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Proceedings of the 2018 International Symposium on Proactive Technologies for Enhancement of Integrated Pest Management of Key Crops

主編 石憲宗 張宗仁

Edited by
Hsien-Tzung Shih and Chung-Jan Chang

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Preface

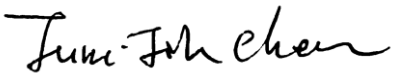
In order to respond to the impacts of climate change that has been intensifying the extreme climate, as well as serious ongoing pest and disease problems on agricultural production system, the Taiwan Agricultural Research Institute has been working with domestic and foreign agricultural research institutions on different themes over the years to reduce the impacts of such problems on the agricultural economies.

In 2012, to tackle the research bottlenecks, investigated all of the programs on the management of crop pests at that time and self-assessment were launched by the Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ) and research institutions, respectively, and new programs included those that need to introduce domestic and foreign experts or new technologies to shorten their research schedules have been followed. In the meantime, TARI also organized “The 2013 International Symposium on Insect Vectors and Insect-Borne Diseases” in 2013, and invited a total of 18 domestic and foreign experts from USA, Japanese, Australian and Taiwan; the lecture topics were divided into taxonomy, biology, ecology and integrated pest management. Through the scientific exchanges, introduce new technologies, mutual visits, etc., researchers in Taiwan have broadened their view and bilateral collaboration on many research items have also been initiated.

This year (2018), a total of 23 domestic and foreign experts to serve as speakers, including 10 Taiwan experts, 7 Japanese experts, 4 US experts, 1 Australian expert and 1 Korean expert have participated in this symposium. The lecture topics of the 2018 International are divided into (A) "Binational collaborative achievements on agricultural pest management between Taiwan and USA" and (B) "Improving the effectiveness on the integrated pest management", which includes (1) the proactive technologies for enhancement of integrated pest management, (2) regulation and application of pesticides, (3) management strategies for important plant diseases, and (4) integrated pest management strategy with regards to climate change.

We look forward to this international symposium, which like the one held in 2013, will become a discussion platform for cooperation between Taiwan and foreign scholars. We sincerely hope that all speakers and other participants can fully engage in the discussion of the specific targeted issues in these three days. Future potential cooperation issues will effectively solve the common research bottlenecks, and look forward to joining the international agricultural science and technology research projects to enhance the vision of Taiwan's

agricultural science and technology research. Finally, I would like to give thanks to the Council of Agriculture for supporting this symposium. My sincere thanks also extended to the staff members of TARI for their endeavor and assistance in planning and preparing the event. I have the confidence that the outcomes of this symposium will certainly be fruitful and successful. Once again, to all the distinguished speakers and participants, welcome to Taiwan and the campus of TARI. To every one of you, thank you for your support and for coming to this very meaningful occasion.



Junne-Jih Chen, Ph.D.
Director General
Taiwan Agricultural Research Institute
Council of Agriculture, Taiwan ROC
September, 2018

序

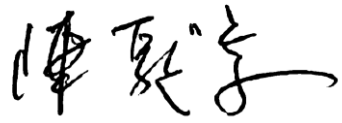
為因應全球區域經濟整合的趨勢、氣候暖化與極端氣候及關鍵病蟲害對農業的影響日益加劇，農業試驗所歷年來一直與國內外農業研究機關，就不同主題共同合作，期能降低病蟲害問題對農業生產所造成的影響。

2012 年起農委會動植物防疫檢疫局及所轄試驗研究單位，透過盤點農業有害生物研究計畫項目及研究瓶頸，啟動新的國際合作的中程議題，期望可強化台灣農業有害生物的研究基礎。為此，農業試驗所舉辦「2013 年媒介昆蟲與蟲媒病害國際研討會」,共計邀請 18 位國內外專家擔任演講者，包括澳洲專家 1 位、日本專家 1 位、美國專家 9 位與台灣專家 7 位。演講主題則可歸類為分類學、生物學、生態學與整合管理。透過這樣的互動，及後續雙方的交流互訪，大幅擴展國內研究人員的視野，也啟動與各國在各個議題的多面向的合作。

今年(2018 年) 的研討會共計邀請 23 位國內外專家擔任演講者，包括台灣專家 10 位、日本專家 7 位、美國專家 4 位、澳洲專家 1 位與韓國專家 1 位參與。所有演講內容可歸類為「分享國際合作重要研究成果」和「可提升有害生物整合管理效能」兩大主軸，其中後者又包括前瞻防治技術、因應氣候變遷的蟲害整合管理策略、重要病害管理與農藥管理等四個課題。

我們期待本次國際研討會，可如 2013 年的國際研討會一樣，成為台灣與國外學者合作研究的討論平台，衷心盼望所有講者與聽眾，可在這三天針對特定的目標議題，充分討論未來的潛在合作議題，有效解決彼此的研究瓶頸，並期待藉此加入國際農業科技的跨國研究項目，提升台灣農業科技研究的視野。最後，感謝農業委員會支持本次研討會，也感謝農業試驗所工作人員為規劃和籌備活動所做的努力和協助。相信本次研討會的成果肯定會取得豐碩成果。也再次向所有演講者和來賓，表達歡迎來到台灣和農業試驗所，感謝您們對此重要會議的支持。

行政院農業委員會農業試驗所
所長



中華民國 108 年 9 月

SYMPOSIUM AGENDA

Date: September 4-6, 2018

Venue: International Conference Hall, Taiwan Agricultural Research Institute (TARI), Taichung, Taiwan, ROC

Sponsor: TARI, Council of Agriculture (COA), Taiwan, ROC

Co-organizers: COA, Executive Yuan, Taiwan, ROC;
Taiwan Entomological Society;
The Plant Protection Society of Republic of China

Schedule	Program/ schedule	Speaker (Institution)	Moderator (Institution)
September 4th (Tues.)			
08:30-09:20	**Registration**		
09:20-09:50	**Opening Session:** **Opening Remarks:** **Welcome Address (Dr. Junne-Jih Chen, Director-General, TARI, COA)**		
09:50-10:10	**Tea Break and Group Photo**		
Session 1: Binational collaborative achievements on agricultural pest management between Taiwan and USA 台美農業有害生物國際合作成果			
10:10-10:30	Introduction to the 2018 international symposium on proactive technologies for enhancement of integrated pest management on key crops	**Dr. Hsien-Tzung Shih** (Applied Zoology Division, TARI, COA, Taiwan, ROC)	**Ms. Ching-Hua Kao, Director** (Applied Zoology Division, TARI, COA, Taiwan, ROC)
10:30-11:20	Identification of the egg parasitoids of Auchenorrhyncha (Hemiptera) of economic importance in Taiwan: collaborative research between Taiwan Agricultural Research Institute and University of California at Riverside scientists	**Dr. Serguei V. Triapitsyn** (Department of Entomology, UC Riverside, CA, USA)	
11:20-12:00	Insights into the biology, diversity, and origins of weedy red rice and the use of phylogeographical structures to control its seed-mediated contamination in Taiwan	**Dr. Dong-Hong Wu** (Crop Science Division, TARI, COA, Taiwan, ROC)	
12:00-13:10	**Lunch Break**		

Session 2: Important, proactive and potential technologies for the enhancement of integrated pest management 提升有害生物整合管理之重要、前瞻與具應用潛力之技術			
13:10-13:50	The eradication projects and the preventative control for quarantine pests in Okinawa, Japan	**Dr. Tsuyoshi Ohishi** (Okinawa Prefectural Plant Protection Center, Naha, Okinawa, Japan)	**Dr. Chien-Chung Chen** (Applied Zoology Division, TARI, COA, Taiwan, ROC)
13:50-14:30	Development of vibrational control methods for grapevine pests in California	**Dr. Rodrigo Krugner** (Crop Diseases, Pests and Genetics Research Unit, San Joaquin Valley Agricultural Sciences Center, USDA-ARS, Parlier, CA, USA)	
14:30-15:10	Identify key virulence gene as a control target to mitigate Pierce's disease of grape	**Dr. Hong Lin** (Crop Diseases, Pests and Genetics Research Unit, San Joaquin Valley Agricultural Sciences Center, USDA-ARS, Parlier, CA, USA)	
15:10-15:30	**Tea Break**		
15:30-16:10	Utilization of electric pulse power for agricultural products cultivation: forest environment control	**Dr. Shoji Ohga** (Department of Agro-environmental Sciences, Faculty of Agriculture, Kyushu University, Japan)	**Dr. Chien-Chung Chen** (Applied Zoology Division, TARI, COA, Taiwan, ROC)
16:10-16:50	Exploitation of new attractants for fruit fly pests of economic importance	**Dr. Ritsuo Nishida** (Department of Applied Life Sciences, Kyoto University, Japan)	
16:50-17:30	**Panel Discussion 1** Moderators: Dr. Chung-Jan Chang; Ms. Ching-Hua Kao, Director; Dr. Chien-Chung Chen		
September 5th (Wed.)			
08:30-09:00	**Registration**		
09:00-09:40	Recent progress in optical control of insect pests with light and color	**Dr. Masami Shimoda** (Institute of Agrobiological Sciences, NARO, Japan)	**Dr. Chien-Chung Chen** (Applied Zoology Division, TARI, COA, Taiwan, ROC)
09:40-10:20	Migration analyses and predictions for migratory insect pests toward Japan	**Dr. Akira Otuka** (Institute of Agricultural Machinery, NARO, Japan)	
10:20-10:30	**Tea Break**		
Session 3: Regulation and application of pesticides 農藥管理與應用			

10:30-11:20	Recent regulations on safe plant protection materials in Taiwan	**Dr. Ming-Hsun Ho** (Taiwan Agricultural Chemicals and Toxic Substances Research Institute, COA, Taiwan, ROC)	**Ms. Ching-Hua Kao, Director** (Applied Zoology Division, TARI, COA, Taiwan, ROC)
11:20-12:00	Potential application of immunoassay for the detection of pesticide residues in agriculture	**Ms. Shu-Chen Chang** (Applied Zoology Division, TARI, COA, Taiwan, ROC)	
12:00-13:10	**Lunch Break**		
13:10-13:50	Development and utilization of plant vaccines in Japan	**Dr. Yasuhiro Tomitaka** (Kyushu Okinawa Agriculture Research Center, NARO, Japan)	**Ms. Ching-Hua Kao, Director** (Applied Zoology Division, TARI, COA, Taiwan, ROC)
13:50-14:30	Application of control-release formulation of biochemical reagent in the integrated pest control	**Dr. Wei-Jyun Chien** (Pheromone Center; Department of Applied Chemistry, Chaoyang University of Technology, Taiwan, ROC)	
14:30-15:10	Insecticide horizontal transfer from Tephritid male lures containing reduced risk pesticides	**Dr. Ming-Yi Chou** (Agricultural Extension Center, National Chung-Hsing University)	
15:10-15:20	**Tea Break**		
Session 4: Management strategies for important plant pests 重要病蟲害管理策略			
15:20-16:00	Control strategy for rice stripe virus transmitted by small brown plant hopper (*Laodelphax striatellus*)	**Dr. Mitsuru Okuda** (Central Region Agricultural Research Center, NARO, Japan)	**Dr. Ruey-Jang Chang, Director** (Plant Pathology Division, TARI)
16:00-16:40	Winter climate and cultivar effects on severity of Pierce's disease in table grapes	**Dr. Lindsey P. Burbank** (Crop Diseases, Pests and Genetics Research Unit, San Joaquin Valley Agricultural Sciences Center, USDA-ARS, Parlier, CA, USA)	
16:40-17:20	**Panel Discussion 2** Moderators: Dr. Chung-Jan Chang; Ms. Ching-Hua Kao, Director; Dr. Chien-Chung Chen; Dr. Ruey-Jang Chang, Director		
September 6th (Thurs.)			
08:30-09:00	**Registration**		
09:00-09:40	Occurrence and management of basal	**Dr. Jin-Hsing Huang** (Plant Pathology Division, TARI, COA, Taiwan,	**Dr. Ruey-Jang Chang, Director** (Plant Pathology

	stalk rot of water bamboo in Taiwan	ROC)	Division, TARI)
09:40-10:20	Application of a bio-control agent for controlling strawberry anthracnose in Taiwan	**Dr. Tsung-Chun Lin** (Plant Pathology Division, TARI, COA, Taiwan, ROC)	
10:20-10:30	**Tea Break**		
Session 5: Integrated pest management strategy with regards to climate change 因應氣候變遷之蟲害整合管理策略			
10:30-11:20	An hierarchical method for identifying current and emerging pest threats under climate change uncertainty	**Dr. Darren Kriticos** (Commonwealth Scientific and Industrial Research Organisation (CSIRO); University of Queensland, Australia)	**Dr. Chung-Jan Chang** (University of Georgia, USA)
11:20-12:00	Integrated management strategy of pests in response to climate change - A case study of oriental fruit fly	**Dr. Yu-Bing Huang** (Applied Zoology Division, TARI, COA, Taiwan, ROC)	
12:00-13:10	**Lunch Break**		
13:10-13:50	Pest distribution model comparisons in integrated management of key crop pests: empirical studies of Taiwan and Southeastern Asia	**Dr. Li-Hsin Wu** (National Pingtung University of Science and Technology, Taiwan, ROC)	**Dr. Chung-Jan Chang** (University of Georgia, USA)
13:50-14:30	Introduction of a model simulation program and its application in a demonstrative expert system to predict optimal spraying time for pest: PopModel1.5 and Pest Forecast System	**Dr. Kyung San Choi** (Rural Development Administration, National Institute of Horticultural and Herbal Sciences, Research Institute of Climate Change and Agriculture, South Korea)	
14:30-15:00	**Panel Discussion 3** Moderators: Dr. Chung-Jan Chang; Ms. Ching-Hua Kao, Director; Dr. Chien-Chung Chen; Dr. Ruey-Jang Chang, Director		
15:00-15:20	**Tea Break**		
15:20-16:30	**Discussion on the international research collaboration among Taiwan, USA, Japan, South Korea and Australia** Moderators: Dr. Chung-Jan Chang; Ms. Ching-Hua Kao, Director; Dr. Chien-Chung Chen; Dr. Ruey-Jang Chang, Director		

Introduction to the 2018 International Symposium on Proactive Technologies for Enhancement of Integrated Pest Management of Key Crops

Hsien-Tzung Shih [1, 2], Yu-Bing Huang [1], Ming-Yaw Chiang [1], and Ching-Hua Kao [1]

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ABSTRACT

The 2018 International Symposium on Proactive Technologies for Enhancement of Integrated Pest Management on Key Crops, including "Binational collaborative achievements on agricultural pest management between Taiwan and USA" and "Improving the effectiveness on the integrated pest management", which includes the proactive technologies for enhancement of integrated pest management, regulation and application of pesticides, management strategies for important plant diseases, and integrated pest management strategy with regards to climate change. We invited 22 experts from Taiwan, Australia, the United States, Japan and South Korea to be our symposium speakers. The sponsors sincerely hope that the symposium will not only provide proactive technologies or innovative pest management strategies for Republic of China, but also serve as a platform for future cooperation between domestic and foreign scholars, which can make a significant contribution to the advancement of Taiwan and global agriculture.

Keywords: agriculture, international cooperation, integrated pest management, proactive technology

Taiwan is located in eastern Asia and is one of the many islands in the East and Southeast Asia island arcs. It covers an area of 36,000 square kilometers and is the 38th largest island in the world. Nearly 70% of the area is mountainous and hilly. The topography and altitude on the western coast vary greatly. Due to the fact that the Tropic of Cancer runs through the middle of Taiwan, the climate of Taiwan covers the tropical and subtropical zones. The north of the Tropic of Cancer is the subtropical monsoon climate and the south is the tropical monsoon

climate. The population of Taiwan has exceeded 23.57 million in May 2018. Based on the gross domestic product (GDP) report released by the International Monetary Fund (IMF) in 2013, Taiwan was the 27th largest in the global economy. Taiwan's economic structure has been shifted to high-tech industries, which replaced labor-intensive industries e.g., plastic products manufacturing, textile, and garment industry, etc., while the proportion of agriculture in GDP fell from 35% in 1952 to 2%.

