo-Cardona, Dr. Anthony M. Shelton, Dr. Myron P. Zalucki, Dr. Michael Furlong, Dr. Sivapragasam Annamalai, Dr. Zhenyu Li, Dr. Franziska Beran, Dr. Inga Mewis, and Dr. Subramanian Sevgan, who assisted me in evolving the Scientific Program of the Conference. I am equally grateful to Dr. Marco Wopereis, Dr. David Johnson, Dr. Yin-Fu Chang and Ms. Maureen Mecozzi of World Vegetable Center who provided great support to organize the conference successfully.

I am very grateful to the financial support from the CropLife Taiwan R.O.C., International Organization for Biological Control (IOBC), the World Vegetable Center and its long-term strategic donors: Taiwan, the Foreign, Commonwealth & Development Office (FCDO) from the UK government, United States Agency for International Development (USAID), Australian Centre for International Agricultural Research (ACIAR), Germany, Thailand, Philippines, Korea, and Japan. Their funds assisted us to bring eminent *Brassica* IPM researchers including young scientists from around the world to attend as Conference participants as well as invited speakers.

I also thank Dr. Paola Sotelo-Cardona (World Vegetable Center), Dr. Myron P. Zalucki (University of Queensland), Dr. Anthony M. Shelton (Cornell University), and Ms. Ariel Wu, Ms. Maureen Mecozzi, Ms. Chen Te-ying (Kathy) and Ms. Amy Chen of the World Vegetable Center for formatting and editing the Proceedings.

Dr. Srinivasan Ramasamy
Lead Entomologist and Flagship Program Leader –
Safe & Sustainable Value Chains, World Vegetable Center
Conference Organizing Secretary

SESSION 1

Diamondback Moth and other Crucifer Pests: The Global Challenge in a Changing Climate

Management of Diamondback Moth and other Brassica Lepidopteran pests: with emphasis on Taiwan

Hsiao, W.F.
EMERITUS PROFESSOR, DEPT. OF PLANT MEDICINE
NATIONAL CHIAYI UNIVERSITY
email: hsiaowf@gmail.com

ABSTRACT

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), the cosmopolitan pest of *Brassica* crops, has been a continuing problem for vegetable growers in Taiwan. The first cropping season usually starts from August to December and the second cropping season is from November to March in Taiwan lowlands. The major lepidopteran pest is diamondback moth (DBM), and the secondary pests are *Pieris rapae crucivora*, *Spodoptera litura*, *Crocidolomia pavonana*, and *Hellula undalis*. The minor pests are *Trichoplusia ni* and *Agrotis ipsilon*. The biology, ecology and control measures of DBM have been studied extensively. A treatment window package was recently proposed by the Taiwan government to manage the insecticide resistance problem of DBM on cabbage. Being the most destructive seedling pest in summer, *H. undalis* could be controlled by applying chemical pesticides and planting resistant varieties. *S. litura* is controlled by applying chemical pesticides and mass trapping with sex pheromone traps. Its population can also be decreased by parasitoid wasps (Ichneumonidae and Braconidae), nucleopolyhedrovirus, entomopathogenic fungi and entomopathogenic nematodes. Other lepidopteran pests such as *P. rapae crucivora*, *C. pavonana*, and *T. ni* are controlled by prophylactic use of insecticides.

Keywords

Diamondback moth, *Brassica*, lepidopterans, Taiwan, treatment window

THE CULTIVATION OF CRUCIFERS IN TAIWAN

Brassicaceae or Cruciferae family contains 372 genera and 4060 accepted species. This family contains species such as *Brassica oleracea* (e.g., broccoli, cabbage, cauliflower, kale, collards), *Brassica rapa* (turnip, Chinese cabbage, etc.), *Brassica napus* (rapeseed, etc.), *Raphanus sativus* (common radish), and *Armoracia rusticana* (horseradish).

In Taiwan, the cultivated cruciferous vegetables have been classified into four types: 1. Leafy type (Chinese mustard, rape, mustard, kale); 2. Heading type (cabbage, Chinese cabbage, heading mustard, kohlrabi); 3. Flower types (Curds) (cauliflower, broccoli); and 4. Root type (radish, turnip). Most of the above crops are planted from autumn to early spring except non-heading type Chinese cabbage and Chinese mustard, which are grown year round. Table 1 provides the statistics of cruciferous crop cultivation in Taiwan during 2017. The planting seasons for the five major cruciferous crops are shown in Table 2. Other four crucifers are grown from fall to next spring. The monthly mean temperature in 2017 ranged between 15.96 and 27.78ºC suggesting that months with temperature below 20ºC are suitable for cruciferous vegetable cultivation in lowlands of Taiwan.

Table 1. Statistics of cruciferous crop cultivation in Taiwan, 2017*

Crops	Planted area (ha)	Production (t)	Value of production (NT$'000)
Chinese Mustard	5263.01	98,351.00	1,924,731
Chinese Cabbage	2,143.67	85,212.00	1,104,345
Cabbage	8,790.00	422,320.00	5,895,591
Leaf Mustard	1,463.43	39,207.00	254,064
Cauliflower	3,018.00	69,963.00	1,937,229
Kohlrabi	341.44	8,006.73	-
Broccoli	1,401.94	30,017.70	-
Kale	1,308.37	22,576.74	-

* http://agrstat.coa.gov.tw/

Table 2. The planting season of five major crucifers in Taiwan (2017)

	Chinese Mustard	Chinese Cabbage	Cabbage	Leaf Mustard	Cauliflower	Broccoli
Jan	○	○	○		○	○
Feb	○	○	○		○	○
Mar	○	○	○			○
Apr	○					
May	○					
Jun	○					
Jul	○					
Aug	○	○				○
Sep	○	○	○	○	○	○
Oct	○	○	○	○	○	○
Nov	○	○	○	○	○	○
Dec	○	○	○	○	○	○

OCCURRENCE OF MAJOR LEPIDOPTERAN PESTS ON CRUCIFERS

Talekar and Lee (1985) had conducted eight-year (1976-1983) monitoring of seasonal incidence of insect pests on Chinese cabbage and common cabbage in southern Taiwan. The results indicated that *H. undalis* infestation occurred during June-October. DBM and *P. rapae crucivora* infested both crops from October to May. *Trichoplusia ni* was also prevalent from October to May, and it preferred common cabbage over Chinese cabbage.

Nine lepidopterans such as diamondback moth (*P. xylostella*), cabbage worm (*P. rapae crucivora*), cabbage webworm (*H. undalis*) and cabbage cluster caterpillar (*C. pavonana*) have been listed as major crucifer crop pests since 1973. Sporadic noctuid species, such as *Spodoptera litura*, *Trichoplusia ni*, *Agrotis ipsilon*, and *A. segetum* are considered as secondary or minor pests. Small cabbage butterfly, *Pieris canidia sordida*, is hardly seen after 1984 (Table 3).

Table 3. Major lepidopterans on crucifers in Taiwan over four decades

		1973	1977	1984	1988	1990	2001	2004	2006
Pieridae	*Pieris rapae crucivova*	○	○	○	○	○	○	○	○
	P. canidia sordida	○	○	○					
Plutellidae	*Plutella xylostella*	○	○	○	○	○	○	○	○
Pyralidae	*Hellula undalis*	○	○	○	○	○	○	○	○
	Crocidolomia pavonana	○	○	○	○		○		○
Noctuidae	*Spodoptera litura*	○	○	○		○			○
	Trichoplusia ni	○	○	○	○	○			
	Agrotis ipsilon	○	○	○	○	○			
	A. segetum	○	○	○	○				

Cabbage butterflies, *Pieris rapae crucivora* and *P. canidia sordida*

Recorded host plants of *P. rapae crucivora* and *P. candia sordida* include 14 species in 3 families (Cheng and Hsu 2003). Larvae attack leaves, head and curds, while in high density the plants could reduce to a partial or complete skeleton. The presence of masses of wet greenish-brown excrement among leaves is indicative of these pests. Both insects have 6-7 generations per year. The adults appeared throughout the year due to the weed, such as *Bidens pilosa* L. var. *radiata* Sch., which provides the food sources for the insect. For the past three decades, *P. canidia sordida* is rarely seen in the lowland according to my personal survey.

Cheng and Hsu (2003) have studied the development of *P. rapae crucivora* at four constant temperatures when fed on *Brassica campestris* leaves. At 15, 20, 25 and 30ºC, the egg stage was 8.2, 6.0, 3.0 and 3.0 days, respectively; larval development took 26.9, 19.1, 14.1 and 11.4 days, respectively; and pupal stage was 15.8, 11.4, 8.1 and 5.9 days, respectively. Adult longevity at 15, 20, 25, 30ºC was 16.4, 19.3, 7.0 and 4.1 days, respectively. Fecundity at 20, 25 and 30ºC was 64.3±39, 46.5±49.8, and 6.3±6.8 eggs/female with a large variation. The development threshold temperatures of egg, larval and pupal stages were 7.38, 4.67 and 7.43ºC. The effective accumulated temperature for the egg, larval and pupal stages were 64.73, 286.50 and 134.60 degree-days (DD).

Biological control agents, *Cotesia*, Pteromalid, and Ichneumonid parasitoids, Granulosis virus (GV), and Reuduviid and Pentatomid predators have been listed as effective. Effective control of cabbage butterfly by granulosis virus in the field was tested by Su (1987a, b). GV was in high incidence in a pesticide-free cabbage field in Silou, Yulin, Taiwan. (Hsiao 2008, unpublished). Granulosis virus-infected 5th instar larvae were found from pesticide-free home garden cabbage plants in Chiayi, Taiwan during 2010 to 2012. In my speculation, GV might be hindered by pesticide application in the commercial crucifer fields.

Biology study and mass rearing of predatory bug, *Eocanthecona furcellata* were conducted by Chu and Chu (1975a, b). The egg, nymph, and adult stages reared at 28ºC were 7, 17-18 and 13-20 days, respectively (Chu and Chu 1975a). The numbers of egg deposited when *E. furcellata* fed on *P. rapae crucivora* and *S. litura* were 156.4 and 148.2 eggs/female, respectively (Chu and Chu 1975b).

A female adult could consume 4.5 larvae of *P. rapae crucivora* per day and the number of larvae consumed increased with an increase in prey density at 25ºC. The predator preferred to feed on larvae of *P. rapae* rather than on those of *S. litura*, B. *mori*, and *Chrysomya megacephala* (Chang and Hsieh 2001). Chang (2002) had released *E. furcellata* in the field and proved that it was able to attack *P. rapae crucivora*, *S. litura*, DBM, and *S. exigua*.

Parasitoid, *Cotesia glomeratus* usually appears from March to May and keeps the cabbage butterfly population in check. Tzou (2008) has indicated that Methyl Jasmonic acid treated radish leaf attracted more *C. glomeratus* adults and this might be helpful for enhancing biological control in the field.

Diamondback moth, *Plutella xylostella*

Since 1973, diamondback moth has become the most destructive pest of cruciferous crops in Taiwan in both the lowland and highland (Kung 1984). Host plants include cruciferous crops and watercress. First instar larva is leaf mining and is difficult to detect and control. The 2nd instar larva feeds on the lower leaf surface and results in irregular patches of damage. Younger larva tends to attack the growing tips at seedling stages.

Diamondback moth adults lay eggs mainly on the upper surface of the cabbage plant's outer leaves. In the inner leaves, they are laid on the lower leaf surface. Egg density decreased from outer to inner leaves. Within a range of 1-11 trichomes per 9 mm^2 leaf area, the number of eggs laid on Chinese cabbage leaves increased with trichome density. Most oviposition took place within 2 h after sunset (Talekar et al. 1994).

Lee and Tseng (1993) indicated that the last larval instar of DBM also preferred the same host plant species that the first three larval instars feed on. However, feeding preference during DBM larval stages was not transferred to adult oviposition preference.

Lu and Lee (1984) found that DBM has 21 generations in a year and overlapping generations were observed. The mean developmental time of egg, larvae, pupae and adults were 3.49±1.20, 8.24±1.80, 5.10±2.09 and 7.30±2.61 d, respectively. The hatching rate of egg was 80.19% and deposited 74.24 eggs/female, while the mean oviposition period was 3.33 d. The development rate was negatively correlated with the temperature.

When DBM was fed on kale and reared at 20, 25 and 30°C, the egg, egg to larval and pupal stage was highest at 20°C, and lowest at 30°C. The fecundity was 299.0±80.0, 143.1±85.4 and 107.2±47.1 eggs/ female (Table 4). At 20, 25, and 30°C, the intrinsic rate of increase (*r*) was 0.1986, 0.2130 and 0.2390/d; mean generation time was 22.69, 15.46 and 12.86 d; net reproduction rate (R_0) was 90.66, 26.96 and 21.59 eggs/ female (Liu et al. 1985).

Table 4. The life cycle of DBM when the larvae were fed on kale at 3 different temperatures (Liu et al. 1985)

Stages		Temperatures (°C)		
		20	25	30
Egg (d)		5.5±0.7	3.0±1.2	2.2±0.6
L1 (d)		3.8±1.0	2.0±0.3	1.8±0.4
L2 (d)		2.6±1.0	1.9±0.7	1.2±0.6
L3 (d)		2.2±0.9	1.6±0.6	1.2±0.4
L4 (d)		3.3±1.1	2.9±1.0	2.0±0.6
Egg-Larva (d)		16.9±1.6a*	11.1±1.8b	8.3±0.8c
Pupa (d)		6.7±0.7a	5.1±0.6b	3.2±0.4c
Adult (d)	female	22.7±2.1	12.0±5.0	7.2±2.3
	male	32.0±11.4	11.8±4.3	6.6±6.6
Fecundity		299.0±80.0a	143.1±85.4b	107.2±47.1c

*The different letters in the same row represent the significant difference at 5% level with Duncan's multiple range test.

The fecundity of DBM when fed on rape at 15, 20 and 25°C was 90.7, 249.8 and 135.0 eggs/ female. When fed on non-heading Chinese cabbage at 15, 20 and 25°C, it was 116.7, 158.9 and 154.9 eggs/ female (Ong and Hsiao, unpublished).

Management

Sex pheromone

DBM sex pheromone trap was suggested to be installed at 30-50 cm above the canopy, at the rate of 20 traps/0.097 ha, and the sex pheromone lures last for 2-3 months (Hung et al. 2017a).

In a mass trapping trial in the cauliflower and cabbage fields in central Taiwan in 2007, the leaf and flower floret infestation rates in the field when set at 8-m intervals for DBM mass trapping were 3.7% and 20.6%, respectively. These rates were significantly lower than those in the regular insecticide application plots (6.1% and 29.3%, respectively) (Hung et al. 2017a).

Hung et al. (2017b) developed a dry trap baited with DBM sex pheromones for diamondback moths. This trap included 2-up traps with a diameter of 8.8 cm, an entrance diameter of 1.0 cm, and ventilators on the lower part of the bottle wall. Hung et al. (2016) also designed one 1-up-PET trap with DBM pheromone lure, which appears to efficiently capture adult moths. Other traps such as wing sticky trap, cylinder sticky trap and double layer PET water trap might also be feasible for trapping DBM moths. Hung et al. (2016) tested five commercial dry and sticky traps baited with sex pheromones for their efficacy to lure DBM moths in cauliflower fields. The results showed that none of them (TDAR trap, Hwang's beauty fly trap, Fly Trap, Golden McPhail fly trap and Victor fly trap) was suitable for trapping DBM adults. Conversely, a 1-up-PET trap appears to efficiently capture DBM moths. In addition, the trapping efficiency of the winged sticky trap was found to be better than that of the Cylinder sticky trap. For the winged sticky trap, the location of the pheromone lure (i.e., either on the bottom layer or on the top layer of the trap) had no influence on trapping efficiency; however, captured moths were mainly found on the bottom layer. The results indicated that the PET water trap (i.e., a double layer PET water trap with two entrances) was also effective for trapping DBM moths.

Biological control

Su (1987a and b) indicated that combination of *P. xylostella* and *P. rapae* granulosis viruses provided an effective control at 1 LE/L concentration when treated at 5 to 7 days intervals. Su (1988) indicated that PxGV and sticker (CS-7) were highly effective to DBM on cabbage in the field.

Hu et al. (2018) indicated that *B. thuringiensis* mixed with chemical insecticides (at full or half of the recommended concentration) showed antagonistic effects; therefore, *B. thuringiensis* should not be mixed with these compounds. If the primary pests only belong to the order Lepidoptera, strong control can be achieved with *B. thuringiensis* alone. Su and Chen (1984) found that protection within 3 weeks after transplanting was necessary to enable cauliflower seedling establishment. Control was needed during 31-45 days after transplanting to keep the pest density under 1 larva/plant. The control threshold thereafter could be increased to 10 larvae/plant. Cost/benefit analysis indicated that 6 - 7 sprays of *B. thuringiensis* could give the maximum profit when the price of cauliflower was no less than 7 NT dollars/Kg.

Tsai et al. (2006) tested 8 isolates of *Beauveria bassiana*. Mycelia can grow at 20-28ºC. The mortalities of *P. xylostella* larvae caused by four of these eight isolates ranged from 99.1% to 99.8%. All *B. bassiana* isolates (except Bb-7) were highly pathogenic to 4th instar larvae of *Spodoptera exigua*, as well. All isolates of *B. bassiana* tested in the field trials showed high control efficacy against *P. xylostella* within 2 weeks.

Three parasitoids (*Cotesia plutellae*, *Diadromus collaris* and *Itoplectis naranyae*) were reported in late 1960s. *C. plutellae* was reported to control DBM with marginal success owing to the widespread use of chemical pesticides (Talekar 1996). *Diadegma semiclausum* was introduced in the mid-1980s and it was established in the highlands and drastically reduced DBM damage. *Oomyzus sokolowskii* was introduced in Taiwan in 1992, which has gradually established in crucifer-growing areas of northern Taiwan (Talekar 1996).

Botanical products

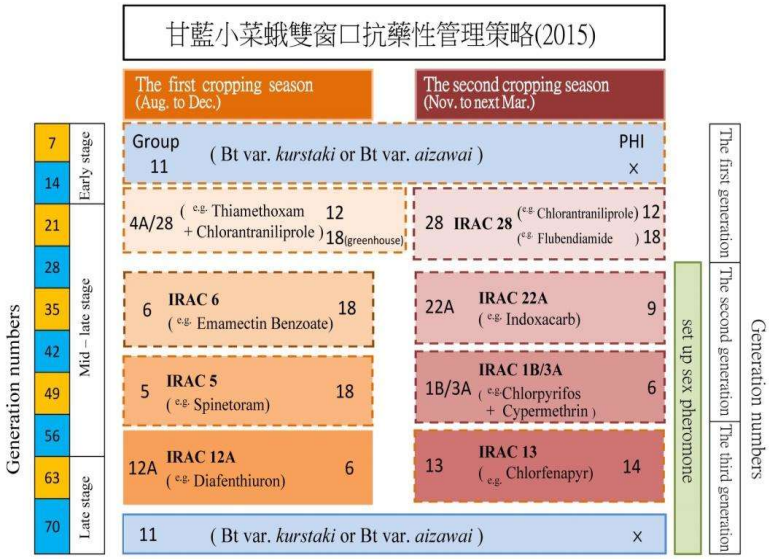
Tomatine is highly toxic to the eggs of the DBM (Chu and Lu 1992). However, it has no significant ovipositional deterrent effect on adult moths (Lu and Chu 1993). Lu and Chu (1992) indicated that α- tomatine treated cabbage leaf has an inhibiting effect on the growth and survival rate of DBM larvae. Hence, this material or tomato leaf extracts could be useful to manage DBM in organic farming.Shih (2008) showed that citronella oil and garlic oil had oviposition deterrent effects on DBM on day 3 but not on day 7.

Insecticides and Resistance Management

Cheng et al. (1996) had conducted 3-year field trials to manage the resistant DBM by alternating the organophosphate, *B. thuringiensis* and cartap in order to avoid cross-resistance. These rotations resulted in a 16.7% increase in crop income, 35% saving in insecticide expenditure, lengthening the spray intervals by 2.1 days (from 5.7 days to 7.8 days), and 1.6 insecticide reduction (from 4.4 to 2.8 insecticides) per tank mixtures.

A "treatment window" package was recently proposed by the government for the management of *P. xylostella* on cabbage. For the first generation of *P. xylostella*, *Bacillus thuringiensis* (IRAC 11) and Thiamethoxam + Chlorantraniliprole (IRAC 4A/28) are recommended for application. For the second generation, Emamectin Benzoate (IRAC 6) and Spinetoram (IRAC 5) are recommended. For the third generation, Diafenthiuron (IRAC 12A) and *B. thuringiensis* (IRAC 11) are recommended to control *P. xylostella* in the first cropping season. For the second cropping season, *B. thuringiensis* (IRAC 11) and Chlorantraniliprole and Flubendiamide (IRAC 28) are recommended to control the first generation. Indoxacarb (IRAC 22A) and Chlorpyrifos + Cypermethrin (IRAC 1B/3A) are recommended for controlling the second generation but Chlorfenapyr (IRAC 13) and *B. thuringiensis* (IRAC 11) are recommended for control of the third generation *P. xylostella*. Pesticides are applied when *P. xylostella* is present on every five of 20 plants (

https://www.baphiq.gov.tw/view_redirect.php?catid=16344).



1. Group: IRAC、Pre-Harvest Interval：need to cautious the PHI.
2. Spray water at dusk to interrupt DBM mating
3. Spray weekly, only when larva presents five every 20 plants.

Cabbage webworm, *Hellulla undalis*

Since 1976, cabbage webworm has become a serious cruciferous pest (Kung 1984). The larvae of cabbage webworm prefer to feed on bud, leaf and flower bud, or mined into leaf stalk. Fecal pellets accumulate on the bore hole, which makes the damaged part wilting, sometimes causing side tillers.

This insect appears in late spring and summer when the heat tolerant crucifers are cultivated. Older larvae pupate in the soil, or wrap the leaves and pupate inside. At 25ºC, the egg, larval, pupal stages are 3.3, 24.6 and 7.6 days respectively, when fed on pak-choy leaf. The adult longevity is 10.3 days with a fecundity of 67.18 eggs/female (Hsiao et al. unpublished). It preferred radish and Chinese cabbage for oviposition than common cabbage or cauliflower (Huang and Hsiao 1999).

Under laboratory conditions, the time required for the development of each growth stage of this insect got shortened with increasing rearing temperature. At 32°C the egg, larval and pupal stages were 3.1, 10.8 and 5.6 days, respectively. With an adult longevity of 4 days, it took 24.1 days to complete one generation. At 16°C, 77.5 days were needed to complete one generation: 8.7 days for eggs, 39.6 days for larvae, 18.7 days for pupae and 10.5 days for adults. At 12°C, the larvae usually die before reaching the 3rd instar. Under room temperature there could be 12 overlapping generations each year. In July to August, egg, larval, pupal and adult stages were 3-4, 11-12, 5-6 and 4 days, respectively, and the entire life cycle took only 24.5 days to complete. Larvae usually underwent four molts. Yet, high temperature would reduce the molts to two. Each female on average laid 154 eggs. Host plants affect the development rate of larvae. At 25°C, larvae reared on pak-choi required 21.8 days to pupate, while those fed on radish pupate after 16.8 days. However, host plants did not significantly affect the pupal stage which was 6-6.7 days. The longevity of adults fed on pak-choi and radish was 3.9 and 7.2 days, respectively (Lin and Chen 1996).

The pathogenicity of the entomopathogenic nematode, *Steinernema carpocapsae, was* evaluated at different temperatures. At 72 h, the mortality of first to fifth instar *H. undalis* at 20ºC was 87, 73, 92, 90, and 88%, respectively. At 25ºC, it was 87-100%, 95-100% at 30ºC. The 2nd instar larva was more susceptible than other larval stages (Hsiao 1997).

Cabbage head caterpillar (*Crocidolomia pavonana*)

Crocidolomia pavonana predominantly occurs from July to November (Hsiao 1984). The eggs are laid in masses on the leaf blade, vein, or petiole of the lower surface of the leaves. Newly hatched larvae feed on the chlorophyll of young leaves creating window-like damage in the leaves and later on the older leaves, buds, and seed pods. Heavily infested plants were completely defoliated and

contaminated with feces (Hsiao 1984).

Hsiao (1984) found that *C. pavonana* consumed less when fed on cabbage, compared to Chinese cabbage and radish.

At 25ºC, the larval, pupal and adult stages were 8.48, 9.76, and 8.33 days, respectively, when fed on cabbage; 9.76, 6.84 and 4.53 days on Chinese cabbage; 11.7, 8.31 and 6.06 days on radish (Hsiao 1984). At 28ºC, the egg stage was 4.3 ± 0.8 d, first to fifth instar larval stages were 1.2 ± 0.4, 1.4 ± 0.5, 3.6 ± 0.8, 3.8 ± 0.7 and 3.0 ± 0.7 days, and pupal stage was 5.6±0.7 days. Male and female longevity were 12.63±4.1 and 7.67±2.9 days, respectively (Hsieh 2004).

The mortality of various larval instars of *C. pavonana* infected by *S. carpocapsae* ranged 71-100% at 20ºC, 74-94% at 25ºC, and 55-84% at 30ºC (Hsiao 1997).

The peak period of egg laying for a female was 5 to 6 days after its emergence. Hsieh (2004) indicated that females preferred to lay eggs on hairy Indian mustard over less hairy cabbage as egg masses were anchored better on leaf hairs and harder to dislodge. However, larvae did not show feeding preference to hairy Indian mustard over less hairy cabbage. Therefore, Indian mustard might be a candidate for the trap crop of *C. pavonana*.

Tobacco cutworm (*Spodoptera litura*)

Tobacco cutworm, *S. litura* is a serious polyphagous pest in Asia, Oceania and the Indian subcontinent. The larvae attack the plants with vigorous eating patterns, often leaving the leaves completely destroyed. Mature larva bores into the head of Chinese cabbage and contaminates the commodity.

The larval stage fed on cauliflower leaf was14±1.0 days at 30ºC. The pupal stage was 8±0.6 days for females and 9±0.6 days for male at 30ºC. The adult longevity was 16±5.6 days for females and 14±7.2 days for male at 30ºC. The fecundity was 1675 egg/ female at 30ºC. The net reproductive rate (R_0) (offspring/female) was 694.55 for 30ºC (Chen and Hsiao 1984).

When *S. litura* was reared on cabbage, the net consumption rate was 439.1 cm^2 (Li 2012). The net reproductive rate, intrinsic rate of increase, and mean generation time were 1893.1 eggs, 0.2374/day, and 31.8 days. All the life parameters of *S. litura* when fed on cabbage were higher than when it was fed on cauliflower.

The performance of *Eocanthecona furcellata* (Hemiptera: Pentatomidae) was tested against *S. litura* (Yeh 2014). *E. furcellata* was feeding more *S. litura* (863.1 larvae) than on *P. xylostella* (644.1 larvae). Yeh (2014) recommended a release rate of one adult or nymph per cabbage plant in the greenhouse.

A local entomopathogenic nematode, *Steinernema taiwanensis* strain T39 was isolated and tested against *S. litura* and *S. exigua* (Tseng et al. 2017a, b). The optimum temperature for *S. taiwanensis* infection was 25-30ºC and did not exhibit any mortality at temperature below 20ºC. The LT_{50} value of fifth instar larvae fed on the diets after inoculation with *S. taiwanensis* at 20 IJs/mL was 37.9 h, whereas those of sixth instar larvae inoculated with the nematode at 25 IJs/mL was 27.0 h. Therefore, *S. taiwanensis* is pathogenic to the larvae of *S. litura*.

The active distance for effective attraction by pheromone traps was estimated as 30-50 m to leeward direction (Chu et al. 1987). Shi and Chu (1995) indicated that the egg mass appeared one week after the adults captured by sex pheromone traps. The economic threshold of the *S. litura* in the earlier, middle, and later growing stage was tentatively estimated as 68, 115, and 157 males/trap/week, respectively in cabbage. Application of insecticides spray should be done when male numbers caught in sex pheromone reached the ETL (Shih and Chu 1995). Lee and Shih (1995) found that neem seed kernel extract possessed a feeding deterrent effect to the second instar larva of *S. litura*.

Cabbage looper, *Trichoplusia ni*

Cabbage looper, *T. ni* is a polyphagous insect, and able to feed on over 160 host plants. Parasitoids (*Apanteles ruficrus* and *Copidosoma truneatellus*) and nucleopolyhedrovirus played important roles for regulating their population and kept this insect as a minor pest.

Black cutworm, *Agrotis ipsilon*

The black cutworm, *A. ipsilon* is also a polyphagous pest. The insect is a nocturnal pest with the larva damaging the basal stems of vegetable seedlings, which would destroy the whole field within a short period. Replanting would make the growth of the crop non-uniform, which eventually posed difficulties during the harvest. It is a serious agricultural pest and feed on nearly several vegetables and other important grains in the world. In the field, it was a localized pest (Hsiao 1995).

Liu and Yang (1987) showed that the life cycle of *T. ni* fed on cabbage at 28 and 32ºC was 45.1 and 36.6 days, respectively. The lower temperature threshold of egg, larva, and pupa was 8.7, 6.33 and 5.6ºC, respectively. Females laid 389-2,458 eggs with an average of 982 eggs.

This insect had five generations in the cabbage field in central Taiwan. The second generation appeared in April and May resulting in the highest plant-cutting rate of up to 90%. The occurrence of other 4 generations was not obvious and the damage was sporadic and slight (Liu and Yang 1987). It was seldom found in tilled fields in Taiwan

and considered as a minor pest. Thus, flooding and tilling would be able to control this pest.

Miscellaneous pests

Agrotis segetum (Denis and Schiffermüller, 1775), *Euproctis taiwana* (Shiraki), slug and birds are sporadic pests of cruciferous crops in Taiwan.

PEST MANAGEMENT AND CONTROL THRESHOLD

Insect pests such as *P. xylostella*, *P. rapae crucivora*, *T. ni* and *S. litura* are the main caterpillar pests defoliating cruciferous crops in Taiwan (Chen and Su 1982). A DBM equivalent unit has been proposed with a table for converting caterpillar counts into DBM-units to facilitate the establishment of control thresholds for these four species by Chen and Su (1982).

The DBM equivalent of 2nd instar larva of *P. rapae*, *T. ni*, *S. litura* were 0.7, 0.7, 2.0; third instar larvae were 5.5, 2.5, 8.0; fourth instar were 15.6, 8.5, 12.6; fifth instar were 22.3, 32.5, 27.0; The sixth instar larva of *S. litura* was 48.0. When there are more than one species appearing at the same time, we could do the transformation, and take action based on DBM control threshold (Table 5).

In Taiwan, control threshold data is available for DBM and TCW, but not for other lepidopterans. Hence, Chen and Su (1982) developed a DBM equivalent unit according to leaf consumption. This unit could help for the conversion of other lepidopteran insect damage in the field for control. Recently, a resistant management strategy based on the "DBM treatment window" has been developed. Pesticide application is suggested to rotate according to IRAC active ingredient, with four categories as follows: (1) Neuromuscular toxins: IRAC 1-6, 9, 14, 19, 22, 28, 29; (2) Insect growth regulators (IGR): IRAC 7, 10, 15-18, 23; (3) Respiratory or metabolic poisons: IRAC 8, 12-13, 20-21, 24-25; and (4) Gut disruption: IRAC 11. The pesticide rotation would help to reduce the development of pesticide resistance.

Finally, the "IPM package" to be offered to the local farmers is composed of: (1) education: Giving IPM course (identify pest and beneficial insects, pest life cycle, behavior, scouting techniques and pest management concept); (2) agro-ecosystems concept: provide the farmers the idea of agro-ecosystems, especially the idea of the interaction of crop and insect pests; and (3) knowledge of good land preparation (GLP) and cultural practices.

Table 5. Conversion of caterpillar counts into diamondback moth larval equivalent units[a] (Chen and Su 1982)

		Mean daily leaf consumption (cm^2/larva)			DBM-units	
Instar	SCW[b]	CL[c]	TAW[d]	SCW	CL	TAW
2	0.27	0.26	0.77	0.7	0.7	2
3	2.2	1.0	3.13	5.5	2.5	8
4	6.23	3.4	5.03	15.6	8.5	12.6
5	6.93	13.0	10.77	22.3	32.5	27
6			19.23			48

a. 1 DBM-unit=leaf consumption by one 3rd-4th instar DBM larva at 15-25℃=0.4 cm^2/larva/day. b. SCW: small cabbage white butterfly. c. CL: cabbage looper. d. TAW: Tobacco cutworm.

References

"Brassicaceae". *The Plant List*.) (http://www.theplantlist.org/1.1/browse/A/Brassicaceae/).

Central Weather Bureau, https://www.cwb.gov.tw/V7/forecast/

Chang CP. 2002. Mass rearing and utilization of the predatory stink bug, *Eocanthecona furcellata*. Proceedings of the symposium on the biological control of agricultural insects and mites. pp 175-181.

Chang CP, Hsieh FK. 2001. Predatory capacity of the predatory stink bug, *Eocanthecona furcellata* (Hemiptera: Pentatomidae) on various prey. Formosan Entomol. 21: 257-267.

Chen CN, Hsiao WF. 1984. Influence of food and temperature on life history traits and population parameters of *Spodoptera litura* Fabricius. Plant Prot. Bull. 26:219-229.

Chen CN, Su WY. 1978. Influence of temperature on the development and feeding amount of Diamondback Moth larvae on cauliflower. Plant Prot. Bull. 20:224-231.

Chen CN, Su WY. 1982. Influence of temperature on development and leaf consumption of three caterpillars on cauliflower. Plant Prot. Bull. 24: 131-141.

Chen CN, Su WY. 1986. Spatial pattern and transformation of field counts of *Plutella xylostella* (L.) larvae on cauliflower. Plant Prot. Bull 28:323-333.

Cheng CL, Shiu CH. 2003. Morphology of *Pieris rapae crucivora* Boisduval (Lepidoptera: Pieridae) and the effects of temperatures on its development. Plant Prot. Bull 45: 271-284.

Cheng EY, Chen CN, Kao CH. 1996. Management of resistant Diamondback Moth and other crucifer pests in Taiwan. J. Agric. Res. China 45:89-104.
Chu YI, Chu CM. 1975a. Life history and the effect of the growth of *Eocanthecona furcellata*. Plant Protec. Bull. 17:99-114.
Chu YI, Chu CM. 1975b. Feeding habit of *Eocanthecona furcellata*. Plant Protec. Bull. 17: 133-141.
Chu YI, Lu FM. 1992. The ovicidal effect of tomatine against deposited eggs of the Diamondback moth, *Plutella xylostella*. Chinese J. Entomol. 12: 213-216. Chu YI, Shih CM, Shih CJ. 1995. The active space to sex pheromone and recaptured velocity of male *Spodoptera litura* (F.). Plant Prot. Bull. 29: 193-197.
Hsiao WF. 1984. The bionomics of cabbage leaf webber, *Crocidolomia binotalis* Zell. (Lepidoptera: Pyralidae). Plant Prot. Bull 26:257-261.
Hsiao WF. 1995. Studies of the relation of infestation of black cutworms to radish and their larval stage. J. Chia-Yi Inst. Agr. 43: 163-170.
Hsiao WF. 1997. Pathogenicity of entomopathogenic nematode, *Steinernema carpocapsae* to cabbage webworm and cabbage pyralid. Plant Prot. Bull. 39: 305-312.
Hsieh MC. 2004. Life habits of *Crocidolomia binotalis* Zeller and the cabbage extracts on the oviposition and feeding attraction. Master thesis of Department of Tropical Agriculture and International Cooperation, 63pp.
Hou RF, Cheng LL, Chen CC. 1998. Effects of a nonsteroidal ecdysteroid agonist on survival and development of the Diamondback moth, *Plutella xylostella*. Plant Prot. Bull. 40: 49-61.
Hsu RC. 2015. Guideline of strategy of insect resistant management — Fundamental chapter (in Chinese). https://www.baphiq.gov.tw/view_redirect.php?catid=16344.
Hu FT, Kuo S, Tzeng CC. 2018. Assessing the effects of mixing *Bacillus thuringiensis* with other pesticides on the biological activities of *Plutella xylostella* and *Spodoptera litura*. Taiwan Pesticide Sci. 4: 21-36.
Huang CC, Hsiao WF. 1999. Primary studies of the oviposition preference of *Hellula undalis* (F.)(Lepidoptera:Pyralidae) on the Crucifers. J. Chia-Yi Inst. Agr. 63:77-84.
Hung JY. 2012. Studies on the pathogenicity of the *Heterorhabditis brevicaudis* on the *Spodoptera exigua*. National Chiayi University, Department of Bioresources, Master Thesis, 62pp.
Hung CC, Wang WL, Wu CY, Chang CH. 2016. Comparing the efficiency of different types of traps for the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae): an evaluation using field data. Taiwan Pestic. Sci. 1: 91-106.
Hung CC, Wang WL, Wu CY, Chang CH, Chang MW. 2017a. Evaluation of integrated management with sex pheromone for mass trapping of the Diamondback Moth, *Plutella xylostella* (Lepidoptera: Plutellidae), in the cauliflower fields of Taiwan. Formosan Entomologist 37: 23-32.
Hung CC, Wang WL, Wu CY, Chang CH, Chang MW. 2017b. Sex pheromone-baited dry trap for diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Taiwan Pestic. Sci. 3: 49-63.
Kao CH, Chiu CS, Cheng EY. 1990a. Field evaluation of microbial and chemical insecticides for Diamondback moth and other lepidopterous pests control on cabbage. J. Agric. Res. China 39: 221-227.
Kung KS. 1984. Changes of economic importance of major insect pests of vegetable and prospects for their control in Taiwan. pp 3-30. In Proceedings of the symposium on the insect control of vegetables in Taiwan. pp3-30. (Hsiao R F., Tien CM and Lin SF editors), 211 pages.
Lee HJ, Tseng YC. 1993. Induction of host plant choice for feeding and oviposition in Diamondback Moth larvae (*Plutella xylostella*). Bull. Institute of Zool. Academia Sinica 32:148-152.
Li NJ. 2012. Population parameters and food consumption of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) on cabbage, taro, and sesbania. National Chung-Hsing University, Department of Entomology, Master thesis. 62 pp.
Lin HC. 2012. Studies on life table and biology of the *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae). National Chiayi University, Department of Bioresources, Master thesis. 62 pp.
Lin CS, Chen CC. 1996. Studies on the life history of *Hellula undalis* Fab. in central Taiwan. Bull. TDARES 51:41-48.
Liu H, Chi H, Chen CN, Kung KS. 1985. The population parameters of the Diamondback Moth, *Plutella xylostella* (L.), on common kale. Plant Prot. Bull. 27:145-153.
Liu TS, Yang YZ. 1987. Life history and field occurrence of *Agrotis ipsilon* in Taichung. Plant Prot. Bull. 29: 255-262.
Lu FM, Chu YI. 1992. Antifeeding effects of α-Tomatine on larvae of the Diamondback moth (*Plutella xylostella* L.). Chinese J. Entomol. 12:135-143.
Lu FM, Chu YI. 1993. Influence of tomatine on oviposition preference of Diamondback moth, *Plutella xylostella* L. Chinese J. Entomol. 13: 379-384.
Lu FM, Lee HS. 1984. Observation of the life history of Diamondback moth, *Plutella xylostella* in the whole year. J. Taiwan Agric. Res. 33: 424-430.
Shih HP. 2008. Effects of plants' secondary compounds on controlling Diamondback moth and turnip aphid. Taoyuan District Agric. Res. and Ext. Res. Bull. 63:27-32.
Shih CJ, Chu YI. 1995. Evaluation of sex pheromone for forecasting the population of Tobacco cutworm, *Spodoptera litura* on cabbage. Plant Prot. Bull. 37: 381-392.
Su CY. 1987a. Utilization of two granulosis viruses for control *Artogeia rapae* and *Plutella xylostella*. Plant Prot. Bull. 29: 293-296.
Su CY. 1987b. The evaluation of granulosis viruses for control *Plutella xylostella* and *Artogeia rapae* at different time intervals. Plant Prot. Bull. 29:403-405.
Su CY. 1988. Utilization of *Plutella xylostella* granulosis virus (PxGV) for control *Plutella xylostella*. Chinese J. Entomol. 8:161-163.
Su WY, Chen CN. 1984. Optimal control of the Diamondback moth on winter cauliflower in Taiwan. Plant Prot. Bull. 26:41-53.
Talekar NS, Lee ST. 1985. Seasonality of insect pests of Chinese cabbage and common cabbage in Taiwan. Plant Prot. Bull. 27: 47-52.
Talekar NS. 1996. Biological control of Diamondback moth in Taiwan--A review. Plant Prot. Bull. 38: 167-189.
Talekar NS, Liu SH, Chen CL, Yiin YF. 1994. Characteristics of oviposition of Diamondback moth (Lepidoptera: Yponomeutidae) on cabbage. Bull. Institute of Zoology, Academia Sinica 33:72-77.
Tang DS, Chi H. 1991. Life table and consumption of the cabbage looper, *Trichoplusia ni*. Formosan Entomol. 11:282-290.
Tang HC. 1998. Studies on the infection of *Spodoptera exigua* with the entomopathogenic fungus, *Nomuraea rileyi.* National Chung Hsing University, Department of Entomology, master thesis.
Tsai YS, Yang P, Hung TH, Kao SS. 2006. Biological characterization of *Beauveria bassiana* isolates from Taiwan. Plant Prot. Bull. 48: 117-128.
Tseng CT, Hou RF, Tang LC. 2017. Pathogenicity of the entomopathogenic nematode, *Steinernema taiwanensis* strain T39, against two noctuids, *Spodoptera litura* and *Spodoptera exigua* (Lepidoptera: Noctuidae). Formosan Entomologist 37: 51-63.
Tseng CT, Hou RF, Tang LC. 2017. Bioassay on the Infectivity of the endogenous entomopathogenic Nematode, *Steinernema taiwanensis* strain T39, against insects and its mass production. Formosan Entomologist 37: 11-22.
Tzou YK. 2008. The effect of *Pieris rapae crucivora* (Boisduval) larval stage and its host plant on development and searching behavior of *Cotesia glomerata*. National Chung Hsing University, Department of Entomology, Master Thesis.
Yeh CC. 2014. Demographic assessment and predation capacity of *Eocanthecona furcellata* fed on *Spodoptera litura* and *Plutella xylostella*. National Chung Hsing University, Department of Entomology, master thesis. 75pp.

Crocidolomia pavonana pest management in Samoa: real IPM is possible!

Furlong, M.J.
SCHOOL OF BIOLOGICAL SCIENCES, THE UNIVERSITY OF QUEENSLAND, ST LUCIA 4072, QUEENSLAND, AUSTRALIA
email: m.furlong@uq.edu.au

Tuivavalagi, P.
SCHOOL OF BIOLOGICAL SCIENCES, THE UNIVERSITY OF QUEENSLAND, ST LUCIA 4072, QUEENSLAND, AUSTRALIA
email: Philipt1978@gmail.com

Uelese, A.
CROPS DIVISION, MINISTRY OF AGRICULTURE AND FISHERIES, NU'U CROPS RESEARCH CENTRE, SAMOA
email: aleni.uelese@maf.gov.ws

Niko, U.P
CROPS DIVISION, MINISTRY OF AGRICULTURE AND FISHERIES, NU'U CROPS RESEARCH CENTRE, SAMOA

Zalucki, M.P.
SCHOOL OF BIOLOGICAL SCIENCES, THE UNIVERSITY OF QUEENSLAND, ST LUCIA 4072, QUEENSLAND, AUSTRALIA
email: m.zalucki@uq.edu.au

ABSTRACT
In the islands of the South Pacific, the large cabbage moth (LCM), *Crocidolomia pavonana* F. (Lepidoptera: Crambidae), can be a greater threat to *Brassica* vegetable crop production than the co-occurring diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae). In Samoa, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) regularly causes high levels of LCM egg mass parasitism on farms where the use of broad-spectrum insecticides is reduced. In insecticide-free experimental *Brassica* crops we measured recruitment of *T. chilonis* to experimental cohorts of LCM eggs and used exclusion cages to assess the impact of the endemic natural enemy complex on egg masses. Predation of egg masses and parasitism by *T. chilonis* lead to significant but highly variable levels of LCM egg mortality. In smaller egg masses (< 35 eggs) a greater percentage (frequently 100%) of eggs within an egg mass was attacked by the parasitoid. In egg masses of > 40 eggs, the proportion of eggs attacked decreased with increasing egg mass size. High *T. chilonis* parasitism may be associated with the presence of alternative host eggs, especially *Nyctemera baulus alba* Pagensteche (Lepidoptera: Erebidae) and *Hypolimnas bolina* (L.) (Lepidoptera: Nymphalidae), laid on the weed *Crassocephalum crepidioides* at crop margins. To exploit the potential ecosystem service offered by *T. chilonis*, adoption of selective insecticides that have lower impact on natural enemies will be required. Regular pest sampling and applications of appropriate insecticides only when necessary could form the beginning of an integrated approach to pest management that would allow farmers to exploit the pest control potential demonstrated by *T. chilonis*. Investigations into the distribution of this species in other South Pacific islands should be explored and its introduction considered if it is found to be absent.

Keywords
Diamondback moth; *Trichogramma chilonis*; predation; alternative hosts; selective insecticide.

INTRODUCTION
Chinese cabbage and head cabbage are the most important *Brassica* vegetables in Samoa. Production has increased in recent years due to awareness programs on the nutritional and health benefits (Ahuja et al. 2010) and the total area of *Brassica* crops grown annually in Samoa is 84 ha (Samoa Bureau of Statistics 2009). The demand for green leafy vegetables has encouraged growers to plant *Brassica* crops year-round, resulting in almost continuous production. As has been found repeatedly, such production regimes in areas with suitable climate can have major insect pest problems (Furlong et al. 2013; Li et al. 2016, 2019).

Farms in Samoa can be classified as either subsistence, semi-subsistence or commercial. Subsistence farms are small portions of land (typically 9- 25 m^2) used to grow a mixture of food crops for day-to-day family consumption. Semi-subsistence farms are typically larger portions of land (≈100 m^2, but size can vary depending on which crops are grown) and, in addition to producing crops for the family's day-to-day use, any extra harvest is sold at market. Typically, 300-500 *Brassica* plants are grown annually on semi-subsistence farms and pest management is rare; that which is practiced is typically based on cultural controls such as hand-picking egg masses of lepidopteran pests. Semi-subsistence farms are very common throughout Samoa and although individual plots are small, they account for substantial production. Commercial farms range in size between 0.5 and 20 ha and grow mixed crops, either in monoculture or in a mixture of two to three vegetable crops in a field. Crops are grown solely for sale at market.

Here we detail how farmers in Samoa perceive constraints on Brassica production and how they have responded to these threats. We build on previous work (Uelese et al. 2014) to show that egg parasitism by *Trichogramma chilonis* has the potential to contribute significantly to the control of key vegetable pests in Samoa in the absence of broad-spectrum insecticides but that its effectiveness can be highly variable in both space and time.

MATERIALS AND METHODS

Survey of pest management practices used by Brassica growers in Samoa

A survey of pest management practices used by *Brassica* growers was conducted on 30 farms located on Upolu, the most populated island of Samoa, between January and March 2010. Thirty farmers were interviewed (8 commercial farmers and 22 subsistence or semi-subsistence farmers). All interviews followed a structured questionnaire (see Tuivavalagi 2016) and were conducted verbally with farmers in the field. Where possible, interviews were pre-arranged but some (n=14) were conducted without prior appointment. The interview technique was informal, and questions were discussed before the farmers' responses were recorded. Interviews took between 45minutes and 2 hours to complete.

Pest phenology and management index surveys on Samoan farms

Eight farms were selected for inclusion in a more detailed study that measured actual farm practices and related them to concomitant pest and natural enemy population dynamics for an entire crop cycle. At each site cabbage plant sampling started one week after seedlings were transplanted to the field and plants were then sampled at 3-4 day intervals until they matured and were ready for harvest. On each sampling occasion 30 cabbage plants were randomly selected and then searched thoroughly, all pests (eggs, larvae and pupae) and arthropod natural enemies on each plant were recorded. The survey to determine management indices for each selected farm was designed by adapting the methods proposed by Taylor et al. (1993) and Furlong et al. (2004) (see Tuivavalagi, 2016) to generate a management index (MI) for each site. The questions were designed to determine whether management practices were consistent with an integrated approach to pest management or whether they were in opposition to it. Practices consistent with the adoption and application of IPM were awarded a positive score, whereas those in direct opposition to the principles of IPM were awarded a negative score; practices that were considered neutral with respect to IPM were awarded a score of zero. Scores were weighted to reflect the importance of a given practice and the MI for each site calculated by summing the individual scores for the different practices. The maximum possible score was 8, reflecting adherence to IPM principles, the minimum possible score -7, reflecting practices inconsistent with IPM.

Estimating the impact of natural enemies on C. pavonana on experimental farms

Between November 2010 and May 2011, 13 natural enemy exclusion experiments were conducted at two sites on Upolu, Samoa: Nu'u Crops Research Centre (approx. 50 ha, elevation of 75 m above sea level, 15 km of the capital city, Apia) and the Chinese Demonstration Farm (approx. 25 ha, elevation of 100m above seas level, 3km east of Nu'u Crops Research Centre). At Nu'u Crops Research Centre head cabbage plants (*Brassica oleracea* var. *capitata* cv. FS) were transplanted to the field at the 5-leaf stage. Plants were arranged in single rows and spaced 0.3 m apart; adjacent rows were also spaced 0.3 m apart. Seedlings were fertilized (N: P: K; 12: 5: 20) at the time of transplanting and subsequent applications of fertilizer were made four and eight weeks later. Plants were hand watered as necessary. Single field plots consisted of 500- 600 plants and 8-10 weeks after seedlings were transplanted another plot was established nearby (≤ 100 m away). The first of three plots was established in the field in early October 2010. Plots were typically maintained for 12 weeks after transplanting, which enabled a continuous crop to be maintained at the research station for most of the duration of the study. The Chinese Demonstration Farm was established as a perma-culture demonstration and is considered organic (although it is not certified) and no synthetic pesticides were used for either pest or weed control. *Brassica* crops covered about 0.25 ha at the farm and *Brassica oleracea* var. capitata cv. FS was the main *Brassica* crop grown. Details of seedling production were not recorded but plant spacing was the same as that at Nu'u Crops Research Centre. Three sequentially planted plots (400- 600 plants in each; spacing the same as that at Nu'u Crops Research Centre) were utilized in the studies and the first was planted in mid-January 2011.

Natural enemy exclusion (NEX) cages were made from plastic-coated wire mesh cylinders (15 cm diameter x 25 cm high) covered by a fine nylon mesh (0.05 mm x 0.05 mm) sleeve that was long enough to extend under plant pots to ensure the exclusion of natural enemies from experimental potted plants. Open control (OC) cages were constructed in the same way but the nylon mesh sleeve did not cover the bottom 10 cm of the central plastic-coated wire mesh cylinder so that natural enemies had access to plants and the egg masses that they supported. The OC cages allowed natural enemy access to the plants but ensured that the plants and egg masses inside each cage treatment were exposed to similar environmental conditions (Furlong et al. 2004).

Field experiments began at Nu'u Crops Research Centre in November 2011 and at the Chinese Demonstration Farm in February 2011. Depending on the experiment, 29-55 plants supporting a single, digitally photographed *C. pavonana* egg mass were allocated to NEX cages and a similar number was allocated to the OC cages. Plants were taken to the field and watered. They were then placed within an appropriate cage type. Pots were not buried in the ground. In NEX cages the open end of the cage was tied with a rubber band and excess netting was tucked under the plant pot, the weight of which was sufficient to hold the cage in place for the duration of the experiment. In OC cages the plant pot was placed directly on the soil surface and the cage secured in place over the plant by

pushing two small wooden stakes through the plastic-coated mesh and into the soil. Potted plants of each treatment were arranged in the experimental plots in a regular grid pattern. The distance between adjacent plants within the grid varied depending on the number of plants in the experiments. Potted plants were allocated to a position in the grid entirely at random. Three days after the caged plants were transferred to the field, the leaf on which the *C. pavonana* egg mass had been laid was removed from the plant, placed in a labeled Petri dish (9 cm diameter) and taken to the laboratory where they were again photographed using the hand-held digital microscope. Eggs masses were then incubated (25 ±2ºC; L: D 12:12h) until eggs hatched or turned black due to parasitism. Egg masses that contained eggs that turned black were photographed again to enable the number of parasitized eggs to be accurately determined. The first experiment was set up at Nu'u Crops Research Centre on 16 November 2010 and five additional runs of the experiment were conducted at this site, the last of which was set up on 25 May 2011. At the Chinese Demonstration Farm the first experiment was set up on 3 February 2011 and six additional runs of the experiment were conducted, the last of which was set up on 23 May 2011.

RESULTS AND DISCUSSION

Survey of pest management practices used by Brassica growers in Samoa

Constraints to Brassica vegetable production:

The major constraint to *Brassica* crop production identified by all farmers interviewed was the control of pests and diseases. All farmers interviewed considered *C. pavonana* a major constraint to production, 83% considered *P. xylostella* a major constraint and 30% considered *Spodoptera litura* (F. (Lepidoptera: Noctuidae) a major constraint, but only one farmer specifically identified a disease (Bacterial wilt) as a significant production issue. A small percentage of farmers identified leaf miners (3%), other insects (3%) and the Giant African Snail (*Achatina fulica* Férussac (Achatinidae) (3%) as constraints to production. Limited water supply was considered to be the next most significant constraint to production with 73% of farmers interviewed expressing this opinion. Other major production constraints identified included costs of pesticides (40%) and seed availability (30%) but only small numbers of farmers identified the wet season (7%) and labour costs (3%) as constraints to production.

Applications of pesticides to crops:

Of the farmers surveyed, 93% applied synthetic insecticides to their crops; but they used only a small number of different insecticides. Ten percent of farmers commented that this was due to the limited range of insecticides available. The vast majority of farmers (80%) used Attack® (a mixture of pyrethroid and organophosphate insecticides) while many (63%) used Match® (an insect growth regulator). There was far lower adoption of the selective insecticide Steward® (indoxacarb), which was used by only 10% of farmers surveyed. One farmer interviewed used no pesticide control methods at all and another applied water to plants daily for insect control. Two farmers applied urea to plants in an attempt at insect control. All farmers using synthetic insecticides reported that the selected products were effective against the target insect pests but one indicated that Match® was sometimes ineffective and, in this case, was replaced by Attack®. The farmers using insecticides (93% of those surveyed) did so as they considered them to improve the quality and quantity of harvested produce. Of the 28 farmers that used synthetic insecticides, 24 (87%) said that they did not use tank mixes of different insecticides but the 4 farmers who did adopt this practice believed it to be effective and considered it to save labour. All farmers that used insecticides applied them at least once a week; 47% said that they adopted a simple calendar approach without checking the crop for insect pests, 33% based their decision to apply insecticide on observation of pests in the crop and 20% determined whether or not to apply insecticide based on pest densities in the crop.

Sources of information on pesticides accessed.

Farmers accessed a range of different sources for information on pesticides for use in *Brassica* pest management. Most farmers (26/30) said they sourced information from the Agricultural Advisory Services within the Ministry of Agriculture and Fisheries (MAF). The next most commonly accessed source of information was that provided by other farmers (14/30); of these, 12 farmers also accessed information elsewhere, ten from MAF and three from pesticide sellers. Only two farmers relied solely on other farmers for information and only five of the 30 farmers interviewed accessed pesticide sellers as sources of information on pesticides, three of these also accessed information from MAF and two accessed information from other farmers (Fig. 2.5). Only one of the 30 farmers interviewed accessed information from MAF, pesticide sellers and other farmers. All farmers interviewed stated that they were aware of the negative impacts that pesticides can have on the environment, non-target organisms and humans.

Knowledge of natural enemies of Brassica crop pests:

Sixty percent of farmers interviewed were aware of natural enemies of *Brassica* pests and of their importance in suppressing pest populations, but the remaining 40% of farmers knew nothing of natural enemies or of their roles in suppressing pest populations.

Cultural controls.

All farmers interviewed practiced crop rotations. Seventy percent of farmers reported that they hand pick pests from their crops.

Pest phenology and management index surveys

Seven out of the 8 farms surveyed returned highly negative MIs (6 farms MI= -6; 1 farm MI= -5) and only one farm returned a positive MI (MI=1). This indicates that in the vast majority of cases, the practices adopted on the commercial *Brassica* farms in Samoa were opposed to the principles of IPM and as such are unlikely to be conducive to the establishment and conservation of natural enemies. At seven of the eight farms investigated, insecticides were routinely applied at a frequency of approximately one application per week throughout the crop. Typically, insecticide applications were weekly from week 1 through to week 8 or 9 after the crop was transplanted to the field (see Figure 1 for representative data). At Field Site-2, although only three applications of insecticide were made (Figure 2) these were made at the start of the crop when the pest densities were low (Figure 2). On all farms, natural enemy abundance was low and no evidence of *T. chilonis* attacking *C. pavonana* was recorded.

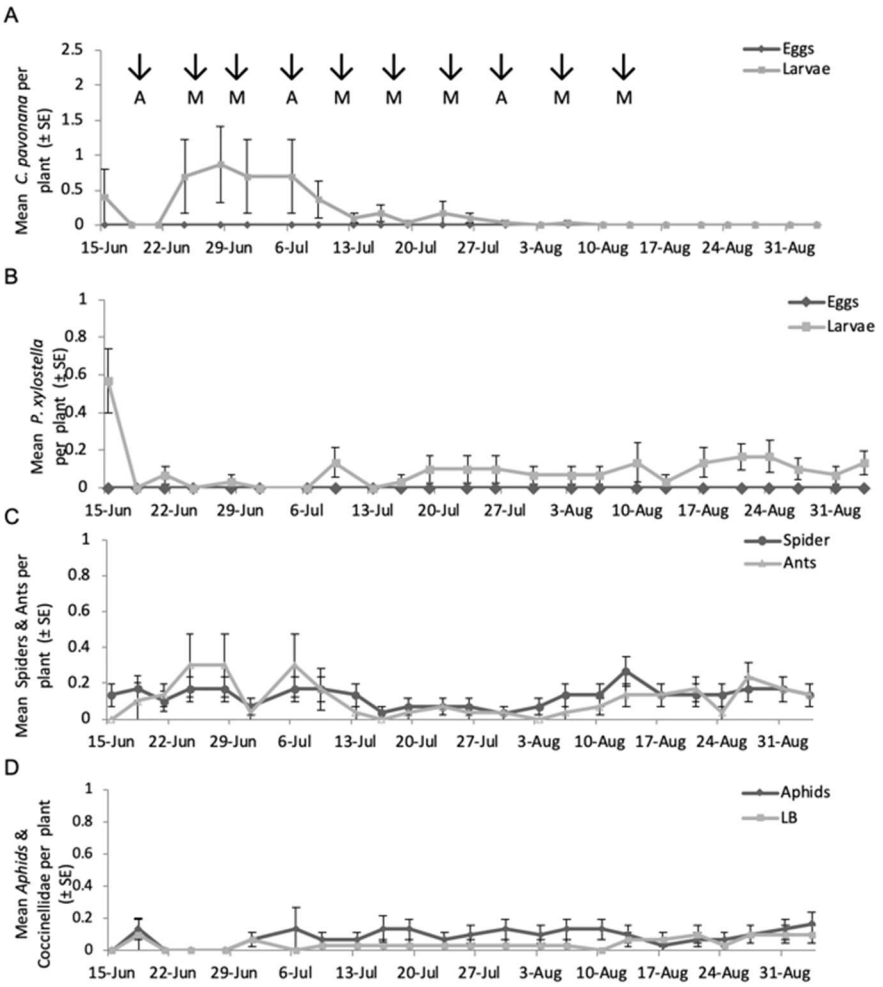


Figure 1. Abundance of pest insects and selected natural enemies at Field Site-1 (MI= -6) during a single head cabbage crop cycle (2010). A) *Crocidolomia pavonana* abundance; B) DBM abundance; C) Foliar abundance of ants and spiders; D) Foliar abundance of aphids and various stages of unidentified species of Coccinellidae (LB). Arrows on panel A represent insecticide applications, A= Attack, M= Match and the time they were applied to the crop

Estimating the impact of natural enemies on *C. pavonana* on experimental farms

The natural enemy exclusion experiments showed that natural enemies caused significant, but variable, mortality to *C. pavonana* eggs at both field sites (Table 1).

At Nu'u, *C. pavonana* mortality was significantly greater in cages that allowed access of natural enemies (OC) than in cages from which natural enemies were excluded (NEX) on all six of the occasions on which the experiment was run (Table 1). *Trichogramma chilonis* parasitized *C. pavonana* eggs in five of the six experiments at Nu'u (Table 1). Egg masses within NEX cages were parasitized by *T. chilonis* on three occasions but parasitism rates were always greater in eggs in OC cages than in eggs in NEX cages (Table 1). The proportion of *C. pavonana* eggs that went missing during the experiments was significantly higher on plants within OC cages than on plants in NEX cages (Table 1); high levels (a proportion of up to 0.256) of egg disappearance were also recorded in NEX cages on occasion (Table 1).

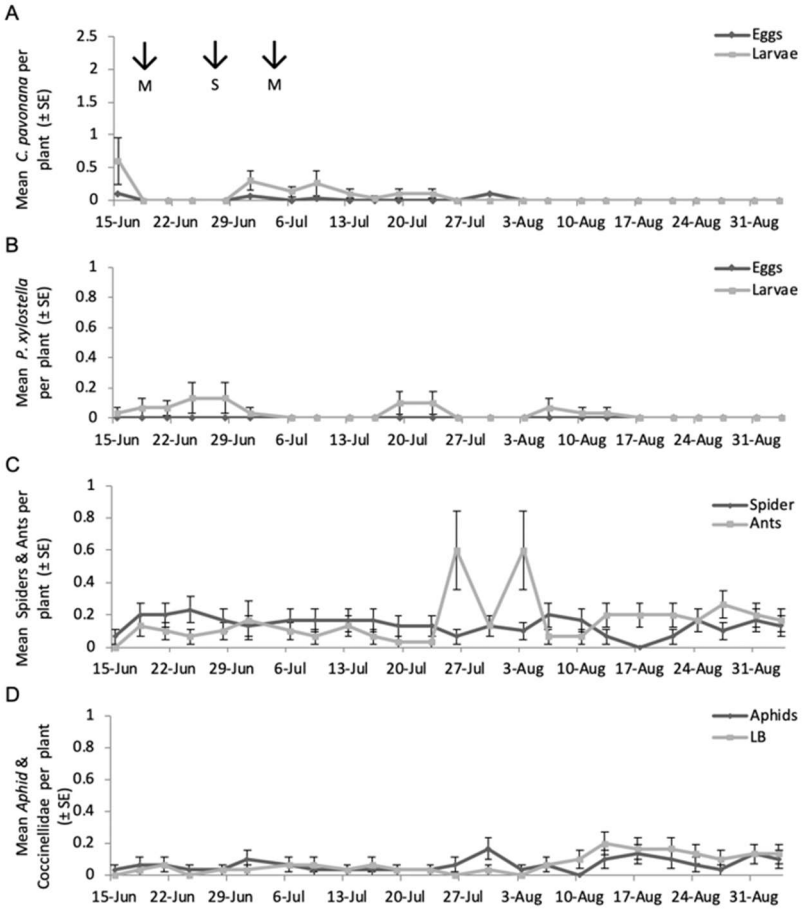


Figure 2. Abundance of pest insects and selected natural enemies at Field Site-2 (MI=1) during a single head cabbage crop cycle (2010). A) *Crocidolomia pavonana* abundance; B) DBM abundance; C) Foliar abundance of ants and spiders; D) Foliar abundance of aphids and various stages of unidentified species of Coccinellidae (LB). Arrows on panel A represent insecticide applications, S= Steward, M= Match and the time they were applied to the crop.

At the Chinese Demonstration Farm, *C. pavonana* mortality was significantly greater in cages that allowed access of natural enemies (OC) than in cages from which natural enemies were excluded (NEX) on all seven of the occasions on which the experiment was run (Table 1). *Trichogramma chilonis* parasitized *C. pavonana* eggs in four of the seven experiments at this site (Table 1). Egg masses within NEX cages were parasitized by *T. chilonis* on two occasions but parasitism rates were always greater in eggs in OC cages than in eggs in NEX cages (Table 1). The proportion of *C. pavonana* eggs that went missing during the experiments was significantly higher on plants within OC cages than on plants in NEX cages (Table 1); but high levels (a proportion of up to 0.299) of egg disappearance were also recorded in NEX cages on occasion (Table 1).

Table 1. Proportion of eggs within an egg mass killed, parasitized by *T. chilonis* and missing for each experiment at each experimental site.

				Mean proportion of eggs per egg mass (±SE)[2]		
Site (Date)	# Egg Masses	Total # Eggs[1]	Cage type	Mortality	*T. chilonis*	Missing
Nu'u						
(16/11/10)	30	828	OC	0.818 (±0.046)a	0.617 (±0.072)a	0.182 (±0.068)a
	30	895	NEX	0.112 (±0.035)d	0.060 (±0.034)d	0.055 (±0.016)d
(15/03/11)	29	997	OC	0.673 (±0.057)a	0.347 (±0.066)a	0.385 (±0.073)a
	29	1183	NEX	0.281 (±0.045)b	0.090 (±0.035)d	0.214 (±0.036)d
(29/04/11)	35	844	OC	0.500 (±0.069)a	0	0.500 (±0.069)a
	35	938	NEX	0.257 (±0.061)d	0	0.256 (±0.061)d
(01/05/11)	33	753	OC	0.716 (±0.050)a	0.338 (±0.061)a	0.410 (±0.079)a
	32	774	NEX	0.174 (±0.048)d	0.006 (±0.004)d	0.161 (±0.048)d
(07/05/11)	30	796	OC	0.713 (±0.051)a	0.322 (±0.056)	0.414 (±0.084)a
	30	862	NEX	0.132 (±0.034)d	0	0.132 (±0.034)d
(25/05/11)	30	723	OC	0.462 (±0.062)a	0.199 (±0.061)	0.300 (±0.058)a
	30	669	NEX	0.061 (±0.018)d	0	0.061 (±0.018)d
Chinese						
(03/2/2011)	25	640	OC	0.812 (±0.040)a	0.616 (±0.072)a	0.299 (±0.067)a
	24	492	NEX	0.143 (±0.045)d	0.060 (±0.045)d	0.093 (±0.021)b
(21/02/11)	42	1002	OC	0.870 (±0.025)a	0.609 (±0.059)	0.339 (±0.058)a
	39	867	NEX	0.143 (±0.019)d	0	0.143 (±0.019)b
(21/03/11)	30	847	OC	0.789 (±0.310)a	0.460 (±0.056)a	0.442 (±0.056)a
	30	860	NEX	0.302 (±0.055)b	0	0.299 (±0.055)b
(25/03/11)	55	1692	OC	0.710 (±0.040)a	0.402 (±0.048)a	0.376 (±0.053)a
	60	1863	NEX	0.181 (±0.022)b	0.058 (±0.020)d	0.129 (±0.015)b
(12/5/2011)	35	708	OC	0.508 (±0.470)a	0	0.508 (±0.047)a
	35	730	NEX	0.167 (±0.070)d	0	0.167 (±0.070)d
(19/05/11)	30	569	OC	0.559 (±0.083)a	0	0.559 (±0.083)a
	30	624	NEX	0.146 (±0.045)d	0	0.146 (±0.045)d
(23/05/11)	30	594	OC	0.607 (±0.080)a	0	0.607 (±0.080)a
	30	541	NEX	0.156 (±0.054)b	0	0.156 (±0.054)d

[1]The total number of eggs (=sum of eggs in all egg masses) exposed to a given treatment in a given run of the experiment at a given site. [2]Mean proportion of eggs within an egg mass killed, parasitized by *T. chilonis* or missing. For each run of the experiment, means for each factor were compared between treatments by t-test. Within an experiment at a site means for a given factor that are marked with a different letter are significantly different (a vs b, $P < 0.05$; a vs c $P < 0.01$ and a vs d $P < 0.001$).

Overall, across all experiments, 55% of egg masses collected were attacked by *T. chilonis*. The proportion of eggs in an egg mass that was parasitized was variable but in many egg masses all the eggs were parasitized by *T. chilonis* (Figure 3) Overall the proportion of eggs in an egg mass that was parasitized declined with increasing egg mass size (Figure 4).

Studies on the specific rates of parasitism within *C. pavonana* egg masses exposed to *T. chilonis* parasitism in the field show that although parasitism rates between egg masses are variable (Figures 3.A and B), a greater proportion of eggs in smaller egg masses is likely to be parasitized than in larger egg masses. No egg masses with more than 35 eggs were completely parasitized by *T. chilonis* (Figure 3) and no more than 40 parasitized eggs were recovered from a single egg mass, even when egg masses containing more than 70 eggs were exposed (Figure 3). This suggests that *T. chilonis* either only has a batch of up to 40 eggs to lay at one time or that there is a possibility that ovipositing parasitoids might get disturbed during the long process of systematically attacking eggs within the *C. pavonana* egg mass. More detailed investigations of *T. chilonis* fecundity and the number of potential hosts that individual females can attack, the characteristics of parasitism within egg masses and *T. chilonis* foraging behaviour would be interesting and provide valuable information that could inform researchers on the suitability of the parasitoid as a possible biological control agent.

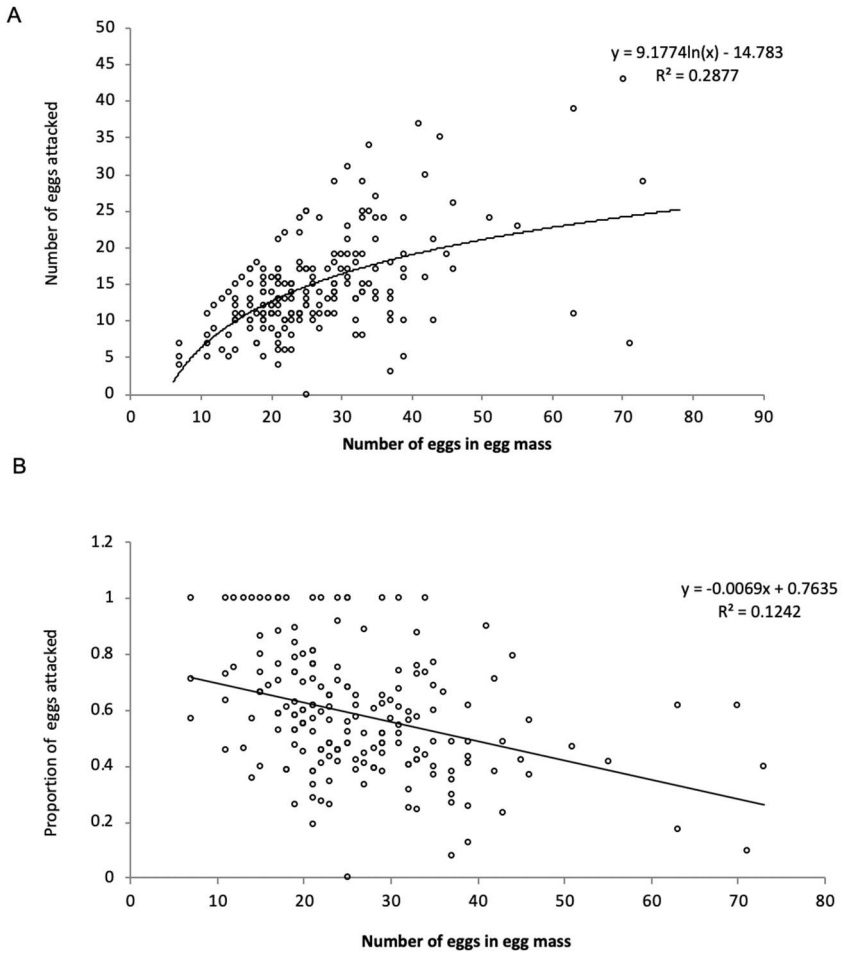


Figure 3. A. Number of *C. pavonana* eggs per egg mass attacked by *T. chilonis*. B. proportion eggs in egg masses attacked by *T. chilonis.*

CONCLUSION

As previously reported and reviewed (Uelese et al. 2014), effective natural enemies of *C. pavonana* are typically lacking in *Brassica* agro-ecosystems. Therefore, the *T. chilonis* that attacks *C. pavonana* in Samoa, and which has been demonstrated to have a significant impact on egg populations, could have positive environmental and economic effects in Samoa, other countries in the Pacific and even places further afield where *C. pavonana* is an important pest. *Trichogramma chilonis* has been recorded from a wide range of Lepidoptera hosts in the Asia-Pacific region and it is found attacking various hosts in other countries where *C. pavonana* is a significant pest of *Brassica* vegetables (Uelese et al. 2014). However, only in Samoa has the parasitoid been reported to cause significant mortality to the pest. Indeed, in neighbouring Fiji extensive sampling of *C. pavonana* eggs has failed to detect any egg parasitoids attacking it. The reasons for this need to be explored in order to better understand the potential of the Samoan population for biological control of *C. pavonana*. Recent work in Samoa has shown that two alternative hosts for *T. chilonis*, *Nyctemera baulus alba* Pagenstacher (Lepidoptera: Arctiidae) and *Hypolimnas bolina* L. (Lepidoptera: Nymphalidae), exist in close proximity to *Brassica* crop fields and that *T. chilonis* attacking these hosts and those attacking *C. pavonana* are genetically identical (Foster et al. 2021). Developing further understanding of the ecological basis for the success of *T. chilonis* against *C. pavonana* in Samoa is fundamental to any attempts to develop the parasitoid as a biological control agent.

Acknowledgements

This work was conducted as part of ACIAR project HORT/2010/090.

References

Ahuja I, Rohloff J, Bones AM. 2010. Defence mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management. In: Sustainable Agriculture, Vol. 2, ed. E. Lichtfouse, M Hamelin, M Navarrete, P Debaeke, pp. 623–70. New York: Springer.

Foster BJ, Hereward JP, Uelese A, Walter GH, Furlong MJ. 2021. *Trichogramma* species in Samoa and their potential as biological control agents against the large cabbage moth, *Crocidolomia pavonana*. *Biological Control* 164: 104781.

Furlong MJ, Shi ZH, Liu YQ, Guo SJ, Lu YB, Liu SS, Zalucki MP.

Furlong MJ, Wright DJ, Dosdal, LM. 2013. Diamondback Moth Ecology and Management: Problems, Progress, and Prospects. *Annual Review of Entomology* 58: 517–541.

Li Z, Feng X, Liu SS, You M, Furlong MJ. 2016. Biology, ecology and management of the diamondback moth in China. *Annual Review of Entomology* 61: 277–296.

Li Z, Furlong MJ, Yonow T, Kriticos DJ, Bao HI, Yin F, Lin Q, Feng X, Zalucki MP. 2019. Management and population dynamics of diamondback moth (plutella xylostella): planting regimes, crop hygiene, biological control and timing of interventions. *Bulletin of Entomological Research* 109: 257-265

Taylor DC, Mohamed ZA, Shamsudin MN, Moyahidin MG, Chiew EFC. 1993. Creating a farmer sustainability index: a Malaysian case study. *American Journal of Alternative Agriculture* 8: 175-184.

Tuivavalagi P. 2016. Investigating the impacts of the natural enemy *Trichogramma chilonis* Ishii on populations of *Crocidolomia pavonana* in Samoa. MPhil thesis. University of Queensland 77p.

Uelese A, Ridland PM, Stouthamer R, He YR, Ang G, Zalucki MP, Furlong MJ. 2014. *Trichogramma chilonis Ishii*: a potential biological control agent of *Crocidolomia pavonana* in Samoa. *Biological Control*, 73: 31-38

Simulation and prediction of diamondback moth biological control dynamics with climate change in the Eastern Afromontane region

Ngowi, B.V.
INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY, NAIROBI, KENYA
email: benastras_x@yahoo.com

Tonnang, H.E.Z.
INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY, NAIROBI, KENYA
email: htonnang@icipe.org

Mwangi, E.M.
SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF NAIROBI, NAIROBI, KENYA
email: emmwangi@uonbi.ac.ke

Ndegwa, P.N.
SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF NAIROBI, NAIROBI, KENYA
email: pndegwa@uonbi.ac.ke

Subramanian, S.
INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY, NAIROBI, KENYA
email: ssubramania@icipe.org

ABSTRACT
Biological control of diamondback moth (DBM), *Plutella xylostella* (L.) with parasitoids, *Diadegma semiclausum* (Hellén) and *Cotesia vestalis* (Haliday), has proved to be highly sustainable in East Africa. The biology and interaction between DBM and its parasitoids and its impact on biological control, is influenced by the prevailing climatic conditions. With climate change, this interaction can potentially be disrupted and hence the sustainability of biological control. Eastern Afromontane regions are vulnerable to climate change, which could disrupt DBM biological control efforts. To understand this, field surveys were conducted over two consecutive years on altitudinal transects along Mount Kilimanjaro and Taita hills to assess the population dynamics of DBM and its parasitoids. The transects were subdivided into low, medium and high zones, based on temperature and altitude, with identified and georeferenced farmer-owned crucifer farms sampled. In each farm, daily temperature and humidity were logged and crucifer plants were sampled for DBM larvae and pupae, which were further observed for parasitoid/DBM emergence in the laboratory. Life history parameters for *D. semiclausum* and *C. vestalis* using lab cultured insects were established at 10, 12.5, 15, 20, 25, 30 and 35°C. Based on the life-history parameters at constant and field fluctuating temperatures, temperature-driven phenology models were developed and validated. All models were developed through Insect Life Cycle Modeling (ILCYM) software. The generated phenology models, the baseline (2013) and future downscaled temperatures (2055) from AFRICLIM; and the digital georeferenced topographical maps of the transects, were loaded in index interpolator, a sub-module of ILCYM for interpolating and mapping growth indices (establishment, generation and activity). Spatial simulations predicted a future decline of DBM population in the low zone of Mt. Kilimanjaro; and in low and medium zones of Taita hills due to increased temperature, which also strengthened its interaction with *C. vestalis.* However, increased temperatures are predicted to weaken the synchrony between DBM and *D. semiclausum* in the low and medium zones. Increased DBM damage in high zones is likely to occur beyond the colonization range of *D. semiclausum*. Linking phenology models with outdoor fluctuating temperature measurements established more realistic distribution models at a local scale than the commonly adopted general regional and global circulation models.

Keywords
Diamondback moth, *Diadegma semiclausum*, *Cotesia vestalis*, Climate change Phenology, Biological control

INTRODUCTION
Weather influences the field incidence of diamondback moth (DBM), with temperature and rain being key environmental factors affecting its biology and ecology (Talekar and Shelton 1993). DBM populations also respond to seasonal variability, vegetation structure of agricultural systems and natural enemies (Kahuthia-Gathu 2011). Changes in temperatures from 7°C to 35°C in laboratory and quality of host plants have been reported to significantly influence the rates of oviposition and survival of DBM (Singh and Singh 1982; Shelton et al. 1991; Syed and Abro 2003; Golizadeh et al. 2007; 2009).

Environmental changes can alter the temporal and spatial population dynamics of insect pests and their natural enemies (Menzel and Dose 2005; Andrew and Hill 2017). Along a spatial gradient, when an insect pest and its natural enemies react to a changing climate by shifting distribution ranges, they might end up colonizing dissimilar ranges. Very often, such shifts can lead to pest outbreaks and increased yield losses (Parmesan 2006). Introduced from 2002 to 2007 and released into different parts of East Africa, the hymenopteran wasps, *Cotesia vestalis* (Haliday) and *Diadegma semiclausum* (Hellén), endoparasitoids of DBM, have proved to be very effective in limiting the field populations of DBM in the low and

high zones, respectively (Löhr et al. 2007; Kahuthia-Gathu 2011; Kahuthia-Gathu et al. 2009; 2017). The two introduced parasitoids have so far outcompeted native parasitoids of DBM (Löhr et al. 2007). It is predicted that with climate change, the low and medium zones in Taita hills will experience substantial increase in temperature as compared to high altitude zones. Increases in temperature in Mt Kilimanjaro are likely to be lower and gradual (Ngowi et al. 2017a). Information on how climate change could influence the population dynamics of DBM and its parasitoids and their effectiveness in biological control, is lacking.

Knowing the biology and ecology of DBM and its parasitoids is indispensable for understanding the sustainability of DBM biological control under changing climate. DBM and parasitoid populations are dynamic and are affected by both biotic and abiotic factors. Generally, insects respond to increasing temperature under global warming by reducing the time taken to acquire enough energy to complete the life cycle (Zalucki et al. 2012; Bahar et al. 2014). However, rising temperatures can affect biological control of DBM, if such a rise leads to phenological mismatch of life cycles of DBM and its parasitoids (Bahar et al. 2012), leading to potential DBM outbreaks. Decline in the number of parasitoid generation and disruption of their host search efficiency with climate change can also impair the biological control of DBM. A recent modeling study has predicted climate change-based shrinkage of the ecological niche of some invasive species (Hill et al. 2017), which could also be possible for the introduced parasitoid species.

The projected reduction of precipitation and temperature rise of 3°C – 4°C by 2080 in most Sub-Saharan Africa (Niang et al. 2014) is predicted to constrain different pest management strategies because of the favorable environmental conditions that will likely support increased pest incidence and changes in their spatio-temporal distributions (Niang et al. 2014). Worldwide, studies that relate laboratory and field-based findings to map the pest activity at spatial scale are very few (Zalucki and Furlong 2011). Distribution models of the pest have been developed based on factors such as, occurrence/absence data (Centre for Agriculture and Bioscience International 2012), timing of the life cycle (Zalucki and Furlong 2011) and pest movement (Harcourt 1986; Honda 1992). The latest comprehensive global distribution map of DBM was created using CLIMEX (Zalucki and Furlong 2011). Hopkinson and Soroka (2010) utilized wind trajectory models to map the potential increase of DBM infestation under a 2°C temperature rise in the Canadian prairies. Closer to East Africa, the spatial distribution of the pest based on temperature and cropping systems has been developed in Ethiopia (Ayalew and Ogol 2006; Ayalew et al. 2007). Increased exposure of the pest to parasitoid attack can lead to increased range expansion (Chapman et al. 2015), which in turn contributes to influencing the extent of parasitism.

This study seeks to understand the potential effect of climate change on the distribution and abundance of DBM and its key parasitoids and the likely implication on the pest-parasitoid interaction and ultimately on the effectiveness of the biological control of DBM. Understanding the potential distribution of DBM and its parasitoids at a local scale under current and future scenarios is important for making meaningful interventions for sustaining biological control under changing climate conditions.

MATERIALS AND METHODS

Study sites

The study was conducted along the south eastern slopes of Mt Kilimanjaro, Tanzania and Taita hills, Kenya. Two altitudinal transects, each 1 km wide, were selected along the windward sides of Mt Kilimanjaro (700 to 1,692 masl) and along the Taita hills (700 to 1,785 masl.) (Fig. 1). Each of these transects was sub-divided into three altitudinal zones based on the mean annual temperature, microclimate and spatially available crucifer vegetables as previously done by Ngowi et al. (2017a, b): low (700 to 1,200 masl.), medium (1,201 to 1,600 masl.) and high (> 1,600 masl.).

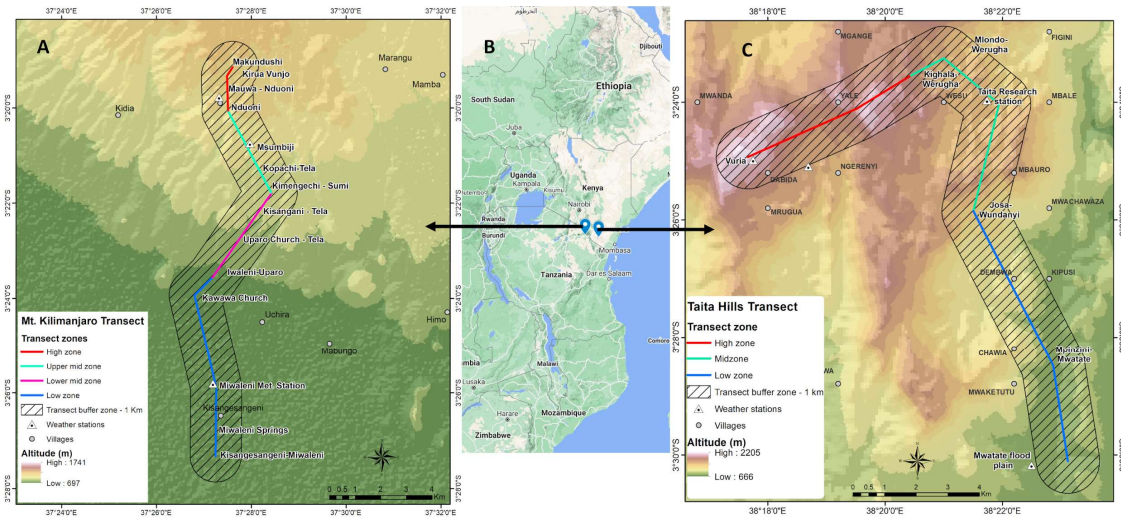


Figure 1: Altitudinal study transects in east Africa (B) along Taita hills, Kenya (C) and Mt Kilimanjaro, Tanzania (A)

Farm selection

Four farms were selected per altitudinal zone of each transect based on whether kale, cabbage and Ethiopian mustard were present on farms, and in adequate abundance for sampling. Along the transects, the farms were selected based on the approximate distance from each other (at least 100 m) and based on their location on either side of the transect. The geographic coordinates and altitudes of the surveyed farms were recorded on site by positioning a handheld Global Positioning System (GPS) receiver (Garmin eTrex 30, Garmin International Inc., Taiwan) in the middle of the farm, about 1 m high above the ground.

