World Vegetable Center

Cornell University

8th International Conference on Management of the Diamondback Moth and Other Crucifer Insect Pests

4-8 MARCH 2019

World Vegetable Center

Shanhua, Taiwan

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

4-8 March 2019

World Vegetable Center

Shanhua, Tainan, Taiwan

Organizers

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Meeting Information

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

4-8 March 2019

World Vegetable Center

Shanhua, Tainan, Taiwan

Meeting Venue

The VIII International Conference on Management of the Diamondback Moth and other Crucifer Insect Pests will be held at the World Vegetable Center, including the Auditorium (Third floor, Administration Building) where the inauguration session will be taking place, and the Multipurpose Hall (Second floor of Cafeteria), for scientific sessions.

Guideline for Oral Presenters

PC and projectors are provided for the meeting rooms. Presentation files should be uploaded to the laptops before the beginning of the sessions. There are 30 minutes for each speaker; 25 minutes for oral presentation and 5 minutes for discussion.

Guideline for Poster Presenters

The posters can be mounted during the respective scientific session. Authors should stand close to their boards to answer questions and facilitate discussions about their work.

Field trip information

Zhutang Vegetable Co-op, Changhua County (彰化縣, 竹塘第九產銷班)

Hankuan Vegetable Co-op, Yunlin County (雲林縣, 漢光果菜生產合作社)

Scientific Committee

Dr. Srinivasan Ramasamy
World Vegetable Center, Taiwan

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Max Planck Institute for Chemical Ecology, Germany

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Humboldt University Berlin, Germany

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International Centre of Insect Physiology and Ecology Kenya

Schedule

Monday, 4 March 2019

Venue: Auditorium (3F, Administration Building)

08:00-09:30	Registration
09:30-09:50	Coffee break (Coffee Lounge)
09:50-10:30	Inaugural session and Welcome
10:30-10:40	A brief introduction to the World Vegetable Center
10:40-11:30	Introduction: Diamondback Moth Management
11:40-11:45	Group Photo
11:50-13:00	Lunch (Cafeteria)

Session 1 Diamondback moth and other crucifer pests: the global challenge in a changing climate

Venue: Multipurpose Hall (2F, Cafeteria)

Session 2 Biology, Ecology and Behavior of Diamondback Moth and Other Crucifer Pests

Tuesday, 5 March 2019

Venue: Multipurpose Hall (2F, Cafeteria)

Session 3 Biological and non-chemical methods of management of crucifer pests (including organic agriculture)

	Badenes-Perez, F.R., et al. (virtual presentation from Spain)
Dinner on your own	

Wednesday, 6 March 2019

Venue: Multipurpose Hall (2F, Cafeteria)

Session 5	**Insecticide Resistance and Management in Crucifer Pests**
08:30-09:00	**Managing Insecticide Resistance in Asia** ………………………………………...21 Knight, S. C. & Parimi, S.
09:00-09:30	**High Resistance *Plutella xylostella* to Spinosad Exhibited Low Cross Resistance to other Insecticide** ………………………………………………………..….22 Nur Adibah, M.I., et al.
09:30-10:00	**Effects of Temperature on the Performance of Deltamethrin-Resistant and Insecticide-Susceptible Field Strains of Diamondback Moth** …………….….23 Wang, L., et al.
10:00-10:30	Coffee break (Cafeteria)
10:30-11:00	**Development, Implementation and Monitoring of an Insecticide Resistance Management Strategy for Diamondback Moth in the South Pacific** …………..24 Atumurirava, F., et al.
11:00-11:30	**Insecticide Resistance Monitoring and Management of Diamondback Moth, *Plutella xylostella*, in Hawaii and Taiwan** ……………………………………..25 Chou, M.Y., et al.
11:30-11:45	**Impact of One Carboxylesterase on Tolfenpyrad Resistance of *Plutella xylostella*** (POSTER) ……………………………………………………………..…27 Ye, T.W. & Huang, Y.B.
11:45-12:00	**Inheritance, Stability and Cross Resistance of Emamectin Benzoate-Resistant *Plutella xylostella*** (POSTER) ……………………………………………...…28 Cheng-Wei Fang & Shu-Mei Dai
12:00-12:15	**Bifenazate Resistance and Mutations in Mitochondrial Cytochrome B of *Tetranychus urticae* Koch (Acari: Tetranychidae)** (POSTER) …………..……..29 Shu-Chen Chang & Ching-Hua Kao
12:15-13:30	Lunch (Cafeteria)
13:30-14:00	**Functional and Genetic Characteristics of Diamide Insecticides Resistance in**

Thursday, 7 March 2019

8:00-17:30	**Field visit** Zhutang Vegetable Co-op, Changhua County (彰化縣, 竹塘第九產銷班) Hankuan Vegetable Co-op, Yunlin County (雲林縣, 漢光果菜生產合作社)
18:30-20:30	Farewell Dinner

Friday, 8 March 2019

Venue: Multipurpose Hall (2F, Cafeteria)

Session 7	Genetic approaches to manage crucifer pests
08:30-09:00	**Development and Validation of a Bio-Pesticide Package for The Control of Major Insect Pests on Pak-Choi in Cambodia** ……………………………….39 Srinivasan, R., et al.
09:00-09:30	**Development and Validation of an Integrated Pest Management Package for the Control of Major Insect Pests on Cabbage in Lao PDR**……….………..41 Srinivasan, R., et al.
09:30-10:00	Coffee break at Demo Garden
10:00-10:30	Genebank visit
10:30-11:30	Group discussion – Management of the Brassica insect pests: the way Forward Venue: Multipurpose Hall (2F, Cafeteria)
11:30-12:30	Official Closing of the VIII International Conference on Management of the Diamondback Moth and other Crucifer Insect Pests
12:30-13:30	Lunch (Cafeteria)
13:30-17:00	Networking/free meetings
	Dinner on your own

Saturday, 9 March 2019

Departure of participants

Session 1: Diamondback Moth and other Crucifer Pests: Global Challenges in a Changing Climate

Management of Diamondback Moth (Lepidoptera: Plutellidae), and Other *Brassica* Lepidopteran Pests: with Emphasis on Taiwan

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ABSTRACT

Diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), the world-wise most destructive Brassica crop pest, has been a continuing problem for vegetable growers in Taiwan. The first cropping season is usually started from August to December and the second cropping season is from November to next March. The major lepidopterans pest is Diamondback moth, and the secondary pests are *Pieris rapae crucivora*, *Spodoptera litura*, *Crocidolomia binotalis*, and *Hellula undalis*. The minor pests are *Trichoplisia ni* and *Agrotis ipsilon*. Other than the basic biology and ecology have studied extensively for the above pests, a “treatment window” package is 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 IRAC 4A/28 (e.g. (Thiamethoxam + Chlorantraniliprole) are recommended to apply. For the second generation, IRAC 6 (e.g. Emamectin Benzoate) and IRAC 5 (e.g. Spinetoram) are recommended. For the third generation, IRAC 12A (e.g. Diafenthiuron) and Bt are recommended to control *P. xylostella* on the first cropping season. On the second cropping season, Bt and IRAC 28 (e.g. Chlorantraniliprole and Flubendiamide) are recommended to control the first generation. IRAC 22A (e.g. Indoxacarb) and IRAC 1B/3A (e.g. Chlorpyrifos + Cypermethrin) are recommended for control the second generation but IRAC 13 (e.g. Chlorfenapyr) and Bt are recommended for control the third generation of *P. xylostella*. Pesticides are applied when *P. xylostella* are presented every five of 20 plants. *H. undalis* is the most destructive seedling pest in summer and can be controlled by applying chemical pesticides and planting resistant varieties. *S. litura* are controlled by applying chemical pesticides, setting sex pheromone trap. The populations can also be decreased by parasitoid wasps (Ichneumonidae and Braconidae), polyhedrosis virus, entomopathogenic fungi and entomopathogenic nematodes. Other lepidopteran pests are controlled by prophylactic use of insecticides.