Although the proportion of agriculture industry has decreased, the deep foundation in science education in the past has enabled Taiwan's agriculture to quickly integrate cross-domain technology when faced with man-made and natural disadvantages. That surely provides a great opportunity to promote and make agriculture an important industry again to Taiwan. In order to respond to the impacts on the global and regional economic integration due to climate change and the intensification of extreme climate, and the aging of rural employment in Taiwan, the Taiwan Agricultural Research Institute (TARI) has been working with domestic and foreign agricultural research institutions on different themes over the years to reduce the impacts of such problems on the agricultural economies. Using the various themes of this 2018 international symposium as an example, it can be traced back to 2012 when Mr. Bao-Liang Chen, the then chief executive of the Animal and Plant Health Inspection and Quarantine Promotion Group in the International Collaboration Field in the Council of Agriculture, Executive Yuan of ROC, entrusted the Taiwan Agricultural Experimental Institute to consult with different agricultural institutes of COA to tackle the research bottlenecks with self-assessment after he has investigated all of the programs on the mnagement of crop pests. The proposed programs included those that need to introduce domestic and foreign experts or new technologies to shorten their research schedules. It is clearly illustrated in the themes of this symposium that fits the purpose of proposed program introducing foreign experts and technologies, which can be used as the basis for handling international cooperation plans.

Therefore, in 2012, we have prioritized five issues as a medium-term plan for international cooperation, including "Insect vectors and insect-borne diseases", "Integrated pest management strategy with regards to climate change", "Proactive technologies for enhancement of integrated pest management", "Regulation and application of pesticides" and "Establish an information platform for transnational and inter-disciplinary pest management and natural enemy applications". Based on the above-metioned five issues, we expect to strengthen the research base of agricultural pests in Taiwan. Afterward, through the program

review, researchers from the different institutes of COA would be sent to the United States, Australia, Japan and other countries for short-term studies ranging from 2 weeks to 2 months, with that we traced the cooperation effects between Taiwan researchers and foreign experts. Besides, we will establish a list of foreign experts as potential international partners in the research of integrated pest management in Taiwan based on the recommendations from the above researchers or other domestic experts. Some experts will be invited to serve as lecturers for specific technologies, and some of them conduct cooperative research through bilateral scientific and technological exchange programs signed by Taiwan and foreign countries, and in the meantime we also organized "**T**he 2013 International Symposium on Insect Vectors and Insect-Borne Diseases" and "2018 International Symposium on Proactive Technologies for Enhancement of Integrated Pest Management on Key Crops" in 2013 and 2018, respectively, by inviting specific domestic and foreign experts. In the 2013 symposium, we invited a total of 18 domestic and foreign experts to serve as speakers, including 7 Taiwan experts, 11 US experts and 1 Australian expert. This year (2018), a total of 23 domestic and foreign experts to serve as speakers, including 10 Taiwan experts, 7 Japanese experts, 4 US experts, 1 Australian expert and 1 Korean expert.

We look forward to this international symposium, which like the one held in 2013, will become a discussion platform for cooperation between Taiwan and foreign scholars. We sincerely hope that all speakers and other participants can fully engage in the discussion of the specific targeted issues in these three days. Future potential cooperation issues will effectively solve the common research bottlenecks, and look forward to joining the international agricultural science and technology research projects to enhance the vision of Taiwan's agricultural science and technology research.

Identification of the Egg Parasitoids of Auchenorrhyncha (Hemiptera) of Economic Importance in Taiwan: Collaborative Research between Taiwan Agricultural Research Institute and University of California at Riverside Scientists

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ABSTRACT

An overview of the three collaborative projects between Taiwan Agricultural Research Institute (TARI), Taiwan, ROC, and University of California at Riverside, USA, scientists is given, as follows: 1) collecting (H.-T. Shih), rearing (S.-H. Huang), and identification (S. V. Triapitsyn) of the egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) of rice leafhoppers and planthoppers (Hemiptera: Cicadellidae and Delphacidae) of economic importance in west central Taiwan; 2) rearing (H.-T. Shih) and identification (S. V. Triapitsyn) of the egg parasitoids (Mymaridae and Trichogrammatidae) of the leafhopper *Kolla paulula* (Walker) (Cicadellidae), vector of the phytopathogenic bacterium *Xylella fastidiosa*; and 3) a preliminary study of the biodiversity and taxonomy of Mymaridae in Taiwan (S. V. Triapitsyn) based largely on the specimens in the insect collection of TARI.

Keywords: rice pests, Cicadellidae, Delphacidae, *Kolla paulula*, vector, Mymaridae, Trichogrammatidae, egg parasitoid, biological control, biodiversity, taxonomy, Taiwan

INTRODUCTION

Since 2013, scientists from the Taiwan Agricultural Research Institute (TARI), Wufeng, Taichung, Taiwan, ROC (H.-T. Shih) and the Entomology Research Museum, University of

California, Riverside, California, USA (S. V. Triapitsyn) have collaborated on the three research projects which are important for applied entomology in Taiwan, particularly for biological control of some key agricultural pests belonging to Auchenorrhyncha (Hemiptera). Several years later, S.-H. Huang (Chiayi Agricultural Experiment Station, TARI) joined the effort on one of these projects, rearing egg parasitoids of rice leafhoppers and planthoppers in Chiayi and Yunlin counties of Taiwan.

As outlined by Triapitsyn [15], the most common egg parasitoids of Auchenorrhyncha belong to two families of Hymenoptera, Mymaridae and Trichogrammatidae of the superfamily Chalcidoidea. Worldwide, they are largely responsible for the natural control of leafhopper (Cicadellidae), planthopper (Delphacidae), and treehopper (Membracidae) species, including some economically important pests. Therefore, knowledge of their taxonomy (for correct identification) and biology is very important for biological control, ecological, and biodiversity studies. Triapitsyn [15] also provided an overview of the history and current status of the taxonomy and biology of these two groups of egg parasitoids in Taiwan.

COLLABORATIVE PROJECTS

1. Collecting, rearing, and identification of egg parasitoids of rice leafhoppers and planthoppers of economic importance in west central Taiwan

Project rationale. Recently, Triapitsyn [20] gave a critical analysis of the history of prior identifications of the egg parasitoids (Mymaridae only) of rice leafhoppers and planthoppers in Taiwan. Chu and Hirashima [3] summarized the earlier Taiwanese literature on the natural enemies of rice leafhoppers and planthoppers, while Hirashima [5] provided information on their survey in Taiwan. Lin [6] and Chen [1] also reported on the egg parasitoids of rice leafhoppers and planthoppers in Taiwan, while Chen and Yu [2] studied *Anagrus* Haliday egg parasitoids of brown rice planthopper, *Nilaparvata lugens* (Stål) (Delphacidae). Unfortunately, almost no voucher specimens from these earlier studies in Taiwan could be found in the museum collections either there (including that of TARI) or in Japan [20], so it was impossible, with a very few exceptions [20], to verify their identity with confidence; it seems likely that most of them were misidentified at species level [20]. Therefore, we decided to conduct a new survey of egg parasitoids of rice leafhoppers and planthoppers in west central Taiwan (Taichung as well as Chiayi and Yunlin counties) with the aim of identifying them properly. The latter, however, has proven to be not a small task by itself as a lot of confusion exists in Asia (in the Oriental

region and southern part of the eastern Palaearctic region) in regard of their identification. For instance, without providing much of critical analysis, Gurr *et al.* [4] summarized many published records of parasitoids of Asian planthopper pests of rice; among the mymarid egg parasitoids they included both valid species and, separately, their several current synonyms, as well as some obvious misidentifications, species of unknown identity such as *Gonatocerus longicrus* (Kieffer), and a genus which is not known to have species that parasitize eggs of either leafhoppers or planthoppers (*Anaphes* spp.).

Specimen collection and preparation. During 2015 and 2016, egg parasitoids of selected species of rice pests belonging to the Auchenorrhyncha were reared in Chiayi and Yunlin counties by S.-H. Huang by exposing their sentinel eggs in rice plants in pesticide-free rice fields. For obtaining sentinel eggs, 30-day-old rice plants were used; each pot contained 3 to 5 tillers. Five gravid female adults each of the green rice leafhopper *Nephotettix cincticeps* (Uhler) (Cicadellidae) or the planthoppers (Delphacidae) *Laodelphax striatella* (Fallén) (small brown planthopper), *Nilaparvata lugens* (Stål) (brown planthopper), and *Sogatella furcifera* (Horváth) (whitebacked planthopper) were released into caged pots with rice plants for oviposition. The host leafhoppers or planthoppers were removed from the cages after 24 hours, and the pots with their sentinel eggs were taken to the rice fields and exposed there for parasitization for 48 hours. Then the rice plants with both unparasitized (Fig. 1) and parasitized (Fig. 2) eggs were taken back to the laboratory. The tillers with host eggs were transferred to vials lined with a moistened filter paper to avoid desiccation. The parasitized host eggs turned reddish (Fig. 2). The emerging parasitoids were collected daily, labeled, and placed in 95% ethanol for storage at -20°C until shipped to H.-T. Shih and/or S. V. Triapitsyn for identification and record keeping.

In Taichung, egg parasitoids of rice leafhoppers and planthoppers were collected using a Malaise trap (Fig. 11) and yellow pan traps in an experimental organic rice field at TARI, Wufeng, during S. V. Triapitsyn's visit in October 2016. Thereafter several Malaise traps were installed in rice fields in Taichung by H.-T. Shih and M.-R. Tzeng, who collected and processed the samples. The obtained data will be used for both parasitoid identification and for obtaining information on their species composition, abundance, and population dynamics. These will be reported elsewhere in a forthcoming joint publication to be submitted to the Journal of Taiwan Agricultural Research.

Most of the collected or reared specimens were identified directly in ethanol. Selected specimens were dried from ethanol using a critical point drier, point-mounted, and labeled; these were then sorted to morphospecies, and some of the specimens were slide-mounted by Vladimir V. Berezovskiy. Both ethanol-preserved, pointed- and slide-mounted voucher specimens were deposited in the insect collection of TARI and the Entomology Research Museum, University of California, Riverside, California, USA.

Specimen identification. The most common and abundant parasitoid reared from sentinel eggs of all four aforementioned host species (one leafhopper and three planthoppers) was *Anagrus nilaparvatae* Pang and Wang (Mymaridae) (Figs 3, 4), both in Chiayi and Yunlin counties of Taiwan. *Anagrus nilaparvatae* was originally described from Wushan (Fuoshan), Guangdong, China, as an egg parasitoid of brown planthopper *Nilaparvata lugens* [(7)]. Since then it was reported, in numerous publications partially summarized by Gurr *et al.* [(4)], from many countries in the Oriental and eastern Palaearctic regions as the main egg parasitoid of several economically important leafhoppers and planthoppers in rice field agroecosystems. As noted by Triapitsyn and Berezovskiy [(22)] and Triapitsyn [(15)], *A. nilaparvatae* is morphologically practically indistinguishable from the common, widespread, polyphagous Palaearctic species *A. incarnatosimilis* Soyka (as *A. incarnatus* Haliday). Its proper taxonomic identity was recently figured out as part of this project [(23)] using both morphometric analysis and genetic studies.

The second most common egg parasitoid of rice leafhoppers in Chiayi and Yunlin counties of Taiwan was *Mymar taprobanicum* Ward (Mymaridae), a rather common and almost cosmopolitan species. Illustrated here is a female of *M. taprobanicum* (Fig. 5) reared from eggs of green rice leafhopper *Nephotettix cincticeps*. Also reared in west central Taiwan were a few specimens of *Gonatocerus aegyptiacus* Soyka (Mymaridae) (Fig. 6) from eggs of *Nephotettix cincticeps* and *Oligosita* sp. (Trichogrammatidae) (Fig. 7) from eggs of small brown planthopper *Laodelphax striatella*.

In the Malaise trap installed in the rice field at TARI, also collected were the rice green leafhopper *Nephotettix nigropictus* (Stål) and the zig-zag leafhopper *Maiestas dorsalis* (Motschulsky) (Cicadellidae) (identifications by S. V. Triapitsyn). The known egg parasitoid *Pseudoligosita nephotetticum* (Mani) (Trichogrammatidae) (Fig. 8) was collected there in yellow pan traps but not in the Malaise trap standing next to them, whereas *Anagrus nilaparvatae* was collected in the same location in both types of traps.

2. Rearing and identification of egg parasitoids of the leafhopper *Kolla paulula* (Walker), vector of *Xylella fastidiosa*

In Taiwan, the cicadelline (Cicadellidae: Cicadellinae: Cicadellini) leafhopper *Kolla paulula* (Walker) is a known vector of the phytopathogenic bacterium *Xylella fastidiosa* [8], the causative agent of Pierce's disease of grapes and similar diseases of other affected plants, and thus of particular economic importance. Two species of parasitoids were reared by W.-F. Hung and H.-T. Shih from its eggs in plant tissue of spreading dayflower, *Commelina diffusa* (Commelinaceae), and then identified by S. V. Triapitsyn as *Pseudoligosita nephotetticum* (Mani) (Trichogrammatidae) (Fig. 9) and one male of *Cosmocomoidea* sp. (Mymaridae) (Fig. 10) [as *Gonatocerus* (*Cosmocomoidea*) sp.] [24]. *Pseudoligosita nephotetticum* was redescribed and illustrated; also provided was a summary of the known records of egg parasitoids (Mymaridae and Trichogrammatidae) of other leafhoppers from the tribe Cicadellini in the entire world [24].

3. A preliminary study of the diversity and taxonomy of Mymaridae in Taiwan

General information. Biogeographically, the fairyfly (Mymaridae) fauna of Taiwan is primarily Oriental, although at high altitudes it fits more that of the Palaearctic ecozone. While generic identifications of most Mymaridae in the region are generally relatively easily available, species identifications are still a major problem. Expensive and time-consuming special preservation and mounting techniques, such as critical point drying from ethanol and making microscopic slides in Canada balsam, are usually required to be able to identify most mymarids to species based on morphology. Overall, prior to this study, taxonomic reports on the diversity of Mymaridae in Taiwan were scarce and either included some random identifications of the Taiwanese specimens as parts of broader generic revisions or those of specimens of interest to biological control projects, mainly of egg parasitoids of rice pests.

Keys and taxonomic revisions of importance. Keys and taxonomic revisions that include Taiwanese specimens of certain fairyfly genera are unfortunately few, but some can be helpful to recognize some species: Taguchi [9, 10, 11, 12, 13] for a number of genera, Triapitsyn [16] for *Nepolynema* Triapitsyn, Triapitsyn [17] for *Himopolynema* Taguchi (a revision of the Taiwanese species with descriptions of several new taxa), Triapitsyn [18] for *Arescon* Walker, Triapitsyn

[19] for *Alaptus* Westwood, Triapitsyn *et al.* [21] for *Zeyanus* Huber, Triapitsyn and Berezovskiy [22] for *Anagrus* Haliday, etc.

Recent achievements. Triapitsyn [20] just published an annotated checklist of Mymaridae of Taiwan, which includes records of 28 genera and 60 named species. Of these, 13 genera and 33 species were newly recorded from Taiwan, including 5 new species described. Information on their distribution and available host records was also provided, along with illustrations of some species and keys to several genera. In addition, a number of the undescribed species were identified in the course of this study; these await thorough taxonomic revisions of the genera to which they belong. The previous published records of mymarid egg parasitoids of rice leafhoppers and planthoppers in Taiwan were critically analyzed.

This checklist was based largely on the collection of Mymaridae in TARI, almost all mounted on individual microscopic slides, captured mostly by sweeping during the 1950s-1970s by Kwei-Shui Lin (1921-2002), a taxonomist and prolific collector of parasitic Hymenoptera, who worked at TARI in Taipei City before moving to Taichung. He collected almost 3,000 specimens of fairyflies throughout Taiwan (the bulk from Taipei City and its environs). These were examined and identified by S. V. Triapitsyn mostly during visits to TARI in August 2013 and October 2016.

Tian *et al.* [14] reported a surprisingly low generic diversity index for the Mymaridae of Taiwan which they suggested, along with Tibet's, to be significantly lower than that for Fujian, China. However, Triapitsyn [20] demonstrated that to be a misleading conclusion because the mymarid fauna of Taiwan was at that time too poorly known. One would expect, due to the large size of the island, diversity of its habitats, and relative proximity to Fujian, a greater fairyfly diversity than reported, along with some possible endemism (particularly in the mountains). Thus, the mymarid fauna of Taiwan could likely be almost as diverse as that of Fujian.