Climate data

Twenty-four thermo-hygrometer data loggers (iButton®, Maxim Integrated Products Inc.) were installed on farms (30 cm high from the ground) and used for recording the baseline daily temperature and relative humidity throughout the survey period.

The projected future temperatures were acquired from African climate database AFRICLIM (Platts et al. 2014). The database is corrected for bias, freely available and accessible at http://www.york.ac.uk/environment/research/kite/resources/. It is loaded with different regional climate models (RCM) developed to project climate in an area as small as 1 km^2. The RCM are based on high resolution climate projections. The potential future local temperatures on-farms were derived from such climate projections through downscaling.

For this study, the daily minimum and maximum temperature values utilized were stored in the RCM projection under the representative concentration pathway 8.5 (RCP 8.5) for the year 2055 as described in Platts et al. (2014). To get specific temperature values on farms, the downloaded temperature raster data were transferred for opening up in Quantum Geographic Information System 2.16.2 (QGIS 2.16.2) (Quantum Geographic Information System Development Team 2009). The difference between the baseline and future projected temperatures was derived from the 'raster calculator' (Sporleder et al. 2008).

Sampling for diamondback moth and its parasitoids

Crucifer vegetables in both transects were observed for DBM and its parasitoids every month for two consecutive years (2013 and 2014). Twenty cabbage, kale or Ethiopian mustard plants were randomly sampled from each individual farm and the leaves physically checked for presence of the larvae and pupae of DBM. The larvae and pupae found on the individual plants were counted and recorded. The larvae and pupae collected from the individual farms were packed into plastic containers (12 X 10.2 X 6.5 cm), fed on crucifer leaves and taken to the laboratory for assessment of DBM parasitism. The emerged adult moths were sexed. The emerged parasitoid species were identified using a dichotomous key (Wahl and Sharkey 1993) and morphometric measurements based on Azidah et al. (2000).

Establishment of diamondback moth and parasitoid colonies

The DBM population obtained from cruciferous vegetables grown in Taita hills transect was utilized for establishment of life tables. The pest colony was initiated and maintained on common cabbage (Gloria, F1 hybrid) at ICIPE in Nairobi, Kenya as described by Kahuthia-Gathu et al. (2008). The collected population was raised for one generation to acclimatize to laboratory conditions. The *C. vestalis* and *D. semiclausum* populations utilized for establishment of life table experiments were obtained from adults that emerged in the laboratory.

Development of temperature – dependent life tables of diamondback moth

A life table was constructed by tracking a population of *n* individuals from eclosion to eclosion of all the progeny of these individuals. Development of DBM life stages, which includes oviposition and mortality, was recorded daily. Freshly laid eggs from the colony were positioned on pieces of cabbage leaf and placed in glass vials (7.5 cm length X 2.5 cm diameter). The vials were lined with paper towels for absorbing excess moisture and covered with a ventilated lid with fine mesh sieve. At 35°C, fresh eggs were individually positioned on young leaves to delay dehydration of the leaves. The stipule ends were enfolded in soaked cotton wool and placed inside a ventilated plastic container (12 X 10.2 X 6.5 cm). The lids of containers were perforated, and the holes were covered by muslin cloth for ventilation as described by Ngowi et al. (2017b; 2019). The experimental set ups were placed in six separate incubators (Sanyo MIR – 554; Sanyo Electric Co. Ltd., Japan), each calibrated at a constant temperature (10°C, 15°C, 20°C, 25°C, 30°C and 35°C, each ± 1°C). A total of 1872 eggs were utilized for life table experiments at all constant temperatures. About a third of these (670 eggs) were utilized at extreme temperatures (10°C and 35°C) to ensure adequate availability of samples following increased mortality. In each life table, at least thirty surviving females were kept for assessment of fecundity. The development, mortality, lifespan and reproduction models were determined under constant temperature as described in detail by Kroschel et al. (2013).

Development of temperature – dependent life tables of parasitoid species

A total of 3,384 second and third instar DBM larvae which had spent the past 24 h exposed to adult parasitoids, were individually placed in glass vials (7.5 cm length X 2.5 cm diameter) for construction of life tables. Specifically, the larvae were attended as described above with a little modification to suit parasitoids: if the host larvae were parasitized, the individual egg and larval stages of parasitoids could not be observed visually except through morphological observation of the host larvae. These stages were thus considered together and were denoted as the combined Egg + Larva (E + L). Moreover, an extra constant temperature (12.5°C) was included for *C. vestalis* after the parasitoid failed to develop at 10°C. The emerged adult parasitoids were paired at a sex ratio of 1:1 and placed inside ventilated plastic containers (12 X 10.2 X 6.5 cm) for mating. A fresh batch of twenty-five second and third instar larvae of DBM (host insect) was daily placed inside the plastic containers for parasitization by *D. semiclausum* or *C. vestalis.* The exercise was repeated daily till death of the female. The development of exposed larvae was recorded daily to assess parasitism levels.

Generation of digital georeferenced topographic data

Development of the distribution and abundance maps was subject to development of the digital georeferenced topographical map of the study site. On the map are points of known geographic coordinates and altitude. Using this information, the digital georeferenced topographical maps of the two transects were developed and obtained stepwise from the Shuttle Radar Topography Mission (SRTM), a database containing high resolution digital 3D topographic maps of the Earth. From the database, the digital georeferenced altitudinal map covering the EABH region was downloaded from the open-sourced CGIAR-CSI server (http://srtm.csi.cgiar.org/SELECTION/inputCoord.asp). The longitudinal coordinates covering the EABH region were specified to be 37.10 and 38.60, while latitudinal coordinates were -3.10 and -3.60. A georeferenced 3D topographical map of the EABH fitting the given geographic coordinates was generated and saved in GeoTIFF file format. As the generated maps covered the two transects and area far beyond these transects the maps were transferred to QGIS 2.16.2 and trimmed to fit in the actual width and length of the Mt Kilimanjaro and Taita hills transects and produced two 'shapefiles' of the transects.

Development of phenology models and analysis

The Insect Life Cycle Modeling (ILCYM version 3.0) software (Sporleder et al. 2012) was employed to produce phenology models. The developed life table datasets were saved in .txt format and loaded in ILCYM to build temperature – dependent development time, development rate, mortality, life span and fecundity phenology models of the insects at constant temperatures.

The projected future pest and parasitoid spatial distribution and abundance

Population growth indices of DBM and its key parasitoids

The insect phenology model, the base year (2013) and projected future (2055) temperature data and shapefiles of the two transects were utilized as inputs for calculating and mapping the population growth indices of DBM, *D. semiclausum* and *C. vestalis.* Three population indices were derived: Establishment Index (EI), Generation Index (GI) and Activity Index (AI). The EI was calculated to identify areas having favorable climate for establishment, survivorship and distribution of the pest and its parasitoid populations. The index value of 1 indicates conditions where all life stages of the insect were able to survive, spread and grow in a given geographical location throughout the year. When an EI = 0.5, it indicates an area where the insect population was able to survive and establish only for six months in a year. Generation index refers to the estimated mean number of generations which DBM, *D. semiclausum* or *C. vestalis* could produce in a

year. Activity index was applied to predict the annual population growth potential of the individual insect species. To do this, a log of products of the daily finite rates of population increase was calculated following the method described by Sporleder et al. (2008). Every increase of the index by one implied the present population increased by 10-fold. An index value of 2 indicated a potential population increase by a factor of 10^2 *i.e.* 100 individuals in a year. The activity index was also used to illustrate the damage potential of DBM and population growth potential of parasitoids. Distribution of the insect population across a spatial scale was mainly addressed by EI while the GI and AI addressed more of the population growth potential of the insect species. Generation of these indices was implemented using the GIS module of ILCYM for spatial analysis and mapping (see below). The corresponding AI values of DBM and parasitoid species represented equilibrium status of the biological control across a spatial scale.

Mapping spatial distribution and abundance of DBM and key parasitoid species

The spatial distributions and abundance of DBM, *D. semiclausum* and *C. vestalis* were mapped individually through a number of steps. The field climate data for the base year 2013 was uploaded and saved in index interpolator, a sub-module of ILCYM. A folder of DBM with its complete phenology was uploaded and saved separately. The shapefile of Mt Kilimanjaro was subsequently uploaded. The spatially–referenced AI index was calculated using the population growth parameter values obtained by linking the loaded temperatures (for base year 2013) and DBM phenology as described in detail by Sporleder et al. (2008), Kroschel et al. (2013) and Tonnang et al. (2013). When plotting, the index interpolator was used to predict the AI values between the sampled farms because of the assumption that spatially distributed objects are spatially correlated.

The plotted AI map of DBM was transferred to QGIS 2.16.2 for better manipulation and visualization. Using the 'Clipper' tool under the 'Extraction' icon, the generated map was trimmed into a shapefile of Taita hills following a number of steps described in Kroschel et al. (2013). A comma delimited (.csv) file containing names, altitudes, latitudes and longitudes of farms located in Mt Kilimanjaro and Taita hills transects was created and saved. By 'turning on' the 'Coordinate Reference System Selector' window, the GPS coordinates of the sampled farms were selected and marked on the developed EI map. The same procedure was used to map AI for the two parasitoids under current and future climate scenarios in Taita hills and Mt Kilimanjaro.

RESULTS

Temperature changes between current and future climate conditions

The future maximum temperature is predicted to increase by 1.6°C in the low zone of Taita hills. Likewise, the minimum temperature was predicted to increase from 1.9°C to 2.7°C from the low to medium zones. The margins of temperature changes along the Mt Kilimanjaro transect will be comparatively lower than those in Taita hills (Ngowi et al. 2017a).

Changes in future abundance of DBM in Mt Kilimanjaro

Between 2013 and 2055, population growth rate of DBM is predicted to decline from the low land farm (KisangesangeniMadukani, - KisaMadukani) upwards with 22.3-fold decline in the low zone (Fig. 2c).

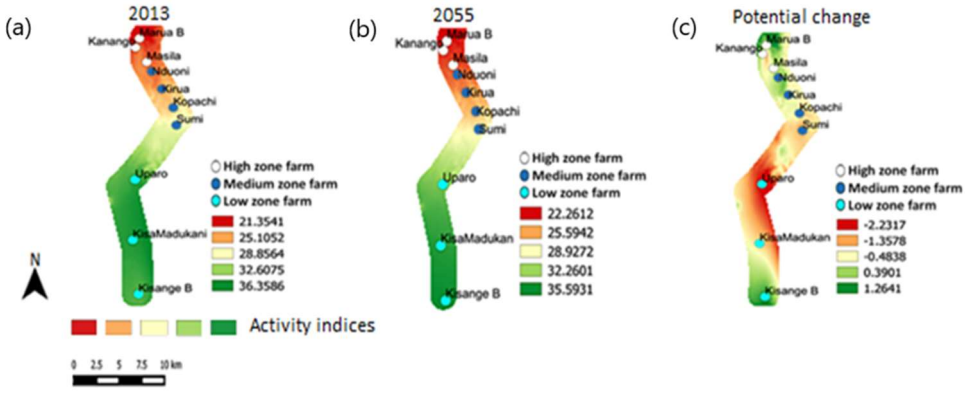


Figure 2: Changes in population growth rates across climate change scenarios of Mt Kilimanjaro (a) Baseline 2013 growth rates of DBM on selected farms; (b) Future 2055 growth rates of DBM; (c) potential change in growth rates between baseline and future scenarios.

Most of the medium zone of Mt Kilimanjaro is predicted to be less favorable to survival and establishment of DBM because the associated change will result in declining the growth rate ($-1.3578 \leq AI \leq -0.4838$) although such

change will be small and not result in population growth rate change in the zone (Fig. 2c).
In contrast, changes in the high zone are predicted to be comparatively favorable for the pest. The rate of increase of DBM is predicted to increase from approximately 3.9 to 12.6-fold.

Changes in future abundance of DBM in Taita hills

The reproduction rate of the pest is predicted to decline significantly in the low zone, adversely affecting the growth rate of DBM population in the zone. The zone is predicted to lose about 92.2-folds of its DBM population between 2013 and 2055 (Fig. 3c).

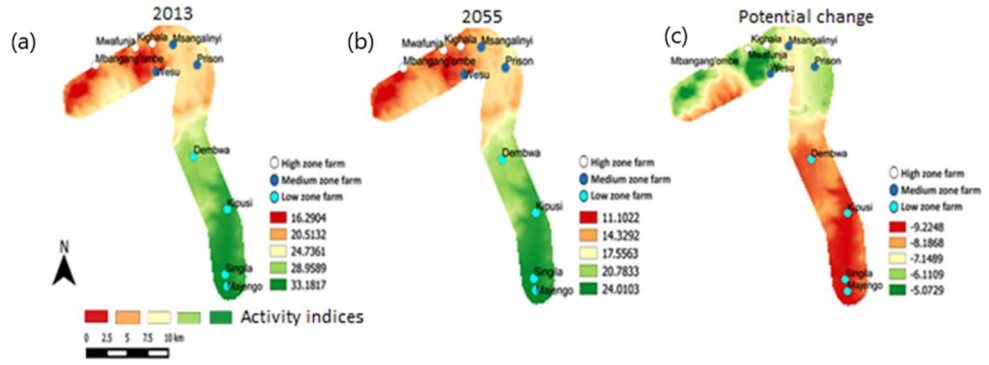


Figure 3: Changes in population growth rates across climate change scenarios of Taita hills (a) Baseline 2013 growth rates of DBM on selected farms; (b) future 2055 growth rates of DBM; (c) potential change in growth rates between baseline and future scenarios.

The model predicted the population growth rate to decline by 7 to 8-fold (Fig. 3c) in the medium zone, This extensive decline will translate to reduced damaging potential of DBM in the zone, The population growth rate in the high zone is predicted to increase marginally (Fig. 3c).

Changes in future distribution and abundance of *C. vestalis* in Mt Kilimanjaro

Between 2013 and 2055, *C. vestalis* population is predicted to increase by a factor of 2.9593 – 4.3261 for most of the zones in Mt Kilimanjaro (Fig. 4c).

The population growth rate of *C. vestalis* in the medium zone is predicted to increase by a range of 15.7 to 22.7-fold (Fig. 4c). Considering changes in the generations in the medium zone, the parasitoid abundance is predicted to increase by a factor of 2.2665 – 2.9593 ($2.2665 \leq AI \leq 2.9593$) (Fig. 4c).

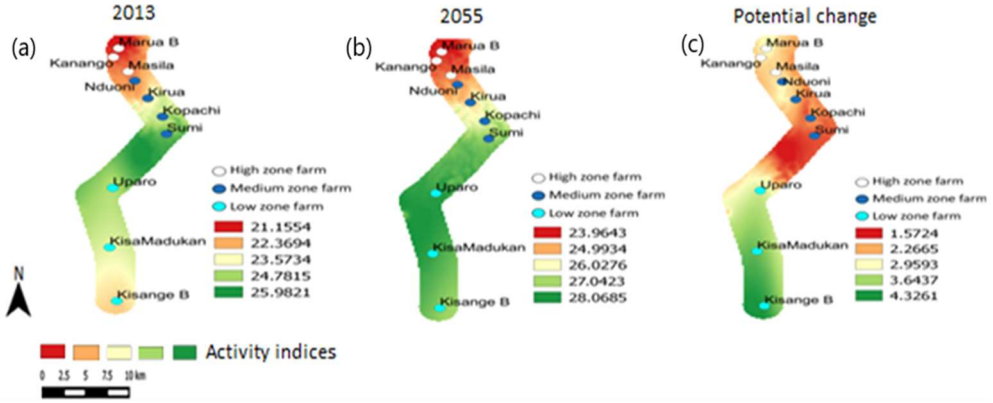


Figure 4: Changes in population growth rates across climate change scenarios of Mt Kilimanjaro (a) Baseline 2013 growth rates of *C. vestalis* on selected farms; (b) Future 2055 growth rates of *C. vestalis*; (c) Potential change in growth rates between baseline and future scenarios.

Changes in future distribution and abundance of *C. vestalis* in Taita hills

Between 2013 and 2055, suitability of the low zone of Taita hills to *C. vestalis* is predicted to change marginally largely due to the added number of generations than the other zones. The added generations will lead to a peak predicted population in the area around Kipusi in the lowland (AI = 3.8344) (Fig. 5c).

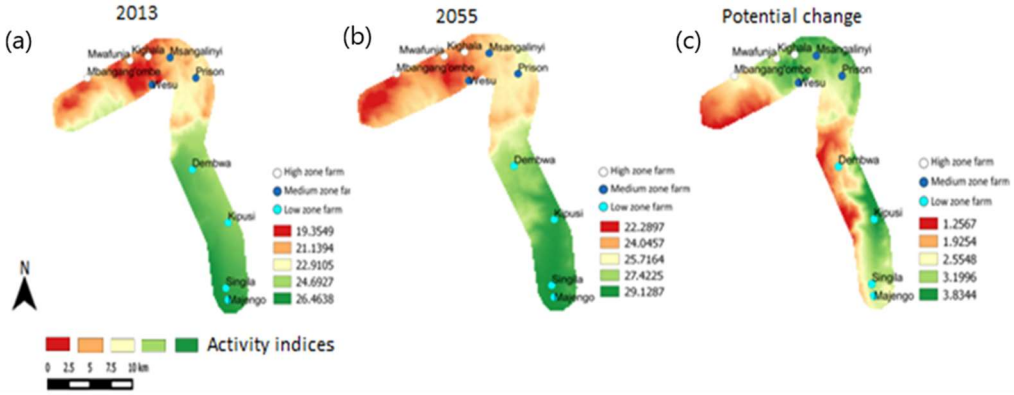


Figure 5: Changes in population growth rates across climate change scenarios of Taita hills (a) Baseline 2013 growth rates of *C. vestalis* on selected farms; (b) Future 2055 growth rates of *C. vestalis*; (c) Potential change in growth rates between baseline and future scenarios.

The corresponding population growth of *C. vestalis* is predicted to increase by a range of 31.9- to 38.3-fold (Fig. 5c). Across the transect, *C. vestalis* population is predicted to increase with a maximum increase of 38.3-fold (AI = 3.8344) (Fig. 5c).

Changes in future distribution and abundance of *D. semiclausum* in Mt Kilimanjaro

Most changes in the potential distribution of *D. semiclausum* between 2013 and 2055 in Mt Kilimanjaro will occur in the low zone. The population growth rate is predicted to shrink by a factor of 3.1885 in the low zone and continue to shrink up to a factor of 5.8554 in Uparo in the medium zone (-5.8554 ≤ AI ≤ -3.1885) (Fig. 6c).

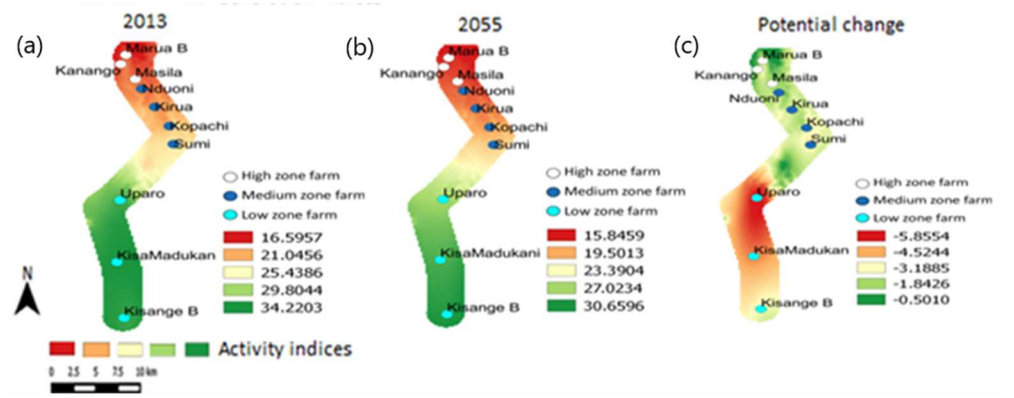


Figure 6: Changed population growth rates across climate change scenarios of Mt Kilimanjaro (a) Baseline (2013) growth rates of *D. semiclausum* on selected farms; (b) Future (2055) growth rates of *D. semiclausum*; (c) Potential change in growth rates between baseline and future scenarios.

A considerable loss in the number of generations of *D. semiclausum* is predicted, especially in the low zone. The overall population growth is predicted to reduce as indicated by the activity index ranging from -3.1885 to -1.8426. The parasitoid is predicted to lose fewer generations per annum in the high zone and hence the population growth is likely to change marginally (Fig. 6c).

Changes in future distribution and abundance of *D. semiclausum* in Taita hills

The number of new generations added to the population will decline overtime and lead to a declining population growth rate of *D. semiclausum* by up to 46-fold (Fig. 7c).

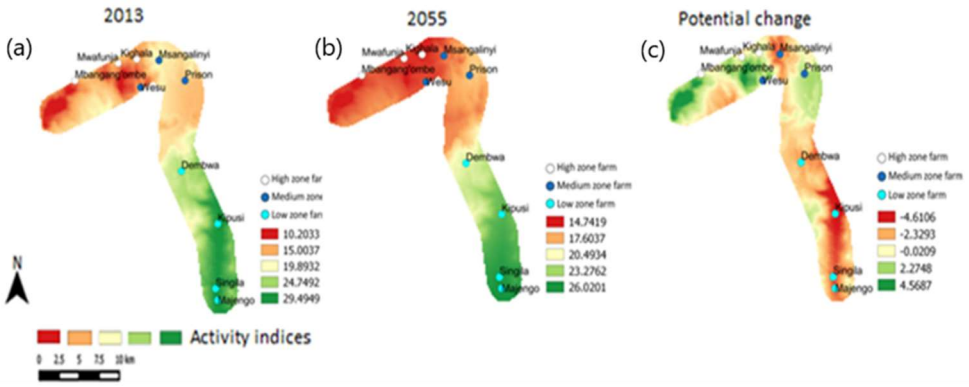


Figure 7: Changed population growth rates across climate change scenarios of Taita hills (a) Baseline (2013) growth rates of *D. semiclausum* on selected farms; (b) Future (2055) growth rates of *D. semiclausum*; (c) Potential change in growth rates between baseline and future scenarios.

Ability of *D. semiclausum* to add new generations will decline through most parts of the zone between 2013 and 2055. Apparently in the mid zone area near Prison, any gain on the *D. semiclausum* population growth is likely to be offset by the loss of generations (Fig. 7c). In the high zone, an overall increase in *D. semiclausum* population growth rate is expected between 22.7- to 45.7-fold ($2.2748 \leq AI \leq 4.5687$) (Fig. 7c).

DISCUSSION

Development and utilization of models for describing the distribution and abundance of insect species is not new. Often, most of these models are developed at a regional, continental or global scale. These models are too generalized and utilize scarce information of the insect biology which frequently limits their applicability in improving management strategies for pests and surveillance of natural enemies in response to climate change at a local scale. Some regional prediction models developed through CLIMEX were utilized successfully to map the distribution of DBM in China. However, CLIMEX based-population modeling is broad and does not explicitly include age structure (Li et al. 2012). Furthermore, most models do not incorporate the local measurements of weather parameters. Predictions of potential effects of climate change on distribution and abundance of DBM and its parasitoids at a local spatial scale requires information on the insect biology and ecology to generate meaningful insights towards improving the pest management strategies.

To understand the potential change of insect distribution and abundance locally under a future climate scenario, the developed phenology models, the georeferenced topographical 3D map and local temperature measurements recorded on farms of Mt Kilimanjaro and Taita hills transects were linked. With climate change, of the two parasitoids, *C. vestalis* is likely to effectively control DBM population in the low zone. This is because the numbers of newly added generations in *C. vestalis* population was greater than those of DBM in the low zone of Taita hills. The parasitoid will likely be very effective in limiting the DBM population in the low zone of Mt Kilimanjaro. In contrast, the growth rate of *D. semiclausum* was predicted to decline substantially in low zones of the two transects. However, higher temperatures in the low zones could also reduce the generation lengths and therefore lead to rapid increase of the insect populations per unit time as observed earlier by Fand et al. (2014). However, this prediction will only hold if the DBM and *D. semiclausum* populations are not exposed for long periods at high temperatures that compromise the vulnerable immature stages. Several studies (Yang et al. 1993; Andrew 2013; Nguyen et al. 2014) have demonstrated when eggs and pupae are subjected to long periods of exposure to temperature above 35°C their development is disrupted.

A substantial increase in the mean minimum and maximum temperatures will be greater in Taita hills than in Mt Kilimanjaro. The change of mountain temperatures is subject to many attributes, including tree coverage and the local topography. The shading effects in the upward slope of Mt Kilimanjaro (Hemp 2006; Fernandes et al. 1984), could lead to less temperature changes in Mt Kilimanjaro. The shading will enable crucifer cropping systems to withstand harsh climates by providing favorable microclimate. Favorable microclimate will benefit DBM and *D. semiclausum* in Mt Kilimanjaro transect than *C. vestalis* as they thrive well under moderate temperatures. A very extensive loss of forest cover due to human activities e.g. agriculture, settlement and logging

in Taita hills (Pellikka et al. 2009) is likely to have contributed to warmer climates compared to Mt Kilimanjaro. Only a third of the forest cover in Mt Kilimanjaro has been lost in the last 70 years (Hemp 2009).

The mean minimum temperatures are predicted to rise substantially in the low zones. Under future climate change scenarios, the areas around Kisangesangeni B in the low zone and most of the medium and high zones of Mt Kilimanjaro will likely face increasing pressures of DBM despite some increase in *D. semiclausum.* The increase in the growth rate of DBM in the low zones of Mt Kilimanjaro highlights the possible effects of Miwaleni springs, which is closest to Kisangesangeni B farm. The springs facilitate continuous cultivation of crucifers with irrigation and provide the microclimate needed for DBM and *D. semiclausum* in a region that is otherwise hotter than the mid and high zones. Elsewhere in the zone, the predicted decline in number of generations and the shrinking of *D. semiclausum* population could lead to future decoupling of DBM and its parasitoid, *D. semiclausum*.

Compared to Mt Kilimanjaro, the models projected a future decline in population growth rates of DBM in the low and medium zones of Taita hills due to a substantial increase of the mean minimum temperatures. Such a declining population of DBM can be associated with potential reduction of crucifer damage in the two zones. Unlike the Chagga homegardens in Mt Kilimanjaro, the agroecological landscape in the medium and high zones of Taita hills is less characterized by tree components, depriving its ability to insulate against the increasing temperature. The increased temperature could become favorable to hot temperature–tolerant pests and may pave the way to potential outbreak of other insect pests such as thrips and aphids (Bergant et al. 2005; Barton and Ives 2014).

The findings suggest that *C. vestalis* can become an increasingly reliable parasitoid for controlling DBM under the warming conditions in the future. With minimum temperature values increasing gradually upward with altitude and towards 2055, the parasitoid is likely to thrive under environmental conditions which could otherwise be relatively unfavorable to temperature-sensitive *D. semiclausum.* Warming conditions with increasing altitude may induce gradual upward shift along the altitude for both parasitoids; *C. vestalis* in response to the favorable warming conditions and *D. semiclausum* in search for more suitably cool conditions. Such a shift could result in potentially weak DBM and its parasitoid interaction in the low and medium zone. In the high zones, currently *D. semiclausum* is solely responsible for DBM biological control, which is predicted to decline with climate change and result in the decoupling of DBM-parasitoid interaction.

In conclusion, the study has highlighted a likely DBM – *D. semiclausum* population mismatch which could lead to increased pest outbreak under future warming conditions. Hence there is a need to investigate, mobilize and mass rearing of temperature tolerant strains of both parasitoids and redistribute them to sustain the effectiveness of DBM biological control. Equally important, linking phenology models with field-collected fluctuating temperature measurements managed to establish more realistic and focused distribution models at a local scale than the commonly adopted general regional circulation models (RCM) and global circulation models (GCM). However, it is recommended to include other factors known to influence insect distributions such as relative humidity, rainfall and host plant diversity to increase validity of the predicted distributions and abundance.

References

Andrew NR. 2013. Population dynamics of insects: impacts of a changing climate, pp 311–324 In: Rohde K (ed.) The Balance of Nature and Human Impact. London, UK, Cambridge University Press.

Andrew NR, Hill SJ. 2017. Effect of climate change on insect pest management. Environmental pest management: challenges for agronomists, ecologists, economists and policymakers, 197.

Ayalew G, Ogol CKPO. 2006. Occurrence of the diamondback moth (*Plutella xylostella* L.) and its parasitoids in Ethiopia: influence of geographical region and agronomic traits. *Journal of Applied Entomology* 130 (6-7): 343–348.

Ayalew A, Baumgärtner J, Ogol CKPO, Löhr B. 2007. Analysis of population dynamics of diamondback moth, (*Plutella xylostella* (Lepidoptera: Plutellidae)) at two sites in central Ethiopia, with particular reference to parasitism. *Biocontrol Science and Technology* 16(6): 607–618.

Azidah AA, Fitton MG, Quicke DLJ. 2000. Identification of the *Diadegma* species (Hymenoptera: Ichneumonidae, Campopleginae) attacking the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Bulletin of Entomological Research* 90: 375–389.

Bahar MH, Soroka JJ, Dosdall LM. 2012. Constant versus fluctuating temperatures in the interactions between *Plutella xylostella* (Lepidoptera: Plutellidae) and its larval parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Environmental Entomology* 41(6): 1653–1661.

Bahar MH, Soroka JJ, Grenkow L, Dosdall LM. 2014. New threshold temperatures for the development of a North American diamondback moth (Lepidoptera: Plutellidae) population and its larval parasitoid, *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Environmental Entomology* 43: 1443–1452.

Barton BT, Ives AR. 2014. Direct and indirect effects of warming on aphids, their predators, and ant mutualists. *Ecology* 95(6): 1479–1484.

Bergant K, Trdan S, Znidarcic D, Crepinsek Z, Kajfez-Bogata L. 2005. Impact of climate change on developmental dynamics of *Thrips tabaci* (Thysanoptera: Thripidae): can It be quantified? *Environmental Entomology* 34(4): 755–766.

Centre for Agriculture and Bioscience International. 2012. *Crop Protection Compendium.* http://www.cabi.org/cpc/Site accessed on 15 April 2018.

Chapman JW, Reynolds DR, Wilson K. 2015. Long - range seasonal migration in insects: mechanisms, evolutionary drivers and ecological consequences. *Ecology,* 287–302.

Fand BB, Tonnang HEZ, Kumar M, Santanu KB, Singh NP, Rao DVKN et al. 2014. Predicting the impact of climate change on regional and seasonal abundance of the mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)

using temperature-driven phenology model linked to GIS. *Ecological Modelling* 288: 62–78.

Fernandes ECM, O'ktingati A, Magembe J. 1984. The Chagga home gardens: a multi-storeyed agroforestry cropping system on Mt Kilimanjaro, northern Tanzania. *Agroforestry Systematics*, 2, 73–86.

Golizadeh A, Kamali K, Fathipour Y, Abbasipour H. 2007. Temperature-dependent development of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) on two brassicaceous host plants. *Insect Science* 14(4): 309–316.

Golizadeh A, Kamali K, Fathipour Y, Abbasipour H. 2009. Life table of the diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on five cultivated brassicaceous host plants. *Journal of Agricultural Science and Technology* 11: 115–124.

Harcourt DG. 1986. Population dynamics of diamondback moth in southern Ontario. Proceedings of the First International Workshop on Management of Diamondback Moth and Other Crucifer Pests, 1–15.

Hemp A. 2006. Vegetation of Kilimanjaro: hidden endemics and missing bamboo. *African Journal of Ecology* 44: 305–328.

Hemp A. 2009. The banana forests of Kilimanjaro: biodiversity and conservation of the Chagga homegardens. *Biodiversity Conservation*, 15(4): 1193–1217.

Hill MP, Gallardo B, Terblanche JS. 2017. A global assessment of climatic niche shifts and human influence in insect invasions. *Global Ecology and Biogeography* 26: 679–689.

Honda K. 1992. Hibernation and migration of diamondback moth in northern Japan, pp 45–50, In: The Management of Diamondback Moth and Other Crucifer Pests. Proceedings of the Second International Workshop on Management of the Diamondback Moth and Other Crucifer Pests. AVRDC-The World Vegetable Centre. Tainan, Taiwan.

Hopkinson RF, Soroka JJ. 2010. Air trajectory model applied to an in-depth diagnosis of potential diamondback moth infestations on the Canadian prairies. *Agricultural and Forest Meteorology* 150: 1–11.

Kahuthia-Gathu R. 2011. Seasonal incidences of *Plutella xylostella* (Lepidoptera: Plutellidae) and its associated natural enemies in major crucifer growing areas of Kenya. *Journal of Applied Biosciences* 46: 3103–3112.

Kahuthia-Gathu R, Löhr B, Poehling HM, Mbugua PK. 2009. Diversity, distribution and role of wild crucifers in major cabbage and kale growing areas of Kenya. *Bulletin of Entomological Research* 99(3): 287–297.

Kahuthia-Gathu R, Löhr B, Poehling HM. 2008. Development and reproductive potential of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) on cultivated and wild crucifer species in Kenya. *International Journal of Tropical Insect Science* 28(1): 19–29.

Kahuthia-Gathu R, Nyambo B, Subramanian S. 2017. Impact of introduced parasitoid *Cotesia vestalis* (Hymenoptera: Braconidae) on *Plutella xylostella* (Lepidoptera: Plutellidae) and its parasitoid guild on kale in semi-arid areas in Kenya. *International Journal of Tropical Insect Science* 37(3): 163–175.

Kroschel J, Sporleder M, Tonnang HEZ, Juarez H, Carhuapoma P, Gonzales JC et al. 2013. Predicting climate change-caused changes in global temperature on potato tuber moth *Phthorimaea operculella* (Zeller) distribution and abundance using phenology modeling and GIS mapping. *Agricultural Forestry and Meteorology* 170: 228–241.

Li ZY, Zalucki MP, Bao HA, Chen HY, Hu ZD, Zhang DY, Lin QS, Yin F, Wang M, Feng X. 2012. Population dynamics and 'outbreaks' of Diamondback moth, *Plutella xylostella*, in Guangdong province, China: climate or the failure of management? *Journal of Economic Entomology* 105: 739–752.

Löhr B, Kahuthia-Gathu R, Kariuki C, Obiero J, Gichini G. 2007. Impact of an exotic parasitoid on *Plutella xylostella* (Lepidoptera: Plutellidae) population dynamics, damage and indigenous natural enemies in Kenya. *Bulletin of Entomological Research* 97: 337–350.

Menzel A, Dose V. 2005. Analysis of long-term time-series of beginning of flowering by Bayesian function estimation. *MeteorologischeZeitschrift* 14: 429–434.

Ngowi BV, Tonnang HEZ, Mwangi EM, Johansson T, Ambale J, Ndegwa PN, Subramanian S. 2017a. Temperature-dependent phenology of *Plutella xylostella* (Lepidoptera: Plutellidae): simulation and visualization of current and future distributions along the Eastern Afromontane. *Plos One,*12(3), e0173590.

Ngowi BV, Tonnang HEZ, Khamis F, Mwangi EM, Nyambo B, Ndegwa PN, Subramanian S. 2017b. Population dynamics of *Plutella xylostella* (Lepidoptera: Plutellidae) and its parasitoids along altitudinal gradients of the eastern Afromontane. International Conference on Biological Control of Arthropods, 15-19 September 2017. Kuala Lumpur, Malaysia.

Ngowi BV, Tonnang HEZ, Khamis F, Mwangi EM, Nyambo B, Ndegwa PN, Subramanian S. 2019. Seasonal abundance of *Plutella xylostella* (Lepidoptera: Plutellidae) and diversity of its parasitoids along altitudinal gradients of the eastern Afromontane. *Phytoparasitica* 47: 375–391.

Nguyen C, Bahar MH, Baker G, Andrew NR. 2014. Thermal tolerance limits of diamondback moth in ramping and plunging assays. *Plos One*, 9(1), e87535.

Niang I, Ruppel OC, Abdrabo MA, Essel A, Lennard C, Padgham J, Urquhart P. 2014. Africa, In: Climate Change 2014: Impacts, Adaptation, and Vulnerability, Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Barros VR, Field CB, Dokken DJ, Mastrandrea MD, Mach KJ, Bilir TE, Chatterjee M, LEbi K, Estrada YO, Genova RC, Girma B, Kissel ES, Levy AN, MacCracken S, Mastrandrea PR, White LL (eds.), Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1199-1265.

Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics* 37: 637–669.

Pellikka PKE, Lötjönen M, Siljander M, Lens L. 2009. Airborne remote sensing of spatiotemporal change (1955−2004) in indigenous and exotic forest cover in the Taita Hills, Kenya. *International Journal of Applied Earth Observation and Geoinformatics* 11(4): 221−232.

Platts PJ, Omeny PA, Marchant R. 2014. AFRICLIM: high‐resolution climate projections for ecological applications in Africa. *African Journal of Ecology* 53(1): 103–108.

Quantum Geographic Information System Development Team. 2009. QGIS Geographic Information System. OpenSource Geospatial Foundation Available: http://qgis.osgeo.org Accessed 9 October 2015.

Shelton AM, Cooley RJ, Kroening MK, Wilsey WT, Eigenbrode SD. 1991. Comparative analysis of two rearing procedures for diamondback moth (Lepidoptera: Plutellidae). *Journal of Entomological Science* 26: 17–26.

Singh SP, Singh D. 1982. Influence of cruciferous host plants on the survival and development of *Plutella xylostella* (L.). *Punjab Agriculture University Research Journal* 19: 100–104.

Sporleder M, Tonnang HEZ, Juarez H, Carhuapoma P, Gonzales JC, Simon R et al. 2012. ILCYM–Insect life cycle modeling (version 3.0): a software package for developing temperature-based insect phenology models with applications to regional and global analysis of insect population and mapping (user manual). International Potato Centre, Lima, Peru, pp 124.

Sporleder M, Simon R, Juarez H, Kroschel J. 2008. Regional and seasonal forecasting of the potato tuber moth using a temperature-driven phenology model linked with geographic information systems, pp15–30, In: Kroschel J, Lacey L (eds) Integrated Pest Management for the Potato Tuber Moth *Phthorimaea operculella* Zeller – A Potato Pest of Global Importance. Weikersheim, Germany, Margraf Publishers.

Syed TS, Abro GH. 2003. Effect of brassica vegetable hosts on biology and life table parameters of *Plutella xylostella* under laboratory conditions. *Pakistan Journal of Biological Sciences* 6(22): 1891–1896.

Talekar NS, Shelton AM. 1993. Biology, ecology, and management of the diamondback moth. *Annual review of Entomology* 38(1): 275-301.

Tonnang EZH, Juarez H, Carhuapoma P, Gonzales JC, Mendoza D, Sporleder M, Simon R, Kroschel J. 2013. ILCYM-insect life cycle modeling. *A software package for developing temperature-based insect phenology models with applications for local, regional and global analysis of insect population and mapping. Int Potato Cent Lima Peru*, *193*.

Wahl DB, Sharkey MJ. 1993. Superfamily Ichneumonoidea. Hymenoptera of the world, an identification guide to families. Agriculture Canada Research Branch Monograph.

Yang JC, Chu YI, Talekar NS. 1993. Biological studies of *Diadegma semiclausum* (Hym; Ichneumonidae), a parasite of diamondback moth. *Entomophaga* 38(4): 579–586.

Zalucki MP, Furlong MJ. 2011. Predicting outbreaks of a migratory pest: an analysis of diamondback moth distribution and abundance revisited, pp. 8–14, In: The Management of Diamondback Moth and Other Crucifer Pests. Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Pests. AVRDC-The World Vegetable Centre. Nakhon Pathom, Thailand.

Zalucki MP, Shabbir A, Silva R, Adamson D, Liu SS, Furlong MJ. 2012. Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella*: Just how long is a piece of string? *Journal of Economic Entomology* 105: 1115–29.

SESSION 2

Biology, Ecology and Behavior of Diamondback Moth and Other Crucifer Pests

Unveiling diamondback moth, *Plutella xylostella*, movement at landscape and regional scales

Perry, K.D.[1,2]
[1]SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE, ADELAIDE, SOUTH AUSTRALIA 5001
[2]SCHOOL OF AGRICULTURE FOOD AND WINE, UNIVERSITY OF ADELAIDE, SOUTH AUSTRALIA 5005
email: kym.perry@sa.gov.au

Baxter, S.W.
SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF ADELAIDE, SOUTH AUSTRALIA 5005
email: simon.baxter@adelaide.edu.au

Keller, M.A.
SCHOOL OF AGRICULTURE FOOD AND WINE, UNIVERSITY OF ADELAIDE, SOUTH AUSTRALIA 5005
email: mike.keller@adelaide.edu.au

ABSTRACT
Integrated management of diamondback moth, *Plutella xylostella* L., depends on understanding its movement ecology at different spatial scales. Local dispersal and long-range migration are well documented, but practical research challenges have led to a gap in knowledge of seasonal *P. xylostella* movements at intermediate landscape and regional scales, which warrants renewed focus. Here, we review a series of recent studies that investigated the colonisation of canola crops by *P. xylostella* at a regional scale in southern Australia. Vast areas of canola grown in temperate southern Australia are seasonally infested by *P. xylostella*, leading to intermittent outbreaks, but the sources of colonising insects were uncertain. We investigated movement of *P. xylostella* from multiple angles. Extensive collections of immature *Plutella* spp. were analysed to identify the geographic distribution, host associations and genetic structure of a cryptic species, *Plutella australiana* Landry and Hebert. It was shown that although the two *Plutella* species co-exist widely in Australia, *P. australiana* poses a minimal threat to *Brassica* crops. Genome-wide analysis using RAD-seq found no genetic structure among Australian *P. xylostella* populations across the Australian cropping zone, regardless of geographic location, brassicaceous host plant or sampling year, reflecting either historical or current gene flow. Field observation and modelling studies over three years elucidated the colonisation of canola crops by *P. xylostella* at a regional scale in South Australia. *P. xylostella* consistently colonised canola early in the cropping cycle, most likely originating from insecticide-resistant populations over-summering locally on wild host plants in the landscape. We synthesise the results and provide perspectives on future directions for dispersal studies in this species.

Keywords
Dispersal, cryptic species, *Plutella australiana*, RAD-seq, CLIMEX.

INTRODUCTION
Better understanding the movement ecology of the diamondback moth, *Plutella xylostella* L., has long been a priority for researchers working to develop integrated management tactics. As a highly mobile species, dispersal is central to the ecological and evolutionary dynamics of *P. xylostella*, but also integral to various management strategies proposed for its control in *Brassica* crops, including biological control, insecticide resistance management, adult trapping, mating disruption, auto-dissemination of entomopathogens, trap cropping, companion planting (see the proceedings of previous workshops), and self-limiting genetic strains (Shelton 2021 this conference).

Dispersal is well-documented in *P. xylostella* and knowledge of its seasonal movements has progressed in recent years (Dosdall et al. 2011; Furlong et al. 2013). We know that moths have little propensity to migrate when host plants, such as *Brassica* vegetable crops, are readily available (Mo et al. 2003). Long-range migration occurs regularly in the Northern Hemisphere, and the species frequently re-colonises higher-latitude regions where winter cold extremes prevent permanent establishment (Chu 1986; Coulson et al. 2002; Dosdall et al. 2004; Furlong et al. 2013; Fu et al. 2014). Migration also occurs in the Southern Hemisphere, though there are few records (Talekar and Shelton 1993; Endersby et al. 2006; Campos et al. 2006; Ridland and Endersby 2008; Shellhorn et al. 2008b). Genetic data from around the world are generally consistent with panmictic populations at intra-continental scales (Endersby et al. 2006; Wei et al. 2013).

Despite its importance, few studies have addressed *P. xylostella* dispersal directly. Mark-capture studies have identified trivial flights at localised scales (< 1km) within *Brassica* vegetable fields and surrounding vegetation (Shirai and Nakamura 1994; Mo et al. 2003; Schellhorn et al. 2008a). Evidence for large-scale migration (> 100s of kms) is mostly circumstantial, but direct evidence is available from two radar studies in the UK (Chapman et al. 2002) and Finland (Leskinen et al. 2011). Some excellent work incorporated trapping networks with wind trajectory analysis to identify source regions of immigrant moths that seasonally infest canola crops on the Canadian Prairies (Dosdall et al. 2004; Hopkinson and Soroka 2010; Dosdall et al. 2011). Similar approaches, together with analysis of data from citizen science portals reporting *P. xylostella* observations in northern Europe, have been used to detect seasonal influxes of moths into the UK (Wainwright 2020). With these exceptions, few studies have addressed *P. xylostella* movement at intermediate

landscape and regional scales (*c.* > one to tens of kilometres or more), leading to a distinct knowledge gap regarding movement among cultivated crops and wild host plant resources in the landscape (Furlong et al. 2013). This probably reflects a lack of suitable methods for ecologists to investigate *P. xylostella* dispersal at this scale. Extrinsic marking techniques are limited to small scales and the insect is too small to carry radio-tracking devices, whilst neutral genetic markers do not differentiate *P. xylostella* populations within regions. Dispersal has received surprisingly little attention at the eight international workshops on management *P. xylostella* held since 1985, comprising only 13 of the total 371 (3.5 %) of papers published in the proceedings (Table 1). This likely reflects the challenges involved (e.g. Nathan 2001; Lushai and Loxdale 2004).

Here, we review a series of recent studies investigating the colonisation of canola crops by *P. xylostella* at a regional scale in southern Australia (Perry 2019). Canola is grown over an area of approximately 3 million hectares per annum in winter rainfall areas of temperate southern Australia. Vast areas of canola are seasonally colonised by *P. xylostella* leading to intermittent outbreaks, but the sources of colonising insects have been uncertain (Furlong et al. 2008). Movement of *P. xylostella* was investigated from multiple angles. First, extensive collections of immature *Plutella* species were analysed to identify the geographic distribution, host associations and genetic structure of a cryptic species, *Plutella australiana* (Landry and Hebert 2013). Second, population genetic studies assessed gene flow and genetic structure among *P. xylostella* populations from different geographic locations and brassicaceous hosts throughout the Australian cropping zone. Third, field sampling and modelling studies over three years investigated the colonisation of canola crops by *P. xylostella* at a regional scale in South Australia. We synthesise the results and provide perspectives on future directions for dispersal studies in this species.

CRYPTIC *PLUTELLA* SPECIES

Undetected cryptic diversity can confound the results of biological research and introduce complexities for pest management (e.g. Miller et al. 2013; Vyskocilova et al. 2019). The reporting of a cryptic *Plutella* species in Australia, *P. australiana* (Landry and Hebert 2013), took diamondback moth researchers by surprise given it was not detected during earlier molecular studies (Endersby et al. 2006; Saw et al. 2006; Delgado and Cook 2009). The absence of information on the biology and ecology of *P. australiana* raised questions about the relative pest status and management implications of the two *Plutella* species in Australian *Brassica* crops.

In a comprehensive study, sympatric Australian populations of *P. xylostella* and *P. australiana* were compared using analysis of multiple complementary datasets from genomics, *Wolbachia* infection screening, inter-species crossing experiments and insecticide bioassays (Perry et al. 2018a). The distribution and host associations of *Plutella* species were investigated through extensive collections of immature *Plutella* from cultivated *Brassica* crops (canola, *Brassica* vegetables, *Brassica* forage crops) and wild brassicaceous species throughout cropping zones of southern Australia in 2014 and 2015. Nearly 1500 individuals from 75 sampled locations were subject to molecular species identification

Table 1. Papers published in the proceedings of international diamondback moth workshops addressing aspects of dispersal.

International Workshop	No. dispersal papers (No. papers)	References (Study aim)
1st, Taiwan 1985	1 (40)	Chu 1986 (Migration)
2nd, Taiwan 1990	1 (62)	Honda 1990 (Migration)
3rd, Malaysia 1996	0 (63)	
4th, Australia 2001	5 (59)	Pichon et al. 2004 (Population variation) Butcher et al. 2004 (Genetic markers) Dosdall et al. 2004 (Migration) Shelton et al. 2004 (Human transport) Mo et al. 2004 (Local dispersal)
5th, China 2006	3 (38)	Zalucki and Furlong 2008 (CLIMEX modelling) Endersby 2008 (Population genetic structure) Ridland and Endersby 2008 (Seasonal phenology)
6th, Thailand 2011	1 (51)	Zalucki and Furlong 2011 (CLIMEX modelling)
7th, India 2015	1 (34)	Perry et al. 2017 (Population genetic structure)
8th, Taiwan 2019	1 (24)	Perry et al. this paper (Regional movement)

using an RFLP-PCR assay of COI (Perry et al. 2017; Perry et. al 2018a), then screened for *Wolbachia* infection to assess the potential effects of these symbionts on mtDNA diversity and reproductive barriers.

Plutella australiana was shown to be widely distributed in Australia but unlikely to pose a significant threat to *Brassica* crops. Immature *P. australiana* were found in approximately one-third of canola crops (n = 39) but absent from *Brassica* vegetable farms (n = 16), and the overall frequency of *P. australiana* among Australia-wide *Plutella* collections was 9–fold lower than *P. xylostella* (Perry et al. 2018a). Insecticide bioassays using synthetic pyrethroids (Group 3A), emamectin benzoate (Group 6), spinetoram (Group 5) and chlorantraniliprole (Group 28) all demonstrated high-level control of *P. australiana*. There was no evidence that this species has evolved any insecticide resistance. These data suggested that current pest management tactics targeting *P. xylostella* do not need to account for the possible presence of *P. australiana*.

The findings across analyses of different experimental datasets provided a coherent hypothesis about how divergence between the two *Plutella* species arose. Although the two species hybridized at low frequency in laboratory crosses, striking contrasts in genomic structure, *Wolbachia* infection frequencies, and phenotypes (insecticide response, host plant use) implied that the two species have come into secondary contact following allopatric divergence (Feder et al. 2013). *Wolbachia* infection was fixed in *P. australiana* but rare in *P. xylostella*, and the two species carried different *Wolbachia* strains (Perry et al. 2018a). The existence of sympatric cryptic *Plutella* species provides a model system for evolutionary biologists interested in hybridisation, genetic and behavioural mechanisms of reproductive isolation and interactions with endosymbionts. *Plutella australiana* can also provide a useful outgroup for molecular phylogenies. Further exploring apparent differences in host use between these *Plutella* species could provide insights into mechanisms of host adaptation in *P. xylostella*. This work identified some introduced brassicas utilised by *P. australiana* in cropping zones. A diversity of wild Brassicales species that occurs across vast non-cropping zones in Australia is likely to include additional host plant refuges for both *Plutella* species.

In future, molecular studies of Australian diamondback moth will require a molecular species identification step. The possible existence of cryptic *Plutella* elsewhere in the world has been implied and requires further investigation (e.g. Robinson and Sattler 2001; Juric et al. 2017). Resolving species identity will remain challenging for field-based ecological research, however researchers should strive to genotype a subset of samples for species identification wherever possible.

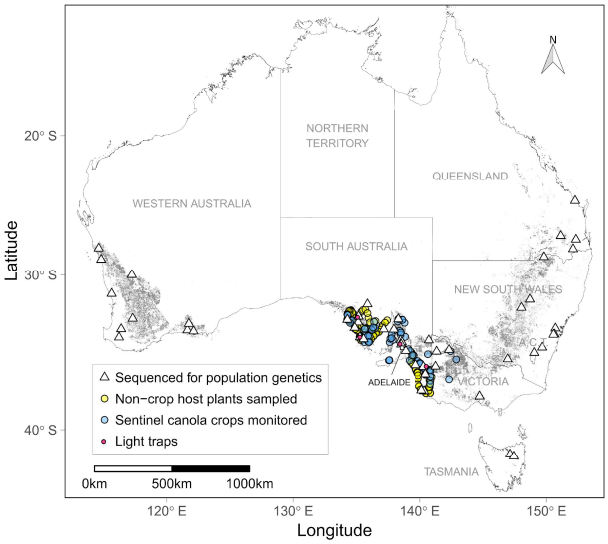


Figure 1. Australian locations sampled for *P. xylostella* between 2014 and 2016 for field and genetic studies. Grey shading denotes cropping areas.

POPULATION STRUCTURE AND GENE FLOW

Studies of population genetic structure can provide insights into patterns of gene flow (arising through successful dispersal of individuals) among geographically distinct insect populations (Roderick 1996). Selectively neutral genetic markers can be analysed to estimate the frequency of dispersal at evolutionary scales indirectly, through its effects on spatial variation in allele frequencies (Ouburg et al. 1999; Broquet and Petit 2009).

Using six microsatellite markers, Endersby et al. (2006) found no genetic differentiation among 17 populations within Australia, but these populations were clearly differentiated from populations from Asia and Africa. Low genetic diversity across nuclear and mtDNA genes suggested a recent genetic bottleneck possibly during its introduction to the Australasia region (Endersby et al. 2006; Saw et al. 2006; Delgado and Cook 2009; Juric et al. 2017; Perry et al. 2018a). Therefore, microsatellite data were unable to reveal current levels of gene flow among *P. xylostella* from different locations or *Brassica* host plants in Australia.

The advent of massively parallel sequencing platforms and associated novel genotyping methods facilitated higher genetic marker densities and unprecedented resolution for population genetic studies (Goodwin et al. 2016; Davey et al. 2011). We re-examined population structure in Australian *P. xylostella* using RAD-seq, a popular method for simultaneous discovery and genotyping of genome-wide SNP markers associated with restriction enzyme cut sites (Baird et al. 2008; Andrews et al. 2016). In earlier work, analysis using RAD-seq generated thousands of useful genome-wide markers in *P.*

xylostella (Perry et al. 2017) and demonstrated genomic divergence among cryptic *Plutella* species (Perry et al. 2018a). The ability to detect weak genetic differentiation among field and laboratory-reared *P. xylostella* colonies (Perry et al. 2017), even though it was the result of inbreeding, provided impetus for a thorough RAD-seq assessment of genetic structure among wild *P. xylostella* populations at an Australia-wide scale (Figure 1). We collected *P. xylostella* from canola crops, wild brassicaceous plants, *Brassica* forage crops and *Brassica* vegetable crops throughout southern Australia in two consecutive years (2014 and 2015). Despite a geographic sampling scale spanning >3000 kilometres, analysis across >1200 genome-wide SNPs confirmed that Australian *P. xylostella* formed a homogenous population across neutral loci, regardless of geographic location, brassicaceous host plant type or sampling year (Perry et al. 2020). These results supported the microsatellite study of Endersby et al. (2006) and given the statistical power of the marker set used, confidently resolved the nature of population structure (Perry et al. 2020). As pointed out by Endersby et al. (2006) and Furlong et al. (2008), neutral molecular markers appear to be uninformative for identifying *P. xylostella* movement in Australia.

These findings raise the questions, "how useful have genetic markers been in resolving *P. xylostella* movement generally, and what directions should be taken in future?" Of the surprisingly few population genetic studies in this species, earlier studies using various genetic markers (reviewed by Endersby et al. 2008), and recent studies using microsatellites and mtDNA (Wei et al. 2013; Yang et al. 2015) and genome-wide SNPs (Perry et al. 2017; Perry et al. 2018a; Perry 2019 pp. 62–81) found little genetic differentiation among *P. xylostella* populations at a regional level, suggesting that gene flow at this scale is common. One microsatellite study reported genetic differentiation among populations from south-eastern China and Taiwan (Ke et al. 2014), but this appears to be an anomalous result given larger-scale studies in the same region showed no population structure across microsatellite loci (Wei et al. 2013; Yang et al. 2015). What can we conclude from a lack of population structure? Even very few migrants per generation can eliminate strong differentiation among populations, particularly where genetic diversity is low, such as in *P. xylostella* in Australia (i.e. genetic drift occurs slowly) (Slatkin 1985; Mills and Allendorf 1996). Even rare long-distance dispersal events through migratory flight (e.g. Chapman et al. 2002) or movement of infested host plant material (Shelton 2004), may be sufficient to eliminate genetic signatures of isolation and drift. The problem remains that spatially uniform allele frequencies cannot reveal dispersal patterns. Data from RAD-seq or other next-generation sequencing methods have potential to detect population structure with greater resolution than microsatellites (Rasic et al. 2015; Vendrami et al. 2017). Whilst RAD-seq was uninformative in Australia, whether similar methods could provide new insights into *P. xylostella* population structure in other countries where the species has higher genetic diversity warrants further assessment.

The use of mtDNA markers has proven more informative for understanding *P. xylostella* movement generally. Mitochondrial DNA evolves more rapidly than nuclear DNA, leading to greater variation, and lack of recombination (maternal inheritance) makes it possible to infer the geographic origins and spread of particular haplotypes (Moritz et al. 1987; Avise 2004). By examining the geographic distribution of *P. xylostella* mtDNA haplotypes across China, two important studies by Wei et al. (2013) and Yang et al. (2015) provided strong evidence that seasonal populations in northern China were migrants from its overwintering range in southern China. Both studies also analysed microsatellite loci, and a lack of differentiation across these markers provided supporting evidence that mtDNA patterns reflected gene flow and not purely other processes, such as local selection. At a smaller scale, Niu et al. (2014), using mtDNA, found that mountain ranges may provide a geographic barrier to gene flow. In Australia, mtDNA haplotype diversity in *P. xylostella* was too low to identify spatial patterns (Saw et al. 2006; Delgado and Cook 2009; Perry et. al 2018a) but the mtDNA data revealed strong evidence for a founder effect and some localised genetic isolation not detected by microsatellites (Saw et al. 2006).

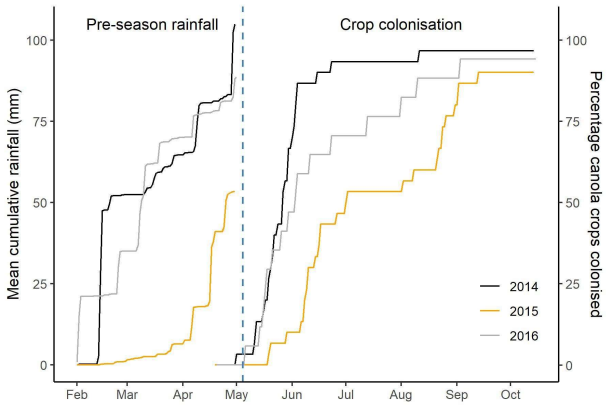


Figure 2. Three-year comparison of cumulative pre-cropping season rainfall averaged across sampling sites (*n* = 181) and subsequent pattern of crop colonisation across a network of sentinel canola crops in South Australia (*n* ≈ 30 crops per year).

These successes indicate that mtDNA markers provide good prospects for detecting large-scale movements of *P. xylostella* where field sampling spans the putative dispersal range of the species. It is known that mtDNA markers can be subject to a range of selective forces, such as interactions with endosymbionts, which can drastically alter patterns of mtDNA diversity and confound mtDNA-based inferences of population structure (Hurst and Jiggins 2005; Delgado and Cook 2009; Perry et al. 2018a). Because the sole use of mtDNA markers can be unreliable, mtDNA-based studies should include corroborating

evidence from independent nuclear markers. Population genetic studies should be carefully designed (see Meirmans 2015), including field sampling at a representative geographic scale, sampling within specific temporal windows to minimise biases caused by migration, and reporting of statistical power using simulation programs such as POWSIM (Ryman and Palm 2006). Overall, evidence that *P. xylostella* displays limited variation across genetic markers suggests that finer-scale information about seasonal movement between crop and non-crop host plants needs to be gathered using other methods.

THE SEASONAL COLONISATION PATTERN IN CANOLA CROPS

We investigated the colonisation of canola crops in South Australia at a regional scale over three years between 2014 and 2016 (Perry 2019, pages 83–106), using multiple approaches. Annually, non-crop brassicaceous plants growing in canola growing areas were sampled for *P. xylostella* by pheromone trapping for moths and sampling plants for immatures during the autumn pre-cropping period. Crop colonisation was then measured across a regional network of sentinel canola crops by experienced field consultants who were engaged as collaborators. Each consultant monitored 1-2 crops weekly until the first detection of immature *Plutella*, indicating establishment of a local breeding population ('colonisation'). We derived a temperature-dependent development model for *P. xylostella* and used it with local temperature data to back-predict initial oviposition in each crop, using the first detection date and developmental life stage as bio-fixes for modelling (R package dbmdev; Perry 2018b). A network of four light traps recorded nightly flight dynamics of male and female moths. Additionally, we used a CLIMEX model for *P. xylostella* (Zalucki and Furlong 2008, 2011; parameters from Li et al. 2012) with seasonal climate observations to predict temporal changes in the distribution and abundance of *P. xylostella* at weekly intervals during the study.

The observational and modelling datasets elucidated the seasonal invasion of canola by *P. xylostella* in South Australia (Perry 2019, pages 83–106). Wild brassicaceous plants, particularly sand rocket, *Diplotaxis tenuifolia*, wall rocket, *D. muralis*, and sea rocket, *Cakile maritima*, supported a low but widespread *P. xylostella* population in canola-growing areas during the pre-cropping season period. These populations had identical insecticide resistance profiles to populations collected from canola crops (Baker 2015). Each year, the majority of canola crops were colonised by *P. xylostella* in May and June soon after germination and nearly all crops (93.5 %) were colonised by early spring (September) (Figure 2).

There were striking differences between years in the pre-season abundance of wild hosts and *P. xylostella*, driven by rainfall patterns (Figure 2). In 2014 and to a lesser extent 2016, substantial rains during February to April promoted the growth of wild host plants, which supported a relatively high incidence of *P. xylostella* across sampled locations (Perry 2019). By contrast, in the pre-season period of 2015, a lack of rainfall led to a scarcity of brassicaceous plants and a very low incidence of *P. xylostella*.

Across years, crop colonisation patterns reflected the pattern of pre-season rainfall (Figure 2). Colonisation of canola by *P. xylostella* occurred earlier on average in 2014 and 2016 when alternative hosts were relatively abundant than in 2015 when such hosts were scarce. These results suggested that greater pre-season rainfall and the associated vegetation response increased the magnitude of the *P. xylostella* invasion of canola. Notably, links between *P. xylostella* abundance and seasonal weather only became apparent across the three-year dataset, highlighting the importance of multi-year datasets in ecological studies.

A key question was where the colonising moths originated. The field sampling and CLIMEX modelling suggested that immigrants came primarily from the surrounding landscape (in the order of $\leq$ tens of km) rather than from long distances away. Most of Australia is climatically unsuitable for the permanent persistence of *P. xylostella*, as shown by vast areas with an Ecoclimatic Index (EI) near zero (Figure 3; for an explanation of CLIMEX, see Zalucki and Furlong 2005).

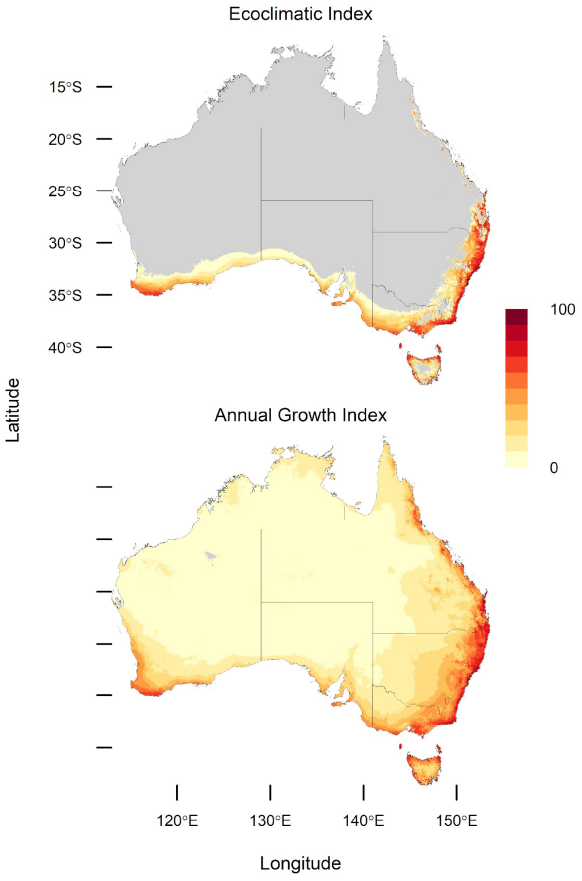


Figure 3. Climate suitability for *P. xylostella* between 2014 and 2016 predicted using a CLIMEX model (parameters from Li et al. 2012) scaled from 0 (grey, unsuitable) to 1 (extremely suitable). The Ecoclimatic Index (upper) shows areas suitable for year-round persistence, accounting for growth and stress indices, and the Annual Growth Index (lower) shows areas suitable for population growth during favourable seasons.

Areas further inland become more suitable for population growth at certain times of the year, as shown by a positive Annual Growth Index. Examining the Weekly Growth Index shows how the distribution and abundance of the insect is expected to change over a season according to weather conditions. Between 2014 and 2016, cropping areas became suitable for *P. xylostella* during the winter cropping season, but became far less suitable during the non-cropping season (Figures 1, 4), when typically hot and dry summer conditions limit *P. xylostella* survival (Furlong et al. 2008). During the non-cropping period, coastal areas proximate to cropping zones were most likely to harbour *P. xylostella* (Figures 3, 4). This suggests source insects that colonised canola were derived from locally over-summering *P. xylostella*. This was supported by light trapping data (Perry 2019) and by good agreement between pre-season weather and *P. xylostella* abundance, and the subsequent crop colonisation patterns. Furthermore, insecticide resistance in *P. xylostella* from unsprayed weedy areas (Baker 2015) demonstrates frequent movement between crops and weeds, and that insecticide resistance alleles can persist locally (Talekar and Shelton 1993).

There were two reasons this work focused on the colonisation process. First, the arrival timing of *P. xylostella* determines the potential for local population increase within canola crops (Dosdall et al. 2011). Second, distinguishing immigrants from locally derived individuals becomes difficult once a local breeding population has established. This problem does not arise at colonisation, because individuals arriving in a newly planted crop must be immigrants from alternative natal hosts. Identifying when immigrants arrived provided a unique chance to infer their origins.

Engaging widely with a network of field agronomists enabled collection of population data at a regional scale. The finding that most crops were consistently colonised by *P. xylostella* soon after crop emergence showed that local population increase can contribute substantially to spring population sizes in canola. The extent to which dispersal from elsewhere supplements spring populations (e.g. Hatami 1996; Ridland and Endersby 2008) remains an open question. Vast areas of brassicaceous plants occur in non-cropping areas during late winter and *P. xylostella* dynamics on these species requires further investigation (Perry et al. 2018a).

SYNTHESIS AND FUTURE DIRECTIONS

Several reviews have highlighted early forecasting of *P. xylostella* populations as a key goal for integrated management (Furlong et al. 2008; Furlong et al. 2013; Li et al. 2016). Achieving this will require greater knowledge of dispersal at local, landscape, regional and continental scales and more "thinking beyond the crop boundary" (Schellhorn 2017; Gurr et al. 2018). The limited number of studies directly addressing *P. xylostella* dispersal reflects the challenge of gathering and interpreting data at scales representing the considerable movement range of the species. Understanding *P. xylostella* seasonal movements among host plant resources in the landscape remains a key knowledge gap (Furlong et al. 2013), and we suggest this area warrants a renewed research focus. The Australian work described here provides a case study for working at this scale, along with the migration research in Canada (Hopkinson and Soroka 2010; Dosdall et al. 2011). More case studies are needed in other regions.

In offering perspectives on future directions for dispersal research in *P. xylostella*, we make several suggestions. First, ambitious studies should be encouraged, including integrating multiple datasets and sampling over wide geographic areas and across geopolitical borders. This will involve more collaboration and coordination across organisations and jurisdictions.

Genetic tools are valuable for identifying *P. xylostella* population structure and gene flow at larger regional and continental scales. Genetic studies should be carefully designed (see Meirmans 2015) and strive for: (a) field sampling across geographic areas spanning the putative dispersal range of the species, and within specific temporal windows to minimise the possibility for migration to affect results (Broquet and Petit 2009); (b) confirmation of species identity in regions where cryptic *Plutella* species are known or suspected, or anomalous results found; (c) analysis of field-collected (F_0) individuals to avoid laboratory inbreeding (Perry et al. 2017); (d) selection of appropriate genetic markers to address dispersal questions at the scale of interest, ideally including analysis of multiple independent markers (Ouborg et at. 1999); (e) considering the possible influence of endosymbionts on mtDNA sequence variation (Delgado and Cook 2009; Perry et al. 2018a); (f) reporting of statistical power. In future, intrinsic biogeographic markers may offer an approach for testing hypotheses about *P. xylostella* movement at larger geographic scales (see Holder et al. 2014; Hobson et al. 2018).

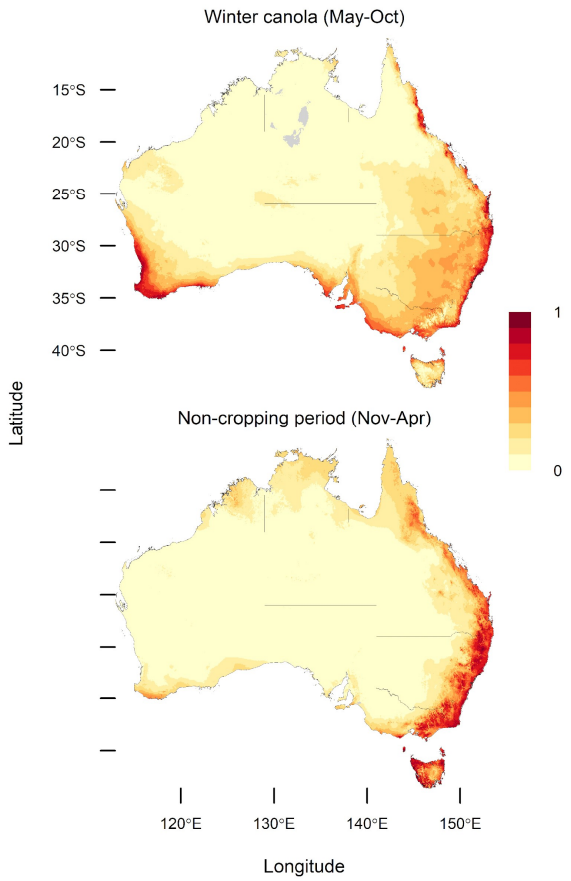


Figure 4. The CLIMEX Weekly Growth index for *P. xylostella* averaged across the canola growing season (upper) and the non-cropping season (lower) between 2014 and 2016. Cropping areas (see Figure 1) become more suitable for *P. xylostella* during the cropping season and less suitable during the summer and autumn non-cropping period.

Field studies should aim to capture population datasets over wide areas to reveal insights into *P. xylostella* spatial dynamics (see Ayalew et al. 2008), including a greater focus on non-cropping vegetation. Sampling networks operated through coordination with collaborators can greatly increase the geographic scope and quantity of data available for study (e.g. Taylor 1986; Sivakoff et al. 2013). Advances in automated pest trapping technologies means that deploying large spatial networks of moth traps is increasingly becoming possible. Similarly, a rapid evolution in digital communication technologies offers greater prospects for pest researchers to harvest routine crop monitoring data collected by others. Population data could be coupled with climate observations to determine relationships.

The CLIMEX modelling software (version 4) now allows researchers to explore the effects of climate on spatio-temporal *P. xylostella* dynamics visualised as weekly map sequences (Kriticos et al. 2015). This approach may be useful for identifying potential source areas for sampling at key times (e.g. Perry 2019, pages 92–95), particularly when coupled with information on the distribution and phenology of host plants, and analysis of wind trajectories (Hopkinson and Soroka 2010).

Acknowledgements

We thank the many agronomists and colleagues in the Australian grains industry who collected biological material, monitored sentinel canola crops and maintained light traps.

References

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics* 17(2):81–92.

Avise JC. 2004. *Molecular markers, natural history and evolution* (2nd edn). Sinauer Associates.

Ayalew G, Sciarretta A, Baumgartner J, Ogol C, Lohr B. 2008. Spatial distribution of diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), at the field and the regional level in Ethiopia. *International Journal of Pest Management* 54(1):31–38.

Baird NA, Etter PD, Atwood, TS, Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE* 3(10).

Baker GJ. 2015. Improving management of diamondback moth in Australian canola: Final report for GRDC, DAS00134. Technical report. South Australian Research and Development Institute.

Broquet T, Petit EJ. 2009. Molecular estimation of dispersal for ecology and population genetics. *Annual Review of Ecology Evolution and Systematics* 40:193–216.

Campos WG, Schoereder JH, DeSouza OF. 2006. Seasonality in neotropical populations of *Plutella xylostella* (Lepidoptera): Resource availability and migration. *Population Ecology* 48(2):151–158.

Chapman JW, Reynolds DR, Smith AD, Riley JR, Pedgley DE, Woiwod IP. 2002. High-altitude migration of the diamondback moth *Plutella xylostella* to the UK: A study using radar, aerial

netting, and ground trapping. *Ecological Entomology*, 27(6):641–650.

Chu YI. 1986. The migration of diamondback moth. In: Talekar NS and Griggs TD (ed.) 1986. *The management of diamondback moth and other crucifer pests: Proceedings of the first international workshop*, 11-15 March 1985, Tainan, Taiwan. The Asian Vegetable Research and Development Center, Shinhau, Taiwan, pp. 77–82.

Coulson S, Hodkinson I, Webb N, Mikkola K, Harrison J, Pedgley D. 2002. Aerial colonization of high Arctic islands by invertebrates: The diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) as a potential indicator species. *Diversity and Distributions* 8(6):327–334.

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics* 12:499–510.

Delgado AM, Cook JM. 2009. Effects of a sex-ratio distorting endosymbiont on mtDNA variation in a global insect pest. *BMC Evolutionary Biology* 9:49.

Dosdall LM, Mason PG, Olfert O, Kaminski L, Keddie BA. 2004. The origins of infestations of diamondback moth, *Plutella xylostella* (L.), in canola in Western Canada. In: Endersby NM and Ridland PM (ed.) 2004. *Management of diamondback moth and other crucifer pests*: *Proceedings of the fourth international workshop*, 26-29 November 2001, Melbourne, Australia. The Regional Institute Ltd., New South Wales, Australia, pp. 95–100.

Dosdall LM, Soroka JJ, Olfert O. 2011. The diamondback moth in canola and mustard: Current pest status and future prospects. *Prairie Soils Crops Journal* 4:66–76.

Endersby NM, McKechnie SW, Ridland PM, Weeks AR. 2006. Microsatellites reveal a lack of structure in Australian populations of the diamondback moth, *Plutella xylostella* (L.). *Molecular Ecology* 15(1):107–118.

Endersby NM. 2008. Population structure and gene flow in diamondback moth in Australia and around the world: Current state of knowledge and directions for the future. In: Shelton AM, Collins HL, Zhang Y and Wu Q (ed.) 2008. *The management of diamondback moth and other crucifer pests: Proceedings of the fifth international workshop*, 21-24 October 2006, Beijing, China. China Agricultural Science and Technology Press, Beijing, China, pp 132–147.

Feder JL, Flaxman SM, Egan SP, Comeault AA, Nosil P. 2013. Geographic mode of speciation and genomic divergence. *Annual Review of Ecology, Evolution, and Systematics* 44:73–97.

Furlong MJ, Spafford H, Ridland PM, Endersby NM, Edwards OR, Baker GJ, Keller MA, Paull CA. 2008. Ecology of diamondback moth in Australian canola: Landscape perspectives and the implications for management. *Australian Journal of Experimental Agriculture* 48(12):1494–1505.

Furlong MJ, Wright DJ, Dosdall LM. 2013. Diamondback moth ecology and management: Problems, progress, and prospects. *Annual Review of Entomology* 58:517–541.

Fu X, Xing Z, Liu Z, Ali A, Wu K. 2014. Migration of diamondback moth, *Plutella xylostella*, across the Bohai sea in northern China. *Crop Protection* 64:143–149.

Goodwin S, McPherson JD, McCombie WR. 2016. Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews Genetics* 17(6):333–351.

Gu H, Fitt GP, Baker GH. 2007. Invertebrate pests of canola and their management in Australia: A review. *Australian Journal of Entomology* 46(3):231–243.

Gurr GM, Reynolds OL, Johnson AC, Desneux N, Zalucki MP, Furlong MJ, Li Z, Akutse KS, Chen J, Gao X, You M. 2018. Landscape ecology and expanding range of biocontrol agent taxa enhance prospects for diamondback moth management. A review. *Agronomy for Sustainable Development* 38(3):23.

Hatami B. 1996. Seasonal occurrence and abundance of diamondback moth, *Plutella xylostella* (L.), and its major parasitoids on brassicaceous plants in South Australia. PhD thesis. The University of Adelaide, October 1996.

Hobson KA, Doward K, Kardynal KJ, McNeil JN. 2018. Inferring origins of migrating insects using isoscapes: A case study using the true armyworm, *Mythimna unipuncta*, in North America. *Ecological Entomology* 43(3):332–341.

Holder PW, Armstrong K, Van Hale R, Millet MA, Frew R, Clough TJ, Baker JA. 2014. Isotopes and trace elements as natal origin markers of *Helicoverpa armigera* - an experimental model for biosecurity pests. *PLoS ONE* 9(3):e92384.

Hopkinson RF, Soroka JJ. 2010. Air trajectory model applied to an in-depth diagnosis of potential diamondback moth infestations on the Canadian Prairies. *Agricultural and Forest Meteorology* 150(1):1–11.

Hurst GDD, Jiggins FM. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: The effects of inherited symbionts. *Proceedings of the Royal Society B-Biological Sciences* 272(1572):1525–1534.

Juric I, Salzburger W, Balmer O. 2017. Spread and global population structure of the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) and its larval parasitoids *Diadegma semiclausum* and *Diadegma fenestrale* (Hymenoptera: Ichneumonidae) based on mtDNA. *Bulletin of Entomological Research* 107(2):155–164.

Ke F, You S, He W, Liu T, Vasseur L, Douglas CJ, You M. 2015. Genetic differentiation of the regional *Plutella xylostella* populations across the Taiwan Strait based on identification of microsatellite markers. *Ecology and Evolution* 5(24):5880–5891.

Kriticos DJ, Maywald GF, Yonow T, Zurcher EJ, Herrmann NI, Sutherst RW. 2015. CLIMEX version 4: Exploring the effects of climate on plants, animals and diseases. CSIRO, Canberra, Australia, 156 pp.

Landry JF, Hebert PDN. 2013. *Plutella australiana* (Lepidoptera, Plutellidae), an overlooked diamondback moth revealed by DNA barcodes. *ZooKeys* 327:43–63.

Leskinen M, Markkula I, Koistinen J, Pylkko P, Ooperi S, Siljamo P, Ojanen H, Raiskio S, Tiilikkala K. 2011. Pest insect immigration warning by an atmospheric dispersion model, weather radars and traps. *Journal of Applied Entomology* 135(1-2):55–67.

Li Z, Zalucki MP, Bao H, Chen H, Hu Z, Zhang D, Lin Q, Yin F, Wang M, Feng X. 2012. Population dynamics and “outbreaks” of diamondback moth (Lepidoptera: Plutellidae) in Guangdong Province, China: Climate or failure of management? *Journal of Economic Entomology* 105(3):739–752.

Li Z, Feng X, Liu S-S, You M, Furlong MJ. 2016. Biology, ecology, and management of the diamondback moth in China. *Annual Review of Entomology* 61:277–296.

Lushai G, Loxdale HD. 2004. Tracking movement in small insect pests, with special reference to aphid populations. *International Journal of Pest Management* 50(4):307–315.

Meirmans PG. 2015. Seven common mistakes in population genetics and how to avoid them. *Molecular Ecology* 24(13):3223–3231.

Miller AD, Skoracka A, Navia D, de Mendonca RS, Szydło W, Schultz MB, Smith CM, Truol G, Hoffmann AA. 2013. Phylogenetic analyses reveal extensive cryptic speciation and host specialization in an economically important mite taxon. *Molecular Phylogenetics and Evolution* 66(3):928–940.

Mills LS, Allendorf FW. 1996. The one-migrant-per-generation rule in conservation and management. *Conservation Biology* 10(6):1509–1518.

Moritz C, Dowling TE, Brown WM. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics*, 18: 269–292.

Nathan R. 2001. The challenges of studying dispersal. Trends in Ecology & Evolution 16(9):481–483.

Niu YQ, Nansen C, Li XW, Liu TX. 2014. Geographical variation of *Plutella xylostella* (Lepidoptera: Plutellidae) populations revealed by mitochondrial COI gene in China. *Journal of Applied Entomology*, 138(9):692–700.

Ouborg NJ, Piquot Y, Van Groenendael JM. 1999. Population genetics, molecular markers and the study of dispersal in plants. *Journal of Ecology* 87(4):551–568.

Perry KD, Pederson SM, Baxter SW. 2017. Genome-wide SNP discovery in field and laboratory colonies of Australian *Plutella* species. In: Srinivasan R, Zalucki MP, Shelton AM, Kumar ARV and Chandrashekara K (ed.) 2017. *The management of diamondback moth and other crucifer pests: Proceedings of the seventh international workshop*. *Mysore Journal of Agricultural Sciences*, 51(A):18–31.

Perry KD, Baker GJ, Powis KJ, Kent JK, Ward CM, Baxter SW. 2018a. Cryptic *Plutella* species show deep divergence despite the capacity to hybridize. *BMC Evolutionary Biology*, 18:77.

Perry KD, Pederson SM, Keller MA. 2018b. https://github.com/kymperry01/dbmdev. Accessed November 2018.

Perry KD. 2019. The colonisation of canola crops by the diamondback moth, *Plutella xylostella* L., in southern Australia. PhD thesis. The University of Adelaide, May 2019.

Perry KD, Keller MA, Baxter SW. 2020. Genome-wide analysis of diamondback moth, *Plutella xylostella* L., from *Brassica* crops and wild host plants reveals no genetic structure in Australia. *Sci Rep* 10, 12047 (2020) https://doi.org/10.1038/s41598-020-68140-w.

Pichon A, Bordat D, Bordat A, Arvanitakis L, Tertois C. 2004. Biological and genetic differences between populations of diamondback moth from different geographic origins. In: Endersby NM and Ridland PM (ed.) 2004. *Management of diamondback moth and other crucifer pests*: *Proceedings of the fourth international workshop*, 26-29 November 2001, Melbourne, Australia. The Regional Institute Ltd., New South Wales, Australia, pp. 79–85.

Rasic G, Schama R, Powell R, Maciel-de Freitas R, Endersby-Harshman NM, Filipovic I, Sylvestre G, Maspero RC, Hoffmann AA. 2015. Contrasting genetic structure between mitochondrial and nuclear markers in the dengue fever mosquito from Rio de Janeiro: Implications for vector control. *Evolutionary Applications* 8(9):901–915.

Ridland PM, Endersby NM. 2008. Seasonal phenology of diamondback moth populations in southern Australia. In: Shelton AM, Collins HL, Zhang Y and Wu Q (ed.) 2008. *The management of diamondback moth and other crucifer pests: Proceedings of the fifth international workshop*, 21-24 October 2006, Beijing, China. China Agricultural Science and Technology Press, Beijing, China, pp. 90–101.

Robinson GS, Sattler K. 2001. *Plutella* in the Hawaiian Islands: Relatives and host-races of the diamondback moth (Lepidoptera: Plutellidae). *Bishop Museum Occasional Papers* 67:1–27.

Roderick GK. 1996. Geographic structure of insect populations: Gene flow, phylogeography, and their uses. *Annual Review of Entomology* 41:325–352.

Ryman N, Palm S. 2006. POWSIM: A computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes* 6(3):600–602.

Saw J, Endersby NM, McKechnie SW. 2006. Low mtDNA diversity among widespread Australian diamondback moth *Plutella xylostella* (L.) suggests isolation and a founder effect. *Insect Science* 13(5):365–373.

Schellhorn NA, Bellati J, Paull CA, Maratos L. 2008a. Parasitoid and moth movement from refuge to crop. *Basic and Applied Ecology* 9(6):691–700.

Schellhorn NA, Macfadyen S, Bianchi FJJA, Williams DG, Zalucki MP. 2008b. Managing ecosystem services in broadacre landscapes: What are the appropriate spatial scales? *Australian Journal of Experimental Agriculture*, 48(12):1549–1559.

Schellhorn NA. 2017. Landscape-level IPM for *Brassica* Pests: Patterns, Problems and Prospects. In: Srinivasan R, Zalucki MP, Shelton AM, Kumar ARV and Chandrashekara K (ed.) 2017. *The management of diamondback moth and other crucifer pests: Proceedings of the seventh international workshop*. *Mysore Journal of Agricultural Sciences*, 51(A):181–184.

Sivakoff FS, Rosenheim JA, Dutilleul P, Carriere Y. 2013. Influence of the surrounding landscape on crop colonization by a polyphagous insect pest. *Entomologia Experimentalis et Applicata* 149(1):11–21.

Shirai Y, Nakamura A. 1994. Dispersal movement of male adults of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae), on cruciferous vegetable fields, studied using the mark-recapture method. *Applied Entomology and Zoology* 29(3):339–348.