Keywords: Diamondback moth, Treatment window, *Bacillus thuringiensis*, IRAC, sex pheromone trap.

Crocidolomia pavonana and other Crucifer Pest Management in Samoa: Real IPM Is Possible!

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ABSTRACT
In the islands of the South Pacific, the large cabbage moth (LCM), *Crocidolomia pavonana* F. (Lepidoptera: Crambidae), is 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. Partial life table analyses of LCM eggs in the presence and absence of natural enemies showed that the marginal rate of parasitism was typically comparable to or higher than mortality due to other biotic factors. In smaller (< 35 eggs) egg masses 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. Frequent 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*. Establishing this species in other South Pacific Islands should be explored.

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

Simulation and Prediction of Dynamics in Diamondback Moth Biological Control under Potential Changing Climate in the Eastern Afromontane

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ABSTRACT

Biological control of diamondback moth (DBM), *Plutella xylostella* L. with parasitoids, *Diadegma semiclausum* and *Cotesia vestalis*, is highly sustainable. The biology and interaction between DBM and its parasitoids, is influenced by their climatic requirements, hence could be disrupted with climate change. Eastern Afromontanes where DBM biological control was implemented, are vulnerable to climate change with possible impacts on biological control. To understand this, Crucifer farms were surveyed over two 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 zones (low, medium and high) based on temperature and altitude. In each zone, crucifer farms were identified, and georeferenced, daily temperature and humidity logged and DBM larvae and pupae sampled. The larvae and pupae were monitored for parasitoid/DBM emergence. Life history parameters for DBM, *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 with Insect Life Cycle Modeling (ILCYM) and validated. The generated phenology models, the downscaled temperatures from AFRICLIM for baseline (2013) and future (2055); and georeferenced topographical maps, were loaded in index interpolator of ILCYM and growth indices (establishment, generation and activity) were mapped. 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. This could strengthen its interaction with *C. vestalis*. However, synchrony between DBM and *D. semiclausum* in the same zones could be weakened. In high zones, DBM damage is likely to increase beyond the colonization range of *D. semiclausum*. Utilization of altitudinal gradients for understanding potential effects of climate change as compared to regional and global circulation models provides better insights for location specific adaptations of biological control of DBM.

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

Session 2: Biology, Ecology and Behavior of Diamondback Moth and Other Crucifer Pests

Seasonal Colonization of Canola by *Plutella xylostella* in Southern Australia Originates from Local Source Populations

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ABSTRACT
The diamondback moth, *Plutella xylostella*, is the principal pest of Australian *Brassica* vegetable crops and a damaging pest of winter canola crops grown in temperate southern regions of Australia. Knowledge of the pest's ecology in Australian canola is limited yet required for effective management. Identifying the regional movement patterns and source populations of *P. xylostella* that seasonally colonize canola remains a priority for enhancing predictive capacity and managing insecticide resistance. We performed comprehensive molecular and field studies to investigate the origins of seasonal colonization of canola crops by *P. xylostella* in southern Australia. *Plutella* was collected from wild and cultivated brassicas throughout southern Australia and genotyped across genome-wide SNP markers using RAD-seq. A statistically powerful SNP marker set revealed no spatial, temporal or host plant-related genetic structure among 59 Australian *P. xylostella* populations despite a geographic scale >3000 kilometers, confirming a previous microsatellite assessment, however low genetic diversity precluded identification of gene flow patterns. Field studies were conducted in South Australia over three years using geographic sampling and trapping networks. Potential host plants were sampled widely in autumn, then subsequent crop colonization was measured across a network of sentinel canola crops and using a temperature-based development model for *P. xylostella* to infer the timing of initial oviposition in each crop. We found that canola crops were consistently colonized soon after germination. There was strong inter-annual variation in the autumn dynamics of *P. xylostella* and its host plants, driven by pre-season rainfall patterns. We used a CLIMEX model to explore spatiotemporal changes in the potential distribution and abundance of *P. xylostella* during our study, and measured male and female flight dynamics using light traps. CLIMEX predictions, light trapping, autumn sampling and crop colonization patterns suggested that insecticide-resistant *P. xylostella* over-summering locally in canola growing areas are the primary source of seasonal invasion of canola crops, with important management implications.

Keywords: Dispersal, colonization, bioclimatic modelling, RAD-seq

The Secret Life of *Plutella australiana*, an Australian Cryptic Diamondback Moth Species

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ABSTRACT
Cryptic species introduce complexities for pest management and ecological research. The recent discovery of a cryptic *Plutella* species endemic to Australia, *Plutella australiana* Landry & Hebert, surprised diamondback moth researchers given previous molecular studies of Australian *P. xylostella*, and raised questions about the ecology, pest status and management implications of the two *Plutella* lineages in Australian *Brassica* crops. We collected *Plutella* from wild and cultivated brassicaceous plants throughout southern Australia and screened individuals to identify mtDNA lineages and *Wolbachia* endosymbiont infections. We genotyped genome-wide SNPs using RADseq in co-existing populations of each species. In addition, we assessed inter-specific reproductive compatibility in laboratory crossing experiments and insecticide susceptibility phenotypes using bioassays. Despite sympatric distributions and the ability for hybridization in controlled crosses, these species appear to be reproductively isolated in the wild. We found striking differences in genetic diversity, *Wolbachia* infections and insecticide susceptibility between these species, consistent with contrasting colonization histories in Australia and reproductive isolation following secondary contact. We conclude that although *P. australiana* is a potential pest of Australian brassicaceous crops, it is of secondary importance to *P. xylostella*.