Looking ahead. Clearly, what is now known about biodiversity and taxonomy of Mymaridae in Taiwan, as summarized recently by Triapitsyn [20], is not enough, primarily because of the lack of systematic collecting efforts of micro-Hymenoptera throughout Taiwan Island and in the surrounding smaller islands using both various modern trapping methods and rearings from known hosts. Likewise, there hasn't been any significant effort to sort, dry, mount, label, and identify any fairyflies from the numerous existing bulk alcohol samples, primarily those from Malaise traps, in any major Taiwanese collections of insects. Thus, more

species are expected to be discovered there once such efforts take place sometimes in the future, provided interest, taxonomic expertise, and appropriate funding become available.

As noted by Triapitsyn [20], at least 5 more genera of Mymaridae are likely to occur in Taiwan, besides those included in that checklist. Indeed, after it was accepted for publication and it was too late to add any taxa to it, one more genus, *Dorya* Noyes and Valentine, was found in Taiwan (John T. Huber, personal communication, material in the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada). Thus, once all or almost all the genera are found and properly recorded from there, the next step would be to compile a key to both sexes of the genera occurring in Taiwan and to identify (and describe, if necessary) species in each genus. The latter, however, may require revising at least the Oriental species of some genera.

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Fig. 1. Unparasitized eggs of brown planthopper *Nilaparvata lugens* (Taiwan).



Fig. 2. Eggs of brown planthopper, *Nilaparvata lugens*, parasitized by *Anagrus nilaparvatae* (Taiwan).



Fig. 3. Female of *Anagrus nilaparvatae*, egg parasitoid of brown planthopper *Nilaparvata lugens* (Lucao, Taiwan).



Fig. 4. Male of *Anagrus nilaparvatae*, egg parasitoid of brown planthopper *Nilaparvata lugens* (Lucao, Taiwan).



Fig. 5. Female of *Mymar taprobanicum*, egg parasitoid of green rice leafhopper *Nephotettix cincticeps* (Gukeng, Taiwan).



Fig. 6. Female of *Gonatocerus aegyptiacus*, egg parasitoid of green rice leafhopper *Nephotettix cincticeps* (Gukeng, Taiwan).



Fig. 7. Male of *Oligosita* sp., egg parasitoid of small brown planthopper *Laodelphax striatella* (Gukeng, Taiwan).



Fig. 8. Female of *Pseudoligosita nephotetticum* (rice field, TARI, Wufeng, Taichung, Taiwan).



Fig. 9. Female of *Pseudoligosita nephotetticum*, egg parasitoid of *Kolla paulula* (Wufeng, Taichung, Taiwan).



Fig. 10. Male of *Cosmocomoidea* sp., egg parasitoid of *Kolla paulula* (Wufeng, Taichung, Taiwan).



Fig. 11. A Malaise trap in the experimental organic rice field (TARI, Wufeng, Taichung, Taiwan).

Insights into the Biology, Diversity, and Origins of Weedy Red Rice and the Use of Phylogeographical Structures to Control its Seed-mediated Contamination in Taiwan

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ABSTRACT

Weedy red rice (WRR) possesses traits, including seed dormancy and shattering, that facilitate its infestation in rice paddies from one crop season to the next. These plants are not only the potential source of pollen-mediated gene flow and hosts for diseases or other pests, but also are competitors for fertilizer due to their vigorous growth. In addition to increased production costs to control this weed, the red pericarp and undesirable eating qualities of WRR lead to reduced product value, consequently putting production constraints on the global rice industry. In rice cultivation regions world-wide, the rice industry is in search of effective ways to control WRR. The rice production system in Taiwan relies on transplanting which is recommended for effective control of weeds in rice fields during the seedling stage. However, the infestation of WRR in rice paddies has become increasingly severe in the past few years in Taiwan. Although WRR occurs at a rate of only 0.5 to 1% in contaminated paddies, it can be spread easily through use of shared field equipment among rice fields. Ratoon cropping or tillage immediately after harvest increases the population densities of WRR, resulting in future yield losses. Effective control strategies for WRR should meet the balance between economic benefit, efficiency, and feasibility. The recommended WRR control measures under a transplanting system begin with irrigating the paddy field after harvest to induce the sprouting of shattered seed, followed by plowing the WRR seedlings into the land. Herbicide is then

applied three times in succession every seven days to kill emerging WRR and to field infestations. The final step is the manual removal of the remaining off-type plants of WRR.

Keywords: weedy red rice, seed shattering, seed dormancy, transplanting system, control strategy

INTRODUCTION

Weeds, soil fertility, availability of fertilizers, and variable climates are the major factors that limit rice growth. Weedy red rice (WRR) and cultivated rice are classified under the same genus and species (*Oryza sativa* L.) and have similar appearance and features; moreover, WRR plants in Taiwan are typically short, making them difficult to distinguish from crop rice, and exhibit rapid growth and high levels of seed shattering and dormancy, all of which are traits that make them invasive weeds. Consequently, once WRR forms a stable population in cultivated fields, controlling it and tracking its distribution patterns become difficult [(29)]. The hazards that WRR causes are detailed as follows: (1) Because of high levels of seed shattering and dormancy, seeds become established readily in the soil seed bank, resulting in plants that become a medium for gene flow as well as pest and disease transmission; (2) The biomass growth advantage of WRR from seedling to maturity leads to nutrient competition; (3) WRR exhibits negative qualities such as a red pericarp and undesirable cooked texture. All of these hazards lead to a sharp rise in rice production costs and decline in the commercial value of cultivated rice, hindering the development of the global rice industry. Hence, governments worldwide have actively searched for solutions to the hazards of WRR [(4)].

In addition, WRR has been an obstacle in the development of rice industries worldwide [(3, 16, 29)]. The invasion of WRR has been particularly problematic in Brazil, South Korea, and the United States of America (USA), where various WRR biotypes are present and rice planting is generally mechanized [(9, 15, 25)]. WRR has reduced rice production in some countries by 10%–50% and prevented subsequent rice planting in some cultivated fields; a density of 35–40 WRR plants per m^2 hampers the production of tall varieties of rice by 60% and that of dwarf varieties by as much as 90% [(22)]. In Japan, where a transplant system is implemented, a WRR density of 5 WRR plants per m^2 reduces rice production by 10% [(20)]. Furthermore, reduced rice production caused by 1–3 WWR plants is equivalent to that of 5–10 plants of barnyardgrass (*Echinochloa crus-galli*). WRR outweighs barnyardgrass in its damage to rice production and is difficult to contain because its appearance is so similar to that of cultivated rice [(21)].

To effectively prevent the invasion, latency, and spread of WRR as well as reinforce the knowledge of rice farmers regarding its detriment to rice industries, this study described the morphological diversity and phylogeographical structures of WRR, and generated control strategies for farmers to reference.

High Morphological Diversity and Adaptability of WRR

WRR is notorious for its high morphological diversity and environmental adaptability. In the *Japonica* rice production zones in Italy, WRR is divided into three variants, namely the long-awned, short-awned, and awnless strains. Long-awned WRR has the longest flag leaves and grains of the three variants; short-awned WRR produces small seeds and exhibits low germination rates; and awnless WRR has wide grains and high germination rates after 30 days of maturity. In particular, long-awned WRR displays the highest morphological diversity and environmental adaptability of all three variants [8]. The WRR in the southern USA has two major variants, namely the strawhull awnless and blackhull awned strains. Strawhull awnless WRR features earlier heading dates than those of cultivated rice, whereas blackhull awnless WRR exhibits later heading dates than those of cultivated rice; both strains have a greater plant height than cultivated rice. In addition to hull color and awn length diversity, the characteristics of the WRR plants vary between strains. The WRR plants having a dense plant architecture and long awns have been noted for their high germination rates and seed vitality, whereas those with a loose plant architecture and no awns were noted for high tolerance to salt [23]. In the *Indica* rice production zones in Thailand, the early generations of WRR were categorized as a mixture between the cultivated crop and weedy biotypes according to the WRR morphology inspection in multiple regions in 2005–2009 [27]. This may have been caused by crossbreeding between the two biotypes [17, 18]; following the accumulation of invading generations, WRR gradually became identical to cultivated *Indica* rice in appearance. Nevertheless, regardless of its generations and regions, WRR exhibits high adaptability to various cultivation methods employed in rice fields because of its early maturity, high levels of seed shattering, and robust productivity [27]. In 2016 Cheng et al. [4] compared the morphological diversity of 521 locally collected WRR strains with that of *Indica* and *Japonica* rice and previously obtained red rice germplasm collections. The results indicated that all 521 WRR plants exhibited earlier maturity, greater seed shattering, more tillers, and shorter plant heights than cultivated rice plants; furthermore, pericarps were consistently red (Fig. 1). In a follow-up principal component analysis, the WRR populations exhibited similar degrees of dispersion with those of the

cultivated rice; their geographic distributions were similar, but the grains of the WRR populations were identical in appearance to the wide and short grains of the early *Indica* crop rice varieties, and profoundly different from the slender grains of recent *Indica* varieties and the short and round grains of *Japonica* varieties. It is speculated that the grain traits of off-type plants were not influenced by human selection when these plants were eradicated intensively from rice nurseries. This indicated that the WRR collections are closely associated with the early rice strains.

Divided Arguments Worldwide on the Origins and Evolution Mechanisms of WRR

The genetic backgrounds of WRR vary considerably between countries, and arguments regarding its origins remain divided among scientific disciplines; however, the various hypotheses pertaining to the evolutionary methods of WRR have been supported by different studies [7]. According to De-Wet and Harlan [6], WRR originated from the adaptation of wild rice to agricultural habitats. In environments where no native wild rice biotypes are present, these can be introduced as a result of seed movement through trade and other means. For example, the WRR in Brazil is hypothesized to originate from Africa during the colonial era [2]. WRR is often closely associated with the genetic background of the rice varieties commonly produced in the areas to which it has been introduced. In Thailand and Malaysia, where rice can be planted every season and the growth areas and heading dates of cultivated rice overlap with those of its wild relatives, the weedy traits of wild rice have been introduced as a result of outcrossing with cultivars [5, 26]; subsequently these descendants evolved into WRR. Moreover, gene flow between wild and cultivated rice has also been identified in the USA [13]. In areas without wild rice, cultivated rice evolves into WRR after the adaptive mutation and de-domestication of the cultivated species and the accumulation of multiple effective mutations [10, 12, 14, 19]. In Brazil, China, South Korea, and the southern USA, WRR apparently originated from a background of closely related *Indica* rice [12, 15, 19, 25], whereas in Italy and California, WRR is speculated to have originated from crossbreeding among the *Japonica* species [8, 29].

According to the whole genome sequencing and domestication-related gene analyses employed by Li *et al.* [12], the WRR in the USA was produced from the de-domestication of cultivated rice and that weedy traits are sustained through selection in a few areas of the genome of the species. Qiu *et al.* [19] compared the de-domesticated genome sections of WRR with the domesticated genome sections of cultivated rice, revealing that the de-domestication of WRR

did not involve changing the genotypes of the cultivated species back into wild genotypes, but it did involve adopting new genetic variations and molecular mechanisms to adapt to changing environments. On the basis of allele frequency distribution, a significant difference was identified among *Indica* and *Japonica* WRR groups in their standing variations and new mutations. Genetic diversity of WRR has been augmented through balancing selections to adapt to complicated survival environments, which is the opposite of the forward selections of cultivated rice. These results enhance existing knowledge of the genetic domestication and de-domestication mechanisms of cultivated crops, enabling an understanding of the environmental adaptation mechanisms of WRR and an exploration of strategies to prevent and eliminate it. However, the origins of WRR vary with its background cultivation ecology. In Taiwan, WRR has become adapted to the transplantation system, which has exacerbated its threat to the rice industry of Taiwan.

Historical Significance, Immediate Causes, and Ultimate Causes of the WRR in Taiwan

In Taiwan's pre-twentieth century agricultural era, cultivated rice primarily consisted of environmentally competitive tall *Indica* cultivars owing to insufficient technology, facilities, and resources. At that time, contamination of cultivated rice with red, black, and glutinous rice landraces, as well as with barnyardgrass, was severe and undermined the quality and appearance of rice products. During 1906–1921, systematic WRR control strategies were conducted to effectively contain the contamination. In 1953, a general survey of the *Indica* rice cultivation area revealed dwarf blackhull long-grain rice as the leading local *Indica* landrace. However, *Indica* red rice, which is drought- and salt-tolerant, was also cultivated by farmers after the primary crop had been harvested in the same year or on arid land; a total of 10 local land races of *Indica* red rice suitable for upland cultivation were recorded to have been planted. In 1959, the local red rice land races became extinct when the highly productive semidwarf "Taichung Native 1" *Indica* variety entered mass production and irrigation infrastructure was improved. Thus, more than 50 years of red rice cultivation was recorded in the rice cultivation history of Taiwan [(11)].

According to a 2005 analysis of rice seed nurseries, most off-type plants in Taiwan were tall, had early maturity, and slender, long grains [(28)]. Further examination revealed that these off-type plants originated from natural crossbreeding, contamination, or unsatisfactory uniformity among the contemporary varieties. Particularly, the samples displayed insufficient

genetic similarities with their neighboring cultivated plants; these samples exhibited large numbers of tillers (36.4), average dwarf height of 73.2 cm, early maturity, and intermediate size grains. Their genetic backgrounds were highly homozygous and were similar to the *Indica* background, rendering the sample plants identical to the recent biotypes of WRR that predominate [(4)].

The rice production and sales policy has recently changed from recommended fallowing (1997) to a system where farmers are subsidized for cultivating leased fallowed lands (2012). Starting from 2003, the Environmental Protection Agency in Taiwan prohibited burning straw to reduce the detrimental effects of high temperature on soil surfaces. However, low-cost production methods such as ratoon stubble and volunteer rice have been applied in some regions during the second cultivation period. After 2012, severe red rice contamination was identified during the second cultivation period even though high-quality rice seed was planted during the previous cultivation period, leading to a drop in the government procurement price for paddy rice. In 2014, rice seed nurseries were invaded by WRR; 5% of registered seed fields (six fields) and 24% of certified seed fields (137 fields) were contaminated with red rice at an average rate of 0.47 ± 0.55%. In 2015, red rice contamination was detected in approximately 43% of the 187 townships in Western Taiwan where paddy rice was harvested for public stocks (data not published). Starting from the second cultivation period in 2014, Taiwan authorities discontinued the procurement of rice produced through perennial roots or seed shattering; however, these low-cost rice production approaches have continued and are a persistent source for the spread of WRR.

Phylogeographical Structures of Weedy Rice

Weedy rice can produce a large number of seeds at maturity and these can be accidentally mixed with cultivated rice seeds during harvest, which may promote the long-distance dispersal of weedy rice seeds due to shipment of seedlings between regions. In Taiwan, farmers grow rice based on a transplanting system, and generally use agricultural machinery during soil preparation, transplanting, fertilizer application and harvesting. The transplanting model is helpful in the control of weeds, and the purchase of seedlings from specialized commercial nurseries should reduce the occurrence of volunteer seedlings. In the field survey of 2015, we collected rice samples from seven counties and found that the contamination of weedy red rice was serious in 83 townships (Fig. 2). The contamination rate of each township is the average

of all paddy fields in the same area. Of the four townships in Taoyuan City, Daxi District had the highest contamination rate of 0.73%, and neighboring Pingzhen, Longtan, and Myrica rubra District possessed red rice contamination rates of 0.17-0.41%. Among the five townships in Miaoli County, contamination rates were highest in Houlong Town, up to 0.51%, and decreased sequentially in the neighboring towns of West Lake, Tofen, Tongxiao, and Yuanli Town, ranging from 0.15 to 0.40%. Similar cases have occurred in Taichung, Changhua, Yunlin, Chiayi and Tainan counties. The contamination rate exceeded 0.6% in Daxi District of Taoyuan City (0.73%), Huatan Township of Changhua County (0.69%), Nantou City of Nantou County (0.74%) and Dongpotential Town of Yunlin County (0.85%). It is worth mentioning that more than half of the eight townships had a contamination rate of 0.20-0.40%, indicating that the invasion of weed type red rice is widespread and serious.