Shelton AM. 2004. Regional outbreaks of diamondback moth due to movement of contaminated plants and favourable climatic conditions. In: Endersby NM and Ridland PM (ed.) 2004. *Management of diamondback moth and other crucifer pests*: *Proceedings of the fourth international workshop*, 26-29 November 2001, Melbourne, Australia. The Regional Institute Ltd., New South Wales, Australia, pp. 101–106.

Slatkin M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16:393–430.

Talekar NS, Shelton A. 1993. Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology* 38:275–301.

Taylor LR. 1986. Synoptic dynamics, migration and the Rothamsted Insect Survey - Presidential Address to the British Ecological Society, December 1984. *Journal of Animal Ecology* 55(1):1–38.

Vendrami DLJ, Telesca L, Weigand H, Weiss M, Fawcett K, Lehman K, Clark MS, Leese F, McMinn C, Moore H, Hoffman JI. 2017. RAD sequencing resolves fine-scale population structure in a benthic invertebrate: Implications for understanding phenotypic plasticity. *Royal Society Open Science* 4(2):160548.

Vyskočilová S, Seal S, Colvin J. 2019. Relative polyphagy of "Mediterranean" cryptic *Bemisia tabaci* whitefly species and global pest status implications. *Journal of Pest Science* 92(3):1071–1088.

Wainwright C, Jenkins S, Wilson D, Elliott M, Jukes A, Collier R. 2020. Phenology of the Diamondback Moth (*Plutella xylostella*) in the UK and Provision of Decision Support for *Brassica* Growers. *Insects* 11(2):118. doi: 10.3390/insects11020118. PMID: 32054105; PMCID: PMC7074397.

Wei SJ, Shi BC, Gong YJ, Jin GH, Chen XX, Meng XF. 2013. Genetic structure and demographic history reveal migration of the diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) from the southern to northern regions of China. *PLoS ONE* 8(4):e59654.

Yang J, Tian L, Xu B, Xie W, Wang S, Zhang Y, Wang X, Wu Q. 2015. Insight into the migration routes of *Plutella xylostella* in China using mtCOI and ISSR markers. *PLoS ONE* 10(6):e0130905.

Zalucki MP, Furlong MJ. 2005. Forecasting *Helicoverpa* populations in Australia: A comparison of regression based models and a bioclimatic based modelling approach. *Insect Science* 12(1):45–56.

Zalucki MP, Furlong MJ. 2008. Predicting outbreaks of a migratory pest: An analysis of DBM distribution and abundance. In: Shelton AM, Collins HL, Zhang Y and Wu Q (ed.) 2008. *The management of diamondback moth and other crucifer pests: Proceedings of the fifth international workshop*, 21-24 October 2006, Beijing, China. China Agricultural Science and Technology Press, Beijing, China, pp. 122–131.

Zalucki MP, Furlong MJ. 2011. Predicting outbreaks of a migratory pest: An analysis of DBM distribution and abundance revisited. In: R. Srinivasan, Anthony M. Shelton, Hilda L. Collins (ed.) 2011. *The management of diamondback moth and other crucifer pests: Proceedings of the sixth international workshop*, 21-25 March 2011, Nakhon Pathom, Thailand AVRDC – The World Vegetable Center, Tainan, Taiwan, Publication No. 11755, pp. 8–14.

SESSION 3

Biological and Non-chemical Methods of Management of Crucifer Pests (Including Organic Agriculture)

Fungal entomopathogens to counter insecticide resistance in diamondback moth, *Plutella xylostella* (L.) in crucifer production systems

Ambethgar, V.
TAMIL NADU RICE RESEARCH INSTITUTE-TNAU, ADUTHURAI-612 101, TAMIL NADU, INDIA
email: drva1965@gmail.com

Azevedo, J.L.
UNIVERSITY OF SÃO PAULO, DEPARTAMENTO DE GENÉTICA (LGN), P.O.83, 3400-970, PIRACICABA, BRAZIL
email: jazevedo@carpa.ciagri.usp.br

Araujo, J.M.
FEDERAL UNIVERSITY OF PERNAMBUCO, RECIFE-CEP 50670-901,BRAZIL

Lima, G.M.S.
FEDERAL UNIVERSITY OF PERNAMBUCO, RECIFE-CEP 50670-901, BRAZIL
email: rosilma23@hotmail.com

ABSTRACT
The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is a most destructive pest of crucifer production systems throughout temperate and tropical climates. Recurrent infestations of DBM have led farmers to spray their crops with insecticides. But, insecticidal management of the DBM is becoming increasingly difficult due to the widespread development of insecticide resistance. Since the first report of DBM resistance to insecticide with DDT witnessed in 1953 in Indonesia, the pest had become resistant to many insecticides across multiple chemical classes including chlorinated hydrocarbons, carbamates, organophosphates and pyrethroids. Resistance to newer insecticide chemistries including spinosad, indoxacarb and emamectin benzoate has also been reported. Over-use of bacterial insecticides including *Bacillus thuringiensis kurstaki* (Btk) also lead to resistance. Insecticides resistance management (IRM) attempts to prevent or delay the development of resistance in target pests. Fungal entomopathogens are promising alternatives and supplements to conventional chemical insecticides for management of DBM in fields. Use of fungal entomopathogens in combination with sub-normal doses of chemical insecticide is suggested to counter insecticide resistance. Integration of promising strains of entomopathogenic fungi with selective insecticides have been reported to improve the control efficiency, besides decreasing the amount of chemical insecticides required, minimizing the risks of environmental contamination and delaying the evolution of insecticide resistance in insect pests. Co-application of any suitable promising formulation, based on *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin, or *Zoophthora radicans* (Brefeld) Batko with a suitable sub-normal concentration of a selective insecticide in a two-in-one tank mix, has been successfully employed to control insect pests in various crops. Such applications also mitigate the selection pressure and help avoid concurrent resistance risks in target pests. The resistance mechanisms in DBM are due to increased rate of detoxifying enzymes- especially hydrolases, transferases and oxygenases. Fungal entomopathogens can increase susceptibility to insecticides in DBM larvae by suppressing enzyme activities and predisposing them for fungal infection. This paper addresses the current state of knowledge on the exploitation of fungal entomopathogens as biological tools to counter insecticide resistance in DBM and enhance sustainable pest management in cruciferous vegetable systems.

Keywords:
Fungal entomopathogens, insecticide resistance, *Plutella xylostella* L., cruciferous vegetables production system

INTRODUCTION
The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is most widely distributed pest in all parts of the world wherever cruciferous crops are grown (Talekar and Shelton 1993). The pest is believed to be of European origin but is now found throughout temperate and tropical climates. It was first observed in North America in 1854 in Illinois, but had spread to Florida and the Rocky Mountains by 1883, and was reported from British Columbia by 1905. In Asia, *P. xylostella* has been reported since 1925 across crucifer production areas in varying intensity. It is regarded as an oligophagous pest and its host plants preference is entirely restricted to cruciferous crops including broccoli, brussels sprouts, cabbage, cauliflower, mustard, rutabaga etc., that contain glucosides (Talekar and Shelton 1993). Almost all these crops are susceptible to attack by DBM across crucifers growing areas. The pest has the ability to migrate and disperse over long distances. In the last four decades, DBM is posing a serious threat to crucifer production in Asia, although its occurrence has been widespread in many other countries, as well. The adults of DBM

overwinter in warmer areas and infest early season crops. The pest has four life stages: egg, larva, pupa and adult. Normally, DBM takes about 32 days to develop from egg to adult. However, the time to complete a generation may vary from 21 to 51 days depending on weather conditions and food availability (Talekar and Shelton 1993). The pest can complete several generations per growing season. In fields, its generations usually overlap and all four life stages may be present at the same time.

DAMAGE POTENTIAL

The damage is caused by the larvae, which feed on succulent leaves, buds, flowers, seed pods, the green outer layer of the stems and occasionally the developing seeds during the whole growing season at any stage of crop's development, but their numbers often increase towards the flowering phase (Talekar and Shelton 1993). The newly hatched first instar larvae feed on the lower leaf surface between the large veins and midribs, leaving the upper epidermis intact creating characteristic white traces due to removal of green tissues. The feeding damage by the mature larvae often causes prominent holes on the lower side of leaves with the upper surface intact which creates a "see through window effect" or "window-panning" effect. The extent of damage varies greatly depending on plant growth stage, larval densities and size. Severe infestations of DBM larvae can cause complete defoliation and substantial yield losses (Verkerk and Wright 1996; Ninsin et al. 2000). In markets, the mere presence of larvae with cosmetic damage in florets can result in complete rejection of produce, leading to colossal economic loss to the growers (Talekar and Shelton 1993). Outbreaks of DBM have occurred frequently in various parts of the world that resulted in severe losses even up to 90 % crop loss (Liu et al. 2002). In 2010, massive outbreak of DBM occurred on cauliflower in Udumalpet Region of Coimbatore District in Tamil Nadu, India (Ambethgar et al. 2011), causing farmers to plough down their field without any harvest.

MANAGEMENT OF DBM

Farmers prefer to use chemical pesticides for controlling pests because synthetic chemicals have an immediate knock-down effect, and are readily available in local markets. It is estimated that brassicas cultivation consumes at least 8-10% of all insecticides used globally. DBM management costs up to US$ 1 billion per year globally (Talekar and Shelton 1993). In India alone, DBM control measures cost about US$ 168 million per annum (Srinivasan et al. 2011), which constitute about 40% of the cost of production of major brassica crops in India. Many pesticides used in brassica production systems pose serious risks to producers, consumers, and the overall health of the environment. Short life-span, high reproductive capacity, overlapping generations and exposure to high selection pressure in the field have resulted in DBM evolving resistance to various types of insecticides. In many countries, chemical control is still the main way to manage DBM. The massive abuse of chemical insecticides resulted in the rapid development of insecticide resistance and eventual control failure of DBM. Therefore, economical production of crucifers has become difficult in certain areas (Lingappa et al. 2000). Crucifer pest management was particularly affected due to insecticide resistance, which was a consequence of excessive use of insecticides on the crop. Insecticide resistance rendered insecticides ineffective, thus increasing the need for repeated applications, waste of resources and consequent environmental contamination and health hazards (Regupathy 1996; Liu et al. 2002). Several efforts have been made worldwide to devise region specific integrated pest management (IPM) systems (Weinberger and Srinivasan 2009; Soleymanzade et al. 2019). However, poor efficacy of insecticides, due to insecticide resistance in DBM and performance inconsistencies of biopesticides and biological control, have made IPM unsustainable.

HISTORY OF INSECTICIDE RESISTANCE IN DBM

Resistance is a "genetic change in response to selection by toxicants (i.e., insecticides) that may impair control of pest insects in the field." Cruciferous crops have high commercial value and are often subjected to intense use of pesticides to ensure maximum profitability. Over the past several decades, cruciferous crops have come to rely on the various groups of conventional insecticides, such as chlorinated hydrocarbons, carbamates, organophosphates and pyrethroids (Kao and Cheng 2001; Miyata et al. 2011). Field populations of DBM have a long history of the evolution of resistance to sprayed insecticides in different countries. The development of insecticide resistance against a range of insecticides has been reported by a number of workers. The first report of DBM resistance to an insecticide was DDT in 1953 in Java, Indonesia (Talekar and Shelton 1993). From the end of World War II to 1980, DBM has become resistant to more than 36 insecticides across multiple chemical classes including chlorinated hydrocarbons, carbamates, organophosphates and pyrethroids (Miyata et al. 2011). The decade 1990-2000 was the most difficult phase for cruciferous pest management. Excessive use of insecticides, especially synthetic pyrethroids led to problems of insecticide resistance in DBM, which further necessitated the repeated application of insecticides (Kao and Cheng 2001). Resistance to

newer insecticide chemistries, including spinosad, indoxacarb and emamectin, benzoate has also been reported (Zhao et al. 2011). By 1990 resistance to abamectin, benzoylphenyl ureas, and various strains of the bacterial insecticide Bacillus thuringiensis have been reported in many parts of the world (Tabashnik et al. 1990; Bhattacharya et al. 2002). Overuse of *Bacillus thuringiensis kurstaki* (Btk) resulted in resistance in the field in Hawaii (Talekar and Shelton 1993; Grzywacz et al. 2010) despite this pesticide having multiple modes of action and preserving beneficial arthropods. To date, DBM has developed resistance to 81 insecticides and has become one of the most difficult pests to control in cruciferous crops globally (Liu et al. 2002). Due to insecticide resistance and eventual control failure of DBM, economical production of crucifers had become difficult in many crucifer production areas (Rahman et al. 2010; Soleymanzade et al. 2019).

INSECTICIDE RESISTANCE MECHANISM

Resistance develops at the population level, not within an individual, as it is an inherited trait. The mechanisms of resistance development within DBM populations are diverse (Ninsin et al. 2000), including acetylcholinesterase insensitivity, reduced penetration, nerve insensitivity and detoxification of insecticides. The presence of multiple mechanisms of resistance suggests that DBM populations become resistant to any class of insecticide given enough time and consistent selection pressure. Therefore any new insecticide chemistries face similar resistance selection problems beginning with their first use. However, the resistance evolution within a population of DBM is regulated via specific gene or combinations of genes (Li et al. 2006). From the perspectives of pest management, specific classes of insecticides are formulated to kill specific groups of target pests. However, toxicants are never totally effective because a percentage of the population may not be susceptible to them (Talekar and Shelton 1993). When the surviving population of DBM mates, individuals with these resistance traits will pass them to their offspring. Eventually, in the subsequent generations, the majority of DBM may become resistant, even when the offspring are continuously exposed to the same toxicant repeatedly over prolonged periods (Wang et al. 2010). The insecticide resistance evolution in DBM populations is illustrated in Figure1.

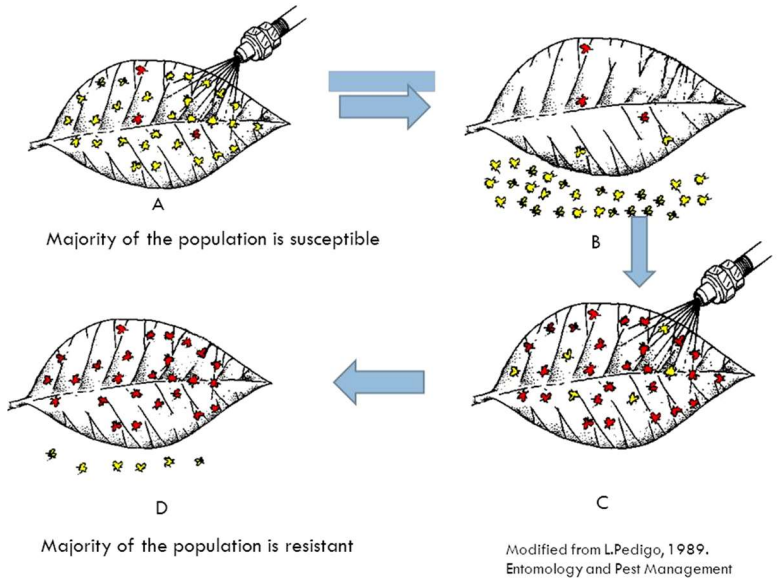


Figure 1. Illustration of insecticide resistance development in DBM population (modified)

Resistance occurs through a complex of mechanisms, especially via metabolic, physical, physiological, behavioral and biochemical mechanisms or their combinations (Talekar and Shelton 1993). It is also affected by the rate or dose, frequency of exposure to insecticides and behavioral characteristics of target pests and other contributing factors (Regupathy 1996; Baker and Kovaliski 1999). Resistance is an inherited ability of an individual insect to survive a concentration of insecticide that is lethal to other individuals that lack this gene (Miyata and Wu 2011). Usually, an insect inherits this resistance gene from its

predecessors. The short life-spans, high reproductive capacity, exposure of overlapping generations to a pesticide or sub-lethal doses of insecticides all contribute to the rapid resistance development in DBM (Talekar and Shelton 1993). With repeated use of insecticides, insects tend to develop resistance due to frequent exposures to toxicants (Miyata and Wu 2011). Thus any new insecticide chemistries may encounter similar resistance selection problems beginning with their first use.

Prior reports reveal that field populations of DBM evolve complex modes of resistance to different insecticides. The massive abuse of pesticides against DBM has resulted in development of cross-resistance and multiple-resistance to different chemical pesticides (Talekar and Shelton 1993; Baker and Kovaliski 1999). For example, resistance to organophosphate insecticides occurs through "esterase resistance" metabolic mechanism because of the overproduction of carboxylesterases sequestered to degrade insecticide esters before the toxicants reach the target sites of the nervous system (Baker and Kovaliski 1999). "Modified acetylcholinesterase (MACE)", involved in target-site mechanism confers immunity to the dimethyl-carbamate (Zhao et al. 2000). The knock-down target-site mechanisms "kdr and super-kdr" confer strong resistance against pyrethroid insecticides. Some insects adapt to 'class-resistance' which occurs in pest populations that develop resistance to any specific class of insecticides such as organophosphates (or) carbamates (or) pyrethroids. The mechanism of 'cross- resistance' occurs when resistance to one insecticide confers resistance to another insecticide, which is illustrated in Fig. 2. The DBM population with cross-resistance traits is hard to manage with even the most powerful insecticides (Zhou et al. 2011; Miyata and Wu 2011).

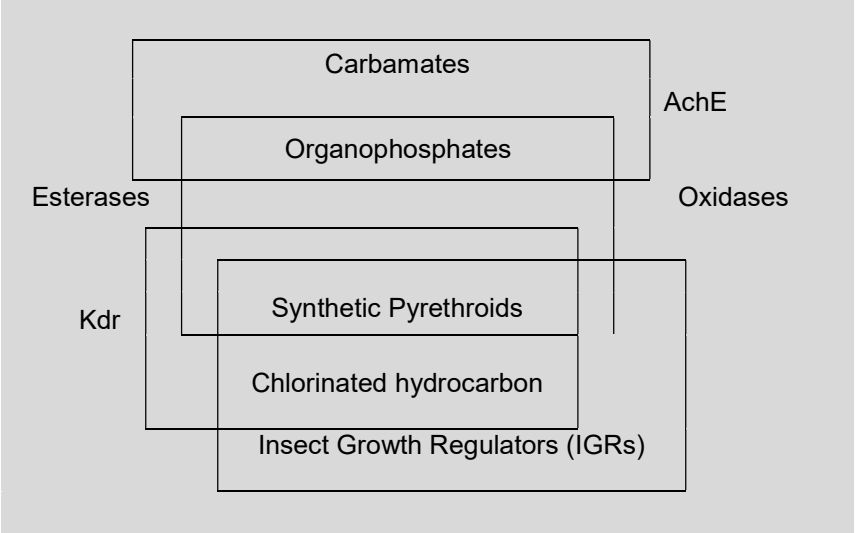


Figure 2. Cross-linking resistance mechanism with different classes of insecticides
IGRs-Insect growth regulators; Kdr-Knockdown resistance; AchE-Acetylcholinesterase
(Source: Ambethgar 2016)

DBM populations that have 'multiple resistance' have independent mechanisms leading to resistance to different chemical families (e.g., carbamates, organophosphates and pyrethroids). On the other hand, DBM populations that tend to 'tolerate' a particular insecticide possess specific physiological or behavioral adaptations for increased survivorship to specific baseline toxicity.

INSECTICIDE RESISTANCE MANAGEMENT IN DBM

Increased emphasis on the development of novel IRM strategies is suggested to obviate insecticide resistance problems. Many new ideas, facts and case studies have been developed over many years to contain DBM problems in crucifers with alternative measures (Zhou et al. 2011). Without proper insecticide resistance management, DBM will continue to overcome an insecticide when used as a solitary control tactic. Due to unsatisfactory insect control due to insecticide resistance, farmers spray repeatedly,

most often with mixtures of insecticides. In the 1990s, DBM resistance to insecticides emerged as a great challenge to DBM management in Asia and Australia. Subsequently, a number of IPM programs, based on a combination of cultural control methods, pheromones and calendar applications of biopesticides / bioagents interspersed with need-based application of insecticides, were initiated in all crucifers growing countries to ensure effective DBM management. However, due to the non-availability of good quality biopesticides and biological control organisms, coupled with sub-optimal efficacy under field conditions, crucifer cultivation came to depend on synthetic insecticides (Baker and Kovaliski 1999). Since insect resistance to insecticides had emerged as a major threat to pest control programs, IPM packages were refined to include Insecticide Resistance Management (IRM) as a major component. Clearly IPM was seen as a proactive method with emphasis on biopesticides and biological control interventions, whereas IRM is meant to overcome the existing 'resistance' crisis through specific strategies to ensure efficient pest control and mitigate the problem of resistance. Many international organizations all over the world provide support for new IRM initiative projects

FUNGAL ENTOMOPATHOGENS FOR IRM

Fungal entomopathogens are important biological control agents throughout the world, have been the subject of intensive research for more than 100 years, and can occur at epizootic or enzootic levels in their host populations (Selman et al. 1997). Use of fungal entomopathogens and their products as mycoinsecticides is an important component of IPM (Pell and Wilding 1992). Fungal entomopathogen in combination with reduced doses of selected insecticide is reported to produce synergistic effect on control of target pests in fields (Ambethgar 2009). Examples of fungal entomopathogens for control of DBM include *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin, and *Zoophthora radicans* (Brefeld) Batko (Wraight et al. 2003; Sarfraz et al. 2005; Ambethgar 2009). These fungi, in combination with selective insecticides at reduced doses, were reported to synergize overall control efficacy and reduced the development of resistance in DBM systems (Ambethgar et al. 2011; Soleymanzade et al. 2019).

MODE OF ACTION OF FUNGI IN INSECTS

Fungal entomopathogens infect insects by direct contact mechanism through the host cuticle (Agarwal 1990). The mode of action and infection cycle of any fungal entomopathogen in insects progresses through a series of metabolic events as illustrated in Figure 3. viz., (a) Spore attachment with the cuticle, (b) Germination of spores on the cuticle, (c) Reaction of cuticle-degrading enzymes, (d) Penetration of fungus into the cuticle, (e) Internal proliferation of blastospores in haemocoel, (f) Reaction of mycotoxins and host death, (g) External sporulation and dissemination of spores (Hajeck and Leger 1994).

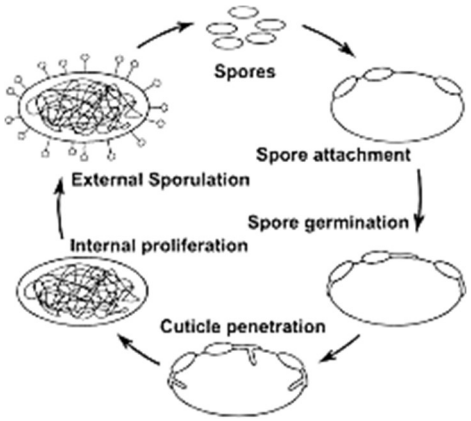


Figure 3. Imaginary view of fungal infection and metabolic events in host insect (Source: Wikipedia)

The infection process of fungi involve mechanical pressure followed by solubilization of cuticle with cuticle-degrading enzymes (Hajek and St. Leger 1994), which facilitates the entry of the fungus in to haemocoel, where the vegetative hyphae produce a complex of toxic metabolites and peptides. Usually, fungi such as *B. bassiana* and *M. anisopliae* release copious amounts of cuticle-lysing enzymes such as

chymoelastase or Pr1, esterases and chitinases to degrade the host cuticle, and the toxins released during fungal metamorphosis paralyze the host insect. At this stage, the infected insect becomes sluggish, reduces feeding efficiency and insensitive to external stimuli. Under conducive high humidity, the vegetative mycelia, proliferate inside the host by utilizing larval haemocoel as nutrients (Hiromori and Nishigaki 2001), destroy host cells, and finally cause death of the host insect. The entire infection cycle of fungal pathogen-induced mortality increases with the fungal infection age. The average time from conidial attachment to death of a host takes less than 10 days.

BENEFITS OF JOINT ACTION OF FUNGI AND INSECTICIDES IN IRM

The main objective of the simultaneous application of insecticide and a fungus agent is to realize synergistic action, in which the insecticide toxicant performs as stress inducer to the target insects, while the fungus act as the major control agent, which invade the host rapidly because of stress created by the toxicants (Anderson and Roberts 1983). Many candidate fungi in conjunction with insecticides discharge powerful enzymes to debilitate the physiological state of host insects and predispose the host insects to infection by inducing stress effects (Hiromori and Nishigaki 2001). Use of sub-lethal concentrations of insecticide tends to readily alter the immune reactions of insects by targeting the humoral defense mechanism, beside enhancing the efficacy of insecticides (Feng and Xiao 2005). The additive advantages of compatibility between pathogens and insecticides have been evaluated in numerous situations with a view to reduce resistance problems in arthropod pests (Anderson and Roberts 1983). Many of these experiments clearly proved that the insecticides compatible with fungal entomopathogens under controlled *in vitro* conditions also showed synergistic action under open field conditions (Furlon and Pell 1996).

Co-application of fungi with sub-lethal doses of selective insecticides forms an ideal alternative measure for insecticide resistance management (Hiromori and Nishigaki 2001). This strategy produces multiple advantages as it requires decreased doses of insecticides, delayed expression of resistance and produces lower residues on commodities (Feng and Xiao 2005). In addition, the pre-lethal effects of fungal infection, reduce feeding propensity, fecundity and crop damage. Sequential application of normal doses of insecticide and entomopathogenic fungi have been reported to prevent or delay insecticide resistance. Studies have also indicated that insecticides, especially at sub-lethal doses, often synergize the speed of fungal infection (Xu et al. 2002). The advantages of combining fungal entomopathogens and insecticides in a two-in-one tank mix method have been shown with DBM in cabbage production (Feng and Xiao 2005; Ambethgar et al. 2011). Initially, the insecticidal toxicant weakens the target pest sufficiently to make it more susceptible for infection by the pathogen.

CASE STUDIES WITH JOINT ACTION OF FUNGI AND INSECTICIDES

Knowledge of fungus-insect association helps to integrate fungal and insecticidal formulation against pestiferous insects in agriculture. In fields, fungal epizootics provide additional fitness cost for the insects. Fungal infection not only restricts the spread of resistance in insects, but also slows the speed of insecticide resistance development in the long run (Feng and Xiao 2005). Besides, combination treatment of selective fungi and insecticides also tends to reduce the expression of insecticide resistance and enhance the persistence of fungal propagules in treated fields (Furlong and Pell 1996). Many potential fungal entomopathogens including *B. bassiana* and *M. anisopliae* have been employed to overcome insecticide resistance in certain agriculturally important polyphagous crop pests (Gupta et al. 2002). Manipulation of technologies to enhance the virulence of fungal propagules can be used to improve the commercial effectiveness of fungal-based control methods. Many researchers have proved the synergistic interactions of several insecticides in sub-lethal doses with *B. bassiana* and *M. anisopliae* preparations in insect control (Feng and Xiao 2005).

Recently, Ambethgar et al. (2011) reported that aqueous suspension of *Z. radicans* together with imidacloprid 17.8 SL at a lower rate (50ml/ha as against its recommended rate of 350 ml/ha) could be incorporated into the DBM management system. *Zoophthora radicans* conidia in combinations with a low rate of imidacloprid were evaluated against DBM on cauliflower in Palladam and Coimbatore of Tamil Nadu state, India. More consistent DBM control was achieved at the high rate of *Z. radicans* + low rate of imidacloprid than the same rate of *Z. radicans* alone, suggesting a possible synergistic effect of the low imidacloprid rate with the pathogen. Several insecticides at sub-lethal concentrations were reported to be compatible with major taxa of fungal entomopathogens such as *B. bassiana*, *M. anisopliae*, *P. farinosus*, *P. fumosoroseus* and *V. lecanii* in different situations (Hiromori and Nishigaki 2001). Reports by Xu et al. (2002) indicated that for practical application, it is important to make use of positive interactions of low-residue insecticides with suitable fungal agents, and also possible to select such insecticides that are biologically compatible with fungi.

FACTORS INFLUENCING JOINT ACTION

Insecticides have a tendency to debilitate the physiological strength of insects, which hastens the infectivity of entomopathogens (Ambethgar 2009). The differential effects of pathogenicity under the exposure of pesticides shown under *in vitro* laboratory and *in vivo* field conditions are complex and influenced by interaction between biotic and abiotic factors. Prior research has indicated that insecticides applied in soil strongly impair the dynamics of fungal entomopathogens and other beneficial soil-microorganisms, which are epizootiologically important elements to establish infections in target pests (Furlong and Pell 1996). Besides the epizootiological factors, type of formulations, carrier materials, emulsifying agents, dosage, edaphological and physiological condition of host plants are reported to influence the joint action of entomofungi and insecticides (Feng and Xiao 2005). The complexities of biotic and abiotic factors are difficult to discriminate in fields. However, thorough laboratory and field experiments could predict the effects of pesticides on entomopathogenic fungi (Ambethgar et al. 2011). Prior investigations indicate that in IPM systems, selective insecticides which are innocuous to fungal formulations may be used in conjunction with them.

ECOLOGICAL IMPLICATIONS

Biodiversity in agro-ecosystems deliver significant ecosystem services to an array of native biological control agents, specifically the entomopathogenic fungi. The virulence of fungal infectivity largely depends on viability of conidia which determine epizootics of native organisms. Fungal propagules that invade non-host insects germinate poorly with aborted infection. Propagules produced from current season infection can survive longer either within the cadavers or in soil to infect later generations of pest populations. However, agro-chemicals applied to control crop pests and pathogens in the same habitat tend to synergize or antagonize towards the resident organisms depending on the prevailing ecological conditions. Feng and Xiao (2005) reported that the anamorphic fungi viz., *B. bassiana* and *M. anisopliae* were reported to tolerate diverse classes of crop protection chemicals and have tendency to persist within the host cadavers. Many researchers have clarified that fungal infectivity is seldom affected at sub-lethal concentrations of pesticides (Wraight et al. 2003). Therefore, pesticides that are harmful to the control *in vitro* environments do not necessarily exhibit the same reaction under open field conditions.

STRATEGIC APPROACHES

Mycopesticides have attained wider utility as an environment benign approach globally, because of their extensive use in diverse agro-ecosystem for the past three decades. Fungi were also exploited as a component of resistance management in the IPM system. Adoption of the following farm manipulative practices may help the farmers achieve effective implementation of insecticide resistance management programs.

- Conservation of naturally occurring myco-pathogens is needed in order to re-establish them in field environments in order to abate concurrent occurrence of resistance build-up in field populations of insect pests.
- Newer insecticides safer to beneficial microorganisms should be screened for potential viability and pathogenicity to target pests.
- Laboratory testing with confirmative field experiments are essential for manipulating compatibility between introduced fungi and insecticides.
- Genetic improvement of fungi entomopathogens for locating insecticide resistant/tolerant strains are needed to be researched to counter the insect resistance to insecticides.
- Biotechnological innovations like genetic engineering may be helpful for strain improvement of fungal entomopathogens for tolerance to exposure of agrochemicals.

Gene manipulations that render overproduction of a cuticle-degrading protease have also been shown to enhance rapid killing of host insects (Feng and Xiao 2005). Similar such studies with selected strains of fungal entomopathogens and insecticides would result in the development of mutant strains with improved virulence against major arthropod pests in order to augment their compatibility with other pest control options for sustainable insecticide resistance management program.

CONCLUSION

Synergism between an array of insecticides and fungal entomopathogens has been documented. Novel technology for joint action of reduced doses of insecticides with promising entomopathogens is suggested for insecticide resistance management. The potential of compatible insecticides that induce physiological fitness of fungal entomopathogens to cause infections in host insects should be identified.

Specific isolates of candidate fungi including *B. bassiana*, *M. anisopliae* and *Z. radicans*, both *in vitro* and *in vivo* environments, have been reported to cause synergistic action with many selected classes of insecticides. Significant reductions in DBM survival and insecticide resistance levels induced by fungal infection support the potential use of fungal entomopathogens and their products in areas where insecticide resistance levels in DBM populations are increasing, potentially adding new product options to the very limited selection of chemicals currently available. Entomopathogenic fungi have a relatively slow speed of kill, but can reduce resistance while killing larvae of DBM before being able to cause economic damage to crops. The susceptibility of insecticide-resistant DBM to fungal pathogens adds weight to the possibility of using mycopesticides within an insecticide resistance management strategy. This could include rotations or mosaics, to slow the spread of resistance. Low-doses of selective insecticides in combination with fungal entomopathogens not only serve to reduce or delay the selection pressure, but such combinations also induce high levels of mortality among resistant DBM populations and reduce the frequency of insecticide applications. While implementing the strategy, adequate care should be taken because insecticides at sub-lethal doses may sometimes lead to the resurgence of less important insect pests. Applied research should be strengthened to explore the practical implications for exploiting fungal entomopathogens as a component in the IRM of DBM in cruciferous vegetables production systems.

References

Agarwal GP. 1990. Entomogenous fungi in India and management of insect pests. Indian Phytopathology, 43 (2): 131-142.

Ambethgar V. 2009. Potential of entomopathogenic fungi in insecticide resistance management (IRM): A Review. Journal of Biopesticides, 2 (2): 177-193.

Ambethgar V, Swamiappan M, Rabindran. 2011. Field assessment of aqueous suspension of Zoophthora radicans conidia and low application rates of imidacloprid for control of diamondback moth Plutella xylostella on cauliflower in Tamil Nadu, India. In: Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC-The World Vegetable Center, P.O. Box 42 Shanhua, Tainan 74199, Taiwan, 182p.

Anderson TE, Roberts DW. 1983. Compatibility of Beauveria bassiana isolate with insecticide formulations used in Colorado potato beetle (Coleoptera: Chrysomelidae) control. Journal of Economic Entomology, 76: 1437-1441.

Baker GJ, Kovaliski J. 1999. Detection of insecticide resistance in Plutella xylostella (L.) (Lepidoptera: Plutellidae) populations in South Australian crucifer crops. Australian Journal of Entomology 38: 132-134.

Bhattacharya RC, Viswakarma N, Bhat SR, Kirti PB, Chopra VL. 2002. Development of insect-resistant transgenic cabbage plants expressing a synthetic crylA (b) gene from Bacillus thuringiensis. Current Science, 83(2): 146-150.

Feng MG, Xiao P. 2005. Time-concentration-mortality modeling of the synergistic interaction of Beauveria bassiana and imidacloprid against Nilaparvata lugens. Pest Management Science, 61: 363-370.

Furlong MJ, Pell JK. 1996. Interactions between the fungal entomopathogen Zoophthora radicans Brefeld (Entomophthorales) and two hymenopteran parasitoids attacking the diamondback moth, Plutella xylostella (L.). Journal of Invertebrate Pathology 68 (1): 15-21.

Grzywacz D, Rossbach A, Rauf A, Russell DA, Srinivasan R, Shelton AM. 2010 Current control methods for diamondback moth and other brassica insect pests and the prospects for improved management with lepidopteran-resistant Bt vegetable brassicas in Asia and Africa. Crop Protection 29: 68-79.

Gupta RBL, Sharma S, Yadava CPS. 2002. Compatibility of two entomofungi Metarhizium anisopliae and Beauveria bassiana with certain fungicides, insecticides and organic manures, Indian Journal of Entomology, 64: 48-52.

Hajeck AE, St. Leger R. 1994. Interactions between fungal pathogens and insect hosts. Annual Review of Entomology, 39: 293-322.

Hiromori H, Nishigaki J. 2001. Factor analysis of synergistic effect between the entomopathogenic fungus, Metarhizium anisopliae and synthetic insecticides. Applied Entomology and Zoology, 36 (2): 231-236.

Kao CH, Cheng EY. 2001. Insecticide resistance in Plutella xylostella L. XI. Resistance to newly introduced insecticides (1990~2001) in Taiwan. Journal of Agricultural Research, China, 50: 80-89.

Lingappa S, Basavanagoud K, Kulkarni KA, Roopa SP, Kambrekar DN. 2000. Threat to vegetable production by diamondback moth and its management strategies. IPM System in Agriculture: 235–248.

Li A, Yang Y, Wu S, Li C, Wu Y. 2006. Investigation of resistance mechanisms to fipronil in diamondback moth (Lepidoptera: Plutellidae). Journal of Economic Entomology 99: 914–919.

Liu SS, Chen FZ, Zalucki MP. 2002. Development and survival of the diamondback moth (Lepidoptera: Plutellidae) at constant and alternating temperatures. Environmental Entomology 31: 221-231.

Miyata T, Wu G. 2011. Management of insecticide resistance development in diamondback moth, Plutella xylostella (L.). In: Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC-The World Vegetable Center, P.O. Box 42 Shanhua, Tainan 74199, Taiwan, 248p

Ninsin KD, Mo, J, Miyata T. 2000. Decreased susceptibilities of four field populations of the diamondback moth, Plutella xylostella (L.) (Lepidoptera: Yponomeutidae), to acetamiprid. Applied Entomology and Zoology, 35: 591-595.

Pell JK, Wilding N. 1992. The survival of Zoophthora radians (Zygomycetes: Entomophthorales) isolates as hyphal bodies in mummified larvae of Plutella xylostella (Lepidoptera: Yponomeutidae). Entomophgaga 37: 649–954.

Rahman MM, Baker G, Powis KJ, Roush RT, Schmidt O. 2010. Induction and transmission of tolerance to a synthetic pesticide emamectin benzoate in field and laboratory populations of diamondback moth Plutella xylostella. Journal of Economic Entomology 103: 1347–1354

Regupathy A. 1996. Insecticide resistance in diamondback moth (DBM) Plutella xylostella(L.): Status and prospects for its management in India. Proceedings of Third International Workshop, Kuala Lumpur, pp. 233-239.

Sarfraz M, Keddie AB, Dosdall ML. 2005 Biological control of the diamondback moth, Plutella xylostella: a review 1. Biocontrol Science and Technology 15, 763-789.

Selman BJ, Dayer S, Hasan M. 1997. Pathogenicity of Beauveria bassiana (Bals.) Vuill. to the larvae of diamondback moth, Plutella xylostella L. (Lepidoptera: Yponomeutidae). Journal of Applied Entomology, 121, 47-49.

Soleymanzade A, Khorrami F, Forouzan M. 2019. Insecticide Toxicity, Synergism and Resistance in Plutella xylostella (Lepidoptera: Plutellidae). Acta Phytopathologica et Entomologica Hungarica, 54 (1)147-154.

Srinivasan R, Lin MY, Hsu YC. 2011. Effects of sub-lethal doses of Bacillus thuringiensis (Bt) δ-endotoxins against natural enemies of diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). In: Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC-The World Vegetable Center, P.O. Box 42 Shanhua, Tainan 74199, Taiwan, 188p.

Tabashnik BE, Cushing NL, Finson N, Johnson MW. 1990. Field development of resistance to Bacillus thuringiensis in diamondback Moth (Lepidoptera: Plutellidae), Journal of Economic Entomology 83: 1671-1676

Talekar NS, Shelton AM. 1993. Biology, ecology and management of the diamondback moth. Annual Review of Entomology, 38: 275-301

Verkerk RHJ, Wright DJ. 1996. Effects of host plant selective insecticide interactions on larvae of Plutella xylostella (Lepidoptera: Yponomeutidae) in the laboratory. Pesticide Science, 44: 171-181.

Wang X, Li X, Shen A, Wu Y. 2010. Baseline susceptibility of the diamondback moth (Lepidoptera: Plutellidae) to chlorantraniliprole in China Journal of Economic Entomology, 103 (3): 843-848.

Weinberger K, Srinivasan R. 2009. Farmers' management of cabbage and cauliflower pests in India and their approaches to crop protection. J.Asia-Pacific Entomology 12: 235-259.

Wraight SP, Galaini-Wraight S, Carruthers RI, Roberts DW. 2003. Zoophthora radicans (Zygomycetes: Entomophthorales) conidia production from naturally infected Empoasca kraemeri and dry-formulated mycelium under laboratory and field conditions. Biological Control, 28 (1): 60-77.

Xu ST, Ying SH, Feng MG. 2002. Biological compatibility of ten commercial pesticides with Beauveria bassiana conidia. Acta Phytophysiologica Sinica, 29: 158-162.

Zhou LJ, Huang JG, Xu HH. 2011. Monitoring resistance of field populations of diamondback moth Plutella xylostella L. (Lepidoptera: Yponomeutidae) to five insecticides in South China: A ten-year case study. Crop Protection, 30: 272-278.

Multi-virus-based biopesticide formulations for crucifer lepidopteran pests

Siti Noor Aishikin, A.H.
AGROBIODIVERSITY AND ENVIRONMENT RESEARCH CENTRE, MALAYSIAN AGRICULTURAL RESEARCH AND DEVELOPMENT INSTITUTE (MARDI), PERSIARAN MARDI-UPM, 43400 SERDANG SELANGOR, MALAYSIA
email: ctaikina@mardi.gov.my

Razali, M.
AGROBIODIVERSITY AND ENVIRONMENT RESEARCH CENTRE, MALAYSIAN AGRICULTURAL RESEARCH AND DEVELOPMENT INSTITUTE (MARDI), PERSIARAN MARDI-UPM, 43400 SERDANG SELANGOR, MALAYSIA
email: zaley@mardi.gov.my

ABSTRACT
Nuclear polyhedrosis virus (NPV) is known for its high epizootic levels, safety to natural enemies due to host specificity and environmental friendly. Previously, formulated *Spodoptera litura* NPV (FNPV) in powdered form was effective for control of *S. litura* larvae in laboratory and field conditions. However, FNPV was specific to *S. litura* whilst in the field condition there were other lepidopteran pest infestations. Therefore, FNPV was upgraded by adding several lepidopteran NPVs, a carrier and UV protectant. Then, the mixed solution was dried and sprayed. The efficacy test was conducted on *S. litura*, *Plutella xylostella*, *Hellula undalis* and *Crocidolomia pavonana* larvae in laboratory and field conditions. The efficacy test was conducted up to twelve months to determine the effectiveness of FMNPV when stored at room temperature. Results showed FMNPV was effective against targeted larvae in laboratory and field conditions.

Keywords
Nucleopolyhedrovirus, *Plutella xylostella, Spodoptera litura, Hellula undalis, Crocidolomia pavonana*

INTRODUCTION
Crucifers are important vegetables in Malaysia. The main crucifers planted in Malaysia are mustard, cabbage, Chinese cabbage and are grown on 19,216 ha with a total production of 317,778 metric tons valued at RM538 million. Most crucifers are planted in Johor, Pahang, Kelantan, Sabah, Perak and Sarawak. Lepidopteran pest infestation by the web-worm (*Hellula undalis*), diamondback moth larvae (*Plutella xylostella*), armyworm (*Spodoptera litura*) and cabbage heart caterpillars (*Crocidolomia pavonana)* cause yield damage between 26 to 100%. To date, chemical-based insecticide applications are the main methods used by farmers to deal with the infestation of these pests.

Environmental and health problems associated with chemical-based insecticides have prompted the search for ecologically acceptable pesticides. Viruses, particularly those belonging to the family Baculoviridae, are one of the most promising biological insecticides. The Baculoviridae family is divided into two genera: Nuclear polyhedrosis viruses (NPVs) and Granulovirus (GVs) that comprise large, double-stranded, circular DNA viruses pathogenic to invertebrates. These viruses occur naturally in insect populations and are capable of causing very high mortality. This family is pathogenic to insects, mostly those in the order Lepidoptera.

The genus nuclear polyhedrosis virus (NPV) produces polyhedral that are proteinaceous crystal bodies occluding a large number of progeny virions in the infected host cell nuclei (Granados and Williams 1986). The NPV is characterised by double-stranded circular DNA genomes ranging from 88 to 153kb (Blissard and Rohrmann 1990). The NPV infection cycle starts upon ingestion of polyhedral by a susceptible larva. The polyhedra are dissolved in the alkaline midgut, thereby releasing infectious virions. Virion replication proceeds at the midgut cell nuclei, then producing a second viral phenotype, the budded viruses (BVs), which are responsible for the secondary infection. At a later stage of infection, occluded progeny viruses are produced (Granados and Williams 1986).

Granulovirus occlusion bodies contain one or rarely two virions and are about 0.16-0.30 µm by 0.30-0.50 µm in size. The nucleocapsid of the virus contains a double-stranded circular-shaped strand of DNA (Fields Virology 2013). Occlusion bodies (Occluded virus, or OV) of granuloviruses contain one or two virions that are wrapped in a protein called granulin (a protein that distinguishes granuloviruses from nuclear polyhedrosis viruses) (Fields Virology 2013).

Prior to death, the viruses-infected larvae hang from an elevated position and liquefy due to the activity of a virus-encoded chitinase (Thomas et al. 1998) and cathepsin-like proteases (Lanier et al. 1996). This behaviour facilitates the dissemination of a large number of polyhedral into the environment.

However, there are certain disadvantages that limit the use of viruses for insect pest control. Compared to the conventional chemical insecticides, they have slower time to death, lower virulence to older instar larvae, narrower host range and are very sensitive to ultraviolet light (UV) (Cunningham 1988). Therefore, several studies had been conducted to overcome these

limitations. The development of a multi-virus-based biopesticide product started in 2013. This biopesticide consists of several baculoviruses and is formulated into a powder with an UV protectant. In this study, the efficacy of the multi-virus-based biopesticide to control lepidopteran pests was determined on conventional cabbage field trials.

MATERIALS AND METHODS

Source of insect and virus:

Larvae of *S. litura, C. pavonana, H. undalis* and *P. xylostella* were obtained from our laboratory colonies at the MARDI Insectary, and maintained on an artificial diet at 26 ± 1°C, 12:12 h light-darkness cycle and 60% relative humidity. The viruses amplification was carried out allowing early third instar larvae to feed on the formalin-free artificial diet, superficially contaminated with 100,000 occlusion bodies (OBs) per mm2. After a 5-day incubation period (at 26 ± 1°C), larvae were removed, homogenised in distilled water and filtered through a muslin cloth. Granules were purified by two cycles of centrifugation in continuous 30–60% (w/w) sucrose gradients at 100,000g for 1 h, at 4°C. The bands containing OBs were removed, pooled, diluted with distilled water and spin down at 8,000g during 1h, at 4°C. OBs were resuspended in distilled water, and stored at 4°C. Viruses suspension were added into a solution which consists of a carrier and UV protectant. Then the virus-solutions were freeze-dried into powder form.

Field trial procedure:

A total of 1435 cabbage plants (311 F1 All Season Cabbage) were planted in Serdang Selangor (lowland). Three treatment groups were conducted in a randomized complete block design (RCBD). The treatment groups were T1 = multi-virus-based biopesticide, T2 = combination application of multi-virus-based biopesticide and Emamectin benzoate and T3 = Emamectin benzoate. The spray frequency was 1 time per week in the evening. The parameters for this study were the number of insects before and after the sprayed and the yield. Data were analysed by using Minitab 18.

RESULTS AND DISCUSSION

There were several types of pests infesting cabbage crops according to its developmental stages. In the early stages, cabbage was infested with flea beetles (56.8%), *H. undalis* and *P. xylostella*. Later, *S. litura* and *C. pavonana* were found to infest the cabbage. Lepidopteran larvae distribution (i.e. *H. undalis*, *P. xylostella, S. litura* and *C. pavonana*) for each treatment group was similar in number with no significant difference (p> 0.05) (Figure 1). However, beneficial insect distribution in T1 was slightly higher compared to T3 with no significant difference (p>0.05) (Figure 1). Multi-virus-based biopesticide is specific for controlling lepidopteran pests and has no effect on beneficial insect including parasitoids and spiders.

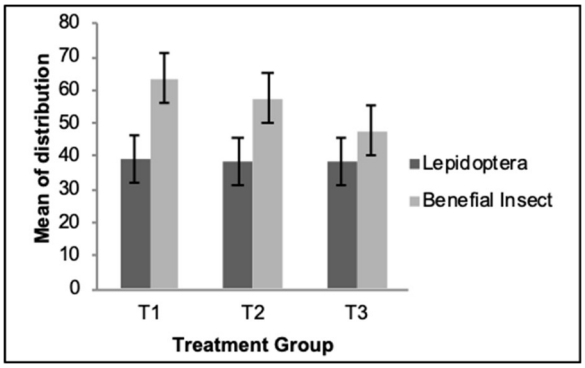


Figure 1. Mean distribution of Lepidopteran pest and beneficial insects between treatment groups.

Percentage of crucifer Lepidopteran pests on cabbage decreased after each spray application per month for all treatment groups (Figure 2). An average percentage of larval reduction in T1 and T2 were 79% and 89% in T3. The first month after transplant, T3 showed 90% reduction of larvae infestation on cabbage compared to 74% and 60% for T2 and T3, respectively. However, T1 showed 90% larvae reduction on cabbage compared to T2 (69%) and T3 (72%) in the second month. Application of Emamectin benzoate (T3) on cabbage reduced larval populations by 98% with 94% and 85% reductions for T2 and T1, respectively.

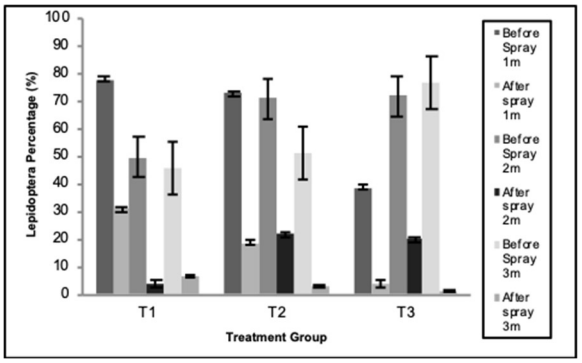


Figure 2. Percentage of crucifer Lepidopteran pest on lowland cabbage for before and after sprayed with T1= Multi virus-based biopesticide, T2= Multi virus-based biopesticide & Emamectin benzoate, T3= Emamectin benzoate

The highest mean number of cabbage heading was in group T3 (126), followed by T2 (112) and T1 (80) (Figure 3). There was a significant difference (p <0.05) between the mean value of T1 and T3. The highest mean number of multiple-headed cabbage was recorded in T1 (72) compared to T2 (40.3) and T3 (15) with significant difference (p <0.05) between T1 and T3. There were no significant differences for the mean

number of cabbage infected with diseases for any treatment groups (Figure 3).

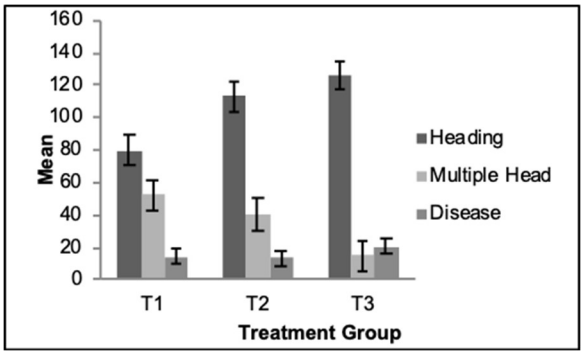


Figure 3. Mean of heading, multiple head and disease occurred in each treatment group

Mean number of cabbage total weight was highest in T3 (20,153g) compared to T2 (18,645g) and T1 (12,499g) with a significant difference ($p<0.05$) between T1 and T3 (Figure 4). From a random observation, the cabbage size in T3 was slightly bigger compared to T2 and T1 (Figure 5). This may be due to the UV protectant in the multi- virus-based biopesticide. However, further studies need to be done to validate it.

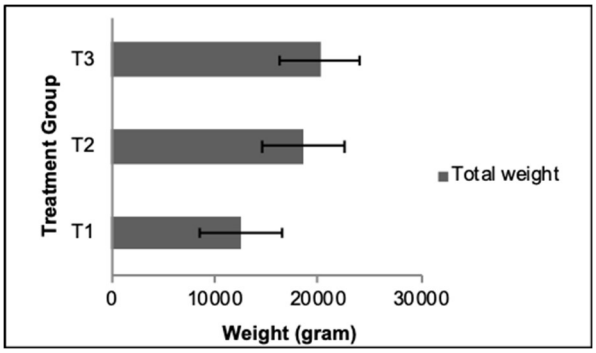


Figure 4. Cabbage total weight between treatment groups.

Figure 5. Harvested cabbage from each treatment groups

The multi-virus-based biopesticide combines NPV and GV which may not have any synergistic or interference effect on major lepidopteran pests in this study. A study by Lowe and Paschke (1968) found that the nucleopolyhedrosis virus and the granulosis virus of *Trichoplusia ni* exhibited neither synergistic nor interference effect upon one another when fed simultaneously as a double inoculum. When inoculated together, the two viruses produced an increased percentage mortality of the test insects.

These multi-virus-based biopesticides could be used with insecticides (e.g. Emamectin benzoate) and showed promising results compared to multi-virus-based biopesticides alone. A study reported that mixtures of NPV and selected insecticides resulted in faster mortality of *S. litura* compared to the NPV alone (Trang and Chaudari 2002).

CONCLUSION

This study demonstrated that the multi-virus-based biopesticide was effective against *H. undalis*, *P. xylostella, S litura* and *C. pavonana* which infested lowland cabbage. This biopesticide also could be used with other insecticides. There are some improvements needed especially the side effect of UV protectant on cabbage size.

Acknowledgements

Authors would like to express gratitude for team members, MARDI and the Malaysian Government for Development Project no. P-RS403.

References

Blissard GW, Rohrmann GF. 1990. Baculovirus diversity and molecular biology. *Annu. Rev. Entomol* 35: 127-155

Cunningham JC. 1988. Baculoviruses: Their status compared to *Bacillus thuringiensis* as microbial insecticides. *Outlook Agric.* 17: 10-17

Fields BN, David MK. 2013. Fields Virology. 6th ed., Vol. 2, Raven Press.

Granados RR, Williams KA. 1986. In vivo infection and replication of baculoviruses. *The Biology of Baculoviruses. In* (R.R. Granados and A. A. Federici (ed.), Vol. I, pp. 89-108. CRC Press, Boca Raton, FL.

Lanier LM, Slack JM, Volkman LM. 1996. Actin binding and proteolysis by the baculovirus AcMNPV: The role of virion associated V-CATH. *Virology* 216: 380-388

Lowe RE, Paschke JD. 1968. Simultaneous infection with the nucleopolyhedrosis and granulosis viruses of *Trichoplusia ni*. *Journal of Invertebrate Pathology* 12: 86-92

Thomas CJ, Brown HL, Hawes CR, Lee BY, Min MK, King LA, Possee RD. 1998. Localization of a baculovirus-induced chitinase in insect cell endoplasmic reticulum. *Journal of Virology* 72: 10207-10212

Trang TTK, Chaudari S. 2002. Bioassay of nuclear polyhedrosis virus (NPV) and in combination with insecticide on *Spodoptera litura* (Fab). *Omonrice* 10: 45-53

Nanoparticles as novel insecticide against diamondback moth, *plutella xylostella* Linnaeus. in cauliflower

Kannan, M.
DEPARTMENT OF PLANT PROTECTION
HORTICULTURAL COLLEGE AND RESEARCH INSTITUTE
PERIYAKULAM- 624625, TAMIL NADU, INDIA
email: kanento@gmail.com

Preetha, S.
DEPARTMENT OF NANO SCIENCE AND TECHNOLOGY,
TAMIL NADU AGRICULTURAL UNIVERSITY,
COIMBATORE- 641 003, TAMIL NADU, INDIA
email: preetha1806@gmail.com

Lokesh, S.
DEPARTMENT OF AGRICULTURAL ENTOMOLOGY,
TAMIL NADU AGRICULTURAL UNIVERSITY,
COIMBATORE- 641 003, TAMIL NADU, INDIA
email: lokeshs808@gmail.com

ABSTRACT
Diamondback moth (DBM), *Plutella xylostella* L. is the important insect pest of cauliflower in India and causes 90% yield loss. DBM has become very difficult to manage because of the development of resistance to currently available insecticides. To overcome resistance, farmers increase the concentration of insecticides which accounts for 30 - 50% of the total cost of production. Nanotechnology has the prospective to revolutionize the existing technologies used in various sectors including insecticides in agriculture. Nanomaterials have potential to be used as crop protection agents. Nanoparticles are absorbed into the cuticle of the insects by physisorption and cause death of insects purely by physical means when applied on leaves and stem surface. Studies were carried out on the toxicity of Nano SiO_2, ZnO, Ag, TiO_2, Zeolite, *Bacillus thuringiensis*, Cry1Ab toxin, Neemazal, Profenofos, Lambda-cyhalothrin and Flubendiamide against *P. xylostella* in cauliflower under laboratory condition. Among the treatments tested for their toxicity (LC_{50}) against the second instar of *P. xylostella*, Flubendiamide was found to be most effective (0.093 µg/ml). The treatment with *Bt* Cry1Ab toxin (0.301), SiO_2 (30.030), Zeolite (38.928), ZnO (40.528), Ag (41.139), TiO_2 (52.051), Lambda-cyhalothrin (113.14), Profenofos (219.75) and Neemazal (889.00) were in the order of their efficacy. Among the nanoparticles SiO_2 caused more mortality to *P. xylostella* next to Flubendiamide and *Bt* Cry1Ab toxin.

Keywords
Nanoparticles, insecticides, Diamondback moth, *Plutella xylostella*, cauliflower

INTRODUCTION
Indian Horticulture is the potentially growing sector, producing a wide range of horticultural crops round the year which facilitates increase in farm income, livelihood security and foreign exchange by export. India accounts for 21% of vegetables in global production and is the second largest producer after China. Cabbage, *Brassica oleracea* var. *capitata* L. and cauliflower, *B. oleracea* var. *botrytis* L. are important crucifers in India with an area of 3,99,000 and 4,53,000 hectares, respectively (Horticultural Statistics at a Glance 2018). Most of the cruciferous vegetables are vulnerable to many insect pests among them the diamondback moth (DBM), *Plutella xylostella* (Linnaeus), is the most important destructive pest of crucifers in India and worldwide causes significant economic losses (92% farmers) up to 50 per cent with an estimate of US$ 4-5 billion million per year (Uthamasamy et al. 2011; Furlong et al. 2013). The main method of control has been the use of conventional insecticides and *P. xylostella* has developed resistance to all pesticides available in the market. Indiscriminate use of chemical insecticides led to resistance, resurgence, residues, eliminating natural enemies, upsetting ecological balance, human being hazards and environmental pollution. Hence, there is an urgent need for alternatives for *P. xylostella* management.

Nanotechnology has the potential to modernize the existing technologies used in various sectors together with agriculture. Nanotechnology refers to the science and technology of the objects that are <100 nm. The prospective uses and benefits are very well explored in fields of electronics, medicines as well as agriculture as crop production inputs. Nanomaterials can be synthesized by top-down or bottom-up approaches. Nanomaterials have a much greater surface area per unit volume ratio compared with the materials made up of bigger particles (Kannan et al. 2020). Nano pesticides represent an emerging technological development in pesticide use and could offer a range of benefits including increased efficacy, durability and reduction in the amounts of active ingredients that need to be used. The nanocides can damage the insect's protective wax coat on the cuticle by absorption and due to the peristaltic movement, it also pierces tissues in the stomach, and finally causes death of insect by desiccation (Bhattacharya et al. 2020). The inorganic Engineered nanoparticles (ENPs), such as metals, metal oxides, and nanoclays

itself may "drive" the biological effect when they used as a pesticide where the active component is the ionic form of respective ENPs (Kookana et al. 2014).

In the present study, nanoparticles such as SiO_2, ZnO, Ag, TiO_2, Zeolite have proven to be a viable alternative to synthetic commercial insecticides for managing arthropod pests of agricultural crops. Nanotechnology has the possibility to overcome the problem in the environment by reduced application of the pesticide.

MATERIALS AND METHODS

Materials

The experiments were conducted with nanoparticles such as SiO_2, ZnO, Ag, TiO_2, Zeolite synthesized and obtained from the Department of Nano Science and Technology, TNAU, Coimbatore. The biological insecticides *Bt* Cry1Ab toxin was obtained from Dr. M. Mohan, Principal Scientist, ICAR- National Bureau of Agricultural Insect Resources - [NBAIR], Bangalore, Karnataka, India. The commercial formulations of chemical insecticides *viz.,* Neemazal (E.I.D. Parry India Limited), Profenofos and Lambda-cyhalothrin (Curacron, Karate, Syngenta India Ltd) and Flubendiamide (Fame, Bayer Crop Science Ltd, Mumbai, India) were purchased from local pesticide market, Coimbatore, Tamil Nadu, India.

Collection and rearing of *P. xylostella*

About 200 larvae were collected from Thondamuthur village of Coimbatore district, Tamil Nadu, India and mass cultured under laboratory condition on mustard and cauliflower at Insectary, Department of Agricultural Entomology, TNAU, Coimbatore to establish the stock culture of *P. xylostella* (with a mean temperature of 27ºC, 60% RH and with a photoperiod of 14:10 (L:D). Larvae were examined regularly to ensure that they remained pathogen-free for rearing and maintained in the laboratory on cauliflower leaves. Pupae were collected and kept in the adult emergence cage. Further, the adult moths emerging from the pupae were allowed in the oviposition cage with 10% honey solution fortified with multivitamins as food for egg laying in mustard seedlings. After egg hatching the neonate larvae fed on the mustard leaves by mining and the second instar larvae were transferred to fresh cauliflower leaves then used for the bioassays to assess the larval mortality.

Bioassay method

Leaf- disk dip bioassay method was used (Tabashnik et al. 1991). The cauliflower leaves were first washed with distilled water containing 0.1% Triton X-100 thoroughly and air-dried. Leaf disc of 6-8 cm diameter were cut and dipped in different concentrations of nanoparticles (SiO_2, ZnO, Ag, TiO_2, and Zeolite), biological (*Bt* Cry1Ab and Neemazal) and chemical insecticides (Profenofos, Lambda-cyhalothrin and Flubendiamide). Each disc was dipped for 5-10 seconds and allowed to air dry under shade for a period of one hour. After complete evaporation, the leaves were transferred to clean bioassay containers over a moistened filter paper. The leaf discs were placed slanting to rest on the side of the container so that larvae can move on either side. Eight to ten second instar larvae were released in each dish and three replicates were maintained per treatment and leaf discs were changed at every three days until pupation in respective doses. A treatment without nanoparticles, biological and chemical insecticides served as control. Larval mortality was recorded every 24 h, consecutively for seven days. All the experiments were carried out in a room with a photoperiod of 12:12 (L: D) and experiments with control mortality more than 20% were discarded and repeated.

Data analysis

The mortality of neonates was observed up to seven days for calculating the lethal concentration values (LC_{50}) and their 95% fiducial limits (FL) against DBM were estimated by Finney (1971). Mortality was corrected by Abbott's formula (Abbott 1925) for each Probit regression analysis.

RESULTS AND DISCUSSION

Toxicity of nanoparticles, biological and chemical insecticides to *P. xylostella*

The results on the toxicity of nano particles (SiO_2, ZnO, Ag, TiO_2, and Zeolite), biological (Cry1Ab and Neemazal) and chemical insecticides (Profenofos, Lamda-cyhalothrin and flubendiamide) to *P. xylostella* were studied by Leaf- disk dip bioassay method and the results of probit regression analysis were shown in Table 1. Among the treatments tested for their toxicity (LC_{50}) against the second instar of *P. xylostella*, Flubendiamide was found to be most effective (0.093 µg/ml). The treatment with *Bt* Cry1Ab toxin (0.301), SiO_2 (30.030), Zeolite (38.928), ZnO (40.528), Ag (41.139), TiO_2, (52.051), Lamda-cyhalothrin (113.14), Profenofos (219.75) and Neemazal (889.00) were in the order of their efficacy. Among the nanoparticles SiO_2, caused more mortality to *P. xylostella* next to Flubendiamide and Bt Cry1Ab toxin. The mean slope of the probit regression for flubendiamide, *Bt* Cry1Ab toxin, SiO_2, Zeolite, ZnO, Ag, TiO_2, Lamda cyhalothrin, Profenofos and Neemazal were reported as 2.01, 1.65, 2.71,2.43, 2.35, 1.77, 1.12, 2.91, 2.68 and 2.60, respectively. In the above bioassays the mortality data fitted the probit model, which were confirmed by the Pearson goodness of fit chi-square test (Table 1). The results of the present study demonstrated that nanoparticles

could kill the larvae of *P. xylostella* and the results are in agreement with findings of Shoaib et al. (2018) in *P. xylostella*; *Lipaphis pseudobrassicae* and *S. litura* (Goswami et al. 2010; Debnath et al. 2012). In addition, Rouhani, et al. (2012) reported that ZnO, TiO_2, Ag NPs revealed insecticidal activity on *Frankliniella occidentalis* (Pergande). The enhanced mortality rate in treatments might be caused by the absorption of water through the disruption of cuticle by nanosilica, which ultimately results to insect death via desiccation. Further, the comparative results obtained on Flubendiamide, *Bt* Cry1Ab toxin, Lambda cyhalothrin, Profenofos and NeemAzal are also congruence with the earlier reports of Shanmugapriya et al. (2019), Kannan et al. (2017) and Abro et al. (2013), respectively.

Table1. Probit regression analysis of mortality data to nanoparticles and insecticides against *P. xylostella*

Sl.No.	Nanoparticles and insecticides	LC_{50} (µgml−1)	LC_{95} (µgml−1)	Slope ± SE	χ2 *
1.	SiO_2	30.030 (24.494 – 36.818)	117.313 (68.977 – 119.521)	2.71± 0.19	2.299
2.	ZnO	40.528 (31.834 – 51.596)	191.532 (87.203 – 420.679)	2.35± 0.15	1.364
3.	Ag	41.139 (34.433 – 49.151)	164.201 (94.966 – 283.919)	1.77 ± 0.13	2.595
4.	TiO_2	52.051 (41.406 – 65.433)	217.799 (109.902– 431.626)	1.12 ± 0.15	2.689
5.	Zeolite	38.928 (31.950 – 47.429)	188.962 (98.292– 363.272)	2.43 ± 0.16	0.443
6.	Cry1Ab	0.301 (0.155 – 0.583)	2.972 (1.759 – 5.023)	1.65 ± 0.18	0.089
7.	Neemazal	889.0 (0.790 – 1.000)	3689.1 (3238.2 – 5294.8)	2.60 ± 0.09	4.63
8.	Profenofos 50EC	219.75 (191.40 – 252.30)	911.83 (687.44 – 1209.46)	2.68 ± 0.12	1.79
9.	Lambda-cyhalothrin 5EC	113.14 (99.79 – 128.27)	414.07 (323.82 – 529.48)	2.91 ± 0.08	3.39
10.	Flubendiamide 20 WG	0.093 (0.072- 0.121)	0.592 (0.245 – 1.432)	2.01 ± 0.04	0.484

* = In each case χ^2 value from the goodness-of-fit test was less than the tabular value, (p = 0.05), indicating that the data fit the probit model.

CONCLUSION

Though insecticides showed a vital role in DBM management, their wide use have resulted in the insect developing resistance to many of the chemical insecticides. In our study, the results concluded that among the treatments tested for their toxicity (LC_{50}) against the second instar of *P. xylostella*, Flubendiamide was found to be most effective (0.093 µg/ml). Among the nanoparticles SiO_2, caused more mortality to *P. xylostella* next to flubendiamide and *Bt* Cry1Ab toxin. Nanoparticles are absorbed into the cuticle of the insects by physisorption and cause death of insects purely by physical means when applied on leaves and stem surface which leads to ecological based sustainable IPM in cruciferous crops.

Acknowledgements

The authors are grateful to the Professor and Head, Department of Agricultural Entomology, Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore for providing us with necessary facilities.

References

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265-267.

Abro GH, Syed TS, Kalhoro AN, Sheikh GH, Awan MS, Jessar RD, Shelton AM. 2013. Insecticides for control of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Pakistan and factors that affect their toxicity. *Crop Protection* 52, 91–96.

Anonymous. 2018. Horticulture Statistics at a Glance. Horticulture Statistics Division, Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, New Delhi, pp, 490.

Bhattacharya A, Epidi TT, Kannan M. 2020. Nano-technology Applications in Pest Management. In: Chakravarthy A (ed.) Innovative Pest Management Approaches for the 21st Century. Springer, Singapore, pp, 391-401.

Debnath N, Mitra S, Das S, Goswami A. 2012. Synthesis of Surface Functionalized Silica Nanoparticles and Their Use as Entomotoxic Nanocides. *Powder Technology* 221: 252–256.

as Entomotoxic Nanocides. *Powder Technology* 221: 252–256.

Finney DJ. 1971. Probit analysis, 3rd ed. Cambridge University Press: London

Furlong MJ, Wright DJ, Dosdall LM. 2013 Diamondback moth ecology and management: Problems, progress and prospects. *Annu. Rev. Entomol. 58*, 517–541.

Goswami A, Roy I, Sengupta S, Debnath N. 2010. Novel Applications of Solid and Liquid Formulations of Nanoparticles against Insect Pests and Pathogens. *Thin Solid Films* 519: 1252–1257.

Kannan M, Elango K, Tamilnayagan T, Preetha S, Kasivelu G. 2020. Impact of Nanomaterials on Beneficial Insects in Agricultural Ecosystems. In: Thangadurai D., Sangeetha J., Prasad R. (ed.) Nanotechnology for Food, Agriculture, and Environment. Nanotechnology in the Life Sciences. Springer, Cham, pp, 379-393.

Kannan M, Lokesh S, Prithiva, JN, Mohan M. 2017. Toxicity and Sublethal Effects of *Bacillus thuringiensis* B. δ-Endotoxin Cry1Ab on the Biological Parameters of Diamondback Moth, *Plutella xylostella* L. *Mysore Journal of Agricultural Sciences* 51(A): 153-158.

Kookana RS, Boxall AB, Reeves PT, Ashauer R, Beulke S, Chaudhry Q, Cornelis G, Fernandes TF, Gan J, Kah M, Lynch I, Ranville J, Sinclair C, Spurgeon D, Tiede K, Van den Brink PJ. 2014. Nanopesticides: guiding principles for regulatory evaluation of environmental risks. *J. Agric. Food Chem.* 62, 4227–4240.

Rouhani M, Samih MA, Kalantari S. 2012. Insecticidal Effect of Silica and Silver Nanoparticles on the Cowpea Seed Beetle, *Callosobruchus Maculatus* F. (Col.: Bruchidae). *Journal of Entomology Research* 4: 297–305.

Shanmugapriya V, Johnson Thangaraj Edward YS, Kannan M, Mohankumar S, Ramanathan S. 2019. Toxicity and sub lethal effects of diamide insecticide (Cyantraniliprole 10.26 OD) on the biology of Diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), *International Journal of Chemical Studies* 7(3): 3524-3527.

Shoaib A, Elabasy A, Waqas M, Lin L, Cheng X, Zhang Q, Shi Z. 2018 Entomotoxic effect of silicon dioxide nanoparticles on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) under laboratory conditions, *Toxicological & Environmental, Chemistry* DOI: 10.1080/02772248.2017.1387786

Tabashnik BE, Finson N, Johnson MW. 1991. Managing resistance to *Bacillus thuringiensis*: Lessons from the diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.,* 84: 49-55.

Uthamasamy S, Kannan M, Senguttuvan K, Jayaprakash SA. 2011. Status, damage potential and management of diamondback moth, Plutella xylostella in Tamil Nadu, India, *Proceedings of the 6th International workshop on Management of the Diamondback moth and other crucifer insect pests*, AVRDC, The world, vegetable, center, Taiwan PP-270-279.

Evaluation of biopesticides along with predatory birds in suppression of diamondback moth, plutella xylostella in cabbage

Kumawat, M.M.
DEPARTMENT OF ENTOMOLOGY, COLLEGE OF AGRICULTURE, AGRICULTURE UNIVERSITY, JODHPUR, RAJASTHAN, INDIA- 342304
email: kumawatmm@gmail.com

Singh, K.M.
CENTRAL AGRICULTURAL UNIVERSITY, PASIGHAT-791102, ARUNACHAL PRADESH, INDIA
email: mamoento@gmail.com

Pandey, A.K.
CENTRAL AGRICULTURAL UNIVERSITY, PASIGHAT-791102, ARUNACHAL PRADESH, INDIA
email: pandey.ajai1@gmail.com

ABSTRACT
The experiments were laid out in 2017-18 to evaluate the biopesticides along with the role of predatory birds for suppression of Diamondback moth (DBM), *Plutella xylostella* of cabbage at Arunachal Pradesh, India. A total of eight bird species were recorded feeding on the DBM in cabbage during the field survey. Grey wagtail bird was the common visitor in the cabbage crop in morning hours as well as afternoon which entered in the field by walking from the margins. During 2017, all the treatments were at par at 30 days after transplanting (DAT) but after 40, 50 and 60 DAT all the treatments are significantly better than the untreated control. The lowest DBM population was recorded in treatment with Cartap hydrocloride 50% SP spray @ 1g/liter of water (0.05%) + predatory birds (open condition) and Cartap hydrochloride 50% SP alone spray @ 1g/liter (0.05%) of water (entry of predatory bird protected by netting) which were significantly better than the treatment predatory birds alone. The similar results were found in the year 2018 where all the treatments provided significantly better control as compared to untreated control. The treatment with Cartap hydrocloride 50% SP spray @ 1g/liter of water (0.05%) + predatory birds (open condition) and Cartap hydrochloride 50% SP alone spray @ 1g/liter (0.05%) of water (entry of predatory bird protected by netting) gave good control of diamondback moth at 30, 40, 50 and 60 days after transplanting. The treatment with *Beauveria bassiana* (wettable powder) alone spaying @ $1x10^8$ cfu/ml of water (entry of predatory bird protected by netting), *Beauveria bassiana* (wettable powder) spray @ $1x10^8$ cfu/ml of water + predatory birds (open condition), *Bacillus thuringiensis* subsp. *kurstaki* (Lipel, 18,000 IU/mg) alone spaying @ 5g/liter of water (entry of predatory bird protected by netting) and *Bacillus thuringiensis* subsp. *kurstaki* (Lipel, 18,000 IU/mg) @ 5g/liter of water + predatory birds (open condition) were at par but these were significantly superior than the predatory birds alone.

Keywords
Diamondback moth, *Plutella xylostella*, biopesticides, predatory birds, Insectivorous

INTRODUCTION
The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive insect pests of *Brassica* crops in tropical and temperate climates all over the world (Talekar and Shelton 1993). The cabbage is cultivated all year round except in areas with no access to irrigation, where it is only produced during the wet season. As a consequence, all life stages of *P. xylostella* can be present at any time of the year. *P. xylostella* easily develops resistance to insecticides. It has now developed resistance to a wide range of insecticides; it has been reported to be resistant to various kinds of insecticides, including recently introduced compounds with new modes of action (Jiang et al. 2015). Insectivorous birds are thought to play a key role in controlling arthropod herbivory, thus having positive indirect effects on plants. Many birds specialized in consuming insects have since long attracted man's attention. The economic value of these birds has often been discussed (Hussain and Afzal 2005; Rajashekara and Venkatesha 2014). It would be convenient to consider all insect-eating birds as beneficial. Studies of intact bird communities show the coexistence of numerous insectivorous birds, each of which developed its own feeding strategy to limit competition pressure from other species (Cody 1974). Insectivorous birds act as important biological control agents of insect pests in agriculture, floriculture, horticulture and forests (Verghese and Sriharan 1993; Dhindsa and Saini 1994; Patankar et al. 2007; Keshavan and Malavannan 2010; Thakur et al. 2010). The present study was carried out to evaluate the role of beneficial birds in combination of safer bio-pesticides in suppression of diamondback moth in cabbage.

MATERIALS AND METHODS

The experiment was carried out during *winter*, 2017 and 2018 at experimental farm, College of Horticulture and Forestry, Pasighat, India in Randomized Block Design with eight treatments and each was replicated 3 times with the plot area of 12 sq. m. to evaluate the role of beneficial birds in suppression of *P. xylostella* of cabbage. The details of the treatment are as follows:

T1- Predatory birds alone (open condition)
T2- *Bacillus thuringiensis* subsp. *kurstaki* (Lipel, 18,000 IU/mg) alone spaying @ 5g/liter of water (entry of predatory bird protected by netting)
T3-*Beauveria bassiana* (wettable powder) alone spaying @ $1x10^8$ cfu/ml of water (entry of predatory bird protected by netting)
T4- Cartap hydrochloride 50% SP alone spray @ 1g/liter of water (0.05%) (entry of predatory bird protected by netting)
T5- *Bacillus thuringiensis* subsp. *kurstaki* (Lipel, 18,000 IU/mg) @ 5g/liter of water + predatory birds (open condition)
T6- *Beauveria bassiana* (wettable powder) spray @ $1x10^8$ cfu/ml of water + predatory birds (open condition)
T7- Cartap hydrochloride 50% SP spray @ 1g/liter of water (0.05%) + predatory birds (open condition)
T8- Control (Un- treated and entry of predatory bird protected by netting)

Pre-treatment observations were taken one day before the spray of insecticides. The post treatment observations were recorded at 40, 50 and 60 days after transplanting (DAT). In all two sprays were given. The first spray was given at 20 DAT and the second spray was given at 39 DAT. The data on population were subjected to analysis of variance after angular transformation.

Population of visiting birds in the field was also counted between 8-10 AM and 3-4 PM every week (3 hrs) during the entire crop season to determine the species diversity in the experimental site. The experimental area is situated between 28^0 07' North latitudes and 95^0 32' East longitude with an altitude of 155 m above MSL. This location has a warm and humid climate with a distinct rainy season spread over 7 months from April to October and irregular mild showers of rain from November to March with an average annual rainfall of 4000 mm. The observation made on encounters of bird species and number of birds was noted as a method described by Verner 1985. Observation was avoided on rainy days. The birds were counted using a Nikon binocular with 8-24x25 zoom and identified. In case of doubtful identification, photographs were taken and the species was identified later by consulting experts. The identification of birds was carried out using standard literature of Ali and Ripley (1983), Grimmett et al. (1998), Ali (2000), and Singh et al. (2013).

RESULTS AND DISCUSSION

Before commencement of the first spray, *P. xylostella* population varied from 3.12 to 5.87 including larva, pupa and adults during both the years i.e., 2017 and 2018. The efficacy of treatments depicted in table 2 and 3 revealed that after first spray, highest pest reduction was observed in T7- Cartap hydrocloride 50% SP spray @ 1g/liter of water (0.05%) + predatory birds (open condition) which reduced the population upto 0.33 DBM/5 leaves. After two sprays, the lowest DBM population was recorded in the treatment of Cartap hydrocloride 50% SP spray @ 1g/liter of water (0.05%)+ predatory birds (open condition) and Cartap hydrocloride 50% SP alone spray @ 1g/liter (0.05%) of water (entry of predatory bird protected by netting) which were significantly better than the treatment predatory birds alone. The similar results were found in the year 2018 where all the treatments provided significantly better control as compared to untreated control. The treatment with Cartap hydrocloride 50% SP spray @ 1g/liter of water (0.05%) + predatory birds (open condition) and Cartap hydrocloride 50% SP alone spray @ 1g/liter (0.05%) of water (entry of predatory bird protected by netting) provided good control of diamondback moth at 40, 50 and 60 days after transplanting. The treatment with *Beauveria bassiana* (wettable powder) alone spaying @ $1x10^8$ cfu/ml of water (entry of predatory bird protected by netting), *Beauveria bassiana* (wettable powder) spray @ $1x10^8$ cfu/ml of water + predatory birds (open condition), *Bacillus thuringiensis* subsp. *kurstaki* (Lipel, 18,000 IU/mg) alone spaying @ 5g/liter of water (entry of predatory bird protected by netting) and *Bacillus thuringiensis* subsp. *kurstaki* (Lipel, 18,000 IU/mg) @ 5g/liter of water + predatory birds (open condition) were at par but these were significantly superior than the predatory birds alone.

Table 1: Efficacy of biopesticides and insectivorous birds against DBM during 2017.

Treatments	Population of DBM per five leaves			
	40 DAT	**50 DAT**	**60 DAT**	**Average**
T1- Predatory birds alone (open condition)	3.33	4.25	5.00	4.19
	(10.51)	(11.89)	(12.92)	(11.81)
T2- *Bacillus thuringiensis* subsp. *kurstaki* (Lipel, 18,000 IU/mg) alone spaying @ 5g/liter of water (entry of predatory bird protected by netting)	1.33	1.50	1.75	1.53
	(6.62)	(7.03)	(7.60)	(7.09)
T3- *Beauveria bassiana* (wettable powder) alone spaying @ $1x10^8$ cfu/ml of water (entry of predatory bird protected by netting)	1.67	3.75	3.87	3.10
	(7.42)	(11.16)	(11.34)	(10.13)
T4- Cartap hydrochloride 50% SP alone spray @ 1g/liter of water (0.05%) (entry of predatory bird protected by netting)	0.25	0.75	0.93	0.64
	(2.86)	(4.96)	(5.53)	(4.60)
T5- *Bacillus thuringiensis* subsp. *kurstaki* (Lipel, 18,000 IU/mg) @ 5g/liter of water + predatory birds (open condition)	1.22	1.33	1.56	1.37
	(6.34)	(6.62)	(7.17)	(6.72)
T6- *Beauveria bassiana* (wettable powder) spray @ $1x10^8$ cfu/ml of water + predatory birds (open condition)	1.87	2.87	3.25	2.66
	(7.86)	(9.75)	(10.38)	(9.39)
T7- Cartap hydrocloride 50% SP spray @ 1g/liter of water (0.05%) + predatory birds (open condition)	0.33	0.67	0.87	0.62
	(3.29)	(4.69)	(5.35)	(4.52)
T8- Control (Un- treated and entry of predatory bird protected by netting)	5.87	6.75	8.25	6.96
	(14.02)	(15.05)	(16.69)	(15.29)
SEM±	**0.101**	**0.112**	**0.138**	**0.118**
CD 5%	**0.311**	**0.329**	**0.401**	**0.344**

*Figures in parentheses are square root transformed values

Table 2: Efficacy of biopesticides and insectivorous birds against DBM during 2018.

Treatments	Population of DBM per five leaves			
	40 DAT	**50 DAT**	**60 DAT**	**Average**
T1- Predatory birds alone (open condition)	3.00	3.87	4.93	3.93
	(9.97)	(11.34)	(12.82)	(11.43)
T2- *Bacillus thuringiensis* subsp. *kurstaki* (Lipel, 18,000 IU/mg) alone spaying @ 5g/liter of water (entry of predatory bird protected by netting)	1.67	1.87	2.00	1.85
	(7.42)	(7.86)	(8.13)	(7.81)
T3- *Beauveria bassiana* (wettable powder) alone spaying @ $1x10^8$ cfu/ml of water (entry of predatory bird protected by netting)	1.93	3.25	4.13	3.10
	(7.98)	(10.38)	(11.72)	(10.14)
T4- Cartap hydrochloride 50% SP alone spray @ 1g/liter of water (0.05%) (entry of predatory bird protected by netting)	0.25	0.66	0.87	0.59
	(2.86)	(4.66)	(5.35)	(4.41)
T5- *Bacillus thuringiensis* subsp. *kurstaki* (Lipel, 18,000 IU/mg) @ 5g/liter of water + predatory birds (open condition)	1.33	1.5	1.67	1.5
	(6.62)	(7.03)	(7.42)	(7.03)
T6- *Beauveria bassiana* (wettable powder) spray @ $1x10^8$ cfu/ml of water + predatory birds (open condition)	2.00	3.25	3.50	2.92
	(8.13)	(10.38)	(10.78)	(9.83)
T7- Cartap hydrochloride 50% SP spray @ 1g/liter of water (0.05%) + predatory birds (open condition)	0.25	0.50	0.67	0.47
	(2.86)	(4.05)	(4.69)	(3.94)
T8- Control (Un- treated and entry of predatory bird protected by netting)	5.67	6.33	9.23	7.08
	(13.77)	(14.57)	(17.68)	(15.42)
SEM±	**0.109**	**0.118**	**0.151**	**0.126**
CD 5%	**0.325**	**0.335**	**0.431**	**0.358**

*Figures in parentheses are square root transformation values

The occurrence of different species of bird communities recorded at the experimental site in the field of cabbage is given in table 3. A total fourteen bird species belonging to 11 genera under 8 families were recorded during the study period. Out of 14 species of birds, 5 species were exclusively predatory in nature and mainly fed on insects and 8 species were depredatory in feeding habits. The depredatory species generally feed upon the insects mainly but sometimes these birds also feed on grains or other vegetation. Only Spotted Dove, *Streptopelia chinensis* was found as a granivorous bird in the field. Of the

recorded bird species, the relative abundance of Grey wagtail, *Motacilla cinerea* was highest (21.28%), whereas it was 0.80% for Indian Roller, *Coracias benghalensis*. Other than *M. cinerea, the* White wagtail, *Motacilla alba*, Yellow wagtail, *Motacilla flava*, Common myna (Starling), *Acridotheres tristis*, House sparrow, *Passer domesticus* and Black headed myna, *Acridotheres fuscus* were also recorded as a excellent insectivorous birds in cabbage field. The wagtail group of birds landed on the margins nearby the field and entered inside by walking. After reaching inside, these birds have reported to feed on larvae and pupae of *P. xylostella.* Rajashekara and Venkatesha (2014) reported that M. *cinerea* and *M. alba* found to feed on larvae of *P. xylostella* and other insects on cabbage. The fact that *A. tristis* feeds on insects' larvae, imagoes and adult insects, pests of agriculture and horticulture. The analysis of the food consumed revealed that *Sturnus contra* fed mostly on insects (chiefly grasshoppers, caterpillars and beetles), earthworms, molluscs and lantana berries (Narang and Lamba 1984). The house sparrow, *P. domesticus* was also found to feed on *P. xylostella*. The previous study reported that *P. domesticus* feed on insects, weed seeds, fruit buds, nectar, etc. (Ali 1996). The variation in abundance of different species of insectivorous bird communities in agro-ecosystems is dependent on the availability of variety of crops, number of nesting sites, and density of perching trees (Rajashekara and Venkatesha 2014). The natural control of insect predation by insectivorous birds was similar to earlier findings by Chakravarthy (1988), Verghese and Sriharan (1993), Dhindsa and Saini (1994), Chakravarthy et al. (2008), Keshavan and Malavannan (2010) and Mehta et al. (2010). Most of the insects are important food sources for many birds of the agricultural landscape.