Keywords: *Plutella australiana*, *Plutella xylostella*, hybridization, *Wolbachia*, insecticide resistance

Plutella xylostella (L.) (Lepidoptera: Plutellidae) Larval Color Polymorphism under Laboratory Conditions

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ABSTRACT

Plutella xylostella (DBM) Color polymorphisms are presented for the first time. Three polymorphisms were observed with the naked eye: green, dark tones (brown) and light tones (yellowish) in a population of larvae from Ambato, Ecuador (S 1° 41’05”, W 78° 40’ 20”). The isolines of the parents of green and brown DBMs, from that population, were made and fed with three host plants: broccoli, cabbage and radish, for a period of five generations. Five larvae of the fourth stage were photographed by insulin, by generation, by the plant. The photographs were followed by a protocol to be subjected to an analysis of pixel intensity in the RGB space and in HSV. The data generated from the 750 images were analyzed with analysis of variance and nonparametric conglomerates, among other analyzers. The results indicate that there are larval color polymorphisms and this was determined by the host plant and not by the paternal phenotype. The pooled analysis showed two groups: the larvae that were fed with cabbage and the larvae that were fed with broccoli and radish. To explain these results, we hypothesize that the color polymorphism could be a crypsis.

Keywords: *Plutella xylostella*, Color, polymorphisms, larvae, RGB, HSV.

Session 3: Biological and Non-Chemical Methods of Management of Crucifer Pests (including Organic Agriculture)

Inoculation and Colonization of Cabbage Seedlings by the Endophytic Entomopathogenic Fungus *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales)

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ABSTRACT

The cabbage plant, *Brassica oleracea* and other brassicas have become very important group of vegetables. Different varieties of cabbages have been bred for productivity, size, and flavor over the years. However, they are still quite vulnerable to pests and diseases. Nearly 100 insect pests and pathogens affect the production and productivity of vegetable brassicas. Chemical control is the primary approach to regulate pests and disease problems. However, resistance to pesticides evolves following heavy and frequent use. Fungal endophytes play an important role in protecting plants against herbivorous insects and plant pathogens. Dual biocontrol potential of both insect pests and plant pathogens has been reported for the fungal entomopathogens, *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales). Evidence has accumulated that *B. bassiana* can endophytically colonize in a variety of plant species, both monocots and dicots. No reports are yet available on the potential of *B. bassiana* to establish as an endophyte in crucifer vegetable crops in Southern India. We investigated an indigenous isolate *B. bassiana* to determine if endophytic colonization could be achieved in cabbage plants through different inoculation methods. Colonization of leaves, stems, and roots by *B. bassiana* was assessed at different intervals 14-days after application of the fungus. Inoculation method based on drenching the soil around cabbage seedlings using conidial suspensions resulted in endophytic colonization of cabbage roots by *B. bassiana*, though it was found in the leaves or stems of the treated cabbage seedlings. In contrast, inoculation either through seed dressing or foliar application with conidia caused no stem or leaf colonization by the fungus in cabbage seedlings. The establishment of *B. bassiana* was detected more often in the proximal end of the root than in the distal end. Colonization levels of *B. bassiana* were higher when plants were sampled at 14-21 days post-inoculation (48-56%) compared to 28-35 days post-inoculation (74-82%), which suggests *B. bassiana* able to persist in the soil or as an endophyte in cabbage roots over time. Differences in colonization success and plant growth were found among the treatments. Further research should focus on the dual control potential of endophytic *B. bassiana* against cabbage pests and diseases

Keywords: *Beauveria bassiana*, endophyte, inoculation, colonization, cabbage seedlings

Potentials of Fungal Entomopathogens to Counter Insecticide Resistance in Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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ABSTRACT

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) has a great economic importance in Brassicaceae crops in many parts of the world. Recurrent infestations of this pest have led farmers to frequently spray their crops with insecticides. However, 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 more than 36 insecticides across multiple chemical classes including chlorinated hydrocarbons, carbamates, organophosphates and pyrethroids. Recently, resistance to newer insecticide chemistries, including spinosad, indoxacarb and emamectin benzoate has also been reported. Even over-use of biorational insecticides including *Bacillus thuringiensis kurstaki* can lead to resistance. Currently, only few registered insecticides can adequately control the DBM. Insecticides resistance management (IRM) offers great promise as a complementary extension of integrated pest management (IPM). IRM attempts to prevent or delay the development of resistance in target pests. Alternatives and supplements to conventional chemical insecticides are being sought for DBM management because of this resistance problem, problems with environmental contamination, and potential hazards to beneficial organisms and human. Interest in the use of fungal entomopathogens in combination with subnormal doses of insecticide to counter insecticide resistance is on the increase. Integration of selected strain entomopathogenic fungi with selective insecticides can improve the control efficiency, besides decrease the amount of insecticides required, minimize the risks of environmental contamination and delay the expression of insecticide resistance in insect pests. Co-application of fungi like *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin, *Nomuraea rileyi* (Farlow) Samson, *Paecilomyces* spp., *lecanicillium (=Verticillium) lecanii* (Zimm.) Veigas, and *Zoophthora radicans* (Brefeld) Batko with suitable subnormal concentration of selective insecticide as *two-in-one tank mix* have been successfully employed against various crop pests to mitigate the selection pressure and to avoid concurrent resistance risks in target pests. Majority of resistance mechanisms in DBM occur through induction of enzymes especially mono-oxygenases and to some extent the esterases. Fungal entomopathogens have ability to induce high degree of susceptibility to insecticides in target pests by suppressing enzyme activities and predispose them for fungal infection. This paper attempts to address the current state of knowledge on the exploitation of fungal entomopathogens as biological tool to counter insecticide resistance in *P. xylostella* population for sustainable pest management systems.

Keywords: Fungal entomopathogens, insecticide resistance management, *Plutella xylostella* L.

Multi Virus Biopesticide Formulation for Crucifer Lepidopteran Pest

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ABSTRACT

Nuclear polyhedrosis virus (NPV) is known for high epizootic levels, safe to natural enemies due to host specificity and environmentally friendly. Previously, formulated *Spodoptera litura* NPV (FNPV) in powdered form was effective to control *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 spray dried by using mini spray dryer. The efficacy test was conducted on *S. litura*, *Plutella xylostella*, *Hellula undalis* and *Crocidolomia binotalis* larvae in laboratory and field conditions. The efficacy test was conducted up to twelve months to determine the effectiveness of FMNPV which stored in room's temperature. Results showed FMNPV was effective to control targeted larvae in laboratory and field conditions.