In order to establish an effective control strategy for weedy red rice, the first priority was to identify the degree to which the invasion in Taiwan occurred through seed-mediated contamination or pollen-mediated contamination, and if the transport distances in these two possibilities were significantly different from each other. In Sri Lanka for example, the abundant within-population genetic diversity coupled with limited population genetic structure and differentiation was likely caused by considerable seed-mediated gene flow of weedy rice along with long-distance exchange of farmer-saved rice seeds between weedy-rice contaminated regions [(10)]. Knowledge of genetic diversity and spatial structure of weedy rice populations will facilitate the design of effective methods to control this weed by tracing its origins and dispersal patterns in a given region. In our study, we used information on genetic diversity and geographical location to understand the transmission patterns of Taiwan's weedy red rice. First, we used molecular markers to determine the genetic clusters of weedy rice populations and then measured the level of genetic diversity among different sub-populations. Second, we plotted the genetic cluster information of weedy rice on a map to reveal the geographical distribution of each group.

WRR Control Strategies Adopted in Japan

The WRR control strategies adopted by Japan, which uses a transplantation system similar to Taiwan, can be applied to treat WRR in Taiwan. These strategies have reduced the WRR contamination rate in fields with a WRR density of 100 plants/ 0.1 ha from 2.3% to 0.1%, fulfilling the safe production standard [(20)]. These cultural practices include submerging fields

with shallow water and plowing before planting, applying a three-phase herbicide treatment, or removing off-type plants manually, which are used on 78%, 20%, and 2%, respectively, of the areas treated for WRR. [20]. Although various strategies are applied to eradicate WRR, a minimum rate of 99.6% depletion is required for lowering the contamination rate annually; a 95% depletion rate causes the contamination rate to rise from 5% to 15% in three years [24].

The focal points of WRR control during each cultivation period as used in Japan are listed as follows [20]:

1. Rice variety purity control and management: Only rice varieties qualified through examination are permitted for use; using self-reserved seed or seedlings of questionable purity raises the risks of contamination.
2. Cultivated seed and rice nursery management: When broken grains and by-products of rice milling are used in the tray medium for growing seedlings, they must decay prior to use. Alternatively, rice mills should enforce the elimination of any residual germs within the pericarps. Use of any seed from contaminated fields is forbidden.
3. Pre-cultivation plow management:
    A. Plowing residual rice plants is avoided. The germination periods of WRR are closely related to its dormancy, maturity, and buried depths. WRR plants that are buried at a depth of 1 cm germinate at day 10 and reach a germination rate of 80% at day 14. Those that are buried at a depth of 3 cm germinate at day 14 and reach a germination rate of 70% at day 40. Those buried at 5 cm display no signs of germination even at day 35. These reports explain the factors leading to the emergence of volunteer plants in cultivated fields. Accordingly, plowing should be avoided before any control strategies are applied to prevent seeds from entering the deep layers of soil.
    B. When crop rotation is applied, gramineous selective herbicides should be used alternatively. The dormancy of residual seeds in the deep layers of soil are disrupted and any residual plants are eliminated when plowing. Seed at the soil surface or during fallowing periods may be consumed by birds, lowering germination rates.
    C. A long-period of flooding should be applied before preparing the soil. The finely prepared soil is covered with a shallow flood for a minimum of 30 days before plowing or from the previous cultivation period to reduce the WRR survival rate to

50%.

D. The soil should be managed after each rice harvest. Furthermore, the flooding treatment should be applied before preliminary plowing. One week after the germination begins during the treatment, at which point the sheath has grown to 20 mm, plowing is performed once to lower the germ survival rate by 10%. Nonselective herbicides are the most effective when used before plowing, raising the WRR elimination rate in a field with a contamination density of 12.7 plants per m^2 to as high as 78% when the soil is plowed after germination.

E. The soil should be cleaned thoroughly using high-temperature steam when environmentally friendly cultivation approaches are applied. A self-propelled high-temperature fumigation machine should be used to clean the cultivated fields, thereby eliminating seed in the shallow soil layers and killing the volunteers.

4. Field management after transplantation: A three-phase herbicide treatment is implemented. Pre-germination and post-germination herbicides are applied to the WRR seeds at various depths of soil, once during transplantation, followed by additional applications at seven and 14 days after transplantation. The herbicides butachlor and pretilachlor can be applied three times to effectively eliminate volunteer plants and improve the WRR elimination rate in a field with a contamination density of 12.7 plants per m^2 by 20%. Attention must be paid to the quality of herbicides, time points of their implementation, and maintenance of flooded fields, all of which affect the effectiveness of the herbicide. Moreover, the growth status of WRR must be considered. Selective herbicides are only effective against volunteer plants before or immediately after their germination, but not against seedlings having one or two leaves emerged.
5. Field management after heading:
   A. Off-type plants must be thoroughly uprooted and removed from cultivated fields.
   B. WRR typically exhibits earlier heading dates and higher morphological variation than cultivated rice does. Therefore, the plants that display inconsistent heading dates and plant traits compared with surrounding plants must be removed.
   C. Off-type plants must be uprooted within 14 days after the heading date. The maturity rate of shattered seeds increases from 10% at 14 days after heading to 50%–70% at 21 days after heading.
6. Sequence of harvest: Harvesters must be thoroughly cleaned after use; moreover,

harvesting crops in less contaminated fields must be prioritized over harvesting those in more contaminated fields.

CONCLUSION

WRR exhibits short growth periods and begins its seed shattering early and at high levels. Although soil preparation is performed before transplantation, a high number of residual seeds remain in the soil; the burial depths of these seeds increase through plowing, delaying and prolonging the growth of WRR plants after transplantation. Therefore, flooding must be implemented to thoroughly remove shattered seeds before plowing. Subsequently, selective herbicides should be applied once every seven days for a total of three times to ensure the delay of volunteer plant growth by a minimum of 21 days, which prevents WRR from entering its heading dates by the harvest period and breaking its seed production. Finally, the WRR plants and any other volunteer rice plants surrounding cultivated fields must be removed manually and comprehensively. Systematically understanding the weedy traits and genetic origins of WRR is critical to eliminate it economically and effectively, mitigating its effect on the development of rice industries.

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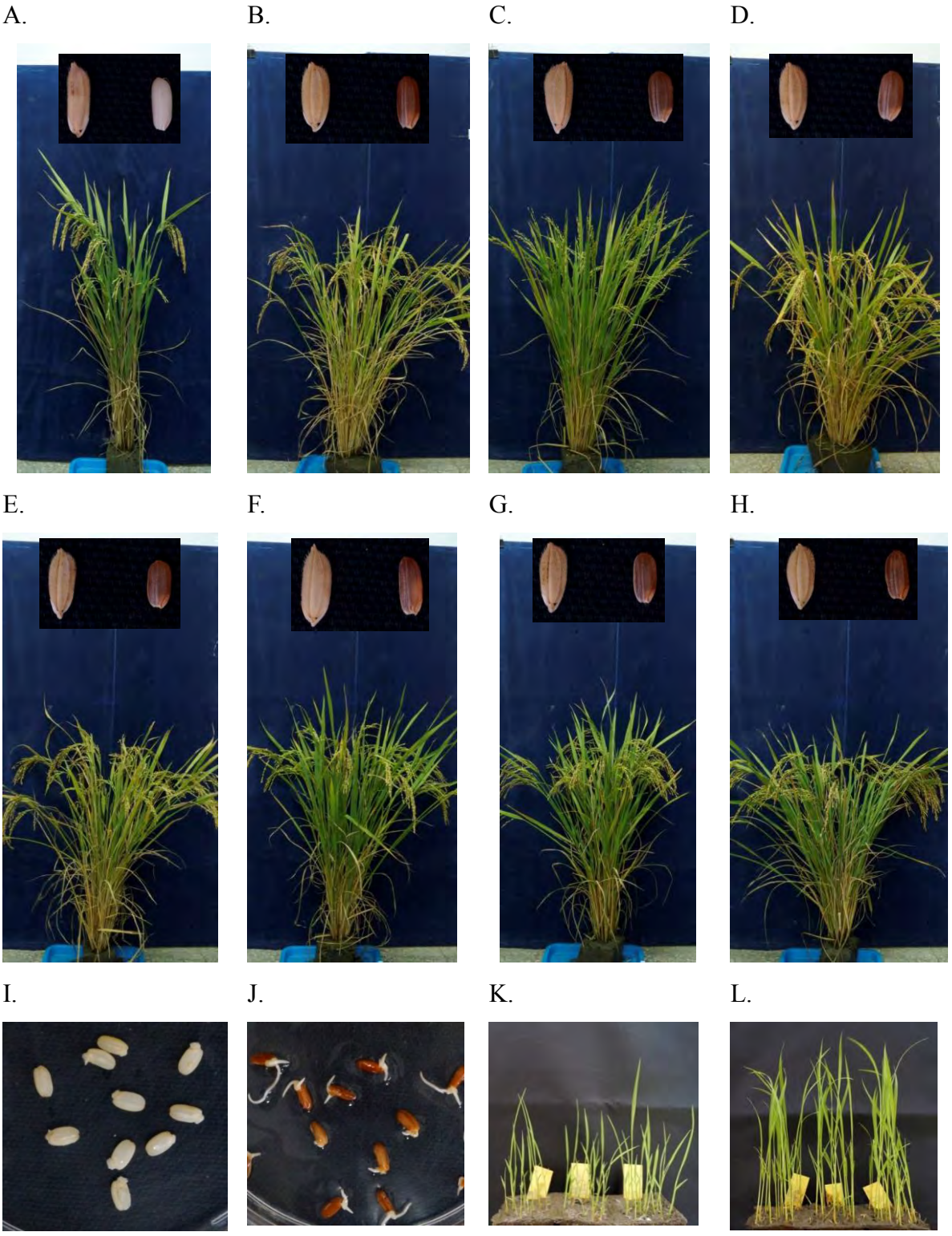


Fig. 1. Morphology, germination and seedling growth of cultivar and weedy red rice. The plant type and grain of "Taichung sen waxy 2" was different compared with the weedy rice accessions (B-H). The check variety "Tainan 11" (I), which was soaked for 3 days obviously germinated slower than weedy rice (J). The seedling growth of the weedy red rice (L) was greater than that of the control "Tainan 11" (K) after 16 days of soaking.

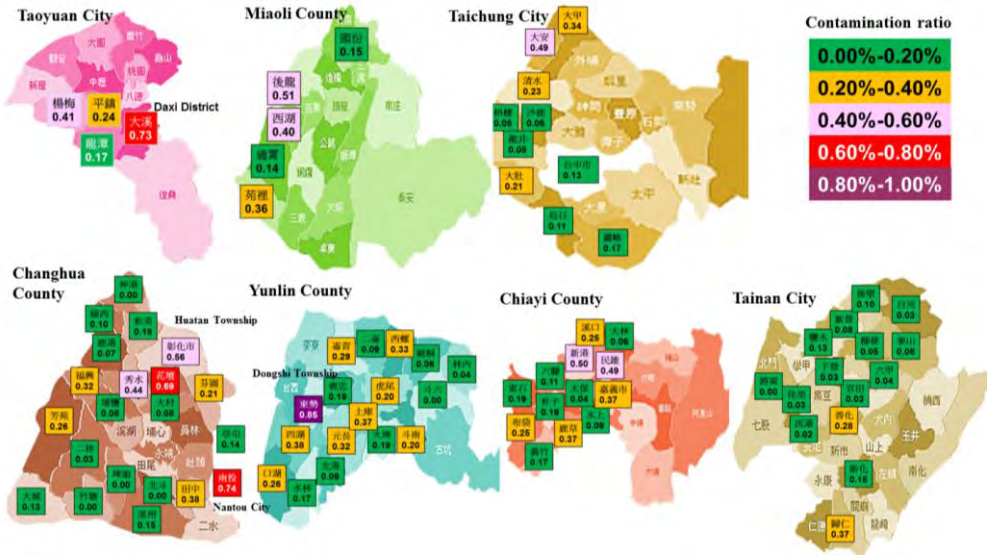


Fig. 2. The contamination ratio of weedy red rice was serious in 83 townships from 7 counties in the field survey of 2015. The contamination rate of each township is the average of all paddy fields in the same area.

The eradication projects and preventative control of quarantine pests in Okinawa, Japan

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ABSTRACT

Of the several exotic insect species in Japan, five quarantine plant pest taxa: the oriental fruit fly, *Bactrocera dorsalis* complex, the melon fly, *Zeugodacus cucurbitae*, the solanaceous fruit fly, *Bactrocera latifrons*, the sweet potato weevil, *Cylas formicarius*, and the West Indian sweet potato weevil, *Euscepes postfasciatus*, have been targeted in eradication programs using the male annihilation technique (MAT) and/or the sterile insect technique (SIT). Although all targeted fruit flies have successfully been eradicated, the incidences of re-invasion by *Bactrocera dorsalis* complex have been increasing in recent years, indicating the necessity for reinforcement and improvement of countermeasures for fruit fly re-invasion and/or recolonization. The program targeted at *C. formicarius* achieved the goal of complete eradication in Kume Island. The program for *E. postfasciatus* has yet to achieve eradication, even though it was initiated at the same time as that for *C. formicarius*. Major technical improvements are required for successful eradication of *E. postfasciatus*.

Keywords: sterile insect technique, male annihilation technique, *Bactrocera dorsalis*, *Zeugodacus cucurbitae*, *Cylas formicarius*, *Euscepes postfasciatus*

INTRODUCTION

Okinawa is located in the southernmost part of Japan, between Kyusyu and Taiwan. Many exotic insects have invaded Okinawa, some of which are now established in this

subtropical region [16, 18]. These invasive insects include some serious quarantine plant pests, and the transport of their host plants from an infested area to the non-infested areas is prohibited. These taxa are capable of inflicting significant economic damage on the agricultural product marketing. Therefore, eradication programs targeting these pests have been conducted in Okinawa, using the sterile insect technique (hereafter, SIT) and/or the male annihilation technique (hereafter, MAT) (Table 1). SIT is an environment-friendly behavioral method [7], wherein a large number of insects are produced, sexually sterilized, and released into the field. The released infertile males compete with the wild males for mating with wild females, resulting in reduction of the wild population via production of sterile eggs [17]. MAT is a target-specific chemical method, where a mixture of male attractant and insecticide is dispersed in the field, resulting in decrease of wild male abundance and the loss of mating opportunities for wild females [60]. Two pest taxa, the oriental fruit fly *Bactrocera dorsalis* complex [6], and the melon fly, (*Zeugodacus cucurbitae* (Coquillett) were eradicated in Japan by 1985 and 1993, respectively [14, 20, 25, 26]. Two other quarantine pests have been successfully eradicated in selected areas. The Solanaceous fruit fly, *Bactrocera. latifrons* (Hendel), was eradicated from Yonaguni Island by 2011 using SIT [8, 19], while the sweet potato weevil, *Cylas formicarius* (Fabricius), was successfully eradicated from Kume Island by 2012 using SIT and MAT [33]. On the other hand, the eradication programs for the West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire) using SIT started in Kume Island and Tsuken Island in 1995 and 2007, respectively [27, 28, 49], but the eradication goals have not yet been achieved, mainly due to limited production of sterile weevils [41].

In this paper, we report the process followed during successful pest eradication programs. We also discuss the risk of re-invasion after eradication, and the counter-measures against them. Finally, we report a current improvement in the mass-rearing system of *E. postfasciatus*, which has been a limiting factor in its eradication program.

Invasion history of and eradication program for *Bactrocera dorsalis* complex and risk of its re-invasion

The oriental fruits fly and its relatives, *Bactrocera dorsalis* complex [6], include several serious pests of fruits and vegetables [2]. The history of invasion and eradication of *B. dorsalis* complex in Japan is summarized in Fig.1. Here, we also show the cases

of Amami Islands (Kagoshima Prefecture) and Ogasawara Islands (Tokyo). *B. dorsalis* complex was first recorded on Okinawa Islands in 1919 [36], on Ogasawara Islands in 1925 [40], and on Amami Islands in 1929 [56]. The eradication program in Amami Islands, using MAT with methyl eugenol as an attractant, begun in 1968, and the eradication was confirmed by Japanese Government in 1980 [64]. In Ogasawara Islands, the eradication program with MAT started in 1975 and the fruit flies were eradicated in 1983 with the assistance of SIT [40]. In Okinawa Islands, the eradication program using MAT was initiated in 1977 and eradication was achieved in 1985 [20, 21].