Table 3: Relative abundance of birds in the cabbage field during the year 2017 and 2018.

Sl. No	Bird Species	Family	Feeding nature	Total birds observed	Relative abundance
1	Indian Roller, *Coracias benghalensis*	Coraciidae	P	14	0.80
2	Green bee-eater, *Merops orientalis*	Meropidae	P	20	1.14
3	Spotted Dove, *Streptopelia chinensis*	Columbidae	G	26	1.49
4	Black Drongo, *Dicrurus macrocercus*	Corvidae	DP	48	2.75
5	Common myna (Starling), *Acridotheres tristis*	Sturnidae	DP	230	13.16
6	Black headed myna, *Acridotheres fuscus*	Sturnidae	DP	156	8.92
7	Asian Pied Starling, *Sturnus contra*	Sturnidae	DP	78	4.46
8	Black bulbul, *Hypsipetes leucocephalus*	Pycnonotidae	DP	28	1.60
9	Red Vented Bulbul, *Pycnonotus cafer*	Pycnonotidae	DP	24	1.37
10	Greater Necklaced Laughingthrush, *Garrulax pectoralis*	Sylviidae	DP	44	2.52
11	House sparrow, *Passer domesticus*	Passeridae	DP	188	10.76
12	Grey wagtail, *Motacilla cinerea*	Passeridae	P	372	21.28
13	White wagtail, *Motacilla alba*	Passeridae	P	284	16.25
14	Yellow wagtail, *Motacilla flava*	Passeridae	P	236	13.50
Grand total				**1748**	**100.00**

P- Predatory; DP- Depredatory, G- Granivorous

CONCLUSION

Globally, birds are critically important for providing humans with valuable economic and ecosystem services by consuming billions of harmful crop-eating insects. The global energy consumption by the insectivorous birds in the form of arthropod prey is substantial. To fulfill these huge energy requirements, the insectivorous birds capture billions of potentially harmful herbivorous insects and other arthropods. The

omnivorous birds in present study such as starlings (Sturnidae) and House sparrow (Passeridae) consume large amounts of arthropods in addition to other types of food. Activity of beneficial birds can be enhanced in the field by putting 'T' perches for sitting of birds in the agricultural ecosystem. The 'T' perches may be removed from the field as the crop attains maturity during grain formation to avoid the damage caused by granivorous and omnivorous birds.

Acknowledgement

The authors are grateful to the Indian Council of Agricultural Research, New Delhi for providing financial assistance to the National Agriculture Higher Education Project at Agriculture University, Jodhpur. The authors are also thankful to World Vegetable Center, Tainan, Taiwan for accepting the research work to present in the 8th International Conference on Management of diamondback moth and other crucifer insect pests. The authors are also grateful to the authorities of Agriculture University, Jodhpur for supporting to present the study at the World Vegetable Center, Tainan, Taiwan.

References

Ali S. 1996. The Book of Indian Birds, 1st edition, Oxford University Press, New Delhi.

Ali S. 2000. The book of Indian birds. 13th edition, Oxford University Press, New Delhi, p.354.

Ali S, Ripley SD. 1983. A pectoral Guide to the Birds of the Indian sub-continent. Bombay Natural History Society, Bombay, India, pp.1-177.

Chakravarthy AK. 1988. Bird predators of pod borers of field bean (Lablab niger Medick). *Tropical Pest Management,* 34(4):395-398.

Chakravarthy AK, Kumar NRP, Praveen MP. 2008. Birds as the biocontrol agents in cultivated ecosystems: a review. *Emerging trends of researches in insect pest management and environmental safety*; 1:197-219.

Cody ML. 1974. Competition and the structure of bird communities. Princeton, New Jersey.

Dhindsa MS, Saini HK. 1994. Agricultural ornithology: an Indian perspective. *Journal of Bioscience*, 19(4): 391-402.

Grimmett R, Inskipp C, Inskipp T. 1998. Birds of the Indian subcontinent. New Delhi: Oxford University Press. p. 528.

Hussain I, Afzal M. 2005. Insectivorous Birds and Their Significance in a Cotton- Wheat Based Agro-ecosystem of Punjab, Pakistan. Pakistan *Journal of Zoology*, vol. 37(2), pp. 133-143.

Jiang T, Wu S, Yang T, Zhu C, Gao C. 2015. Monitoring Field Populations of *Plutella xylostella* (Lepidoptera: Plutellidae) for Resistance to Eight Insecticides in China. *Florida Entomologist*, 98(1): 65-73.

Keshavan PC, Malavannan S. 2010. Green to evergreen revolution: ecological and evolutionary perspectives in Pest Management. *Current Science*, 99:908-914.

Mehta KS, Patyal SK, Rana RS, Sharma KC. 2010. Ecofriendly techniques for the management of *Helicoverpa armigera* (Hubner) in tomato. *Journal of Biopesticides*, 3 (special Issue): 296-303.

Narang ML, Lamba BS. 1984. A contribution to the food habits of some Indian mynas (Aves). *Records of the Zoological Survey of India*. Occasional paper no. 44: 1-76.

Patankar P, Desai I, Shinde K, Suresh B. 2007. Ecology and breeding ecology of the Cattle egret, Bubulcus ibis in an industrial area at Vadodara, Gujarat. *Zoo's Print*, 22: 2885-2888.

Rajashekara S, Venkatesha MG. 2014. Insectivorous bird communities of diverse agro-ecosystems in the Bengaluru region, India. *Journal of Entomology and Zoology Studies*, 2 (5): 142-155.

Singh KM, Kumawat MM, Phurailatpam A, Rao VV, Pandey AK. 2013. Birds of Arunachal Pradesh (A compilation). College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh, India, p. 206.

Talekar NS, Shelton AM. 1993. Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology*, 38: 275-301.

Thakur ML, Mattu VK, Lal HS, Sharma VN, Raj H, Thakur V. 2010. Avifauna of Arki Hills, Solan (Himachal Pradesh), India. *Indian Birds*, 5:162-166.

Verghese A, Sriharan TP. 1993. Insectivorous birds and their use as biological control agents. *In*: Bird Conservation Strategies for the Nineties and Beyond. Verghese A, Sridhar S, Chakravarthy AK, eds. Bangalore: Ornithological Society of India; 207-211.

Verner J. 1985. Assessment of counting technique. *Current Ornithology*, 2: 247-463.

Evaluation of efficacy of entomopathogenic fungi for suppression of aphid infested in mustard

Sundria, M.M.
AGRICULTURAL RESEARCH STATION, MANDOR, AGRICULTURE UNIVERSITY, JODHPUR, RAJASTHAN, INDIA- 342304
email: sul79man76@gmail.com

Sanp, R.K.
ADAPTIVE TRIAL CENTRE, RAMPURA, JODHPUR, RAJASTHAN, INDIA- 342305
email: Yadav.ento84@gmail.com

Pandey, S.
AGRICULTURAL RESEARCH STATION, MANDOR, AGRICULTURE UNIVERSITY, JODHPUR, RAJASTHAN, INDIA- 342304
email: pandeyshalini80@gmail.com

ABSTRACT
An experiment was carried out during *rabi*, 2017-18 and 2018-19 to evaluate the efficacy of entomopathogenic fungi for the management of mustard aphid, *Lipaphis erysmi* at experimental fields of Agricultural Research Station (ARS), Mandor, Adaptive Trial Center (ATC) and Cultivated Fields (CF) Jodhpur, Rajasthan, India. Based upon observations, it was concluded that among the entomopathogenic fungi, *Verticillium lecanii* was the most effective fungi for the control of mustard aphid, *L. erysimi*. The results depicted that the mustard aphid population declined significantly after application of this fungus. During the years, an aphid infestation index of 1.2 (on a 5-point scale) was reduced to 0.3 and 0.1, respectively after a week and 10 days of spray with $1x10^8$ spore suspension of *V. lecanii*. Use of surfactant in the form of castor oil @ 0.1% was very useful for uniform spread of the fungal spore formulation.

Keywords
Mustard Aphid, *Lipaphis erysimi,* Entomopathogenic Fungus, *Verticillium Lecanii*

INTRODUCTION
Rapeseed and mustard are important oilseed crops of the family Cruciferae and occupy prominent place among them. Brassica (rapeseed-mustard) is the second most important edible oilseed crop in India after groundnut and accounts for nearly 30% of the total oilseeds produced in the country. It is grown on an area of about 6.4 mha with production of 8.02 mt and productivity is 1262 kg/ha (Patel and Singh 2017). It has an oil content ranging from 35-45% (Patel et al. 2017). It also contains an adequate amount of two essential fatty acids, linoleic and linolenic acids. Besides being used as edible oil, the seeds and oil of mustard have peculiar pungency, thus used as condiment in preparation of pickles, flavoring curries and vegetables. Mustard (*Brassica juncea* (L.) Czern and Coss) is damaged by a number of insect-pests. *viz.,* sawfly (*Athalia lugens*), aphid (*Lipaphis erysimi*), painted bug (*Bagrada hilaris* L.) and leaf miner (*Phytomyza horticola*). According to Sachan and Purwar (2007), rapeseed and mustard is attacked by more than 43 insect species, out of which mustard aphid, *Lipaphis erysimi* (Kalt.) is the key pest of the crop in India. Both nymphs and adults suck the sap from tender leaves, buds and pods. Curling may occur in infested leaves and at an advanced stage, plants may wither and die. Plants remain stunted and sooty molds grow on the honeydew excreted by the insects which affects the photosynthesis process and resulting in reduction of yield.

MATERIALS AND METHODS
The experiment was laid out in a randomized block design with six treatments including control (untreated) and replicated four times with the plot area of 12 m^2 during both the years (Fig 1). One botanical based insecticide and four entomopathogens were used as foliar spray. The untreated (control) plots were also maintained for the comparison. The spray was done by using a "Knapsack sprayer". The suspension was sprayed only at a time when economic threshold level of mustard aphid was attained. The quantity of spray solution used was 500 to 750 liters per hectare. The data of aphid population were recorded from five randomly selected and tagged plants in each treatment from the 10 cm terminal portion of central shoot of a plant using magnifying lens Fig 2. Pre-treatment count was taken just one day before the application of insecticide/ entomopathogens. The post treatment counts were recorded on the 7^{th} and 10^{th} days after application. Seed yield was also recorded on harvesting of the crop.

Figure 1. Field view of experiment

Figure 2. Aphid infestation on mustard.

The observation on aphid infestation was recorded as aphid infestation index (AII) on a 0–5-point scale and per cent reduction in aphid population /10 cm of central twig (Rana 2005). The data obtained on aphid population was converted into percent reduction by using correction factor given by Henderson and Tilton (1955) referring it to be a modification of Abbot's (1925) formula:

Percentage reduction =

$$100 \times \left[1 - \frac{Ta \times Cb}{Tb \times Ca}\right]$$

where,

T_a – Number of insects after treatment of spray

T_b - Number of insects before treatment of spray

C_a - Number of insects in untreated control after treatment of spray

C_b - Number of insects in untreated control before treatment of spray

The data obtained on percent reduction of aphid population were transformed into angular values and subjected to statistical analysis and seed yields were converted into qha^{-1}.

RESULTS AND DISCUSSION

Among the tested treatments, *V. lecanii* ($1x10^8$) @ 5.0 g/l was found most effective and significantly superior over other treatments (Table 1).

Table 1. Efficacy of entomopathogenic fungi against mustard aphid at Agricultural Research Station (ARS), Mandor

Treatments	Aphid Infestation Index (AII)				Yield
	Before spray	7 DAS	10 DAS	Average	(q ha-1)
M. anisopliae ($1x10^8$) @ 5.0 g/l	2.44	1.76^{c}	1.15^{d}	1.46^{b}	16.26^{c}
B. bassiana ($1x10^8$) @ 5.0 g/l	2.35	1.86^{bc}	1.36^{c}	1.61^{b}	17.79^{b}
V. lecanii ($1x10^8$) @ 5.0 g/l	2.42	0.30^{a}	0.10^{a}	0.20^{a}	20.21^{a}
P. lilacinus ($1x10^8$) @ 5.0 g/l	2.39	1.96^{b}	1.42^{c}	1.69^{b}	17.89^{b}
Neem based insecticide (0.15%)	2.36	0.36^{a}	0.46^{b}	0.41^{c}	19.43^{a}
Control	2.38	3.12^{d}	3.86^{e}	3.49^{d}	14.35^{d}
SEm±	0.26	0.05	0.04	0.05	0.47
CD at 5%	NS	0.17	0.12	0.16	1.43
CV (%)	10.26	9.47	11.31	9.85	12.56

Figures super scribed with same letter are at par with one another

Maximum seed yield (20.21 q ha^{-1}) was obtained from the plots treated with *V. lecanii* ($1x10^8$) @ 5.0 g/l followed by neem-based insecticide 0.15% (19.43 q ha^{-1}) and both treatments are at par to each other. Minimum seed yield was recorded in control (14.35 q ha^{-1}) followed by the plots treated with *M. anisopliae* ($1x10^8$) @ 5.0 g/l (16.26 q ha^{-1}).

At Adaptive Trial Center (ATC) and Cultivated Fields (CF)

Data revealed that after two sprays of *V. lecanii* ($1x10^8$) @ 5.0 g/l was found most effective in reducing aphid population followed by neem-based insecticide (0.15%) and significantly superior than other treatments. The treatments *M. anisopliae* ($1x10^8$) @ 5.0 g/l and *B. bassiana* ($1x10^8$) @ 5.0 g/l were recorded least effective and these were found at par to each other.

Maximum seed yield (19.33 and 15.00 q ha^{-1}) was obtained from the plots treated with *V. lecanii* ($1x10^8$) @ 5.0 g/l and minimum (12.25 and 11.81 q ha^{-1}) was found in control plot at Adaptive Trial Center and Cultivated Fields, respectively (Table 2).

Similar findings reported by Singh et al. (2008) evaluated *V. lecanii* @ 10^8 spores/ml against mustard aphid in the field and found some promising results. Similarly, Meena et al. (2013) also reported that significantly low (10.55 - 30.03 aphids/plant) population of mustard aphid was recorded in the treatments viz., *V. lecanii* @ 5 g per litre of water, *B. bassiana* @ 5 g per litre of water, *M. anisopliae* @ 5 g per litre of water, cow urine @ 50 litre per ha, tobacco extract @ 5%, onion extract @ 5%, NSKE @ 5% and dimethoate 30 EC @ 300 g a.i/ha over control (170.75 aphid/plant).

In contrary to the present findings, Deka et al. (2017) reported that the entomopathogenic fungi *Metarhizium anisopliae* (1×10^8) was found most effective and recorded highest mortality (69.20%) followed by *B. bassiana* (≥ 50% mortality from the 5th day onwards) and *Verticillium lecanii* (60%> mortality obtained at 6th day). Ujan and Shahzad (2012) tested the efficacy of *V. lecanii*, *M. anisopliae* and *B. bassiana* and recorded 98.00, 72.00 and 88.00% mortality of mustard aphid after 3 days at 10^7 spore ml^{-1}. Saranya et al. (2010) reported *V. lecanii* (10^7 spores ml^{-1}), *M. anisopliae* (10^7 spores ml^{-1}) and *B. bassiana* (10^7 spores ml^{-1}) caused 100.00, 83.30 and 61.50% mortality, respectively of *Aphis craccivora* after 7 days. The difference in virulence of the fungal isolates may be attributed to genetic variation in fungal strains, difference in bioassay methods, aphid species and to different abiotic and biotic factors

Table 2. Efficacy of entomopathogenic fungus against mustard aphids at Adaptive Trial Center (ATC) and Cultivated Fields (CF)

Treatments	PTP	First spray		PTP	Second spray		Yield (q ha^{-1})	
		Per cent reduction in aphid population /10 cm of central twig			Per cent reduction in aphid population /10 cm of central twig			
		After 7 DAS	After 10 DAS		After 7 DAS	After 10 DAS	ATC	CF
M. anisopliae ($1x10^8$) @ 5.0 g/l	47.65	19.88 (26.48)	25.15 (30.10)	46.05	23.60 (29.06)	25.34 (30.22)	14.75	12.99
B. bassiana ($1x10^8$) @ 5.0 g/l	47.65	23.23 (28.81)	26.24 (30.82)	43.70	26.93 (31.26)	27.03 (31.33)	15.08	13.30
V. lecanii ($1x10^8$) @ 5.0 g/l	50.35	46.56 (43.03)	61.08 (51.40)	42.50	59.56 (50.56)	64.92 (53.68)	19.33	15.00
P. lilacinus ($1x10^8$) @ 5.0 g/l	48.05	20.98 (27.26)	28.59 (32.32)	46.75	25.58 (30.38)	28.92 (32.53)	14.00	12.90
Neem based insecticide (0.15%)	50.05	38.93 (38.60)	53.04 (46.74)	41.80	47.62 (43.64)	54.44 (47.55)	17.00	14.21
Control	52.50	--	--	86.25	--	--	12.25	11.81
SEm±	--	0.84	0.71	--	0.64	0.88	59.26	--
CD at 5%	--	2.60	2.18	--	1.98	2.71	182.6	--
CV (%)	--	9.54	9.27	--	10.96	9.03	14.39	--

*Figures in parenthesis are angular transformed value, ATC= Adaptive Trial Center, CF= Cultivated Field, PTP-= Pretreatment population

CONCLUSION

Among all the treatments, *Verticillium lecanii* was the most effective fungi for the control of mustard aphid, *L. erysimi*. Maximum seed yield was obtained from the plots treated with *V. lecanii* ($1x10^8$) @ 5.0 g/l.

Acknowledgements

The authors are thankful to University Authorities for their support, guidance and providing necessary facilities to conduct experiments.

References

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

Deka AC, Goswami NK, Sarma I. 2017. Biocontrol Prospects of Entomopathogenic Fungi for Management of Mustard Aphid (*Lipaphis erysimi* Kalt.) on Rapeseed-Mustard. Advances in Applied Science Research 8(4): 21-29.

Henderson CF, Tilton EW. 1955. Tests with acaricides against the brown wheat mite. Journal of Economic Entomology 48:157-161.

Meena H, Singh SP, Nagar R. 2013. Evaluation of microbial agents and bio-products for the management of mustard aphid, *Lipaphis erysimi* (Kalt.). The Bioscan 8 (3): 747-750.

Patel S, Singh CP. 2017. Development of weather based forewarning model for mustard aphid, *Lipaphis erysimi* (Kalt.) in Tarai region of Uttarakhand. Journal of Agrometeorology 19 (4):382.

Patel S, Yadav SK, Singh CP. 2017. The incidence of painted bug, *Bagrada hilaris* (Burmeister) on *Brassica spp*. and *Eruca sativa* with respect to the date of sowing. Journal of Entomology and Zoology Studies 5(1):774-776.

Rana JS. 2005. Management of mustard aphid, *Lipaphis erysimi* (Kalt.) by use of an entomopathogenic fungi, *Verticillium lecanii* under field conditions. Department of Entomology, CCS Haryana Agricultural University Hisar, 2005.

Sachan GC, Purwar JP. 2007. Integrated insect pest management in rapeseed and mustard. In: Entomology novel approaches (Eds. Jain P.C. and Bhargava M.C), New India Publishing Agency, New Delhi, 550.

Sandhu SS, Sharma AK, Beniwal V, Goel G, Batra P, Kumar A, Jaglan S, Sharma AK, Malhotra S. 2012. Myco-biocontrol of insect pests: Factors involved, mechanism and regulation. Journal of Pathogens doi:10.1155/2012/126819.

Saranya S, Ushakumari R, Sosamma J, Philip BM. 2010. Efficacy of different entomopathogenic fungi against cowpea aphid *Aphis craccivora* (Koch). Journal of Biopesticides 3: 138-142.

Shah FA, Butt TM. 2005. Influence of nutrition on the production and physiology of sectors produced by the insect pathogenic fungus *Metarhizium anisopliae*. FEMS Microbiology Letter 250: 201-207.

Singh YP, Singh SP, Meghwal HP. 2008. Evaluation of bioagents against mustard aphid, *Lipaphis erysimi* (Kalt.) (Homoptera: Aphididae), under net covered condition in the field. Journal of Biological Control. 22: 321-326.

Thakur R, Sandhu SS. 2010. Distribution, occurrence and natural invertebrate hosts of indigenous entomopathogenic fungi of Central India. Indian Journal of Microbiology 50: 89-96.

Ujan AA, Shahzad S. 2012. Use of entomopathogenic fungi for the control of mustard aphid (*Lipaphis erysimi*) on Canola (*Brassica napus* L.). Pakistan Journal of Botany 44: 2081-2086.

SESSION 4

Insect Plant Interactions, Host Plant Resistance, and Chemical Ecology of Crucifer Pests

Colonization of organic cabbage by key pests and beneficial insects in the presence of companion plants

Zulaikha, M.
HORTICULTURE RESEARCH CENTER,
MALAYSIAN AGRICULTURAL RESEARCH AND DEVELOPMENT INSTITUTE, 43400 SERDANG, SELANGOR, MY
email: zulaikha@mardi.gov.my

Liburd, O.E.
ENTOMOLOGY AND NEMATOLOGY DEPARTMENT,
UNIVERSITY OF FLORIDA
BLDG 970 NATURAL AREA DRIVE
GAINESVILLE, FL 32611
USA
email: oeliburd@ufl.edu

ABSTRACT
Current management strategies to control major pests of cabbage including diamondback moth (DBM), *Plutella xylostella* (L), rely heavily on conventional insecticides. The overuse of these insecticides have led to the development of resistance and negative effects on non-target organisms. The purpose of this study was to evaluate alternatives to chemical control for sustainable management of insect pests of cole crops. The colonization of cabbage pests and their natural enemies were investigated for 2 years in cabbage intercropped with different companion plants. Five treatments were evaluated including cabbage intercropped with 1) marigold (*Tagetes patula*), 2) collards (*Brassica oleracea* var. *acephala*), 3) roselle *Hibiscus sabdariffa*, 4) cabbage treated with Entrust® (Spinosad), and 5) untreated cabbage. All treatments were replicated four times in a randomized complete block design. During 2016 with the exception of marigold, all treatments reduced the population of DBM below the control. There were no other significant cabbage pests recorded. In 2017, three major lepidopteran pests, cabbage worm *Pieris rapae* L., cabbage looper *Trichoplusia ni* (Hübner) and DBM were recorded. DBM larvae were significantly fewer in cabbage treated with Entrust® compared with other treatments. Natural enemy populations were high in cabbage intercropped with companion plants with 10 and 20 families of predators and parasitoids recorded, respectively. Higher numbers of anthocoridae and carabid beetles were recorded in plots treated with marigold. More parasitoids were also collected from plots interplanted with marigold and roselle compared to the control and Entrust® treatment. Among the companion plants, roselle and marigold appear to have the best potential for integrating with Entrust® to manage lepidopteran pests in organic cabbage.

Keywords
Companion plants, roselle, marigold, IPM

INTRODUCTION
Companion planting is one of the cultural practices used to manage insect populations with some success (Parker et al. 2013). This is in part due to several mechanisms including a diverse cropping system, which provides a wider array of resources (food and oviposition sites) for natural enemies (Root 1973). Insect 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. Dover (1986) intercropped garden sage (*Salvia officinalis* L.), white clover (*Trifolium repens* L.) and other herbs with Brussels sprouts to reduce DBM oviposition and damage on brassicas.

Collards (*Brassica oleracea*) and marigolds (genus *Tagetes*) are commonly planted intercropped with cabbage in organic fields in southeastern US. Marigold is known to attract 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 several different species of parasitoid wasps (Gredler 2001). Another plant that has potential to be used as a companion plant with brassicas is roselle (*Hibiscus sabdariffa* L.). Roselle had been previously intercropped in tomatoes where it increased parasitoid diversity but did not reduce pest population (Smith et al. 2001).

Implementation of companion planting into traditional monocrop systems may show potential for sustainable management of cabbage pests, and consequently reduce the frequency and amount of insecticides and enhance natural enemy populations. Therefore, two years of field experiments were conducted involving the integration of roselle, collard, and marigold as companion planting for cabbage. The objectives of this study were to 1) evaluate the colonization of key pests on organic cabbage including DBM, imported cabbageworm (CW) *Pieris rapae* L. (CW), cabbage looper *Trichoplusia ni* (Hübner) (CL), and aphids in the presence of companion plants, and 2) determine how selected companion plants and an insecticide Entrust® (spinosad) labeled for organic use affect natural enemy populations and marketable yields in a 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 done at the University of Florida Plant Science Research and Education Unit (PSREU) in Citra, (location: 29.410868N, 82.141572W) Marion County, Florida, USA.

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* (Seed Area, 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. 2015). 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 their respective treatment beds.

In 2017, cabbage and collard seeds were planted in seedling trays in the SFVIPM greenhouse (as described in 2016) but seedlings were transplanted at 7 weeks. Marigolds and roselle were also initially grown for 4 weeks in the SFVIPM laboratory 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 (between cabbage rows). In 2016, each bed was 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 without competing with cabbage for mineral salts and water.

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® (Spinosad) (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, Nature Safe, Irving, TX) was applied weekly at 236 liters per ha.

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.

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 for pest populations. Visual counting was conducted starting from the second week after transplanting and ended a week before harvest.

Estimating Natural Enemies Population

Yellow sticky traps (14 cm x 11.5 cm) were deployed on each bed and used to observe the presence of natural enemies (predators and parasitoids). Traps were collected after 5 d in the field and replaced each week. In 2016, sampling was conducted from Mar 18 through May 6 and in 2017 data were collected from Mar 15 through May 17. Traps were brought back to SFVIPM and assessed under a 10X dissecting microscope. The number of natural enemies (predators and parasitoids) and cabbage pests caught on the traps were recorded and identified to the family. Additionally, these traps gave relative density information on other minor pests and predators including alate aphids, adult whiteflies, thrips, adult minute pirate bug, and adult big-eyed bug.

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 a 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.

Marketable Cabbage

In both seasons, twenty cabbage heads were harvested randomly from each treatment. Cabbage heads with no observable insect injury to minor injury (no damage after removing 4 folded leaves) were rated as marketable, while cabbage heads with obvious to severe injuries were rated as non-marketable. 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 were 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 by 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 (Fig. 1).

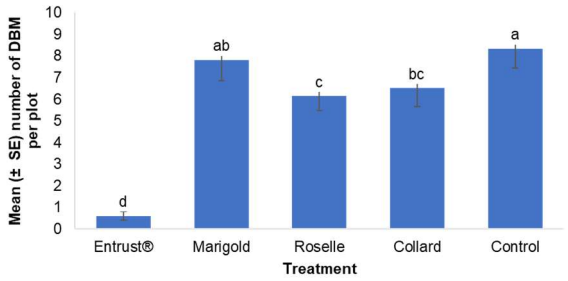


Figure 1. Overall mean ± SE number of diamondback moth (DBM) recorded over an eight-week period for the companion planting study in 2016. Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD). Entrust® application rate (0.29 L per ha).

Weekly *in-situ* counts for DBM in 2016 showed that significant differences varied throughout the 8 weeks of sampling (Fig. 2). 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 treated with roselle planted as a companion plant had significantly fewer DBM than the control (F = 4.63; df = 4,95; P = 0.002) (Fig 2).

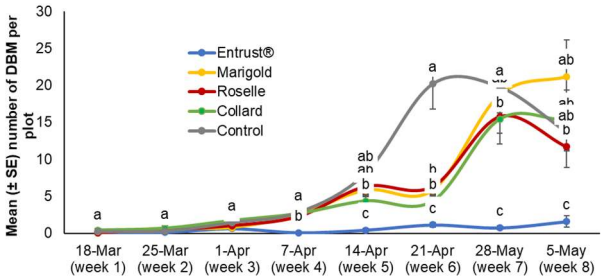


Figure 2. Mean ± SE number of diamondback moth (DBM) population observed weekly during *in-situ* counts over an eight-week period for the companion planting study in 2016. Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD). Entrust® application rate (0.29 L per ha).

In-situ Counts in 2017

Three major lepidopteran pests including DBM, CW, and CL were recorded (Table 1). Significant differences among the treatments were observed in the number of DBM ($F = 9.50$; $df = 4,950$; $P < 0.0001$) and CW ($F = 5.28$; $df = 4,950$; $P = 0.0003$). 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 1). Significantly fewer CW larvae were recorded in plots treated with Entrust® compared with other treatments. 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. Both treatments (marigold and roselle) had numerically lower numbers of CW compared with control. There was no significant difference among the treatments for CL over time (Table 1).

Table 1. Mean ± SE number of lepidopteran pests observed during *in-situ* counts over a ten-week period for the companion planting study in 2017.

Treatment	DBM	Cabbage worm	Cabbage looper
Entrust®	0.07 ± 0.03 b	0.03 ± 0.01 c	0.01 ± 0.01 a
Marigold	0.34 ± 0.05 a	0.16 ± 0.04 ab	0.06 ± 0.02 a
Roselle	0.38 ± 0.06 a	0.16 ± 0.03 ab	0.07 ± 0.03 a
Collard	0.49 ± 0.07 a	0.12 ± 0.03 b	0.04 ± 0.01 a
Control	0.45 ± 0.08 a	0.21 ± 0.04 a	0.05 ± 0.02 a

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

Secondary Cabbage Pests

Aphids

Aphid species included the green peach aphid *Myzus persicae* (Sulzer) and the cabbage aphid *Brevicoryne brassicae* (L.). There were no significant differences among treatments for aphids observed on yellow sticky traps in 2016 ($F = 0.41$; $df = 4,600$; $P = 0.80$) and in 2017 ($F = 0.61$; $df = 4,280$; $P = 0.66$) (Fig. 3).

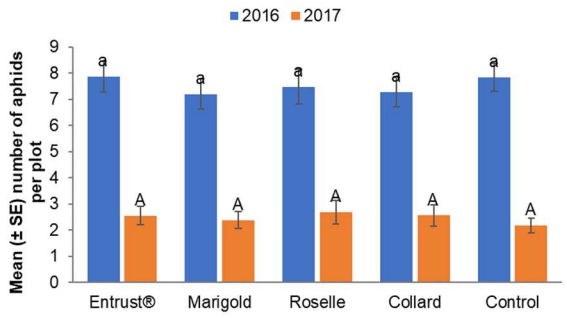


Figure 3. Mean ± SE number of aphids collected from yellow sticky traps for the companion planting study in 2016 and 2017. Treatments with the same letter are not significantly different *P* ≤ 0.05 (LSD). Entrust® application rate (0.29 L per ha).

Whiteflies

The whitefly species recorded was sweet-potato whitefly biotype B *Bemisia tabaci* (Gennadius). There were no significant differences among treatments for adult whiteflies observed on yellow sticky traps in 2016 ($F = 0.47$; $df = 4,600$; $P = 0.76$) (Fig. 4). In 2017, significantly fewer adult whiteflies collected in cabbage plots treated with marigolds compared to the other treatments. Significantly fewer adult whiteflies ($F = 11.88$; $df = 4,280$; $P < 0.0001$) were also collected in cabbage plots treated with roselle compared to plots treated with Entrust® and interplanted with collards (Fig. 4).

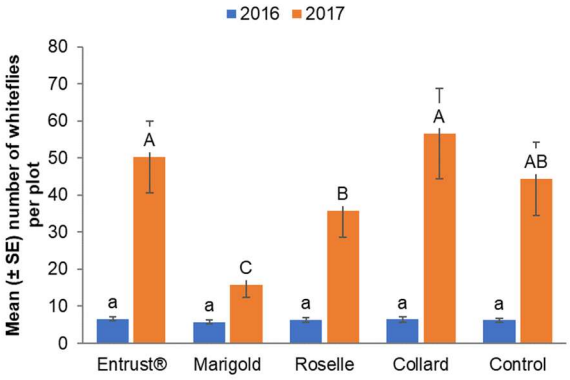


Figure 4. Mean ± SE number of whiteflies collected from yellow sticky traps for the companion planting study in 2016 and 2017. Treatments with the same letter are not significantly different *P* ≤ 0.05 (LSD). Entrust® application rate (0.29 L per ha).

Thrips

The principal thrips species recorded was Florida flower thrips, *Frankliniella bispinosa* (Morgan). There were no significant differences among treatments for thrips recorded on yellow sticky traps in 2016 ($F = 1.27$; $df = 4,600$; $P = 0.28$) (Fig. 5). In 2017, the number of thrips showed significant difference by treatment ($F = 11.03$; $df = 4,280$; $P < 0.0001$). Plots interplanted with marigolds had significantly higher thrips population compared with the other treatments (Fig. 5).

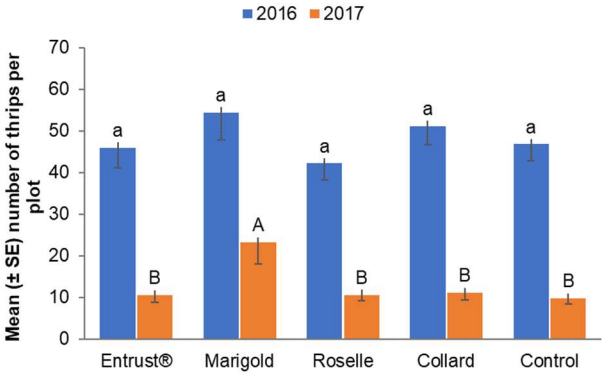


Figure 5. Mean ± SE number of thrips collected from yellow sticky traps for the companion planting study in 2016 and 2017. Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD). Entrust® application rate (0.29 L per ha).

Beneficial Insects

Predators

The predators observed on yellow sticky traps include ants (Formicidae), green lacewings (Chrysopidae), brown lacewings (Hemerobiidae), 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). The predators that were captured in the pitfall trap include ground beetles (Carabidae), spiders, ants, ladybird beetles, and dragonflies (Coenagrionidae).

In 2016, there were significant differences among treatments for the overall number of predators (F = 3.21; df = 4,600; P = 0.013) captured on yellow sticky traps (Fig. 6). Significantly higher numbers of predators were recorded on cabbage plots interplanted with marigolds compared with Entrust®, roselle, and collards (Fig. 6). Among the predators captured in pitfall traps, only ground beetles showed significant differences in treatments (F = 2.93; df = 4,20; P = 0.03) (Fig. 7). Cabbage plots interplanted with roselle had significantly higher populations of ground beetles compared to the other treatments except for the control plots. Also, cabbage plots interplant with collard had fewer ground beetles than the control (Fig. 7).

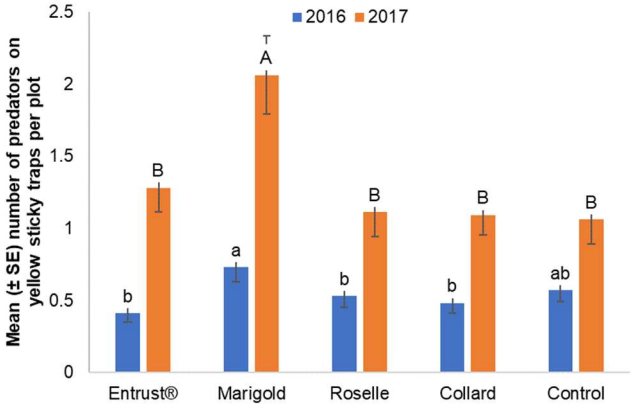


Figure 6. Overall mean ± SE number of predators collected from yellow sticky traps for the companion planting study in 2016 and 2017. Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD). Entrust® application rate (0.29 L per ha).

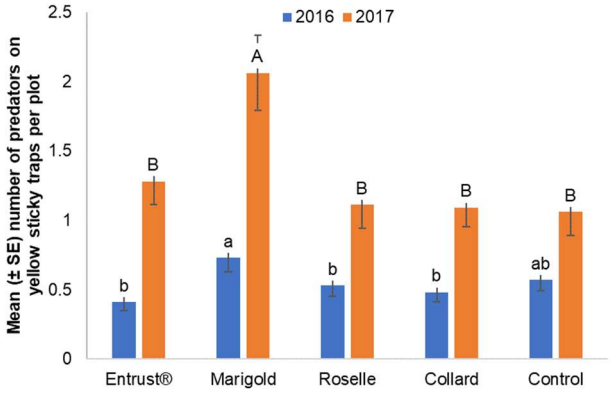


Figure 7. Mean ± SE number of ground beetles collected from pitfall traps for the companion planting study in 2016 and 2017. Treatments with the same letter are not significantly different *P* ≤ 0.05 (LSD). Entrust® application rate (0.29 L per ha).

In 2017, predators observed on yellow sticky traps (ants, including green lacewings, minute pirate bugs, ladybird beetles, spiders, predatory thrips, ground beetles, rove beetles, big-eyed bugs, and tachinid flies) showed significant differences in treatment (F = 6.20; df = 4,600; P < 0.0001) (Fig. 6). There were significantly more predators, specifically minute pirate bugs (F=29.62; df = 4,280; P < 0.0001) recorded in plots interplanted with marigolds compared to the other treatments (Fig. 8).

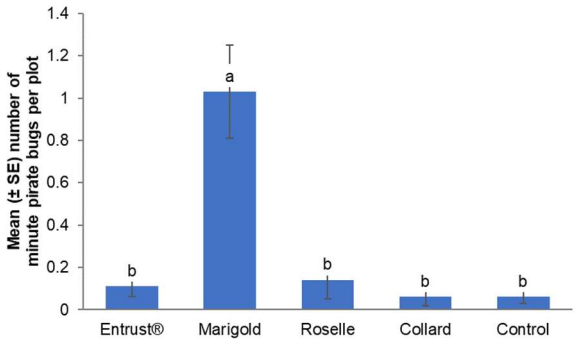


Figure 8. Mean ± SE number of minute pirate bugs (anthocoridae) collected from yellow sticky traps over a ten-week period for the companion planting study in 2017. Treatments with the same letter are not significantly different *P* ≤ 0.05 (LSD). Entrust® application rate (0.29 L per ha).

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 (F = 4.70; df = 4,60; P = 0.002) (Fig. 7).

Parasitoids

Twenty families of parasitoids were recorded on yellow sticky traps in 2016 and 2017. Six families were known to parasitize DBM including Braconidae, Chalcididae, Eulophidae, Pteromalidae, Trichogrammatidae, and Ichneumonidae. Other parasitoids families observed include Aphelinidae, Encyrtidae, Eupelmidae, Eurytomidae, Mymaridae, Signiphoridae, Ceraphronidae, Megaspilidae, Bethylidae, Figitidae, Evaniidae, Mymarommatidae, Platygastridae, and Diapriidae.

In 2016, there were significant differences between treatment in the overall number of parasitoids sampled by yellow sticky traps (F = 58.16; df = 4,280; P < 0.0001). Significantly more parasitoids were recorded in the control plots compared with all the other treatments. We 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 (Fig. 9).

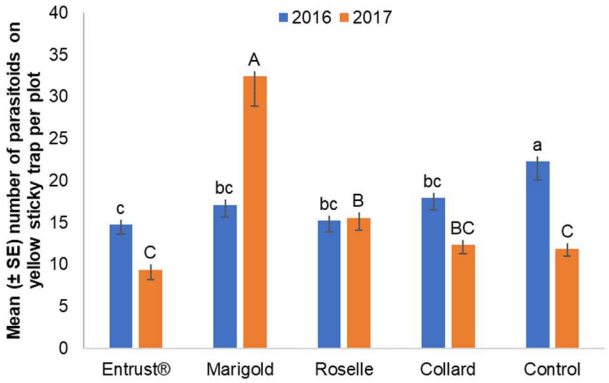


Figure 9. Overall mean ± SE number of parasitoids collected from yellow sticky traps for the companion planting study in 2016 and 2017. Treatments with the same letter are not significantly different $P \leq 0.05$ (LSD).

In 2017, there were significant differences among treatments for overall number of parasitoids captured using yellow sticky traps ($F = 58.16$; df = 4,280; $P < 0.0001$) (Fig. 9). Significantly more parasitoids were recorded in plots interplanted with marigolds compared to the other treatments. Additionally, significantly more parasitoids were also collected from plots interplanted with roselle compared to thec control and Entrust® treatment. Cabbage plots interplanted with collards, treated with Entrust® and the control had fewer parasitoids and were not significantly different from each other (Fig. 9).

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 (week 4: $F = 2.90$; $df = 4,35$; $P = 0.04$), (week 5: $F = 4.07$; $df = 4,35$; $P = 0.008$), (week 6: $F = 10.22$; $df = 4,35$; $P < 0.0001$), (week 7: $F = 11.16$; $df = 4,35$; $P < 0.0001$) (week 9: $F = 28.67$; $df = 4,35$; $P < 0.0001$), (week 10: $F = 24.47$; $df = 4,35$; $P < 0.0001$). Parasitoid populations peaked at 64 d (week 7) after planting in all treatments except for roselle, which peaked later at 78 d (week 9) (Fig. 10).

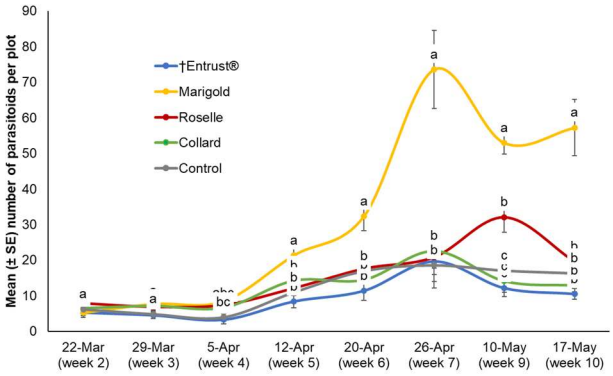


Figure 10. Mean ± SE number of parasitoids collected from yellow sticky traps over a ten-week period for the companion planting study in 2017.

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 ($F = 4.07$; $df = 4,15$; $P = 0.02$) (Fig. 11). 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.

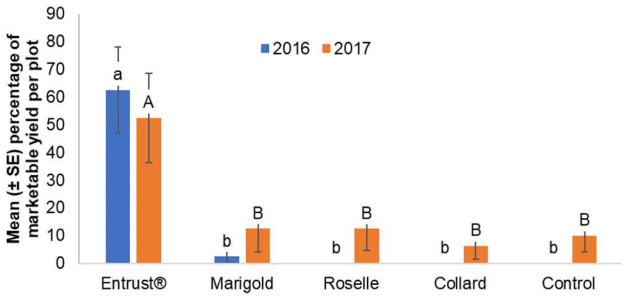


Figure 11. Overall mean ± SE percentage of marketable yield for the companion planting study in 2016 and 2017. Treatments with the same letter are not significantly different *P* ≤ 0.05 (LSD). Entrust® application rate (0.29 L per ha).

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. Maxwell and Fadamiro (2006) also reported that Entrust® provided the most consistent and lowest mean damage ratings against lepidopteran pests in cole crops. 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 must rotate Entrust® with other insecticides or pest management tactics for management of lepidopteran complex in cole crops. The active ingredient in Entrust® is spinosad and the formulation is labelled for organic use. Entrust® belongs to a relatively new class of insecticides called Naturalytes™. The pesticide effects are produced through a fermentation process of naturally occurring bacteria (*Saccharpolyspora spinosa*). Spinosad acts through a novel site in the nicotinic receptor which is different from other insecticides including neonicotinoids (Dripps et al. 2008).

Interplanting insect deterring plants such as roselle or marigold are alternatives to relying exclusively on insecticides that are labeled for organic use. In our 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 treatment; however, Al-Mamun et al. (2011) recorded 100% mortality against *Tribolium castaneum* Herbst (Tenebrionidae) using fruit extracts from roselle. 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 laboratory bioassay we 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 (O.E.L unpublished data). 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 Malvaceae 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) 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 a 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 were found to be effective in reducing DBM damage (Mitchell et al. 1997, 2000; Badenes-Perez et al. 2004; Musser et al. 2005).

In 2017, the lowest number of cabbage pests (total insect pest population) was recorded on cabbage plots interplanted with marigold. Volatiles emitted from marigolds 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 were reported to reduce aphid reproduction (Tomova et al. 2005). Finally, the flowers produced by marigold were found to attract the most predators and parasitoids into our research plots. Similar findings were reported by Silveira et al. (2009).

The absence of cabbage yield in 2016 caused us to modify the 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 or a rotation spray to Entrust®, a grower standard.

Only a few 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 (Fig. 12). During the early cabbage season, mustards on the adjacent research plot were flowering (Fig. 13), promoting high densities of CW in our field plots. CWs have strong preference for bright yellow flowers and the floral scent from mustard flowers may stimulate the frequent visitation of these pests on mustard (Ômura et al. 1999).

Figure 12. Strawberry research plot next to cabbage research plots.

Figure 13. Imported cabbage worm, *Pieris rapae* adult on Mustard *Brassica rapa* flowers.

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) evaluated the potential to reduce whitefly population in common bean, *Phaseolus vulgaris* L., by intercropping with poor and non-host plants that included 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 host for these 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.

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, Razze et al. (2016) also reported that aphid densities were reduced in squash production systems when intercropped with buckwheat, *Fagopyrum esculentum* Moench.

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, the 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 hypothesis is that marigolds provided more ground covered areas that may have influenced predation activities for these beetles. Previous studies using similar systems 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 beetle activities. Increasing the possibilities of the prey to be encountered by this predator. In 2017, minute pirate bug populations were also high 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). Peshin (2014) indicated that marigolds, particularly *T. tenuifolia*, positively influenced minute pirate bugs and parasitoid populations.

Parasitoid abundance was positively correlated with the flowering phase of marigold and roselle in both growing seasons. Higher parasitoid densities were 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 these wasps recorded on untreated plots may be associated with the high DBM pressure on this plot. In 2017, the parasitoid in the Platygastridae 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 enhance 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 injury was caused by imported cabbage worms, 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 suggests 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 pests 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 labeled for organic use, they have the potential of providing sufficient protection for cabbage pests and reducing economic damage.

CONCLUSION

Integrating flowering companion plants could enhance natural enemy population by providing additional groundcover and extra floral nectar to predators and parasitoids. These plants positively influence the parasitism and predation activities on cabbage pests (Balmer et al. 2013). Roselle was found to have a deterrent effect on DBM. 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 systems.

Acknowledgements

We would like to thank the University of Florida Plant Science, Research and Education Unit (PSREU) in Citra, Florida for providing space, preparation, and maintenance of the field for this research. We thank the members of the University of Florida Fruits and Vegetables Laboratory for their help and support.

References

Adedipe F, Park YL. 2010. Visual and olfactory preference of *Harmonia axyridis* (Coleoptera: Coccinellidae) adults to various companion plants. *J. Asia. Pac. Entomol.* 13: 319–323.

Al-Mamun M, Khatun H, Nesa ML, Islam MR, Munira MS. 2011. In vitro evaluation of the antibacterial, cytotoxic and insecticidal activities of *Hibiscus sabdariffa* fruits. *Libyan Agric. Res. Center J. Int.* 2: 144–149.

Armstrong G, McKinlay RG. 1997. Vegetation management in organic cabbages and pitfall catches of carabid beetles. *Agric. Ecosyst. Environ.* 64: 267–276.

Asare-Bediako E, Addo-Quaye AA, Mohammed A. 2010. Control of diamondback moth (*Plutella xylostella*) on cabbage (*Brassica oleracea* var capitata) using intercropping with non-host crops. *Am. J. Food Technol.* 5: 269–274.

Badenes-Perez FR, Shelton AM, Nault BA. 2004. Evaluating trap crops for diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *J. Econ. Entomol*. 97: 1365–1372.

Bird TG, Hedin PA, M. L. Burks ML. 1987. Feeding deterrent compounds to the boll weevil, *Anthonomus grandis* Boheman in Rose-of-Sharon, *Hibiscus syriacus* L. *J. Chem. Ecol.* 13: 1087–1097.

Björkman M, Hambäck PA, Hopkins RJ, Rämert B. 2010. Evaluating the enemies hypothesis in a clover-cabbage intercrop: Effects of generalist and specialist natural enemies on the turnip root fly (*Delia floralis*). *Agric. For. Entomol.* 12: 123–132.

Cai H, You M, Lin C. 2010. Effects of intercropping systems on community composition and diversity of predatory arthropods in vegetable fields. *Acta Ecol. Sin*. 30: 190–195.

Cortesero AM, Stapel JO, Lewis WJ. 2000. Understanding and manipulating plant attributes to enhance biological control. *Biol. Control*. 17: 35–49.

Ditner N, Balmer O, Beck J, Blick T, Nagel P, Luka H. 2013. Effects of experimentally planting non-crop flowers into cabbage fields on the abundance and diversity of predators. *Biodivers. Conserv*. 22: 1049–1061.

Dover JW. 1986. Effect of labiate herbs and white clover on *Plutella xylostella* oviposition. *Entomol. Exp. et Appl.* 42: 243 -247.

Dripps J, Olson B, Sparks T, Crouse G. 2008. Spinetoram: How artificial intelligence combined natural fermentation with synthetic chemistry to produce a new spinosyn insecticide. *Plant Health Progress* doi:10.1094/PHP-2008-0822-01-PS

Faizi S, Khan RA, Azher S, Khan SA, Tauseef S, Ahmad A. 2003. New antimicrobial alkaloids from the roots of *Polyalthia longifolia* var. pendula. *Planta Med.* 69: 350–355.

Funderburk J, Stavisky J, Olson S. 2000. Predation of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in field peppers by *Orius insidiosus* (Hemiptera: Anthocoridae). *Environ. Entomol.* 29: 376–382.

Gredler G. 2001. Encouraging beneficial insects in your garden. [Covallis, Or.]: Oregon State University Extension Service. http://hdl.handle.net/1957/38715 (Accessed Jan 2017).

Honda H, Bowers WS. 1996. Feeding and oviposition deterrent activities of flower buds of globemallow, *Sphaeralcea emoryi* Torrey, against boll-weevil, *Anthonomus grandis* Boheman (Coleoptera, Curculionidae). *J. Chem. Ecol.* 22: 139–150.

Jankowska B. 2010. Effect of intercropping white cabbage and pot marigold (*Calendula officinalis*) on diamondback moth (*Plutella xylostella* L.) population density and it's parasitoid complex. Veg. *Crop. Res. Bull.* 73: 107–117.

Jankowska B, Poniedziałek M, Jędrszczyk E. 2009. Effect of intercropping white cabbage with French Marigold (*Tagetes patula* nana L.) and Pot Marigold c*Calendula officinalis* L.) on the colonization of plants by pest insects. *Folia Hortic.* 21: 95–103.

Maxwell EM, Fadamiro HY. 2006. Evaluation of several reduced-risk insecticides in combination with an action threshold for managing Lepidopteran pests of cole crops in Alabama. *Florida Entomol.* 89: 117–126.

Mitchell ER, Hu G, Johanowicz D. 2000. Management of diamondback moth (Lepidoptera: Plutellidae) in cabbage using collard as a trap crop. *Hortsci.* 35: 875–879.

Mitchell ER, Hu GY, Okine JS. 1997. Diamondback Moth (Lepidoptera: Plutellidae) infestation and parasitism by *Diadegma insulare* (Hymenoptera: Ichneumonidae) in collards and adjacent cabbage fields. *Florida Entomol.* 80: 54.

Musser FR, Nault BA, Nyrop JP, Shelton AM. 2005. Impact of a glossy collard trap crop on diamondback moth adult movement, oviposition, and larval survival. *Entomol. Exp. et Appl.* 117: 71–81.

Ômura H, Honda K, Hayashi N. 1999. Chemical and chromatic bases for preferential visiting by the cabbage butterfly, *Pieris rapae*, to rape flowers. *J. Chem. Ecol.* 25: 1895–1906.

Parker JE, Snyder WE, Hamilton GC, Rodriguez C. 2013. Companion planting and insect pest control. In *Tech. Open Access.* http://dx.doi.org/10.5772/55044.

Peshin R. 2014. Integrated pest management. *Integr. Pest Manag. Pestic. Probl*. 3.

Rahat S, Gurr GM, Wratten SD, Mo J, Neeson R. 2005. Effect of plant nectars on adult longevity of the stinkbug parasitoid, *Trissolcus basalis*. *Int. J. Pest Manag.* 51: 321–324.

Razze JM, Liburd OE, Webb SE. 2016. Agroecology and sustainable food systems intercropping buckwheat with squash to reduce insect pests and disease incidence and increase yield intercropping buckwheat with squash to reduce insect pests and disease incidence and increase yield. *Agroecol. Sust. Food.* 40: 863–891.

Root RB. 1973. Organization of a plant-arthropod association in simple and diverse habitats: fauna of collards (Brassica oleracea). *Ecological Monographs* 43: 95-120.

Silveira LCP, Berti Filho E, Pierre LSR, Peres FSC, Louzada JNC. 2009. Marigold (*Tagetes erecta* L.) as an attractive crop to natural enemies in onion fields. *Sci. Agric.* 66: 780–787.

Smith HA, Mcsorley R, Arnoldo J, Izaguirre S. 2001. Effect of intercropping common bean with poor hosts and nonhosts on numbers of immature whiteflies (Homoptera: Aleyrodidae) in the Salamá Valley, Guatemala. *Environ. Entomol.* 30: 89–100.

Smith S. 1996. Biological control with Trichogramma: advances, successes, and potential of their use. *Annu. Rev. Entomol.* 41: 375–406.

Tolulope M. 2007. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. *J. Med. Plants Res*. 1: 9–13.

Tomova BS, Waterhouse JS, Doberski J. 2005. The effect of fractionated Tagetes oil volatiles on aphid reproduction. *Entomol. Exp. et Appl.* 115: 153–159.

Zotarelli L, Dittmar PJ, Ozares-Hampton M, Dufault NS, Webb SE, Wang Q, Miller C. 2015. Cole crop production. Vegetable production handbook for Florida. 33-51.

Effect of flowering yellow rocket (Brassicaceae) on crucifer insect pests and beneficial insects

Badenes-Pérez, F.R.
INSTITUTE OF AGRICULTURAL SCIENCES (CSIC), 28006 MADRID, SPAIN
email: frbadenes@ica.csic.es

Parrado Márquez, B.
INSTITUTE OF AGRICULTURAL SCIENCES (CSIC), 28006 MADRID, SPAIN

Petitpierre, E.
UNIVERSITAT DE LES ILLES BALEARS, 07122 PALMA DE MALLORCA, SPAIN
email: eduard.petitpierre@uib.es

ABSTRACT
This study tests whether the presence of flowering yellow rocket, *Barbarea vulgaris* R. Br. (Brassicaceae), affects different insect pests and beneficial insects in an adjacent cauliflower crop. Flowering *B. vulgaris* did not change the densities of insect pests found in adjacent cauliflower, except for *Eurydema ornata* L. (Hemiptera: Pentatomidae), which had reduced densities. Flowering reduced the attractiveness of *B. vulgaris* to *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), making it lose its effectiveness as a trap crop for this insect pest. However, flowering *B. vulgaris* increased *P. xylostella* parasitism by *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae). The lady beetles *Adalia bipunctata* L. and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), and several species of flea beetles of the genus *Phyllotreta* (Coleoptera: Chrysomelidae) were more abundant on flowering *Barbarea* than on cauliflower plants. Flowering *B. vulgaris* was visited by several species of aphidophagous hoverflies (Diptera: Syrphidae) and by mining bees (Hymenoptera: Andrenidae). Being biennial, *B. vulgaris* could be used as a trap crop for *P. xylostella* the first year, and to lower the populations of *E. ornata*, increase parasitism of *P. xylostella*, and attract aphidophagous hoverflies and pollinators, when the plant flowers the second year.

Keywords
Barbarea vulgaris, *Diadegma insulare*, *Eurydema ornata*, lady beetles, *Plutella xylostella*

INTRODUCTION
An increase in vegetational diversity can often reduce insect pest incidence in crops, but there are also reports of no changes and increases in insect pest densities with increased vegetational diversification (Ratnadass et al. 2012; Veres et al. 2013). A reduction in insect pests through vegetational diversity can be achieved for example through trap cropping. One of the plant species proposed as a trap crop for the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is *Barbarea vulgaris* R. Br. (Brassicaceae), commonly known as yellow rocket (Badenes-Pérez et al. 2004; 2005 2014; Idris and Grafius 1996; Lu et al. 2004; Shelton and Nault 2004; Shelton and Badenes-Pérez 2006). *Barbarea vulgaris* is also very attractive to the flea beetles *Phyllotreta cruciferae* Goeze and *P. striolata* F. (Coleoptera: Chrysomelidae), suggesting that this plant could also be used as a trap crop for flea beetles (Root and Tahvanainen 1969). For other economic pests nothing is known about the effect that flowering *B. vulgaris* could have on their abundance on an adjacent cauliflower crop.

Laboratory experiments have shown that both flowering and non-flowering *B. vulgaris* are attractive to ovipositing *P. xylostella* (Lu et al. 2004). Flowering *B. vulgaris* is also a good nectar source for *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae) and for bees and hoverflies (Dailey and Scott 2006; Idris and Grafius 1995, 1997). Thus, our hypothesis is that flowering *B. vulgaris* could be used as a trap crop for different insect pests, in conservation biological control, and to attract pollinators.

MATERIALS AND METHODS
Experiments were conducted in field plots near Madrid (Spain). Cauliflower, cultivar 'Snowball' and G-type *B. vulgaris* var. *arcuata* were used. Four plots of each treatment were set up. A control treatment of cauliflower was compared to a treatment with cauliflower that had flowering *B. vulgaris* planted in two opposite borders. The control plots had 8 rows, while the plots with flowering *B. vulgaris* had two additional rows with flowering *B. vulgaris*. Each row had 20 plants. Rows were separated by 1.0 m with 0.5 m spacing between adjacent plants within rows (Badenes-Pérez et al. 2017). The insects sampled included the lady beetles *Adalia bipunctata* L. and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae); cabbage red bug, *Eurydema ornata* L. (Hemiptera: Pentatomidae); flea beetles in the genera *Phyllotreta* and *Psylliodes* (Coleoptera: Chrysomelidae); cabbage whitefly, *Aleyrodes proletella* L. (Hemiptera: Aleyrodidae); cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae); the cabbage white butterflies *Pieris*

brassicae L. and *P. rapae* L. (Lepidoptera: Pieridae); and *P. xylostella*.

To compare parasitism rates in *P. xylostella* in plots with and without flowering *B. vulgaris*, pupae of *P. xylostella* were collected in June (Badenes-Pérez et al. 2017).

To measure the attractiveness of *B. vulgaris* to pollinators, floral visitors were recorded in May, at the peak of bloom in *B. vulgaris*, by observing sets of 5 plants for 5 minutes on three different sampling dates. Insect visits were recorded between 9:00 and 12:00 h.

RESULTS AND DISCUSSION

Except for *E. ornata*, we found no significant differences in insect densities between cauliflower plots with flowering *B. vulgaris* and control cauliflower plots. *Eurydema ornata* was found in lower densities on the cauliflower plants in the plots with flowering *B. vulgaris* than on the control plots (Wald $\chi 2 = 6.28$; df = 1; P = 0.012). When comparing insect densities on flowering *B. vulgaris* and adjacent cauliflower, *A. proletella*, *B. brassicae*, *E. ornata*, *P. brassicae*, *P. rapae*, and *P. xylostella* were more abundant on cauliflower than on flowering *B. vulgaris* plants. *Adalia bipunctata* and *C. septempunctata*, as well as *Psylliodes* spp. and *Phyllotreta* spp. were significantly more abundant on flowering *B. vulgaris* than on cauliflower (Badenes-Pérez et al. 2017). The higher densities of the lady beetles on flowering *B. vulgaris* compared to cauliflower were not due to higher densities of aphids, whiteflies and other prey, which were more abundant on cauliflower. The higher densities of lady beetles on flowering *B. vulgaris* were probably due to the presence of pollen that they feed upon in flowering *B. vulgaris* plants.

We found two parasitoids in *P. xylostella* pupae: *D. insulare* and *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae). Parasitism by *D. insulare* was 22.8 and 38.4% for pupae collected in control plots and in plots with flowering *B. vulgaris*, respectively (z = 2.23; P = 0.013). Parasitism by *D. collaris* was 1.8 and 7.2% for *P. xylostella* pupae collected in control plots and in plots with flowering *B. vulgaris*, respectively (z = 1.43; P = 0.076) (Badenes-Pérez et al. 2017).

The most common floral visitor of *B. vulgaris* flowers was the hoverfly *Sphaerophoria scripta* L. (Diptera: Syrphidae) (0.4 insect visits/plant/min). Other floral visitors included the pollen-feeding beetles *Heliotaurus ruficollis* F. (Coleoptera: Tenebrionidae) and *Psilothrix viridicoerulea* Geoffroy (Coleoptera: Melyridae); mining bees of the genus *Andrena* (Hymenoptera: Andrenidae); and the hoverfly *Episyrphus balteatus* De Geer (Diptera: Syrphidae). Besides being pollinators as adults, larvae of the hoverflies *S. scripta* and *E. balteatus* feed mainly on aphids (Bargen et al. 1998; Jauker and Wolters 2008; Khan et al. 2016).

Presence of flowering *B. vulgaris* did not significantly alter the abundance of insects on adjacent cauliflower, except in the case of *E. ornata*. As *E. ornata* adults and egg masses were not found on *B. vulgaris* plants, the decrease in *E. ornata* densities could not be caused by a preference for *B. vulgaris*, but rather by an interference with finding its cauliflower host. The reduced attraction to *P. xylostella* in flowering *B. vulgaris* could be due to a reduction in glucosinolate content in this plant with the onset of flowering. As *B. vulgaris* can be used as a trap crop for *P. xylostella* in a vegetative, pre-flowering stage on its first year (Badenes-Pérez et al. 2005), being biennial, *B. vulgaris* could be used as a trap crop for *P. xylostella* the first year, and to reduce infestations of *E. ornata* and attract beneficial insects when it flowers the spring of the following year.

CONCLUSION

Flowering *B. vulgaris* lowered densities of *E. ornata* in adjacent cauliflower plants and it increased parasitism of *P. xylostella* by *D. insulare*. Furthermore, flowering *B. vulgaris* was attractive to lady beetles, aphidophagous hoverflies, pollen-feeding beetles, and mining bees. On the other hand, with the onset of flowering, *B. vulgaris* lost its effectiveness as a trap crop for *P. xylostella*.

Acknowledgements

Funding was provided by the Spanish Ministry of Science and Innovation (grant AGL2010-18151). Thanks to Dr. Niels Agerbirk for providing *B. vulgaris* seeds.

References

Badenes-Pérez FR, Shelton AM, Nault BA. 2004. Evaluating trap crops for diamondback moth, *Plutella xylostella* (Lepidoptera : Plutellidae). *Journal of Economic Entomology* 97(4): 1365-1372.

Badenes-Pérez FR, Shelton AM, Nault BA. 2005. Using yellow rocket as a trap crop for diamondback moth (Lepidoptera : Plutellidae). *Journal of Economic Entomology* 98(3):884-890

Badenes-Pérez FR, Reichelt M, Gershenzon J, Heckel DG. 2014. Using plant chemistry and insect preference to study the potential of *Barbarea* (Brassicaceae) as a dead-end trap crop for diamondback moth (Lepidoptera: Plutellidae). *Phytochemistry* 98(1): 137-144.

Badenes-Pérez FR, Márquez BP, Petitpierre E. 2017. Can flowering *Barbarea* spp. (Brassicaceae) be used simultaneously as a trap crop and in conservation biological control? *Journal of Pest Science* 90(2): 623-633.

Bargen H, Saudhof K, Poehling HM. 1998. Prey finding by larvae and adult females of *Episyrphus balteatus*. *Entomologia Experimentalis et Applicata* 87(3): 245-254.

Dailey, TB, Scott PE. 2006. Spring nectar sources for solitary bees and flies in a landscape of deciduous forest and agricultural fields: production, variability, and consumption. *The Journal of the Torrey Botanical Society* 133(4): 535-547.

Idris AB, Grafius E. 1996. Effects of wild and cultivated host plants on oviposition, survival, and development of diamondback moth (Lepidoptera: Plutellidae) and its parasitoid *Diadegma insulare* (Hymenoptera: Ichneumonidae). *Environmental Entomology* 25(4): 825-833.

Idris AB, Grafius E. 1995. Wildflowers as nectar sources for *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Yponomeutidae). *Environmental Entomology* 24(6): 1726-1735.

Idris AB, Grafius E. 1997. Nectar-collecting behavior of *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Plutellidae). *Environmental Entomology* 26(1): 114-120.

Jauker F, Wolters V. 2008. Hover flies are efficient pollinators of oilseed rape. *Oecologia* 156(4): 819-823.

Khan AA, Shah MA, Majid S. 2016. Functional response of four syrphid predators associated with green apple aphid (Hemiptera: Aphididae) in laboratory. *Journal of Economic Entomology* 109(1): 78-83.

Lu JH, Liu SS, Shelton AM. 2004. Laboratory evaluations of a wild crucifer *Barbarea vulgaris* as a management tool for the diamondback moth *Plutella xylostella* (Lepidoptera : Plutellidae). *Bulletin of Entomological Research* 94(6): 509-516.

Root RB, Tahvanainen J. 1969. Role of winter cress, *Barbarea vulgaris*, as a temporary host in seasonal development of crucifer fauna. *Annals of the Entomological Society of America* 62(4): 852-855.

Shelton AM, Badenes-Perez FR. 2006. Concepts and applications of trap cropping in pest management. *Annual Review of Entomology* 51: 285-308.

Shelton AM, Nault BA. 2004. Dead-end trap cropping: a technique to improve management of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Crop Protection* 23(6): 497-503.

SESSION 5
Insecticide Resistance and Management in Crucifer Pests

Managing Insecticide Resistance in Asia

Knight, S.C.
SYNGENTA ASIA PACIFIC PTE. LTD.; NO. 1 HARBOUR FRONT AVENUE, #03-03 KEPPEL BAY TOWER; 098632; SINGAPORE
email: susan.knight@syngenta.com

Elias, J.
SYNGENTA CROP PROTECTION AG, SYNGENTA RESEARCH CENTER STEIN SCHAFFHAUSERSTRASSE 101 4332 STEIN; SWITZERLAND
email: jan.elias@syngenta.com

ABSTRACT
Resistance to crop protection products is increasingly recognized as a threat to agricultural productivity in Asia. The risk of insecticide resistance is particularly high in fruit and vegetable crops, since they may receive multiple sprays in a single season. The challenge of managing resistance in Asia is formidable. In many countries, farmers lack a basic understanding of resistance management. Effective management of resistance requires cooperation among insecticide manufacturers, with the involvement of the distribution and retail channel, governments, academics, and other stakeholders.

The Asia Insecticide Resistance Action Committee (AIRAC) was formed in 2017, supported by CropLife Asia. Its role is to drive better resistance management throughout Asia, principally by supporting the establishment of national IRACs, and through driving educational and communication initiatives. CropLife Asia is also working with governments to encourage the inclusion of mode-of-action information on product labels. There is no "silver bullet" for managing insecticide resistance, but the guidelines promoted by the AIRAC will help to support farmers in adopting sustainable insect management programs. The diamondback moth is a worldwide pest, and is notorious for its propensity to develop resistance to insecticides. Rigorous adherence to resistance management guidelines is essential, to prolong the durability of existing insecticide active ingredients, and avoid over-reliance on too few modes of action. Adoption of non-chemical practices is an important component of a good resistance management program, for example crop rotation, trap cropping, and the use of light traps or pheromone traps.

Keywords
Insecticide, resistance, IRAC, CropLife, diamondback moth

INTRODUCTION
Insecticide resistance risk is high in many Asian countries, as a consequence of several factors. The tropical or subtropical climate favors year-round insect development, and farmers may exacerbate this favorable environment by planting the same crop continuously, with limited crop rotation. Furthermore, most farmers are unfamiliar with resistance management principles. Therefore there is poor adherence to the resistance management guidelines that are designed to delay the onset of resistance. The diamondback moth (DBM), *Plutella xylostella*, is notorious for developing resistance to insecticides, and has developed resistance to almost all known modes of action (Whalon et al. 2008). Over-reliance on products from a limited number of mode-of-action groups has fueled a vicious circle, in which populations rapidly evolve resistance to newly introduced modes of action.

The Insecticide Resistance Action Committee (IRAC) was established in 1984. It is a specialist technical group of the plant science industry association, CropLife, and provides a coordinated industry response to prevent or delay the development of resistance in insect and mite pests. The IRAC mission is "to facilitate communication and education on resistance to insecticides and insect-resistant traits, and to promote and facilitate development and implementation of resistance management strategies to maintain efficacy and support sustainable agriculture and improved public health" (https://www.irac-online.org/).

There is a network of IRAC global and national groups; however, until recently, IRAC groups were not active in many Asian countries. In this paper, we review the activities of the newly established Asia IRAC, with particular focus on the endeavors to support farmers in the implementation of insecticide resistance management. This includes the communication of information on an insecticide's mode of action.

Diamide insecticides such as chlorantraniliprole and flubendiamide offer excellent control of DBM, and have been widely used since their introduction in 2007 (Hirooka et al. 2007; Nauen 2006; Teixeira and Andaloro 2013). These insecticides belong to IRAC Group 28, and affect insect muscle contraction by binding to ryanodine receptors (RyR) and interfering with the regulation of cellular calcium levels. Sensitivity monitoring for the diamide insecticides has been conducted worldwide for several pests, and results are shared from a study carried out in Japan in 2018.

MATERIALS AND METHODS

Diamide insecticide resistance monitoring was conducted in *P. xylostella* using a Taqman genotyping assay to detect the frequency of the target site mutation, G4946E.

A total of 50 second to third instar larvae of *P. xylostella* were sampled from each of four locations in Japan: Tahara (Nakayama), Tahara (Minamikanbe) and Toyohashi (Jinno) in Aichi prefecture, and Minamiawaji (Shindai) in Hyogo prefecture. The samples were stored in 70% ethanol and shipped for molecular analysis in the Syngenta genotyping laboratory located in Singapore. Genomic DNA of 30 samples per population was extracted using KAPA Express Extract Kit (Axon Lab) and samples were tested through a previously designed Taqman genotyping assay using the primers described in Table 1.

Table 1. Primers used in genotyping assay

Primer name	Sequence
PxTaqMan_F	5'-CGC CGC TCA TCT GTT GGA-3'
PxTaqMan_R	5'-GCG TGA CAG ACT GCA AGA TAG T-3'
PxTaqMan_WT	5'-VIC-TG GCT GTT GGG TTC AA-3'
PxTaqMan_Mut 1	5'-FAM-TG GCT GTT GAG TTC AA-3'

PCR reactions (10 µL) contained 2 µL of genomic DNA, 5 µL of SensiMix II Probe (Bioline, London, UK), 800 nM of each primer and 200 nM of each probe. Reactions were run on a 7900HT fast real-time PCR system (Life Technologies, Carlsbad, CA, USA) using cycling conditions of 10 min at 95°C, followed by 40 cycles of 95°C for 10 sec and 60°C for 45 sec.

RESULTS

The target site mutation G4946E was present in all four populations of DBM (Figure 1). Homozygous resistant (RR) individuals are associated with a high resistance factor (RF) (Steinbach et al. 2015), and diamide insecticides would be expected to give reduced efficacy against populations with a high homozygous resistant mutation frequency. These results are aligned with earlier studies reporting the presence of the G4946E mutation in Japanese populations of DBM (Sonoda and Kataoka 2016)

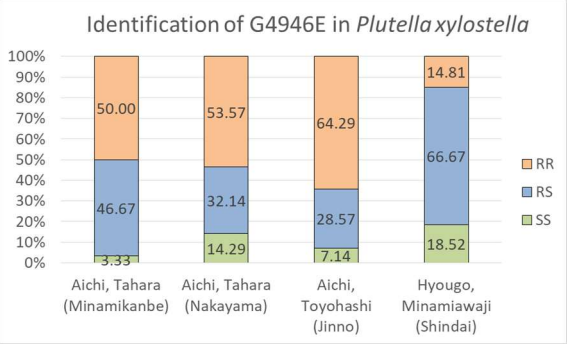


Figure 1. Results of sensitivity monitoring showing allele frequency of G4946E in diamondback moth in four sites in Japan

DISCUSSION

Communicating resistance management guidelines to hundreds of millions of farmers is a formidable challenge. To tackle this, and to improve the coordination of insecticide resistance management in Asia, the Asia Insecticide Resistance Action Committee (AIRAC) was formed in 2017, strongly supported by CropLife Asia and IRAC International. Its role is to support Asian countries in the implementation of insecticide resistance management. Since its inception, the AIRAC has successfully led the establishment of several national resistance management working groups in Asia. Together with these national groups, the AIRAC has organized workshops to facilitate dialogue among government representatives, academics, and all those who provide advice to farmers. The principal outcome of these workshops has been the agreement of national priorities for insecticide resistance management (for e.g., based on pest, crop, and geography), as well as plans for the dissemination of resistance management guidelines to growers.

One of the ultimate goals of the AIRAC is to provide practical and simple guidelines to farmers on how to manage insecticide resistance. Insecticide resistance management is based on the rotation of different mode-of-action groups, in particular by reducing the risk that successive generations of a pest will be exposed to insecticides belonging to the same mode-of-action group. Over-reliance on any mode of action increases the risk that resistant populations will evolve. The IRAC International mode of action team is responsible for assigning all insecticidal and acaricidal active ingredients to a mode-of-action group. The classification is based on data that demonstrate a clear target effect (activation, inhibition, or modulation) at concentrations that would be delivered by an application of the insecticide to a crop. These data may be corroborated by physiological and/or symptomatology studies to link insect mortality to the effect on the target site. This team is also responsible for introducing new groups,

or reallocating active ingredients, when new information becomes available, as well as for developing educational resources. A unique number and letter code is assigned to each mode of action group.

A serious impediment to the adoption of resistance management guidelines is that farmers, retailers and advisors are often unaware of the mode-of-action group of the insecticides that they use. To address this, CropLife International (CLI) member companies have made a commitment to include mode-of-action codes on product labels by 2023. This labelling will provide clear information on the type of pesticide and its mode of action group, and will enable farmers to identify products with the same mode of action that should not be used repetitively on successive generations of the pest. To maximize the benefit of this initiative for farmers, CLI is encouraging all pesticide manufacturers to include mode of action information on their labels (Figure 2). CLI is also encouraging regulatory authorities to consider the mandatory use of mode of action labelling.

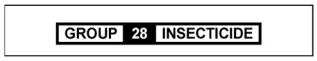


Figure 2. Example of mode of action labeling following CropLife International guidelines

The DBM is notorious for its propensity to evolve resistance to insecticides. There are several factors that contribute to the rapid development of resistance. Resistance risk is typically higher in organisms with a high number of generations per crop, short lifecycle and large numbers of progeny. The DBM is a migratory pest, which means that dispersion of resistant individuals may facilitate the spread of resistance across large distances. Furthermore, the pest has few natural enemies, and has a fairly narrow host range, the cruciferous vegetables, leading to scenarios of intense and prolonged exposure to insecticides. Resistance has been reported to most classes of insecticide, with target site and non-target site resistance to 95 different active ingredients across multiple mode of action groups according to the arthropod pesticide resistance database (APRD 2019).

The diamide insecticides demonstrate high levels of activity against Lepidopteran pests, high selectivity, and low risk to non-target organisms when used as directed. Not surprisingly, the diamides have been regarded as a panacea by farmers around the world struggling to control Lepidopteran pests, and have been rapidly incorporated into insect management regimes since their launch in 2008. However, over-reliance on this group of insecticides has inevitably led to resistance in some target species, with the DBM being the first insect pest to develop resistance. Diamide resistance has now been reported in the DBM in several countries worldwide (Elias 2016). Studies have confirmed that a glycine to glutamic acid substitution (G4946E) in the diamide binding site of the RyR is correlated with a high resistance factor (RF) in the DBM. This has enabled the design of a pyrosequencing-based diagnostic assay for monitoring resistance in populations of DBM (Troczka et al. 2012) and a Syngenta in-house Taqman genotyping assay.

An important component of responsible resistance management is the implementation of sensitivity monitoring studies, to assess the sensitivity of a particular species to an insecticide. These results can be used to advise farmers on appropriate resistance management programs. Evaluating sensitivity before commercialization of a new active ingredient sets a baseline for detection of subsequent sensitivity shifts. Early identification of sensitivity shifts may help with designing or adjusting resistance management strategies to halt or delay further deterioration in sensitivity, ideally before the effectiveness of an insecticide is compromised.

Sensitivity monitoring programs have been conducted worldwide for the diamide insecticides in many important insect pests, including the DBM. This information is being used by manufacturers and advisors to guide resistance management strategies. In locations with predominantly resistant populations, farmers should avoid using diamide insecticides for control of DBM; in locations with low or moderate frequencies of the resistant allele, resistance management guidelines should be rigorously followed. In Japan, resistance monitoring using a molecular assay has confirmed that there is widespread diamide resistance in the DBM, resulting from intensive use of diamide insecticides.

Fall armyworm (FAW), *Spodoptera frugiperda*, is an invasive pest, present in Asia since 2018, and has expanded rapidly throughout South- and Southeast Asia. Based on its lifecycle and behavior, it is assumed to represent a somewhat lower insecticide risk than the DBM, in particular because its host range is not restricted to the intensively cultivated cruciferous crops. Nevertheless, it is a migratory pest with multiple generations per crop cycle, and has a history of evolving insecticide resistance in the Americas (from where the species originates) (Carvalho et al. 2013; Farias et al. 2014; Gutiérrez-Moreno et al. 2019). At present, it is not known whether the FAW populations in Asia are already resistant to insecticides, but resistance management principles must be followed. Asian farmers urgently need advice and support with managing this new pest, and guidance must include recommendations for managing insecticide resistance.

CONCLUSION

Resistance to crop protection products is increasingly recognized as a threat to agricultural productivity in Asia. In many countries, farmers lack a basic understanding of the principles of resistance management. Effective management of resistance requires cooperation among insecticide manufacturers, as well as the distribution and retail channel, governments, academics, and other stakeholders. The purpose of the new AIRAC is to drive better resistance management throughout Asia, by working with governments, academics, farmer associations, local industry associations and advisors to provide practical guidelines to farmers. The inclusion of mode-of-action information on product labels will help to guide farmers in the design of resistance management programs.

There is no "silver bullet" for managing insecticide resistance, but adherence to resistance management guidelines will prolong the durability of existing insecticide active ingredients, and will reduce over-reliance on too few modes of action. Adoption of non-chemical practices is an important component of a good resistance management program, for example crop rotation, trap cropping, and the use of light traps or pheromone traps.

The DBM is notorious for its propensity to develop resistance to insecticides. All those who provide information and advice to farmers must collaborate to provide farmers with practical insecticide resistance management guidelines that can be incorporated into local crop management programs, to delay the development of resistance and prolong the usefulness of all insecticide mode of action groups.

Acknowledgements

The authors would like to acknowledge the contribution of all those who have been instrumental in establishing the Asia IRAC and supporting insecticide resistance management in Asia, particularly Russell Slater and the global IRAC; the Asia IRAC members (Victor Alpuerto, Gajendra Babu, Luis Camacho, Mao Chen, Arnold Estrada, Jinsuk Hong, Al Hsiao, Susan Knight, Srigiriraju Lakshmipathi, Derrick Liu, Charlie Ni, Srinivas Parimi, David Penna, Uwe Pluschkell, Sianghee Tan and Yanqing Wang).

References

APRD (2019). Arthropod Pesticide Resistance Database (https://www.pesticideresistance.org/index.php: Michigan State University).

Carvalho RA, Omoto C, Field LM, Williamson MS, Bass C. (2013). Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*. PLoS One *8*, e62268.

Elias J. (2016). Global diamide resistance and mechanisms. Presented at: Twenty-Fifth International Congress of Entomology (Orlando).

Farias JR, Andow DA, Horikoshi RJ, Sorgatto RJ, Fresia P, Dos Santos AC, Omoto C. (2014). Field-evolved resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. Crop Protection *64*, 150-158.

Gutiérrez-Moreno R, Mota-Sanchez D, Blanco CA, Whalon ME, Terán-Santofimio H, Rodriguez-Maciel JC, Difonzo C. (2019). Field-Evolved Resistance of the Fall Armyworm (Lepidoptera: Noctuidae) to synthetic insecticides in Puerto Rico and Mexico. Journal of Economic Entomology *112*, 792-802.

Hirooka T, Nishimatsu T, Kodama H, Reckmann U, Nauen R. (2007). The biological profile of flubendiamide, a new benzenedicarboxamide insecticide. Pflanzenschutz Nachrichten Bayer *60*, 183-202.

Nauen R. (2006). Insecticide mode of action: return of the ryanodine receptor. Pest Management Science *62*, 690-692.

Sonoda S, Kataoka Y. (2016). Genotyping for the G4946E site of ryanodine receptor gene in *Plutella xylostella* (Lepidoptera: Yponomeutidae) considering gene duplication. Applied Entomology and Zoology *51*, 195-204.

Steinbach D, Gutbrod O, Lümmen P, Matthiesen S, Schorn C, Nauen R. (2015). Geographic spread, genetics and functional characteristics of ryanodine receptor based target-site resistance to diamide insecticides in diamondback moth, *Plutella xylostella*. Insect Biochemistry and Molecular Biology *63*, 14-22.

Teixeira LA, Andaloro JT. (2013). Diamide insecticides: Global efforts to address insect resistance stewardship challenges. Pesticide Biochemistry and Physiology *106*, 76-78.

Troczka B, Zimmer CT, Elias J, Schorn C, Bass C, Davies TG, Field LM, Williamson MS, Slater R, Nauen R. (2012). Resistance to diamide insecticides in diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is associated with a mutation in the membrane-spanning domain of the ryanodine receptor. Insect Biochemistry and Molecular Biology *42*, 873-880.

Whalon ME, Mota-Sanchez D, Hollingworth RM. (2008). Global pesticide resistance in arthropods. CABI, UK. pp. 169.

Plutella xylostella Resistant to Spinosad Exhibits Low Cross Resistance to Other Insecticides

Nur Adibah, M.I.
DEPARTMENT OF AGRICULTURE TECHNOLOGY, FACULTY OF AGRICULTURE, UNIVERSITI PUTRA MALAYSIA
email: ajlaa_911@yahoo.com

Norida, M.
DEPARTMENT OF AGRICULTURE TECHNOLOGY, FACULTY OF AGRICULTURE, UNIVERSITI PUTRA MALAYSIA
LABORATORY CLIMATE-SMART FOOD CROP PRODUCTION
INSTITUTE TROPICAL AGRICULTURE AND FOOD SECURITY (ITAFOS), UNIVERSITI PUTRA MALAYSIA
email: noridamz@upm.edu.my

Dzolkhifli, O.
DEPARTMENT OF CROP PROTECTION, FACULTY OF AGRICULTURE, UNIVERSITI PUTRA MALAYSIA
email: zolkifli@upm.edu.my

Lau, W.H.
DEPARTMENT OF CROP PROTECTION, FACULTY OF AGRICULTURE, UNIVERSITI PUTRA MALAYSIA
email: lauweih@upm.edu.my

ABSTRACT
Insecticides are widely applied to Brassicaceae in the field in order to manage the infesting pests especially *Plutella xylostella* (Diamondback moth, DBM). However, the effectiveness of insecticides is greatly compromised by the resistance developed by DBM. In Malaysia, despite the popular use of insecticides in the field, there is a lack of information regarding the insecticides resistance status of this important pest. The present finding focused firstly on the resistance status of DBM to spinosad insecticide. The DBM strain originated from an organic farm in Semenyih, Selangor, Malaysia was selected with spinosad until generation 15 (G15), producing spinosad-selected (Spi-Sel) strains. The LC_{50} of G15 was 490.63 ppm, giving 42.81 fold resistance ratio (RR) when compared to 11.5 ppm of the LC_{50} Parent generation. The population was continuously selected with spinosad and at G27, the selection was terminated to produce spinosad-decaying (Spi-Dec) strain. Instability of the resistance was recorded from G1 until G5 of the Spi-Dec strain. The LC_{50} ranged from 547.33 to 132.57 ppm with no significant difference detected among the generations. The cross resistance potential of the Spi-Sel strain was tested by exposing it to emamectin benzoate, deltamethrin and chlorantraniliprole. The new population was also exposed to spinosad as the reference strain for cross resistance study and at G5 recorded 9.5-fold RR to the spinosad. Moderate cross resistance was recorded towards emamectin and deltamethrin (RR = 3.8 and RR = 3.49, respectively) and low cross resistance recorded towards chlorantraniliprole (RR = 1.16). Rapid degeneration in spinosad resistance accompanied by moderate to low cross resistance to other insecticides suggested that resistance to spinosad in DBM can be delayed by alternating the spinosad with insecticides of different classes and mode of action.