Keywords: Nuclear polyhedrosis virus (NPV), Biopesticide, *Plutella xylostella, Spodoptera litura, Hellula undalis, Crocidolomia binotalis*

Exploring the Biodiversity and Virulence of *Beauveria bassiana* for the Management of Diamondback Moth Infecting Crucifers

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ABSTRACT

Biodiversity of fungal entomopathogens is very high across the country in different isolates. Extensive survey was conducted and 15 different indigenous isolates of *Beauveria bassiana* was collected. The genetic diversity of the isolates was identified by the sequence analysis of the internal transcribed spacer (ITS) region and BLAST similarity searches. All the fifteen *B. bassiana* isolates were grouped together under Clade – 1 and the out-group members combined together as Clade – 2. Clade – 1 was further subdivided into two subclades. BbI3, BbI4, BbI5, BbI6, BbI7, BbI8, BbAI4, BbAI18 and BbAI19 isolates were grouped together in subclade – 1 and BbI1, BbI2, BbAI5, BbAI6 and BbAI10 in subclade – 2. Virulence nature among the isolates varied significantly though they belong to the same clade. Effective strains BbI8 and BbI3 was further mass multiplied in optimized media and blended with different carrier materials as formulated product. The shelf life of the product at different concentrations was recorded as 23.67 x 10^8 g^{-1}, 22.67 x 10^{12} g^{-1}, 14.67 x 10^{16} g^{-1} and 7.33 x 10^{20} g^{-1} CFU, respectively after 180 days of storage. The formulated product was evaluated for its efficacy against DBM under both pot culture and field conditions. Under pot culture experiments the highest cumulative mean population reduction of 74.85 and 69.66 per cent was recorded in cabbage and cauliflower, respectively. In field conditions NMBbB at 6 g L^{-1} was significantly superior and recorded the highest cumulative reduction of 68.65 and 69.91 per cent in cabbage during two seasons respectively. Next to Chlorantriniprole 18.5 SC standard check, the highest per cent reduction of 68.64 and 62.12 in cauliflower was recorded during two seasons by NMBbB at 6 g L^{-1}.

Keywords: *Beauveria bassiana*, Diamondback moth, Biodiversity, Entomopathogens.

Nanoparticles as Novel Insecticide against Diamondback Moth, *Plutella xylostella* L. in Cauliflower

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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 potential to revolutionize the existing technologies used in various sectors including insecticides in agriculture. Nanomaterials, have potential to be used as crop protection agents. Nanoparticles absorbed into the cuticle of the insects by physiosorption and cause death of insects purely by physical means when applied on leaves and stem surface. Studies were carried out on the synthesis, characterization and toxicity of Nano SiO_2, ZnO, Ag, TiO_2, Zeolite, *Bacillus thuringiensis*, Cry1Ab toxin, NeemAzal, Profenofos, Lamda cyhalothrin and Flubendiamide against *P. xylostella* in cauliflower. The above nano particles were synthesized and characterized by Particle Size Analyzer, scanning electron microscope (SEM), Transmission electron microscope (TEM), Energy Dispersive X-Ray Spectroscopy (EDX), X- ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). 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 (38.928), 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 nano particles SiO_2, caused more mortality to *P. xylostella* next to flubendiamide and Bt Cry1Ab toxin.

Keywords: Nanoparticles, Insecticides, Diamondback Moth, *Plutella xylostella*, Cauliflower

The Evolutionary Study of two DBM Parasitoids with its Virus from *Diadegma fenestrale* and *Diadegma semiclausum*

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ABSTRACT

The genus Diadegma is a well-known parasitoid group and some are known to have symbiotic virus so called polydnavirus (PDV, more specifically Ichnovirus, IV). The presence of the IV in the Diadegma species has already been identified more than a decade ago. Previously we reported a DfIV, 62 genomic segments, 247kb from *D. fenestrale* which parasitized wide range of lepidopteran species (Generalist). However, DsIV from *D. semiclausum* which parasitized in the *P. xylostella* (Specialist) has 48 genomic segments, 208kb. Finally, 123 ORFs were re-annotated (repeat element protein, 41; cysteine motif protein, 11; viral innexin, 6; viral ankyrin, 8; polar residue rich protein, 7; N gene, 3; Neuromodulin protein, 2 and not assigned gene, 45). DsIV also have most of lepidopteran immunosuppression gene families and 103 ORFs annotated (repeat element protein, 36; cysteine motif protein, 8; viral innexin, 7; viral ankyrin, 6; polar residue rich protein, 7; N gene, 3; and not assigned gene, 36).
Certainly, although viral species-specific segments exist, two IVs showed high similarity in most of segments. However, DfIV have a greater number of genes such as cysteine motif protein and viral ankyrin. Two Diadegma species also showed difference in mitochondrial genome structure. Therefore, we concluded that two species have their own evolutionary linage depending on the lepidopteran hosts with its own symbiotic virus.

Keywords: *P. xylostella*; *D. fenestrale*; *D. semiclausum*; Polydnavirus; Ichnovirus

Relative Efficacy of Some Microbial Bio-Pesticides against *Spodoptera litura* (Lepidoptera: Noctuidae)

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ABSTRACT

Spodoptera litura (F.) causes considerable economic damage to brassica crops including cabbage and cauliflower in many countries. In the present study, two bacterial fermented derivatives such as Suspend 5 SG (Emamectin benzoate) and Ambush 1.8 EC (Abamectin) and three entomopathogens for example Bacillus thuringiensis (Bt), Beauveria bassiana and S-NPV were evaluated against *S. litura* larvae through different application methods (such as topical, leaf-dip and combination) with different concentrations. Among two bacterial fermented derivatives, Suspend 5 SG was found very effective even in low concentrations against *S. litura* while Ambush 1.8 EC was found less effective at recommended dose. Ambush 1.8 EC provided better control when applied with 4-5 times higher dose compared to recommended dose. Among three entomopathogens, S-NPV and Bt were found very effective against *S. litura* larvae while *B. bassiana* was found moderately effective. For all five bio-pesticides, the mortality of *S. litura* larvae dependent on the dose, post exposure time and application methods. Leaf-dip method is found effective for Suspend 5 SG, S-NPV and Bt, while this application method was not found suitable for achieving higher mortality using Ambush 1.8 EC. However, *B. bassiana* was found less effective in all the application methods. From this study it can be concluded that Suspend 5 SG, S-NPV and Bt can be applied to control *S. litura* larvae effectively.

Keywords: Emamectin benzoate, Abamectin, *Bacillus thuringiensis*, *Beauveria bassiana*, S-NPV

Evaluation of Biopesticides along with Predatory Birds in Suppression of Diamondback Moth, *Plutella xylostella* in Cabbage

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ABSTRACT

The experiments were laid out in 2017-18 to evaluate the biopesticides along with role of predatory birds for suppression of Diamond back moth, *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 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 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) 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: Diamond back moth, *Plutella xylostella*, biopesticides, predatory birds

Evaluation of Efficacy of Entomopathogenic Fungus for Suppression of Aphids Infested in Mustard

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ABSTRACT

Rapeseed mustard is one of the cruciferous oilseed crops grown in India. Numbers of limiting factors are responsible for the low yield of this oilseed and the insect pests are one of the major constraints for the poor yield followed by diseases. Entomopathogenic fungi have been considered to be pathogenic to a wide range of insect-pests including aphids, thrips and white fly. Its efficacy as a tool of biological control of mustard aphid, *Lipaphis erysimi* was tested under field conditions. Based upon observations, it was concluded that among the entomopathogenic fungus, *Verticillium lecani* was most effective fungi for the control of mustard aphid, a predominant species of aphid in rapeseed and mustard crop in subtropical regions of Indian subcontinent. The results depicted that the mustard aphid population declined significantly a week after applications of this fungus. During the year 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. lecani*. 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*, *entomopathogenic fungus*, management.