However, several re-invasion events have been detected in Okinawa with the help of monitoring traps (hereafter, trap) baited with methyl eugenol [24]. Ohno et al [43] reported that more than 300 adult flies were captured by the traps from 1987 to 2008, showing frequent re-invasions even after the eradication. In fact, the numbers of the male captured by the trap and the incidences of recolonization (i.e., detection of infested fruit) have been increasing in Sakishima Islands, especially after 2010 (Fig. 2). During this period, recolonization occurred ten times (Fig. 2).

One of the possible sources of re-invasion is windborne immigration from distribution areas adjacent to Okinawa, such as Taiwan, the Philippines, and mainland China [54]. Summer is considered the season with high risk of re-invasion by wind in Okinawa Islands and Sakishima Islands [4, 43, 54].

To quickly counter the re-invasion, we implement two ways of monitoring measures: i) trap survey, where c.a. 500 traps are checked every two weeks, and ii) a biannual host fruits survey, where c.a. 100,000 fruits are collected and inspected. As a preventive control, pieces of wood fiber boards (hereafter, fiber board) soaked with a mixture of methyl eugenol and insecticide are placed in the area four times a year (in total, 400,000 pieces/year).

In all cases of recolonization, the fruit flies were re-eradicated within a few months, using extra countermeasures [32, 44, 51]. A mixture of protein bait and insecticide were sprayed within a 100- to 200-m radius from the point where infested fruits were detected, host fruits within a 500-m radius were removed, and extra pieces of the fiberboard were distributed within a 2-km radius, at a density of 2–3 pieces per hectare.

As seen above, the risk of re-invasion and recolonization of the oriental fruit fly has been increasing in Okinawa. This indicates the necessity for reinforcement and

improvement of countermeasures for controlling the fruit fly re-invasions and/or recolonizations.

Invasion history of and eradication program for the melon fly, *Zeugodacus cucurbitae* (Coquillett), and risk of its re-invasion

The melon fly, *Zeugodacus cucurbitae* (Coquillett), is distributed widely in temperate, tropical, and sub-tropical regions of the world, with India considered its native area [5]. The fly has been reported to cause damage to 81 host plants, and is a major pest of cucurbitaceous vegetables, particularly bitter gourd (*Momordica charantia*), muskmelon (*Cucumis melo*), and snake gourd (*Trichosanthes anguina*) [31, 35].

The history of invasion and eradication of *Z. cucurbitae* in Japan is summarized in Fig.3. Here, we also show the cases of Amami Islands (Kagoshima Prefecture). The melon fly was first discovered in the Yaeyama Islands in 1919 [36], and 10 years later, it was reported in the Miyako Islands [23]. In 1970, it was discovered on Kume Island [59], and it spread to Okinawa Islands in 1972 [11]. It further expanded its distribution to Amami Islands in 1974 [23].

Prior to the start of the melon fly eradiation project, an experimental SIT project was started on Kume Island in 1972 [12]. Following the successful eradication project on Kume Island, an eradication project for the entire Okinawa Prefecture was initiated in 1979 [14, 21]. By releasing more than 200 million sterile flies per week, the eradication on Miyako Islands, Okinawa Islands, and Yaeyama Islands was achieved in 1987, 1990, and 1993, respectively [4].

As Okinawa is located near South East Asia, where the melon fly currently occurs (i.e., Taiwan), there is always a risk of re-invasion. To prevent re-colonization by accidental invasion from infested areas, the preventive release of 45–70 million sterile flies per week is being carried out continuously. The monitoring has been conducted in the same way as the oriental fruit fly. So far, only a few cases of re-invasion have been detected on the Yonaguni Island. Single male each was caught by monitoring trap in 1993, 1995, and 1996, and a female emerged from a fruit collected in regular host plant survey (Table 2).

Eradication of the solanaceous fruit fly, *Bactrocera latifrons* (Hendel), from Yonaguni Island

The solanaceous fruit fly, *Bactrocera latifrons* (Hendel), is known as a serious pest of solanaceous crops [66]. It is widely distributed throughout South and South-East Asia [66], and has invaded Hawaii [65] and Africa [3]. In Japan, it was first detected in Yonaguni Island, located in the westernmost part of Japan (only 100 km apart from Taiwan) in 1984 [15]. Since there was a concern that *B. latifrons* could spread over the southern islands of Japan by hitchhiking, transportation of infested fruits, and/or the dispersion of adult flies, eradication project for *B. latifrons* using SIT was urgently implemented in 2004. Firstly, protein bait spray and clean culture methods (removing the host fruit) were applied, to suppress the wild population before implementation of the SIT. Mass release of sterile flies was initiated in 2007. About 400,000 sterilized insects were released throughout the island every week. The last detection of wild *B. latifrons* was in March 2009 in a host fruit survey [19]. The Japanese Government confirmed the eradication of *B. latifrons*, based on the surveys of host fruits in 2011 [8]. To check the re-invasion in Yonaguni Island, the host fruits are surveyed two times per year. After the eradication, no fruit infested by *B. latifrons* was detected in the island until 2017.

Invasion history of and eradication program for the sweet potato weevil, *Cylas formicarius* (Fabricius), from Kume Island, Okinawa, Japan

The sweet potato weevil, *Cylas formicarius* (Fabricius), is a destructive pest of sweet potato, *Ipomoea batatas* (L.) Lam. [67]. It also infests wild convolvuaceous plants, such as blue morning glory, *I. indica*, and beach morning glory, *I. pes-caprae*, in Japan [37]. The weevil is distributed in subtropical and tropical area of the world [55, 62], including the Ryukyu Islands [69] and Ogasawara Islands [56]. The weevil is assumed to have originated from the Indian subcontinent [67] or northwestern South America [1]. In Japan, the weevil was first recorded on Okinawa Island [56]. It was also detected on the Yoron Island and Amami-Oshima Island in 1915, and on the Kikai Island in 1940 [56].

The eradication project for the weevil using MAT and SIT was initiated on Kume Island in 1994 [27, 28]. To suppress the wild population of the weevil to the level at which SIT was applicable, MAT was first applied from 1994 to 1999 [28], wherein fiberboard soaked with a mixture of synthetic sex pheromones [9] as a male attractant and insecticide [57] was used. Eight-to-sixteen fiberboards were distributed per hectare per

month over the Kume Island, except over meadows and catchments [28]. The release of sterile weevils was initiated in 1999 [28]. With the progress of the project, the number of released sterile weevil was increased from 30,000 to 500,000 per week, because of the expansion of the productive capacity of the mass-rearing facility [28]. To evaluate the control efficacy, traps with synthetic sex pheromones were used as an attractant [61], and the host plant stems were examined [28, 33]. To assess the infestation rate, we collected the stems of host plants (*I. batatas*, *I. pes-caprae*, and *I. indica*), dissected them, and counted the number of larvae, pupae, and adults of the weevil therein. The wild weevil was last detected in the island in 2012 [52]. Based on the host plant surveys, the Japanese Government confirmed the world's first eradication of this pest weevil using SIT in 2012 [33]. After the eradication, the weevil has not been detected in either host plant surveys or monitoring traps until 2017. The weevil eradication program started in 2007 is still going on in Tsuken Island [49].

Invasion history of and eradication program for the West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire), and improvement of mass-rearing system

The West Indian sweet potato weevil, *Euscepes postfasciatus* (Fairmaire), is one of the serious pests of sweet potato, *I. batatas*, in the South Pacific, Caribbean basin, and some countries of Central and South America [55]. The weevil has established throughout Ryukyu Islands (Okinawa Prefecture) and the Amami Islands (Kagoshima Prefecture) after World War II [56, 69]. The eradication program for *E. postfasciatus* using SIT was initiated on Kume Island in 1994 [28] and on Tsuken Island in 2007 [49].

In Kume Island, the number of the released weevil was increased to about 3,000,000 per week by 2004 [41], but was drastically reduced later due to a decreased production of the weevil, which was mass-reared on sweet potato roots [30, 41]. As a cause of the decline, Morita et al. [34] found that almost all mass-reared weevils were infected with an undescribed neogregarine *Farinocytis*-like protozoa (*Farinocystis* sp.) which reduces longevity and fecundity of the weevil [29]. Since it was difficult to eliminate the protozoa from the mass-reared strain of the weevil being reared on sweet potato roots, a novel mass-rearing system was developed using an artificial diet. In this system, the weevil eggs are washed and disinfected before being inoculated on the artificial diet, which is considered free from the protozoa [29, 42, 58]. However, protozoan infection has

not been completely eliminated in the artificial diet system [39]. Moreover, as the productivity of the artificial diet system was not enough to replace the sweet potato root system, a combination of both systems has now been adopted (Fig. 4). Recently, a rearing method to reduce the horizontal transmission of *Farinocystis* sp. between adult weevils was developed [63]. The reformation of the ingredients of the artificial diet [38] and egg-inoculation density [10] also helped improve the quantity and quality of the weevil. Owing to these developments, the number of sterile weevil release has recovered to the level of about 1,500,000 per week at present [53]. However, this number is still considerably lower than the desirable number of weevils for release, in both Tsuken Island and Kume Island. Thus, the release has been suspended in Kume Island [30, 50], and the release program is currently running only on Tsuken Island, the area of which is much smaller than that of the Kume Island [50]. Clearly, further improvements in the mass-rearing system are necessary to achieve sufficient quantity and quality of mass-reared weevil for the SIT program in Kume Island.

Future prospects

All target fruit flies have been successfully eradicated; however, the incidences of re-invasion of *B. dorsalis* complex have been increasing in recent years. While all these incursions have been controlled within several months, the risk of re-invasion by these species would further increase because of the ongoing climatic change and increasing opportunities of human travel and goods movement. Therefore, we may have to consistently revise and upgrade the trap and the countermeasures for these fruit flies.

Eradication of *C. formicarius* from Kume Island took 19 years, which is much longer compared with the case of the melon fly. This indicates that the required costs (time, money, and manpower) for the eradication of *C. formicarius* are much higher than those for fruit flies. It goes without saying that the eradication program for *E. postfasciatus* is still ongoing, even though it was initiated at the same time as that of *C. formicarius*. Therefore, it is essential to keep improving the techniques used in the eradication programs and revise the strategy for the programs based on these improvements.

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Table 1. Quarantine pest eradication projects in Japan

Target Pests	Area	Period	Methods	References
The oriental fruits fly and its relatives *Bactrocera dorsalis* complex	Okinawa	1977 – 1985	MAT	Koyama et al. (1985)
	Amami Isls.	1968 – 1980	MAT	Ushio et al. (1982)
	Ogasawara Isls.	1975 – 1984	MAT and SIT[a]	Ohkawa (1985)
The melon fly *Zeugodacus* cucurbitae, (Coquillett)	Okinawa	1975 - 1993	SIT	Kuba et al. (1996)
	Amami Isls.	1985 - 1987	SIT	Koyama (1994)
The solanaceous fruit fly *Bactrocera latifrons* (Hendel)	Yonaguni Is.	2004 - 2011	SIT	Kohama & Matsuyama (2010) Fukugasako & Okamoto (2012)
The sweet potato weevil *Cylas formicarius* (Fabricius)	Kume Is.	1994 - 2012	MAT and SIT	Matsuyama (2010)
	Kikai Is.	2001 -	SIT	Yamaguchi et al. (2010)
	Tsuken Is.	2007 -	MAT and SIT	Okinawa Prefecture (2008)
The West Indian sweet potato weevil *Euscepes postfasciatus* (Fairmaire)	Kume Is.	1995 -	SIT	Kuba et al. (2005)
	Tsuken Is.	2007 -	SIT	Okinawa Prefecture (2008)

[a] SIT was applied to complement MAT in Ogasawara Islands.

Table 2. Record of re-invasion for *Zeugodacus* cucurbitae, (Coquillett) after eradication in Okinawa Prefecture, Japan.

Locality	Period	Method	No. of fly	Reference
Okinawa Is.	1993. Sep. 30-Oct. 14	Surveillance trap	1 male	Okinawa prefecture (1993)
Yonaguni Is.	1995. Jun. 30-Jul. 14	Surveillance trap	1 male	Okinawa prefecture (1995)
Okinawa Is.	1996. Jun. 20-Jul. 4	Surveillance trap	1 male	Okinawa prefecture (1996)
Yonaguni Is.	2004. Jun. 8-Jul. 29	Host fruits survey*	1 female	Okinawa prefecture (2005)

*Emerged from a cherry tomato, *Solanum Lycopersicum* L.

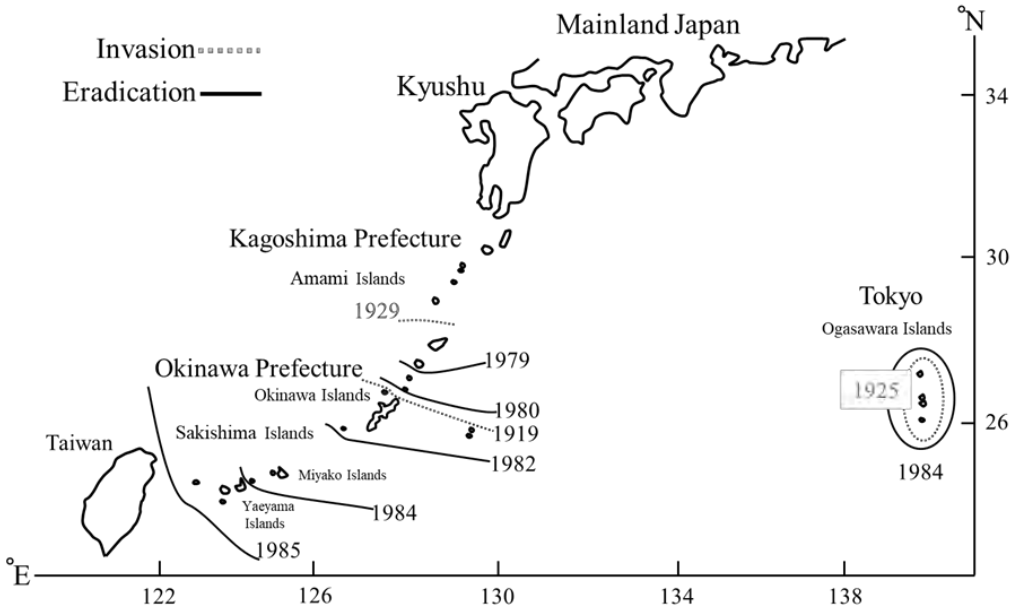


Fig. 1. Invasion and eradication of *Bactrocera dorsalis* complex in Ryukyu Islands and Ogasawara Islands, Japan.

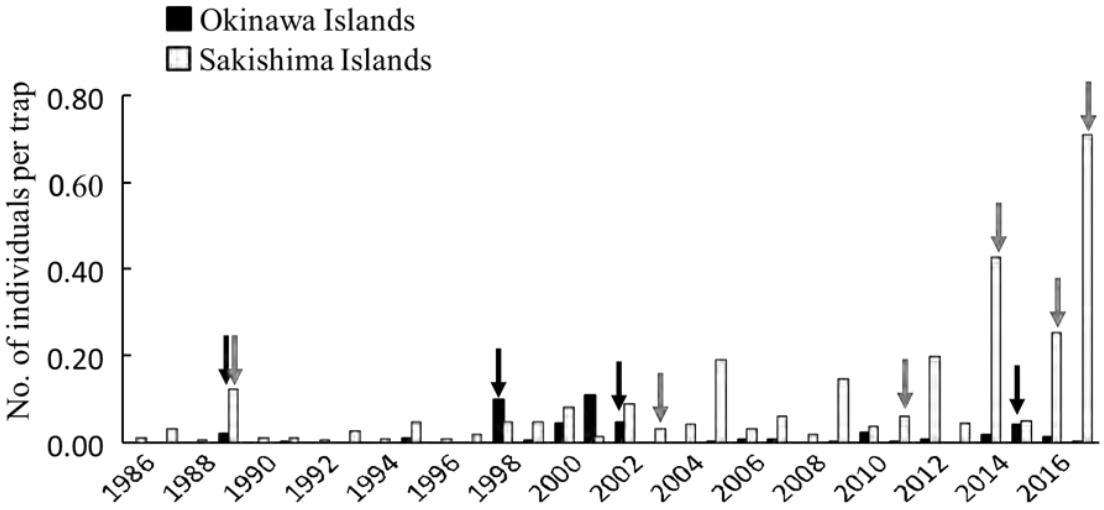


Fig. 2. Records of *Bactrocera dorsalis* complex males captured by surveillance trap in Okinawa and Sakishima Islands. For the male attractant, methyl eugenol (ME) alone was used from 1986 to 1990, while a mixture of ME and cue-lure (male attractant of *Zeugodacus cucurbitae*) with 9:1 blend has been used since 1991. Black and Gray arrows show detection of infested fruits in Okinawa and Sakishima Islands, respectively.