Keywords
Diamondback moth, spinosad, selection, cross resistance

INTRODUCTION
Plutella xylostella (L.) (Lepidoptera: Plutellidae), commonly known as diamondback moth, is an oligophagous insect with the larvae feeding exclusively on Brassicaceae (crucifers) including cauliflower (*Brassica. oleracea* var. *botrytis*), cabbage (*B. oleracea* var. *capitata*) and Chinese mustard (*B. juncea*). Diamondback moth is the most destructive pest of crucifers worldwide (Talekar and Shelton 1993; Regupathy 1996; Verkerk and Wright 1996; Zhao et al. 2006).

In the southeastern USA, DBM makes up more than 90% of the defoliating lepidopterans of canola (Ramachandran et al. 2000). Mazlan and Mumford (2005) reported that local production of cabbage in Malaysia was 206,102 metric tons, however due to intensified farming in Cameron Highland, the crop was heavily infested by DBM. Previously, damage caused by DBM has been estimated to be approximately US$ 1 billion (Talekar and Shelton 1993) and that figure was routinely quoted (Shelton et al. 2000; Sayyed et al. 2008) until Zalucki et al. (2012) provided an update.

Insecticides have been the main tactic in controlling DBM. Although there are some significant drawbacks of insecticides, they are still the most preferred method by the farmers especially in developing countries (Thuy et al. 2012; Muriithi et al. 2016). Spinosad is a natural, broad spectrum insecticide derived from a soil actinomycete *Saccharopolyspora spinosa*, firstly introduced in Japan in 1997. It is widely used in the field and had been a very promising chemistry back in the earlier times of introduction due to the strong insecticidal activity. However, not long after its introduction, a high level of resistance was detected in several DBM populations; California (Zhao et al. 2002), Malaysia (Sayyed et al. 2004) and

Georgia (Zhao et al. 2006). Insecticides applications aided by the high fecundity of DBM enhances selection pressure in the DBM and eventually leads to its resistance to a wide range of insecticides.

Following resistance, cases of cross resistance were also recorded in DBM. Previous studies showed that DBM resistance to Cry1A *Bacillus thuringiensis* subsp. *kurstaki* confers cross – resistance to Cry1F (Tabashnik et al. 1994). Spinosad-resistant DBM CH_1 strain conferred a high level of cross resistance to abamectin (Sayyed et al. 2004). Cross resistance to spinosyn analogs (3'-O- ethyl spinosyn) and spinetoram are known in spinosad resistance DBM strain (Sparks et al. 2012). One of the important factors that could mitigate the effect of DBM resistance in the field is to investigate the stability of the resistance of DBM towards any given insecticide. Unstable resistance will reduce the frequency of resistant individuals in a population therefore securing an effective resistance management program.

In Malaysia, although DBM was abundantly found in the field and one of the major threats to crucifers' production, there are few studies on this pest, specifically ones that focus on the resistance and cross resistance. Therefore, this study was conducted to investigate the resistance status of the DBM to spinosad and its potential of cross resistance to other insecticides. We also looked into the stability of the spinosad resistance in the spinosad-selected DBM.

MATERIALS AND METHODS

Rearing DBM in Insectary

The rearing procedure followed Qian et al. (2008) with some modifications. The DBM was initially collected from an organic farm in Semenyih, Selangor, Malaysia. It was then reared in glasshouse Ladang 2 at Universiti Putra Malaysia (UPM). Upon collection from the field, the larvae and pupae were released in rectangular cages each containing a pot of *Brassica rapa* var. *chinensis* (white pak choy). The host plant was observed daily and replaced whenever necessary. Adults of DBM were provided with 10% honey solution on cotton wool.

Leaf-dip Bioassay Method

Leaf-dip bioassay method was conducted following the procedure of Sayyed et al. (2008) with some modifications. The experiment was conducted at Toxicology Lab, Department of Crop Protection, UPM. Bioassays used early third instar (L3) of DBM from original strain collected from the field; labelled as susceptible (SS) and spinosad-selected (Spi-Sel) strain. Healthy cabbage leaves were used and cleaned with water and cut round (70 mm in diameter). For the purpose of determining LC_{50} of spinosad to the strains, five concentrations of spinosad solution (10, 15, 25, 35 and 50 ppm) were prepared together with distilled water that served as control. Surfactant Triton X-100 was added as an emulsifier and mixed into the control and spinosad solutions. Four replications were used for each concentration. Cabbage leaves were dipped in the solutions for 10 seconds. They were placed on the corrugated aluminium foil to allow air dry for approximately 2 hours.

Upon drying, one cabbage leaf was placed in a round 70 mm petri dish and 10 larvae were placed in each petri dish. The larva was firstly starved for two hours prior feeding on spinosad-soaked leaves. A small opening was made on the cover of petri dishes and covered with fine muslin cloth to provide aeration inside the petri dishes. The dishes were sealed with parafilm to prevent larvae from escaping and were maintained at 25°C at 11:13 (L:D) hr photoperiod. Mortality of the larvae was recorded after 48h of exposure and was confirmed when there is no movement of larvae when disturbed with tips of tissue.

Selection with Spinosad

Resistance selection began at generation 12 (G12) upon field collection of susceptible (SS) strain by feeding the larvae with spinosad-sprayed host plant. The G12 was labelled as Parent (P) generation as in resistance selection study. Prior to the selection experiment, a preliminary study using leaf-dip bioassay method was conducted to determine the suitable concentration of spinosad to be exposed to the larvae. The LC_{50} of the preliminary study was sprayed on the host plant of DBM and air-dried. Once the spinosad solution dried, the host plant was placed inside the DBM (SS) cage to feed the larvae of Parent generation. Upon reaching the L3 stage, leaf-dip bioassay method was conducted and the LC_{50} of Parent generation was used to spray onto host plants throughout the selection process. The strain selected with spinosad was labelled as spinosad-selected (Spi-Sel) strain. The selection process was carried out throughout the study and leaf-dip bioassay method was conducted in each generation until generation 15 (G15) unless the population was not sufficient to be tested.

Cross Resistance Study

At fifth generation of the selection, Spi-Sel and SS strain was bioassayed with emamectin benzoate, deltamethrin and chlorantraniliprole. In order to determine the cross resistance potential, LC_{50} values of both strains to each insecticides was compared to the obtained resistance ratio (RR).

Resistance Stability

In order to determine spinosad resistance stability, Spi-Sel strain was reared free from the insecticide starting from generation 27. The population deselected with spinosad was labelled as spinosad-decaying (Spi-Dec) strain. Bioassay was conducted from the first generation of deselection until four subsequent generations. Resistance ratio was obtained in each generation by dividing LC_{50} values of n^{th} generation with LC_{50} of the first generation.

Statistical Analysis

Probit analysis using POLOPLUS software (LeOra Software 2003) was used to analyse the mortality data of the DBM and determine the LC_{50}, 95% fiducial limit (FL) and the slope (±SE) of the log-dose probit line. LC_{50} between the generations were significantly different when their 95% limits did not overlap. DBM resistance to spinosad and to other insecticides as well in the present study was determined by resistance ratio (RR), calculated by dividing the LC_{50} values of the progenies with LC_{50} of the parent (P) generation. In cross resistance study, the LC_{50} values of the Spi-Sel strain preceding exposure to other insecticides was divided with LC_{50} values of the SS strain exposed to the same insecticide. In resistance stability study, the LC_{50} values of n^{th} generation were divided with LC_{50} values of the first generation of the deselection with spinosad.

RESULTS AND DISCUSSION

The continuous selection of susceptible (SS) DBM strain in the laboratory with spinosad for 15 generations produced a spinosad-selected (Spi-Sel) strain. The LC_{50} value from Parent until G15 ranged from 11.46 – 490.63 ppm (Table 1). At G3 and G4, the resistance ratio was around the value of 1, indicating no resistance developed in the generations. At G5, low resistance began to develop in the population (RR=3.59), of which the rate of resistance increased gradually until G13. Between G13 and G15, a huge increment in resistance was observed, with a resistance ratio (RR) of 42.81 fold from Parent until G15. Significant difference in resistance ratio was only detected between the last two generations (based on non-overlapping fiducial limit; 95% FL). The concentration-mortality curves estimated in each generation fitted to the probit model (χ^2 not significant, $P > 0.05$).

Table 1: Selection history of spinosad-resistant (Spi-Sel) diamondback moth strain

Generation	n[a]	LC_{50} (ppm) (95%FL)	Fit of probit line			RR[b]
			Slope ± SE	X^2	df	
Parent	240	11.46 (3.51 - 16.90)	1.19 ± 0.37	1.519	3	-
G3	240	12.59 (9.03 - 15.50)	2.41 ± 0.42	2.060	3	1.10
G4	240	14.37 (0.50 - 23.72)	0.81 ± 0.36	1.964	3	1.25
G5	240	41.14 (30.68 - 76.18)	1.54 ± 0.39	1.066	3	3.59
G7	240	79.67(49.51 - 361.50)	1.47 ± 0.42	1.207	3	6.95
G8	200	103.65 (n.a)	1.29 ± 0.68	1.445	3	9.04
G10	180	108.46(87.04-204.23)	2.64 ± 0.77	2.044	2	9.46
G13	240	125.42(112.46-144.26)	3.86 ± 0.89	2.098	3	10.94
G15	240	490.63(160.10-n.a)	0.90 ± 0.44	2.691	3	42.81

[a] Total number larvae used in bioassay including control
[b] Resistance ratio: LC_{50} of n^{th} generation divided by LC_{50} of Parent

The ability to develop resistance varies, depending on the insect species in question, genetic background (Alphey et al. 2011), selection intensity (Shad et al. 2010) and history of insecticides application (Attique et al. 2006). In the present study resistance in DBM to spinosad was lower than in previous studies. A field-collected *Spodoptera exigua* (beet armyworm) displayed a resistance ratio of 345.5 times in only five generations of selection to spinosad (Wang et al. 2006) whereas the spinosad resistance in *Spodoptera litura* increased up to 3921 times after 11 generations as compared to the susceptible strain (Rehan and Freed 2014).

High resistance recorded in the above studies was primarily contributed by pre-exposure of spinosad and other insecticides in the field as most of the populations were collected in the field prior to selection in the laboratory. The pre-existence of resistant alleles in the population, thus contributed to a high resistance when being selected in the laboratory. A study by Zhao et al. (2002) supported the fact that after spinosad introduction in the region, it was heavily dependent on as other insecticides gave poor control. However, within only two years of its introduction, failure of spinosad in controlling DBM

occurred with high resistance in the region. In contrast to the previous populations, the population in the present study originated from an organic farm in Selangor, Malaysia without a history of insecticide exposure, and this explained the moderate resistance in the Spi-Sel strain compared to the previous studies.

The rate of resistance development depends on the dominance of resistant genes in the population (Afzal et al. 2015), where the rate is reduced in the recessive genes. Although no attempt was made to observe the initial frequency of resistance genes in the study, the finding suggests that the resistance could be associated with the initial frequency of resistant genes when compared with the previous studies, taking pre-exposure history in the field into account.

At G27, the selection pressure of spinosad was removed from the Spi-Sel strain in order to study stability of spinosad resistance, producing the spinosad decaying (Spi-Dec) strain. After five generations rearing without spinosad exposure, resistance in Spi-Dec strain decreased significantly (non-overlapping 95% FL between G1 and G6) (Table 2), reflecting unstable resistance. While the LC_{50} of the strain in the G1 after the removal of the selection was 547.328 ppm, the value in the G2 increased slightly to 743.702 ppm. Afterwards the LC_{50} decreased drastically at G3 to approximately 5-fold and remained steady within the rate in the succeeding generations.

Table 2: Stability of spinosad resistance in spinosad-decaying (Spi-Dec) DBM

Generation	LC50 (ppm) (95%FL)	Fit of Probit Line			RR
		Slope±SE	X^2	df	
1	547.33(234.62-11520.69)	0.80±0.26	6.386	3	1
2	743.70 (N.A.)	0.45±0.36	8.125	3	1.36
3	150.43(83.76-569.25)	0.70±0.25	2.553	3	0.28
4	153.53(94.31-337.57)	0.98±0.24	1.541	3	0.28
5	132.57(93.85-215.63)	1.24±0.23	2.363	3	0.24

Earlier, findings have found resistance instability in the lab-reared insects including medical vectors, urban and agriculture pests as well as beneficial insects upon removal of selection pressure from the population (Khan et al. 2014; Ejaz et al. 2017; Mansoor et al. 2017). In the present study, the reduction in the resistance within three generations after the final insecticide treatment reflected that that resistance is not stable in the early generations. After an insecticide is withdrawn from a population, the tendency for the resistance to deteriorate is high, especially when the initial resistance gene frequency is rare, fitness/resistant cost is dominant and resistance is recessive (Acharya et al. 2017). As a consequence insect susceptibility will be achieved by restoring the original regime. Rapid and substantial reversion rate of the resistance implied a high cost paid off by the Spi-Sel strain during resistance development. Often, the reversal of the resistance in the insects are thought to be associated with the fitness cost in the insect, though the cost varies (Raymond et al. 2001).

Cross resistance of spinosad to other insecticides (emamectin, deltamethrin and chlorantraniliprole) was determined by testing the Spi-Sel and SS strain to the insecticides. Spi-Sel strain displayed LC_{50} of 321.015, 97.957, 350.811 and 86.13 ppm when treated with spinosad, emamectin, deltamethrin and chlorantraniliprole, respectively (Table 3). When these values were further compared to those of SS, highest resistance ratio was determined upon testing the DBM with spinosad (RR = 9.5), followed by moderate resistance to emamectin and deltamethrin (RR=3.8 and 3.49, respectively) and lowest resistance to chlorantraniliprole (RR=1.16). Relative to resistance ratio displayed by the Spi-Sel strain to spinosad, the resistance ratio of the strain to other insecticides suggested moderate cross resistance to emamectin and deltamethrin, with very low cross resistance between spinosad and chlorantraniliprole.

Table 3: Cross resistance spectrum in susceptible (SS) and spinosad-selected (Spi-Sel) DBM

Insecticide	Strain	LC_{50} (ppm) (95%FL)	Fit of Probit Line			RR
			Slope±SE	X^2	df	
Spinosad	SS	33.78 (13.98-54.67)	1.02±0.24	0.57	3	
	Spi-Sel	321.02(175.89-1450.88)	0.89 ± 0.23	2.86	3	9.5
Emamectin	SS	26.05 (5.03-47.87)	0.73±0.22	4.82	3	
	Spi-Sel	97.96 (44.48-308.75)	1.80 ±0.40	7.84	3	3.8
Deltamethrin	SS	100.59 (61.18-197.84)	0.85±0.22	0.95	3	
	Spi-Sel	350.81(176.01– 427.66)	0.85±0.25	3.71	3	3.5
Chlorantraniliprole	SS	73.94(57.73-94.59)	1.87±0.25	4.35	3	
	Spi-Sel	86.13(7.24–10284.44)	1.97±0.60	15.97	3	1.2

High resistance ratio in Spi-Sel strain relative to other insecticides when tested with spinosad was expected since the strain was continuously selected with the insecticide in this study. Continuous selection pressure in DBM eventually resulted in decreasing susceptibility to the spinosad. Parallel to the finding, a spinosad-resistant oriental fruit fly population exhibited an outstanding resistance level (RR=>408) than other insecticides when it was subjected to spinosad treatment in cross resistance study (Hsu and Feng 2006). In Agreste of Pernambuco region in Brazil where the pesticides was being overused on DBM, the low initial DBM resistance to spinosad (RR=<5) (Oliveira et al. 2011) elevated significantly in 5 years' time (RR=200) (Neto et al. 2017) as a result of high selection pressure of spinosad in the agriculture areas of the region. Although the resistance development in this study was not as rapid as the previous studies, the resistance revealed is important since the Spi-Sel strain was first subjected to selection pressure during the rearing process in insectary in UPM.

Cross resistance in DBM is another issue to be tackled in the agriculture field due to DBM ability to develop cross resistance to classes of insecticides. Cases of cross resistance associated with DBM either within the same insecticide classes such as between fufenozide and methoxyfenozide (Sun et al. 2012) and phenthoate and parathion (Park et al. 2004) or among different classes including indoxacarb and metaflumizone (Zhang et al. 2017) and cypermethrin and flubendiamide (Sunitha et al. 2014) have been documented. Therefore, the moderate cross resistance of spinosad to emamectin and deltamethrin in this study should not be underestimated because like chlorantraniliprole, the insecticides tested in this study were all grouped in different insecticide classes, which reflects different target sites of the insecticides, hence initially cross resistance was not expected. Cross resistance between spinosad to emamectin and

deltamethrin suggests involvement of metabolic resistance.

CONCLUSION

Selection of the Spi-Sel population with spinosad revealed the potential of the strain to develop resistance even within a short period of time. Nevertheless, the moderate resistance suggested a potential of an effective resistance monitoring as spinosad resistance was unstable after removal of selection pressure. Out of three other insecticides tested, chlorantraniliprole showed unlikely cross resistance, demonstrating that it is a good candidate to be rotated with spinosad.

Acknowledgements

I would like to thank FRGS Grant no:5524522 for the grant given to conduct the study. I also highly appreciate the travel grant awarded by SEARCA for me to come and join this conference.

References

Acharya B, Head GP, Price PA, Huang F. 2017. Fitness costs and inheritance of Cry2Ab2 resistance in *Spodoptera frugiperda* (JE Smith). *Journal of Invertebrate Pathology 149*: 8-14.

Afzal MBS, Shad SA, Basoalto E, Ejaz M, Serrao JE. 2015. Characterization of indoxacarb resistance in *Phenacoccus solenopsis* Tinsley (Homoptera: Pseudococcidae): cross-resistance, stability and fitness cost. *Journal of Asia-Pacific Entomology 18*(4): 779-785.

Alphey N, Bonsall MB, Alphey L. 2011. Modeling resistance to genetic control of insects. *Journal of Theoretical Biology 270*(1): 42-55.

Attique MNR, Khaliq A, Sayyed AH. 2006. Could resistance to insecticides in *Plutella xylostella* (Lep., Plutellidae) be overcome by insecticide mixtures? *Journal of Applied Entomology 130*(2): 122-127.

Ejaz M, Afzal MBS, Shabbir G, Serrão JE, Shad SA, Muhammad W. 2017. Laboratory selection of chlorpyrifos resistance in an Invasive Pest, *Phenacoccus solenopsis* (Homoptera: Pseudococcidae): Cross-resistance, stability and fitness cost. *Pesticide Biochemistry and Physiology 137*: 8-14.

Hsu JC, Feng HT. 2006. Development of resistance to spinosad in oriental fruit fly (Diptera: Tephritidae) in laboratory selection and cross-resistance. *Journal of Economic Entomology 99*(3): 931-936.

Khan HAA, Akram W, Shad SA. 2014. Genetics, cross-resistance and mechanism of resistance to spinosad in a field strain of *Musca domestica* L. (Diptera: Muscidae). *Acta Tropica 130*: 148-154.

Mansoor MM, Raza ABM, Abbas N, Aqueel MA, Afzal M. 2017. Resistance of green lacewing, *Chrysoperla carnea* Stephens to nitenpyram: cross-resistance patterns, mechanism, stability, and realized heritability. *Pesticide Biochemistry and Physiology 135*: 59-63.

Mazlan N, Mumford J. 2005. Insecticide use in cabbage pest management in the Cameron Highlands, Malaysia. *Crop Protection 24*(1): 31-39.

Muriithi BW, Affognon H., Diiro GM, Kingori SW, Tanga CM, Nderitu PW, Mohamed SA, Ekesi S. 2016. Impact assessment of Integrated Pest Management (IPM) strategy for suppression of mango-infesting fruit flies in Kenya. *Crop Protection 81*: 20-29.

Neto L, Escócio J, Siqueira HÁAD. 2017) Selection Of *Plutella xylostella* (L.)(Lepidoptera: Plutellidae) To Chlorfenapyr Resistance: Heritability And The Number Of Genes Involved. *Revista Caatinga 30*(4): 1067-1072.

Oliveira ACD, Siqueira HÁAD, Oliveira JVD, Silva JED, Michereff Filho M. 2011. Resistance of Brazilian diamondback moth populations to insecticides. *Scientia Agricola 68*(2): 154-159.

Park NJ, Oh SC, Choi YH, Choi KR, Cho KY. 2004. Inheritance and cross resistance of phenthoate-selected diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Journal of Asia-Pacific Entomology 7*(2): 233-237.

Qian L, Cao G, Song J, Yin Q, Han Z. 2008. Biochemical mechanisms conferring cross-resistance between tebufenozide and abamectin in *Plutella xylostella*. *Pesticide Biochemistry and Physiology 91*(3): 175-179

Ramachandran S, Buntin GD, All JN. 2000. Response of canola to simulated diamondback moth (Lepidoptera: Plutellidae) defoliation at different growth stages. *Canadian Journal of Plant Science 80*(3): 639-646.

Raymond M, Berticat C, Weill M, Pasteur N, Chevillon C. 2001. Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation?. In *Microevolution Rate, Pattern, Process* (pp. 287-296). Springer, Dordrecht.

Regupathy A. 1996. Insecticide resistance in diamondback moth (DBM), *Plutella xylostella* (L.): status and prospects for its management in India. Pesticide resistance mechanisms and resistance management strategies in *Proceedings: The Management of Diamondback Moth and Other Crucifer Pests,* 1996, pp. 233– 239.

Rehan A, Freed S. 2014. Selection, mechanism, cross resistance and stability of spinosad resistance in *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Crop Protection 56*: 10-15.

Verkerk RHJ, Wright DJ. 1996. Multitrophic interactions and the diamondback moth: implications for pest management in *Proceedings: The Management of Diamondback Moth and Other Crucifer Pests,* 1996, pp 243-248

Sayyed AH, Omar D, Wright DJ. 2004. Genetics of spinosad resistance in a multi-resistant field-selected population of *Plutella xylostella*. *Pest Management Science: formerly Pesticide Science 60*(8): 827-832.

Sayyed AH, Saeed S, Noor-Ul-Ane M, Crickmore N. 2008. Genetic, biochemical, and physiological characterization of spinosad resistance in *Plutella xylostella* (Lepidoptera: Plutellidae). *Journal of Economic Entomology 101*(5): 1658-1666.

Shad SA, Sayyed AH, Saleem MA. 2010. Cross-resistance, mode of inheritance and stability of resistance to emamectin in *Spodoptera litura* (Lepidoptera: Noctuidae). *Pest Management Science 66*(8): 839-846.

Shelton AM, Tang JD, Roush RT, Metz TD, Earle ED. 2000. Field tests on managing resistance to Bt-engineered plants. *Nature Biotechnology 18*: 339.

Sparks TC, Dripps JE, Watson GB, Paroonagian D. 2012. Resistance and cross-resistance to the spinosyns–a review and analysis. *Pesticide Biochemistry and Physiology 102*(1), 1-10.

Sun J, Liang P, Gao X. 2012. Cross-resistance patterns and fitness in fufenozide-resistant diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Pest Management Science 68*(2): 285-289.

Sunitha V, Singh TVK, Satyanarayana J. 2014. Cross-Resistance Variation among Different Populations of Diamondback Moth, *Plutella xylostella* (Linn.) to New Insecticides. *International Journal of Bio-resource and Stress Management 5*(4): 522-529.

Tabashnik BE. 1994. Evolution of resistance to Bacillus thuringiensis. *Annual Review of Entomology 39*(1): 47-79.

Talekar NS, Shelton AM. 1993. Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology 38*(1): 275-301.

Thuy PT, Van Geluwe S, Nguyen VA, Van der Bruggen B. 2012. Current pesticide practices and environmental issues in Vietnam: management challenges for sustainable use of pesticides for tropical crops in (South-East) Asia to avoid environmental pollution. *Journal of Material Cycles and Waste Management 14*(4): 379-387.

Wang W, Mo J, Cheng JA, Zhuang P, Tang Z. 2006. Selection and characterization of spinosad resistance in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology 84*(3): 180-187.

Zalucki MP, Shabbir A, Silva R, Adamson D, Liu SS, Furlong MJ. 2012. Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella* (Lepidoptera: Plutellidae): just how long is a piece of string? *Journal of Economic Entomology 105*(4): 1115-1129.

Zhang S, Zhang X, Shen J, Li D, Wan H, You H, Li J. 2017. Cross-resistance and biochemical mechanisms of resistance to indoxacarb in the diamondback moth, *Plutella xylostella*. *Pesticide Biochemistry and physiology 140*: 85-89.

Zhao JZ, Collins HL, Li YX, Mau RFL, Thompson GD, Hertlein M, Andaloro JT, Boykin R, Shelton AM. 2006. Monitoring of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad, indoxacarb, and emamectin benzoate. *Journal of Economic Entomology 99*(1): 176-181.

Zhao JZ, Li YX, Collins HL, Gusukuma-Minuto L, Mau RFL, Thompson GD, Shelton AM. 2002. Monitoring and Characterization of Diamondback Moth (Lepidoptera: Plutellidae) Resistance to Spinosad. *Journal of Economic Entomology*. 95(2): 430-436

Effects of deltamethrin resistance on development in diamondback moths

Wang, L.
SCHOOL OF BIOLOGICAL SCIENCES, THE UNIVERSITY OF QUEENSLAND, ST LUCIA 4072, QUEENSLAND, AUSTRALIA
email: leyun.wang@uq.net.au

Walter, G.H.
SCHOOL OF BIOLOGICAL SCIENCES, THE UNIVERSITY OF QUEENSLAND, ST LUCIA 4072, QUEENSLAND, AUSTRALIA
email: g.walter@uq.edu.au

Furlong, M.J.
SCHOOL OF BIOLOGICAL SCIENCES, THE UNIVERSITY OF QUEENSLAND, ST LUCIA 4072, QUEENSLAND, AUSTRALIA
email: m.furlong@uq.edu.au

ABSTRACT
Worldwide, use of the pyrethroid insecticide deltamethrin has resulted in the selection of deltamethrin-resistant field populations of diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). A deltamethrin-resistant DBM population was field-collected in southeast Queensland, Australia and split into two populations. One population (= non-selected) was maintained with no further exposure to any insecticides while the other (= deltamethrin-selected) was maintained under a regime of intermittent laboratory selection with deltamethrin. Colonies of each population were reared at constant temperatures of 10, 15, 20, 25, 30, and 35◦C (light: dark = 12 hr: 12 hr), and the effect of rearing temperature on various traits was investigated. When the study was conducted, the deltamethrin-selected population was 20-fold more resistant to deltamethrin than the non-selected population. Generally, development time, adult life span, female pupal weight and fecundity of moths in both populations declined significantly with increasing temperature. Overall, the deltamethrin-selected insects developed significantly faster than non-selected insects and a significant interaction was detected between rearing temperature and deltamethrin resistance status on development time. Similarly, female pupal weight was significantly affected by temperature and deltamethrin resistance status, with a significant interaction between these main effects. Although deltamethrin-selected insects and non-selected insects laid eggs when reared at 10◦C, few of these eggs were fertile. While non-selected female moths reared at 10◦C or 25◦C could lay fertile eggs following mating with male moths reared at 25◦C, no female moths could lay fertile eggs following mating with male moths that had been reared at 10◦C, suggesting that rearing at this low temperature affected male fertility in a manner that is yet to be determined. The study provides important information on how insecticide resistance status and abiotic stresses can interact, which will be useful for refining models that predict DBM population dynamics.

Keywords
Plutella xylostella, pyrethroids, deltamethrin, resistance, temperature

INTRODUCTION
The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a serious pest of *Brassica* vegetables and canola. DBM is one of the most widespread insects globally, and it has colonized both tropical and temperate regions (Lim 1986; Talekar and Shelton 1993). Inappropriate insecticide use, rapid generation times, and high reproductive rates of DBM promote the development of insecticide resistance and the pest has developed resistance to all classes of insecticide (including crystalline toxins of the bacterium *Bacillus thuringiensis*) deployed against it in the field (Furlong et al. 2013).

The increased frequency of the alleles that confer resistance in DBM may affect associated fitness traits in the absence of the insecticide (Crow 1957). Typically, a fitness cost will reduce the level of resistance in a population in the absence of the insecticide, as the negative effects of pleiotropy become more strongly expressed. For example, a field-evolved chlorantraniliprole-resistant DBM population showed longer larval and pupal durations and male life span, higher hatching rates, and lower larval weight and fecundity in the absence of the insecticide (Ribeiro et al. 2014). The survival, fecundity, and fertility of a *B. thuringiensis*-resistant DBM strain declined in the absence of *B. thuringiensis* (Groeters et al. 1994), and lower reproduction rates were reported in a tebufenozide-resistant DBM strain (Cao and Han 2006). Extreme temperatures could interact with these effects and even increase the fitness costs, as shown in a spinosad-resistant DBM strain (Li et al. 2007). However, insecticide resistant insects do not inevitably incur a fitness cost, for example, fenvalerate-resistance did not affect developmental time, mortality, or fecundity in a DBM population collected in Thailand (Motoyama et al. 1992).

Deltamethrin is a broad-spectrum synthetic pyrethroid insecticide that paralyses and kills insects by targeting the α subunit of voltage-gated sodium channels (Soderlund and Bloomquist 1989). Resistance to pyrethroids can be conferred by both metabolic and non-metabolic mechanisms in DBM (Furlong et al. 2013) and more than one mechanism might function in individuals (Schuler et al. 1998; Lambkin and Furlong 2011).

Deltamethrin is widely used against agricultural and urban pests, and we used a field-evolved deltamethrin-resistant DBM strain in the study. The field collected insects were divided into two subpopulations that were maintained separately; one was maintained without exposure to insecticides so that insecticide-resistance attenuated, while the other was intermittently selected with deltamethrin to maintain expression of deltamethrin-resistance. As the two experimental populations had the same genetic background, comparisons of fitness traits across them, at different temperatures, were expected to reveal any costs of deltamethrin-resistance under different thermal conditions. These results have considerable significance for understanding the individual performance, population dynamics, and geographical limits of this highly migratory pest.

MATERIALS AND METHODS

Common cabbage (*Brassica oleracea* L. *capitata* cv. sugarloaf) was grown in individual pots (15-cm-diameter) with organic potting medium and an organic fertilizer (Osmocote, N: P: K=16: 35: 10, Scotts Australia, Baulkham Hills, NSW, Australia) in a glasshouse at the University of Queensland, St. Lucia, QLD, Australia. The DBM were collected from commercial cabbage crops in Gatton (27°33'S, 152°18'E), south-east Queensland in 2014. One generation after collection from the field the DBM population showed increased susceptibility to deltamethrin when compared to the standard susceptible Waite population in laboratory bioassays (Etebari et al. 2015). The population was divided in two, and both cultures were maintained on fresh cabbage leaf material at 23 ± 1°C and photoperiod of 16hr light: 8hr dark. One of these subpopulations (the deltamethrin selected, resistant strain) was intermittently selected with deltamethrin (Suncis, 25g AI/kg) while the other (the unselected, susceptible strain) was not exposed to insecticide. At the time of experimentation, the resistant strain was 20-fold more resistant to deltamethrin than the susceptible strain (Barbosa 2019).

The survival and developmental time of all immature developmental stages (eggs, all larval stages and pupae), pupal weight, adult life span, and fecundity of the deltamethrin-resistant and susceptible strains were investigated. For both strains, individuals on pieces of cabbage leaf were placed in separate Petri dishes (6 cm diameter) containing a moistened filter paper and then incubated at one of six constant rearing temperatures of 10, 15, 20, 25, 30, and 35°C (temperatures ± 1°C; light: dark = 12 hr: 12 hr; RH = 50-70%). Depending on previously reported survival at different temperatures (Liu et al. 2002), each treatment began with 26- 159 eggs of each DBM strain. Each individual was observed twice per day at 0800 hr and 2000 hr to record the time of death, the stage of development through to pupation, and adult emergence. Each pupa was weighed on a microbalance (METTLER TOLEDO, XS3DU, Switzerland, d = 1 μg/10 μg) within 24 hr of developing. Once an adult emerged, its sex was determined, and a cotton roll soaked with water was put into the Petri dish to prevent the moth dehydrating. Water was supplied daily until the adult died, and adult life span was recorded. Newly emerged adults of the same strain were paired with individuals of the opposite sex that had been reared at the same temperature, and used to investigate the effect of rearing temperature on fecundity in both strains at 25 ± 1°C (light: dark = 12 hr: 12 hr; RH = 50-70%). Moths were paired in Petri dishes (6 cm diameter) with a small piece of common cabbage leaf as an oviposition substrate and a cotton roll soaked with water. Leaf pieces were replaced every 24 hr, and the number of eggs laid on each was recorded until the female died. The leaf portion was then placed into another Petri dish (6 cm diameter) that was sealed with Parafilm® and incubated (25 ± 1°C; light: dark = 12 hr: 12 hr) and the number of eggs that hatched on each leaf was recorded after 5 d (Liu et al. 2002).

Statistical analysis was conducted in R (R Core Team 2018). Before conducting ANOVA, the homogeneity of variances was examined by Bartlett's tests. Normality of distribution of dependent variables was also examined. The effects of temperature and deltamethrin-resistance status on the duration of the life cycle and adult life span were tested with two-way ANOVA with White-corrected covariance matrices applied; post-hoc analyses were conducted with Games-Howell tests. The effects of deltamethrin-resistance status on duration of the life cycle at each temperature were compared with one-way ANOVA. The impacts of temperature and deltamethrin-resistance status on pupal weight and fecundity were tested with two-way ANOVA; post-hoc analyses were conducted with Tukey's HSD tests.

RESULTS AND DISCUSSION

Insecticide resistance usually results in increased "fitness costs" in resistant insects due to the higher energetic costs associated with metabolic mechanisms of resistance and/or changes to the frequency of alleles conferring resistance that may also affect fitness (Crow 1957). In the absence of selection pressure, susceptibility to insecticides is likely to increase through time (Muggleton 1983). If insecticide resistance does not carry a fitness cost, then the insecticide-resistant phenotype may be maintained without a selection pressure (ffrench-Constant and Bass 2017). This study aimed to identify the difference in the developmental time, adult life span, pupal weight, and fecundity across the deltamethrin-resistant DBM strain and its homogenous susceptible field strain at different temperatures. Resistance significantly reduced the time taken to complete a generation (Table 1; Fig. 1a), but it had no effect on adult lifespan (Table 1; Fig. 1b). Although resistance had an inconsistent effect on female pupal weight (Table 1; Fig. 1c), it did not affect fecundity (Table 1; Fig. 1d).

Table 1. Summary of ANOVA statistics for the effects of temperature and deltamethrin-resistance status on the duration of the life cycle, adult life span, pupal weight, and fecundity of *Plutella xylostella*.

Source	d.f.	*F*	*P*
Duration of a Life Cycle			
Temperature	4	13026.26	< 0.001
Resistance	1	31.72	< 0.001
Temperature x Resistance	4	6.17	< 0.001
Residuals	384		
Adult Life Span			
Temperature	4	230.12	< 0.001
Resistance	1	0.10	0.753
Temperature x Resistance	4	2.50	< 0.05
Residuals	353		
Pupal Weight (Females)			
Temperature	4	155.36	< 0.001
Resistance	1	4.32	< 0.05
Temperature x Resistance	4	5.75	< 0.001
Residuals	191		
Fecundity			
Temperature	4	40.67	< 0.001
Resistance	1	0.18	0.674
Temperature x Resistance	4	2.04	0.094
Residuals	114		

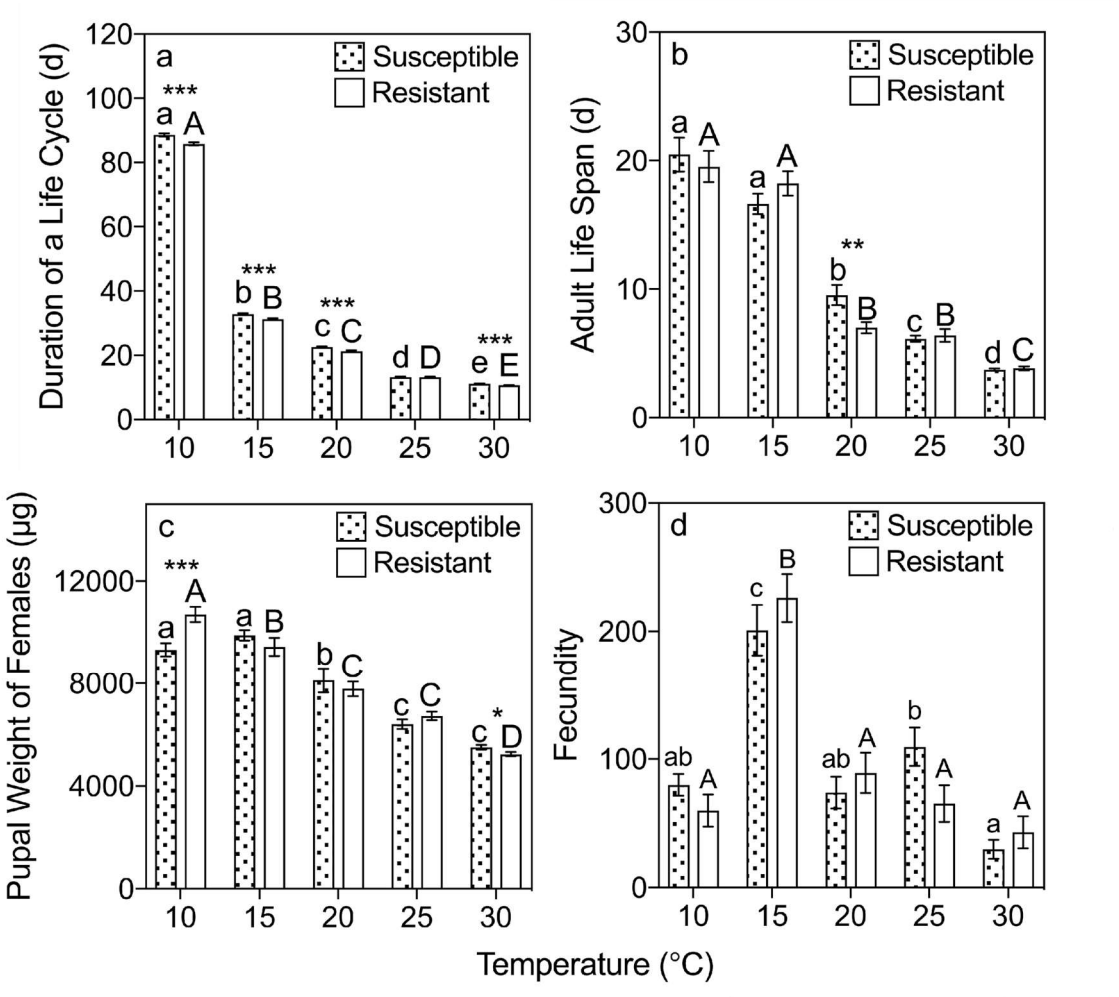


Figure 1 Mean (± SE) duration of the life cycle (a), adult life span (b), female pupal weight (c), and fecundity (d) of the susceptible strain (dotted columns) and the deltamethrin-resistant strain (empty columns) of *Plutella xylostella* at different constant temperatures. Characters above columns show the results of post-hoc analyses. Lowercase letters represent comparisons within the susceptible strain, and uppercase letters represent comparisons within the resistant strain among different temperatures (*CI* = 95%). Asterisks above characters indicate the p-value (no stars: *P* > 0.05; *: 0.01 < *P* < 0.05; **: 0.001 < *P* < 0.01; *: *P* < 0.001) of one-way ANOVA comparing the parameter across these two strains at a given temperature.**

Both the deltamethrin-resistant strain and the susceptible strain could complete development at constant temperatures of 10, 15, 20, 25, and 30°C. However, neonate larvae of both strains died very soon after hatching at 35°C. Surprisingly, resistance decreased the total development time (Fig. 1a; Table 1) as it is more common that resistant strains take longer than their susceptible counterparts to complete development (e.g. Sayyed et al. 2008; Ribeiro et al. 2014). In our study, resistance to deltamethrin did not impede development, rather it accelerated it. If metabolic resistance is involved, in the deltamethrin-resistant DBM, enhanced development rate may have been influenced by one or other of the metabolic enzymes. Alternatively, changes to the frequency of the associated alleles may have had an influence. Previous studies have shown that insecticide-resistance can interact with environmental conditions and thus influence survival rates differently. A fenvalerate-resistant line of DBM had significantly smaller eggs and a lower survival rate than the susceptible line (Chen et al. 2006a), and the resistant line reverted to a susceptible state after 10 generations if exposed to unfavourable dry and hot environments without selection pressure (Chen et al. 2006b). The intrinsic rates of population increase of highly and moderately *B. thuringiensis*-resistant sub-populations of DBM were similar to one another (Sayyed and Wright 2001).

The adult life span of the deltamethrin-resistant and susceptible strains were similar to one another (Fig. 1b; Table 1). However, resistance and its interaction with temperature had significant effects on the pupal weight of females (Fig. 1c; Table 1). The pupal weight of resistant females was significantly higher at 10°C, but lower at 30°C than that of the susceptible females (Fig. 1c). This might suggest that deltamethrin-resistant females can cope better with cold conditions and that susceptible females can cope better with higher temperature extremes in terms of gaining biomass. Resistance status did not affect the fecundity at either of these temperatures (Fig. 1d; Table 1), suggesting that larger females do not necessarily produce more eggs. Although deltamethrin-resistant insects and susceptible insects laid eggs when reared at 10°C, few of these eggs were fertile. While susceptible female moths reared at 10°C or 25°C could lay fertile eggs following mating with male moths reared at 25°C, no female moths could lay fertile eggs following mating with male moths that had been reared at 10°C (unpublished data, L. Wang et al.), suggesting that rearing at this low temperature affected male fertility in a manner that is yet to be determined.

CONCLUSION

The study provides important information on the complexities of the outcomes of the interactions between deltamethrin-resistance in DBM and ambient temperature. Ectotherm genotypes (in this case insecticide-resistance status) and abiotic stresses can interact and then generate unpredictable outcomes. Current models predicting DBM population dynamics and relative abundance in different locations do not consider different thermal biologies of different genotypes. This work shows the dramatic effects of the environment on many parameters used in these models and will help to enhance their accuracy, and thus their utility.

Acknowledgements

This project was funded in part by ACIAR projects HORT/2010/090 and HORT/2016/185. There is no conflict of interests.

References

Barbosa MG. 2019. *Strategies in the resistance management of Plutella xylostella (Lepidoptera: Plutellidae) and Spodoptera frugiperda (Lepidoptera: Noctuidae): Rotation of insecticides and biological control with parasitoid.* PhD, Federal University of Ceara.

Cao G, Han Z. 2006. Tebufenozide resistance selected in *Plutella xylostella* and its cross-resistance and fitness cost. *Pest Management Science* 62**:** 746-751.

Chen XD, Sanada-Morimura S, Yanagi S, Nakasuji F. 2006a. Genetic relationships between development of insecticide resistance and reduction of egg size as a negative effect on the fitness of the diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Applied Entomology and Zoology* 41**:** 479-486.

Chen XD, Sanada-Morimura S, Yanagi S, Nakasuji F. 2006b. Rapid recovery of susceptibility under harsh environmental conditions in fenvalerate-resistant strains of the diamondback moth, *Plutella xylostella* (Lepidoptera : Yponomeutidae). *Applied Entomology and Zoology* 41**:** 641-650.

Crow JF. 1957. Genetics of insect resistance to chemicals. *Annual Review of Entomology* 2**:** 227-246.

Etebari K, Furlong MJ, Asgari S. 2015. Genome wide discovery of long intergenic non-coding rnas in diamondback moth (*Plutella xylostella*) and their expression in insecticide resistant strains. *Scientific Reports* 5**:** 14642.

ffrench-Constant RH, Bass C. 2017. Does resistance really carry a fitness cost? *Current Opinion in Insect Science* 21:39-46.

Furlong MJ, Wright DJ, Dosdall LM. 2013. Diamondback moth ecology and management: Problems, progress, and prospects. *Annual Review of Entomology* 58**:** 517-541.

Groeters FR, Tabashnik BE, Finson N, Johnson MW. 1994. Fitness costs of resistance to *Bacillus thuringiensis* in the diamondback moth (*Plutella xylostella*). *Evolution* 48**:** 197-201.

Li ZM, Liu SS, Liu YQ, Ye GY. 2007. Temperature-related fitness costs of resistance to spinosad in the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *Bulletin of Entomological Research* 97**:** 627-635.

Lim GS. Biological control of diamondback moth. Diamondback Moth Management. Proceedings of the First International Workshop, Tainan, Taiwan, 11-15 March, 1985., 1986. Asian Vegetable Research and Development Center, 159-171.

Liu SS, Chen FZ, Zalucki MP. 2002. Development and survival of the diamondback moth (Lepidoptera : Plutellidae) at constant and alternating temperatures. *Environmental Entomology* 31**:** 221-231.

Muggleton J. 1983. Relative fitness of malathion-resistant phenotypes of *Oryzaephilus surinamensis* L. (Coleoptera, silvanidae). *Journal of Applied Ecology* 20**:** 245-254.

R Core Team. 2018. R: A language and environment for statistical computing. Version 3.5.0. Vienna, Austria: R Foundation for Statistical Computing.

Ribeiro LM, Wanderley-Teixeira V, Ferreira HN, Teixeira AA, Siqueira HA. 2014. Fitness costs associated with field-evolved resistance to chlorantraniliprole in *Plutella xylostella* (Lepidoptera: Plutellidae). *Bulletin of Entomological Research* 104**:** 88-96.

Sayyed AH, Saeed S, Noor-Ul-Ane M, Crickmore N. 2008. Genetic, biochemical, and physiological characterization of spinosad resistance in *Plutella xylostella* (Lepidoptera: Plutellidae). *Journal of Economic Entomology* 101**:** 1658-1666.

Sayyed AH, Wright DJ. 2001. Fitness costs and stability of resistance to *Bacillus thuringiensis* in a field population of the diamondback moth *Plutella xylostella* L. *Ecological Entomology* 26**:** 502-508.

Soderlund DM, Bloomquist JR. 1989. Neurotoxic actions of pyrethroid insecticides. *Annual Review of Entomology* 34**:** 77-96.

Talekar NS, Shelton AM. 1993. Biology, ecology, and management of the diamondback moth. *Annual Review of Entomology* 38**:** 275-301.

Development, implementation and monitoring of an insecticide resistance management strategy for diamondback moth in the South Pacific.

Atumurirava, F.
PLANT HEALTH, LAND RESOURCES DIVISION, PACIFIC COMMUNITY, NERERE, FIJI ISLANDS.
email: FeretiA@spc.int

Nand, N.
PLANT HEALTH, LAND RESOURCES DIVISION, PACIFIC COMMUNITY, NERERE, FIJI ISLANDS.
email: NiteshN@spc.int

Ahoafi, E.
MINISTRY OF AGRICULTURE FOOD AND FORESTRY, VAINI RESEARCH STATION, VAINI, KINGDOM OF TONGA.
email: ahoafieme@gmail.com

Foliaki, S.
MINISTRY OF AGRICULTURE FOOD AND FORESTRY, VAINI RESEARCH STATION, VAINI, KINGDOM OF TONGA.
email: sionekelo@gmail.com

Uelese, A.
CROPS DIVISION, MINISTRY OF AGRICULTURE AND FISHERIES, NU'U CROPS RESEARCH CENTRE, NU'U SAMOA
email: aleni.uelese@maf.gov.ws

Caucau, A.
MINISTRY OF AGRICULTURE RURAL AND MARITIME DEVELOPMENT, WATERWAYS AND ENVIRONMENT, KORONIVIA RESEARCH STATION, KORONIVIA, FIJI ISLANDS.
email: Anare.caucau@agriculture.gov.fj

Furlong, M.J.
SCHOOL OF BIOLOGICAL SCIENCES, THE UNIVERSITY OF QUEENSLAND, ST LUCIA 4072, QUEENSLAND, AUSTRALIA
email: m.furlong@uq.edu.au

ABSTRACT
In the South Pacific, management of the diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae), is constrained by resistance to broad-spectrum insecticides and access to selective products. Beginning in 2013, the susceptibility of DBM field populations to a range of insecticides was monitored in Fiji, Samoa and Tonga over a 5-year period. In 2014 an affordable *Bacillus thuringiensis* formulation, "AgChem-Bt", was launched in Fiji in close collaboration with a local pesticide retail company. The product was introduced to *Brassica* vegetable farmers through a series of "Pesticide Fora" and it was established as the cornerstone of an insecticide resistance management (IRM) strategy. The IRM strategy was based on the "window" principle advocated by the Insecticide Resistance Action Committee (IRAC); fundamentally, the rotation of insecticides with different modes of action in discrete temporal windows (=duration of pest life-cycle, ≈18 days for DBM in Fiji) to minimize exposure of successive generations to similarly acting insecticides. Constrained by the insecticides available to farmers in 2014, indoxacarb, lufenuron, abamectin, AgChem-Bt and chlorantraniliprole were incorporated into the IRM strategy; pyrethroid and organophosphate insecticides, although widely used, were excluded. In 2013, DBM populations from farms across Fiji demonstrated high levels of resistance to deltamethrin, indoxacarb and chlorantraniliprole, but all were very susceptible to AgChem-Bt. Annual collections of DBM populations from crops through to 2017 demonstrated significant reductions in resistance to these key products and the susceptibility of field collected insects to AgChem-Bt and abamectin remained high. The overall change in practice that has been achieved is demonstrated by the change in insecticide use by farmers in the Sigatoka Valley, Fiji. Prior to the IRM strategy, pyrethroid insecticides accounted for 21% of applications but this has declined to 7% and the products recommended by the strategy now account for 93% of insecticide applications against DBM.

Keywords
Bt, abamectin, chlorantraniliprole, selective insecticide, pyrethroid

INTRODUCTION
The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a destructive pest of *Brassica* crops throughout the Pacific (Furlong 2016), and is the major constraint to production. In Fiji, Tonga and Samoa, broad spectrum-insecticides have historically been readily available to farmers whereas safer, selective insecticides have not. In Fiji and Tonga, broad-spectrum compounds have been used intensively and there are reports of resistance to some of them in Fiji (Atumurirava and Furlong 2011; Atumurirava et al. 2016). In this longitudinal study, we investigated the susceptibility of DBM field-populations to a range of broad spectrum and selective insecticides in the three countries. In Fiji, an insecticide resistance management (IRM) strategy was designed and implemented to mitigate resistance to the limited number of selective insecticides that

were already available and preserve their long-term effectiveness. A key element of the overall strategy was the development of a close working relationship with a local pesticide retailer to establish selective insecticides in the local marketplace. Particular attention was given to importation, local testing, repackaging (in small, affordable quantities) and the provision of training on the use of a *Bacillus thuringiensis* subsp. *aizawai* product. This paper reports on the development and implementation of the IRM strategy, the monitoring of its effects and the change in insecticides used for the management DBM in the Sigatoka valley, Fiji's major vegetable production area.

MATERIALS AND METHODS

Insecticides

Deltamethrin (Suncis®, 25g $aikg^{-1}$), indoxacarb (Steward®, 200g aiL^{-1}) and chlorantraniliprole (Prevathon®, 50g aiL^{-1}) and *Bacillus thuringiensis* subsp. *aizawai* (AgChem Bt) were supplied by AgChem Fiji Ltd. Lufenuron (Match®, 50g aiL^{-1}) was supplied by Morris & Hedstrum Fiji Ltd. All commercial insecticide formulations were stored at room temperature.

Plants

Chinese cabbage, *Brassica rapa* var. *chinensis* cv Pak choy, was used in all experiments and for all *P. xylostella* rearing. Seeds were sown into a mixture of alluvial soil mixed with poultry manure in seed beds. Four-week old seedlings were transplanted into potting bags filled with the same soil-manure medium and grown to the 7-8 leaf stage in a shade-house before use in bioassays or for maintaining the insect cultures.

Insects

Culture methods

All DBM strains were reared separately at room temperature (25°C). Adult moths were held in wooden framed muslin covered oviposition cages (45 x 45 x 45 cm) containing a fresh potted Chinese cabbage plant (7-8
leaf stage) and an adult food source (20% w/v aqueous honey solution). Plants were exposed to adult moths for 24 h and replaced daily. Each egg-laden plant was labeled with the *P. xylostella* strain and oviposition date; plants containing eggs of different strains were kept separately from each other. When the eggs on a plant hatched, the plant was cut at its base, transferred to a ventilated plastic box (10 x 20 x 30 cm) and covered with freshly excised Chinese cabbage leaves. Old dry leaves were removed daily and replaced with fresh leaves. When the insects developed to pupae, they were carefully removed from the rearing box and stored in labeled Petri dishes (9 cm diameter) before being used to maintain the culture.

Leaf-dip bioassays

A standard leaf disc bioassay method, which was modified from previous studies (Furlong et al. 1994; Atumurirava et al. 2016), was used for all test insecticides. Test-solutions of each insecticide were prepared from commercial formulations using distilled water and 0.03% Tween-80 as a surfactant. Leaf discs (4.8 cm diameter) were cut from the middle leaves of Chinese cabbage plants and then immersed into the test solution for 10 seconds; excess solution was allowed to drip off from the leaf discs for another 10 seconds and then treated leaf discs were carefully placed, abaxial surface uppermost, on corrugated aluminium foil to dry for 1 h. Control leaf discs were treated by immersion in distilled water containing surfactant (0.03% Tween-80) only and then drained and dried in the same manner as insecticide treated leaf discs. Four leaf discs were treated with each test solution and the control. When dry, treated leaf discs were placed individually into Petri dishes (5 cm diameter) lined with moist Whatman No.1 filter papers; 10 early 3rd instar larvae were then carefully introduced to each Petri dish. In a given bioassay, six to seven test insecticide solutions and a Tween-80 control were tested. Petri dishes were taped together, placed in a ventilated plastic container and incubated at 25 (±2°C) with 12:12 (L:D). Treated leaf discs were removed after 48 h and replaced with fresh untreated Chinese cabbage leaves. Assessment of mortality varied between test insecticides: mortality caused by deltamethrin, indoxacarb, abamectin and chlorantraniliprole was assessed after 72 h, while mortality caused by lufenuron and Bt was assessed after 96 h. Mortality was determined by prodding each larva gently with a paint brush; any larva that did not respond to touch was regarded as dead.

Regional status of insecticide resistance

The susceptibility of field populations of DBM to commonly used and recently introduced selective insecticides was monitored in Fiji (2013-2017), Tonga (2013-2015) and Samoa (2013-2015). Head cabbage or Chinese cabbage crops were hand searched and large larvae and pupae were collected and taken back to the laboratory. A minimum of 50 individuals was collected per site (= small number of separate farms in a location), but typically >>100 individuals were collected. Insects from a single site were transferred to an oviposition cage to mate and lay eggs. Typically, second instar larvae from the next generation of insects was used in tests of the susceptibility of the population to a range of given insecticides but in some cases egg numbers produced were low and numbers had to be built up over one or more further generations before they could be tested in leaf dip bioassays. In all

cases, the susceptibility of the field population was compared to that of the Waite population, a standard insecticide susceptible DBM population that has been kept in the lab for more than 200 generations and which showed very stable responses to all insecticides over the course of the study (data not shown). For any given field population, the resistance ratio (RR) to a given insecticide was calculated by dividing the LD_{50} of that insecticide against that population by the LD_{50} of the insecticide against the Waite population.

Concomitant with the annual collections of insects in Fiji (2013-2017) in 2015 and 2017, farming communities completed a short, written survey detailing the insecticides that they used to manage *P. xylostella*.

Design and implementation of Insecticide Resistance Management (IRM) strategy in Fiji.

An insecticide resistance management (IRM) strategy was designed based on the principles of the Insecticide Resistance Action Committee (IRAC) (www.irac-online.org). Fundamentally the strategy is based upon the rotation of insecticides with different modes of action in a manner that minimises the probability that individuals of successive generations will be exposed to insecticides with the same mode of action. In Fiji, design of the strategy was constrained by very limited farmer access to selective insecticides. Bt, which is fundamental to sustainable management of *P. xylostella* (Furlong et al. 2013), was not available through commercial outlets. To ensure reliable availability of Bt in Fiji (and later more widely in the region) we worked closely with a local retailer, AgChem Fiji, to facilitate the importation, field testing (to satisfy Fiji Ministry of Agriculture requirements), registration, packaging and promotion of a Bt-aizawai product from Wuhan, China. The product, marketed as "AgChem Bt" was launched in Fiji in July 2014, and was sold with a leaflet on how it should be applied in a manner consistent with the basic rules of the IRM strategy; the launch was accompanied by a series of "Pesticide Forums" across Viti Levu and Vanua Levu, which introduced the IRM strategy to farmers and extension officers. The basic principles of the IRM strategy are:

- selective insecticides to be used in "windows" <1 DBM generation (≈ 18 days in Fiji)
- available insecticides with different modes of action should be strategically alternated between windows
- of the insecticides available in Fiji in 2014, Steward (indoxacarb), Match (lufenuron), Multiguard (abamectin), AgChem Bt (Bt) and Prevathon (chlorantraniliprole) were considered appropriate for incorporation into the overall IPM/ IRM strategy. All pyrethroid and organophosphate insecticides were considered incompatible with the strategy's goals, and were expressly excluded.

RESULTS AND DISCUSSION

In 2013, DBM field populations tested in Fiji demonstrated significant resistance to deltamethrin (30-fold) and indoxacarb (26-fold) and moderate resistance to chlorantraniliprole (13-fold) but were susceptible to the other selective insecticides tested (Figure 1A). Similarly, field populations from Tonga demonstrated significant resistance to deltamethrin (68-fold) but were susceptible to all other insecticides tested (Figure 1C); in Samoa all populations tested were susceptible to all insecticides (Figure 1B). In Tonga and Samoa, susceptibility of field populations did not change during the study (Figures 1B and 1C).

Following implementation and farmer adoption of the IRM strategy in Fiji in 2014, the susceptibility of DBM field populations to indoxacarb and chlorantraniliprole declined sharply and they were maintained at these levels throughout the rest of the study period (Figure 1A). Unfortunately, no further testing of field populations with deltamethrin was conducted after 2013, but it is anticipated that levels fell further with reduced use of the insecticide (Atumurirava et al. 2016). In addition to the reduced levels of resistance to indoxacarb and chlorantraniliprole, the adoption of the IRM strategy maintained the effectiveness of abamectin and AgChem Bt, two selective insecticides that were introduced as core elements of the IRM strategy. Similarly, susceptibility to lufenuron, which was incorporated because of its selective properties, its availability in Fiji and its wide use by farmers, was also maintained.

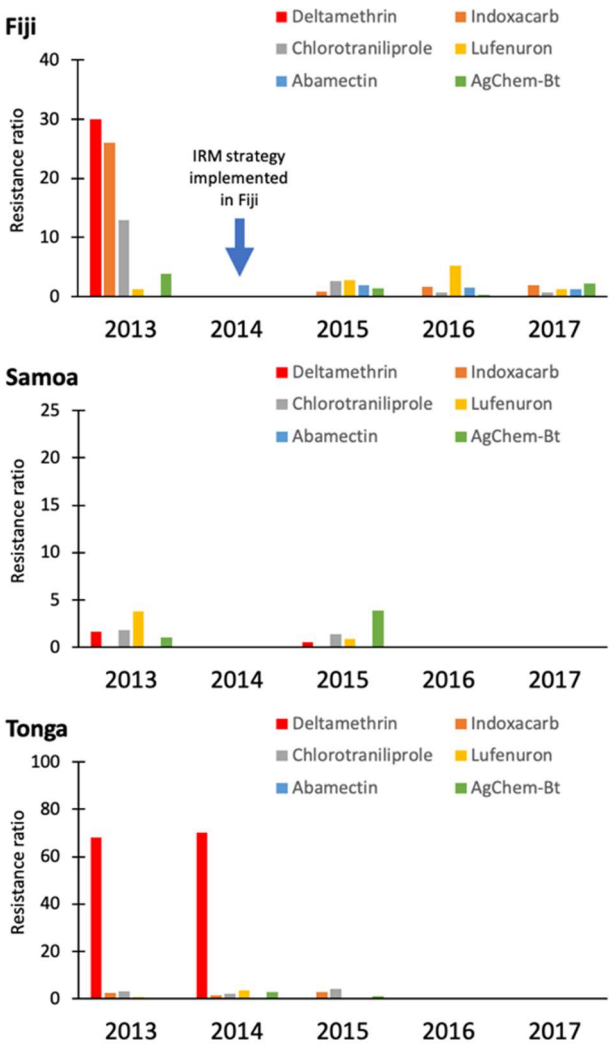


Figure 1. Resistance ratios (LC_{50} of test population/ LC_{50} of laboratory insecticide-susceptible population) of field-collected DBM populations to insecticides in A) Fiji, B) Samoa and C) Tonga. In 2014, an insecticide resistance management (IRM) strategy was implemented to mitigate resistance and facilitate the sustainable adoption of *Bacillus thuringiensis* and abamectin by *Brassica* growers in Fiji.

Farmer education and awareness (understanding the fundamental hazards of pesticide use, promoting safe and effective insecticide application methods, appreciation that organophosphate and pyrethroid insecticides are incompatible with IPM, effective application methods, the unsustainability of intensive and continued use of single or a narrow range of insecticides and the need to strategically rotate those insecticides that are used) and the provision of reliable source of selective insecticides in the marketplace were fundamental to the successful approach. The national insecticide awareness forums that were held in 2014 and the provision of information leaflets that detailed how to prepare and apply Bt as part of the IRM strategy resulted in a change of practice and a significant shift in the types of insecticide that were applied to *Brassica* crops in the Sigatoka valley in Fiji (Figure 2).

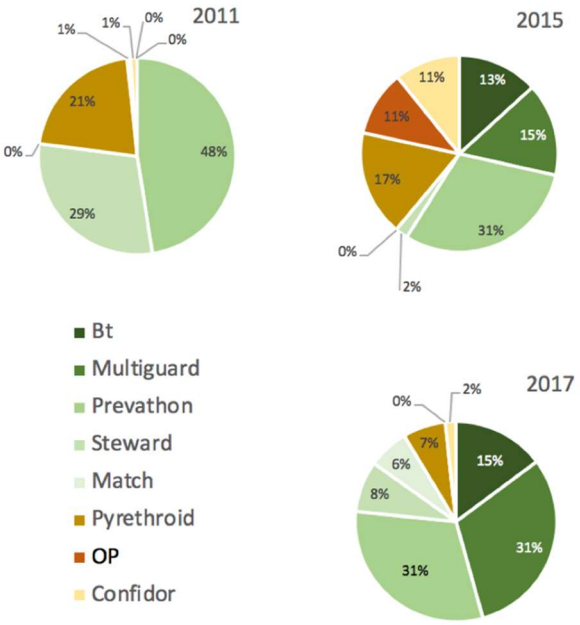


Figure 2. The contribution of different insecticides to the overall insecticide use against *P. xylostella* in the Sigatoka valley, Fiji 2011-2017.

In a 2011 survey that predated this study, *Brassica* farmers in the Sigatoka valley relied almost exclusively on chlorantraniliprole (Prevathon) (48% of applications), indoxacarb (Steward) (29%) and pyrethroid insecticides (21%) to manage DBM. Intensive use of these insecticides undoubtedly contributed to the resistance to these products that was measured at the start of this study in 2013 (Figure 1). By 2015, 1 year after the introduction of AgChem Bt and promotion of abamectin (Multiguard) and the IRM strategy, chlorantraniliprole use had declined to 31% of applications, indoxacarb to only 2% of applications and the combined use of Bt and abamectin contributed to 28% of applications. Further changes were apparent by 2017 when Bt and abamectin accounted for 46% of all applications, chlorantraniliprole for 31% and pyrethroids only 7% of applications, allowing reintroduction of indoxacarb (7%) following the return of susceptibility in DBM field populations (Figures 1 and 2). In the 5-year timespan of this study, the introduction and promotion of Bt as an integral part of an IRM strategy has been successful, allowing the continued use of older selective products (chlorantraniliprole, indoxacarb and lufenuron) and its effective use alongside abamectin. Improvement of effectiveness of the stagey can likely be achieved by the incorporation of other selective insecticides (e.g., Spinosad, Bt-*kurstaki*) and the possible elimination of lufenuron, which has been linked to toxic effects in mammals and non-target arthropods (EFSA 2009). Regardless of this initial success, farmers and their support networks must remain vigilant. DBM has a demonstrated propensity to develop resistance rapidly when any insecticide is used inappropriately in the tropics (Furlong et al. 2013) and the tendency of farmers to over-use products that they view as particularly effective is clear in this and other (Furlong et al. 2013; Li et al. 2016) studies. The susceptibility of DBM field populations and the use of different insecticides should be monitored frequently to note any changes in insecticide susceptibility in the pest population and over reliance on any given insecticide by farmers. If this is done, and provided that education and awareness campaigns are maintained, then the strategy promises to be sustainable, and should be extended to other countries in the region, especially Tonga, where resistance to pyrethroids has been detected.

Acknowledgements

This work was conducted as part of ACIAR project HORT/2010/090.

References

Atumurirava F, Furlong MJ. 2011. Diamondback moth resistance to commonly used insecticides in Fiji. In: R. Srinivasan, Anthony M. Shelton and Hilda L. Collins (ed.) Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests. *Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests*, Nakhon Pathom, Thailand, (216-221).

Atumurirava F, Nand N, Furlong MJ. 2016. Diamondback moth resistance to insecticides and its management in the Sigatoka Valley, Fiji. In: *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): International Symposium on Horticulture in Developing Countries and World Food Production*, Brisbane, Australia, (125-130). doi:10.17660/ActaHortic.2016.1128.17

EFSA- European Food and Safety Authority 2009. Conclusion regarding the peer review of the pesticide risk

assessment of the active substance lufenuron. Volume 7. DOI: 10.2903/j.efsa.2009.189r

Furlong MJ, Verkerk RHJ, Wright DJ. 1994. Differential-Effects of the Acylurea Insect Growth-Regulator Teflubenzuron On the Adults of 2 Endolarval Parasitoids of *Plutella xylostella*, *Cotesia plutellae* and *Diadegma* semiclausum. *Pesticide Science* 41: 359-364. doi:10.1002/ps.2780410412

Furlong MJ, Wright DJ, Dosdall LM. 2013. Diamondback moth ecology and management: problems, progress, and prospects. *Annual Review of Entomology* 58: 517-541. doi:10.1146/annurev-ento-120811-153605

Furlong MJ. 2016. Exploiting ecosystems services for biological control of pests and diseases. In *Mainstreaming ecosystem services and biodiversity into agricultural production and management in the Pacific Islands* (pp. 56-65) Rome, Italy: Food and Agriculture Organization of the United Nations.

Li Z, Feng X, Liu SS, You M, Furlong MJ. 2016 Biology, ecology, and management of the diamondback moth in China. *Annual Review of Entomology* 61: 277-296. doi:10.1146/annurev-ento-010715-023622

Insecticide resistance monitoring and management of diamondback moth, *Plutella xylostella* in Hawaii and Taiwan

Chou, M.Y.
NATIONAL CHUNG HSING UNIVERSITY, AGRICULTURAL EXTENSION CENTER
145 XING DA RD., SOUTH DISTRICT, TAICHUNG, 402, TAIWAN R.O.C.
email: mingyichou@nchu.edu.tw

Shimabuku, R.
UNIVERSITY OF HAWAII AT MANOA, HAWAII COOPERATIVE EXTENSION SERVICE
310 KAAHUMANU AVE., BLDG. 214 KAHULUI, HI, 96732, U.S.A.
email: ShimabukuR@ctahr.hawaii.edu

Sugano, J.
UNIVERSITY OF HAWAII AT MANOA, HAWAII COOPERATIVE EXTENSION SERVICE
910 CALIFORNIA AVE. WAHIAWA, HI, 96786-2124, U.S.A.
email: suganoj@ctahr.hawaii.edu

Uyeda, J.
UNIVERSITY OF HAWAII AT MANOA, HAWAII COOPERATIVE EXTENSION SERVICE
910 CALIFORNIA AVE. WAHIAWA, HI, 96786-2124, U.S.A.
email: juyeda@hawaii.edu

Hamasaki, R.
UNIVERSITY OF HAWAII AT MANOA, HAWAII COOPERATIVE EXTENSION SERVICE
67-5189 KAMAMALU ROAD KAMUELA, HI, 96743, U.S.A.
email: rth@hawaii.edu

Tavares, K.
UNIVERSITY OF HAWAII AT MANOA, HAWAII COOPERATIVE EXTENSION SERVICE
310 KAAHUMANU AVE., BLDG. 214 KAHULUI, HI, 96732, U.S.A.
email: Kylielw@hawaii.edu

Silva, J
UNIVERSITY OF HAWAII AT MANOA, HAWAII COOPERATIVE EXTENSION SERVICE
955 Kamehameha Highway, Pearl City, HI, 96782-3344
email: jhsilva@hawaii.edu

Huang, Y.B.
TAIWAN AGRICULTURAL RESEARCH INSTITUTE, COUNCIL OF AGRICULTURE
189, ZHONGZHENG RD., WUFENG DIST., TAICHUNG CITY 41362, TAIWAN (R.O.C.)
email: ybhuang@tari.gov.tw

Kao, C.H.
TAIWAN AGRICULTURAL RESEARCH INSTITUTE, COUNCIL OF AGRICULTURE
189, ZHONGZHENG RD., WUFENG DIST., TAICHUNG CITY 41362, TAIWAN (R.O.C.)
email: chkao@tari.gov.tw

Mau, R.F.L.
UNIVERSITY OF HAWAII AT MANOA, HAWAII COOPERATIVE EXTENSION SERVICE
3550 MAILE WAY, GILMORE 310, HONOLULU, HI 96822, U.S.A.
email: maur@ctahr.hawaii.edu

ABSTRACT

Diamondback moth populations are exposed to constant insecticide selection pressure under year-round crop production in tropical areas such as Hawaii and Taiwan. A window based insecticide rotation program has been implemented in Hawaii since 2003 after multiple crop failures caused by over reliance on a single active ingredient over time. Recommendations of effective active ingredients were made to growers according to semi-annual laboratory bioassays using diagnostic dose, LC_{99} value obtained from susceptible population, to monitor insecticide resistance in three main production areas on the island of Oahu, Maui and Hawaii. The key elements for the success of the decade long program were the involvement of growers and extension personnel. Growers have input into the monthly rotations in organizing the 6-month class rotation cycles, which increase their commitment to the program. A similar program was initiated in the early 90s in Taiwan with 29 farmers' associations participating in the pilot program. Monitoring programs continued in Kaohsiung (Luzhu) and Changhua (Xihu) to provide information to growers on insecticide susceptibility for local *P. xylostella* population. Leaf-dip bioassay data from 2013 to 2016 field population showed that spinetoram, emamectin benzoate and tolfenpyrad were three active ingredients, which obtained >60% of DBM mortality at these two locations. An extension program was launched in 2018 with collaboration from growers in the Changhua area to record *P. xylostella* insecticide susceptibility in rotation (Zhutang) versus cocktail mixing (Xizhou) management using a two-window management approach. Significantly higher (two sample t-test, $p<0.05$) insecticide susceptibility was recorded from *P. xylostella* collected from area adapting rotation program (Zhutang) in Fall for BT, diafenthiuron, spinosad and tolfenpyrad compared to the beginning of the program in Spring 2018. Laboratory data indicated that 6 to 10-month suspension from treatment is required to improve *P. xylostella* susceptibility to insecticide compounds. Rotation of spinetoram, emamectin benzoate, flubendiamide and

chlorfenapyr is adapted in the Zhutang area for 2019 production season.

Keywords
pesticide treatment window, insecticide resistance, *Plutella xylostella*

INTRODUCTION
Mild climate in the tropics and subtropics provides a suitable environment for year-round crucifer vegetable production and consistent pest pressure challenges. Cabbage is cropped weekly throughout the year in Hawaii with a total of 300 ha in production annually. There are two groups of cabbage producers who face different challenges in managing insecticide resistance problems. Year-round planting on small acreage (average 2 ha production per grower) provides consistent insecticide selection pressure for diamondback moth (DBM), *Plutella xylostella* to develop resistance traits. Growers who have access to large land tracts that are located in different parts of the island (>100 ha production) use distance as a barrier to plant various crucifer crops but kept in distance to lessen the importance of DBM movement from plot-to-plot.

Insecticide resistance in DBM has been a continuing problem. DBM is the key pest of crucifers due to its propensity for selection of genetic resistance. Imported cabbageworm (*Pieris rapae*), cabbage webworm (*Hellula undalis*), and cabbage looper (*Trichoplusia ni*) are of lesser importance because they are easily controlled by most labeled insecticides. DBM resistance to efficacious insecticide classes commonly occurs within two to three years after its introduction. Genetically resistant populations have occurred to many insecticide classes described by the Insecticide Resistance Action Committee (IRAC) classification system. Genetic insecticide resistance in Hawaii's DBM has occurred to insecticides in the following groups: organophosphates and carbamates (IRAC Class 1A and B), pyrethroids (IRAC Class 3), spinosyns (IRAC Class 5), *Bacillus thuringiensis* (IRAC Class 11), novaluron (IRAC Class 15), indoxacarb (IRAC Class22), and diamides (IRAC Class28) (Tabashnik et al. 1987; Mau and Gusukuma-Minuto 2001 ; Zhao et al. 2006).

Development of Hawaii DBM insecticide class rotation program. In 2000, spinosad resistance was discovered at several farms on Oahu Island, Hawaii after less than two years of its introduction. Resistance ratios (RR) of 600 to 1,000 fold higher in DBM than from a susceptible DBM colony from Cornell University at farms at 3 distant locations across the island (Zhao et al. 2002). In discussing options, an IRAC representative revealed that there was a genetic basis for resistance in DBM populations and the possibility of remediating populations by not using the insecticide class. Since growers had already experienced the development of resistance to organophosphate, organochlorine, and pyrethroid insecticides prior to onset to that in spinosad, growers agreed to a program proposed by the University of Hawaii Cooperative Extension Service. Spinosad use was disallowed and resistance levels were monitored. A threshold for reintroduction of spinosad was set at a LC_{50} dose of 2 ppm. Spinosad RR ratio was reintroduced for use in about two years and a rudimentary insecticide class rotation system of four insecticide classes (emamectin benzoate, indoxacarb, *B. thuringiensis*, and spinosad) was implemented on Maui and Hawaii islands in 2002 and on Oahu in 2003. Insecticide rotation window durations were arbitrarily set initially; a month-long rotation window was established in 2006 to instruct growers following the pesticide label restrictions.

Intensive planting and small growers are most common for agriculture production in Asia. Diverse crucifer crop varieties and highly unsynchronized management schedule among producers in the area provide a unique challenge for DBM insecticide resistance management. The average cabbage production is at 0.2 ha per grower with repeated up to 3 plantings in Taiwan. Annual planting of cabbage in central Taiwan is 8,000~10,000 ha, not including Chinese cabbage, broccoli, cauliflower, radish and other leafy crucifer crops. There is limited extension personnel available to provide up-to-date DBM insecticide susceptibility information to thousands of producers in the area. Local ag-chemical suppliers are often the main source of information when growers need recommendations on pest control products. Conventional practice relies on weekly treatment of cocktail mixture of broad spectrum insecticides with one to two novel classes of insecticides to provide blemish free produce.

DBM insecticide resistance monitoring and management program in Taiwan was initiated in the 80s with collaboration between Taiwan Agricultural Research Institute and 29 farmers' associations in production areas around the island. The recommended sampling plan was one population every 5,000 ha (50 km^2) area (Cheng 1981). However, with the increase of novel classes of active ingredients available in the market during the last two decades, the complexity of insecticide use pattern and selection pressure for DBM insecticide resistance also increased dramatically. Widespread resistance to organophosphate, fipronil, pyrethroids, abamectin, emamectin benzoate, chlorfenapyr, cartap, chlorfluazuron and diamide were recorded among DBM populations in Taiwan (TARI 2018). Spinosad, tolfenpyrad and indoxacarb provided good field performance in some areas.

Key elements in Hawaii DBM insecticide resistance management program include routine single dose bioassays (diagnostic dose) of the active ingredients in the rotation program. The calculated LC_{99} value from the response of susceptible colonies provides early detection of signs of resistance build-up in the field population (Zhao and Grafius 1993). The program was implemented since 2003 when spinosyns, indoxacarb and emamectin benzoate were the only three effective active ingredients available for DBM control. Extension personnel conduct 2-4 bioassays annually to provide growers the most up-to-date information. Grower meetings are conducted after each bioassay to offer growers the opportunity to input their thoughts on scheduling of the rotation program. After the rotation program is determined for the following season, local ag-chemical suppliers are informed to distribute the advice to growers who participated in the program.

In Taiwan, efforts of educating growers about insecticide rotation were conducted through field demonstration trials, grower collaboration projects and laboratory research (Cheng et al. 1996; Kao and Cheng 2001). Long term insecticide resistance monitoring was conducted in three production areas (Changhua, Yunlin and Kaohsiung) using label rate to provide growers the DBM susceptibility information on more than 20 insecticide active ingredients. Although label rate bioassays provide a field situation for the tested active ingredients, it is often a late warning for resistance detection. In addition, lack of information for local pest populations is one of the main hurdles for growers to adapt the resistance management program.

An education program launched in 2018 in one of the cabbage production areas in Changhua with 10 growers (total planting: 10+ ha) participated in DBM resistance management by limiting the number of active ingredients applied in the area. Insecticide susceptibility information of the local population was provided to the growers to determine the most effective products for rotation. The group voluntarily removed organophosphate from the treatment program after bioassay revealed low DBM mortality. The results and impact of the program are discussed in detail in the following sections.

MATERIALS AND METHODS

Program Essentials.

Hawaii DBM resistance management program was a region-based, area-wide program that utilized month-long windows for insecticide class rotations. We have used a program similar to that described by Zhao and Grafius (1993). A diagnostic single dose bioassay of representative insecticides from different insecticide classes (IRAC insecticide classes) are conducted every six months. Insecticides that provide 70% or greater mortality are used in the monthly-use windows. The monthly insecticide class rotations are selected to assure high mortality rates every other month to assure that DBM populations do not increase for two consecutive months.

Two methods were used to determine the insecticide single-dose assay concentration. With insecticides that were never used against Hawaii field DBM populations, we performed multi-dose insecticide assays with one Hawaii field population to determine insecticide toxicity and analyzed results using POLO probit analysis software. The predicted LC_{99} was chosen as the diagnostic dose. If the insecticide had already widespread use in Hawaii, we selected the highest field-use rate allowed by the EPA product label. Test concentrations were based on a 60 gallons per acre treatment rate and an appropriate spray adjuvant was used in the bioassay treatment preparation.

Collection of Geographic Populations.

Populations of DBM were collected twice a year from major crucifer growing regions on Oahu, Maui, and Hawaii islands. Due to the natural occurrence of DBM larval parasitoids, 150 -200 DBM pupae were collected from each region to form a cohort for insecticide bioassay evaluations. Collections were done during a week period prior to the occurrence of the full moon. The pupae were placed in two 15 cm diameter, cylindrical plexiglass tubes that stood vertically on end. Each tube carried approx. 100 DBM pupae and supplied with a vial of 10% honey-water solution fitted with a piece of cotton dental wick for adult feeding. Each cage was covered with a sheet of paper towel covered by a sheet of nylon organdy that was secured by a polyethylene band. After adult DBM emergence, chunks of cabbage were placed on the nylon organdy to induce DBM oviposition on the paper towel layer. Paper towel layers were replaced daily for 3 days. Each day's egg collection was secured in a zip-lock polyethylene bag and refrigerated at about 4ºC. One to two days prior to egg eclosion, the paper towels were placed on the upper surfaces of large rape plants grown in 10 cm diameter pots. Second and third instar DBM larvae were used in the insecticide leaf dip DBM bioassays and mortality was determined after 3-5 days depending on the insecticide class. Moribund individuals were considered dead.

Susceptibility was determined using the IRAC Susceptibility Test Method 18 for *Plutella xylostella*. Leaf dip bioassays consisted of using ten 50 mm diameter head cabbage leaf disks treated at the discriminating dose for each insecticide. The insecticides tested were spinetoram, emamectin benzoate, novaluron, and indoxacarb. The tested diagnostic doses for each was 1.08, 1.0, 75, and 50 ppm, respectively. The following diamide insecticides chlorantraniliprole, cyantraniliprole and flubendiamide were tested, and the discriminating doses were 175 (field rate), 130 (field rate), and 1.68 ppm, respectively.

Field rate monitoring program in Taiwan adopted a similar protocol to the Hawaii program. DBM samples were collected every 6 months from three to five closed-by fields (<5 km in distance) at each of the monitoring areas. Leaf dip bioassays were conducted according to Kao et al. (2001). Bioassays conducted in 2017 included abamectin, cartap, chlorantraniliprole, chlorfenapyr, chlorfluazuron, delta-methrin, emamectin benzoate, fenvalerate, fenpropathrin, fipronil, flubendiamide, indoxacarb, permethrin, profenofos, spinosad and tolfenpyrad. Field rate and 2X field rate were tested to determine the susceptibility of field collected DBM populations. Two bioassays were conducted in 2018 with Zhutang and Xizhou populations to determine the effects of adopting resistance management program on the DBM susceptibility to the following active ingredients: tolfenpyrad, indoxacarb, *B. thuringiensis*, abamectin, spinosad, spinetoram, emamectin benzoate and flubendiamide. Ten replicated samples, containing ten 2~3 instars DBM larvae each were evaluated at field rate to determine the susceptibility of DBM populations. The statistical difference of DBM susceptibility in spring and fall bioassays were determined with two sample t-test.

RESULTS AND DISCUSSION

Hawaii.

Emamectin benzoate (IRAC Class 6), tolfenpyrad (IRAC Class 21A) and spirotetramat (IRAC Class 23) resistance have not been detected since the products were launched in Hawaii (Table 1). Effective DBM suppression was achieved by limiting the use of each active ingredient to <3 non-consecutive months per year. The genetic traits of insecticide resistance in the populations did not affect the field control during the 10+ years of program.

Table 1. Resistance status and total numbers of windows allowed for Hawaii DBM resistance management program

Active Ingredient	Resist Status	IRAC Class	Total Used: Windows/yr
Spinosyn	R	5	0-2
Emamectin benzoate	N	6	3
Bacillus thuringiensis	R	11	Use when needed
Novaluron	R	15	1-3
Tolfenpyrad	N	21A	2-3
Indoxacarb	R	22	1-2
Spirotetramat	N	23	2
Diamides	R	28	1-2

Determination of the Regional Monthly Insecticide Order.

Based on the regional bioassay insecticide mortality results, insecticides are either added or removed from the next six-month insecticide treatment windows. Insecticides with mortality that is less than 70% are not scheduled for use. The remaining insecticides are placed in monthly windows following three rules. 1. The most effective insecticide classes are scheduled when we have historically the greatest DBM pressure; 2. There are no consecutive months with products that provide marginal control of the DBM; 3. No insecticide is used for two consecutive months. In addition, if additional treatments for other lepidopteran pests are needed during a monthly insecticide spray window, growers should use *B. thuringiensis* or pyrethroid insecticides (Table 2).

Table 2. Insecticide rotation schedule for Maui Spring, 2017

Month	IRAC	Active ingredient (mortality from leaf-dip bioassay)
January	22	Indoxacarb (69%)
February	5	Spinetoram (90%) or Spinosad
March	6	Emamectin benzoate (100%)
April	15	Novaluron (85%)
May	28	Flubendiamide (100%) or Chlorantraniliprole
June	5	Spinetoram (90%) or Spinosad

Taiwan.

DBM field populations collected from Changhua (Xihu) and Kaohsiung (Luzu) between 2012 to 2016 showed low level of response to organophosphate, fipronil, pyrethroids, abamectin and emamectin benzoate, chlorfenapyr, cartap, chlorfluazuron and diamides (Table 3). The use of profenofos and fipronil on crucifer crops were removed in 2019 and 2018, respectively. Further studies will be needed to record the recovery of the field populations. Spinosyn, tolfenpyrad and indoxacarb were novel classes of active ingredients, which provided >60% DBM larval mortality from the two tested locations during 2013-2016. Location based monitoring and management programs are urgently needed to provide growers information on suitable rotation schedules for the area.

Table 3. Field rate bioassay for Taiwan DBM populations, 2013-2016

MoA	Active ingredient	%Mortality (Luzu / Xihu)			
		2013	2014	2015	2016
1A	*Carbofuran	17 / 5	8 / 8	9 / 6	19 / 11
1B	*Profenofos	49 / 62	15 / 37	17 / 46	62 / 30
2B	*Fipronil	4 / 0	4 / 4	4 / 21	31 / 8
3A	*Permethrin	0 / 0	6 / 6	4 / 4	7 / 9
5	Spinosad	60 / --	20 / 20	44 / 98	80 / 61
	Spinetoram	91 / 83	92 / 92	94 / 64	98 / 98
6	*Emamectin benzoate	77 / 91	77 / 77	49 / 61	33 / 44
	*Abamectin	6 / 0	13 / 13	4 / 41	9 / 7
13	*Chlorfenapyr	20 / 16	16 / 16	36 / 67	48 /24
14	*Cartap	68 / 43	48 / 48	70 / 94	48 / 46
15	*Chlorflua-zuron	26 / 8	16 / 16	9 /35	14 / 5
21A	Tolfenpyrad	71 / 82	73 / 73	66 / 98	68 / 35
22A	Indoxacarb	83 / 50	35 / 35	76 / 60	66 / 51
28	*Chloran-traniliprole	6 / 0	0 / 0	13 / 38	16 / 9
	*Fluben-diamide	66 / 34	35 / 35	46 / 53	48 / 30

Active ingredients with * were rated as ineffective to the two populations tested. Data were summarized from the TARI annual report (2017).