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

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ABSTRACT

Current management strategies to control major pests of cabbage including diamondback moth (DBM), *Plutella xylostella* (L), rely heavily on insecticides. There are concerns that overuse of insecticides will lead to the development of resistance and negative effects on non-target organisms. The purpose of this study was to evaluate alternatives to chemical control 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, 2) collards, 3) roselle, 4) cabbage treated with Entrust®, 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 Diamondback moth (DBM), *Plutella xylostella* (L), below the control. 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 with companion plants with 10 and 20 families of predators and parasitoids were recorded, respectively. In the family *Trichogrammatidae*, plots treated with Entrust® had significantly lower counts than all the other treatments. Higher numbers of anthocorids and carabids beetle were recorded in plots treated with marigold. 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

Insect Bioassays and Identification of Resistance Sources to Major Insect Pests in Chinese cabbage

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ABSTRACT

Brassica breeding in the past decades has been made considerable efforts in increasing yield and quality but did not focus on improving insect resistance. Availability of accurate and efficient techniques to assess the resistance or susceptibility of plant germplasm would facilitate identification of resistance sources which is the first step toward developing insect resistant cultivars. Choice and no-choice bioassays are used to evaluate insect resistance, which allowing for determination of antixenosis and/or antibiosis and thus the selection of plants with the highest levels of resistance. However, these are very time and labor intensive and not easily applicable to large plant populations required in breeding for polygenic traits. Developing a rapid and valid screening protocols would be very useful for identification of resistant sources and improve Brassica breeding. At World Vegetable Center (WorldVeg), 700 Brassica accessions were evaluated for resistance to three major insect pests: aphids, diamondback moth, and cabbage webworm. Moderate resistance to aphids was detected in four accessions belonging to *Brassica juncea* (B464, B487, B488, and B490). In addition, two accessions belonging to *B. campestris* ssp. *chinensis* (B95, B583) were moderate resistance to diamondback moth, and four Chinese cabbage accessions were low resistance to cabbage webworm. Both *B. juncea* and *B. campestris* ssp. *chinensis* can be crossed with heading Chinese cabbage *B. campestris* ssp. *kinensis* and introduced resistance genes into heading cabbage type. More efforts are required for screening more WorldVeg Brassica germplasm to identify higher levels of resistance for pre-breeding programs aiming to develop insect resistant lines.

Keywords: Aphids, cabbage webworm, choice and no-choice bioassays, diamondback moth, genetic sources

Impact of Trap Type, Trap Color, Trap Height and Pheromone Lure-Distance for Trapping Diamondback Moth, *Plutella xylostella* in Cabbage Ecosystem

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ABSTRACT

The diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Plutellidae: Lepidoptera) is the most devastating insect pest of cruciferous crops throughout the world, infesting particularly cabbage, broccoli and cauliflower. In the present study, number of parameters were evaluated that affect pheromone-based trapping, including different traps, trap color, trap height and effect of pheromone lure-distance in trapping. During the study, water traps trapped maximum males than cross vane, delta, funnel and wing vane traps. In a color choice test, *P. xylostella* adults preferred green over blue, yellow or white colored water traps. Green colored traps with pheromone lures caught more adults than identical traps without lures, suggesting that DBM is influenced by both olfactory and visual cues. Trap height experiment revealed that maximum catches were obtained when the traps were set at 0.3 m above the crop canopy level. In effect of pheromone lure-distance test, traps located at 0, 5 and 10 m from cabbage field attracted more male moths than those at 15 and 20 m. It is evident from the studies that pheromone baited green colored water traps (Wota - T) installed at 0.3 m above the crop canopy at 10 m distance were most effective in trapping maximum male DBM adults in cabbage. An attempt was also made on the effectiveness of the pheromones in combination with need-based insecticides under farmers' field conditions revealed that a trap density of 40 traps/acre recorded the highest number of moths trapped with highest per cent population reduction at both Bangalore and Kolar locations with a maximum cabbage head weight and yield. Pheromone traps installed plots had also significantly increased *Cotesia plutellae* (parasitoid) population with highest C: B ratio when compared to farmer's practice (only insecticides), indicating the greater potential for mass trapping and integrated pest management programs on DBM.

Keywords: Sex pheromones, *Plutella xylostella*, Standardization of trapping technique and Cabbage

Preliminary Test of Attract Efficiency by Spray Glue Mixed Odor for the Striped Flea Beetle, *Phyllotreta striolata* (Fabricius)

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ABSTRACT

The Striped flea beetle, *Phyllotreta striolata* (Fabricius) is one of the important pests of cruciferous vegetables in Southeast Asia and in Taiwan, which often causes major economic losses. Integrating many pest control methods, in addition to chemical control, yellow sticky paper is a very common method to catch the flea of adults. Because the basic ecological behavior research for the flea is inadequate, using yellow sticky paper is still not obvious for prevention. According to previous study, some components of cruciferous plants contain mustard to lure the striped flea beetle. Therefore, this study tempted to use spray glue mixed with mustard mixture, directly sprayed on plastic plate or treasure bottle. It is easy to lure the adult flea by the odor of mustard mixture. After three weeks of test, the treatment containing mustard oil composition was significantly 2 times higher than that of the yellow sticky paper ($p < 0.05$). The result showed the potential of industrial application by spray glue with the mixture. The follow-up research should be developed.

Keywords: Striped Flea Beetle, Attract, Spray Glue.

Effect of Flowering Yellow Rocket (Brassicaceae) on Crucifer Insect Pests and Beneficial Insects

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ABSTRACT

This study tests whether the presence of flowering yellow rocket, *Barbarea vulgaris* R. Br. (Brassicaceae), could affect 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 densities were reduced by flowering *B. vulgaris*. Flowering reduced the attractiveness of *B. vulgaris* to *P. xylostella*, 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 ladybugs *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, *Barbarea* plants 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 plants flower the second year.

Keywords: *Barbarea vulgaris*, *Coccinella septempunctata*, *Diadegma insulare*, *Eurydema ornata*, *Plutella xylostella*

Session 5: Insecticide Resistance and Management in Crucifer Pests

Managing Insecticide Resistance in Asia

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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 between insecticide manufacturers, as well as 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 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.

The authors would like to acknowledge the CropLife Asia and AIRAC members who have been instrumental in establishing the Asia IRAC (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, Yanqing Wang).