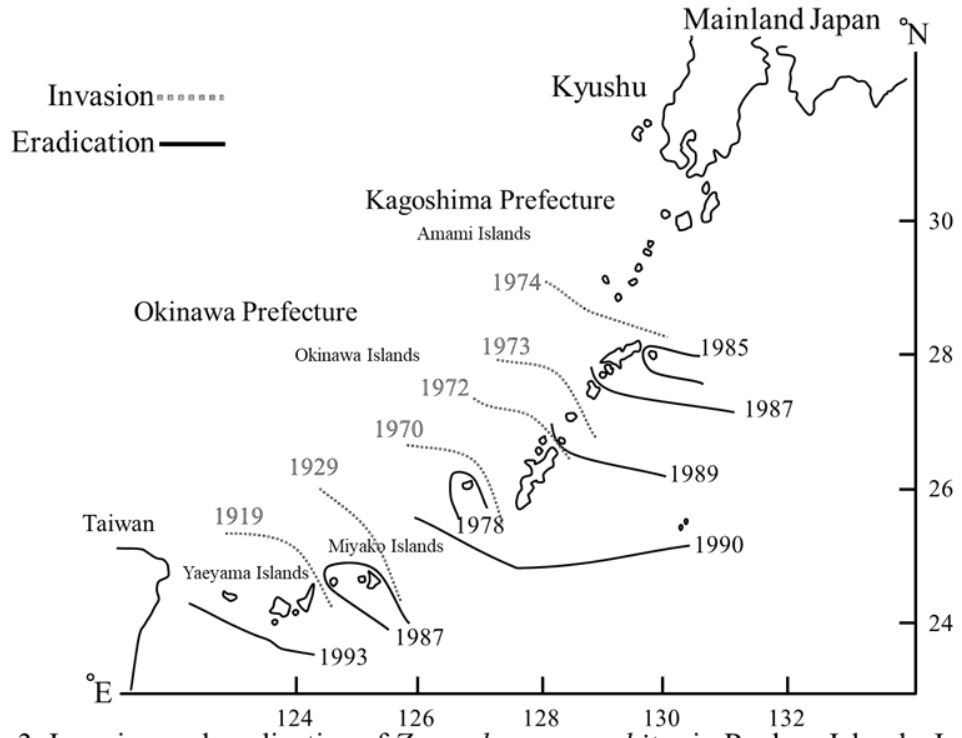


Fig. 3. Invasion and eradication of *Zeugodacus cucurbitae* in Ryukyu Islands, Japan.

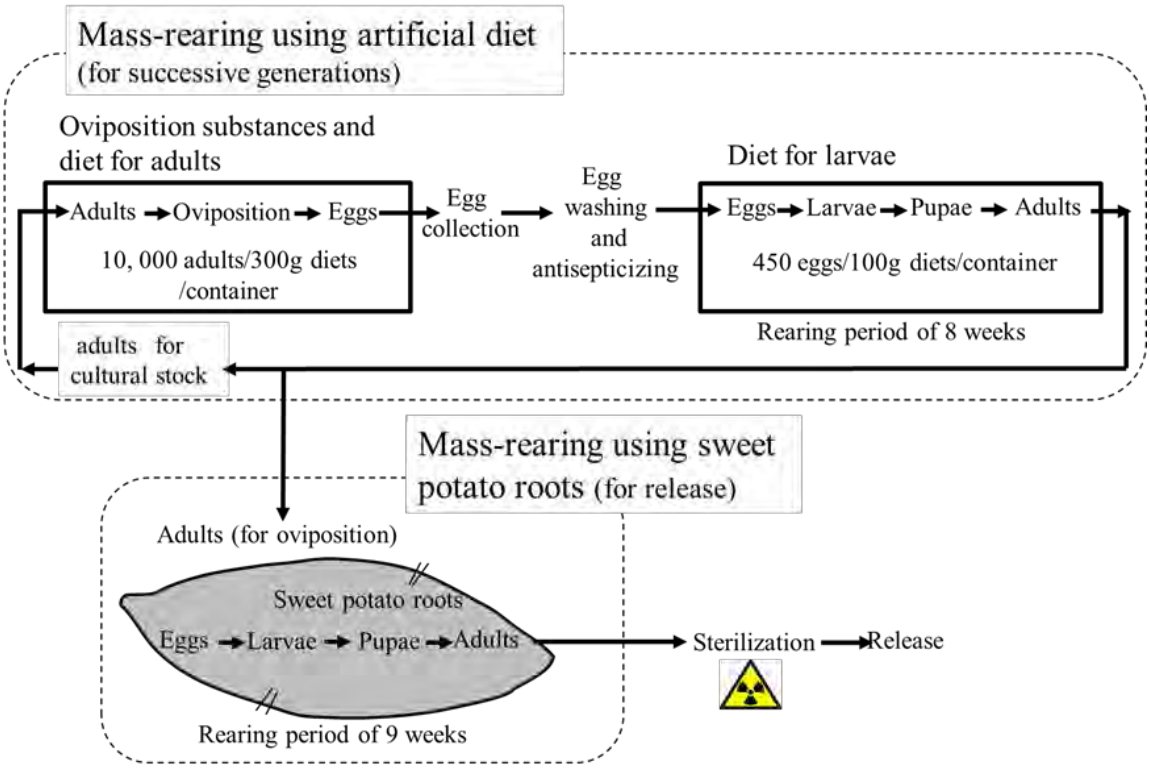


Fig. 4. Schema of the mass-rearing methods of *Euscepes postfasciatus*. Modify the figure of Ohishi et al. (2016).

Development of Vibrational Control Methods for Grapevine Pests in California

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ABSTRACT

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), is an important vector of the bacterium *Xylella fastidiosa*, the causal agent of Pierce's disease of grapevine. GWSS communicate by exchanging mating calls that are transmitted through host plants as vibrational signals. Interference with GWSS communication by playback of disruptive signals should lead to reduced population growth, but existing knowledge on mating behavior was insufficient to develop a vibrational control method for this pest. A collaborative research between the United States Department of Agriculture in Parlier, California, and Fondazione Edmund Mach, Italy, led to the description of GWSS mating communication, identification of several candidate disruptive signals for playback interference, and evaluation of the efficacy of a novel vibrational signal playback method in disrupting GWSS mating under field conditions. Results showed that playback of vibrational signals through vineyard trellis significantly reduced mating of GWSS on grapevines compared to control. Although further studies are needed prior to method implementation, data from these studies continue to support application of vibrational mating disruption as a novel method to control GWSS populations.

Keywords: *Xylella fastidiosa*, Pierce's disease, biotremology, mating disruption, vibrational communication

INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), is a xylem fluid-feeder that transmits *Xylella fastidiosa* (Wells et al. 1983), a xylem-limited bacterium that causes Pierce's disease of

grapevines [3]. Grapes are one of the most economically important crops in California ($5.5 billion/year) with over 340,000 ha of vineyards distributed throughout the state [16]. Pierce's disease was first detected in California in 1884 in southern California and the first records of the disease in the San Joaquin Valley occurred in 1917 [7]. Disease management practices have included removal of infected grapevines and control of insect vectors.

A key component in Pierce's disease management has been an area-wide insecticide application program to suppress GWSS populations in vineyards, citrus orchards, and urban areas [1, 8, 13, 17]. The systemic neonicotinoid imidacloprid has been the primary insecticide used to control GWSS populations, but despite constant surveys and insecticide applications re-infestations by GWSS are commonly reported [2]. In addition, GWSS resistance to imidacloprid has been reported from populations under aggressive insecticide treatments [15]. Moreover, insecticide applications to suppress GWSS populations in citrus orchards have the potential to eliminate the GWSS parasitoids [10] and to disrupt biological control of citrus pests such as the cottony cushion scale, *Icerya purchasi* (Williston) (Hemiptera: Margarodidae) [6]. Therefore, novel methods to control GWSS are needed.

On grapevines in California, GWSS reproduce from spring to fall, producing at least two generations per year. Previous research identified vibrational signal playback as a novel pest management strategy for *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) by transmitting a synthetic *S. titanus* male "disturbance noise" through wires of vineyard trellis [4, 14]. In field trials, mating in virgin male-female pairs was suppressed by about 90%. These promising results opened the floodgates for studying the feasibility of this method to disrupt mating of other pests, including the GWSS and other pests of grapevines. For GWSS, the approach encompassed three main objectives: 1) to identify and describe the substrate-borne signals associated with GWSS communication, 2) design and test candidate disruptive signals in laboratory, and 3) evaluate efficacy of a disruptive signal under field conditions.

Identification and description of the substrate-borne signals associated with GWSS communication

Insects used in the experiments described below were obtained from colonies maintained year-round at the USDA-ARS in Parlier, California. Briefly, late-instar

GWSS obtained from colonies were separated by gender in cages to generate virgin adult individuals. A series of laboratory studies were conducted to describe vibrational signals used in GWSS communication. First, virgin males and females were placed on host plants individually to identify common and unique signals produced by each gender. Second, a male and female was paired on a host plant to identify signals used in advertisement and species recognition, male-female duetting that results in oriented movement of one individual to another, and courtship. Third, groups of individuals (males and females together and males and females separately) were placed on plants to identify potential rivalry or distress signals. Insects were monitored via video surveillance and vibrational signals produced by individuals were recorded and measured using laser Doppler vibrometry [12].

Analyses of spectral and temporal characteristics of vibrational signals in parallel with behavioral observations showed that GWSS mating communication involved the emission of three male and two female signals, with specific roles in two distinct phases of mating behavior, identification and courtship. Mating success depended on vibrational duets between genders, which were temporarily interrupted in the presence of male rivalry. Male rivalry behavior involved the emission of three distinct rivalry signals. Two rivalry signals resemble female signals and were associated with replacement of the female in the duet by the rival male. The third rivalry signal was emitted by competing males. Data suggested that rival males used mimicry and hostile signals to interrupt the ongoing duet and gain access to a female. Knowledge acquired from this study was essential to initiate research reported below.

Design and test candidate disruptive signals in laboratory.

Once GWSS mating communication and associated vibrational signals were described, male and female behaviors were targeted separately to identify candidate disruptive signals for each gender. First, because GWSS males search for a stationary female after the onset of signaling by the female, different disruptive signals specifically aimed at interfering with the male search behavior were tested. The frequency pattern of a GWSS female signal was modified to create different versions of the female signal. Signal modification was conducted using the FTT filter function of Adobe Audition to reduce intensity of signal components and remove signal components. Modified signals were transmitted into the plant to 1) identify intrinsic

spectral features of female signal that elicit male signaling response, 2) to design disruptive signals that alter male perception and acceptance of a female signal, and (3) to determine the minimum threshold of signal intensity required for an effective application of disruptive signals. Results showed that male responses to playback of modified female signals were significantly reduced by 60-75% when part of the female signal spectral components above or below 400 Hz were deleted. Playback of vibrations to plants showed that transmission of an 80 Hz pure frequency tone completely suppressed male signaling to female signal playback, even if the disruptive signal amplitude was lower than the female signal playback [(11)].

On the second study, playback of white noise, pre-recorded female signals, and artificial female noise (continuously overlapping female signals) to GWSS male-female pairs were evaluated to determine the efficacy of candidate signals in disrupting mating of GWSS under laboratory conditions. White noise was artificially generated spanning the main frequency range of GWSS mating signal (1 to 1000 Hz) to overpower any natural signal on the plant. Natural female signals were created from a previously recorded female and included a 34-min loop of a natural female signaling pattern; the loop included 120 signals with no repeats and natural spacing of 3 to 61 sec and delivered to plants as an attempt to suppress female signaling activity or confuse males. Female noise was composed of two different female signals overlapped (50% overlap in time) creating a continuous noise (similar to white noise), but with greater signal intensity in the relevant signaling frequencies. Playback of white noise, pre-recorded female signals, and artificial female noise (continuously overlapping female signals) significantly reduced mating of GWSS when compared to silent control mating trials [(5)].

Evaluate efficacy of a disruptive signal under field conditions

Once candidate signals were identified, designed, and tested under laboratory conditions, the next objective was to evaluate the efficacy of playback of vibrational communication signals for disrupting mating of GWSS in a natural vineyard setting and evaluate spectral properties of signal transmission through vineyard trellis. A modified version of GWSS female signals was used in this experiment. The female signals were transmitted to wires used in the vineyard trellis by a custom-made electronic playback system consisting of a control unit and tuned emitters (CBC

(Europe) Srl - Nova Milanese, Italy) specifically designed for research studies in vibrational mating disruption. At the vineyard, a virgin male and a female were paired and assigned to one of two treatments: silence (control) or disruptive signal (female signal). The pair was kept together on the vine between 0800 and 1630 h and visually inspected every 2 hr to determine whether they mated or not. About 13 male-female pairs were tested per day in each treatment (silence and disruptive playback) using one pair per vine. Trials were repeated in 10 different days totaling 134 male-female pairs per treatment. Results showed that playback reduced mating of GWSS on grapevines [9]. A total of 28 (out of 134) male-female pairs mated in the control treatment (silence) and only one (out of 134) pair mated when treated with the vibrational signal playback. Playback of vibrational signals through vineyard trellis was affected by distance from signal source, with frequency composition being the highest at the signal source and lowest on vines positioned away from the source. Frequency composition in canes housing test insects decreased exponentially as distance from the source increased, whereas the relative amplitude of analyzed frequencies decreased linearly.

SUMMARY AND CONCLUSION.

Results showed that GWSS mating communication is vulnerable to playback of vibrational signals, suggesting that mating disruption methods under field conditions may be implemented upon identification of pest-specific disruptive signals that result in little to no non-target effects. In this study, GWSS mating communication and behaviors were described, candidate signals for vibrational disruption were identified, and efficacy of signals in disrupting mating of GWSS was evaluated in laboratory and field conditions. As for delivery of desirable signal characteristics to areas with variable size, vineyard trellis systems, and horticultural practices, the energy requirements for signal transmission has to be assessed. Access to and cost of electricity likely will be determinants when evaluating the efficiency of vibrational disruption compared to other control methods. With that, a reduced-energy signal (described under Objective 2 above) suppressed signaling activity of GWSS males in laboratory, but efficacy of the signal in disrupting mating has not been tested under field conditions. If proven successful, such signal would substantially reduce the energy required to implement playback in the field. While white noise and female noise treatments interfered with GWSS communication and suppressed mating, there is a great likelihood for non-target effects

of broadband substrate vibrations on natural enemies that use this frequency range to communicate. Therefore, further research on design of disruptive signals to interfere with pest communication should focus on natural signals or more narrow frequency bands. In conclusion, this research demonstrated that synthetic playback of frequency-specific vibrations through vineyard trellis at intensities above and below the natural signaling of females disrupted mating of GWSS, which ultimately should suppress population growth in vineyards. Although further studies are needed prior to method implementation, data from this study continue to support integration of vibrational mating disruption with current methods to suppress GWSS populations and support expansion to include other grapevine pests that use vibrational communication.