Education program introduced to growers in the Zhutang and Xizhou area in 2018 showed different DBM susceptibility responses to the tested compounds in spring and fall seasons. Both groups of growers were provided with data from spring bioassay, and were free to decide whether to participate in the management program by restricting the use of pesticide to recommended compounds. Growers in Xizhou decided not to participate the program and results from bioassays conducted in fall showed that susceptibility of DBM to spinetoram, spinosad, tolfenpyrad, indoxacarb and abamectin did not have significant changes between spring and fall (Figure 1). Susceptibility of DBM to flubendiamide and BT decreased significantly but increased significantly to emamectin benzoate. Growers who adapted the idea of resistance management by limiting the active ingredients applied in the area.

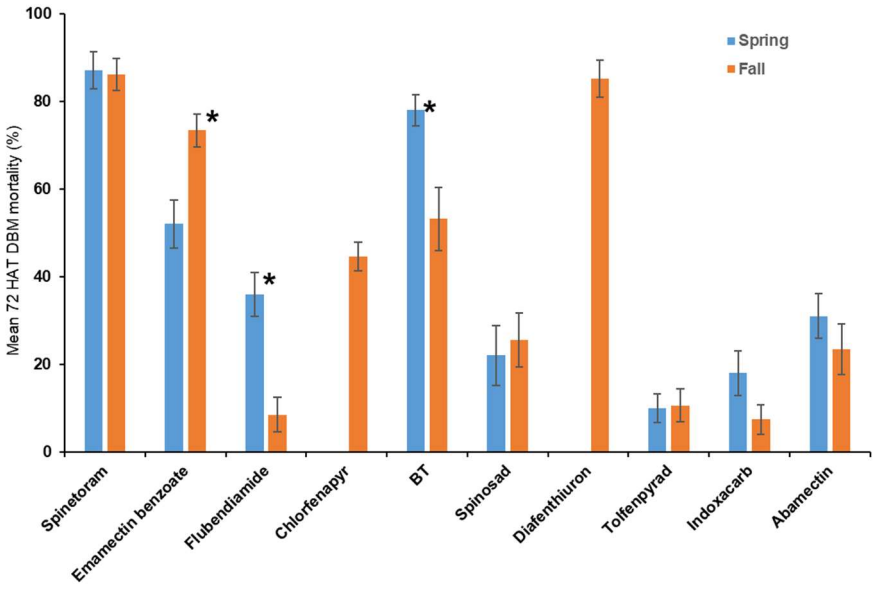


Figure 1. Field rate leaf dip bioassays of Xizhou DBM populations in spring and fall 2018. Means with * were significantly different according to two sample t-test ($p < 0.05$).

Growers in Zhutang limited the use of insecticide to spinetoram, emamectin benzoate, flubendiamide and chlorfenapyr in spring and fall. Significant increased DBM mortality to BT, tolfenpyrad, spinosad and diafenthiuron was recorded in fall (Figure 2). No significant difference was found among all other tested compounds.

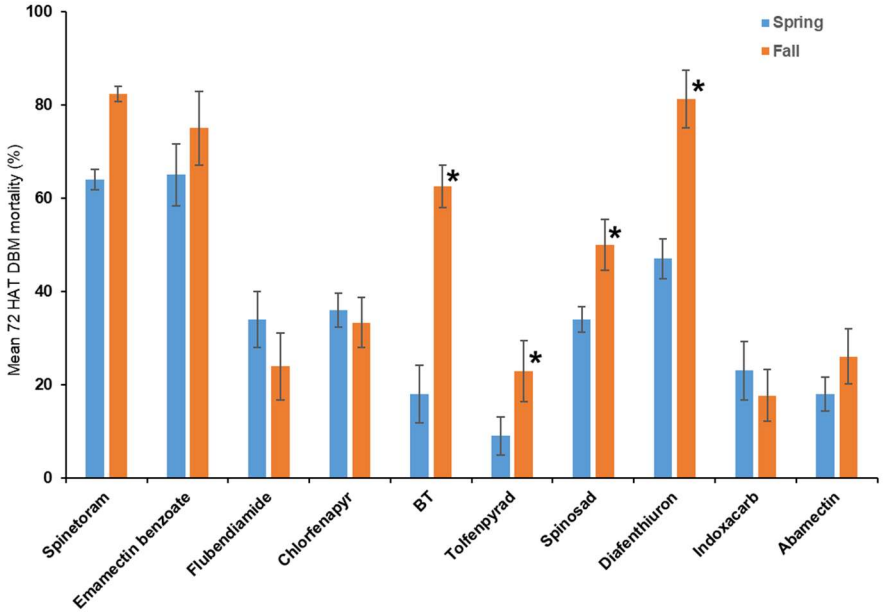


Figure 2. Field rate leaf dip bioassays of Zhutang DBM populations in spring and fall 2018. Means with * were significantly different according to two sample t-test (p<0.05).

Bioassays conducted with the recommended products from local ag-chemical suppliers revealed low DBM response to thiodicarb and surprisingly high response to cyromazine, a dipteran IGR product labeled for leaf miner control (Table 4). A resistance management program including spinetoram, emamectin benzoate, flubendiamide, chlorfenapyr and cyromazine was established for 2019 following discussion with the grower group. The use of thiodicarb was voluntarily removed for DBM control.

Table 4. DBM mortality at 72 h after treatment from leaf dip bioassays of commonly used pesticides in Changhua area

IRAC	Treatment	ppm	N	Mortality (Mean ± S.E.)
	CK		10	1±1
1A	Thiodicarb	300	10	20±4.94
17	Cyromazine	111	10	81±4.33
	Cyromazine	222	10	99±1

Establishing a window based insecticide rotation program for Taiwan DBM management was initiated by BAPHIQ (Hsu and Chang 2016). The program was designed with the consideration of protecting natural enemies in the environment by using BT and other bio-pesticides in the first window after transplanting. Pheromone trapping and evening water sprinkler were recommended as an integrated pest management package. However, limited extension service was available to provide ground level information, update and modifications to growers in different production areas. Studies conducted in the Zhutang and Xizhou area in 2018 showed growers who adopted a resistance management program by limiting insecticide usage significantly increased the response of DBM to some compounds, which were not in the recommended list in the following season.

CONCLUSION

Resistance mitigation program history.

Grower participation in resistance mitigation programs have been excellent since it was to their advantage to stop use of a genetically challenged insecticide. Resistance mitigation for spinosad, indoxacarb, and novaluron took two to three years. The Hawaii DBM resistance management program has succeeded because of grower participation, the insecticide bioassay program, and due to the number of insecticide classes where remediation occurred relatively quickly.

Regulatory support and limitations.

One of the key components in successful insecticide resistance management is clear regulation guidelines for applicators to follow and adapt the practice. The input of each insecticide active ingredient in the U.S. is restricted by insecticide label. US-EPA has developed voluntary pesticide resistance management labeling guidelines based on target site/ mode of action (MoA) since 2001 (US-EPA 2001). The policy supports the maximum amount and number of treatments allowed and therefore provides the foundation for applicators to adapt an insecticide rotation practice. In contrast, insecticide labels in Taiwan regulate the field rate without the limitation on the total input nor maximum number of treatments allowed for each active ingredient. The essential framework is absent to provide guidelines for the applicators to implement an insecticide resistance management program.

Acknowledgements

Data reported for the Taiwan extension project for 2018 was funded by the Council of Agriculture, Taiwan, R.O.C. (Project # 107- 16.1.1).

References

Cheng EY. 1981. Insecticide resistance study in *Plutella xylostella* L.- I . Developing a sampling method for surveying. Journal of Agricultural Research China 3: 277-284.

Cheng EY, Chen CN, Kao CH. 1996. Management of resistant diamondback moth and other crucifer pests in Taiwan. Journal of Agricultural Research China 45: 89-104.

Hsu JC, Chang CC. 2016. Instruction to pesticide resistance management. 58 pp. Hsu JC eds. Pub: Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan. Taipei, Taiwan.

Kao CH, Cheng EY. 2001. Insecticide resistance in *Plutella xylostella* L. XI. Resistance to newly introduced insecticides in Taiwan (1990~2001). Journal of Agricultural Research China 50(4): 80~89.

Mau RFL, Gusukuma-Minuto L. 2001. Diamondback moth resistance to spinosad in Hawaii: confirmation, review of causal factors and establishment of a mitigation plan, pp. 75-80. In Proceedings of 5th International Seminar on Technology of Cole Crops Production, 17-18 May 2001, University de Celaya, Celaya, Guanajuato, Mexico

Tabashnik BE, Cushing NL, Johnson MW. 1987. Diamondback moth (Lepidoptera: Plutellidae) resistance to insecticides in Hawaii: intra-island variation and cross-resistance. Journal of Economic Entomology 80(6):1091-9.

TARI 2017. Pesticide Research. Annual Report of Taiwan Agricultural Research Institute 2016: 84-86.

TARI 2018. Pesticide Research. Annual Report of Taiwan Agricultural Research Institute 2017: 75-76.

US-EPA (United States Environmental Protection Agency). 2001. Guidance for Pesticide Registrants on Pesticide Resistance Management Labeling (PR Notice 2001–5) posted at http://www.epa.gov/PR_Notices/pr2001–5.pdf

Zhao JZ, Grafius E. 1993. Assessment of different bioassay techniques for resistance monitoring in the diamondback moth (Lepidoptera: Plutellidae). Journal of Economic Entomology 86(4): 995-1000.

Zhao JZ, Li YX, Collins HL, Gusukuma-Minuto L, Mau RL, Thompson GD, Shelton AM. 2002. Monitoring and characterization of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad. Journal of Economic Entomology 95(2): 430-436.

Zhao JZ, Collins HL, Li YX, Mau RFL, Thompson GD, Hertlein M, Andaloro JT, Boykin R, Shelton AM. 2006. Monitoring of diamondback moth (Lepidoptera: Plutellidae) resistance to spinosad, indoxacarb, and emamectin benzoate. Journal of Economic Entomology 99(1): 176-181.

Bifenazate resistance and mutations in cd1 helix of mitochondrial cytochrome *b* of *Tetranychus urticae* Koch (Acari: Tetranychidae)

Chang, S.C.
APPLIED ZOOLOGY DIVISION, TAIWAN AGRICULTURAL RESEARCH INSTITUTE, COUNCIL OF AGRICULTURE
189 CHUNGCHENG ROAD, WUFENG, TAICHUNG CITY, TAIWAN, REPUBLIC OF CHINA
email: scchang@tari.gov.tw

Kao, C.H.
FORMER RESEARCH FELLOW, APPLIED ZOOLOGY DIVISION, TAIWAN AGRICULTURAL RESEARCH INSTITUTE, COUNCIL OF AGRICULTURE
189 CHUNGCHENG ROAD, WUFENG, TAICHUNG CITY, TAIWAN, REPUBLIC OF CHINA
email: kao767989@gmail.com

Dai, S.M.
DEPARTMENT OF ENTOMOLOGY, NATIONAL CHUNG HSING UNIVERSITY
145 XINGDA ROAD, SOUTH DIST., TAICHUNG CITY, TAIWAN, REPUBLIC OF CHINA
email: sdai5497@dragon.nchu.edu.tw

ABSTRACT
The two-spotted spider mite, *Tetranychus urticae* Koch, is responsible for yield losses in many crops due to its short life cycle, abundant progeny, and ability to develop resistance to acaricides rapidly. Many newly developed acaricides appear to affect mitochondrial respiration like electron transport chain disruption. In Taiwan, bifenazate was registered for watermelon mite control in 2010, and later extended to several fruits and vegetables for mite control before 2018. To understand the possible resistance profile of bifenazate in two-spotted spider mite, toxicity resistance selection against mite population collected from Chiayi county of Taiwan was conducted every two weeks under laboratory conditions. The resistance ratios of progeny were 23.6 times after 25-month selection and 267.5 times after 30-month selection in comparison with the parental population. One nucleotide substitution, which resulted in amino acid substitution of I128T, was detected in cd1 helix of mitochondrial cytochrome *b* Qo pocket of bifenazate-resistant BCR strain. The frequency of the other two nucleotide substitutions also increased with the increase of resistance. Three and half years after the termination of bifenazate selection, the above three nucleotide substitutions still existed and the resistance ratio did not decrease, which indicates the bifenazate resistance obtained through nucleotide substitutions was stable. To slow down the development of bifenazate resistance of the two-spotted spider mite population, we suggest that bifenazate should not be used repeatedly and should be rotated with acaricides of different modes of action to ensure the control efficacy.

Keywords
bifenazate resistance, mite, mitochondrial cytochrome *b*, nucleotide substitution.

INTRODUCTION
The acaricide “bifenazate” was discovered in 1990 and first commercialized in 1999 (Dekeyser and McDonald 1994; Grosscurt and Avella 2005). It belongs to an acaricidal group of hydrazine derivatives (Fig. 1), and used worldwide for the control of tetranychid mites on several crops. The two-spotted spider mite, *Tetranychus urticae* Koch, is responsible for yield losses in many crops due to its short life cycle, abundant progeny, and ability to develop resistance to acaricides rapidly. It takes only 7.59 days from egg to adult, and each female can lay 112.2 eggs at 30°C (Ho and Lo 1979). Nine years after commercial production, *T. urticae* appeared severely resistant to bifenazate in the Netherlands in 2008 (Van Leeuwen et al. 2008). In Taiwan, this acaricide was registered for watermelon mite control in 2010, and extended to mite control of many fruits and vegetables before 2018.

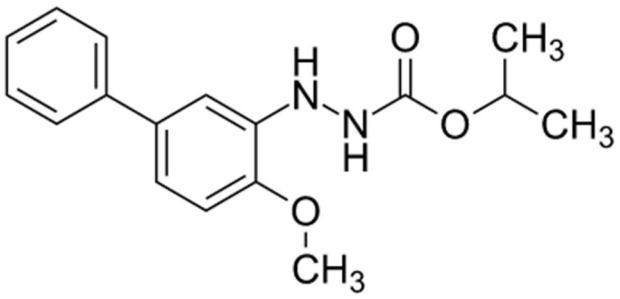


Figure 1. Chemical structure of bifenazate.

Bifenazate is a pro-acaricide that needs to be metabolized to its active component, and ester hydrolysis is considered to be the first step in this process. Diazene is the principal active metabolite of bifenazate in soil, plants and by photolysis, and is more toxic than bifenazate (US/EPA 2003; Ochiai et al 2007). Although organophosphates and carbamates are acetylcholinesterase inhibitors, bifenazate is not suggested to be used together with organophosphate and carbamate insecticides in the field (Van Leeuwen et al. 2007). Moreover, repeated application of organophosphates and bifenazate pose a threat of developing resistance to these chemicals (Van Leeuwen et al. 2007). The mode of action of bifenazate was initially suggested as a gamma-amino butyric acid (GABA) agonist in a Uniroyal Chemical technical data sheet

(Dekeyser 2005; Hiragaki et al. 2012), and categorized in IRAC group 25 until 2007 (Van Leeuwen et al. 2012). However, recent studies revealed that bifenazate resistance is linked with mutations in the Qo site of cytochrome *b* in complex III of the electron transport chain (Van Leeuwen et al. 2008; Van Nieuwenhuyse et al. 2009), hence bifenazate was recategorized to IRAC group 20 in 2019. To understand the resistance profile of bifenazate in two-spotted spider mites in Taiwan, toxicity bioassays and resistance selection were conducted and discussed.

MATERIALS AND METHODS

Mite preparation, bioassay and resistance selection

The commercial formulations of bifenazate (Acramite®, 43.2% SC, Valdosta, USA) was purchased from United Phosphorus Ltd. Two-spotted spider mites were collected from a rose field in Chiayi county of Taiwan where several pesticides had been used, and maintained on potted common bean plants, *Phaseolus vulgaris* var. *communis* Aeschers, in a climatically controlled room at 25 ± 1 °C, 65 ± 10% and 12/12 h (L/D) photoperiod.

Toxicity bioassays against mite population were conducted as follows. Twenty adult female mites were placed on 5-cm^2 leaf discs and then sprayed with 0.6 mL of bifenazate in a Potter spray tower (Burkard Manufacturing, UK) at 5 psi pressure, three replicates. The treated leaf discs and mites were transferred into petri dishes with wet cotton, and then placed at 25 ± 1 °C, 65 ± 10% and 12/12 h (L/D) photoperiod. The mortality was recorded at 24 h, and median lethal concentration (LC50) values and their 95% confidence limits were calculated using a probit analysis program developed by Chi (1997).

The recommended concentration of bifenazate applied for watermelon mite control has been 288 μg/mL in Taiwan since 2010. To understand the possible resistance profile of bifenazate in two-spotted spider mites in Taiwan, resistance selection for bifenazate was carried out at this concentration against mite population maintained on potted kidney bean plants every two weeks in the laboratory. After 24-selection cycles within 11 months, the selection pressure rose to 576 μg/mL, 1,152 μg/mL after 34-selection cycles in 16 months, and a constant concentration of 1,500 μg/mL after 56 selection cycles about 26 months. This bifenazate-selected strain was given a name of BCR strain. In general, one generation according to the egg-adult development time is about two weeks at 25℃ (Ho and Lo 1979).

Mutations in mitochondrial cytochrome *b* of resistance strain

To compare the sequences of cd1 helix of mitochondrial cytochrome *b* Qo pocket in the offspring of resistant BCR strain and their parents, ten individuals of BCR F34, F52, F61, and the generation after 3.5-year relaxation were sequenced. Genomic DNA of each individual mite was extracted by the Easy DNA high-speed extraction tissue kit (Fisher Biotec, Perth, Australia) with a modified procedure as described below. Individual female mites were crushed with a plastic pestle in a 1.5 mL microcentrifuge tube containing 16 μL of solution 1A and 4 μL of solution 1B. After adding 5 μL of solution 2, the sample was incubated for 20 min at 90 °C. Finally, the samples were centrifuged at 10,000 g for 1 min and the suspension was used as the template in PCR reactions. Primers were designed to amplify cd1 helix of mitochondrial cytochrome *b* Qo pocket from 7917-7938th and 8443-8465th mitochondrial sequences of LS-VL (Genebank EU345430), CB3 (5' AAACAAATGTGAATTTCAGGGA 3') and CB4 (5' TCCTCCAATTTTTCTTGGTACAG 3'), respectively. The cd1 helix was amplified by Advantage® 2 PCR kit (Clontech, CA, USA). PCR assays were conducted in a 20-μl mix liquid containing 3 μL purified DNA extracts, 2 μL Advantage 2 PCR buffer (10X), 0.5 μL dNTP, 0.5 μl Advantage 2 polymerase mix (50X), 0.5 μL of each primer, and 13μL sterile water on GeneAmp PCR System 2400 (Waltham, MA, USA). PCR conditions were as follows: 1 cycle of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min followed by 30 cycles of 94°C for 20 s, 60°C for 20 s and 72°C for 20 s, with a final extension step of 7 min at 72°C. PCR products were analyzed in 1.4% agarose gels, and purified using PCR Clean-Up and Gel Extraction Kit (GeneDireX, USA). The purified fragment was then cloned with TOPO TA cloning kit (Invitrogen, California, USA) and sequenced. DNA was analyzed using Vector NTI® Suite 11.0 software (Invitrogen, California, USA).

RESULTS

Selection of bifenazate resistance of *T. urticae*

To understand the resistance profile of bifenazate in two-spotted spider mites in Taiwan, resistance selection against mite populations collected from rose fields in Chiayi county of Taiwan were conducted every two weeks in the laboratory. The resistance of *T. urticae* to bifenazate developed gradually in the first two years. After 25 months of selection, the resistance ratio of F52 progeny was 23.6-fold compared to the parent population, and the resistance ratio of F61 increased significantly to 267.5-fold after 30 months of selection. The LC_{50} values were raised from 42.1 μg/mL to 11,260.5 μg/mL, and the slopes were ranging from 0.62 of parent to 4.90 of F61 progeny (Table 1). Three and half years after the termination of bifenazate selection, the resistance ratio was still maintained more than 400-fold.

Table 1. Response of *Tetranychus urticae* to bifenazate under selection in the laboratory

BCR strain[1)]	Selection period (month)	Slope	LC50 (μg/mL)(95% CI)	Resistance ratio
P	0	0.62 ± 0.08	42.1 (32.4-51.8)	1.0
F20	10	1.31 ± 0.18	411.6 (317.9-505.3)	9.8
F52	25	2.27 ± 0.25	994.5 (925.0-1,064.0)	23.6
F54	26	3.19 ± 0.70	1,746.4 (1,676.4-1,874.8)	41.5
F61	30	4.90 ± 0.50	11,260.5 (11,042.9-11,478.1)	267.5
3.5 years after relaxation from bifenazate selection		4.25 ± 0.73	16,998.5 (16,282.1-17,394.2)	403.8

1) The BCR strain was artificially selected for bifenazate resistance every two weeks in the laboratory.

Mutations in mitochondrial cytochrome *b* of resistance strain

One DNA fragment of 549 bp was amplified with primers BC3/BC4 used in the PCR amplifications of genomic DNA extracted from the mite. The sequences obtained from parent and progeny of F61 were submitted to GenBank databases, the accession numbers are MT514659 and MT514660, respectively. Comparison of aligned sequences of cd1 helix of mitochondrial cytochrome *b* Qo pocket from offspring of resistant BCR strain and their parent showed one main nucleotide substitution, which resulted in 100% amino acid substitution from hydrophobic isoleucine to hydrophilic threonine at 128th position in F61, but only 90% of F52 carried this amino acid substitution. Two other substitutions were also discovered with the increase of resistance. At the 133th amino acid, 30.8% of the parental population were alanine and 69.2% were valine, while 100% of F52 and F61 were valine. At the 139th amino acid, 15.4% of parents were threonine and 84.6% were isoleucine, but 100% of F52 and F61 were isoleucine (Table 2). It is speculated that the 128th amino acid substitution is the main reason for the bifenazate resistance of the two-spotted spider mite. Three and half years after relaxation from bifenazate selection, the above three nucleotide substitutions still exist and the resistant level does not decrease, which indicates the bifenazate resistance obtained through nucleotide substitutions is stable.

Table 2. Relationship between bifenazate resistance and genotype of cd1 helix of cytochrome *b* Qo pocket of *Tetranychus urticae*

BCR strain[1)]	Selection period (month)	Resistance ratio	cytochrome *b* genotype (%)[2)]					
			I128T		A133V		T139I	
P	0	1.0	100.0	0	30.8	69.2	15.4	84.6
F34	16	17.7	100.0	0	20.0	80.0	10.0	90.0
F52	25	23.6	10.0	90.0	0	100.0	0	100.0
F61	30	267.5	0	100.0	0	100.0	0	100.0
3.5 years after relaxation from bifenazate selection		403.5	0	100.0	0	100.0	0	100.0

1) The BCR strain was artificially selected for bifenazate resistance every two weeks in the laboratory.
2) The substitution rate of the cd1 helix of mitochondrial cytochrome *b* Qo pocket.

DISCUSSION

Bifenazate is an acaricidal group of hydrazine derivatives discovered in 1990 and commercialized in 1999. The bifenazate resistance was first detected in *T. urticae* populations from the Netherlands in 2008 (Van Leeuwen et al. 2008). An acaricide susceptible strain (LS-VL) of *T. urticae* from roses in Belgium was selected for bifenazate resistance in the laboratory, and extremely high resistance ratio (RR) of >164,000 was obtained from the resistant strain (MR-VL) after 36 generations (Van Leeuwen et al. 2006). Enzyme assays and synergism studies revealed that the well-known detoxification routes were most likely not involved in the resistance (Van Leeuwen et al. 2006).

Reciprocal crosses between susceptible and resistant strain revealed that bifenazate resistance was only inherited maternally (Van Leeuwen et al. 2006), which indicates mitochondria may be the target site of bifenazate. Comparison of the mitochondrial genome sequences between LS-VL and BR-VL strains, only 3 nucleotide substitutions, all resulted in amino acid substitutions, were detected in the mitochondrial cytochrome *b*. Among these 3 amino acid substitutions, G126S, S141F and D161G, two former substitutions located on cd1 helix and the last one located between cd1 helix and ef helix (Van Leeuwen et al. 2008, 2015). A further comparison has shown that three out of four bifenazate resistant BR-VL strains in Netherlands, HOL1, HOL2, and HOL4, contained the G126S mutation, revealed G126S is the key mutation, only HOL3 strain showed P262T substitution on the highly conserved PEWY motif at the ef helix (Van Leeuwen et al. 2008). G126S substitution also plays essential roles in acequinocyl resistance of field population *T. urticae* in Japan (Sugimoto and Osakabe 2019).

To understand the possible resistance profile of bifenazate in two-spotted spider mites in Taiwan, we conducted the bifenazate resistance selection every two weeks using the field recommendation concentration. After 30 months of selection, the bifenazate resistance ratio of this BCR strain reached 267.5 times, and the frequency of the I128T mutation located on the cd1 helix near the mutation above was from 0 to 100%. The other two mutations, A133V and T139I, appeared in the parental population also, but the probability of mutation increases with the selection time. Moreover, three and half years after the termination of bifenazate selection, the above three nucleotide substitutions still exist and the resistance ratio does not decrease, which indicates the bifenazate resistance obtained through nucleotide substitutions is very stable. Based on the above results, the possible resistance profile of bifenazate in two-spotted spider mite in Taiwan may be also the mutation of cd1 helix of mitochondrial cytochrome *b* Qo pocket, and the rotation of different mode of action acaricides applied in field must be very careful.

CONCLUSION

Bifenazate is tightly linked with mutations in the mitochondrial cytochrome *b* Qo pocket, and the mutation is very stable even many years after relaxation from bifenazate selection. To delay or postpone the development of bifenazate resistance of the two-spotted spider mite population, we suggest that do not use bifenazate subsequently, and rotate bifenazate with acaricides of different modes of action to ensure the control efficacy.

Acknowledgements

This study was supported by the Council of Agriculture Executive Yuan (103AS-10.2.1-CI-C3 and 104AS-10.6.2.CI-C2). The authors would like to express their gratitude to Ms. Yu-Chen Yang for her assistance with mite rearing and related analyses.

References

Chi H. 1997. Computer program for probit analysis. National Chung Hsing University, Taichung, Taiwan.

Ho CC, Lo KC. 1979. Influence of temperature on life history and population parameters of *Tetranychus urticae*. *Journal of Agricultural Research of China* 28:261-271. (in Chinese with English abstract)

Dekeyser MA, McDonald PT. 1994. Insecticidal phenylhydrazine derivatives, US Patent US 5367093.

Dekeyser MA. 2005. Acaricide mode of action. *Pest Management Science* 61:103-110.

Grosscurt A, Avella L. 2005. Bifenazate, a new acaricide for use on ornamentals in Europe and Africa. Proceedings of the BCPC Int. Congress-Crop Science and Technology, 2005, pp 49-56.

Hiragaki S, Kobayashi T, Ochiai N, Toshima K, Dekeyser MA, Matsuda K, Takeda M. 2012. A novel action of highly specific acaricide; bifenazate as a synergist for a GABA-gated chloride channel of *Tetranychus urticae* [Acari: Tetranychidae]. *NeuroToxicology* 33:307-313.

Ochiai N, Mizuno M, Mimori N, Miyake T, Dekeyser M, Canlas LJ, Takeda M. 2007. Toxicity of bifenazate and its principal active metabolite, diazene, to *Tetranychus urticae* and *Panonychus citri* and their relative toxicity to the predaceous mites, *Phytoseiulus persimilis* and *Neoseiulus californicus*. *Experimental and Applied Acarology* 43:181-197.

Sugimoto N, Osakabe M. 2019. Mechanism of acequinocyl resistance and cross-resistance to bifenazate in the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). *Applied Entomology and Zoology* 54:421-427.

US/EPA. 2003. Bifenazate http://www.epa.gov/fedrgstr/EPA-PEST/2003/January/Day-15/p850.htm

Van Leeuwen T, Tirry L, Nauen R. 2006. Complete maternal inheritance of bifenazate resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae) and its implications in mode of action considerations. *Insect Biochemistry and Molecular Biology* 36:869-877.

Van Leeuwen T, Van Pottelberge S, Nauen R, Tirry L. 2007. Organophosphate insecticides and acaricides antagonise bifenazate toxicity through esterase inhibition in *Tetranychus urticae*. *Pest Management Science*: formerly *Pesticide Science* 63:1172-1177.

Van Leeuwen T, Vanholme B, Van Pottelberge S, Van Nieuwenhuyse P, Nauen R, Tirry L, Denholm I. 2008. Mitochondrial heteroplasmy and the evolution of insecticide resistance: Non-Mendelian inheritance in action. *Proceedings of the National Academy of Sciences*. 105:5980-5985.

Van Nieuwenhuyse P, Van Leeuwen T, Khajehali J, Vanholme B, Tirry L. 2009. Mutations in the mitochondrial cytochrome *b* of *Tetranychus urticae* Koch (Acari: Tetranychidae) confer cross-resistance between bifenazate and acequinocyl. *Pest Management Science* 65:404-412.

Van Nieuwenhuyse P, Demaeght P, Dermauw W, Khalighi M, Stevens CV, Vanholme B, Tirry L, Lümmen P, Van Leeuwen T. 2012. On the mode of action of bifenazate: New evidence for a mitochondrial target site. *Pesticide Biochemistry and Physiology* 104:88-95.

Van Leeuwen T, Tirry L, Yamamoto A, Nauen R, Dermauw W. 2015. The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. *Pesticide Biochemistry and Physiology* 121:12-21.

Diamides resistance status and management strategy of Spodoptera exigua (Lepidoptera: Noctuidae) in South Korea

Kim, J.
PROGRAM OF APPLIED BIOLOGY, DIVISION OF BIO-RESOURCE SCIENCES, COLLEGE OF AGRICULTURE AND LIFE SCIENCE, KANGWON NATIONAL UNIVERSITY, CHUNCHEON 24341, REPUBLIC OF KOREA
HIGHLAND AGRICULTURE RESEARCH INSTITUTE, NATIONAL INSTITUTE OF CROP SCIENCE, RDA, 25342 PYEONGCHANG, REPUBLIC OF KOREA
email:forweek@kangwon.ac.kr

Nam, H.Y.
PROGRAM OF APPLIED BIOLOGY, DIVISION OF BIO-RESOURCE SCIENCES, COLLEGE OF AGRICULTURE AND LIFE SCIENCE, KANGWON NATIONAL UNIVERSITY, CHUNCHEON 24341, REPUBLIC OF KOREA
HIGHLAND AGRICULTURE RESEARCH INSTITUTE, NATIONAL INSTITUTE OF CROP SCIENCE, RDA, 25342 PYEONGCHANG, REPUBLIC OF KOREA

Kwon, M.
HIGHLAND AGRICULTURE RESEARCH INSTITUTE, NATIONAL INSTITUTE OF CROP SCIENCE, RDA, 25342 PYEONGCHANG, REPUBLIC OF KOREA

Kim, G.H.
DEPARTMENT OF PLANT MEDICINE, COLLEGE OF AGRICULTURE, LIFE AND ENVIRONMENT SCIENCES, CHUNGBUK NATIONAL UNIVERSITY, 28644, CHEONGJU, REPUBLIC OF KOREA

ABSTRACT
Kimchi cabbage, the main ingredient of Kimchi, is one of the most important crops in Korea. The main Lepidopteran pests of Kimchi cabbage are diamondback moth (*Plutella xylostella*), cabbage butterfly (*Pieris rapae*), cabbage moth (*Mamestra brassicae*), and beet armyworm (*Spodoptera exigua*). Among these pests, especially, *S. exigua* is one of the key pests developing diamide resistance. Since chlorantraniliprole and flubendiamide were first registered in Korea in 2008, diamide insecticide is widely used to control lepidopteran pests nowadays. Diamide resistance has already been reported although the insecticide has been used only for 10 years in Korea. Due to frequent applications of chlorantraniliprole and flubendiamide, the Korean field strain of *S. exigua* showed a high level of LC_{50} ratio exceeding 28,950 and 135,286-fold compared to a susceptible strain in 2019. Nevertheless, G4946E mutation in the ryanodine receptor, which is one of the well-known diamide resistant mechanisms, was not found in resistant populations in Korea. Rather, I4790M mutation, which was identified in *P. xylostella* and *S. frugiperda* and related to resistant allele-specific InDel, was found. Resistant allele-specific Indel region was used as a resistant allele diagnostic primer and developed LAMP (lamp loop-mediated isothermal amplification) to diagnose resistance. A broad range of DNA concentration was workable in LAMP assay, in which the minimum detectable DNA concentration was 100 pg. Moreover, a DNA releasing method was newly used, which took only five minutes of incubation at 95℃, and some pieces of larvae and adult samples were needed. This simple and accurate LAMP assay, which took only 100 minutes, can be applied in the intensive field monitoring of the diamide resistance and integrated resistance management of *S. exigua*.

Keywords
Spodoptera exigua, diamide resistance, ryanodine receptor, LAMP, integrated resistance management

INTRODUCTION
Diamide insecticides are one of the major classes of insecticides worldwide, which target insect ryanodine receptors (RyRs). These insecticides were introduced to the market to control a broad range of herbivorous pests, particularly active against lepidopterans (Nauen 2006; Sattelle 2008). The anthranilic diamides, including chlorantraniliprole and cyantraniliprole, were discovered and developed commercially by Dupont (Cordova et al. 2005; Lahm et al. 2005; Froster et al. 2012; Liu et al. 2018). The third systemic anthranilic diamide, cyclaniliprole, was developed by Ishihara Sangyo Kaisha (ISK) Biosciences Corporation in 2004. The phthalic acid diamide flubendiamide was discovered by Nihon Nohyaku and co-developed by Bayer (Tohnishi et al. 2005). These insecticides were commercialized and rapidly gained market share exceeding the US $ 1.4 billion, representing approximately 8% of the insecticide market in 2013 (Sparks and Nauen 2015). Currently, a total of five types of diamide insecticides have been registered and sold, including tetraniliprole, which most recently registered in 2018. When diamide insecticide was first supplied to the market in Korea, farmers used it extensively because it was able to control various lepidopteran pests, such as *Plutella xylostella, Pieris rapae, Mamestra brassicae, Spodoptera exigua, Agrotis segetum, Agrotis ipsilon, Spodoptera litura, Helicoverpa armigera, Trichoplusia ni, Hellula undalis, Melanchra persicariae,* a

nd *Sarcopolia illoba* in Kimchi cabbage. However, in less than 10 years since the introduction of diamide insecticides in Korea, resistance was reported in some pests. In 2015, resistance was confirmed in *P. xylostella*, which was collected from Goesan-gun, Chungcheonbuk-do, the central region of Korea. With continuous insecticide selection using chlorantraniliprole, a 2,000-fold higher level of resistance was shown in *P. xylostella*. Also, chlorantraniliprole-resistant strain exhibited a high level of cross-resistance with flubendiamide. However, in the bioassay of the four populations of *P. xylostella* in Korea within the recommended concentration, almost 100% was controlled in most populations except Pyeongchang. On the other hand, five populations of *S. exigua* in Korea were difficult to control effectively within the recommended concentrations (Cho et al. 2018). Therefore, we conducted studies on elucidating the resistance mechanism, and developed a diagnostic method because it is necessary to understand the resistance status of *Spodoptera exigua* in Korea and to establish an effective management system for it.

Diamide resistant status of *Spodoptera exigua*

The beet armyworm, *Spodoptera exigua* (Hübner), is a major pest of global importance on numerous cultivated crops, including Kimchi cabbage (CABI 2020). Insecticide resistance has been constantly monitored, since *S. exigua* is a major pest in cabbage in Korea. Recently, S. *exigua* was collected in Gangneung in 2018, then reared for one generation in the lab at Highland Agriculture Research Institute, and tested with 21 types of insecticides. As a result, only six insecticides (fluxametamide, broflanilide, indoxacarb, spinetoram, pyridalyl, and chlorfenapyr) recorded more than 95% of the mortality rate after 72 h (Fig. 1), whereas other insecticide showed less than 70% mortality. No mortality was recorded for deltamethrin and diazinon. All the five diamide insecticides showed a mean mortality of less than 60% (Fig. 1). Obviously, only one population cannot represent the overall level of insecticide resistance in Korea, but it is a fact that the resistance level of *S. exigua* in Korea is already high.

Besides Gangneung area, chlorantraniliprole recorded 100% of mortality rate in all tested local populations at recommended concentration in 2014 (Fig. 2). However, in 2017, mortality rate ranged between 7.5% (Jindo) and 37.8% (Yeonggwang), which confirmed rapid development of resistance within 3-4 years (Fig. 2). In the case of Miryang and Geochang, the mortality rate was 92% and 60%, respectively, which indicated the regional variations in resistance level (Cho et al. 2018). Examination of six regional populations in Korea in 2019 confirmed a significant level of resistance to chlorantraniliprole and flubendiamide, compared to the susceptible strain (Table 1). These results warrant that resistant management is immediately needed in Korea.

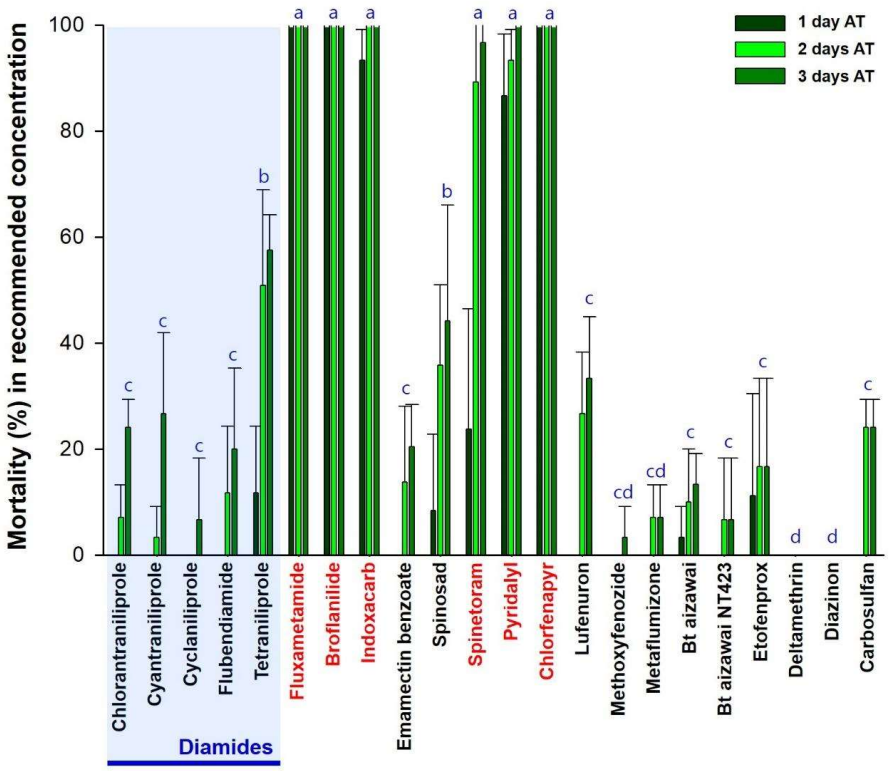


Fig. 1. Susceptibility of a field population of *Spodoptera exigua* against 21 insecticides at recommended c oncentrations. Third instar larvae were used and insecticides were treated in the artificial diet. The bars w ith the same letter(s) are not significantly different at P < 0.05.

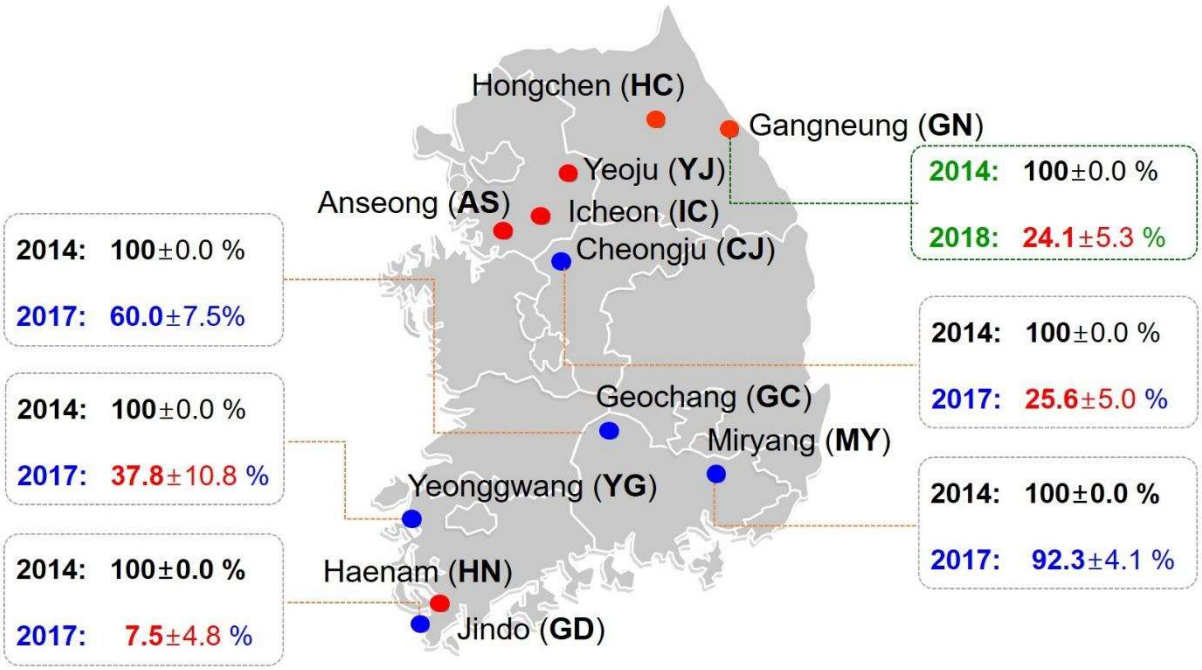


Fig. 2. Field population collection sites for bioassay in 2017 (blue dots, modified from Cho et al. 2018) and later (red dots, modified from Kim et al. 2020a).

Table 1. Susceptibility of six field populations which collected in 2019 and a lab strain of *Spodoptera exigua* to two diamide insecticides

Strains	Chlorantraniliprole		Flubendiamide	
	LC_{50}(mgL-1) (95% CL) [a]	RR [b]	LC_{50}(mgL-1) (95% CL) [a]	RR [b]
Susceptible	0.002	1	0.0007	1
Anseong	8 (5.3 - 12.5)	4,000	0.3 (0.2 - 0.5)	428
Cheongju	1.2 (0.3 - 2.7)	600	10.5 (7.0 - 14.4)	14,957
Gangneung	6.6 (5.3 - 8.2)	3,300	210.1 (71.7 - 295.1)	300,143
Icheon	4.6 (2.3 - 7.0)	2,300	52.31 (32.1 - 70.0)	74,729
Jindo	13.4 (7.6 - 25.3)	6,700	27.9 (24.1 - 32.2)	39,929
Yeoju	21.2 (9.9 - 498.0)	12,500	90.4 (67.8 - 132.0)	129,186

[a] CL: Confidence limits.
[b] Resistance ratio = LC_{50} of the resistant strain or local populations / LC_{50} of the susceptible strain.

Diamide resistant mechanism of *Spodoptera exigua*

The target site of diamide, ryanodine receptor (RyR) is the ligand-gated calcium channel found in the sarcoplasmic/endoplasmic reticulum membrane in muscle and nervous tissue (Sun and Xu 2019). RyR controls the release of calcium from intracellular stores and regulates a variety of cellular and physiological activities, such as gene expression, neurotransmitter release, hormone secretion, muscle contraction, cell proliferation, and finally insect death (Coronado et al. 1994; Nauen and Steinbach 2016).

The target-site mutation, amino acid substitution G4946E, was first identified in the diamide-resistant strain of *P. xylostella* from the Philippines and Thailand (Troczka et al. 2012), and subsequently detected in field populations collected in many countries (Guo et al. 2014a, 2014b; Steinbach et al. 2015). Three additional substitutions (I4790M, E1338D, and Q4594L) connected with diamide resistance were found in a field population of *P. xylostella* (Guo et al. 2014a). The high level of diamide resistance of *S. exigua* in addition to many lepidopteran pests is mostly based on the mutations in target sites of RyR (Troczka et al. 2012; Roditakis et al. 2015; Boaventura et al. 2020). And these mutations were functionally confirmed via CRISPR/Cas9 or expression system (Troczka et al. 2015; Douris et al. 2017; Zuo et al. 2017). Even though metabolic resistance mechanisms to diamides in *S. exigua* remain largely unknown (Nauen and Steinbach 2016), there is no doubt that mutation in RyR is one of the major mechanisms in diamide resistance.

Linkage analysis was performed to analyze the main factors, which are affecting the development of resistance in *S. exigua*. As a result, resistance strain showed 28,950 and 135,286-fold higher resistance than that of susceptible strain against chlorantraniliprole and flubendiamide, respectively. (Kim et al. 2020a). F1 hybrid was produced by single pair mating each resistance and susceptible strain, and then treated with chloran traniliprole at the recommended concentration. Statistically, the difference was not manifested between five mating combinations of susceptible strain (female) and resistant strain (male), and five mating combinations of susceptible strain (male) and resistant strain (female). Therefore, we identified that the resistance gene may be present on the autosome, not on the sex chromosome. In addition, F2 was produced by mass mating of F1 hybrid families and then treated with the recommended concentrations of the five diamides (chlorantraniliprole, cyclaniliprole, flubendiamide, cyantraniliprole, tetraniliprole). As a result, less than 50% of the resistance levels were shown in F2 (Fig. 3). We realized that a resistant allele is involved in cross-resistance and it is possibly inherited as incomplete recessive.

As mentioned earlier, RyR is one of the major target sites in diamide resistance and G4946E mutation is one of the well-known diamide resistance mechanisms in *S. exigua* (Zuo et al. 2017). However, G4946E was not found in resistant populations in Korea. Instead, the I4790M mutation was detected, and this mutation was found not only in Korean *S. exigua* population but also in Chinese *S. exigua* (Zuo et al. 2019), *Spodoptera frugiperda* (Boaventura et al. 2020), and *P. xylostella* (Guo et al. 2014a). Moreover, resistant allele-specific InDel was identified, and was linked to the I4790M mutation (Fig. 4). Despite using the same primer set, resistant allele-specific InDel was able to confirm the difference between resistance and susceptibility in PCR products (Fig. 4A). By using the primer set, which is capable of detecting the resistance-speci

fic InDel in the intron, the resistance-specific InDel w as verified in both gDNA and cDNA (Fig. 4B). The e xistence of a resistant-specific section was identified by aligning partial sequencing (Fig. 4C), which confir med that the resistant-specific InDel present in an intr on can be expressed in any form. Certainly, there wer e case studies showing that the changes in genomic le vel such as InDel are involved in insecticide resistanc e (Faucon et al. 2015; Berger et al. 2016; Lucas et al. 2019). However, further research is needed to deter mine whether it is involved in long non-coding RNA form or participated in resistance by another unknow n mechanism (Kim et al. 2020a).

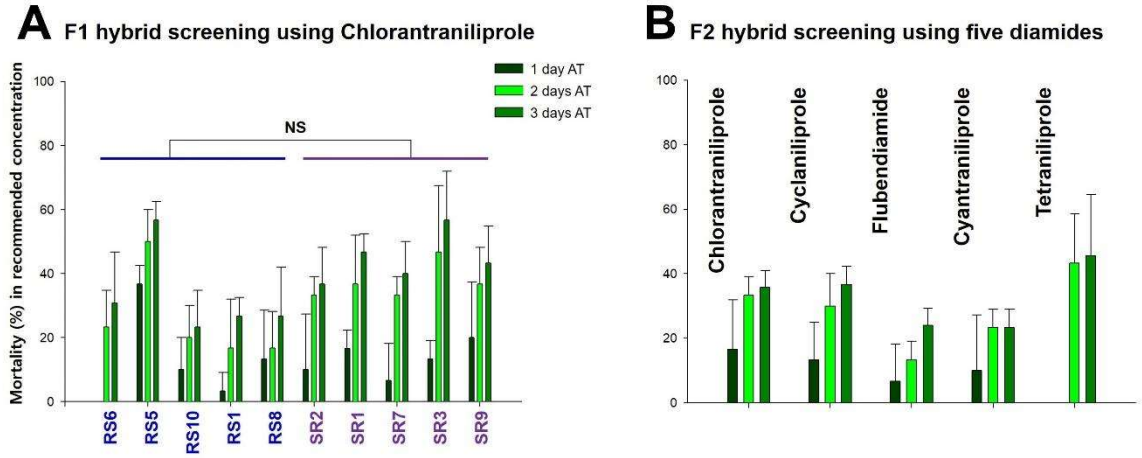


Fig. 3. Diamide resistant and susceptible strain (A) F1 and (B) F2 hybrid screening results. (A) Chlorantra niliprole was used for F1 hybrid screening in recommended concentration. Five families of RS (R♂ × S♀) and SR (S♂ × R♀) were used, respectively. (B) F2 hybrid was generated via F1 families mass mating. Thir d instar larvae were used with three replications.

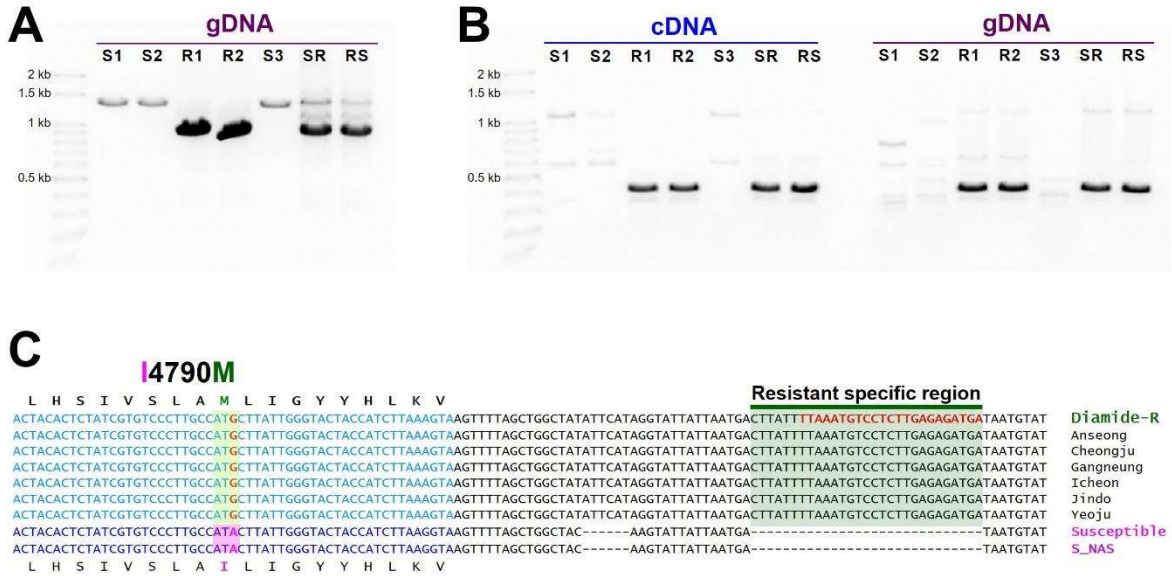


Fig. 4. (A) Diamide resistant strain specific transcript variant and existence of InDels were confirmed via g el electrophoresis of PCR results using gDNA as a template. (B) Resistant allele was amplified from cDNA and gDNA template of diamide resistant strain and its F1 hybrids, SR (male susceptible X female resistan t strain) and RS (male resistant X female susceptible strain). F1 hybrids generated by single pair mating u sing susceptible and diamide resistant strains. (C) Partial RyR sequence alignment including I4790M mut ation site and some part of resistant specific InDel, intron. Red colored sequences in a resistant specific r egion means a diagnostic priming region (modified from Kim et al. 2020a).

Development of diamide resistant diagnostic methods

Prior information on the level of resistance development is essential for the selection of insecticides. Diagnosis of diamide resistance is important because diamide insecticide is one of the most widely used groups to control lepidopteran pests, not only in Korea but also worldwide. Therefore, we developed an effective method, LAMP (Kim et al. 2020a), which can be applied directly in the field without a technical DNA extraction process (Fig. 5). DNA was detected in both larvae and adults by DNA releasing technique, which signifies that diagnosis is possible in the field if there is only a heat block which can control the temperature without DNA extraction. LAMP reduces time and effort, and is recently utilized in various fields, such as ecology, medicine, and also to diagnose plant viruses in insect bodies or to identify the pest species like *S. frugiperda* (Lee et al. 2017; Kim et al. 2020b). Moreover, LAMP can be used to diagnose insecticide resistance allele (Badolo et al. 2012; Badolo et al. 2015; Choi et al. 2018). Besides LAMP, resistance diagnosis can be performed using multiplex PCR and other techniques. Depending on the combination of primers, these diagnosis methods can detect homo- or hetero- traits, but this can only be performed at the laboratory level.

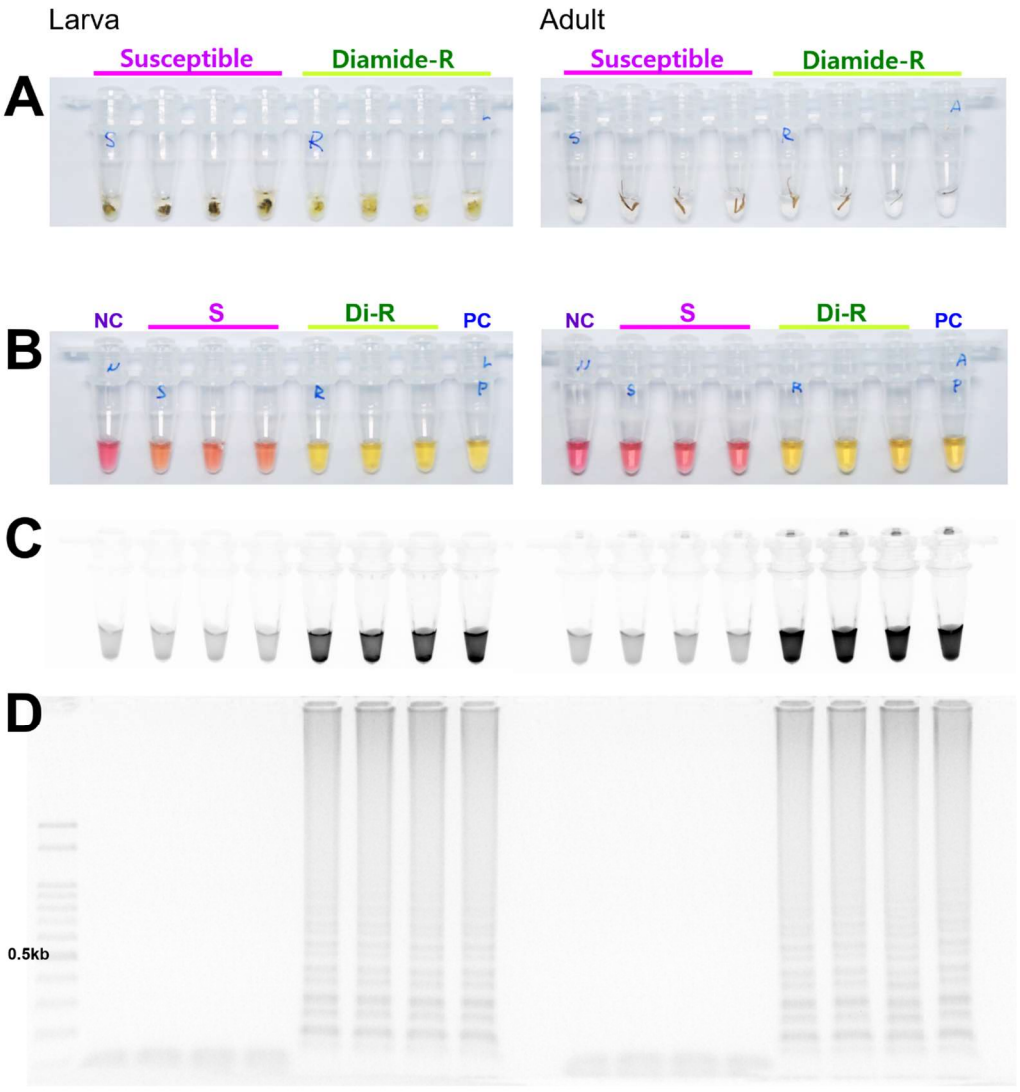


Fig. 5. Sensitivity of the LAMP assay results with the DNA releasing technique from insect tissue. (A) Around 10 mg of larval tissue or adult leg (or antenna) were incubated in 95℃ for 5 min. (B) LAMP products under visible light, (C) ultraviolet light with Cyber Green, and (D) gel electrophoresis. NC: negative control, S: susceptible strain and Di-R: diamide resistant strain, PC (positive control, isolated DNA from diamide resistant strain, Di-R).

Prospects of diamide management strategy

As recommended by IRAC, the most effective way is to select an insecticide with excellent control effect and alternately spray the insecticides, which have different MoA (IRAC 2019), but it is not easy to select which insecticide is more effective. On the other hand, diagnosing insecticide resistance is a more effective way to determine the level of resistance. In that respect, it is necessary to develop and apply a molecular diagnostic method that is inexpensive, simple, and quick, compared to a bioassay, which requires a lot of money and time (Van Leeuwen et al. 2020).

Methods such as LAMP, which can be applied and diagnosed directly in the field without a technical DNA extraction process are being developed and utilized rather than analyzing mutations after extracting DNA or RNA in the past (Lee 2017; Kim et al. 2020b). Fluxametamide and broflanilide, recently registered as lepidopteran insecticides, are IRAC group 30, whose target site is GABA-gated chloride channels (Nakao and Banba 2016; Asahi et al. 2018; Katsuta et al. 2019). It would be good, if novel insecticide would continue to be developed, but we estimate that it will be more difficult in the future. Also, there are various advantages of diamide insecticides except for the development of resistance (Jeanguenat 2013).

Therefore, the optimal pest management strategy involves the diagnosis of insecticide resistance for the currently used insecticides such as diamides and control with effective insecticide in suitable time (Wang et al. 2016; Wang et al. 2018).

Acknowledgments

This study was supported by the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ01358801 and PJ0135880 2), the Rural Development Administration, Republic of Korea.

References

Asahi M, Kobayashi M, Kagami T, Nakahira K, Furukawa Y, Ozoe Y. 2018. Fluxametamide: A novel isoxazoline insecticide that acts via distinctive antagonism of insect ligand-gated chloride channels. Pestic Biochem Physiol, 151, 67-72. doi:10.1016/j.pestbp.2018.02.002

Badolo A, Bando H, Traoré A, Ko-Ketsu M, Guelbeogo WM, Kanuka H, Ranson H, Sagnon N, Fukumoto S. 2015. Detection of G119S ace-1 (R) mutation in field-collected *Anopheles gambiae* mosquitoes using allele-specific loop-mediated isothermal amplification (AS-LAMP) method. Malar J. 14:477. https://doi.org/10.1186/s12936-015-0968-9.

Badolo A, Okado K, Guelbeogo WM, Aonuma H, Bando H, Fukumoto S, Sagnon N, Kanuka H. 2012. Development of an allele-specific, loop-mediated, isothermal amplification method (AS-LAMP) to detect the L1014F kdr-w mutation in *Anopheles gambiae*. Malar J. 11:227. https://doi.org/10.1186/1475-2875-11-227.

Berger M, Puinean AM, Randall E, Zimmer CT, Silva WM, Bielza P, Field LM, Hughes D, Mellor I, Hassani-Pak K, Siqueira HA, Williamson MS, Bass C. 2016. Insecticide resistance mediated by an exon skipping event. Mol Ecol. 25(22): 5692-5704. https://doi.org/10.1111/mec.13882

Boaventura D, Bolzan A, Padovez FEO, Okuma DM, Omoto C, Nauen R. 2020. Detection of a ryanodine receptor target-site mutation in diamide insecticide resistant fall armyworm, *Spodoptera frugiperda*. Pest Manag Sci 76:47–54. https ://doi.org/10.1002/ps.5505

CABI. 2020. Invasive species compendium. https://www.cabi org/isc/search/index. Accessed 04 May 2020

Cho SR, Kyung Y, Shin S, Kang WJ, Jung DH, Lee SJ, Park GH, Kim SI, Cho SW, Kim HK, Koo HN, Kim GH. 2018. Susceptibility of field populations of *Plutella xylostella* and *Spodoptera exigua* to four diamide insecticides. Korean J Appl Entomol 57(1):43–50. https://doi.org/10.5656/KSAE.2018.02.0.009

Cordova D, Benner EA, Sacher MD, Rauh JJ, Sopa JS, Lahm GP, Selby TP, Stevenson TM, Flexner L, Gutteridge S, Rhoades DF, Wu L, Smith RM, Tao Y. 2006. Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. Pestic Biochem Physiol, 84(3), 196-214. https://doi.org/10.1016/j.pestbp.2005.07.005

Coronado R, Morrissette J, Sukhareva M, Vaughan DM. 1994. Structure and function of ryanodine receptors. Am J Physiol, 266(6), C1485-C1504. https://doi.org/10.1152/ajpcell.1994.266.6.C1485

Douris V, Papapostolou KM, Ilias A, Roditakis E, Kounadi S, Riga M, Nauen R, Vontas J. 2017 Investigation of the contribution of RyR target-site mutations in diamide resistance by CRISPR/Cas9 genome modification in Drosophila. Insect Biochem Mol Biol 87:127–135. https ://doi.org/10.1016/j.ibmb.2017.06.013

Faucon F, Dusfour I, Gaude T, Navratil V, Boyer F, Chandre F, Sirisopa P, Thanispong K, Juntarajumnong W, Poupardin R, Chareonviriyaphap T, Girod R, Corbel V, Reynaud S, David JP. 2015 Identifying genomic changes associated with insecticide resistance in the dengue mosquito *Aedes aegypti* by deep targeted sequencing. Genome Res. 25(9): 1347-59. https://doi.org/10.1101/gr.189225.115

Foster SP, Denholm I, Rison JL, Portillo HE, Margaritopoulis J, Slater R. 2012. Susceptibility of standard clones and European field populations of the green peach aphid, *Myzus persicae*, and the cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae), to the novel anthranilic diamide insecticide cyantraniliprole. *Pest Manag Sci*, *68*(4), 629-633. https://doi.org/10.1002/ps.2306

Guo L, Liang P, Zhou X, Gao X. 2014a. Novel mutations and mutation combinations of ryanodine receptors in a Chlorantraniliprole resistant population of *Plutella xylostella* (L.). Sci Rep 4:6924. https ://doi.org/10.1038/srep0 6924

Guo L, Wang Y, Zhou X, Li Z, Liu S, Pei L, Gao X. 2014b. Functional analysis of a point mutation in the ryanodine receptor of *Plutella xylostella* (L.) associated with resistance to chlorantraniliprole. Pest Manag Sci 70(7):1083–1089. https ://doi.org/10.1002/ps.3651

Insecticide Resistance Action Committee. 2019. IRAC Online. https ://www.irac-onlin eorg/about /resistance /mechanisms /. Accessed 01 May 2020

Jeanguenat A. 2013. The story of a new insecticidal chemistry class: the diamides. Pest Manag Sci, 69(1), 7-14. https: //doi.org/10.1002/ps.3406

Kang WJ, Koo HN, Jeong DH, Kim HK, Kim J, Kim GH. 2017. Functional and genetic characteristics of Chlorantraniliprole resistance in the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). Entomol. Res., Vol.47 ; pp.394-403. https://doi.org/10.5656/KSAE.2018.02.0.009

Katsuta H, Nomura M, Wakita T, Daido H, Kobayashi Y, Kawahara A, Banba S. 2019. Discovery of broflanilide, a novel insecticide. J Pestic Sci, 44(2), 120-128. doi:10.1584/jpestics.D18-088

Kim J, Nam HY, Kwon M, Choi JH, Cho SR, Kim GH. 2020. Development of a diamide resistance diagnostic method u

sing LAMP based on a resistance-specific indel in ryanodine receptors for *Spodoptera exigua* (Lepidoptera: Noctuidae). Biorxiv. https://doi.org/10.1101/2020.05.19.103507

Kim J, Nam HY, Kwon M, Kim H, Yi HJ, Hänniger S, Unbehend S, Heckel DG. 2020. Development of a simple and accurate molecular tool for *Spodoptera frugiperda* species identification using LAMP. Biorxiv. https://doi.org/10.1101/2020.04.07.029678

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Data sets. Mol Biol Evol, 33(7), 1870-1874. https://doi:10.1093/molbev/msw054

Lahm GP, Cordova D, Barry JD. 2009. New and selective ryanodine receptor activators for insect control. Bioorg Med Chem 17(12), 4127-4133. https://doi.org/10.1016/j.bmc.2009.01.018

Lee PL. 2017. DNA amplification in the field: move over PCR, here comes LAMP. Mol Ecol Resour, 17(2), 138-141. https://doi:10.1111/1755-0998.12548

Liu JB, Li FY, Dong JY, Li YX, Zhang XL, Wang YH, Xiong LX, Li ZM. 2018. Anthranilic diamides derivatives as potential ryanodine receptor modulators: Synthesis, biological evaluation and structure activity relationship. Bioorg Med Chem, 26(12), 3541-3550. https://doi.org/10.1016/j.bmc.2018.05.028

Lucas ER, Miles A, Harding NJ, Clarkson CS, Lawniczak MKN, Kwiatkowski DP, Weetman D, Donnelly MJ. 2019. Anopheles gambiae 1000 Genomes Consortium (2019) Whole-genome sequencing reveals high complexity of copy number variation at insecticide resistance loci in malaria mosquitoes. Genome Res. 29(8):1250-1261. https://doi.org/10.1101/gr.245795.118

Nakao T, Banba S. 2016. Broflanilide: A meta-diamide insecticide with a novel mode of action. Bioorg Med Chem, 24 (3), 372-377. doi:10.1016/j.bmc.2015.08.008

Nauen R. 2006. Insecticide mode of action: return of the ryanodine receptor. Pest Manag Sci, 62(8), 690-692. https://doi.org/10.1002/ps.1254

Nauen R, Steinbach D. 2016. Resistance to Diamides in Lepidopteran Pests. In: Horowitz A, Ishaaya I (eds) Advances in insect control and resistance management (pp. 219-240). Springer, Dordrecht

Richardson EB, Troczka BJ, Gutbrod O, Davies TGE, Nauen R. 2020. Diamide resistance: 10 years of lessons from lepidopteran pests. J Pest Sci. https://doi.org/10.1007/s10340-020-01220-y

Roditakis E, Vasakis E, Grispou M, Stavrakaki M, Nauen R, Gravouil M, Bassi A. 2015. First report of *Tuta absoluta* resistance to diamide insecticides. J Pest Sci 88(1):9–16. https ://doi.org/10.1007/s1034 0-015-0643-5

Sanderson MJ, Wojciechowski MF. 2000. Improved bootstrap confidence limits in large-scale phylogenies, with an example from Neo-Astragalus (Leguminosae). Syst Biol 49(4), 671-685. https ://doi:10.1080/106351500750049761

SAS Institute 2013. SAS/STAT User's Guide: Statistics, Version 9.4. SAS Institute, Cary, N.C., U.S.A.

Sattelle DB, Cordova D, Cheek TR. 2008. Insect ryanodine receptors: molecular targets for novel pest control chemicals. *Invert Neurosci 8*(3), 107-19. https://doi.org/10.1007/s10158-008-0076-4

Sparks TC, Nauen R. 2015. IRAC: mode of action classification and insecticide resistance management. Pestic Biochem Physiol 121:122–128. https ://doi.org/10.1016/j.pestbp.2014.11.014

Steinbach D, Gutbrod O, Luemmen P, Matthiesen S, Schorn C, Nauen R. 2015. Geographic spread, genetics and functional characteristics of ryanodine receptor based target-site resistance to diamide insecticides in diamondback moth, *Plutella xylostella*. Insect Biochem Mol Biol 63:14–22. https ://doi.org/10.1016/j.ibmb.2015.05.001

Sun L, Qiu G, Cui L, Ma C, Yuan H. 2015. Molecular characterization of a ryanodine receptor gene from *Spodoptera exigua* and its upregulation by Chlorantraniliprole. Pestic Biochem Phys 123:56-63. https ://doi.org/10.1016/j.cbd.2018.07.001

Sun Z, Xu H. 2019. Ryanodine Receptors for Drugs and Insecticides: An Overview. Mini Rev Med Chem, 19(1), 22-33. https://doi.org/10.2174/1389557518666180330112908

Tohnishi M, Nakao H, Furuya T, Seo A, Kodama H, Tsubata K, Fujioka S, Kodama H, Hirooka T, Nishimatsu T. 2005. Flubendiamide, a novel insecticide highly active against lepidopterous insect pests. J Pestic Sci, 30(4) 354- 360. https://doi.org/10.1584/jpestics.30.354

Troczka B, Zimmer CT, Elias J, Schorn C, Bass C, Davies TGE, Field LM, Williamson MS, Slater R, Nauen R. 2012. Resistance to diamide insecticides in diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) is associated with a mutation in the membrane-spanning domain of the ryanodine receptor. Insect Biochem Mol Biol 42(11):873–880. https ://doi.org/10.1016/j.ibmb.2012.09.001

Troczka BJ, Williams AJ, Williamson MS, Field LM, Lüemmen P, Davies TE. 2015. Stable expression and functional characterization of the diamondback moth ryanodine receptor G4946E variant conferring resistance to diamide insecticides. Sci Rep 5, 14680 (2015). https://doi.org/10.1038/srep14680

Van Leeuwen T, Dermauw W, Mavridis K, Vontas J. 2020. Significance and interpretation of molecular diagnostics for insecticide resistance management of agricultural pests. Curr Opin Insect Sci, 39, 69-76. doi:10.1016/j.cois.2020.03.006

Wang X, Chen Y, Gong C, Yao X, Jiang C, Yang Q. 2018. Molecular identification of four novel cytochrome P450 genes related to the development of resistance of Spodoptera exigua (Lepidoptera: Noctuidae) to chlorantraniliprole. Pest Manag Sci 74(8):1938–1952. https ://doi.org/10.1002/ps.4898

Wang XG, Gao XW, Liang P, Shi XY, Song DL. 2016. Induction of Cytochrome P450 Activity by the Interaction of Chlorantraniliprole and Sinigrin in the Spodoptera exigua (Lepidoptera: Noctuidae). Environ Entomol. 45(2):500-7. https://doi.org/10.1093/ee/nvw007

Zuo YY, Wang H, Xu Y, Huang J, Wu S, Wu Y, Yang Y. 2017. CRISPR/Cas9 mediated G4946E substitution in the ryanodine receptor of *Spodoptera exigua* confers high levels of resistance to diamide insecticides. Insect Biochem Mol Biol 89:79–85. https ://doi.org/10.1016/j.ibmb.2017.09.005

Zuo YY, Ma HH, Lu WJ, Wang XL, Wu SW, Nauen R, Wu YD, Yang YH. 2019. Identification of the ryanodine receptor mutation I4743M and its contribution to diamide insecticide resistance in *Spodoptera exigua* (Lepidoptera: Noctuidae). Insect Sci. https ://doi.org/10.1111/1744-7917.12695

Strategies to manage insecticide resistance: insights from modeling and greenhouse tests

Shelton, A.M.
CORNELL UNIVERSITY/NYSAES
GENEVA, NEW YORK 14456, USA
email: ams5@cornell.edu

Roush, R.T.
PENNSYLVANIA STATE UNIVERSITY
UNIVERSITY PARK, PENNSYLVANIA 16802, USA
email: Rtr10@psu.edu

Zhao, J.Z.
CORNELL UNIVERSITY/NYSAES
GENEVA, NEW YORK 14456, USA
email: zhaojz@yahoo.com
(Current address: Corteva Agriscience, Johnston, Iowa 50131, USA)

ABSTRACT
Insecticides are valuable tools for integrated pest management (IPM). However, resistance is a threat to an insecticide's continued use. To avoid resistance, it is important that a product be used in an insecticide resistance management (IRM) strategy. Common strategies include using a product in a rotation with other insecticides in the same area, using multiple insecticides during the same time period but in different spaces, or mixing it with another insecticide. Insecticides can also be applied in traditional methods (e.g. foliar sprays) or expressed in plants. Using modeling studies and greenhouse experiments, we discuss the advantages and disadvantages of these IRM strategies.

Keywords
Resistance, insecticides, pest management

INTRODUCTION
Insects and mites cause an estimated annual $470 billion in losses to agricultural crops globally (Culliney 2014). Insecticides and miticides remain the dominant control tactic used against pest arthropods and have an estimated annual market value of > $16 billion (Statistica 2019). Development, registration and launch of a new insecticide and miticide may take 10 years and cost > $250 million. Preserving the effectiveness of a new insecticide or miticide is essential for the company and for the farmer who uses it. The threat of a species evolving resistance to a new insecticide is real. Currently, there are 586 insect species documented to be resistant to one or more insecticides (Sparks and Nauen 2015). The diamondback moth (DBM) is one of the leaders in evolving resistance, with 866 cases reported (Mota-Sanchez and Wise 2019) and covering most all insecticide classes.

The Insecticide Resistance Action Committee (IRAC) defines "resistance as the selection of a heritable characteristic in an insect population that results in the repeated failure of an insecticide product to provide the intended level of control when used as recommended." Knowledge of the insecticide and the insect are helpful for designing insecticide resistance management (IRM) strategies to prevent or delay resistance. Each insecticide has a particular mode of action and knowledge of it is helpful in designing how the insecticide should be used in an IRM strategy. From the standpoint of the insect's biology, knowledge of the genetic basis for evolving resistance is also helpful: e.g., number of genes involved and their allele frequencies in the population, the mechanism(s) of resistance including via increased metabolism or less sensitive target site, whether genes are dominant or recessive, and whether they carry a fitness cost. Unfortunately, generally these factors are not known prior to the insecticide being released into the market. Therefore, it is usually necessary to introduce IRM strategies without full knowledge of the genetics involved.

METHODS
While there are many variations for how and when insecticides can be applied (e.g. systemic or contact, high or low dose, with or without synergists, targeting a specific stage of the insect, etc.), there are three general IRM strategies that have engendered the most discussion for IRM: rotations, mosaics and mixtures. These strategies can be applied to traditionally applied insecticides or to insecticidal proteins expressed in plants.
We have tested these three strategies using: 1) mathematical modeling and 2) greenhouse trials using foliar applied insecticides and plants expressing insecticidal protein from *Bacillus thuringiensis*.

Rotations
Rotational strategies are based on the sequence over time of two or preferably more insecticide classes with different modes of action. The rotation strategy allows any resistance selected to the first insecticide to decline over time (due to costs of resistance alleles to pest fitness, and/or dilution by in-migrating susceptible individuals) while the subsequent insecticides are deployed. Rotations of insecticides can be accomplished using a "window" strategy in which an insecticide is applied in an area for a specific time period before another insecticide is introduced

during the next window of time. This strategy can continue with a third insecticide, then a fourth, etc. Use of this strategy requires a suite of insecticides that are unlikely to share cross-resistance (a common target site or metabolic resistance mechanism) but also requires coordination and compliance by growers to be effective. Ideally, such a window strategy should be initiated at the first introduction of an insecticide and coordinated with multiple insecticides already in the market. Another critical question about rotations is how often the rotation should occur, such as the number of insect generations or during specific months, but a general guideline is a best fit into a cropping pattern (e.g., to divide a growing year into 2-4 windows for species with multiple generations, or alternate years for species with only one generation annually).

Mosaics

A mosaic strategy is similar to a rotation but allows the use of multiple insecticides during the same time period but in different spaces. For example, one farmer may use insecticide A while another may use insecticide B, but both could have adjacent fields or be in the same county. The landscape and spatial factors are important considerations when using a mosaic strategy.

Mixtures

A mixture is a single formula that combines more than one insecticide, each with a different mode of action, or the application of two or more insecticides in the same time frame. This approach assumes that, if resistance to each insecticide is rare, then multiple resistance will be extremely rare.

RESULTS

Each of these strategies has advantages in some circumstances, and disadvantages in others. Modeling studies, greenhouse evaluations and longer-term field studies have provided some general conclusions about the effectiveness of these three IRM strategies.

Modeling Studies

Rotations vs Mosaics

Results from modeling studies (Roush 1989) always showed that rotation of insecticides was never worse than mosaics and sometimes much better. The primary reason for this is that in a mosaic there is often interbreeding of individuals between the areas in the mosaic. This is especially characteristic of mobile insects that move freely between fields, such as the DBM. In practice it may be a single meta-population that is moving between fields and being treated over a certain area. Furthermore, treatment of less than half of the population with one insecticide will tend to delay resistance to that compound, but not to the other, and will result in no overall increase in the durability of both compounds.

Mixtures

Results from modeling studies (Roush 1989) indicate that mixtures of insecticides can significantly delay resistance compared to rotations only when each of the insecticides kills at least 95% of the target insects when used individually, and are more likely to be disruptive to non-target species. The main reason for the poorer results of mixtures is that each insecticide will often have a different decay rate. Specifically, if pesticide A continues to kill susceptible individuals after pesticide B has dissipated, the residue of the mixture will no longer effectively delay resistance to A.

Greenhouse Studies

Foliar Insecticides

Using large cages within greenhouses, we conducted studies over nine generations of DBM that compared rotation of three insecticides to use of a mosaic in which all three insecticides were applied to different sets of plants in the same cage (Zhao et al. 2010). Furthermore, for the rotation strategy we compared the time between sprays (every generation vs every third generation). Each insecticide used had a different mode of action based on IRAC classification (shown in parentheses): indoxacarb (22), spinosad (5) and Dipel (11, a product containing Bt). Although sets of plants in a particular cage might have been treated differently, larvae could not move between plants, but adults could. The population of DBM had a known frequency of resistance to each insecticide and the changes in resistance and population levels were assessed over time.

Results of both population density and resistance development indicated that an insecticide rotation every generation was better for IRM than if the insecticide was rotated every third generation. The mosaic strategy generated the most rapid resistance to all three insecticides and provided the least consistent control of larvae.

Bt Plants

We used our system of broccoli plants expressing different Bt proteins and populations of DBM with a known level of resistance to each insecticide to test IRM strategies. Using large cages within greenhouses, we tested the evolution of resistance using rotations, mixtures and mosaics.

In one set of experiments using Bt broccoli and populations of DBM, we tested the durability of two-gene plants to single gene plants when they were deployed in a mosaic or in a sequence (=rotation). Two gene plants could be considered as "mixtures" because each protein expressed had a different target site. After 24 generations of selection, resistance to the two-gene plants was significantly delayed compared with resistance to single-gene plants deployed in mosaics and or a rotation (Zhao et al. 2003).

In another set of experiments, we tested single Bt gene plants vs dual Bt gene plants (Zhao et al. 2005). After 24–26 generations of selection in the greenhouse, the concurrent use of one- and two-gene plants resulted in control failure of both types of Bt plants. When only two-gene plants were used in the selection, none or few insects survived when tested on one- or two-gene Bt plants. These results indicated that concurrent use of transgenic plants expressing a single and two Bt genes (as in a mosaic strategy) will select for resistance to two-gene plants more rapidly than the use of two-gene plants alone.

DISCUSSION

The studies discussed here provide some insights into IRM strategies for foliar insecticides and for plants expressing Bt proteins.

In Hawaii, Mau and his colleagues have developed and implemented an effective IRM strategy for DBM using a "window" approach for foliar insecticides in which one class of insecticides is used in a particular month and then rotated with other insecticide classes in different months (Chou et al. 2021). Growers had experienced resistance to newer insecticides (Zhao et al. 2006) and were desperate to develop a more sustainable practice. The success of their window-based IRM program has been due to the involvement of the growers and extension personnel.

Our program has developed and extensively tested Bt crucifers (Cao et al. 1999; Metz et al. 1995). Bt crucifers and Bt-resistant DBM (Zhao et al. 2001) provided an insightful model system to study IRM strategies such as the use of refuges (Shelton et al. 2000; Tang et al. 2001), and the durability of single and dual Bt gene plants (Zhao et al. 2003; Zhao et al. 2006). Additionally, there was hope that Bt crucifers would be able to enter the market for control of DBM (Grzyacz et al. 2010), but this has not yet occurred.

It is worth noting that dual Bt gene plants are much more durable than single gene plants when "95% kill by each toxin" is achieved (Zhao et al. 2003; Zhao et al. 2006) while the same cannot be said of mixtures of traditional insecticides (Roush 1989). The reason for this is that a Bt plant continually (or should) produce both proteins, whereas with a traditional insecticide mixture each insecticide residue would decline over time, often at different rates.

CONCLUSION

The development and regulatory process for a new insecticide to come to market is a time-consuming and expensive affair and it is in the best interest of a company and the grower to ensure the durability of the product through an IRM strategy. IRM programs have been derived from basic studies on the mode of action of insecticides, the genetic basis of resistance in insects and the ecology of the system. While complete knowledge of all these specific aspects is usually not known prior to the commercialization of the product, experience has provided some guidelines to help ensure a products longevity:

- Use other strategies to reduce the overall insect population. These include crop rotation, plowing crop residue, adjusting planting time, enhancing biological control, etc.
- Use a product only when needed to avoid economic loss
- For foliar insecticides, a window strategy is the best bet for delaying resistance in most situations
- For Bt plants, use of multiple genes will provide longer durability
- For traditional insecticides and for Bt crops, having a refuge which can harbor susceptible insects should be encouraged.

Acknowledgements

We thank the many members of our team who have provided their knowledge and skills to help us manage insecticide resistance.

References

Cao J, Tang JD, Strizhov N, Shelton AM, Earle ED. 1999. Transgenic broccoli with high levels of *Bacillus thuringiensis* Cry1C protein control diamondback moth larvae resistant to Cry1A or Cry1C. *Mol. Breeding* 5:131-141.

Chou MY, Shimbuju R, Sugano J, Uyed J, Hamasaki R, Tavares K, Silva J, Huang YB, Kao CH, Mau RFL. 2021. Insecticide resistance monitoring and management of diamondback moth, *Plutella xylostella,* in Hawaii and Taiwan. (this Proc issue)

Culliney TW. 2014, Crop losses to arthropods. *Integrated Pest Management: Pesticide Problems, Vol. 3* doi:10.1007/978-94-007-7796-5_8.

Grzywacz D, Rossbach A, Rauf A, Russell D, Srinivasan R, Shelton AM. 2010. Current control methods for diamondback moth and prospects for improved management with lepidopteran-resistant Bt vegetable brassicas in Asia and Africa. *Crop Protection* 29: 68-79.

IRAC. https://www.irac-online.org/modes-of-action/

Metz TD, Roush RT, Tang JD, Shelton AM, Earle ED. 1995. Transgenic broccoli expressing a *Bacillus thuringiensis* insecticidal crystal protein: implications for pest resistance management strategies. *Mol. Breeding* 1: 309-317.

Mota-Sanchez D, Wise JC. 2019. The Arthropod Pesticide Resistance Database. Michigan State University. On-line at: http://www.pesticideresistance.org

Roush RT. 1989. Designing resistance management programs: how can you choose? *Pestic. Sci* 26: 423–441.

Shelton AM, Tang JD, Roush RT, Metz T, Earle ED. 2000. Field tests on managing resistance to Bt-engineered plants. *Nature Biotech.* 18: 339-342.

Sparks TC, Nauen R. 2015. IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochem. Physiol.* 121:122–128.

Statistica 2019. Market value of insecticides worldwide from 2013 to 2022 (in billion U.S. dollars). https://www.statista.com/statistics/606103/value-of-the-global-insecticide-market/. Accessed 27 August 2019.

Tang JD, Collins HL, Metz TD, Earle ED, Zhao J, Roush RT, Shelton AM. 2001. Greenhouse tests on resistance management of Bt transgenic plants using refuge strategies. *J. Econ. Entomol.* 94: 240-247.

Zhao JZ, Collins HL, Shelton AM. 2010. Testing insecticide resistance management strategies: mosaics vs rotations. *Pest Management Sci.* 66: 1101-1105.

Zhao J, Cao J, Li Y, Collins HL, Roush RT, Earle ED, Shelton AM. 2003. Plants expressing two *Bacillus thuringiensis* toxins delay insect resistance compared to single toxins used sequentially or in a mosaic. *Nature Biotech.* 21: 1493-7.

Zhao JZ, Li Y, Collins HL, Cao J, Earle ED, Shelton AM. 2001. Different cross-resistance patterns in the diamondback moth resistant to *Bacillus thuringiensis* toxin Cry1C. *J. Econ. Entomol.* 94: 1547-1552.

Zhao J, Cao J, Collins HC, Bates SL, Roush RT, Earle ED, Shelton AM. 2005. Concurrent use of transgenic plants expressing a single and two Bt genes speeds insect adaptation to pyramided plants. *Proc. Natl. Acad. Sci. USA* 102: 8426-8430.

Zhao JZ, Collins HL, Li YX, Mau RF, Thompson GD, Hertlein MS, Andaloro JT, Boyken R, Shelton AM. 2006. Monitoring of diamondback moth resistance to spinosad, indoxacarb and emamectin benzoate. *J. Econ. Entomol.* 99: 176-181.