Keywords: Insecticide, resistance, IRAC, AIRAC, diamondback moth

High Resistance *Plutella xylostella* to Spinosad Exhibited Low Cross Resistance to other Insecticides

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ABSTRACT

Insecticides are vastly applied to Brassicaceae family in the field in order to manage the infesting pests especially the *Plutella xylostella* (Diamondback moth, DBM). However, the effectiveness of the insecticides is greatly compromised by the resistance developed by DBM. In Malaysia, despite the popular use of insecticides in the field, there is lacking of information regarding the insecticide's 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) strain. The LC_{50} of the strain was determined whenever possible. The LC_{50} of G15 was 490.63 ppm, giving 42.81-fold resistance ratio (RR) when compared to 11.5 ppm of LC_{50} Parent generation. The population was allowed to 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} ranging 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 further verified 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 in the present finding 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

Effects of Temperature on the Performance of Deltamethrin-Resistant and Insecticide-Susceptible Field Strains of Diamondback Moth

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ABSTRACT

Worldwide, excessive 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 collected from the field in southeast Queensland, Australia and divided into two sub-populations in the laboratory. One population (= non-selected) was maintained with no further exposure to any insecticides while the other population (= deltamethrin-selected) was maintained under a regime of intermittent selection with deltamethrin in the laboratory. Both populations were reared at constant temperatures of 10, 15, 20, 25, 30 and 35 C (12: 12, L: D) and the effect of rearing temperature on various traits was investigated. At the time of experimentation, the deltamethrin-selected population was >15-fold less susceptible to deltamethrin than the non-selected population. Development time, adult longevity, male pupal weight, female pupal weight and fecundity of both populations declined significantly with increasing temperature. Overall, the deltamethrin-selected insects developed more quickly than non-selected insects and there was a significant interaction between rearing temperature and deltamethrin resistance status on development time. Similarly, female pupal weight was significantly affected by temperature and deltamethrin resistance status and there was a significant interaction between these main effects. Although deltamethrin-selected insects and non-selected insects laid eggs when reared at 10 C, $\leq$ 1% of these eggs were fertile. While female non-selected 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*, temperature, abiotic stress; fecundity, fertility.

Development, Implementation and Monitoring of an Insecticide Resistance Management Strategy for Diamondback Moth in the South Pacific.

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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 2103, the susceptibility DBM field populations to 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, information on which was supplied with every sale. 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 chlorotraniliprole 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 chlorotraniliprole, but all were very susceptible to AgChem-Bt. Annual collections of DBM populations from crops through to 2017 demonstrated significantly reduced levels of resistance to these key products and the susceptibility 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, chlorotraniliprole, selective insecticide, pyrethroid.

Insecticide Resistance Monitoring and Management of Diamondback Moth, *Plutella xylostella*, in Hawaii and Taiwan

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ABSTRACT

Diamondback moth populations are exposed to constant insecticide selection pressure under year-round crop production in the tropical areas such as Hawaii and Taiwan. A window-based insecticide rotation program was implemented in Hawaii since 2003 after multiple crop failures caused by over reliance on 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 farmer associations in production areas participated the pilot program. Monitoring program 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 show 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 at Changhua area to record *P. xylostella* insecticide susceptibility in rotation (Zhutang) versus cocktail mixing (Xizhou) management using two-window management approach. Significantly higher (two sample t-test, $p < 0.05$) insecticide

Impact of One Carboxylesterase on Tolfenpyrad Resistance of *Plutella xylostella*

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ABSTRACT

The diamondback moth (*Plutella xylostella*) is currently considered the biggest threat to cruciferous plants, as it has developed resistance to many insecticides. This study utilizes insecticides commonly used in the field to select resistant moths. Field moths collected from the wild (Xihu in Changhua) showed increased susceptibility about long periods of time without insecticide contact, and this can be used as a reference for duration of insecticide cessation. Using Next Generation Sequencing (NGS) data, we compared four resistant strains (chlorantraniliprole, emamectin benzoate, tolfenpyrad, and flubendiamide) with our current F1 lab strain. Four metabolic enzyme genes with more than 3-fold difference in FPKM values were chosen as candidate gene, and the four insecticides were used for selection. Expression levels of candidate genes in the fifth generation of these strains were tested using quantitative real-time PCR (qPCR) and expression levels of one carboxylesterase was shown to be heightened in tolfenpyrad-resistant strains. Esterase inhibitor TPP (triphenyl phosphate) given during synergism tests was shown to significantly increase tolfenpyrad susceptibility in tolfenpyrad-resistant strains, thereby proving that esterase inhibitors can increase tolfenpyrad susceptibility. Finally, dsRNA interference of carboxylesterase expression showed a 4.9-fold decrease in expression levels after 72 hours in comparison with the control group. The test group was given tolfenpyrad after RNA interference and a post-72h comparison with the control group showed that mortality rates increased from 4.1% to 43.8% under the diagnostic dose and LC_{50} mortality rates of tolfenpyrad increased from 46.9% to 85.4%, proving that reduction of expression levels in this carboxylesterase also decreased diamondback moth resistance to tolfenpyrad.

Keywords: Diamondback moth, Insecticide resistance, Resistance selection, Tolfenpyrad.

Inheritance, Stability and Cross Resistance of Emamectin Benzoate-Resistant *Plutella xylostella*

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ABSTRACT

Diamondback moth (DBM, *Plutella xylostella* L.) is one of the main insect pests of cruciferous vegetables. It has developed insecticide resistance to almost all insecticide ingredients. To postpone or delay the development of insecticide resistance in and to extend life of insecticide use in *P. xylostella*, rotated use of insecticide with different mode of action is the main tactics in insecticide resistance management (IRM). The first step to implement IRM strategies is to understand the potential of insecticide-resistance development, and inheritance, stability and cross resistance of insecticide resistance in field diamondback moth. In this study, the inheritance, stability and cross resistance of emamectin benzoate-resistant (Em-R) *P. xylostella* were conducted. The results showed that resistance of emamectin benzoate in *P. xylostella* is inherited as an incomplete dominant and autosomal trait governed by polygene. In the absence of selection pressure, the emamectin benzoate resistance declined very fast, decrease from 414-fold to 22-fold within 10 generation. There was relatively low cross resistance of Em-R *P. xylostella* to chlorantraniliprole and flubendiamide (~7-fold), no cross resistance to metaflumizone, spinosad, fipronil and mevinphos (≦3-fold), and low negative cross resistance to chlorpyrifos (-2.2-fold). These results are important for management of emamectin benzoate resistance in the field *P. xylostella*.

Keywords: *Plutella xylostella*, emamectin benzoate resistance, inheritance, cross resistance.

Bifenazate Resistance and Mutations in Mitochondrial Cytochrome B of *Tetranychus urticae* Koch (Acari: Tetranychidae)

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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 extended to many fruits and vegetables for mite control before 2018. To understand the resistance profile of bifenazate in two-spotted spider mite, toxicity bioassays and resistance selection against mite population collected from Chiayi county of Taiwan were conducted every two weeks in the laboratory. After 25 months of selection, the resistance ratio of F52 compared with the parent was 23.6 times; after 30 months of selection, the resistance ratio increased significantly to 267.5 times. Comparison of the cd1 helix of mitochondrial cytochrome b Qo pocket alignment of sequences from 10 individuals of the resistant strain with its parent showed one nucleotide substitution, which all resulted in 128th amino acid substitution from hydrophobic isoleucine to hydrophilic threonine of F61; but only 90% of F52 carried this amino acid variation. Two other nucleotide substitutions were also discovered with the increase of resistance. At the 133th amino acid, 30.8% of parent are alanine and 69.2% are valine, while 100% of F52 and F61 are valine. At the 139th amino acid, 15.4% of parent are threonine and 84.6% are isoleucine, but 100% of F52 and F61 are isoleucine. 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 the termination of bifenazate selection, the above three nucleotide substitutions still exist and the resistance does not decrease, which indicates the bifenazate resistance obtained through nucleotide substitutions is stable. To decelerate the development of bifenazate resistance of the two-spotted spider mite population, we suggest that do not use bifenazate continuously, and rotate bifenazate with acaricides of different mode of action to ensure the control efficacy.