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Identify key virulence gene as a control target to mitigate Pierce's disease of grape

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ABSTRACT

Xylella fastidiosa (*Xf*) is an important phytopathogen that infects a number of economically important crops including citrus, almonds, coffee and olive. The *Xf* Temecula1 strain causes Pierce's disease of grapevines, a devastating disease to the viticulture industry. We deleted the *pilG* in *Xf* (*XfΔpliG*) and found that the mutant strain was avirulent. Type IV pili of *Xyella fastidiosa* are regulated by *pilG*, a response regulator protein putatively involved in chemotaxis-like operon sensing stimuli through signal transduction pathways. Results demonstrated that *XfΔpliG* showed significant reduction in cell-matrix adherence and biofilm production compared with wild type *X. fastidiosa*. *In planta* experiments showed that no Pierce's disease (PD) symptoms were observed in grapevines inoculated with *XfΔpilG,* whereas grapevines inoculated with the wild type *X. fastidiosa* and complemented strain *XfΔpilG*-C developed typical PD symptoms. These results suggest that *pilG* plays a key role in *X. fastidiosa* virulence. To develop a target-basis of therapeutic control of *X. fastidiosa* virulence, we evaluate the effect of putative anti-virulence molecules on the target gene. Our studies identified several small molecules that exhibit effective suppression on twitching motility and virulence traits under *in vitro* and *in planta* evaluation. This study facilitates the development of a novel target-basis strategy to mitigate the Pierce's disease of grapevines.

Keywords: *Xylella fastidiosa*, Pierce's disease, virulence genes, antivirulence

INTRODUCTION

X. fastidiosa is a Gram-negative non-flagellated bacterium and limited to the water-conducting xylem vessels. Pierce's disease of grapevines (PD) results in the blockage of xylem vessels, water stress and nutritional deficiencies [(5)]. Genome analysis indicates

that *X. fastidiosa* is involved in the mechanisms associated with pathogenicity and virulence such as toxins, antibiotics and ion sequestration systems, as well as bacterium-bacterium and bacterium-host interactions mediated by a range of proteins. Like most phytopathogens, *X. fastidiosa* possesses a wide array of virulence factors to invade their hosts and develop diseases. These virulence factors are precisely regulated to reduce unnecessary expression cost and to maximize fitness. To this regard, the pathogens use environmental cues to regulate their virulence gene expression. Therefore, a dynamic regulation of virulence factor expression is a key strategy utilized by pathogens. Twitching motility is a flagella-independent form of bacterial translocation over moist surfaces. It occurs in a manner of jerky motility. Twitching is mediated by the activity of filament-like structure called type IV pili which extend from the cell's exterior. Active movement mediated by the twitching system has been shown to be an important component of the pathogenic mechanisms.

The twitching motility of *X. fastidiosa* has been microscopically characterized [7, 9]. The colonization of xylem vessels is dependent on the ability of *X. fastidiosa* to migrate within xylem vessels. At least about twenty genes in *X. fastidiosa* genome have been putatively identified to be associated with type IV pili. The *pilB*, *pilQ*, and *pilR* mutants, for example, result in the defect of type IV pili and non-twitching phenotypes that showed reduced disease symptoms in grapevines [7, 9]. These suggest that twitching motility provides *X. fastidiosa* not only a means for long-distance intra-plant movement and colonization but also contributes toward virulence.

The activity of twitching motility of *X. fastidiosa* was controlled by a chemotaxis-like regulatory system [2,14], Pil-Chp operon, similar to that in *P. aeruginosa* and *E. coli* [3, 4]. Like *P. aeruginosa* CheIV (Pil-Chp) cluster, *X. fastidiosa* possesses a single predicated chemosensory system, Pil-Chp operon that regulates the twitching motility of type IV pili [4, 15]. Pil-Chp operon of *X. fastidiosa* encodes proteins involved in signal transduction pathways including *pilG, pilI, pilJ, pilL, chpB* and *chpC* as in *P. aeruginosa* and *E. coli* [2, 4]. Upon binding of the chemical stimuli in the periplasmic domain, the transmembrane chemoreceptors activate a signalling cascade in the cytoplasmic portions and ultimately control bacterial twitching motility [2]. A phospho-shuttle protein PilG in Pil-Chp operon of *X. fastidiosa* is homologous to CheY, a response regulator in chemotaxis systems of *E. coli* and *P. aeruginosa*, in which CheY interacts with the flagellar motor proteins [3, 4]. Recent studies indicated that the

homologue of chemotaxis regulator, PilG is required for the twitching motility of *X. fastidiosa* since the *pilG*-deleted *X. fastidiosa* strain was deficient in twitching motility [14]. The critical roles of the Pil-Chp operon in the virulence in *X. fastidiosa* were examined recently [2, 14].

The mobility mediated by pili genes were reported to play important roles in the pathogenicity of animal and human bacterial pathogens including *Vibrio cholera*, *Neisseria meningitides*, and alkalophic *Bacillus* strains [6, 8, 16]. Recently, small molecule inhibitors targeting to bacterial motility were reported [6, 8, 16]. These molecules specifically bind the domains of the target genes and result in disrupting the function of virulence factors. For example, a small molecule amiloride was found to be able to target the extracellular Na^+-driven flagellar motor resulting in the inhibition of the motility of alkalophic *Bacillus* strains [13, 16]. Rasmussen et al. [13] demonstrated that small molecules Quinazoline and its analogs effectively inhibited the expression of the cholera toxin and the toxin-coregulated pilus responsible for motility but did not affect the cell growth *in vitro*. In *X. fastidiosa,* the twitching motility *mediated by* type IV pili contributes toward virulence via the long-distance intra-plant movement and colonization. Thus, the disruptions of the functions of the type IV pilus genes via small molecule inhibitors that block the twitching motility of *X. fastidiosa* could be a promising strategy to disarm pathogenicity and prevent and/or block diseases development. The goal of this study was to determine the roles of *pilG* in cell growth, attachment, biofilm formation, pathogenicity and to evaluation of the effects of small molecular inhibitors on the twitching motility and the pathogenicity of *X. fastidiosa.* Our results demonstrate that the disrupting the function of the target gene by antivirulence molecules would suppress virulence of *X. fastidiosa*. Therefore, this strategy could provide a novel control method for grape PD management.

RESULTS AND DISCUSSION

The roles of pilG in cell growth, attachment, biofilm formation, and pathogenicity

pilG-knock-out strain *XfΔpilG* and its complemented strain *XfΔpilG*-C were obtained as described previously [14]. The expression of *pilG* was not detected in *XfΔpilG* but was in complemented *XfΔpilG*-C (Data not shown). No significant difference in cell growth was observed between wild-type, *XfΔpilG* and *XfΔpilG*-C strains grown in liquid culture. The growth curves of the *XfΔpilG* mutant and

complemented *XfΔpilG*-C strains paralleled wild-type, all three strains showed similar growth curves suggesting that deletion of *pilG* does not affect cell growth under rich cultural media. However, bacterial populations of mutant *XfΔpilG* strain are significantly lower than that of wild type and complemented strains in infected grapevines, indicating mutation causes the reduction of fitness in host. *In vitro* study showed that *X. fastidiosa* and *XfΔpilG*-C strains attached to the inner surface of walls of the tubes and formed a wide ring whereas no cell-attached ring was observed in *XfΔpilG* cells (Fig. 1A). The biofilm formation of *XfΔpilG* was about 5-6 fold less than that of wild-type and *XfΔpilG*-C strain ($P<0.01$) (Fig. 1B).

In planta pathogenicity assessment further confirmed that grapevines inoculated with *XfΔpilG*-C developed typical PD symptoms with a severity comparable to wild type. In contrast, grapevines inoculated with *XfΔpilG* exhibited no visible symptoms in greenhouse experiments (Fig. 2). The titer of *X. fastidiosa* was well correlated with the severity of disease symptoms. Grapevines inoculated by mutant strain had significantly lower of *Xf* populations than those inoculated by wt while complemented strains showed similar levels to wt (data not shown). Twitching motility is one of the important virulence factors. Several *X. fastidiosa* twitching motility-associated mutants have been reported [7, 9]. Most of these were found only in partial reduction in virulence and PD symptoms [1, 9]. In this study, however, we found that the pathogenicity was completely knocked-out in *XfΔpilG*. To this regard, based on our *in vitro* and *in planta* data we conclude that *pilG* could have critical roles involving multiple regulatory functions and pathogenicity, therefore, it is a central virulence factor in mediating PD development.

Effect of anti-virulence molecules on virulence

Many pathogenic bacteria use a conserved membrane histidine sensor kinase (QseC) to respond to external signals or stimulus in order to promote the expression of virulence factors. Rasko et al [11] identified a small molecule that inhibits the binding of signals to QseC and prevented its autophosphorylation and consequently inhibited QseC-mediated activation of virulence gene expression. This type of small molecule usually is not toxic but specifically disrupt functional domain of the target genes. In addition, such small molecules (< 500 Daltons) are ready to be delivered into the cells. Since molecules only target to functional domains of virulence genes they don't likely impose selection pressure on cell growth. In addition, such molecules usually do not

repress pathogen growth but selectively inhibit the target virulence of pathogens in vitro and in vivo in animal [11]. Similarly, several studies demonstrated that small molecules inhibitors had functional roles in inhibiting the pilus assembly and suppressing bacterial motility [8, 13, 17]. For example, the inhibition of motility with phenamil in *V. cholera* has been shown to have effects on virulence gene expression and mitigation of the disease development [17]. Consequently, these findings suggest that small molecules inhibitors could exert anti-virulence action on virulence traits of pathogens. Results from our study demonstrated that *pilG* mutant exhibited deficiency in twitching motility, reduction in biofilm formation, and virulence [14]. To examine whether antivirulence molecules are capable of disrupting twitching motility and therefore disarm pathogenicity of *X. fastidiosa*, we constructed a custom chemical library (ChemBridge Corp, San Diego, USA) consisting of two thousand putatively small molecule inhibitors. We examined the peripheral fringe of cell morphology, an indication of the capability of type IV pilus-mediated twitching motility [14], and assessed the inhibitory effect of small molecules on the peripheral fringe morphologies of *X. fastidiosa*. From chemical library screening, we have identified several compounds that showed promising inhibitory effects on bacterial twitching motility. For example, one of the compounds, DL-3-Amino butyric acid exerts notable effective inhibition on peripheral fringes at a concentration of as low as 5μM. (Fig. 3). A microfluidic chamber time-lapse recording system further confirmed the suppression of twitching motility treated by selected anti-virulence molecules (data not shown).

To further confirm the effect of small molecular inhibitors on the pathogenicity of *X. fastidiosa,* greenhouse-grown *X. fastidiosa*-infected tobacco plants were used for pathogenicity assay. Plants were mechanically inoculated with *X. fastidiosa.* Two weeks post-inoculation, plants in treatment group were foliar-sprayed with selected inhibitor compounds while plants in control group were sprayed with water. Spraying continued once a week for 4 weeks. Our results indicated that leaves of tobacco plants developed chlorosis and necrosis five weeks post inoculation with *X. fastidiosa* and symptoms continued up to 12 weeks while plants treated with DL-3-Amino butyric acid showed significantly alleviated symptoms (Fig. 4A) indicating treatment of virulence inhibitor mitigate *X. fastidiosa* infection and disease development. Anti-virulence treatment also resulted in lower bacterial titers compared to those of untreated tobacco

plants (Figure. 4B). These results are consistent with the data obtained from *in vitro* evaluations.

CONCLUSIONS

X. fastidiosa possesses a multitude of virulence factors that enable the pathogen to invade to and disseminate of pathogens in host and subsequently cause systemic infections. The virulence factors including cell-to-cell auto-aggregation, biofilm formation, and twitching motility are critical requirements for pathogenicity of *X. fastidiosa* [5, 10, 15] (Purcell and Saunders 1999; Hopkins 1989; Simpson et al. 2000). A conventional strategy to combat bacterium-associated diseases is to kill bacteria by antibiotics or bactericides which directly disrupt essential metabolic pathways such as preventing protein synthesis, cell wall synthesis and DNA replication [12]. While these approaches are usually effective, they impose strong selection pressure on bacteria which facilitates the selection for development of antibiotic resistance populations [12]. Anti-virulence molecular approach specifically targets bacterial virulence genes or factors and selectively disarm of bacterial pathogenicity without affecting bacterial survival. This approach is unlikely to impose strong selection pressure on bacteria for the development of resistance [12]. Taking these results together, the application of virulence-target-based strategy provides a novel means for controlling *X. fastidiosa* or other bacterium-associated economically significant crop diseases.

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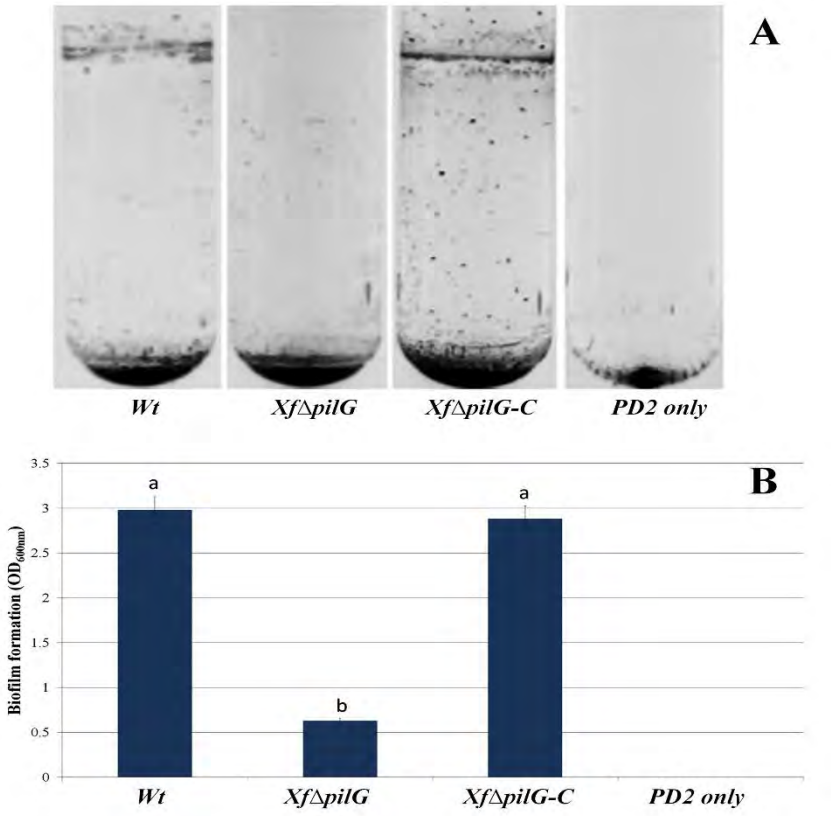


Fig. 1. A. Representative cell attachment of *X. fastidiosa* wild type, *XfΔpilG* and *XfΔpilG*-C in PD2 broth. *X. fastidiosa* wt cells showed an attached ring inside wall of the glass tube, no ring was formed in *XfΔpilG* cells under the same cultural conditions. Complemented strain *XfΔpilG*-C restored ring formation. *Xf*-free PD2 medium was served as a negative control. **B.** Quantitative measurement of biofilm formation of *X. fastidiosa* wild type, *XfΔpilG* and *XfΔpilG*-C trains. Data are the average of three replications with error bars indicating standard deviation. Bars with the same lowercase letter are not significantly different ($P< 0.01$). The experiments were repeated three times.



Fig. 2. Pathogenicity assays on Chardonnay grapevines inoculated with PBS (negative control), *X. fastidiosa* wild type, *XfΔpilG* and *XfΔpilG*-C in the greenhouse, respectively. Twenty weeks post-inoculation grapevines inoculated with wt and *XfΔpilG*-C developed typical PD systems while vines infected with *XfΔpilG* showed very mild or no symptoms. The experiments were repeated three times with at least six plants per treatment group.