SESSION 6
Genetic Approaches to Manage Crucifer Pests

Diversity of cruciferous pests: genetic analysis of flea beetle and *Pieris rapae* populations from southeast asia based on mitochondrial *coxI* gene

Senthil Kumar, R.
WORLD VEGETABLE CENTER, P.O.BOX 42, SHANHUA, TAINAN, 74151 TAIWAN
email: drkumaragribiotech@gmail.com

Srinivasan, R.
WORLD VEGETABLE CENTER, P.O.BOX 42, SHANHUA, TAINAN, 74151 TAIWAN
email: srini.ramasamy@worldveg.org

ABSTRACT
Flea beetles and cabbage butterfly (*Pieris rapae*) are the most destructive and devastating pests of cruciferous crops worldwide. Individually, they may cause yield losses as much as 100% in brassicas and therefore cripple the economy of smallholder farmers especially in Southeast Asia. In order to understand the genetic diversity, phylogeography and intraspecific genetic variation among these pest populations, mitochondrial *cytochrome c oxidase 1* (mtcox1) gene was used. We first explored 89 individuals of *Pieris* and 187 individuals of flea beetle populations from five different geographically distinct regions including Taiwan, Cambodia, Vietnam, Thailand and Laos. Individual haplotype diversities based on *cox1* gene showed 10 and 35 different haplotypes of *Pieris* and flea beetles, respectively. Furthermore, Tajima's *D*, Fu's *Fs* and Analysis of Molecular Variance (AMOVA) of pairwise comparison (F_{st}) tests were performed across all *Pieris* and flea beetle populations in target countries. The results showed low nucleotide diversity, high genetic differentiation and gene flow, which suggest recent population expansion especially between Taiwan and Thailand populations of flea beetles. Similarly, isolated populations of *P. rapae* have higher haplotype diversity (h>28) and lower nucleotide diversity (0.00033) in Vietnam and Thailand. In addition, genetic differentiation results showed that the Taiwan population could be a separate species. Thus, our results provided better understanding on the genetic diversity of flea beetle and cabbage butterfly populations in Southeast Asia.

Keywords
Mitochondrial *cytochrome c oxidase 1* (mtcox1), haplotype diversity, nucleotide diversity, population genetics, integrated pest management

INTRODUCTION
Vegetable brassicas are an important crop in Southeast Asia, which is grown over 148,182 ha with an annual production of 3.25 million (FAO 2019). The productivity in Southeast Asia (19 t/ha) is lower than the Asian average of 25 t/ha. It also widely varies among the countries in the region. For instance, the marketable yield of leafy mustard is only 5.6 t/ha in Laos, whereas it is 15 t/ha in the neighboring Cambodia (Schreinemachers et al. 2017). Insect pests are one of the major limiting factors, causing up to 90% yield loss as reported in Vietnam (Nhung et al. 2008). Besides diamondback moth (*Plutella xylostella* L.), which is the dominant pest in brassicas, cabbage butterfly (*Pieris rapae* L.) and striped flea beetle (*Phyllotreta striolata* Fab.) cause marketable yield losses of up to 100% in leafy brassicas in Southeast Asia (Ungsa and Vanharn 1995; Nhung et al. 2008).

Brassica farmers heavily rely on indiscriminate, repeated application of chemical pesticides to prevent crop losses by pests. A study found that farmers in Cambodia, Laos, and Vietnam on average sprayed pesticides on brassicas weekly as a prophylactic measure, using about 1 kg/ha/week (Schreinemachers et al. 2017). About 75% of these growers mixed different pesticides together in a single spray. Indiscriminate pesticide use can adversely affect human and environmental health, besides creating high-risk perceptions among consumers due to high pesticide residues in the harvested vegetables (Praneetvatakul et al. 2013; Nguyen et al. 2020). In addition, pests experiencing tremendous pressure from pesticides increase the likelihood of developing resistance to the pesticides. Resistance of *P. striolata* to commonly used pesticides has been reported in Southeast Asia (Feng et al. 2000; Ulrichs et al. 2001).

Development of suitable pest control measures requires a thorough understanding of the population of the target pest species, because the population structure and dynamics usually vary from region to region and effective control strategies, such as pheromones and biological control have to be considered accordingly. DNA barcoding using the mitochondrial gene cytochrome c oxidase I (*coxI*) has been used to identify the species, since it is highly conserved across species (Hebert et al. 2003). Understanding the genetic diversity and a comparative phylogeographic studies using different types of markers has important economic implications (Hewitt 2001; Taberlet 1998) Molecular phylogeny using mitochondrial and/or nuclear gene sequences has been well established in thrips (Kadirvel et al. 2013), whiteflies (Ramasamy et al. 2013; Ram Kumar et al. 2017) and *Maruca vitrata* (Malini et al. 2019; Periasamy et al. 2015). Hence, the main objectives of this paper were to identify genetic differences among flea beetle and cabbage butterfly populations through partial mitochondrial *coxI* DNA sequences and to better understand phylogenetic

relationships among the pest populations predominantly occurring in selected Southeast Asian countries.

MATERIALS AND METHODS

Geographical location and sampling collection

The flea beetle and the *P. rapae* insects were collected from selected sites in Taiwan, Laos, Cambodia, Thailand and Vietnam (Tables 1 and 2). The collected insects were stored in 95% ethanol at a temperature of -20˚C before DNA extraction.

Table 1. List of *P. rapae* samples with their host plants and geographical origin

Sample and Sequence id	*Host Plant*	*Geographical Locations*
Pieris rapae		
Taiwan		
PR-BRC_Tw-1 to PR-BRC_Tw-5	Broccoli	WorldVeg HQ, Shanhua, Tainan
Lao PDR		
PR-Lao-Cb8a-1 to PR-Lao-Cb8a-6	Cabbage	Nong, Kasi district, Vientiane province
PR-Lao-Cb9a-1 to PR-Lao-Cb9a-5	Cabbage	Ban Pha Tang, Vang Vieng, Vientiane province
PR-Lao-Cb46-1 to PR-Lao-Cb46-4	Cabbage	Phaxang village, Kasi district, Vientiane province
PR-Lao-pk37-1 to PR-Lao-pk37-5	Pakchoy	Nadao village, Vang Vieng, Vientiane province
Vietnam		
PR-SVT-CC-2-7	Common cabbage	Mai Son, Son la province
PR-SVT-FR-20-1 to PR-SVT-FR-20-4	Forage radish	Moc chau district, Son la province
PR-VT-C3C-1 to PR-VT-C3C-5	Cauliflower	Khuyen Luong, Thanh tri, Hanoi
PR-VT-Cb-2a-1 to PR-VT-Cb-2a-5	Cabbage	Quynh Luong, Quynh Luu, Nghe An
PR-VT-Cb5B-1 to PR-VT-Cb5B-5	Cabbage	Co do Moc chau district, Son la province
PR-VT-CC-1-1 to PR-VT-CC-1-5	Common cabbage	Giam Lam district, Hanoi
PR-VT-CC2F-1 to PR-VT-CC2F-5	Cauliflower, Cabbage	Song Phuong, Hoai Duc, Hanoi
PR-VT-CC-10-1, PR-VT-CC-10-2, PR-VT-CC-10-4, PR-VT-CC-10-5	Common cabbage	Thanh tri district, Hanoi
PR-VT-Cf6a-1 to PR-VT-Cf6a-5	Cauliflower	Da Mai, Bac Giang city
PR-VT-Lb9B-1 to PR-VT-Lb9B-5	Leafy Brassica	Mai Son, Son La province
PR-VT-LM4B-1 to PR-VT-LM4B-5	Leafy mustard	Tay Tuu, Tu Liem district, Hanoi
Thailand		
PR-Th-1 to PR-Th-5	Cabbage	WorldVeg, Kamphaeng Saen, Nakhon Pathom

Table 2. List of flea beetle samples with their host plants and geographical origin

Sample and Sequence id	Host Plant	Geographical Locations
Taiwan		
SFB-M_Tw1-2 to SFB-M_Tw1-5	Mustard	WorldVeg HQ, Shanhua, Tainan
SFB-R_Tw2-1 to SFB-R_Tw2-5	Radish	WorldVeg HQ, Shanhua, Tainan
Cambodia		

CaM_SFB-5-2, CaM_SFB-5-10	Pak choi	Krong Stung Treng, Stung Treng province
CaM_SFB-10-1 to CaM_SFB-10-10	Leafy mustard	Anlong veng, Oddar Meanchey province
CamJ06_2-1_MV to CamJ06_2-10	Leafy mustard	Samkhuoy, Stung Treng province
CaMM06_SFB-16-1, CaMM06_SFB-16-2, CaMMJ06_SFB-5-1	Radish	Stueng village, Kandal province
SFB-13-1 to SFB-13-5	Pak-choi	Preah Sdach district, Prey Veng province
Thailand		
SFB-Th_pk1-1 to SFB-Th_pk1-5	Pak-choi	Kamphaeng Phet province
Lao PDR		
Lao_SFB8c-1, Lao_SFB8c-2, Lao_SFB8c-4, Lao_SFB8c-5	Cabbage	Nong, Kasi district, Vientiane province
Lao_SFB9c-1, Lao_SFB9c-2, Lao_SFB9c-4, Lao_SFB9c-5	Cabbage	Ban Pha Tang, Vang Vieng, Vientiane province
SFB-6a-1, SFB-6a-3, SFB-6a-4, SFB-6a-5	Cabbage	Na Then, Kasi district, Vientiane province
Fb-4_1 to Fb-4_14	Leafy mustard	Homtai Village, Hatsayphong district, Vientiane Capital
Fb13_1 to Fb-13_10	Cauliflower	Koksay village, Hatsayphone district, Vientiane Capital
Fb-14_1 to Fb-14_10	Green mustard	Don village, Hatsayphone district, Vientiane Capital
Fb-15_1, Fb-15_2, Fb-15_6 to Fb-15_10	Cabbage	Nonetea village, Xaythany district, Vientiane Prefecture
Fb-16_1 to Fb-16_10	Cauliflower	Sitantai village, Hatsayphone district, Vientiane Capital
FB-2a-1, FB-2a-2	Chinese cabbage	Luang Prabang
Vietnam		
VT_FB-1c-1, VT_FB-1c-3	Chinese cabbage	Quynh Luong, Quynh Luu, Nghe An
VT_FBCM-10-3, VT_FBCM-10-4	Chinese mustard	Mai Son, Son La province
VT_SFB1b-1, VT_SFB1b-5	Mustard	Quynh Luong, Quynh Luu, Nghe An
VT_SFBCC-7B-2	Chinese cabbage	Van Duc, Gia Lam district, Hanoi
VT_SFBLB-7A-1 to VT_SFBLB-7A-5	Leafy brassica	Thuan Chau district, Son La Province
VT_SFBLB-8B-1 to VT_SFBLB-8B-5	Leafy brassica	Mai Son, Son La province
VT_SFBLB-10B-1 to VT_SFBLB-10B-5	Leaf brassica	Ham Duc, Ham Thuan Bac district, Bình Thuận Province
VT_SFBLB-11B-1 to VT_SFBLB-11B-5	Leaf brassica	Xuan Loc district, Đồng Nai Province
VT_SFBLB-12A-1 to VT_SFBLB-12A-5	Leaf brassica	Buon Me Thuot city, Dak Lak province
VT-_SFBPLM-6B-4, VT-_SFBPLM-6B-5	Pak-choy	Da Mai, Bac Giang city, Bac Giang Province
VT-FB-1c-4, VT-FB-1c-5	Chinese cabbage	Quynh Luong, Quynh Luu, Nghe An
VT-FBCM-10-1	Chinese mustard	Mai Son, Son la province
VT-SFBLB-7A-5	Leaf brassica	Mai Son, Son la province
VT-SFBLF-1B-1 to VT-SFBLF-1B-5	Pak- choy	Tien Phong Me Linh district, Hanoi
VT-SFBLM-4C-1 to VT-SFBLM-4C-5	Leafy mustard	Tay Tuu, Tu Liem district, Hanoi
VT-SFBLM-5B-1 to VT-SFBLM-5B-5	Leafy mustard	Co do, Moc Chau district, Son la province

Vt_SFBCC-3-1 to Vt _SFBCC-3-5	Common cabbage	Co Noi, Mai son, Son la province
Vt _SFBLM-4-2, Vt _SFBLM-4-3, Vt _SFBLM-4-5	Leafy mustard	Co Noi, Mai son, Son la province
Vt -SFBCC-5-1 to Vt -SFBCC-5-3	Chinese cabbage	Co Noi, Mai son, Son la province
Vt -SFBR-15-1, Vt -SFBR-15-2, Vt -SFBR-15-5	Radish	Muong Bon, Mai Son, Son la province

DNA Extraction and Sequencing

Extraction of genomic DNA was done using the individual larva or adult tissues of *P. rapae* and flea beetle using Geneaid genomic DNA mini kit (Taipei, Taiwan) following the procedure outlined by (Liu et al. 2011). The mitochondrial *coxI* gene fragment was amplified using universal *cox1* primers (HCO2198, 5'-TAAACTTCAGGGTGACCAAAAAATCA-3', and LCO1490, 5'-GGTCAACAAATCATAAAGATATTGG-3') (Folmer et al. 1994) (Folmer et al. 1994), following PCR protocol: 95°C for 10 min followed by 4 cycles of 95°C for 30s, 55°C for 45s and 72°C for 1.30 min, followed by 30 cycles of 95°C for 30s, 50°C for 45s, with the final extension at 72°C for 8 min. The PCR products were sequenced at Genomics Bioscience and Technology Company Limited, Taiwan.

Molecular divergence and population genetic analyses

The *coxI* sequences were aligned and edited using BioEdit version 7.0 (Hall 1999). c*ox1* sequences of *P. rapae* from Canada (KT140090.1, KM547147.1), Australia (KF404991.1), Korea EU105213.1, Japan (LC090567.1), Spain (JN827888.1), Romania (HQ004953.1), South Korea (EU105296.1), Kazakhstan (FJ663942.1), Germany (JF415723.1), China (JQ996397.1), USA (JF283398.1), France (KX041897.1), Norway (KX048703.1), Finland (KM573502.1), and flea beetle from Spain (KF656309), Finland (KJ966332), Germany (KM450461), Spain (KF654007), Canada (MG054786), Italy (MH323316.1), Sweden (JX243022), and Finland (KJ962687) were obtained from National Center for Biotechnology Information (NCBI) GenBank. The number of haplotypes, nucleotide diversity and haplotype diversity were calculated for investigating the *coxI* sequence diversity using DnaSP 5.10 software (Librado and Rozas 2009). Statistical tests of Tajima's *D* and Fu's *Fs* values were also conducted using DnaSP 5.10.

The genetic structure of flea beetle and *P. rapae* populations based on *coxI* sequences was examined by Analysis of Molecular Variance (AMOVA) using Arlequin 3.5 software (Excoffier and Lischer 2010). Maximum-likelihood (ML) phylogenetic analysis was used to identify major clades and to evaluate the relationships among the haplotypes of the *coxI* sequences. The appropriate model of sequence evolution, including model parameters, were calculated using corrected Akaike Information Criterion (AICc value) with MEGA 7 (Tamura et al. 2011). The clustering probabilities of each resulting phylogenetic tree node were statistically tested by a bootstrap method consisting of 1000 replicates. *Pieris melete* (Syn: *Artogeia melete*) (Lepidoptera: Pieridae) was used as an outgroup for cabbage butterfly. *Chrysolina fastuosa* (Coleoptera: Chrysomelidae) was used as an outgroup for flea beetles.

Sequence data

Sequence data that support the findings of this study has been deposited in NCBI GenBank with accession numbers MZ032867- MZ032876 for *Pieris rapae* and MZ033002 – MZ033036 for flea beetle.

RESULTS AND DISCUSSION

We examined the genetic diversity and structure of *P. rapae* and flea beetles based on the c*oxI* gene. A total of 89 individuals of *P. rapae* and 187 individuals of flea beetles from five countries (Taiwan, Cambodia, Vietnam, Thailand and Laos) were examined. We successfully amplified 690 bp utilizing *coxI* universal primers. After trimming and editing, the final length consisted of approximately 582 bp for *P. rapae* and 584 bp for flea beetles. A total of 10 *coxI* haplotypes were identified in 83 *P. rapae* samples based on sequence similarity (Table 3). The largest haplotype contained nine individuals from Vietnam. This was followed by four haplotypes from Laos.

Table 3. List of number of samples studied, number of haplotypes, haplotype diversity (h), nucleotide diversity (π), Tajima's *D* and Fu's *Fs* tests for *Pieris rapae* populations from Southeast Asia

Country	No. of Samples	No. of haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)	Tajima's *D*	Fu's *Fs*
Taiwan	5	1	-	-	-	-
Laos	24	4	0.5688*	0.02623*	2.95875 *	2.2939*
Vietnam	49	9	0.4804	0.00891	- 1.21535*	0.2245
Thailand	5	2	0.3333*	0.00057*	-0.93302*	-0.9647*
All countries	83	10	0.5631	0.0149	0.5408	0.9384

The haplotype diversity is a measure of the uniqueness of a particular haplotype in a given population (Nei and Tajima 1981), whereas nucleotide diversity is used to measure the degree of polymorphism within a population (Nei and Li 1979). The total haplotype diversity value of all *P. rapae* population from sampled countries was 0.5631, whereas the total nucleotide diversity of all *P. rapae* population from sampled countries was 0.0149 (Table 3). The highest haplotype and nucleotide diversity values were recorded in Laos, whereas the lowest values were recorded in Thailand. However, no nucleotide and haplotype diversity values of *P. rapae* were recorded for the Taiwan population. This can be due to the absence of haplotypes.

Tajima's *D* and Fu's *Fs* tests were used to calculate neutrality indices (Fu 1995; Fu 1997; Tajima 1989). Negative Tajima's *D* values were recorded for the Thailand and Vietnam populations (Table 3). The negative Tajima's *D* values in these countries indicated that the *P. rapae* population began to expand recently. However, the significantly positive Tajima's *D* value for Laos indicated that the *P. rapae* population may have suffered a recent sharp decline in its size (bottleneck). Such studies are lacking for the brassica pests in Southeast Asia. However, Tajima's *D* test was used to study the population structure of legume pod borer (*Maruca vitrata*) in Southeast Asian countries (Periasamy et al. 2015). A significantly positive value of Fu's *Fs* for Laos's population is evidence for the deficiency of alleles due to a recent population decrease. Thus, both Tajima's *D* and Fu's F_S values for *P. rapae* population in Laos indicated the population bottleneck events. Although the positive value of Fu's *Fs* was recorded for the P. rapae population in Vietnam, it was not significant. Besides Tajima's *D*, a negative value of Fu's *Fs* for Thailand *P. rapae* population is evidence for a possible recent population expansion or genetic drift due to random sampling.

The F_{ST} values of all population pairwise comparisons ranged from -0.06 to 1.00 (Table 4). Negative F_{ST} values can be interpreted as no genetic differences between the two populations compared, due to imprecision of the algorithm used (Jaramillo et al. 2001), as shown between Taiwan and Vietnam *P. rapae* populations. The low and non-significant F_{ST} values (0.13-0.24) among Taiwan vs Laos and Vietnam, Laos vs Vietnam and Thailand, and Vietnam vs Thailand populations showed less genetic differences among them. However, the Thailand population recorded a higher F_{ST} value (1.00) when compared with Taiwan population and hence making it unique and maybe a separate species (Roderick 1996), which warrants further studies including morphological characterization. This can be also explained by the fact that absence of heterozygote in the sub-population relative to the target populations could be the major factor for the complete genetic differentiation in Taiwan (Nei et al. 1975; Roderick 1996). Such genetically less diverse populations were recorded in previous studies with *P. xylostella* and *Xyleutes ceramicus* (Li et al. 2006; Panyamang et al. 2018).

Table 4. Pairwise F_{ST} values comparing populations of *Pieris rapae*

Population	Taiwan	Laos	Vietnam	Thailand
Taiwan	0			
Laos	0.19	0		
Vietnam	-0.06	0.24	0	
Thailand	**1.00 ***	0.24	0.13	0

The phylogenetic pattern of *P. rapae* was developed utilizing all haplotypes obtained in the present study. According to the Maximum likelihood (ML) phylogenetic tree, two different clades were formed for *P. rapae* (Figure 1). Clade I contained the majority of the samples from Laos, Taiwan and Vietnam; interestingly, Thailand population and one sample from the highlands of Vietnam (Son la province) were assembled as a subclade with 61% bootstrap value within this clade I. Similarly, two samples

from lowlands of Vietnam also formed another subclade with 64% bootstrap value within clade I. It should be noted that all the reference samples of *P. rapae* from NCBI aligned with clade I. Clade II contained two populations from Laos (Kasi and Vangvieng districts of Vientiane province) and two populations from Vietnam highlands (Moc Chau and Mai Son of Son la province). These results indicated the genetic dissimilarity of *P. rapae* populations originating, especially from Laos and Vietnam. This could be possibly due to speciation events occurring in those regions (Coyne 1992; Avise 2000), although it may not be so obvious in the adult butterflies. Hence, additional samples from these regions should be obtained to validate the findings of this study, especially using the nuclear regions, since mitochondrial DNA is often unable to identify recently emerged species because of the time required to separate the intraspecific variation from interspecific divergence (barcoding gap). In addition, adult samples should also be collected for morphological characterization.

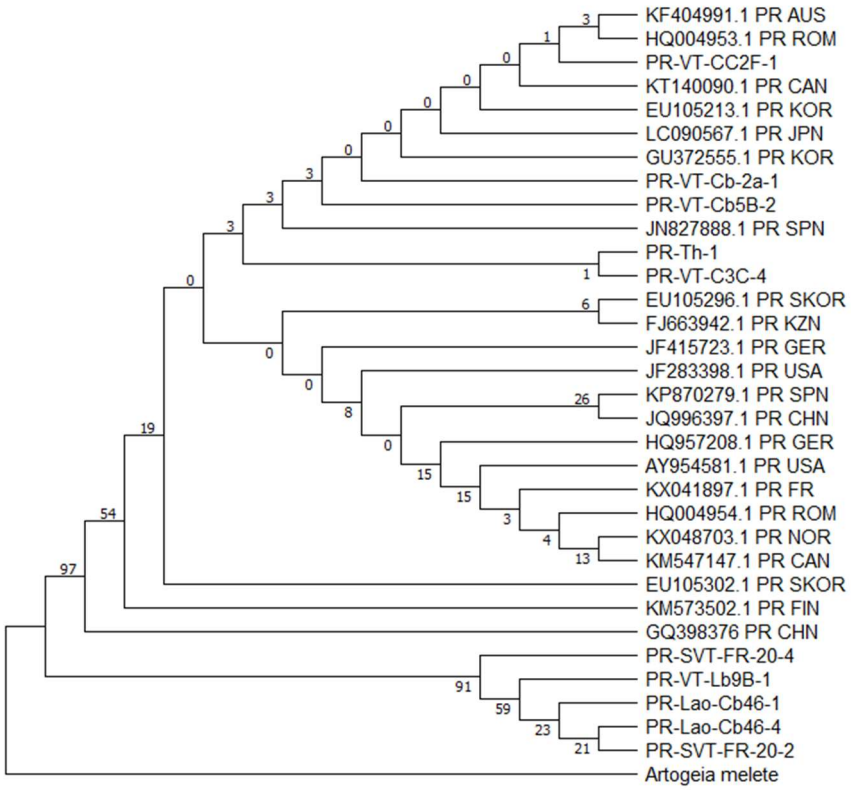


Figure 1. Phylogenetic relationship based on partial mtcox1 sequence data of *Pieris rapae* populations from South-East Asia.

The evolutionary history was inferred using the maximum likelihood method employing the best fit Tamura 3-parameter model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown. This analysis involved 33 nucleotide sequences. Evolutionary analyses were conducted in MEGA 7 (Tamura et al. 2011)

A total of 36 *coxI* haplotypes were identified in 187 flea beetle samples based on sequence similarity (Table 5). The largest number of haplotypes occurred in Vietnam, followed by 11 haplotypes in Laos. The total haplotype diversity value of all flea beetle population from sampled countries was 0.82, whereas the total nucleotide diversity of all flea beetle population from sampled countries was 0.00601 (Table 5). The haplotype diversity values were almost the same for both Laos (0.855) and Vietnam (0.858), and they were significant. Similarly, Vietnam recorded the significantly highest nucleotide diversity value (0.00729). The lowest haplotype and nucleotide diversity values were recorded in Taiwan.

Table 5. List of number of samples studied, number of haplotypes, haplotype diversity (h), nucleotide diversity (π), Tajima's *D* and Fu's *Fs* tests for flea beetle populations from Southeast Asia

Country	No. of Samples	No. of haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)	Tajima's *D*	Fu's *Fs*
Taiwan	10	2	0.2	0.00034 *	-1.11173 *	-0.339 *
Laos	26	11	0.855 *	0.004	-1.40894 *	-3.981 *
Vietnam	109	28	0.858 *	0.00729 *	-2.70033 *	-9.497 *
Thailand	5	2	0.4	0.00205	-1.04849 *	1.688
Cambodia	37	7	0.643	0.00333	-0.28919 *	-0.201 *
All countries	187	35	0.82	0.00601	-2.65988	-18.104

The negative Tajima's *D* values for all the countries indicated that the flea beetle population began to expand recently. Besides Tajima's *D*, significant and negative values of Fu's *Fs* for all the countries except Thailand are also an evidence for a possible recent population expansion or genetic drift due to random sampling.

The F_{ST} values of all flea beetle population pairwise comparisons ranged from -0.079 to 0.78502 (Table 6). Negative F_{ST} values between Cambodia and Thailand flea beetle populations confirmed the absence of genetic differences. The low and non-significant F_{ST} values among Laos and Vietnam (0.01407), Vietnam and Thailand (0.06434), and Taiwan and Vietnam (0.0026) populations showed less or negligible genetic differences among them. However, significantly higher F_{ST} values among Taiwan and Cambodia (0.46616), and Taiwan and Thailand (0.78502) populations can be considered as a different subspecies or species, which warrant further molecular studies using nuclear regions and morphological characterization. It should be noted that migration by means of anthropogenic activities played a critical role in shaping the flea beetle population structure in Taiwan (Lee et al. 2011). The maximum likelihood (ML) tree was constructed for *cox1* gene using *Phyllotreta* reference sequences from Europe (Sweden, Italy, Germany, Finland, and Spain), North America (the USA and Canada) and Oceania (Australia). According to the phylogenetic analysis (Figure 2), all populations are more related to *P. striolata* suggesting the presence of close genetic relationship and are not distinct with bootstrap value 1000.

Table 6. Pairwise F_{ST} values comparing populations of flea beetle

Population	Taiwan	Cambodia	Laos	Vietnam	Thailand
Taiwan	0				
Cambodia	0.46616 *	0			
Laos	0.2983	0.2817	0		
Vietnam	0.0026	0.1086	0.01407	0	
Thailand	0.78502 *	-0.079	0.31787	0.06434	0

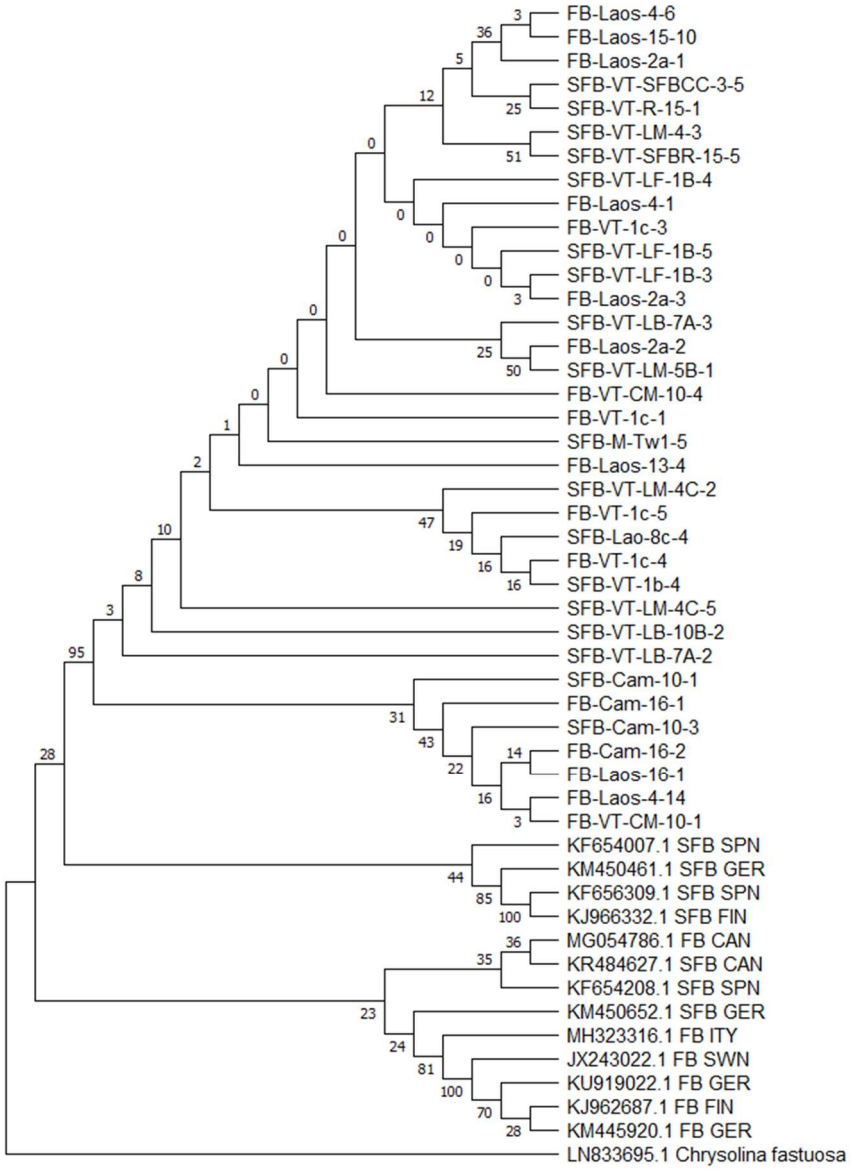


Figure 2. Phylogenetic relationship based on partial mtcox1 sequence data of Flea beetle populations from South-East Asia.

The evolutionary history was inferred using the maximum likelihood method employing best fit Hasegawa-Kishino-Yano model (Hasegawa et al. 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown. This analysis involved 49 nucleotide sequences. Evolutionary analyses were conducted in MEGA 7 (Tamura et al. 2011)

CONCLUSIONS

The study was carried out to understand the phylogenetic relationship among geographically different flea beetles and *P. rapae* populations in Southeast Asia using the *cox1* gene. No such studies were carried out in Southeast Asia in the past. The results showed that both *Phyllotreta* flea beetles and *Pieris rapae* had more haplotypes in Laos and Vietnam. A part of the *P. rapae* population from Vientiane province in Laos and the Son la highlands in Vietnam formed a separate clade in the phylogenetic tree. The majority of the *Phyllotreta* flea beetles in target countries were identified as *P. striolata*. Additional studies are suggested to validate the findings of this study, especially using the nuclear regions, since mitochondrial DNA is often unable to identify recently emerged species. It is imperative to understand the genetic differences or similarities existing among the flea beetle and *P. rapae* populations in Southeast Asia in order to introduce effective pheromone-based and biological control options to manage these pests, and thus reducing the indiscriminate use of hazardous pesticides.

Acknowledgements

This work was supported by the Federal Ministry for Economic Cooperation and Development (GIZ Project number 13.1432.7-001.00), Germany.

References

Avise JC. 2000. Phylogeography: the history and formation of species. Harvard University Press.

Coyne JA. 1992. Genetics and speciation. Nature 355:511-515.

Excoffier L, Lischer HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567.

FAO. 2019. Crops. FAOSTAT. http://www.fao.org/faostat/en/#data/QC

Feng H, Huang YJ, Hsu J. 2000. Insecticide susceptibility of cabbage flea beetle (*Phyllotreta striolata* (Fab.)) in Taiwan. Plant Protection Bulletin (Taipei), 42(1): 67-72.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294-299.

Fu YX. 1995. Statistical properties of segregating sites. Theoretical Population Biology 48:172-197.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915-925.

Hasegawa M, Kishino H, Yano T. 1985. Dating the human-ape split by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160-174.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NTNucleic acids symposium series: [London]: Information Retrieval Ltd., c1979-c2000. pp 95-98.

Hebert PD, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. Proceedings. Biological Sciences 270:313-321.

Hewitt GM. 2001. Speciation, hybrid zones and phylogeography - or seeing genes in space and time. Molecular Ecology 10:537-549.

Jaramillo C, Montana MF, Castro LR, Vallejo GA, Guhl F. 2001. Differentiation and genetic analysis of *Rhodnius prolixus* and *Rhodnius colombiensis* by rDNA and RAPD amplification. Memorias do Instituto Oswaldo Cruz 96:1043-1048.

Kadirvel P, Srinivasan R, Hsu YC, Su FC, De La Pena R. 2013. Application of cytochrome oxidase I sequences for phylogenetic analysis and identification of thrips species occurring on vegetable crops. Journal of Economic Entomology 106:408-418.

Lee C, Chang H, Wang CL, Chen WS. 2011. A review of *Phyllotreta* Chevrolat in Taiwan (Coleoptera: Chrysomelidae: Galerucinae: Alticini). Zoological Studies 50:525-533.

Li J, Zhao F, Choi YS, Kim I, Sohn HD, Jin BR. 2006. Genetic variation in the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) in China inferred from mitochondrial COI gene sequence. European Journal of Entomology 103:605-611.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

Liu L, Liu J, Wang Q, Ndayiragije P, Ntahimpera A, Nkubaye E, Yang Q, Li Z. 2011. Identification of *Bactrocera invadens* (Diptera: Tephritidae) from Burundi, based on morphological characteristics and DNA barcode. African Journal of Biotechnology 10(62): 13623-13630.

Malini P, Ramasamy S, Schafleitner R, Muthukalingan K. 2019. Pheromone-binding proteins based phylogenetics and phylogeography of *Maruca* spp. from Asia, Africa, Oceania, and South America. Ecology and Evolution 9:9239-9272.

Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America 76: 5269-5273.

Nei M, Tajima F. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics. 97(1): 145–163.

Nei M, Maruyama T, Chakraborty R. 1975. The Bottleneck Effect and Genetic Variability in Populations. Evolution. International Journal of Organic Evolution 29:1-10.

Nguyen T, Mai H, Boulom S, Voe P, Heang C, Ha D, Chialue L, Bor B, Phommaluesa S, Pham D. 2020. Consumers' risk perception of vegetables in Southeast Asia: Evidence from Laos, Cambodia, and Viet Nam. APN Science Bulletin, 10(1); doi:10.30852/sb.2020.1130.

Nhung NT, Viet TQ, Nghiep NT. 2008. Insect pests on Crucifers and their control by chemicals at Vo cuong, Bac Ninh Province, *Proceeding of the 6th Vietnam National Conference on Entomology, May -10, 2008*. Agricultural Publishing House, Hanoi, Vietnam.

Panyamang A, Duangpakdee O, Rattanawannee A. 2018. Genetic structure of teak beehole borer, *Xyleutes ceramicus* (Lepidoptera: Cossidae), in northern Thailand. Agriculture and Natural Resources 52:66-74.

Periasamy M, Schafleitner R, Muthukalingan K, Ramasamy S. 2015. Phylogeographical Structure in Mitochondrial DNA of Legume Pod Borer (*Maruca vitrata*) Population in Tropical Asia and Sub-Saharan Africa. PloS One 10:e0124057.

Praneetvatakul S, Schreinemachers P, Pananurak P, Tipraqsa P. 2013. Pesticides, external costs and policy options for Thai agriculture. Environmental Science & Policy 27:103-113.

Ram Kumar N, Chang JC, Narayanan MB, Ramasamy S. 2017. Phylogeographical structure in mitochondrial DNA of whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) in southern India and Southeast Asia. Mitochondrial DNA. Part A, DNA Mapping, Sequencing, and Analysis 28:621-631.

Ramasamy S, Hsu YC, Palchamy K, Lin MY. 2013. Analysis of *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex in Java Island, Indonesia based on mitochondrial cytochrome oxidase I sequences. The Philippine Agriculturist 96:290-295.

Roderick GK. 1996. Geographic structure of insect populations: gene flow, phylogeography, and their uses. Annual Review of Entomology 41:325-352.

Schreinemachers P, Chen HP, Nguyen TTL, Buntong B, Bouapao L, Gautam S, Le NT, Pinn T, Vilaysone P, Srinivasan R. 2017. Too much to handle? Pesticide dependence of smallholder vegetable farmers in Southeast Asia. The Science of the Total Environment 593-594:470-477.

Taberlet P. 1998. Biodiversity at the intraspecific level: The comparative phylogeographic approach. Journal of Biotechnology 64:91-100.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-595.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28:2731-2739.

Ulrichs C, Mewis I, Schnitzler WH, Burleigh JR. 2001. Effectivity of synthetic insecticides against *Maruca vitrata* F. and the parasitoid *Bassus asper* Chou & Sharkey in the Philippines. Mitteilungen der Deutschen Gesellschaft fur Allgemeine und Angewandte Entomologie. 13(1-6): 279–282.

Ungsa M, Vanharn H. 1995. Vegetable Production, Research and Policy in Cambodia. *In*: AVRDC.1995. Vegetable research and development in Cambodia, Lao PDR and Vietnam; Proceedings of a workshop. Asian Vegetable Research and Development Center. Shanhua, Tainan, Taiwan. Publication no. 94-431.130 p.

Estimation of molecular dates separating four *Plutella* species

Ward, C.M.
SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF ADELAIDE, ADELAIDE AUSTRALIA
email: christopher.ward@adelaide.edu.au

Landry, J.F.
CANADIAN NATIONAL COLLECTION OF INSECTS, ARACHNIDS, AND NEMATODES, AGRICULTURE AND AGRI-FOOD CANADA, OTTAWA RESEARCH AND DEVELOPMENT CENTRE, OTTAWA, CANADA.
email: jean-francois.landry@canada.ca

Baxter, S.W.
SCHOOL OF BIOSCIENCES, UNIVERSITY OF MELBOURNE, MELBOURNE, AUSTRALIA
email: simon.baxter@unimelb.edu.au

ABSTRACT
Plutella xylostella L., the diamondback moth, has successfully colonized agricultural regions across the world, and is now recognized as the foremost pest of cruciferous crops. In contrast, other members of the *Plutella* genus have limited observed pest potential and dispersal. Here we sequence and assemble genomes of three neglected species, *P. armoraciae* and *P. porrectella* and the leek moth, *Acrolepiopsis assectella*, then in conjunction with existing data from *P. xylostella* and *P. australiana*, apply a molecular clock to estimate their divergence. All *Plutella* and *Acrolepiopsis* mitochondrial genomes show similar sizes (~15-16 kb) and a conserved gene order that includes two rRNA, 13 protein coding and 22 tRNA genes. *Plutella armoraciae* and *P. porrectella* show an average 91.95 and 91.08% homology to *P. xylostella* across mitochondrial protein coding genes. We then construct a chronogram using complete COX1 and 16S sequences, dating the crown node of *Plutella* to 5.46 (95% HPD 7.05 – 4.10) MYA, and find the topology is concordant with a species tree constructed from 104 nuclear single copy orthologs. This work provides complete mitochondrial genomes and nuclear genome resources for future comparison within the genus *Plutella* to understand why *P. xylostella* has become such a widespread and serious pest, relative to its sister taxa.

Keywords
Plutella, mitochondrial genome, molecular clock, genome

INTRODUCTION
The genus *Plutella* are microlepidopterans within the superfamily Yponomeutoidea (Sohn et al. 2013) containing a total of 26 recognized species worldwide (Baraniak 2007; Søli et al. 2018). *Plutella* moths are specialist herbivores of crucifers, which include many plants of economic importance such as canola and cabbage (Bonnemaison 1965; Smith and Sears 1984; Zalucki et al. 2012). Mitochondrial gene trees of cytochrome oxidase I (COX1) have previously provided species topologies of 11 *Plutella* species (Landry and Hebert 2013; Søli et al. 2018). However, extensive molecular research has only been carried out on a single species of economic importance, *P. xylostella* (Talekar and Shelton 1993; Furlong et al. 2013).

Plutella xylostella, diamondback moth, is an invasive agricultural pest species throughout the world with an annual estimated cost of $4-5 billion (Zalucki et al. 2012; Furlong et al. 2013). Recognized as the major insect pest of cruciferous crops, *P. xylostella* routinely develops resistance to insecticides (Zalucki et al. 2012; Furlong et al. 2013), and is highly dispersive (Chapman et al. 2002; Wei et al. 2013a; Fu et al. 2014; Perry et al. 2018). Lack of population structure at the continent level occurs in Australia (Endersby et al. 2006; Perry et al. 2018) and China (Wei et al. 2013a; Fu et al. 2014), enabling resistance alleles to spread between populations. Generational time is highly dependent on temperature, with generations per year ranging from greater than 10 in tropical climates to 2-4 in regions with harsh winters (Bonnemaison 1965; Harcourt 2012). Due to its economic importance, extensive research has been carried out on the life history traits (Bigger and Fox 1997; Philips et al. 2014; Garrad et al. 2015), ecology (Talekar and Shelton 1993; Furlong et al. 2013; Philips et al. 2014) and genetics (Heckel et al. 2007; You et al. 2013; Ward and Baxter 2018) of *P. xylostella*. In contrast, its neglected allies generally show low pest potential and consequently aspects of their biology and evolutionary history are underrepresented in the scientific literature.

Recent research has focused on a cryptic ally of *P. xylostella* (Landry and Hebert 2013), *P. australiana,* revealing key biological and genetic differences (Perry et al. 2018; Ward and Baxter 2018). *Plutella australiana* lacks population structure across its native range of Australia, has low pest potential and shows low tolerance to many insecticides (Perry et al. 2018). *Plutella xylostella* and *P. australiana* do have the ability to hybridize in laboratory crosses (Perry et al. 2018) raising concerns that insecticide resistance alleles may be exchanged. However, genome-wide scans have failed to identify widespread gene flow, supporting reproductive isolation in the field (Ward and Baxter 2018).

Literature surrounding other species of *Plutella* is generally limited to their taxonomic descriptions and brief details regarding their host plant ranges (Baraniak 2007). A single species, *P. porrectella,* has detailed life history described (Smith and Sears 1984) with descriptions of another, *P. armoraciae*, currently being carried out (Paul Abram, *pers. comm.*). In contrast to *P. xylostella, P. porrectella* appears to have limited dispersal and no more than two generations per year with developmental diapause at the larval stage during winter (Smith and Sears 1984). Larvae produce large quantities of silk, linking together leaves and feeding inside these concealed structures, unlike *P. xylostella* which feeds openly. Likewise, *P. armoraciae* adults have lower activity and longer generational times than *P. xylostella* (Paul Abram, *pers. comm.*) suggesting these traits may be the norm for ancestral *Plutella* species.

Genomic resources for *Plutella* species are mostly limited to *P. xylostella* with both nuclear (You et al. 2013) and mitochondrial (Wei et al. 2013b; Dai et al. 2016a) genomes available. The notable exception is in its closest known relative, *P. australiana,* whose complete mitochondrial genome has been assembled (Ward and Baxter 2018). Here we sequence the genomes of two *Plutella* species, *P. porrectella* and *P. armoraciae*, along with the outgroup *Acrolepiopsis assectella*, for comparison with *P. xylostella* (Wei et al. 2013b; You et al. 2013; Dai et al. 2016a) and *P. australiana* (Ward and Baxter 2018) datasets. We then assemble the complete mitochondrial genomes of these three species in order to date molecular divergence among these representatives of the *Plutella* genus.

MATERIALS AND METHODS

Sample collection and sequencing

Plutella armoraciae, P. porrectella and *A. assectella* samples were provided by J.-F.L. from laboratory colonies. *Acrolepiopsis assectella* was considered an outgroup species due to their presence within the same superfamily (Yponomeutoidea) as *Plutella*. DNA was extracted from single individuals of *P. armoraciae, P. porrectella* and *A. assectella* using the Qiagen DNeasy kit according to the manufacturer's protocol. DNA was then sequenced using an Illumina NextSeq High Output kit (2x150bp) to an estimated 20-30X coverage, based on the assumption of a similar genome size to *P. xylostella* ~339 Mb (Baxter et al. 2011). Quality assessment of short read sequence data was performed using fastqc (Version 0.11.6) (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and ngsReports (Ward et al. 2020). Short read WGS data for each species can be found on the Sequence Read Archive with accessions: SAMN17577696 (*P. porrectella*), SAMN17577695 (*P. armoraciae*) and SAMN18035365 (*A. assectella*).

Mitochondrial and nuclear genome assembly

Mitochondrial genomes for *A. assectella*, *P. porrectella* and *P. armoraciae* were assembled using NOVOplasty (Dierckxsens et al. 2016) and error corrected using Pilon (Walker et al. 2014). Annotation of the circularized mitochondrial genomes was then carried out by homology to *P. xylostella* mitochondrion annotations (KM023645) for protein coding genes (Dai et al. 2016a) and using MITOS web server (Bernt et al. 2013) for rRNA and tRNA. Mitochondrial genome assembles can be found under GenBank accessions: MW662613 (*Plutella armoraciae*), MW662614 (*Plutella porrectella*) and MW662615 (*Acrolepiopsis assectella*).

Nuclear genome assembly was carried out for *P. australiana* (SRR6505270), *P. armoraciae, P. porrectella* and *A. assectella* using MaSuRCA (Zimin et al. 2013) under default settings. Draft genomes were then polished with Pion (Walker et al. 2014) by mapping the short-read data to their respective genome using BWA mem (Li 2013). Heterozygous contigs with >97% identity were removed using the Redundans pipeline (Pryszcz and Gabaldón 2016). Insectav9 Benchmarking Universal Single Copy Orthologs were then annotated on the assembled genomes and the *P. xylostella* reference genome DBM FJ v1.1 (You et al. 2013) using BUSCOv3 (Waterhouse et al. 2017).

Nuclear divergence and phylogenetic inference

Single copy orthologs with complete open reading frames were aligned with Geneious v11.0 (Kearse et al. 2012) using amino-acid sequence to inform the alignment. Maximum likelihood gene trees were constructed for each of the single copy orthologs using RAxML (Stamatakis 2014) with a GTR substitution model and gamma rate heterogeneity. The species tree was then inferred from gene trees using ASTRAL2 (Zhang et al. 2018).

Genetic distance between *P. xylostella* and the four other species assembled was calculated for each of the single copy ortholog alignments using Geneious v11.0 (Kearse et al. 2012). Codons within the alignment were partitioned based on their position: 1st plus 2nd codon positions, 3rd position of all codons and 3rd position of codons that show four-fold degeneracy.

Estimation of species divergence dates

Cytochrome oxidase I and 16S rRNA genes were extracted from each of the assembled mitochondrial genomes and aligned to their corresponding genes in *P. australiana* (MG787473) (Ward and Baxter 2018) and *P. xylostella* (KM023645) (Dai et al. 2016a) mitochondrial genomes with Geneious v11.0 using the MAFFT algorithm. BEAST2 v2.5.1 (Bouckaert et al. 2018) was used to estimate the mitochondrial divergence date. Substitution models for each gene were estimated using the bModelTest package (Bouckaert and Drummond 2017). Unlinked lognormal relaxed clocks were applied to both COXI and 16S with priors set to 0.0177 (±0.0019) and 0.0064 (±0.0009) substitutions Myr^{-1} according to Papadopoulou et al. (2010). Two independent MCMC chains of $2.5x10^8$ were carried out, sampling every 10,000. Tracer v1.6 (Rambaut et al. 2018) was then used to visually inspect log files for model mixture and to determine burn-in (16%).

RESULTS AND DISCUSSION

Mitochondrial genome description

The complete circular mitochondrial genomes of *P. armoraciae, P. porrectella* and *A. assectella* were 15,569, 16,196 and 15,369 bp long, respectively (Figure 1), which is similar to the length of *P. xylostella* (16,014 bp) (Dai et al. 2016a) and *P. australiana* (15,962 bp) (Ward and Baxter 2018). The genomes of each of the species contained two rRNA, 13 protein coding and 22 tRNA genes in the conserved gene order for lepidopterans (Sun et al. 2017) (Figure 1).

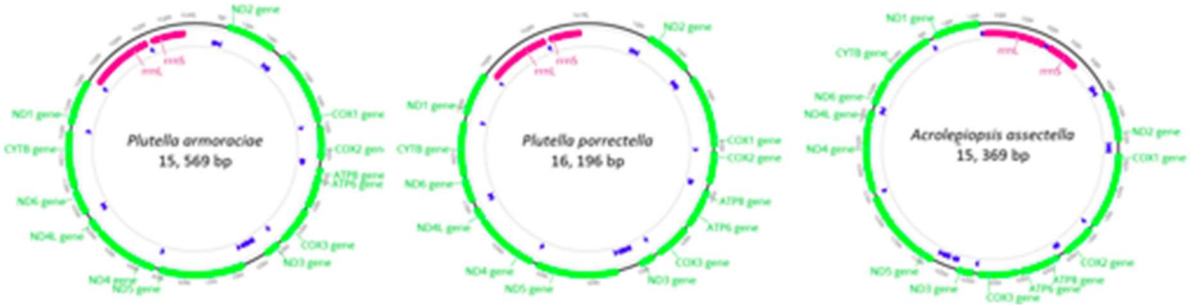


Figure 1: Mitochondrial genome assemblies and annotations of *Plutella armoraciae, P. porrectella* and *Acrolepiopsis assectella*. Annotated protein coding (n = 13, green), ribosomal RNA (n = 2, red), and transfer RNA (n = 22, blue) are highlighted along the circular mitochondrial

Each of the genomes contained an A-T rich D-loop of variable length. D-loop size correlated with total mitochondrial genome size, the longest being *P. porrectella* (1,179 bp). *Plutella armoraciae* and *A. assectella* had similar D-loop sizes of 579 and 490 bp. The A-T skew of the D-loop was similar between all species with an average of 93.3% A-T (±0.0086), consistent with reports from other lepidopterans (Dai et al. 2016b; Meng et al. 2016; Sun et al. 2017).

Protein coding genes made up 71.9% (11,198 bp), 69.1% (11,198 bp) and 72.6% (11,159 bp) of the total genome size in *P. armoraciae, P. porrectella* and *A. assectella*, respectively. Twelve mitochondrial protein coding genes contained a conventional start codon (ATN), whereas COX1 is initiated with CGA. Genetic distance between the mitochondrial genome of *P. xylostella* and genomes the other species analysed here was 9.43% (*P. porrectella*) and 8.06% (*P. armoraciae*). As expected, the outgroup *A. assectella*, showed the highest overall genetic distance to *P. xylostella* (16.8%).

Mitochondrial divergence of *Plutella*

Genetic distances between DNA sequences of individual mitochondrial protein coding genes were compared. The genetic distance between *P. xylostella* and *P. armoraciae* ranged from 6.19-11.9%, with an average of 8.05%. The genetic distance between *P. xylostella* and *P. porrectella* was 5.93-17.26% and had an average distance of 8.92% (Table 1). In both sequenced *Plutella* species, ATP8 showed the highest genetic distance to *P. xylostella*. The average genetic distance to *P. xylostella* among protein coding gene regions was lower than the complete mitochondrial genome, reported above (Table 1).

Table 1: Genetic distance (%) and alignment length of the DNA sequences for the 13 mitochondrial protein coding genes to *P. xylostella*.

		Genetic distance to *P. xylostella*		
Gene name	Alignment Length	*Plutella armoraciae*	*Plutella porrectella*	*Acrolepiopsis assectella*
ATP6	678	6.64	7.96	15.63
ATP8	168	11.9	17.26	16.07
COX1	1531	7.7	9.86	12.34
COX2	679	6.63	7.51	10.75
COX3	789	8.88	9.38	14.83
CYTB	1152	9.03	7.64	14.94
ND1	942	8.07	8.07	13.06
ND2	1019	8.06	7.28	19.92
ND3	354	6.21	5.93	14.97
ND4	1339	7.02	7.99	14.94
ND4L	291	6.19	7.56	13.4
ND5	1724	7.83	7.19	15.16
ND6	540	10.49	12.36	23.52
Average	862	8.05	8.92	15.49

A relaxed molecular clock was applied to cytochrome oxidase I (0.0177±0.0019 MYA) and 16S (0.0064±0.0009 MYA) nucleotide alignments in order to date the mitochondrial divergence time. The topology agreed with mitochondrial gene trees produced by Landry and Hebert (2013) and Søli et al. (2018).

Plutella xylostella and *P. australiana were* shown to be in reciprocal monophyly with *P. armoraciae* and *P. porrectella*. An estimated crown node age of 5.46 (95% HPD 7.05 – 4.10) MYA was generated using COX1 and 16S clock rates (Figure 2). Median divergence times of 2.68 (3.65 – 1.83) and 3.85 (5.43 – 2.57) MYA (95% HPD) were estimated for the *P. australiana*/*P. xylostella* and *P. armoraciae*/*P. porrectella* splits (Figure 2). The *P. australiana*/*P. xylostella* divergence date overlaps with the previously reported 1.96 (± 0.175) MYA (Ward and Baxter 2018). The discrepancy in divergence dates is likely due to the application of a relaxed log normal clock informed by both the 16S and COXI substitution rates in this study.

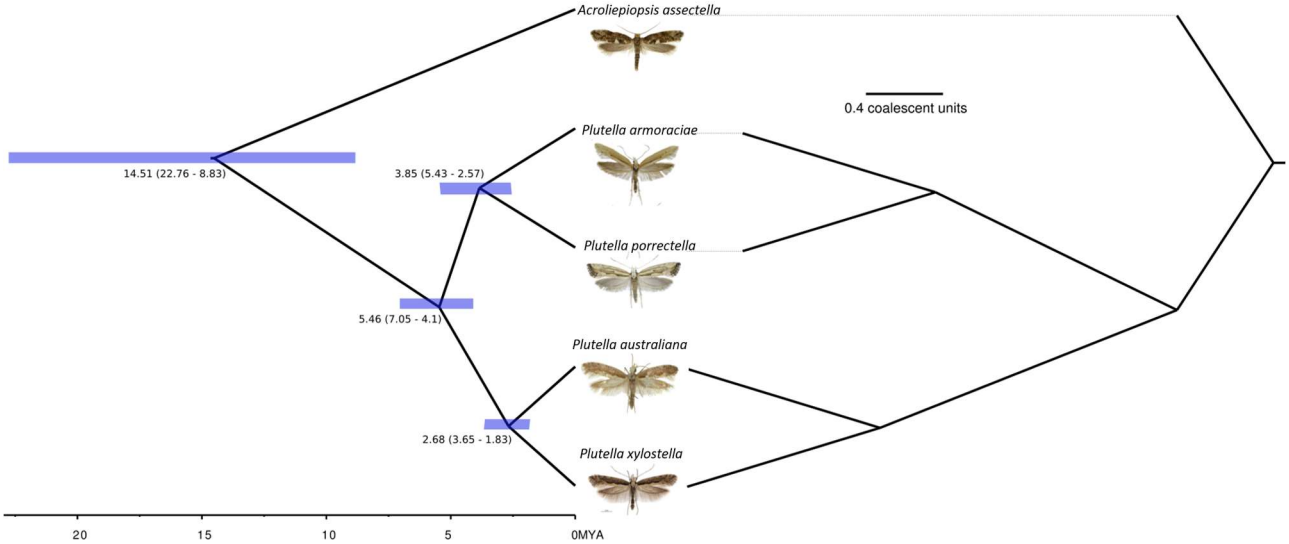


Figure 2: Mitochondrial (left) chronogram and nuclear species tree (right0 for four *Plutella* species and outgroup *Acrolepiopsis assectella*. LEFT: COX1 and 16S were used to estimate the mitochondrial divergence date of four *Plutella* species. Divergence is shown in millions of years and the 95% highest posterior density (HPD) intervals are indicated with brackets. RIGHT: 104 nuclear single copy orthologs were used to construct a maximum likelihood phylogeny using Astral III. All nodes had complet (1.0) posterior probability support.

Nuclear divergence of *Plutella*

Mitochondrial and nuclear genomes commonly show discordant topologies and divergence times (Shaw 2002, Fisher-Reid and Wiens 2011, Zheng et al. 2011, Wallis et al. 2017). Therefore, we carried out nuclear genome assembly on the data and constructed a Maximum-Likelihood phylogeny from single copy orthologs.

Nuclear genome assemblies of *P. armoraciae*, *P. armoraciae* and *A. assectella* were all highly fragmented with N_{50} values, (the shortest contig

length needed to cover 50% of the genome) ranging from 4,017-6,157 bp. Of 1,658 complete benchmarking single copy orthologs (BUSCO) genes present in the insecta orthoDB v9 dataset, 931 were recovered in *P. armoraciae*, 1,163 in *P. porrectella*, 1,225 in *A. assectella*, and 1,300 in *P. australiana*. Only 383 BUSCO genes were both single copy and present across all five genome assemblies. These were then filtered such that all contained complete open reading frames, leaving 104 BUSCOs spanning 76.5 kb for analysis. Gene trees were then constructed using each of the 104 single copy orthologs and used to reconstruct a species tree. The species tree showed an identical topology to the mitochondrial chronogram (Figure 2). Genetic distance across codon partitions were then calculated between each of the species and *P. xylostella* (Table 2). This revealed *P. xylostella* nuclear genes share greater homology with *P. porrectella* than *P. armoraciae* across all codon partitions (Table 2), in contrast to the mitochondrial genome (Table 1). Genetic distance was greatest in the third position of four-fold degenerate codons (Table 2) as these are essentially neutral (Obbard et al. 2012).

Table 2: Genetic distance (%) of each codon partition (1st+2nd, 3rd) to *P. xylostella* for nuclear single copy ortholog nucleotide alignments. Genetic distance at the 3rd position of four-fold degenerate codons are shown separately.

	Genetic distance to *Plutella xylostella*		
Species	1st+2nd	3rd	3rd 4-fold
P. australiana	1.22	9.47	14.42
P. armoraciae	3.98	21.45	31.39
P. porroctella	3.97	21.15	30.86
A. assectella	13.3	47.24	61.9

CONCLUSION

Complete circularized mitochondrial genomes of two *Plutella* species and an outgroup Yponomeutoidean were successfully assembled and annotated. All genomes were similar in size to *P. xylostella* and contained two rRNA, 13 protein coding genes and 22 tRNA conserved throughout Insecta. The topology of the *Plutella* species sequences were congruent between the mitochondrial and nuclear genomes. Furthermore, the topology was consistent with published literature. We also place the crown node age of *Plutella* at 5.46 (95% HPD 7.05 – 4.10) MYA.

Acknowledgements

We thank Paul Abram (Agriculture & Agri-Food Canada, Agassiz Research and Development Centre, British Columbia) and Peter Mason (Agriculture & Agri-Food Canada, Ottawa Research and Development Centre) for founding the *P. armoraciae* culture used, as well as providing unpublished results on the life history of *P. armoraciae*. CMW is funded by The Commonwealth Hill Trust and Grains Research Development Council.

References

Baraniak E. 2007. Taxonomic revision of the genus Plutella Schrank, 1802 (Lepidoptera: Plutellidae) from the Palaearctic Region with notes on its phylogeny. Pismo Entomologiczne.

Baxter SW, Davey JW, Johnston JS, Shelton AM, Heckel DG, Jiggins CD, Blaxter ML. 2011. Linkage Mapping and Comparative Genomics Using Next-Generation RAD Sequencing of a Non-Model Organism. PLOS ONE 6:e19315.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: Improved de novo metazoan mitochondrial genome annotation. Molecular Phylogenetics and Evolution 69:313-319.

Bigger DS, Fox LR. 1997. High-density populations of diamondback moth have broader host-plant diets. Oecologia 112:179-186.

Bonnemaison L. 1965. Insect Pests of Crucifers and Their Control. Annual Review of Entomology 10:233-256.

Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, Heled J, Jones G, Kühnert D, Maio ND, Matschiner M, Mendes FK, Müller NF, Ogilvie H, Plessis LD, Popinga A, Rambaut A, Rasmussen D, Siveroni I, Suchard MA, Wu CH, Xie D, Zhang C, Stadler T, Drummond AJ. 2018. BEAST 2.5: An Advanced Software Platform for Bayesian Evolutionary Analysis. BioRxiv:474296.

Bouckaert RR, Drummond AJ. 2017. bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC evolutionary biology 17:42.

Chapman JW, Reynolds DR, Smith AD, Riley JR, Pedgley DE, Woiwod IP. 2002. High-altitude migration of the diamondback moth Plutella xylostella to the U.K.: a study using radar, aerial netting, and ground trapping. Ecological Entomology 27:641-650.

Dai LS, Zhu BJ, Qian C, Zhang CF, Li J, Wang L, Wei GQ, Liu CL. 2016a. The complete mitochondrial genome of the diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). Mitochondrial DNA Part A 27:1512-1513.

Dai LS, Zhu BJ, Zhao Y, Zhang CF, Liu CL. 2016b. Comparative Mitochondrial Genome Analysis of Eligma narcissus and other Lepidopteran Insects Reveals Conserved Mitochondrial Genome Organization and Phylogenetic Relationships. Scientific Reports 6:26387.

Dierckxsens N, Mardulyn P, Smits G. 2016. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Research 45:e18-e18.

Endersby NM, McKechnie SW, Ridland PM, Weeks AR. 2006. Microsatellites reveal a lack of structure in Australian populations of the diamondback moth, Plutella xylostella (L.). Molecular Ecology 15:107-118.

Fisher-Reid MC, Wiens JJ. 2011. What are the consequences of combining nuclear and mitochondrial data for phylogenetic analysis? Lessons from Plethodon salamanders and 13 other vertebrate clades. BMC evolutionary biology 11:300-300.

Fu X, Xing Z, Liu Z, Ali A, Wu K. 2014. Migration of diamondback moth, Plutella xylostella, across the Bohai Sea in northern China. Crop Protection 64:143-149.

Furlong MJ, Wright DJ, Dosdall LM. 2013. Diamondback Moth Ecology and Management: Problems, Progress, and Prospects. Annual Review of Entomology 58:517-541.

Garrad R, Booth DT, Furlong MJ. 2015. The effect of rearing temperature on development, body size, energetics and

fecundity of the diamondback moth. Bulletin of Entomological Research 106:175-181.
Harcourt DG. 2012. Biology of the Diamondback Moth, Plutella maculipennis (Curt.) (Lepidoptera: Plutellidae), in Eastern Ontario. II. Life-History, Behaviour, and Host Relationships. The Canadian Entomologist 89:554-564.
Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics (Oxford, England) 28:1647-1649.
Landry JF, Hebert PDN. 2013. Plutella australiana (Lepidoptera, Plutellidae), an overlooked diamondback moth revealed by DNA barcodes. ZooKeys 327:43-63.
Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv preprint arXiv:1303.3997.
Meng Z, Lei C, Chen X, Jiang S. 2016. Complete mitochondrial genome sequence of Heliconius melpomene rosina (Insecta: Lepidoptera: Nymphalidae). Mitochondrial DNA Part A 27:3911-3912.
Obbard DJ, Maclennan J, Kim KW, Rambaut A, O'Grady PM, Jiggins FM. 2012. Estimating Divergence Dates and Substitution Rates in the Drosophila Phylogeny. Molecular Biology and Evolution 29:3459-3473.
Papadopoulou A, Anastasiou I, Vogler AP. 2010. Revisiting the Insect Mitochondrial Molecular Clock: The Mid-Aegean Trench Calibration. Molecular biology and evolution 27:1659-1672.
Perry KD, Baker GJ, Powis KJ, Kent JK, Ward CM, Baxter SW. 2018. Cryptic Plutella species show deep divergence despite the capacity to hybridize. BMC Evolutionary Biology 18:77.
Philips CR, Fu Z, Kuhar TP, Shelton AM, Cordero RJ. 2014. Natural History, Ecology, and Management of Diamondback Moth (Lepidoptera: Plutellidae), With Emphasis on the United States. Journal of Integrated Pest Management 5:D1-D11.
Pryszcz LP, Gabaldón T. 2016. Redundans: an assembly pipeline for highly heterozygous genomes. Nucleic Acids Research 44:e113-e113.
Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Systematic Biology 67:901-904.
Shaw KL. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. Proceedings of the National Academy of Sciences 99:16122.
Smith DB, Sears MK. 1984. Life history of Plutella porrectella, a relative of the Diamondback moth, Plutella xylostella (Lepidoptera: Plutellidae). The Canadian Entomologist 116:913-917.
Sohn JC, Regier JC, Mitter C, Davis D, Landry JF, Zwick A, Cummings MP. 2013. A Molecular Phylogeny for Yponomeutoidea (Insecta, Lepidoptera, Ditrysia) and Its Implications for Classification, Biogeography and the Evolution of Host Plant Use. PLOS ONE 8:e55066.
Søli G, Aarvik L, Magnussen T. 2018. Plutella polaris Zeller, 1880 (Lepidoptera, Plutellidae) rediscovered at Svalbard, Norway, with comments on its taxonomic position. Nota Lepidopterologica 41:129-137.
Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics (Oxford, England) 30:1312-1313.
Sun Y, Chen C, Gao J, Abbas MN, Kausar S, Qian C, Wang L, Wei G, Zhu BJ, Liu CL. 2017. Comparative mitochondrial genome analysis of Daphnis nerii and other lepidopteran insects reveals conserved mitochondrial genome organization and phylogenetic relationships. PLOS ONE 12:e0178773.
Talekar NS, Shelton AM. 1993. Biology, Ecology, and Management of the Diamondback Moth. Annual Review of Entomology 38:275-301.
Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: An Integrated Tool for Comprehensive Microbial Variant Detection and Genome Assembly Improvement. PLOS ONE 9:e112963.
Wallis GP, Cameron-Christie SR, Kennedy HL, Palmer G, Sanders TR, Winter DJ. 2017. Interspecific hybridization causes long-term phylogenetic discordance between nuclear and mitochondrial genomes in freshwater fishes. Molecular Ecology 26:3116-3127.
Ward CM, Baxter SW. 2018. Assessing Genomic Admixture between Cryptic Plutella Moth Species following Secondary Contact. Genome Biology and Evolution 10:2973-2985.
Ward CM, To TH, Pederson SM. 2020. ngsReports: a Bioconductor package for managing FastQC reports and other NGS related log files. Bioinformatics 36:2587-2588.
Waterhouse RM, Seppey M, Simão FA, Manni M, Ioannidis P, Klioutchnikov G, Kriventseva EV, Zdobnov EM. 2017. BUSCO applications from quality assessments to gene prediction and phylogenomics. Molecular biology and evolution 35:543-548.
Wei SJ, Shi BC, Gong YJ, Jin GH, Chen XX, Meng XF. 2013a. Genetic Structure and Demographic History Reveal Migration of the Diamondback Moth Plutella xylostella (Lepidoptera: Plutellidae) from the Southern to Northern Regions of China. PLOS ONE 8:e59654.
Wei SJ, Shi BC, Gong YJ, Li Q, Chen XX. 2013b. Characterization of the mitochondrial genome of the diamondback moth Plutella xylostella (Lepidoptera: Plutellidae) and phylogenetic analysis of advanced moths and butterflies. DNA and cell biology 32:173-187.
You M, Yue Z, He W, Yang X, Yang G, Xie M, Zhan D, Baxter SW, Vasseur L, Gurr GM, Douglas CJ, Bai J, Wang P, Cui K, Huang S, Li X, Zhou Q, Wu Z, Chen Q, Liu C, Wang B, Li X, Xu X, Lu C, Hu M, Davey JW, Smith SM, Chen M, Xia X, Tang W, Ke F, Zheng D, Hu Y, Song F, You Y, Ma X, Peng L, Zheng Y, Liang Y, Chen Y, Yu L, Zhang Y, Liu Y, Li G, Fang L, Li J, Zhou X, Luo Y, Gou C, Wang J, Wang J, Yang H, Wang J. 2013. A heterozygous moth genome provides insights into herbivory and detoxification. Nature Genetics 45:220-225.
Zalucki MP, Shabbir A, Silva R, Adamson D, Shu-Sheng L, Furlong MJ. 2012. Estimating the Economic Cost of One of the World's Major Insect Pests, Plutella xylostella (Lepidoptera: Plutellidae): Just How Long Is a Piece of String? Journal of Economic Entomology 105:1115-1129.
Zhang C, Rabiee M, Sayyari E, Mirarab S. 2018. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. BMC bioinformatics 19:153.
Zheng Y, Peng R, Kuro-o M, Zeng X. 2011. Exploring Patterns and Extent of Bias in Estimating Divergence Time from Mitochondrial DNA Sequence Data in a Particular Lineage: A Case Study of Salamanders (Order Caudata). Molecular biology and evolution 28:2521-2535.
Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. 2013. The MaSuRCA genome assembler. Bioinformatics 29:2669-2677.

Phylogeographical structure in mitochondrial DNA of Diamondback Moth (*Plutella xylostella*) population in Southeast Asia

Malini, P.
#812, 60 YI MING LIAO
WORLD VEGETABLE CENTER
SHANHUA, TAINAN 74151, TAIWAN
email address: malini.biotechnology@gmail.com

Wong, Y.T.
FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
SANDAKAN CAMPUS, 90509 SANDAKAN, SABAH
MALAYSIA
email address: shixuan0827@gmail.com

Srinivasan, R.
ENTOMOLOGY GROUP
WORLD VEGETABLE CENTER
SHANHUA, TAINAN 74151, TAIWAN
email address: srini.ramasamy@worldveg.org

ABSTRACT

Diamondback moth, *Plutella xylostella* L. (Plutellidae: Lepidoptera) is one of the most important insect pests, constraining brassica production worldwide. Since it has developed resistance to chemical and bio-pesticides, no single approach can provide satisfactory control of this notorious pest. Hence, integrated pest management strategies are warranted. In order to use the control options such as bio-control agents and sex pheromone lures, a thorough understanding of the population structure of the target pest is highly imperative. Hence, this study was undertaken to assess the genetic diversity of the *P. xylostella* population in Southeast Asia, especially in Cambodia, Lao PDR, Malaysia, Thailand, Taiwan and Vietnam. For comparison, a population from West Asia (Syria) was also included. The cytochrome c oxidase subunit 1 (*cox1*) gene was used to understand the phylogenetic relationship of geographically different *P. xylostella* population from Southeast Asia. Extensive sampling was done from different host plant species (broccoli, cabbage, cauliflower, Chinese cabbage, Chinese kale, green mustard, pak-choi, radish, and kohlrabi) in target countries. A total of 52 different populations containing 245 individuals were used in the study. An amplicon of 709 bp was produced by polymerase chain reaction, and editing resulted in a consensus sequence of 643 bp across all *P. xylostella* population. A total of 77 haplotypes were identified in 245 *P. xylostella* individuals. Phylogenetic analysis showed no difference among most of the *P. xylostella* population from different host plants, except few populations from Thailand and Vietnam, which formed a separate cluster. The high F_{ST} values (0.44-0.45) of *P. xylostella* population in Taiwan compared to Cambodia and Malaysia population seem to indicate the presence of genetically diverse populations, which are yet to be confirmed by additional sampling. The negative Tajima's *D* and Fu's F_S values showed the recent demographic expansion of the *P. xylostella* population in Cambodia, Lao PDR and Vietnam. Thus, this study showed the likely presence of genetically diverse *P. xylostella* in Southeast Asia, but it requires additional studies with more populations, especially from Cambodia, Lao PDR, Vietnam, Thailand and Taiwan.

Keywords

Plutella xylostella, mitochondrial cytochrome c oxidase I, phylogeny, population structure

INTRODUCTION

Vegetable brassicas are an important group of vegetables cultivated and consumed in Southeast Asia. Cabbages and other brassicas are cultivated in an area of over 123,000 ha, with an annual production of 2.89 million t in this region (FAO 2019). Cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), broccoli (*B. oleracea* var. *italica*), Chinese cabbage (*Brassica rapa* var. *pekinensis*), pak-choi (*B. rapa* var. *chinensis*), choi sum (*B. rapa* var. *parachinensis*), Chinese kale (*B. oleracea* var. *alboglabra*), green mustard (*Brassica juncea*), radish (*Raphanus sativus*) and kohlrabi (*B. oleracea* var. *gongylodes*) are some of the most commonly cultivated brassica crops in Southeast Asia. These vegetables provide vitamins, minerals, and dietary fiber. They are also high value crops, since they provide quick (short-duration and repeat cycle crops) and higher incomes compared to staple crops.

Insect pests and plant diseases severely limit the productivity of brassicas and stimulate the overuse of pesticides in Southeast Asia. In the specific case of the Diamondback moth (*Plutella xylostella*), this is the dominant pest in brassicas, although *Spodoptera litura, Pieris rapae* and *Phyllotreta striolata* caused marketable yield losses of up to 100% in brassicas in Cambodia, Lao PDR and Vietnam (Ungsa and Vanharn 1995; Nhung et al. 2008; World Bank 2012; Srinivasan et al. 2019). In addition, the productivity of brassicas is comparatively lower in Southeast Asia (23.41 t/ha) than the world (28.68 t/ha) or East Asia

(35.04 t/ha) average (FAO 2019). The productivity significantly varies among the countries within Southeast Asia. For instance, the yield of green mustard is only 5.6 t/ha in Lao PDR, whereas it is 12.4 t/ha in Vietnam and 15 t/ha in Cambodia (Schreinemachers et al. 2017). Hence, the farmers predominantly rely on chemical pesticides to reduce the incidences of pests and diseases and thus increasing the yield and income. Unless these biotic constraints are addressed through a comprehensive integrated pest management (IPM) approach, it will not be possible to significantly reduce overall pesticide use on the brassica crops.

In order to use the most effective pest control options especially bio-control agents and sex pheromone lures, a thorough understanding of the population structure of the target pest is highly imperative. Studies in Australia have revealed the presence of a cryptic species *Plutella australiana*, besides *P. xylostella* (Landry and Hebert 2013; Perry et al. 2018). However, a recent study showed that there was no genetic differentiation among *P. xylostella* populations in Australia irrespective of geographic location, host plant or sampling year, and no evidence for isolation-by-distance (Perry et al. 2020). Little genetic differentiation was found among the *P. xylostella* populations from China and Korea (Li et al. 2006). Similarly, there was no genetic differentiation found among the *P. xylostella* populations and no correlation between genetic and geographical distance was found. However, pairwise analysis of the mitochondrial genes indicated that *P. xylostella* populations from the southern region of China were more differentiated than those from the northern region (Wei et al. 2013). A recent study on spatial genetic structure analysis in China also revealed three genetic clusters of *P. xylostella* in the southern provinces (Chen et al. 2021). However, there was no study assessing the populations of *P. xylostella* in Southeast Asia. Therefore, this study was undertaken to assess the genetic diversity of the *P. xylostella* population in Southeast Asia, especially in Cambodia, Lao PDR, Malaysia, Thailand, Taiwan and Vietnam.

MATERIALS AND METHODS

Insect sampling

For this study, a total of 52 different *P. xylostella* populations from nine host plants (cabbage, cauliflower, broccoli, Chinese cabbage, Chinese kale, green mustard, pak-choi, radish and kohlrabi) in six countries, *viz*., Cambodia (11°50'N 105°01'E), Lao PDR (19°05'N 102°24'E to 19°51'N 102°06'E, and 17º49'N 102º41'E to 18º07'N 102º42'E), Malaysia (5°59'N 116°34'E), Taiwan (23°12N 120°30'E), Thailand (14°01'N 99°57E) and Vietnam (21°07'N 105°49"E to 21°16'N 105°76"E, 20°51'N 109º36"E, 19°09'N 105°43'E, 19°12'N 105°30'E, and 14°50'N 105°49"E) were collected from the field, and preserved in 95% ethanol. For comparison, a population from West Asia (Syria) was also included.

DNA extraction

The whole insect was placed on Whatman filter paper, washed with double distilled water, and allowed to dry for 10 min. A small part of the larva was cut and transferred to eppendorf tubes containing 50 µl UniversAll™ extraction solution. The samples were vortexed for 15 s, incubated in a dry heater for 23-25 min at 98°C. The samples were left to cool down in room temperature and then centrifuged at 3000 rpm. The DNA solution was treated with RNase and stored in aliquots at -20°C.

Polymerase chain reaction (PCR) and DNA sequencing

Cytochrome c oxidase subunit 1 (*cox1*) gene was selected for this study. The universal *cox1* primer pair (HC02198 5Rev'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' and LCO1490: 5'For-GGT CAA CAA ATC ATA AAG ATA TTG G-3') reported by Folmer et al. (1994) was used to amplify the partial sequence of the *cox1* gene of *P. xylostella* larvae. PCR was performed in a total reaction volume of 25 µl containing 2.0 µl 10x PCR buffer, 1.5 µl 0.15 µM dNTPs, 2.0 µl 5 µM of each forward and reverse primer, 0.25 µl 0.015 unit/µl *Taq* polymerase, 16.45 µl of double distilled water (ddH_2O), and 0.8 µl of DNA template. PCR was performed in a MJ Research Thermocycler (PTC200 DNA Engine Cycler, Bio-Rad Laboratories, Inc.) following the profile: 95°C for 10 min followed by 4 cycles of 95°C for 30 s, 50°C for 45 s, 72°C for 1 min, followed by 30 cycles of 95°C for 30 s, 65°C for 45 s, 72°C for 45 s with the final extension at 72°C for 8 min. After amplification, 5 µl of the PCR product of each sample was analyzed by electrophoresis on 1.5% agarose gel containing ethidium bromide. Bands were revealed and photographed under ultraviolet light. After electrophoresis, the remaining PCR products were used for sequencing with the previously mentioned forward and reverse primers, using ABI 3730XL systems at Genomics Bioscience and Technology Company Limited, Taiwan.

Molecular divergence and population genetic analyses

The *cox1* sequences were aligned and edited by using BioEdit version 7.0 (Hall 1999). The obtained sequences were aligned with mitochondrion genome reference sequences from National Center for Biotechnology Information (NCBI) GenBank (GenBank accession number NC_025322.1) to confirm that the amplified gene region is located in the

mitochondria only. The sequences were also examined for polymorphism among the *P. xylostella* population collected from different locations or host plants. Reference sequences were obtained from the NCBI GenBank database. The number of variable nucleotide sites, number of haplotypes, nucleotide diversity and haplotype diversity were calculated for investigating the *cox1* sequence diversity using DnaSP 5.10 software (Librado and Rozas 2009). Statistical tests of Tajima's *D* and Fu's F_S values were used to detect the deviation from the neutral model of evolution using DnaSP 5.10. Pairwise F_{ST} values used to appraise the genetic structure among population were obtained with 1000 permutations and at the significance level of 0.05 using the Kimura 2-parameter (K2P) model (Kimura 1980).

Phylogenetic analysis

The FASTA formatted *cox1* sequences were imported into MEGA X software package sequence alignment application and a multiple sequence alignment was performed with ClustalW algorithm using default parameters (Tamura et al. 2011). The insects that showed 100% nucleotide similarities were designated as a single *cox1* haplotype and the others showing different sequence polymorphism were designated as different *cox1* haplotype. *cox1* sequence of *P. xylostella* from China obtained from NCBI GenBank was used as the reference sequence. The aligned sequences were used for phylogenetic analysis. Maximum Likelihood (ML) phylogenetic analysis was used to identify major clades and to evaluate the relationship among the haplotypes of the *P. xylostella cox1* sequences. The appropriate model of sequence evolution, including model parameters, was calculated using corrected Akaike Information Criterion (AICc value) with MEGA X, and resulted in T92+G+I as the best model (Tamura et al. 2011). The model was also selected based on partitioning by codon position. With those settings, a heuristic search was performed (nearest neighbor interchange algorithm starting tree obtained via neighbor joining). Non-uniformity of evolutionary rates among sites was modeled by using a discrete Gamma distribution (+G) with 5 rate categories and by assuming that a certain fraction of sites are evolutionarily invariable (+I). Whenever applicable, estimates of gamma shape parameters were included. The clustering probabilities of each resulting phylogenetic tree node were statistically tested by a bootstrap method consisting of 1000 replicates. The tree was rooted by the outgroup *Plutella australiana* (KF370868).

RESULTS AND DISCUSSION

The universal *cox1* primer pair (HC02198 and LCO1490) successfully amplified PCR products of 709 bp size in different *P. xylostella* population. Although the sequence alignment and editing resulted in a consensus sequence of 643 bp across all *P. xylostella* population, we used 632 bp consensus sequences to include the reference sequence in this study.

A total of 77 *cox1* haplotypes were identified in 245 *P. xylostella* individuals (Table 1). The largest haplotype (Haplotype 9) contained 44 *P. xylostella* individuals, which were collected from Cambodia, Lao PDR, Vietnam and Syria. Haplotype 6 with 43 *P. xylostella* individuals from Cambodia, Lao PDR, Malaysia, Vietnam and Syria formed the second largest haplotype group. Haplotype 13 contained 20 *P. xylostella* from Lao PDR and Vietnam. A total of 42 haplotypes occurred within the populations from Vietnam, followed by 37 in Lao PDR, 8 in Cambodia and 4 each in Thailand and Malaysia.

Table 1. List of identified *Plutella xylostella* haplotypes with their geographical origin

Country	No. of samples	No. of haplotypes	Haplotypes
Cambodia	31	8	H6, H9, H44 to H49
Lao PDR	84	37	H1, H6, H9 to H43
Malaysia	10	4	H3, H4, H5, H6
Taiwan	5	2	H1, H2
Thailand	13	4	H1, H6, H7, H8
Vietnam	99	42	H1, H2, H6, H9, H11, H13, H14, H16, H18, H21, H25, H32, H37, H43, H46, H50 to H76
Syria	3	3	H6, H9, H77
All countries	245	77	

Nucleotide diversity is used to measure the degree of polymorphism within a population (Nei and Li 1979). The nucleotide diversity of *P. xylostella* population from Syria was the lowest (0.00181) with Malaysia being the highest (0.00995), followed by Thailand, Vietnam, Lao PDR, Taiwan and Cambodia (Table 2). The total nucleotide diversity of

all *P. xylostella* population from sampled countries was 0.00637. The haplotype diversity is a measure of the uniqueness of a particular haplotype in a given population (Nei and Tajima 1981). The haplotype diversity value was lowest in Taiwan, followed by Cambodia; it was highest in Lao PDR, followed by Vietnam. The total haplotype diversity value of all *P. xylostella* population from sampled countries was 0.926 (Table 2).

Tajima's *D* test showed positive values but not significant for Syria, Thailand and Taiwan populations (Table 2). However, Tajima's *D* test showed negative values for all other samples including the overall population. The negative Tajima's *D* values indicated that the *P. xylostella* population in Cambodia, Lao PDR, Malaysia and Vietnam began to expand recently, and they provide evidence for purifying selection at this locus. However, positive Tajima's *D* value for Syria, Thailand and Taiwan indicated that the *P. xylostella* population may have suffered a recent sharp decline in its size (bottleneck). Besides Tajima's *D*, a significant negative value of Fu's F_S especially for Cambodia, Lao PDR, Syria and Vietnam population is evidence for a possible recent population expansion or genetic drift due to random sampling. A positive value of Fu's F_S for Taiwan and Thailand population is evidence for the deficiency of alleles due to a recent population decrease.

Table 2. List of number of samples studied, number of haplotypes, haplotype diversity (*h*), nucleotide diversity (π), Tajima's *D* and Fu's F_S tests for *Plutella xylostella* populations from 7 countries in Southeast Asia, and West Asia

Country	Haplotype diversity (h)	Nucleotide diversity (π)	Tajima's *D*	Fu's F_S
Cambodia	0.686	0.00256	-1.87799*	-5.239**
Lao PDR	0.946	0.00604	-1.16204	-25.774**
Malaysia	0.889	0.00995	-0.45258	0.466
Thailand	0.744	0.00817	0.08186	4.149
Vietnam	0.914	0.00721	-0.97574	-25.584**
Taiwan	0.600	0.00373	1.64070	3.022
Syria	0.833	0.00181	0.00000	-1.216*
All countries	0.926	0.00637	-1.55611	-32.696

The F_{ST} values of all population pairwise comparisons were ranged from -0.07 to 0.45 (Table 3). Negative F_{ST} values can be interpreted as no genetic differences between the two populations compared, due to imprecision of the algorithm used (Jaramillo et al. 2001). The genetic difference of Taiwan population from Cambodia or Malaysia population was highly significant based on pairwise F_{ST} values (0.44–0.45; $p<0.01$). Surprisingly, Syrian *P. xylostella* population has little genetic differentiation from Southeast Asian population, except Malaysia and Taiwan.

The intraspecific phylogenetic relationships based on the *cox1* sequences of *P. xylostella* are shown in Fig 1. Phylogenetic analysis based on partial *cox1* sequences was used to classify *P. xylostella* collected from different crops in different locations of selected countries in Southeast Asia. According to the maximum likelihood phylogenetic tree in the current study, there was no difference among most of the *P. xylostella* population from different host plants, except a few populations from Thailand and Vietnam, which formed a separate cluster. Population genetic structure of *P. xylostella* has been assessed in various countries including Australia, China, Korea and India. Another study assessed genetic differentiation among 14 *P. xylostella* populations from the USA, Brazil, Japan, the Philippines, Uzbekistan, France, Benin, South Africa, Reunion Island, and Australia (Pichon et al. 2006).