Keywords: Bifenazate Resistance, Mite, Mitochondrial Cytochrome *B*, Nucleotide Substitution.

Functional and Genetic Characteristics of Diamide Insecticides Resistance in *Plutella xylostella*

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ABSTRACT

The diamondback moth (*Plutella xylostella*) is a globally distributed and important economic pest, and it has developed resistance to all conventional insecticide classes used in the field. The Chlorantraniliprole is a new chemical class of insecticide, diamide that acts as a conformation-sensitive activator of the insect ryanodine receptor (RyR). In the present study, a field strain (16.3-fold resistance to chlorantraniliprole) was collected in Korea and lab-selected with chlorantraniliprole for more than one year. The resulting strain presented 2,157-fold resistance to chlorantraniliprole. A point mutation (G4946E) in the RyR gene was observed at a high frequency in the resistant strain. Enzyme assays indicated that glutathione S-transferase (GST) and P450 activity in the resistant strain were 2.4- and 1.96-times higher than that of the susceptible strain, respectively. The expression of the RyR, GST and CYP321E1 gene was higher in the resistant strain than in the susceptible strain. The F1 progeny resulting from reciprocal crosses did not reveal maternal effects or a diamide-susceptible phenotype, which suggests an autosomal nearly recessive mode of inheritance. The resistant strain exhibited high cross-resistance to flubendiamide (5,910 fold) and showed no cross-resistance to spinetoram, spinosad, indoxacarb, and metaflumizone. These results can serve as an important basis for guiding the use of insecticides in the field

Keywords: Diamide; Chlorantraniliprole; Ryanodine receptor; Inheritance; Cross-resistance

Strategies to Manage Insecticide Resistance

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ABSTRACT

Insects and mites cause an estimated annual $470 billion in losses to agricultural crops globally. Insecticides and miticides remain the dominant control tactic used against pest arthropods and have an estimated annual market value of > $16 billion. 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 of more insecticides.

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 essential 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 helpful (i.e., number of genes involved and their frequency in the population, the mechanism(s) of resistance including via increased metabolism or less sensitive target site, whether they are dominant or recessive, and whether they carry a fitness cost). Unfortunately, generally these factors in the insect 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. While there are many variations in how insecticides can be applied (e.g. high or low doses, with or without synergists, etc.), there are three general IRM strategies that have engendered the most discussion for IRM: rotations, mosaics and mixtures. Rotational strategies are based on the rotation 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 when the subsequent insecticides are deployed. A mosaic strategy is similar to a rotation but allows the use of multiple insecticides during the same time period but in different spaces. 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. 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. Rotation of a single effective insecticide over some period of time is always as good or better than use of a mosaic strategy. 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. 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 window in which a different insecticide is used, then a fourth window, etc. Use of this strategy requires a suite of insecticides that act differently on the target insect (preferably with different modes of action) but also requires coordination and compliance by growers to be effective. Successful examples of the window strategy will be presented.

Keywords: Genetic engineering, biotechnology, pest management

Field Monitoring of Pesticide Residues and Integrated Management Strategy for Diamondback Moth

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ABSTRACT

Diamondback moth (DBM), *Plutella xylostella* L., is an important pest of cruciferous vegetables. It is difficult to control due to the pesticide resistance has been quickly developed. In views of this, the related researches have been conducted in Taiwan Agricultural Research Institute (TARI) from 1973, and the resistance and interaction between different pesticides are elucidated. The results from previous studies indicated that carbamates, synthetic pyrethroids and insect growth regulators are susceptible to decomposition by multi-functional oxidases in DBM, in consequence of quickly developing of resistance. In order to provide information for the management of DBM, we have surveyed the field resistance of DBM population continuously in the main vegetable producing areas. The results from recent years monitoring shown that good insecticidal effects of Spinetoram, Emamectin Benzoate, Indoxacarb, Tolfenpyrad and Cartap are observed. However, the chlorantraniliprole is almost in-effective to DBM. There is significant difference in pesticide resistance among different regions, this indicated that the habits of pesticide using of farmers are significantly associated with resistance. In order to alleviate the resistance of DBM to various types of pesticide, it is important to simplify the types of pesticide and use different mechanisms to avoid resistance development. The resistance of DMB to pesticides in the field can be delayed by careful selecting effective pesticide and taking turns in different mechanisms, to achieve sustainable and effective prevention and control.

Keywords: Diamondback moth (DBM), pesticide resistance, field monitoring, integrated management strategy

Session 6: Genetic Approaches to Manage Crucifer Pests

Genetically Engineered, Self-Limiting Diamondback Moths: Results from Greenhouse, Field and Modeling Studies

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ABSTRACT
Biologically-based pest management approaches for diamondback moth (DBM) are sorely needed to ensure sustainable production of crucifer crops with minimal hazard to humans and the environment. A promising approach utilizes genetic engineering to create 'self-limiting' insects that carry a gene that, after mating with wild counterparts, prevents female offspring from surviving. Sustained releases of self-limiting males will lead to pest population declines. We have used a self-limiting strain of DBM (OX4319L) in a series of studies conducted in the laboratory, greenhouse and open-field to explore its potential for pest management.

Laboratory studies found no differences in mating competitiveness or population growth rates between the OX4319L and a wild-type strain. Few differences in longevity were found between strains. Studies in a wind tunnel indicated that OX4319L males responded to a synthetic DBM pheromone in a similar manner as individuals from three wild-type strains of DBM. Collectively, these laboratory studies indicated OX4319L males were similar to wild-type males, except for the formers' self-limiting trait.

In greenhouse experiments, introductions of OX4319L males into wild-type populations led to rapid pest population decline, and then elimination. In separate experiments on broccoli plants, relatively low-level releases of OX4319L males in combination with broccoli expressing Cry1Ac (Bt broccoli) suppressed population growth and delayed the spread of Bt resistance.

In a series of mark-release-recapture field studies with OX4319L males and its wild-type counterpart, the dispersal, persistence and field survival of each strain were measured. In most cases, no differences were detected in these parameters.

Using results from these studies, mathematical models were developed that indicate release of OX4319L males will offer efficacious pest management.

Keywords: Genetic engineering, biotechnology, pest management

Diversity of Cruciferous Pests: Genetic Analysis of Flea Beetle and *Pieris rapae* Populations from South-East Asia Based on Mitochondrial Cox1 Gene

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ABSTRACT
The Coleopterous Flea beetle and Lepidopterous *Pieris rapae* are the most destructive and devastating pests that threaten 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, mitochondrial *cytochrome c oxidase 1* (mt*cox1*) marker gene was utilized. Here, 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 36 different haplotypes of *Pieris* and Flea Beetles, respectively. Furthermore, Tajima's *D*, Fu's *Fs* and Analysis of Molecular Variance (AMOVA) of pair wise comparison (*Fst*) 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>9) and lower nucleotide diversity (0.00891) in Vietnam. In addition, no genetic differentiation between Taiwan and Thailand populations of *Pieris rapae* was reported through AMOVA analysis. Interestingly, phylogenetic analysis revealed two putative individuals of *Pieris* from Laos and Vietnam grouping together with *P. canidia*, which are genetically distinct across all other *P. rapae* populations. Overall, the information gleaned from this analysis based on mt*cox1* sequences, has broad implications for quarantine and designing integrated pest management strategies especially in target countries of South-east Asia where it is recently expanding.

Keywords: Flea Beetles, *Pieris Rap*ae, Population Genetics, Phylogenetics, Integrated Pest Management.

Tissue Specific Transcriptomic Response to Legume Feeding in Diamondback Moth

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ABSTRACT
Diamondback moth (DBM, *Plutella xylostella*) is a major pest of Brassica crops, however, in 1999 DBM were unexpectedly reported infesting sugar-snap pea (*Pisum sativum*) in Kenya. Host plant range expansion of this key agricultural pest represents the potential to severely impact agriculture outside the Brassicaceae. Here we perform tissue-specific transcriptome sequencing on Kenyan pea-adapted and wild-type DBM 4th instar larvae, to identify differentially expressed genes that may be associated with adaptation to legumes

Eggs of the two stains were collected and placed on either *Pi. sativum* or *Brassica napus* and reared to the 4th instar. Transcriptome libraries were then prepared for larval head capsules, three pools of 20 samples, and midgut tissue, three pools of five samples. RNA sequencing was performed on the Illumina platform and produced an average of 24 million paired end reads per library.

Differential gene expression analysis revealed large numbers of genes were differentially expressed between pea-adapted and wild-type head capsules (n = 1485) and midgut (n = 1584) tissues when reared on *B. napus*. However, when pea-adapted DBM were reared on alternate host plants, a total of 629 and 759 genes showed significant (corrected p<0.05) differential expression in head capsule and midgut tissues respectively. Furthermore, both tissue types displayed differential expression of key genes involved in secondary metabolite detoxification and digestion pathways.

These results signify an important step in identification of key genes and pathways that may contribute to host plant preference and range expansion in diamondback moth.

Keywords: Diamondback moth, Adaptation, Host plant preference, RNAseq

Phylogeographical Structure in Mitochondrial DNA of Diamondback Moth (*Plutella xylostella*) Population in Southeast Asia

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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 *P. xylostella* population in Southeast Asia, especially in Cambodia, Laos, 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, Mustard green, pak-choi, radish, and Kohlrabi) in target countries. A total of 52 different population 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. However, the results suggested that *P. xylostella* has formed two putative subspecies (which are yet to be differentiated based on morphological characters) in Southeast Asia, as indicated by the high pairwise F_{ST} values (0.31–0.45). The high F_{ST} values (0.44-0.45) of *P. xylostella* population in Taiwan compared to Cambodia and Malaysia population seem to indicate a different sub-species. The negative Tajima's *D* and Fu's F_S values showed the recent demographic expansion of *P. xylostella* population in Cambodia, Laos and Vietnam. Thus, this study confirmed the presence of two putative sub-species in *P. xylostella* in Southeast Asia. Hence, the genetic differences in *P. xylostella* population should be carefully considered while designing the pest management strategies in different geographical regions.

Keywords: *Plutella xylostella*, mitochondrial cytochrome c oxidase I, phylogeny, population structure

Session 7: At the Farm and Landscape Level: Barriers to and Innovations for Management of Crucifer Pests

Development and Validation of a Bio-Pesticide Package for the Control of Major Insect Pests on Pak-Choi in Cambodia

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ABSTRACT

Leafy brassicas including pak-choi are the most important vegetables, which are extensively grown and consumed in Cambodia. Lepidopteran caterpillars especially diamondback moth (*Plutella xylostella*) and common armyworm (*Spodoptera litura*) and flea beetles (*Phyllotreta* spp.) are the most devastating pests of pak-choi. Hence, pak-choi producers overwhelmingly rely on the application of chemical pesticides in an attempt to produce blemish free produce. In order to reduce the pesticide misuse and/or over-use, 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) on pak-choi in Kandal, Kampong Chhnang, Svay Rieng and Prey Veng provinces of Cambodia during 2015- 2018. The results showed that *B. thuringiensis* formulations were able to reduce the population of *P. xylostella* on pak-choi. *M. anisopliae* formulation, Real M-62® was also found to be effective against *P. xylostella* and *S. litura* in most of the trials on pak-choi. Interestingly, Real M-62® was also effective against *P. striolata* beetles in most of the trials in the current study. However, neem leaf extract did not show consistent results in the current study. The yield of pak-choi was significantly higher in *B. thuringiensis* and *M. anisopliae* treated plots consistently in 2015 trials, but the yield did not differ significantly in 2016 trials. The IPM package demonstrated the pest suppression consistently in pak-choi during 2016-2018 trials. It also led to significant yield increases. Hence, the IPM package can be a better alternative to chemical pesticides in managing the key insect pests on pak-choi in Cambodia. After validation in major brassica production locations, these IPM packages can be promoted for large-scale adoption.

Keywords: Bio-Pesticides, Neem leaf extract, IPM, Diamondback Moth, Common Armyworm, Flea Beetles

Development and Validation of an Integrated Pest Management Package for the Control of Major Insect Pests on Cabbage in Lao PDR

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ABSTRACT

Brassicas vegetables are the most important crops, which are 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. Hence, brassica producers mostly rely on the application of chemical pesticides to control the pests, and in fact they sprayed more frequently than the farmers in Cambodia and Vietnam. In order to reduce the pesticide misuse and/or over-use, 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 results showed that the IPM package was able to reduce the shot-hole damage by flea beetle, and the populations of *S. litura* as well as *P. xylostella* significantly on cabbage across the locations. The effectiveness of IPM package was on par with Farmers' practice in reducing *C. pavonana* on cabbage in all the locations. Interestingly, *P. rapae* population was the lowest in abamectin treatment in most of the trials, and it was followed by the IPM package. The yield of cabbage was also significantly higher in abamectin treated plots (51.88 t/ha), followed by the IPM package (48.38 t/ha). The IPM package demonstrated its effectiveness in reducing the pest damage in cabbage, besides yield increases. Hence, the IPM package can be a better alternative to chemical pesticides in managing the key insect pests on cabbage in Lao PDR. After validation in major brassica production locations, the IPM package can be promoted for large-scale adoption.

Keywords: IPM, abamectin, diamondback moth, common armyworm, cabbage cluster caterpillar, imported cabbage worm, striped flea beetle

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