Table 3. Pairwise F_{ST} values (below diagonal) and the statistical significance (above diagonal) comparing populations of *Plutella xylostella* based on *coxI*

Population	Cambodia	Lao PDR	Malaysia	Thailand	Vietnam	Taiwan	Syria
Cambodia	0.000	**	**	**	**	**	ns
Lao PDR	0.093	0.000	**	**	*	ns	ns
Malaysia	0.222	0.190	0.000	**	**	**	*
Thailand	0.358	0.148	0.257	0.000	**	ns	ns
Vietnam	0.085	0.010	0.145	0.106	0.000	**	ns
Taiwan	0.445	0.173	0.448	0.013	0.147	0.000	*
Syria	-0.021	-0.070	0.277	0.135	-0.067	0.310	0.000

However, there was no study assessing the populations of *P. xylostella* in Southeast Asia, especially in Cambodia, Lao PDR, Malaysia, Thailand, Taiwan and Vietnam. Hence, the current study was conducted using *P. xylostella* populations from these countries. Our results from phylogenetic analysis showed that the few *P. xylostella* populations from Thailand and Vietnam formed a separate clade in the ML phylogenetic tree. Otherwise, the majority of the populations formed a single clade, without showing significant genetic variations. Earlier results from Australia also showed that there was no genetic differentiation among *P. xylostella* populations irrespective of geographic location or distance, host plant or sampling year (Perry et al. 2020), although a cryptic species *P. australiana*, was known to occur in Australia (Landry and Hebert 2013; Perry et al. 2018). Similarly, little genetic differentiation was found among the *P. xylostella* populations from China and Korea (Li et al. 2006; Wei et al. 2013). Another study from India, which involved *P. xylostella* populations from 13 provinces demonstrated that all the populations were highly interrelated based on *cox1* gene (Ojha et al. 2016). However, a recent study on spatial genetic structure analysis revealed three genetic clusters of *P. xylostella* in the southern provinces of China (Chen et al. 2021).

Based on Random Amplified Polymorphic DNA (RAPD) markers, considerable genetic variation was found among *P. xylostella* populations from hilly regions of Himachal Pradesh and the Indo-Gangetic plains in India (Arvind Kumar et al. 2018). Therefore, the *P. xylostella* populations from Thailand and Vietnam that showed some genetic variations should be studied further to understand if the degree of differentiation varies within a population of *P. xylostella* due to migrations or reduction of size due to environmental conditions.

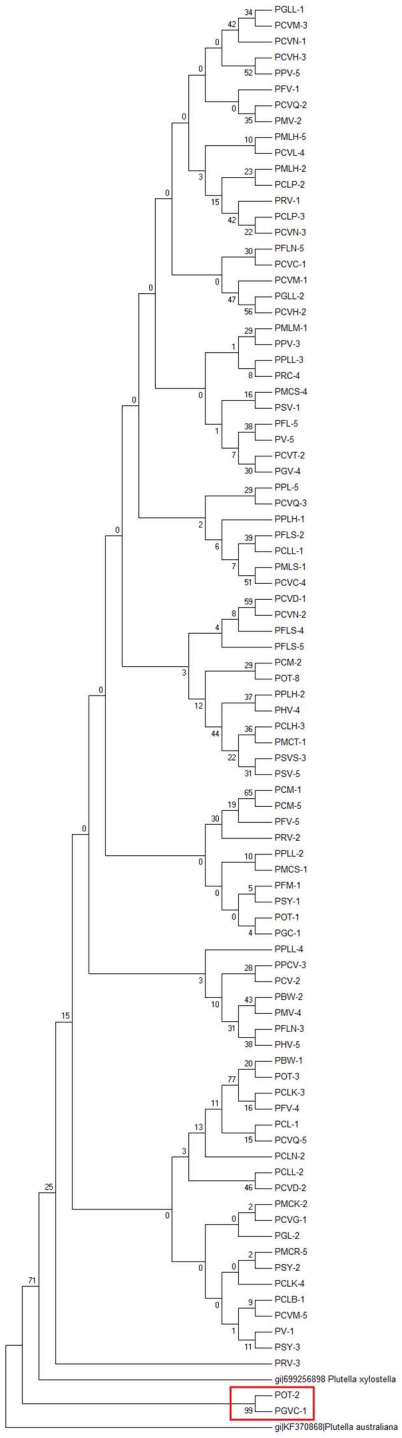


Fig. 1. Phylogenetic relationship among *Plutella xylostella* populations based upon a 643 bp mitochondrial *coxI* gene fragments using maximum likelihood (ML) algorithm

In our study, the *P. xylostella* population may have experienced a recent population expansion, indicated by the negative Tajima's *D* and Fu's F_S values for Cambodia, Lao PDR and Vietnam populations. Negative values of Tajima's *D* are associated with selective sweeps or population expansion after a recent sharp decline (Tajima 1989). Similarly, negative values of Fu's F_S are usually caused by an excess of singletons in a population expansion event (Fu 1995; Fu 1997). In consequence, *P. xylostella* population in Cambodia, Lao PDR and Vietnam could have experienced recent demographic expansion events. The statistically non-significant numbers indicating recent population growth could have been confined mostly by local geographical regions, except in Cambodia (Liao et al. 2010). It should be noted that even subspecies could be sharply genetically differentiated using genetic differentiation (F_{ST}) values. The pair-wise estimates of F_{ST} among subspecies of beach mice was found to be 0.23-0.63 (Mullen et al. 2009). Putative subspecies of *M. vitrata*, which cannot be differentiated based on morphological characters in Asia and sub-Saharan Africa was indicated by the high pairwise F_{ST} values of 0.44–0.85 (Malini et al. 2015). Hence, a pairwise F_{ST} value of 0.44–0.45 among the Taiwan, Cambodia and Malaysia populations of *P. xylostella* in the current study should be carefully considered for additional sampling and further analysis to determine if there are genetically distinct *P. xylostella* populations are existing in these countries, since the pest management strategies should be precisely developed and adopted according to the genetic differences of the pest. It should be noted that pairwise analysis of the mitochondrial genes indicated that *P. xylostella* populations from the southern region of China were more differentiated than those from the northern region (Wei et al. 2013). Consequently, it is possible to expect genetically distinct *P. xylostella* populations within Southeast Asia.

In conclusion, this study confirmed the presence of genetically distinct *P. xylostella* in Southeast Asia, but it requires additional studies with more populations, especially from Cambodia, Lao PDR, Vietnam, Thailand and Taiwan. Therefore, the genetic differences in *P. xylostella* population should be carefully considered while designing the pest management strategies in different geographical regions.

Acknowledgements

This work was supported by the Federal Ministry for Economic Cooperation and Development (GIZ Project number 13.1432.7-001.00), Germany.

References

Arvind K, Rana RS, Sharma KC, Chandel VGS, Kaur M, Sharma S. 2018. Genetic diversity of diamondback moth, *Plutella xylostella* populations from different host plants and across locations in North India. Journal of Entomology and Zoology Studies. 6(1): 1482-1486.

Chen MZ, Cao LJ, Li BY, Chen JC, Gong YJ, Yang Q, Schmidt TL, Yue L, Zhu JY, Li H, Chen XX, Hoffmann AA, Wei SJ. 2021. Migration trajectories of the diamondback moth, *Plutella xylostella* in China inferred from population genomic variation. *Pest Management Science* 77(4): 1683–1693.

FAO. 2019. FAOSTAT – crops and livestock products. http://www.fao.org/faostat/en/#data/QCL

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol*. 3: 294–299.

Fu YX. 1995. Statistical properties of segregating sites. Theor Popul Biol. 48: 172–197.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 147: 915–925.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*. 41: 95–98.

Jaramillo C, Montaña M, Castro L, Vallejo G, Guhl F. 2001. Differentiation and genetic analysis of *Rhodnius prolixus* and *Rhodnius colombiensis* by rDNA and RAPD amplifications. Memorias Instituto Oswaldo Cruz. 96(8): 1043–1048.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*. 16: 111–120.

Landry JF, Hebert PDN. 2013. *Plutella australiana* (Lepidoptera, Plutellidae), an overlooked diamondback moth revealed by DNA barcodes. *Zookeys*. 327: 43–63.

Li J, Zhao F, Soo CY, Kim I, Sohn HD, Jin BR. 2006. Genetic variation in the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) in China inferred from mitochondrial *COI* gene sequence. *Eur. J. Entomol.* 103(3): 605-611.

Liao PC, Kuo DC, Lin CC, Ho KC, Lin TP, Hwang SY. 2010. Historical spatial range expansion and a very recent bottleneck of *Cinnamomum kanehirae* Hay. (Lauraceae) in Taiwan inferred from nuclear genes. BMC Evol Biol. 10: 124.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25: 1451–1452.

Malini P, Schafleitner R, Muthukalingan K, Ramasamy S. 2015. Phylogeographical structure in mitochondrial DNA of legume pod borer (*Maruca vitrata*) population in tropical Asia and Sub-Saharan Africa. PLoS ONE. 10(4): e0124057.

Mullen LM, Vignieri SN, Gore JA, Hoekstra HE. 2009. Adaptive basis of geographic variation: genetic, phenotypic and environmental differences among beach mouse populations. Proc. R. Soc. B. 276: 3809–3818.

Nei M, Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *PNAS*. 76(10): 5269–5273.

Nei M, Tajima F. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics*. 97(1): 145–163.

Nhung NT, Viet TQ, Nghiep NT. 2008. Insect pests on Crucifers and their control by chemicals at Vo cuong, Bac Ninh Province, *Proceeding of the 6th Vietnam National Conference on Entomology, May -10, 2008*. Agricultural Publishing House, Hanoi, Vietnam.

Ojha R, Jalali SK, Poorani J, Srinivasa Murthy K. 2016. Genetic variation among different Indian populations of cabbage diamondback moth (*Plutella xylostella*; Lepidoptera: Plutellidae) based on mitochondrial DNA. Molecular Entomology. 7(2): 1-7.

Perry KD, Baker GJ, Powis KJ, Kent JK, Ward CM, Baxter SW. 2018. Cryptic *Plutella* species show deep divergence despite the capacity to hybridize. *BMC Evol. Biol.* 18, 77.

Perry KD, Keller MA, Baxter SW. 2020. Genome-wide analysis of diamondback moth, *Plutella xylostella* L., from *Brassica* crops and wild host plants reveals no genetic structure in Australia. *Sci Rep*. 10: 12047.

Pichon A, Arvanitakis L, Roux O, Kirk AA, Alauzet C, Bordat D, Legal L. 2006. Genetic differentiation among various populations of the diamondback moth, *Plutella xylostella* (Lepidoptera Yponomeutidae). Bull Entomol Res. 96(2): 137-144.

Schreinemachers P, Chen HP, Nguyen TTL, Buntong B, Bouapaoe L, Gautam S, Le NT, Pinnh T, Vilaysone P, Srinivasan R. 2017. Too much to handle? Pesticide dependence of smallholder vegetable farmers in Southeast Asia. Sci. Total Env. 593-594, 470–477.

Srinivasan R, Bhanu KRM, Lin MY, Yule S, Su FC, Huang CC, Khumsuwan C, Heng CH, Sarika S, Hai VM, Hien NTT, Khanh LD, Trang VTT, Diep NX, Phimchai V, Chansamone P, Soukhavong K. 2019. Evaluation of novel pheromone lures against striped flea beetles (*Phyllotreta striolata* Fab.) on brassicas and bean pod borer (*Maruca vitrata* Fab.) on yard-long bean in Southeast Asia. *Acta Horticulturae*. 1257: 37–46.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 123: 585–595.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 28: 2731–2739.

Ungsa M, Vanharn H. 1995. Vegetable Production, Research and Policy in Cambodia. In: AVRDC.1995. Vegetable research and development in Cambodia, Lao PDR and Vietnam; Proceedings of a workshop. Asian Vegetable Research and Development Center. Shanhua, Tainan, Taiwan. Publication no. 94-431 .130 p.

Wei SJ, Shi BC, Gong YJ, Jin GH, Chen XX, Meng XF. 2013. Genetic structure and demographic history reveal migration of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) from the southern to northern regions of China. *PLoS ONE* 8(4): e59654.

World Bank. 2012. Annex L: Pest Management Plan, p. L1-L18. http://siteresources.worldbank.org/INTLAOPRD/Resources/annex-l.pdf

SESSION 7
At the Farm and Landscape Level: Barriers to and Innovations for Management of Crucifer Pests

Development and validation of an integrated pest management strategy for the control of major insect pests on pak-choi in Cambodia

Srinivasan, R.
WORLD VEGETABLE CENTER
SHANHUA, TAINAN, TAIWAN
email: srini.ramasamy@worldveg.org

Sotelo-Cardona, P.
WORLD VEGETABLE CENTER
SHANHUA, TAINAN, TAIWAN
email: paola.sotelo@worldveg.org

Lin, M.Y.
WORLD VEGETABLE CENTER
SHANHUA, TAINAN, TAIWAN
email: mei-ying.lin@worldveg.org

Heng, C.H.
DEPARTMENT OF PLANT PROTECTION SANITARY AND PHYTOSANITARY
GENERAL DIRECTORATE OF AGRICULTURE
PHNOM PENH, CAMBODIA
email: chhunhyheng@gmail.com

Kang, S.
DEPARTMENT OF PLANT PROTECTION SANITARY AND PHYTOSANITARY
GENERAL DIRECTORATE OF AGRICULTURE
PHNOM PENH, CAMBODIA
email: kangsareth_bsc@yahoo.com

Sarika, S.
DEPARTMENT OF PLANT PROTECTION SANITARY AND PHYTOSANITARY
GENERAL DIRECTORATE OF AGRICULTURE
PHNOM PENH, CAMBODIA.
email: sorsarika@yahoo.com

ABSTRACT
Pak-choi is one the most important leafy vegetables, which is extensively grown and consumed in Cambodia. Insect pests, especially lepidopteran caterpillars and the flea beetles are one of the major production constraints in pak-choi. This has forced the vegetable farmers to heavily rely on calendar-based application of chemical pesticides. In order to develop an effective alternative to harmful pesticides, we evaluated the effectiveness of microbial pesticides (*Bacillus thuringiensis* and *Metarhizium anisopliae* formulations), and neem leaf extract alone and in combination (as an IPM package) against diamondback moth, common armyworm, cabbage webworm and striped flea beetle on pak-choi in three different provinces of Cambodia during 2015-early 2018. *Bacillus thuringiensis* and *M. anisopliae* formulations reduced the incidence of diamondback moth, common armyworm, cabbage webworm and the damage by striped flea beetle to the levels equivalent to chemical pesticide (abamectin). Yield was significantly higher in bio-pesticide treated plots than untreated plots in most of the trials. The performance of the IPM package was on par with Farmers' practice (calendar-based application of chemical pesticides) in reducing the damage by target pests, leading to significantly higher yield. Hence, the IPM package can be piloted and scaled out as an effective alternative to chemical pesticides to manage the insect pests on pak-choi in Cambodia.

Keywords
IPM, diamondback moth, common armyworm, cabbage webworm, striped flea beetle

INTRODUCTION
Pak-choi (*Brassica rapa* var. *chinensis*) is among the most important vegetables grown and consumed in Cambodia (Genova et al. 2010). Brassica vegetables including pak-choi are excellent sources of calcium, fiber, vitamin A (in the form of β-carotene) or pro-vitamin A, and vitamin C (Fahey 2016). Leafy brassicas are high value, short-duration and repeat-cycle crops that could lift small-scale farmers out of poverty. Since leafy vegetables account for 64% of the total vegetable area in Cambodia, their contribution to overall livelihoods is significant (Genova et al. 2010). However, their productivity is limited, mainly due to insect pests and plant diseases. Besides diamondback moth (*Plutella xylostella*), which is the dominant pest of brassicas, common armyworm (*Spodoptera litura*) and striped flea beetle (*Phyllotreta striolata*) can cause up to 100% yield losses in leafy brassicas (Srinivasan et al. 2019).

Vegetable farmers in Cambodia rely heavily on indiscriminate, repeated application of chemical pesticides to prevent the damages by pests and diseases. About 94% of the farmers in Cambodia solely rely on chemical pesticides, and the use of bio-pesticides in vegetable brassicas is almost nil (Schreinemachers et al. 2017). At least 71% of Cambodian spray applicators mixed different pesticides together in a single spray (Schreinemachers et al. 2017). Such a misuse and overuse of pesticides raises concerns for human and environmental health. In addition, development of resistance to pesticides and the pesticide residues in the harvested produce add additional dimensions to pesticide misuse. For instance, detectable levels of organophosphate and carbamate pesticides were reported in vegetable brassicas sampled from Cambodian markets (Neufeld et al. 2010). Hence, there is an urgent need to develop alternative options to reduce the use of pesticide in production of vegetable brassicas.

Bio-pesticides are an important component in integrated pest management (IPM) approaches, which can reduce the reliance on chemical pesticides. Very few studies have been carried out to assess the impact of bio-pesticides on insect pests of brassicas in Cambodia (Srinivasan et al. 2020). Bio-pesticides such as *Bacillus thuringiensis*, *Metarhizium anisopliae* and neem extract were shown to reduce the incidence of diamondback moth, common armyworm, cabbage webworm and the damage by striped flea beetle to levels equivalent to chemical pesticide (abamectin) on Chinese mustard in Cambodia (Srinivasan et al. 2020). A bio-based IPM package was demonstrated to be on par with farmers' practice of calendar-based pesticide application in reducing the damage by key pests of Chinese mustard, leading to significant yield gains in farmer participatory trials. Hence, the current study was carried out to evaluate different microbial pesticides and neem either alone or in combination (sequential applications) against major insect pests on pak-choi in different provinces of Cambodia.

MATERIALS AND METHODS

Study sites

Field studies were conducted in four Cambodian provinces (Kandal, Kampong Chhnang, Svay Rieng and Prey Veng) (Table 1) to evaluate the efficacy of individual bio-pesticides during July-September in 2015 and February-April in 2016 on pak-choi.

Table 1. Provinces and experimental locations for bio-pesticide evaluation in pak-choi during 2015-2016 in Cambodia

Province	Experimental site
Kandal	Kbal Koh Vegetable Research Station, Kbal Koh Commune, Kien Svay District
	Praek Thmey Village, Praek Thmey Commune, Koh Thum District
Kampong Chhnang	Sdork Reach Village, Andoung Snay Commune, Rolea B'ier District
	Chrey Bak Village, Chrey Bak Commune, Rolea B'ier District
Svay Rieng	Khuoch Village, Krol Kor Commune, Svay Chrum District
Prey Veng	Sdao Village, Krang Svay Commune, Preah Sdach District

Subsequently, field trials were conducted in three provinces (Kandal, Kampong Chhnang and Prey Veng) (Table 2) during October-December in 2016 and October 2017-January 2018 to evaluate the efficacy of an IPM package based on bio-pesticides in comparison with Farmers' practices, which is mainly based on calendar-based spraying of chemical pesticides.

Table 2. Provinces and experimental locations for comparing IPM vs. Farmers' practices in pak-choi in Cambodia during 2016-2018

Province	Experimental site
Kandal	Kandal Village, Banteay Daek Commune, Kien Svay District
	Praek Thmey Village, Praek Thmey Commune, Koh Thum District
Kampong Chhnang	Preal Village, Banteay Preal Commune, Rolea B'ier District
	Andong Preng Village, Krang Leav Commune, Rolea B'ier District
Prey Veng	Sdao Village, Krang Svay Commune, Preah Sdach District
	Porlos Vegetable Research Station, Preah Sdach District

Treatment and data collection

Bio-pesticide trials

Field trials were conducted during 2015 and 2016 to evaluate the efficacy of *Bacillus thuringiensis* and *Metarhizium anisopliae* formulations, and neem leaf extract against *P. xylostella*, *S. litura* and *P. striolata* on pak-choi. Seven treatments, *viz*., four bio-pesticide formulations [Xentari® (*B. thuringiensis* subsp. *aizawai*), Crymax® and E-911® (*B. thuringiensis* subsp. *kurstaki*), Real M-62® (*M. anisopliae*)], neem leaf extract, abamectin (chemical pesticide, a "positive" control), and an untreated check were used in each trial during 2015. The 2016 trials included six treatments, Crymax® was dropped. There were three replications for each treatment, and each replication was imposed on a 2 to 5 m^2 plot (depending on the farm size in the farmers' fields or in the research station) following a randomized complete block design (RCBD) with a 1 m distance between plots. The crop was monitored for damage by target pests, and the bio-pesticide treatments were initiated from one to three weeks after planting depending on the pest incidence and continued at weekly intervals. The number of larvae of *P. xylostella* and *S. litura* on five randomly selected plants in each replicate plot were counted, whereas the number of 'shot-holes' in a 4 cm^2 area in two younger leaves from each plant were counted on five randomly selected plants in each replication for *P. striolata* damage. Marketable yield was recorded during harvest.

IPM trials

Two field trials in each province were conducted during 2016 to early 2018 to evaluate the efficacy of an IPM package against *P. xylostella, S. litura*, *H. undalis* and *P. striolata* on pak-choi. The IPM package consisted of the sequential application of bio-pesticides and the chemical pesticide. The spraying order was designed based on the incidence of target pests in a given season and the location. Three treatments, *viz*., IPM package, Farmers' practice (alternate spraying of abamectin and cypermethrin) and an untreated control were used in all the trials, with six replications for each treatment, following RCBD. The individual replication size was 2.5-3.75 m^2. The crop was monitored for damage by target pests, and the treatments were initiated from one to three weeks after planting depending on the pest incidence and continued at weekly intervals until a week before the harvest. The data collection was similar to the bio-pesticide trials.

Data analysis

The data were averaged for each plot and analyzed using a combined analysis approach of several experiments (Petersen 1994; Moore and Dixon 2015). Preliminary analysis of variance was completed for each individual analysis (each location in each season), experimental errors were examined for heterogeneity and Shapiro-Wilkinson test for normality was performed in each individual analysis. The data was then analyzed using analysis of variance (ANOVA) with the Proc GLM MIXED of SAS, version 9.1 (SAS Institute, Cary, NC, USA). Each year/province/experimental site was considered a particular environment for the combined analysis. Random effects were considered for years and locations whereas treatments were fixed effects. When significant treatment differences were identified, means were separated by Tukey's HSD Test (SAS) (differences were considered significant at $\alpha = 0.05$). Data on pest incidence and *P. striolata* damage were arcsine transformed. Non-transformed data are presented in the results section.

RESULTS

Bio-pesticide trials on pak-choi (2015)

Interaction effects (Treatment*Location) showed significant difference for *P. xylostella* incidence and *P. striolata* shot-hole damage (Table 3). The *P. xylostella* population was significantly reduced by *B. thuringiensis* and neem extract, which were on par with abamectin and followed by Real M-62® in Kandal (Figure 1). In Kampong Chhnang, Xentari®, E-911® and Real M-62® recorded the lowest infestation of *P. xylostella.* Real M-62® led to significant reduction of *P. xylostella*, which was followed by *B. thuringiensis* formulations in Svay Rieng. The *P. striolata* damage (Figure 2) was significantly reduced by the E-911® treatment in Svay Rieng, which was on par with abamectin, and the bio-pesticide effects in general across locations were not so obvious in 2015 trials.

All the bio-pesticides reduced the *S. litura* incidence to significantly lower levels compared to untreated control plots (Table 4). The yield was affected by location and treatments, but not their interaction. Average yield in Svay Rieng was significantly higher (23.94 ± 3.79 t/ha), compared to Kampong Chhnang (18.64 ± 2.31 t/ha) and Kandal (16.19 ± 3.54 t/ha). Regarding the effect of treatment, the yield was significantly higher in E-911®, and Real M-62® treatments, compared to untreated control plots (Table 4). The other treatments recorded an intermediate yield and were similar to either of the extremes.

Table 3. Analyses for incidence of *P. xylostella* and *S. litura*, damage of *P. striolata* (shot-holes / 4 cm^2) and pak-choi marketable yield for target provinces in Cambodia during 2015

Source		Location	Treatment	Treatment* Location
DF		2	6	12
No. of *P. xylostella* larvae/plant	F value	53.25	38.94	5.60
	Pr>F	0.0002	<.0001	*<.0001*
No. of *S. litura* larvae/plant	F value	0.72	3.69	0.92
	Pr>F	0.526	*0.006*	0.539
No. of shot-holes/4 cm^2	F value	6.11	9.34	2.13
	Pr>F	0.036	<.0001	*0.040*
Marketable yield (t/ha)	F value	13.94	3.13	0.64
	Pr>F	*0.006*	*0.014*	0.790

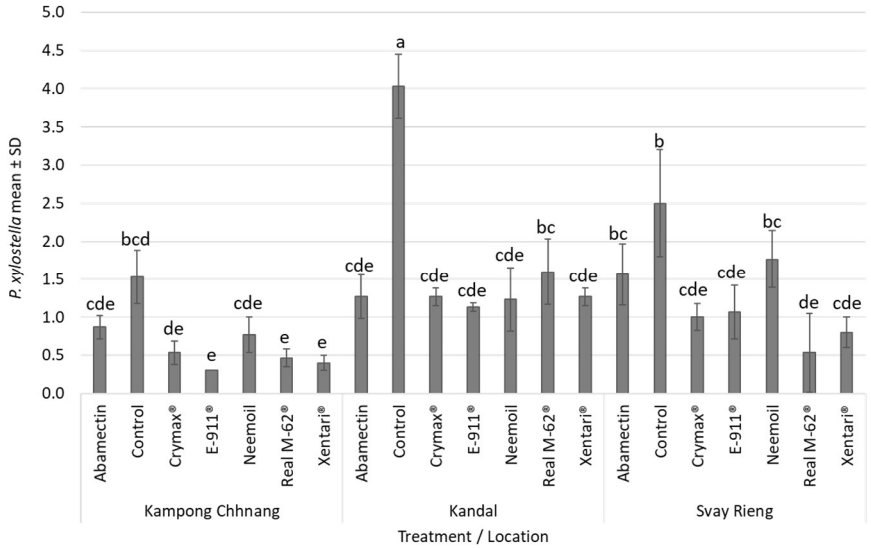


Figure 1. Mean (±SD) number of *P. xylostella* population on pak-choi in three provinces in Cambodia during 2015. Significant differences are presented for the interaction Locations*treatment. Treatments with the same letter(s) did not differ statistically across locations.

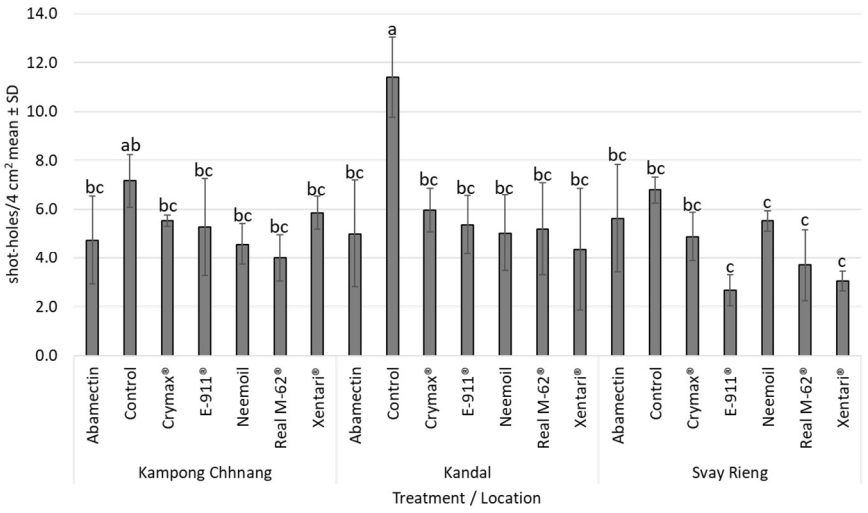


Figure 2. Mean (±SD) number of shot-holes/4 cm^2 caused by *P. striolata* on pak-choi in three provinces in Cambodia during 2015. Significant differences are presented for the interaction Locations*treatment. Treatments with the same letter(s) did not differ statistically across locations.

Table 4. Mean (±SD) number of *S. litura* and pak-choi marketable yield in Cambodia during 2015

Treatment	No. of *S. litura* larvae/plant	Marketable yield (t/ha)
Xentari®	0.24 (0.16) b	20.37 (3.95) ab
Crymax®	0.19 (0.15) b	19.01 (4.08) ab
E-911®	0.31 (0.23) b	21.06 (3.77) a
Real M-62®	0.31 (0.18) b	20.93 (4.97) a
Neem oil	0.28 (0.20) b	20.41 (5.74) ab
Abamectin	0.30 (0.21) b	19.09 (5.03) ab
Control	0.63 (0.38) a	16.28 (3.83) b

Means followed by the same letter(s) in a column are not significantly different ($p<0.05$) by Tukey's HSD

Bio-pesticide trials on pak-choi (2016)

Since the analyses for each variable (except 'shot-holes' by *P. striolata* beetles) showed significant differences among the treatments across the target provinces in 2016 (Table 5), the treatment effects in each province have been presented separately. In Kandal, E-911® treated plots recorded significantly lower *P. xylostella* larvae, followed by Real M-62® and Xentari® treatments (Figure 3). However, the treatment effects in reducing the *P. xylostella* population were not so obvious in Kampong Chhnang. In Prey Veng, Real M-62® and Xentari® treated plots recorded significantly lower *P. xylostella* population, which were followed by E-911® treated plots. *H. undalis* was absent in Prey Veng, and negligible in Kandal (Figure 3). Hence, the low incidence of *H. undalis* in some of the bio-pesticide treated plots cannot be attributed to treatment effects (Figure 4). However, *B. thuringiensis* treatments recorded significantly lower *H. undalis* larvae which were on par with abamectin treated plots in Kampong Chhnang. The shot-hole damage caused by *P. striolata* beetles was significantly higher in Kandal compared to Svay Rieng. In terms of bio-pesticide treatment, damage was lower in Real M-62® treated plots, compared to untreated control plots, whereas the other treatments recorded an intermediate damage and similar to either of the extremes (Figure 5). Significantly higher yields were recorded in E-911® and neem leaf extract treated plots in Kandal. Lower yields were recorded in the untreated control and in E-911® in Kampong Chhnang, whereas the other treatments recorded an intermediate yield (Figure 6).

Table 5. Analyses for incidences of *P. xylostella* and *H. undalis*, damage of *P. striolata* (shot-holes / 4 cm^2) and pak-choi marketable yield for each target province in Cambodia during 2016

Source		Location	Treatment	Treatment* Location
DF		2	5	10
No. of *P. xylostella* larvae/plant	F value	4.66	16.59	3.24
	Pr>F	0.060	<.0001	*0.006*
No. of *H. undalis* larvae/plant	F value	72.05	1.76	2.83
	Pr>F	<.0001	0.152	*0.013*
No. of shot-holes/4 cm²	F value	68.54	2.71	1.23
	Pr>F	*<.0001*	*0.039*	0.315
Marketable yield (t/ha)	F value	10.10	2.30	2.20
	Pr>F	0.012	0.070	*0.047*

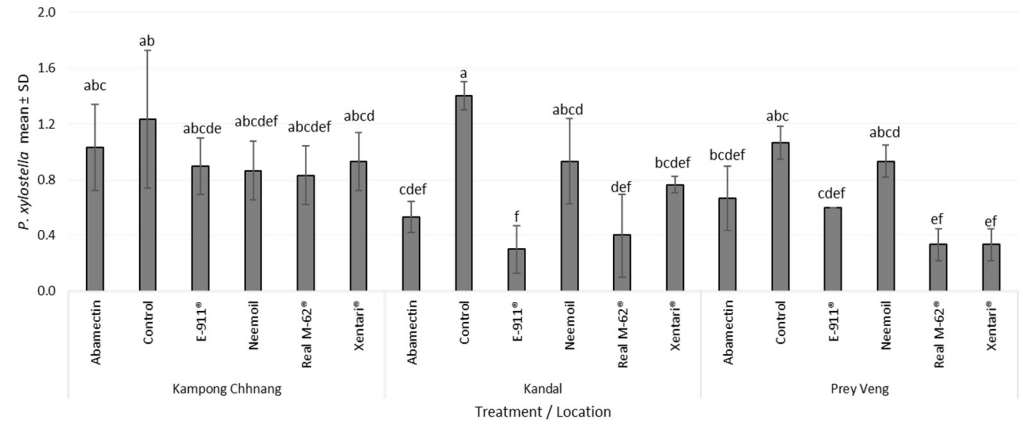


Figure 3. Mean (±SD) number of *P. xylostella* on pak-choi in three provinces in Cambodia during 2016. Significant differences are presented in the locations, where treatments differed statistically. Treatments with the same letter(s) did not differ statistically across locations.

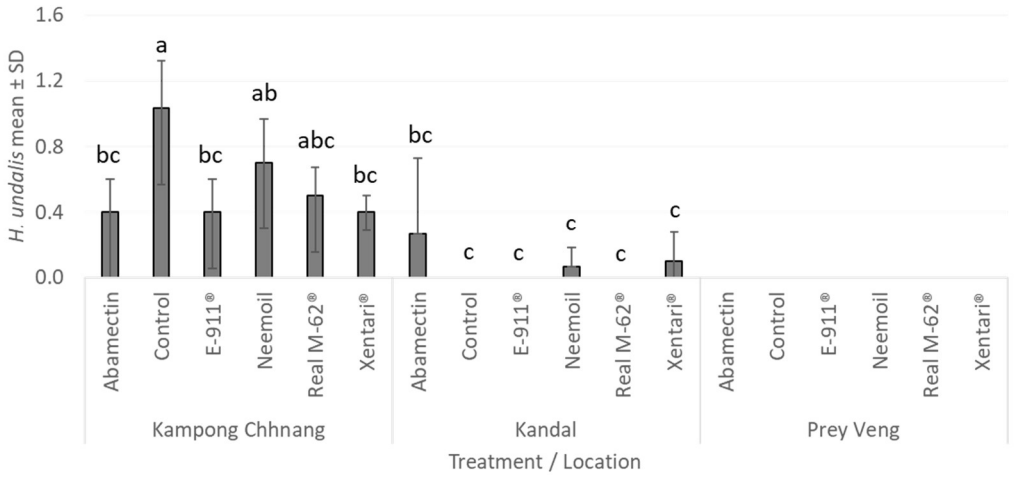


Figure 4. Mean (±SD) number of *H. undalis* on pak-choi in three provinces in Cambodia during 2016. Significant differences are presented in the locations, where treatments differed statistically. Treatments with the same letter(s) did not differ statistically across locations.

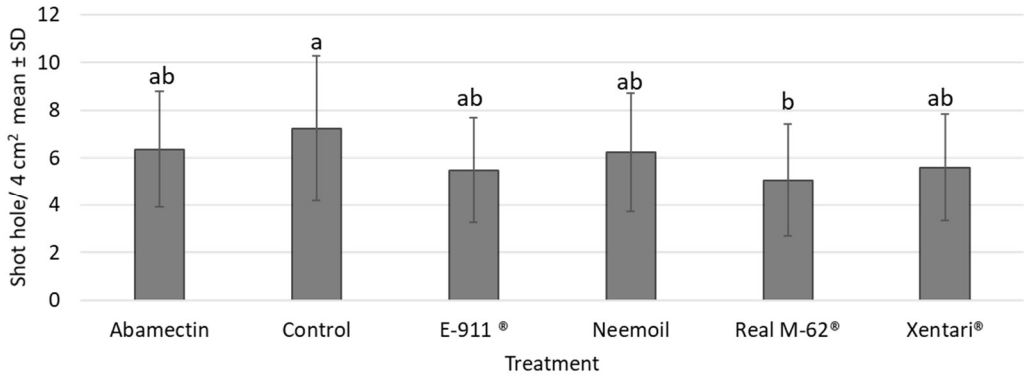


Figure 5. Mean (±SD) number of *P. striolata* shot-holes on pak-choi in target provinces in Cambodia during 2016. Significant differences are presented in the locations, where treatments differed statistically. Treatments with the same letter(s) did not differ statistically across locations.

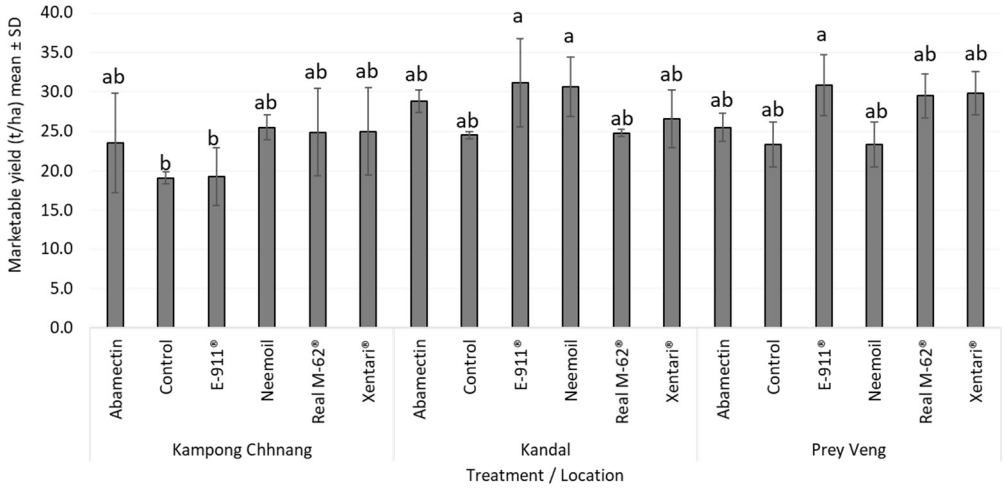


Figure 6. Mean (±SD) marketable yield of pak-choi in three provinces of Cambodia during 2016. Significant differences are presented in the locations, where treatments differed statistically. Treatments with the same letter(s) did not differ statistically across locations.

IPM trials on pak-choi

Based on the combined analyses for each evaluated variable, only direct effects are presented (Table 6) since no interaction effects were found in the analyses. Regarding the effect of different years and provinces, Kampong Chhnang and Prey Veng recorded less *P. xylostella* population in 2017 compared to Kampong Chhnang and Kandal in 2016 (Table 7). No. *H. undalis* populations were observed at any of the study provinces in 2017. Prey Veng showed less shot-hole damage by *P. striolata* in 2017 compared to Kampong Chhnang in 2016, where damage was two-fold higher. Interestingly, Pak-choi yield was significantly higher in Prey Veng in 2016, but 36% less in 2017 than the previous year (Table 7). The IPM treatment effects were mostly on par with the Farmers' practices. IPM treatment significantly reduced the larval population of *P. xylostella*, *S. litura* and *H. undalis*, and the shot-hole damage by *P. striolata* compared to the untreated control plots (Table 8). The marketable yield was also significantly higher in IPM treated plots than the untreated control plots.

Table 6. Combined analyses for infestation of *P. xylostella*, *S. litura*, and *H. undalis*, shot-holes damage by *P. striolata* and yield in pak-choi for six locations (2 years, 3 provinces, 1 sites/province) in Cambodia during 2016-2018

Source		Location	Treatment	Treatment* Location
DF		5	2	10
No. of *P. xylostella* larvae/plant	F value	9.44	140.82	1.69
	Pr>F	*<.0001*	*<.0001*	0.1031
No. of *S. litura*/plant	F value	0.81	14.44	0.64
	Pr>F	0.5513	*<.0001*	0.7754
No. of *H. undalis* larvae/plant	F value	8.88	3.57	0.99
	Pr>F	*<.0001*	*0.0343*	0.4582
No. of shot-holes/4 cm^2	F value	44.94	47.06	1.20
	Pr>F	*<.0001*	*<.0001*	0.3096
Marketable yield (t/ha)	F value	19.56	5.53	0.75
	Pr>F	*<.0001*	*0.0062*	0.6761

Table 7. Effect of locations [mean (±SD)] on *P. xylostella*, *H. undalis*, shot-holes damage by *P. striolata* and yield on pak-choi in Cambodia during 2016-2018

Year	2016			2017-2018		
Provinces	Kampong Chhnang	Kandal	Prey Veng	Kampong Chhnang	Kandal	Prey Veng
No. of *P. xylostella*/plant	2.62 (2.04) a	2.83 (1.50) a	2.22 (1.13) ab	1.68 (1.02) bc	2.24 (1.76) ab	1.35 (0.83) c
No. of *H. undalis*/ plant	0.41 (0.27) a	0.70 (0.15) 1	0.45 (0.25) a	0.00 (0.00) b	0.00 (0.00) b	0.00 (0.00) b
No. of shot-holes / 4 cm^2	9.67 (0.81) a	6.54 (1.17) c	7.95 (1.09) b	5.04 (0.65) de	5.88 (1.33) cd	4.57 (0.85) e
Marketable yield (t/ha)	24.67 (1.29) ab	25.24 (2.39) ab	27.48 (2.11) a	22.67 (1.20) b	24.61 (0.74) ab	17.44 (0.48) c

Means followed by the same letter(s) in a column are not significantly different ($p<0.05$) by Tukey's HSD
Figures in parentheses are *arcsine* transformed values

Table 8. Effect of IPM treatment [mean (±SD)] on *P. xylostella*, *S. litura*, *H. undalis*, shot-holes damage by *P. striolata* and yield on pak-choi in Cambodia during 2016-2018

Treatment	Control	Farmers' practice	IPM	
No.	36	36	36	
No. of *P. xylostella*/plant	3.74 (1.22) a	1.47 (0.68) b	1.26 (0.60) b	$F_{2,107}$ = 140.82; P<.0001
No. of *S. litura*/plant	1.11 (0.78) a	0.52 (0.56) b	0.46 (0.46) b	$F_{2,107}$ = 14.44; P<.0001
No. of *H. undalis*/plant	0.38 (0.57) a	0.22 (0.42) ab	0.18 (0.38) b	$F_{2,107}$ = 3.57; P=0.0343
No. of shot-holes/4 cm^2	7.51 (2.04) a	6.71 (1.92) b	5.61 (1.99) c	$F_{2,107}$ = 47.06; P<.0001
Marketable yield (t/ha)	22.37 (3.32) b	24.02 (4.99) ab	24.67 (4.42) a	$F_{2,107}$ = 5.53; P=0.0062

Means followed by the same letter(s) in a column are not significantly different (p<0.05) by Tukey's HSD

DISCUSSION

Bio-pesticides are effective against various lepidopteran pests. *Bacillus thuringiensis* formulations were able to reduce the population of *P. xylostella* and *S. litura* on pak-choi. A study on Chinese mustard also demonstrated similar efficacy in Cambodia in the study provinces (Srinivasan et al. 2020). An earlier study confirmed that *P. xylostella* and *S. litura* were susceptible to Xentari®, Crymax® and E-911® in Taiwan (Srinivasan et al. 2017a), even though *B. thuringiensis* formulations are widely used by the brassica farmers. However, the use of *B. thuringiensis* formulations is not common in Cambodia. Hence *P. xylostella* and *S. litura* populations in our study areas were seemingly susceptible to *B. thuringiensis* foliar spraying, which suppressed the pests. Although *P. xylostella* had already developed resistance to *B. thuringiensis* formulations in different parts of the world (Díaz-Gomez et al. 2000; Ferré et al. 1991; Ghosh et al. 2011; Hama 1992; Mohan and Gujar 2003; Pérez and Shelton 1997; Shelton et al. 1993; Syed 1992; Tabashnik et al. 1990; Zhao et al. 1993), the *P. xylostella* populations resistant to *B. thuringiensis* subsp. *kurstaki* were susceptible to *B. thuringiensis* subsp. *aizawai* (Talekar and Shelton 1993; Syed 1992), since the latter produces additional toxins such as Cry1C. Hence, use of different formulations such as Xentari®, Crymax® and E-911® can last longer in field conditions in Cambodia.

Metarhizium anisopliae formulation (Real M-62®) was also found to be effective against both *P. xylostella* and *S. litura* on pak-choi. A study on Chinese mustard demonstrated similar results in Cambodia (Srinivasan et al. 2020). *Metarhizium anisopliae* isolates and formulations were found to be most effective against *P. xylostella* in Taiwan (AVRDC 1999), and against egg and/or larval stages of *S. litura* in China (Lin et al. 2007), India (Borkar et al. 2013), and Pakistan (Asi et al. 2013). We too found that *M. anisopliae* formulation can be used to manage *P. xylostella* and *S. litura* on leafy brassicas in Cambodia. In addition, Real M-62® was found to reduce the damage by *P. striolata* beetles in the current study. Few studies have demonstrated the effectiveness of entomopathogenic fungi against flea beetles on brassicas in the USA (Reddy et al. 2014) and in Cambodia (Srinivasan et al. 2020). Since the immature stages of *P. striolata* are found in the soil, future studies should focus on application of entomopathogenic fungi in soil in brassica fields. The current study found that Real M-62® could reduce the lepidopteran species (*P. xylostella* and *S. litura*) as well as the coleopteran species (*P. striolata*) on pak-choi, and hence *M. anisopliae* can be included in an overall management program for *P. xylostella*, *S. litura* and *P. striolata* on leafy brassicas in Cambodia.

Neem leaf extract reduced the population of *P. xylostella* and *S. litura* and *P. striolata* damage in 2015, but not in 2016 trials. Similar inconsistencies were recorded on Chinese mustard in Cambodia earlier (Srinivasan et al. 2020). However, neem seed kernel extract was found to be effective in reducing *P. xylostella* damage (Jayadevi and Kumar 2011), while neem was found to reduce the damage of *P. cruciferae* (Boopath et al. 2010; Reddy et al. 2014) and *P. striolata* (Hou et al. 2003). Hence, the effectiveness of neem extracts and formulations might depend on the location and climatic conditions. The yield of pak-choi was significantly higher in *B. thuringiensis* and *M. anisopliae* treated plots consistently in 2015 trials, but it did not differ significantly in 2016 trials. Hence, the bio-pesticides were incorporated into an IPM strategy instead of using them individually throughout the season, and then validated during 2016-2018.

The efficacy of an IPM package was shown to be consistent in reducing pest damage on pak-choi, which led to significant yield increases. Similar results were recorded on Chinese mustard (Srinivasan et al. 2020). A pesticide window strategy was proven effective for managing *P. xylostella* in the Asia-Pacific region (Baker 2011; Ridland and Endersby 2011; Walker et al. 2011; Feng et al. 2011; Mau and Gusukuma-Minuto 2001). Since these window strategies mainly rotated the chemical pesticides with different modes of action, bio-pesticides were substituted to reduce the amount of chemical pesticides, which was also found to be effective in lowlands of Taiwan (Srinivasan et al. 2017a). Hence, the current IPM package in Cambodia was enriched with microbial and neem pesticides, along with the chemical pesticide, and applied them sequentially. This approach achieved pest suppression levels and yields equivalent to the Farmers' traditional practice of calendar-based pesticide spraying. The reduction in the amount of chemical pesticides in the IPM fields can augment the natural enemies in brassica fields, as shown in Vietnam and Laos (Srinivasan et al. 2017b). Hence, this IPM package can be promoted for large-scale adoption, after validation in major brassica production locations in Cambodia.

Acknowledgements

This work was supported by the Federal Ministry for Economic Cooperation and Development (GIZ Project number 13.1432.7-001.00), Germany and by core donors to the World Vegetable Center: Republic of China (Taiwan), UK aid from the UK government, United States Agency for International Development (USAID), Australian Centre for International Agricultural Research (ACIAR), Germany, Thailand, Philippines, Korea, and Japan. We also thank the Real IPM (now a member of the Biobest Group), Kenya for providing the *Metarhizium anisopliae* formulations for the trials.

References

Asi MR, Bashir MH, Afzal M, Zia K, Akram M. 2013. Potential of entomopathogenic fungi for biocontrol of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). J. Anim. Plant Sci. 23, 913–918.

AVRDC. 1999. AVRDC Report 1998. Asian Vegetable Research and Development Center, Shanhua, Tainan, Taiwan. vii+148 P.

Baker GJ. 2011. Crucifer vegetable insecticide resistance management strategies and issues in Australia,in: Srinivasan, R., Shelton, A. M., Collins, H. L. (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC – The World Vegetable Center, Publication No. 11755. AVRDC – The World Vegetable Center, Taiwan. pp. 241-247.

Boopath T, Pathak KA, Ngachan SV, Nabajyoti D. 2010. Evaluation of neem oil and insecticides against Phyllotreta cruciferae on broccoli. Ann. Plant Prot. Sci. 18: 236 -237.

Borkar AN, Deotale RO, Undirwade DB, Thakre DR. 2013. Efficacy of entomopathogenic fungal pesticides under laboratory condition against *Spodoptera litura*. J. Soils Crops. 23, 409-412.

Díaz-Gomez O, Rodríguez JC, Shelton AM, Lagunes TA, Bujanos MR. 2000. Susceptibility of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations in Mexico to commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 93, 963–970.

Feng X, Li ZY, Wu QJ, Chen AD, Wu YD, Hou YM, He YR, Li JH, Xie SH, Zhang JM, Wie F, Ma CS. 2011. Rising to the challenge: a national project on sustainable control of DBM in China, in: Srinivasan, R., Shelton, A. M., Collins, H. L. (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC – The World Vegetable Center, Publication No. 11755. AVRDC – The World Vegetable Center, Taiwan. pp. 15-18.

Fahey JW. 2016. Brassica: characteristics and properties. Encyclopedia of Food and Health. pp. 469-477. http://dx.doi.org/10.1016/B978-0-12-384947-2.00083-0

Ferré J, Real MD, van Rie J, Jensens S, Peferoen M. 1991. Resistance to *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. PNAS. 88, 5119–5123.

Genova C, Weinberger K, Acedo AL Jr. 2010. An assessment of market opportunities for leafy vegetables in the upland areas of Cambodia, Laos, and Vietnam. Final project report, RETA 6376. AVRDC Publication No. 10-734. AVRDC – The World Vegetable Center, Taiwan. 47 p.

Ghosh SK, Chaudhary M, Kumar P. 2011. Myco-Jaal: a novel formulation of *Beauveria bassiana* for managing diamondback moth (*Plutella xylostella*) in subtropical and tropical crucifer production systems. in: Srinivasan, R., Shelton, A.M., Collins, H.L. (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, 21-25 March 2011, Kasetsart University, Nakhon Pathom, Thailand. AVRDC – The World Vegetable Center, Publication No. 11-755. AVRDC – The World Vegetable Center, Taiwan. pp. 153-158.

Hama H. 1992. Insecticide resistance characteristics of the diamondback moth, in: Talekar, N.S. (Ed.), Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop. Shanhua, Taiwan: Asian Vegetable Research and Development Center, pp. 455-463.

Hou Y, Pang X, Liang G, You M. 2003. Evaluation of azadirachtin against striped flea beetle, *Phyllotreta striolata* (F.). Ying Yong Sheng Tai Xue Bao= J. Appl. Ecol. 14, 959-962.

Jayadevi HC, Kumar ARV. 2011. Why is a crude extract of neem superior to commercial neem formulations? A field test against *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in cabbage, in: Srinivasan, R., Shelton, A.M., Collins, H.L., (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, 21-25 March 2011, Kasetsart University, Nakhon Pathom, Thailand. AVRDC – The World Vegetable Center, Publication No. 11-755. AVRDC – The World Vegetable Center, Taiwan. pp. 172-181.

Lin HF, Yang XJ, Gao YB, Li SG. 2007. Pathogenicity of several fungal species on *Spodoptera litura*. J. Appl. Ecol. 18, 937–940.

Mau RFL, Gusukuma-Minuto L. 2001, Diamondback moth, *Plutella xylostella* (L.), resistance management in Hawaii, in: Ridland, P.M., Endersby, N.M. (Eds.), Proceedings of the Fourth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, Melbourne, Victoria, Australia. pp. 307-311.

Mohan M, Gujar GT. 2003. Characterization and comparison of midgut proteases of *Bacillus thuringiensis* susceptible and resistant diamondback moth (Plutellidae: Lepidoptera). J. Invert. Pathol. 83, 1–11.

Moore JK, Dixon PM. 2015. Analysis of combined experiments revisited. Agron. J. 107, 763-771.

Neufeld DSG, Savoeun H, Phoeurk C, Glick A, Hernandez C. 2010. Prevalence and Persistence of Organophosphate and Carbamate Pesticides in Cambodian Market Vegetables. Asian J. Water Environ. Poll. 7, 89-98.

Pérez CJ, Shelton AM. 1997. Resistance of *Plutella xylostella* (Lepidoptera: Plutellidae) to *Bacillus thuringiensis* Berliner in Central America. J. Econ. Entom. 90, 87–93.

Petersen RG. 1994. Agricultural field experiments: Design and analysis. Marcel Dekker, New York.

Reddy GVP, Tangtrakulwanich K, Wu S, Miller JH, Ophus VL, Prewett J. 2014. Sustainable Management Tactics for Control of *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae) on Canola in Montana. J. Econ. Entomol. 107, 661-666.

Ridland PM, Endersby NM. 2011. Some Australian populations of diamondback moth, *Plutella xylostella* (L.) show reduced susceptibility to fipronil, in: Srinivasan, R., Shelton, A. M. Collins, H. L. (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC – The World Vegetable Center, Publication No. 11755. AVRDC – The World Vegetable Center, Taiwan. pp. 207-215.

Schreinemachers P, Chen HP, Nguyen TTL, Buntong B, Bouapaoe L, Gautam S, Le NT, Pinnh T, Vilaysone P, Srinivasan R. 2017. Too much to handle? Pesticide dependence of smallholder vegetable farmers in Southeast Asia. Sci. Total Env. 593-594, 470–477.

Shelton AM, Wyman JA, Cushing NL, Apfelbeck K, Dennehy TJ, Mahr SER, Eigenbrode SD. 1993. Insect resistance of diamondback moth (Lepidoptera: Plutellidae) in North America. J. Econ. Entomol. 86, 11-19.

Srinivasan R, Lin MY, Su FC, Huang CC. 2017a. Towards developing an integrated pest management strategy for cabbage production systems in lowlands of Taiwan, Republic of China. Mysore J. Agric. Sci. 51, 185–197.

Srinivasan R, Lin MY, Hien NTT, Hai VM. 2017b. Biological control in vegetable brassica pest management in tropical Asia: where do we currently stand? In: Mason PG, Gillespie DR, Vincent C, eds. 2017. *Proceedings of the 5th International Symposium on Biological Control of Arthropods*, 11-15 September 2017, Langkawi, Malaysia. pp. 144-146.

Srinivasan R, Bhanu KRM, Lin MY, Yule S, Su FC, Huang CC, Khumsuwan C, Heng CH, Sarika S, Hai VM, Hien NTT, Khanh LD, Trang VTT, Diep NX, Phimchai V, Chansamone P, Soukhavong K. 2019. Evaluation of novel pheromone lures against striped flea beetle (*Phyllotreta striolata* Fab.) on brassicas and bean pod borer (*Maruca vitrata* Fab.) on yard-long bean in Southeast Asia. *Acta Horticulturae*. 1257: 37–46. doi: 10.17660/ActaHortic.2019.1257.6

Srinivasan R, Sotelo P, Lin MY, Heng CH, Kang S, Sarika S. 2020. Validation of a bio-based integrated pest management package for the control of major insect pests on Chinese mustard in Cambodia. *Crop Protection*. 135: 104728 https://doi.org/10.1016/j.cropro.2019.02.004

Syed AR. 1992. Insecticide resistance in the diamondback moth in Malaysia, in: Talekar, N.S. (Ed.), Management of Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop. Shanhua, Taiwan: Asian Vegetable Research and Development Center, pp. 437-442.

Tabashnik BE, Cushing NL, Finson N, Johnson MW. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83, 1671-1676.

Walker GP, Macdonald FH, Wallace AR. 2011. Recent developments in the management of diamondback moth in New Zealand, in: Srinivasan, R., Shelton, A.M., Collins, H. L. (Eds.), Proceedings of the Sixth International Workshop on Management of the Diamondback Moth and Other Crucifer Insect Pests, AVRDC – The World Vegetable Center, Publication No. 11755. AVRDC – The World Vegetable Center, Taiwan. pp. 234-240.

Zhao JZ, Zhu GR, Ju Z, Wang W. 1993. Resistance of diamondback moth to *Bacillus thuringiensis* in China. RPMN. 5,11-12.

Development and validation of an integrated pest management package for the control of major insect pests on cabbage in Lao PDR

Srinivasan, R.
ENTOMOLOGY GROUP
WORLD VEGETABLE CENTER
SHANHUA, TAINAN 74151, TAIWAN
email: srini.ramasamy@worldveg.org

Yule, S.
ENTOMOLOGY GROUP
WORLD VEGETABLE CENTER
EAST AND SOUTHEAST ASIA REGIONAL OFFICE
KAMPHAENG SAEN, NAKHON PATHOM 73140, THAILAND
email: sopana.yule@worldveg.org

Vilaysone, P.
CLEAN AGRICULTURE STANDARDS CENTER
DEPARTMENT OF AGRICULTURE
VIENTIANE CAPITAL, LAO PEOPLE'S DEMOCRATIC REPUBLIC
email: vphimchai@yahoo.com

Phommachan, C.
CLEAN AGRICULTURE STANDARDS CENTER
DEPARTMENT OF AGRICULTURE
VIENTIANE CAPITAL, LAO PEOPLE'S DEMOCRATIC REPUBLIC
email: cphommachan@yahoo.com

Khodsimouang, S.
CLEAN AGRICULTURE STANDARDS CENTER
DEPARTMENT OF AGRICULTURE
VIENTIANE CAPITAL, LAO PEOPLE'S DEMOCRATIC REPUBLIC
email: soukhavong2000@yahoo.com

Sotelo-Cardona, P.
ENTOMOLOGY GROUP
WORLD VEGETABLE CENTER
SHANHUA, TAINAN 74151, TAIWAN
email: paola.sotelo@worldveg.org

Lin, M.Y.
ENTOMOLOGY GROUP
WORLD VEGETABLE CENTER
SHANHUA, TAINAN 74151, TAIWAN
email: mei-ying.lin@worldveg.org

ABSTRACT
Brassicas vegetables are the most important crops grown for domestic consumption in Lao PDR. Lepidopteran caterpillars including diamondback moth (*Plutella xylostella*), common armyworm (*Spodoptera litura*), cabbage cluster caterpillar (*Crocidolomia pavonana*) and imported cabbage worm (*Pieris rapae*) and striped flea beetle (*Phyllotreta striolata*) are the most devastating pests of brassica vegetables in Lao PDR. Brassica producers mostly rely on the application of chemical pesticides to control these pests. In order to reduce pesticide overuse, we evaluated the effectiveness of an integrated pest management (IPM) package (*Bacillus thuringiensis* and neem oil formulations and a chemical pesticide, plus installation of *P. xylostella* and *S. litura* pheromone lures) on cabbage in Kasi district, Vientiane province during January–March 2016. The IPM package was compared with Farmers' practice (calendar-based application of abamectin) and an untreated control. The IPM package was able to reduce shot-hole damage by flea beetle, and the populations of *S. litura*, *P. xylostella*, *C. pavonana* and *P. rapae* significantly on cabbage across the locations. However, the IPM package was not as effective as Farmers' practice. The yield of cabbage was significantly higher in Farmers' practice plots, followed by the IPM package. The IPM package needs to be strengthened, before being validated in major brassica production locations, and then promoted for large-scale adoption.

Keywords
Brassicas, IPM, biopesticides, lepidopteran pests, striped flea beetle

INTRODUCTION
Brassica vegetables are the most important crops grown for domestic consumption in Laos, and also for exports within the region (Chanthasombath et al. 2012). Besides cabbage, leafy brassicas including green mustard, Chinese cabbage, Chinese kale, and pak-choi are also grown mainly for domestic consumption in Laos (Genova et al. 2010). Cabbage is cultivated for domestic and export markets as a high value crop. For instance, 460,000 t of cabbage (worth USD$74 million) was imported into Thailand from Laos. About 32% of income in the agriculture sector comes from cabbage alone, in major cabbage producing regions such as Champasak. However, the annual net profit from cabbage cultivation is only about USD 700 per hectare, and hence the production efficiency of cabbage is at a low level in Laos

(Supawadee 2013). Pests and diseases continue to be a major production constraint in cabbages and other leafy brassicas in Laos.

Lepidopteran caterpillars including diamondback moth (*Plutella xylostella*), the common armyworm (*Spodoptera litura*), the cabbage cluster caterpillar (*Crocidolomia pavonana*) and the imported cabbage worm (*Pieris rapae*), as well as the striped flea beetle (*Phyllotreta striolata*) are the most devastating pests of brassica vegetables in Laos (Schreinemachers et al. 2017; Srinivasan et al. 2019). Severe incidences of multiple pests limit the productivity of brassica vegetables. For instance, the marketable yield of leafy mustard is much lower in Laos (5.6 t/ha), compared to Cambodia (15 t/ha) and Vietnam (12.4 t/ha) (Schreinemachers et al. 2017). Hence, brassica producers mostly rely on the application of chemical pesticides to control the pests, with short spray intervals (two to three days) and the combination of more than ten types of insecticides per season (Mazlan and Mumford 2005; Grzywacz et al. 2010). A recent study found that 83% of the farmers used synthetic pesticides to manage the pests on leafy brassicas, and no farmers used any bio-pesticides (Schreinemachers et al. 2017). Farmers in Laos sprayed more frequently than their counterparts in Cambodia and Vietnam, and 63% of farmers mixed different pesticides in a single spray. Another study documented that 59% of the sampled farmers in Laos overused pesticides (Schreinemachers et al. 2020).

Integrated pest management (IPM) packages can be a better alternative to managing the key insect pests on vegetable brassicas. A bio-pesticide (*Bacillus thuringiensis* and *Metarhizium anisopliae* formulations, and neem leaf extract) based IPM package was similar in efficacy with farmers' practice of calendar-based pesticide application in reducing damage by key insect pests on Chinese mustard, leading to significant yield gains in Cambodia (Srinivasan et al. 2020). However, no such IPM packages have been tested on vegetable brassicas in Laos. Therefore, the objective of the current study was to evaluate an IPM package against major insect pests on cabbage in the Vientiane province of Laos.

MATERIALS AND METHODS

Study sites

Field trials were conducted in Vientiane province (Phaxang village, Kasi district) to evaluate the efficacy of an IPM package based on bio-pesticides in comparison with Farmers' practices (calendar-based spraying of chemical pesticides) and an untreated check during January–March 2016.

Treatment and data collection

Four field trials were conducted to evaluate the efficacy of an IPM package against *P. xylostella, S. litura*, *C. pavonana*, *P. rapae* and *P. striolata* on cabbage. The package consisted of the sequential application of bio-pesticides [Zitarback F.C.™ (*Bacillus thuringiensis* subsp. *aizawai*), Redcat™ (*B. thuringiensis* subsp. *kurstaki*) and neem (Thai neem™) and the chemical pesticide (abamectin). The spraying order was designed based on the incidence of target pests in a given field. Pheromone lures of *P. xylostella* and *S. litura* were also installed in the IPM treatment. Three treatments, *viz*., IPM package, Farmers' practice (calendar-based application of abamectin) and an untreated control were used in all the trials, with four replications for each treatment, following a Completely Randomized Block Design. The individual replication plot size was 12 m^2. The crop was monitored for damage by target pests, and the treatments were initiated from one to three weeks after planting depending on pest incidence and continued at weekly intervals until a week before harvest. The number of larvae on five randomly selected plants in each plot were counted for *P. xylostella*, *S. litura*, *C. pavonana*, and *P. rapae*, whereas the number of 'shot-holes' in a 4 cm^2 area in two younger leaves from each plant were counted on five randomly selected plants in each replication for *P. striolata* damage. Marketable yield was recorded during harvest.

Data analysis

The data was averaged for each plot and analyzed using a combined analysis approach of several experiments (Petersen 1994; Moore and Dixon 2015). Preliminary analysis of variance (ANOVA) was completed for each individual analysis (each location), experimental errors were examined for heterogeneity and Shapiro-Wilkinson test for normality was performed in each individual analysis. The data was then analyzed using ANOVA with the Proc GLM MIXED of SAS, version 9.1 (SAS Institute, Cary, NC, USA). Each experimental site was considered a particular environment for the combined analysis. Random effects were considered for locations, whereas fixed effects were considered for treatments. When significant treatment differences were identified, means were separated by Tukey's HSD Test (SAS) (differences were considered significant at $\alpha = 0.05$). Data on pest incidences and

P. striolata damage were arcsine transformed. Non-transformed data are presented in the results section.

RESULTS

For *P. striolata* damage, *P. xylostella* larval population, and yield both location and treatment were highly significant, whereas *C. pavonana* population was only affected by treatment. In addition, the *P. rapae* larval population showed an interaction effect between location and treatment (Table 1). *Spodoptera litura* larval population was not significantly affected by location and treatment effects. Among the experimental locations, Sakhone farm was the most affected by *P. striolata* damage, but it did not have any significant impact on the marketable cabbage yield, since the same location was the one that recorded the highest yield compared to the other three locations (Table 2). In terms of *P. xylostella* larval population, Ot farm recorded almost twice as much larval incidence as observed in the other locations.

Table 1. Analyses for damage of *Phyllotreta striolata*, incidences of *Spodoptera litura*, *Crocidolomia pavonana*, *Plutella xylostella*, and *Pieris rapae*, and marketable yield of cabbage in Laos during 2016.

Source	df	*No. of P. striolata* shot-holes / leaf		*No. of S. litura* larvae/plant		*No. of C. pavonana* larvae/plant		*No. of P. xylostella* larvae/plant		*No. of P. rapae* larvae/plant		Marketable yield (t/ha)	
		F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
Model	23	10.75	*<.0001*	3.04	*0.005*	2.89	*0.006*	19.32	*<.0001*	22.50	*<.0001*	21.63	*<.0001*
Location	3	20.49	*<.0001*	0.69	0.577	3.41	0.053	25.23	*<.0001*	3.86	*0.038*	7.17	*0.005*
Treatment	2	21.88	*<.0001*	3.29	0.055	8.57	*0.002*	105.88	*<.0001*	224.15	*<.0001*	203.72	*<.0001*
Location * Treatment	6	1.88	0.13	2.42	0.057	0.74	0.624	1.27	0.307	3.09	*0.022*	1.84	0.134

Table 2. Mean (±SD) damage of *Phyllotreta striolata*, *Plutella xylostella* larval incidence, and marketable yield by location in Laos during 2016.

Location	N	*P. striolata* shot-holes / leaf	*P. xylostella* larvae/plant	Cabbage yield (t/ha)
Buaodam farm	12	0.24 ± 0.06 b	0.90 ± 0.11 b	48.42 ± 0.99 ab
Mon farm	12	0.13 ± 0.05 b	0.69 ± 0.11 b	46.00 ± 1.33 bc
Ot farm	12	0.14 ± 0.07 b	1.38 ± 0.17 a	45.17 ± 2.02 c
Sakhone farm	12	0.42 ± 0.06 a	0.78 ± 0.12 b	48.92 ± 1.52 a

Means followed by the same letter(s) in a column are not significantly different (p<0.05) by Tukey's HSD

Among the treatments, farmers' practice significantly reduced *P. striolata* damage, and *P. xylostella* larval population, compared to the IPM treatment, but they were significantly higher in untreated control (Table 3). However, both IPM and the farmers' practice significantly reduced the *C. pavonana* larval population, compared to the untreated control. Similarly, the *P. rapae* larval population was significantly higher in untreated control plots compared to IPM and farmers' practice plots, where incidence was low to intermediate (Fig. 1). The marketable yield of cabbage was significantly higher in the farmers' practice (7% higher) compared to IPM plots, which recorded about 18% higher yield compared to the untreated control plots (Table 3)

Table 3. Mean (±SD) damage of *Phyllotreta striolata*, incidence of *Crocidolomia pavonana* and *Plutella xylostella*, and marketable cabbage yield by treatment in Laos during 2016.

Treatment	N	*P. striolata* shot-holes / leaf	*C. pavonana* larvae/plant	*P. xylostella* larvae/plant	Cabbage yield (t/ha)
Control	16	0.31 ± 0.08 a	0.15 ± 0.10 a	1.31 ± 0.13 a	41.12 ± 1.99 c
Farmers' practice	16	0.16 ± 0.03 c	0.05 ± 0.04 b	0.63 ± 0.09 c	51.87 ± 1.17 a
IPM	16	0.23 ± 0.07 b	0.06 ± 0.05 b	0.87 ± 0.16 b	48.37 ± 1.24 b

Means followed by the same letter(s) in a column are not significantly different ($p < 0.05$) by Tukey's HSD.

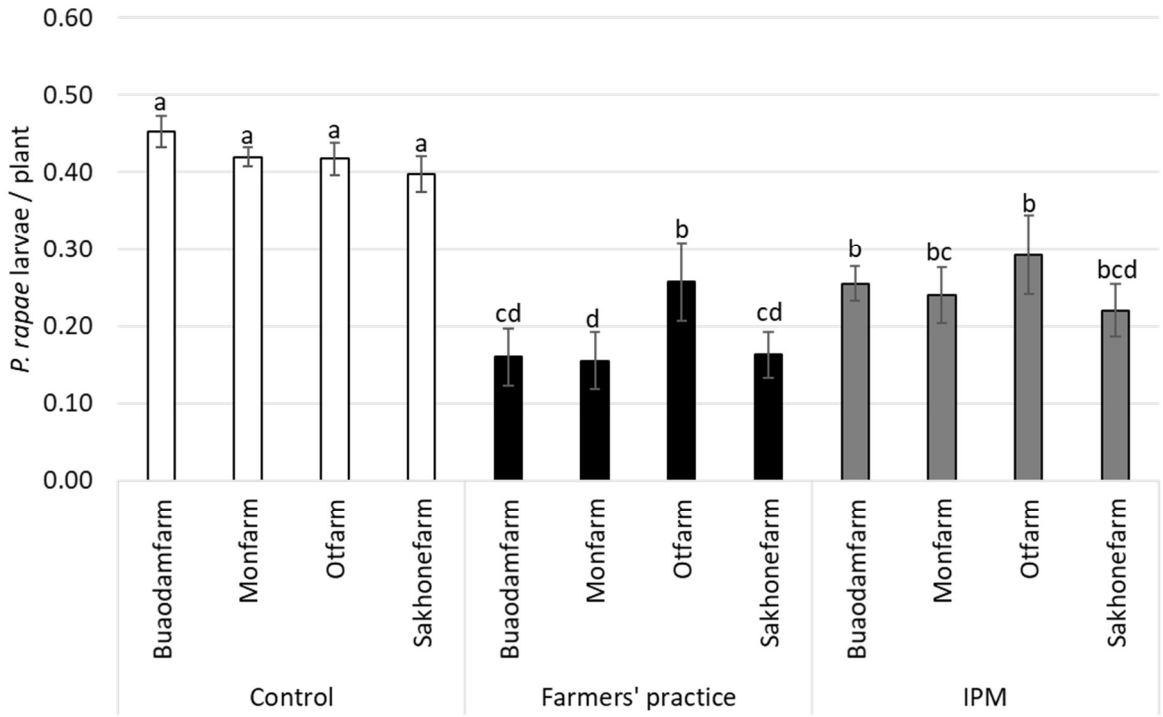


Fig. 1. Mean (±SD) number of *Pieris rapae* on cabbage in four locations in Laos during 2016. Significant differences are presented for the interaction Location*treatment. Locations with the same letter did not differ statistically across treatments.

DISCUSSION

The IPM package was shown to be consistent in reducing pest damage on cabbage, which led to significant yield increases across the four experimental sites in Laos. The IPM package, which predominantly consisted of *B. thuringiensis* and neem oil formulations, provided significant pest control on cabbage in Laos. It should be noted that use of bio-pesticides is highly uncommon in Laos (Schreinemachers et al. 2017), and the farmers have very limited or no access to quality bio-pesticides. Thus, the farmers in our trial locations have never applied bio-pesticides before. Hence, the target insects on cabbage were seemingly susceptible to the bio-pesticide formulations in the IPM package. In neighboring Cambodia, it was demonstrated that the major insect pests of vegetable brassicas were susceptible to various bio-pesticide formulations including *B. thuringiensis*, *M. anisopliae* and neem leaf extract (Srinivasan et al. 2020). Because of the no

or limited application of bio-pesticides in those locations in Cambodia, the study confirmed that the insects on Chinese mustard have not developed any resistance and the bio-pesticides were able to provide significant control in field conditions. Due to the analogous situation in Laos, we conclude that the *B. thuringiensis* and neem oil formulations are able to provide adequate control of key insect pests on cabbage.

However, the efficacy and yield from the IPM package were less than the farmers' practice of calendar-based pesticide application. This was contradictory to the results from Cambodia, which showed that the IPM package performed on par with the farmers' practice in Chinese mustard (Srinivasan et al. 2020). It should be noted that the IPM packages in Laos and Cambodia were not the same. Besides *B. thuringiensis* and neem leaf extract, *M. anisopliae* was included in the IPM package in Cambodia. Since we did not have access to *M. anisopliae* in Laos, it was not included in the IPM package. Hence, the current results clearly revealed the need for the inclusion of additional components in the IPM package in Laos. Besides providing better pest control, an improved IPM package should also lead to better or similar yield to farmers' practice. The latter is extremely important to convince farmers to adopt IPM packages, since about 84% of the growers in Laos thought that bio-pesticides were not as effective as synthetic pesticides (Schreinemachers et al. 2017). In addition, the ecosystem services provided by the adoption of IPM packages should be clearly explained to the brassica producers, which could also convince them to reduce the application of hazardous pesticides. It was already documented in Laos that the reduction in the number of chemical pesticides in the IPM fields could augment the natural enemies in brassica fields (Srinivasan et al. 2017). Finally, it is equally important to increase the local availability of bio-pesticides in Laos. Since the Laos Government has planned to increase the organic agriculture and Good Agricultural Practices farms to 70,000 and 100,000 by 2025, respectively (Hoonthong and Manowalailao 2016), the policy environment is highly conducive for the introduction of bio-pesticides.

In conclusion, the IPM package based on *B. thuringiensis* and neem oil formulations provided significant control of *P. striolata*, *C. pavonana*, *P. xylostella* and *P. rapae* damage on cabbage in Laos, and increased the yield. However, the efficacy of the IPM package was less than the farmers' practice and had lower yield. Hence, this IPM package needs to be strengthened with additional bio-pesticide components, before being validated in major brassica production locations and promoted for large-scale adoption in Laos.

Acknowledgements

This work was supported by the Federal Ministry for Economic Cooperation and Development (GIZ Project number 13.1432.7-001.00), Germany and by core donors to the World Vegetable Center: Republic of China (Taiwan), UK aid from the UK government, United States Agency for International Development (USAID), Australian Centre for International Agricultural Research (ACIAR), Germany, Thailand, Philippines, Korea, and Japan.

References

Chanthasombath T, Srilaong V, Wasusri T, Acedo AL Jr, Kanlayanarat S. 2012. Current Supply Chains of Cabbage in Lao PDR. Acta Hort. 943: 265-270.

Genova C, Weinberger K, Acedo AL Jr. 2010. An assessment of market opportunities for leafy vegetables in the upland areas of Cambodia, Laos, and Vietnam. Final project report, RETA 6376. AVRDC Publication No. 10-734. AVRDC – The World Vegetable Center, Taiwan. 47 p.

Grzywacz D, Rossbach A, Rauf A, Russell DA, Srinivasan R, Shelton AM. 2010. Current control methods for diamondback moth and other brassica insect pests and the prospects for improved management with lepidopteran-resistant Bt vegetable brassicas in Asia and Africa. Crop Protection, 29 (1) 68-79.

Hoonthong P, Manowalailao R. 2016. Planting 'Clean Agriculture' for future Laotian. https://www.asean-agrifood.org/planting-clean-agriculture-for-future-laotian/

Mazlan N, Mumford J. 2005. Insecticide use in cabbage pest management in the Cameron Highlands Malaysia. Crop Prot. 24, 31–39.

Moore JK, Dixon PM. 2015. Analysis of combined experiments revisited. Agron. J. 107, 763–771.

Peterson RG. 1994. Agriculture field experiments. Design and Analysis. Marcel Dekker, New York.

Schreinemachers P, Grovermann C, Praneetvatakul S, Heng P, Nguyen TTL, Buntong B, Le NT, Pinn T. 2020. How much is too much? Quantifying pesticide overuse in vegetable production in Southeast Asia. *Journal of Cleaner Production*, 244: 118738. https://doi.org/10.1016/j.jclepro.2019.118738

Schreinemachers P, Chen HP, Nguyen TTL, Buntong B, Bouapaoe L, Gautam S, Le NT, Pinnh T, Vilaysone P, Srinivasan R. 2017. Too much to handle? Pesticide dependence of smallholder vegetable farmers in Southeast Asia. Sci. Total Env. 593-594, 470–477.

Srinivasan R, Bhanu KRM, Lin MY, Yule S, Su FC, Huang CC, Khumsuwan C, Heng CH, Sarika S, Hai VM, Hien NTT, Khanh LD, Trang VTT, Diep NX, Phimchai V, Chansamone P, Soukhavong K. 2019. Evaluation of novel pheromone lures against striped flea beetle (*Phyllotreta striolata* Fab.) on brassicas and bean pod borer (*Maruca vitrata* Fab.) on yard-long bean in Southeast Asia. *Acta Horticulturae*. 1257: 37–46. doi: 10.17660/ActaHortic.2019.1257.6

Srinivasan R, Lin MY, Hien NTT, Hai VM. 2017. Biological control in vegetable brassica pest management in tropical Asia: where do we currently stand? In: Mason PG, Gillespie DR, Vincent C, eds. 2017. *Proceedings of the 5th International Symposium on Biological Control of*

Arthropods, 11-15 September 2017, Langkawi, Malaysia. pp. 144-146.

Srinivasan R, Sotelo P, Lin MY, Heng CH, Kang S, Sarika S. 2020. Validation of a bio-based integrated pest management package for the control of major insect pests on Chinese mustard in Cambodia. *Crop Protection*. 135: 104728 https://doi.org/10.1016/j.cropro.2019.02.004

Supawadee K. 2013. Benefits and Efficiency of Cabbage Production of Lao Farmers under Thai-Laos Contract Farming. China-USA Business Review, 12(7): 646-654.

Scientific Committee

	Name	Affiliation	Country / Region
1	Dr. Anthony M. Shelton ams5@cornell.edu	Cornell University	USA
2	Dr. Franziska Beran fberan@ice.mpg.de	Max Planck Institute for Chemical Ecology	Germany
3	Dr. Inga Mewis inga@entomology.de	Humboldt University Berlin	Germany
4	Dr. Michael Furlong m.furlong@uq.edu.au	University of Queensland	Australia
5	Dr. Myron P. Zalucki m.zalucki@uq.edu.au	University of Queensland	Australia
6	Dr. Paola Sotelo-Cardona paola.sotelo@worldveg.org	World Vegetable Center	Taiwan
7	Dr. Sivapragasam Annamalai a.siva@cabi.org	CABI Southeast Asia	Malaysia
8	Dr. Srinivasan Ramasamy srini.ramasamy@worldveg.org	World Vegetable Center	Taiwan
9	Dr. Subramanian Sevgan ssubramania@icipe.org	International Centre of Insect Physiology and Ecology	Kenya
10	Dr. Zhenyu Li zhenyu_li@163.com	Guangdong Academy of Agricultural Sciences	China

Participants

Participant	Email	Organization
Abdul Hamid, Siti Noor Aishikin	ctaikina@mardi.gov.my	Malaysian Agricultural Research and Development Institute (MARDI)
Atumurirava, Fereti	FeretiA@spc.int	Pacific Community
Badenes-Pérez, Francisco Rubén	frbadenes@ica.csic.es	Institute of Agricultural Sciences (CSIC)
Bruin, Jan	jan.bruin@syngenta.com	Syngenta Seeds
Chang, Sandra	sandra.chang@croplifetaiwan.org	CropLife Taiwan
Chang, Shu Chen	SCCHANG@tari.gov.tw	Taiwan Agricultural Research Institute (TARI)
Chiang, Ming Yao	mingyaw@tari.gov.tw	Taiwan Agricultural Research Institute (TARI)
Chou, Ming Yi	mingyichou@dragon.nchu.edu.tw	National Chung Hsing University
Dai, Shu Mei	sdai5497@dragon.nchu.edu.tw	National Chung Hsing University
Gyawali, Purushottam	gyawalipg93@gmail.com	World Vegetable Center (Intern)
Hsiao, Wen Feng	wfhsiao@mail.ncyu.edu.tw	National Chiayi University
Hsiao, Chia Chang	al.hsiao@syngenta.com	Syngenta Asia Pacific Pte. Ltd.
Hu, Ruey Chuan		World Vegetable Center
Huang, Chun Te	ted.huang@syngenta.com	Syngenta Taiwan Ltd.
Huang, Chun Chu	amy.huang@worldveg.org	World Vegetable Center
Janarthanan Senthilkumar, Prashanth Kumar	prashanth.thriller@gmail.com	World Vegetable Center (Intern)
Kao, Ching Hua	chkao02@tari.gov.tw	Taiwan Agricultural Research Institute (TARI)
Khan, Mohammed Abul Monjur Khan	khan@bau.edu.bd	Bangladesh Agricultural University
Kim, Juil	forweek@korea.kr	National Institute of Crop Science, RDA
Knight, Susan	susan.knight@syngenta.com	Syngenta Asia Pacific Pte. Ltd.
Ku, Chia Yin	chiainnku@gmail.com	National Chung Hsing University
Kumawat, Madan Mohan	kumawatmm@gmail.com	Mandor Agriculture University
Liang, Yuan		World Vegetable Center
Lin, Jing Huan		National Chung Hsing University
Lin, Feng Chyi	FCLin@tari.gov.tw	Taiwan Agricultural Research Institute (TARI)
Lin, Mei Ying	mei-ying.lin@worldveg.org	World Vegetable Center
Liu, Derrick	derrick.liu@basf.com	BASF Taiwan Ltd.
Liu, Tim	tim.liu@croplifetaiwan.org	CropLife Taiwan

Participant	Email	Organization
Lu, Yen Cheng	exmoon0308@knownyou.com	KnownYou Seed Co., Ltd
Luther, Greg	gregory.c.luther@gmail.com	Agricultural Development Initiatives, Inc.
Malaisamy, Kannan	kanento@gmail.com	Tamil Nadu Agricultural University
Mau, Ronald	maur@ctahr.hawaii.edu	University of Hawaii
Mazlan, Zulaikha	zulaikha@mardi.gov.my	Malaysian Agricultural Research and Development Institute (MARDI)
Mohd Ishadi, Nur Adibah	ajlaa_911@yahoo.com	Universiti Putra Malaysia
Nand, Nitesh	NiteshN@spc.int	Pacific Community
Onkarappa, Dhanyakumar	dhanyakumarento@gmail.com	World Vegetable Center (Intern)
Periasamy, Malini	malini.biotechnology@gmail.com	Independent consultant
Perry, Kym	kym.perry@sa.gov.au	South Australian Research and Development Institute
Rajendran, Senthil Kumar	drkumaragribiotech@gmail.com	National Taiwan University
Rakha, Mohamed	mohamed.rakha@worldveg.org	World Vegetable Center
Ramasamy, Srinivasan	srini.ramasamy@worldveg.org	World Vegetable Center
Ridland, Peter	p.ridland@unimelb.edu.au	University of Melbourne
Shelton, Anthony	ams5@cornell.edu	Cornell University /NYSAES
Shih, Hsien Tzung	htshih@tari.gov.tw	Taiwan Agricultural Research Institute (TARI)
Shimabuku, Robin	ShimabukuR@ctahr.hawaii.edu	University of Hawaii
Sim, Khay Chuan	kcsim@ancomcropcare.com.my	Ancom Crop Care SDN. BHD
Sopana, Yule	sopana.yule@worldveg.org	World Vegetable Center
Sotelo-Cardona, Paola	paola.sotelo@worldveg.org	World Vegetable Center
Su, Fu Cheng	fu-cheng.su@worldveg.org	World Vegetable Center
Subramanian, Sevgan	ssubramania@icipe.org	icipe
Sundria, Man Mohan	sul79man76@gmail.com	Mandor Agriculture University
Suwa, Akiyuki	suwa-akiyuki@nichino.co.jp	Nihon Nhoyaku Co., Ltd.
Tavares, Kylie	kylielw@hawaii.edu	University of Hawaii
Topagi, Sanjay	sanjay.topagi@gmail.com	University of Agricultural Sciences, GKVK
Tsai, Chin Chih	tfsongg@gmail.com	Ching Long Seed Co., Ltd
Van Herwijnen, Zeger	z.van.herwijnen@rijkzwaan.nl	Rijk Zwaan Breeding B.V.
Vellaisamy, Ambethgar	drva1965@gmail.com	Tamil Nadu Agricultural University
Wang, Leyun	leyun.wang@uq.net.au	University of Queensland
Ward, Christopher	christopher.ward@adelaide.edu.au	University of Adelaide
Wong, Hong Ren	hrwong@ancomcropcare.com.my	Ancom Crop Care SDN. BHD

Participant	Email	Organization
Wu, Tsung Jung		National Chung Hsing University
Yang, Chung Ming		National Chung Hsing University
Ye, Ting Wei	vic1991527@hotmail.com	National Taiwan University
Zalucki, Myron	m.zalucki@uq.edu.au	University of Queensland

Author Index