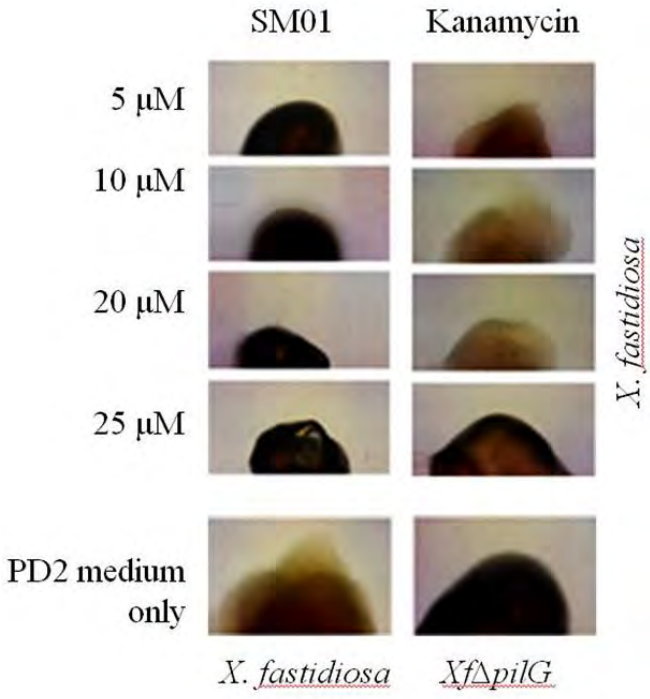


Fig. 3. The peripheral fringes were observed in *X. fastidiosa* colonies grown on PD2 agar medium while *pilG* mutant *Xf*Δ*pilG* showed smooth colony morphologies. When PD2 medium was supplemented with 5μM, 10μM, 20μM and 25 μM of small molecular inhibitor SM01 (DL-3-Amino butyric acid), no peripheral fringes were observed on *X. fastidiosa* colonies. In contrast, the effective concentrations on suppression of peripheral fringe structure were observed on PD medium supplemented with at least 25μM Kanamycin. The experiments were repeated three times.

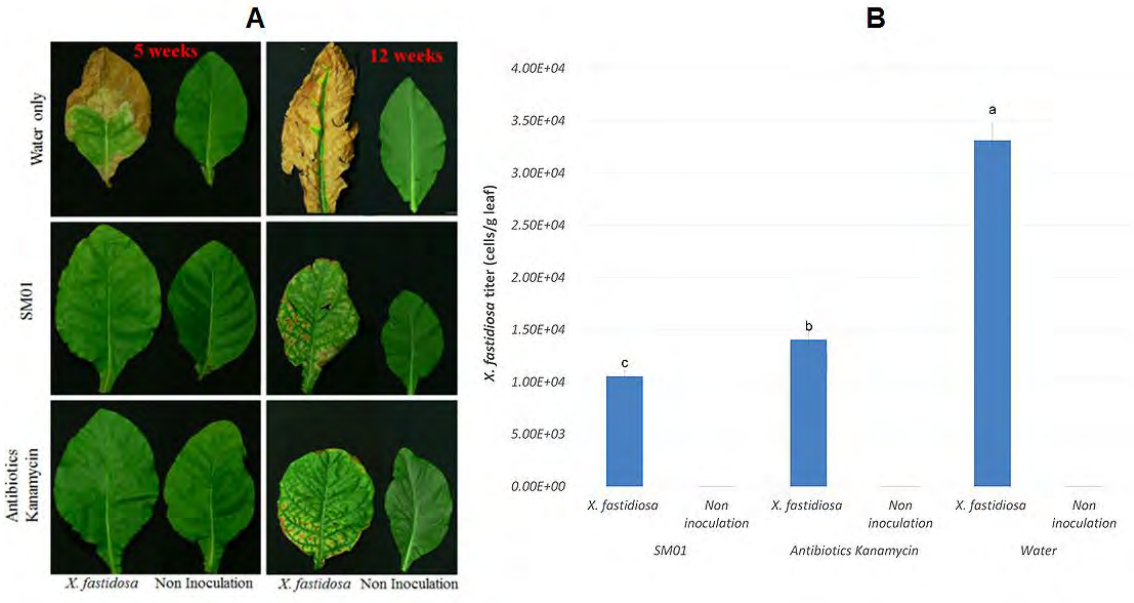


Fig. 4. Pathogenicity evaluation on tobacco plants inoculated with *X. fastidious*. **A.** Progressive development of leaf symptoms on the experimental tobacco plants 5 weeks and 12 weeks after inoculation with *X. fastidiosa* and foliar-sprayed with SM01(DL-3-Amino butyric acid), water or Kanamycin, respectively, Foliar spray was conducted once a week at 50μM for four weeks in the greenhouse. Tobacco plants developed disease symptoms from mild to severe while treatment groups showed alleviated symptoms. Greenhouse experiments were repeated three times. **B)** *X. fastidiosa* titers of tobacco leaves were estimated by ELISA two months post-inoculation. Data were means from five replications. Different letters indicate statistical significance ($P < 0.05$).

Utilization of electric pulse power for agricultural products cultivation: forest environment control

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ABSTRACT

Effect of electric pulse stimulation was tested on the fruiting body formation of valuable mushroom *Tricholoma matsutake* in the field of its natural habitat. After applying the electric stimulator to the specific area of pine forest we found that the treatment stimulated the fructification of *T. matsutake.* The most valuable findings of the study was that only our treatment plots showed fruiting body formation whereas the control plots and the whole natural habitats of our study area showed zero production of this mushroom during the same time. From the point of view of mushroom production, our experiment shows that fruiting body production can be upgraded by using pulsed power as an electrical stimulation in the field of its natural habitat. These findings confirm the effectiveness and the significance of pulsed power technology for the improvement of *T. matsutake* fruiting body production in its natural habitat.

Keywords: ectomycorrhizal, *Tricholoma matsutake*, fruit body, electric pulse stimulation

Exploitation of new attractants for fruit fly pests of economic importance

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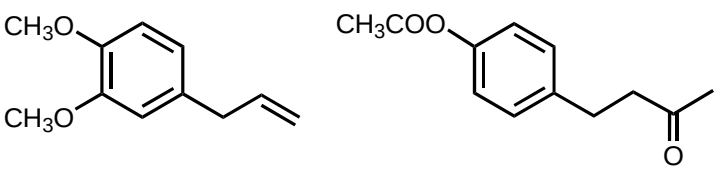
ABSTRACT

The fruit fly tribe Dacini (Tephritidae: Diptera) comprises more than 700 species including many fruit pests of economic importance, particularly in the two genera, *Bactrocera* and *Zeugodacus*. Males of many dacine species show strong affinity either to methyl eugenol (ME) or cue-lure (CL). However, there are over a hundred species that show no affinity to these lures. We have been seeking new attractants for these 'nonresponding' species particularly of endemic agricultural importance based on the following three criteria, (1) by observing their mating sequences, (2) their natural interactions with plants and (3) by analyzing specific chemicals the wild male flies accumulate in their secretory organ (rectal gland) associated with pheromone production. Orchid flowers of many *Bulbophyllum* species attract fruit fly males by emitting specific volatiles for pollination, in the rain forests of Southeast Asia. Several species produce either ME or raspberry ketone (RK), and some species use zingerone (ZN) to attract both ME-sensitive and RK-sensitive males. ZN was found as a specific male attractant for *B. jarvisi*. A specific attractant for the solanaceous fruit fly, *B. latifrons*, was characterized as 3-hydroxy-α-ionone from a specific variety of eggplants. Several 3-oxygenated α-ionone analogs were developed as monitoring agents for *B. latifrons* in Okinawa, Japan. Furthermore, wild *Bactrocera* males that have fed on attractants from certain plant sources selectively accumulate/biotransform compounds in the rectal gland possibly for sex pheromone to attract females. A sesquiterpene, β-caryophyllene, was identified from the rectal gland in wild *B. correcta* males. β-Caryophyllene was found to be more attractive than ME to males, and thus, potentially serves as a new monitoring agent for *B. correcta*. These exemplify the importance of a multidisciplinary study including behavioral, physiological, biochemical and chemoecological aspects to develop new fruit fly attractants.

Keywords: *Bactrocera*, *Zeugodacus*, Dacini, Tephritidae, Diptera, fruit fly, methyl eugenol, cue-lure, raspberry ketone, zingerone, 3-oxo-α-ionone, β-caryophyllene, phenylpropanoid, phenylbutanoid, terpenoid, attractant, pheromone, allomone, synomone, floral fragrance, rectal gland, fruit fly orchid, pollination, sequestration, biotransformation, male annihilation technique, sterile-insect technique

INTRODUCTION

The fruit fly tribe Dacini is one of the most species-rich clades within the dipteran family Tephritidae (Diptera) comprising more than 700 species, which includes many fruit pest species of economic importance, particularly in the two genera, *Bactrocera* and *Zeugodacus* [e.g. oriental fruit fly, *B. dorsalis*; banana fruit fly, *B. musae*; guava fruit fly, *B. correcta*; carambola fruit fly, *B. carambolae*; solanaceous fruit fly, *B. latifrons*; peach fruit fly, *B. zonata*; Queensland fruit fly, *B. tryoni*; Japanese orange fly, *B. tsuneonis*; melon fly, *Z. cucurbitae*; cucumber fly, *Z. cucumis*; pumpkin fruit fly, *Z. tau*] [2,16]. Males of many dacine fruit fly species show strong affinity to either methyl eugenol (ME) or cue-lure (CL) (Fig. 1) [20, 42]. Male annihilation technique (MAT) has been developed by using ME as a powerful trapping agent in the eradication and suppression programmes of one of the most destructive species, the oriental fruit fly, *B. dorsalis*, in various countries [15, 21, 32]. In the case of the eradication programme of melon fly, *Z. cucurbitae*, in Okinawa, Japan, CL was used successfully to suppress the initial field population of males prior to release of sterile flies in the sterile-insect technique (SIT) [43]. These lure chemicals are effective only on the male flies (females usually do not respond to the chemicals), but the lure chemicals are potent enough to reduce the male population to such a low level that mating does not occur [32, 43].



Methyl eugenol (**ME**) Cue-lure (**CL**)

Fig. 1. Two major male attractants of *Bactrocera* and *Zeugodacus* fruit flies

The lure activity of ME and CL to male flies are extremely powerful and highly specific to a number of *Bactrocera* and *Zeugodacus* species [20, 42]. However, there are several important pestiferous dacine species, which do not respond to these lures – such as *Bactrocera atrisetosa*, *B. decipiens*, *B. jarvisi*, *B. latifrons*, *B. minax*, *B. pyrifoliae*, *B. tsuneonis* and *Zeugodacus cucumis* (Table 1). We have been seeking new attractants for those 'non-responding' species, particularly those that are endemic agricultural pests. In order to develop new attractants for those male lure-unknown species, we need to understand the nature of fruit flies' life histories, particularly by observing their natural interactions with plant chemicals that have strong affinity with those pest species. Furthermore, we need to study their sexual communication systems, which may involve pheromone precursor materials arisen from specific phytochemicals selectively accumulated by male flies during foraging as in a strong association between *B. dorsalis* and ME-producing plants.

Table 1. Pestiferous *Bactrocera* and *Zeugodacus* fruit fly species – responsive and non-responsive to specific male lures

Methyl-eugenol (ME)-responsive	Cue-lure (CL)-responsive	Non-responsive to ME and CL
B. carambolae	*B. albistrigata*	*B. jarvisi*
B. correcta	*B. frauenfeldi*	*B. latifrons*
B. dorsalis	*Z. caudata*	*B. minax*
B. musae	*Z. cucurbitae*	*B. pyrifoliae*
B. umbrosa	*Z. tau*	*B. tsuneonis*
B. zonata	*Z. tryoni*	*Z. cucumis*

Herein I give an overview of the nature of specific male attractants in association with sex pheromone system, and the flowers as lure source, particularly focusing on synomonal communication between some fruit fly species and so-called "fruit fly orchids" via specific male attractants. Then, I introduce some cases of exploitation of new attractants from plant sources in relation to the male pheromone gland components.

A large part of this study has been conducted as a collaboration project with Dr. Keng-Hong Tan (Malaysia), particularly on fruit fly species of economic importance.

1. Sex pheromone system and male lures

Males of many pestiferous dacine species are known to possess a glandular sac associated with rectum, which is supposed to store and release sex pheromone to attract conspecific females during courtship [6, 42]. The volatile chemical constituents in the rectal glands have been extensively studied in many *Bactrocera* and *Zeugodacus* species, which include aliphatic amides, alcohols, ketones, esters, lactones, spiroacetals, terpenoids, pyrazines, and some aromatic compounds [7]. Many of these volatiles are supposed to be biosynthesized endogenously by males and stored in the rectal gland during sexual maturation. However, the biological role(s) of the male rectal volatiles has not been well understood.

In the case of *B. dorsalis,* the male rectal glands are almost empty with some minor quantities of *N*-3-methylbutylacetamide and a series of fatty acid esters, when the flies were cultured in the laboratory. However, most of the male flies captured in the field sites were found to contain large amounts of two phenylpropanoid compounds, 2-allyl-4,5-dimethoxyphenol (DMP) and (*E*)-coniferyl alcohol (ECF) in large quantities often over 100μg/male (Fig. 2) [24]. DMP and ECF also accumulated in the rectal glands when laboratory-reared males were fed with pure ME [24].

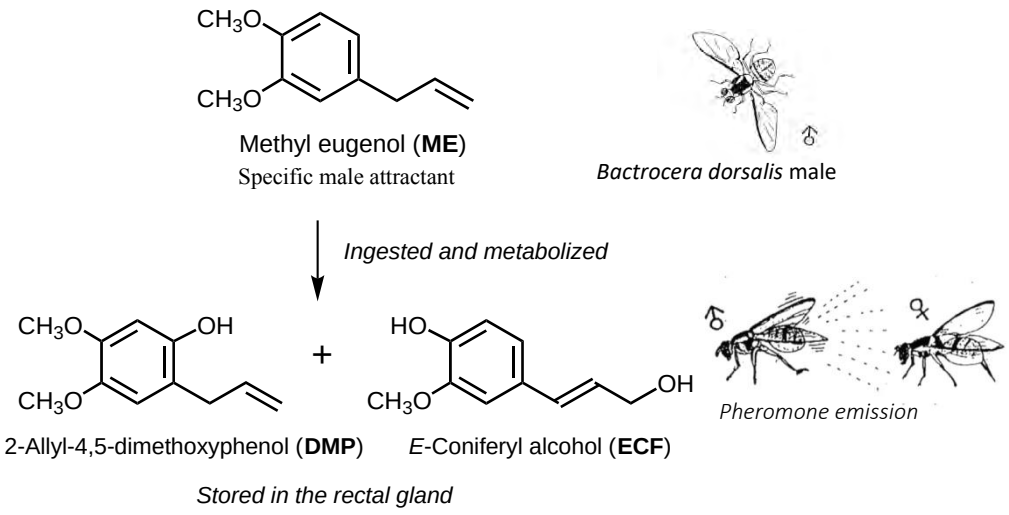


Fig. 2. Methyl eugenol (ME) as a precursor for sex pheromone of the oriental fruit fly, *Bactrocera dorsalis*. Males incorporate ME and convert it to two metabolites

(DMP + ECF) and release them as a "pheromone smoke" to attract and arrest a female during courtship.

These ME-metabolites are released with vigorous wing-fanning action to the air as the "pheromone smoke" from the rectal gland at the sunset when the mating takes place (Fig. 2)[23, cf. 17]. Laboratory studies have shown that *B. dorsalis* males that have fed on pure methyl eugenol have a mating advantage over males denied this compound[9,30,33]. Females are sexually stimulated by the male rectal pheromone components, often exhibiting "ovipositor-extending behavior", possibly a sign of an acceptance of the courting male[33]. However, field trap tests revealed that the rectal metabolites are attractive rather to males but not to females, suggesting that the females are responsive to these compounds only in a very short distance during the courtship period; and males may be triggered to aggregate by the smoke substances (aggregation pheromone) possibly to form a lek prior to courtship[24, 33]. Besides, males that fed on ME were shown to gain a defensive measure (allomone) by sequestering DMP and ECF in their body tissues[23, 34].

A series of related phenylpropanoids, including (*Z*)-coniferyl alcohol (ZCF) and (*Z*)-3, 4-dimethoxycinnamyl alcohol, have been characterized from rectal glands of *B. correcta* and *B. zonata* males compulsively fed on ME[41], suggesting their pheromonal role in their mating sequence. In these instances, the male pheromone systems are suggested to have advanced within the context of sexual selection – the female's preference for the specific components linked to fitness-related defensive compounds[22]. By contrast, males of *B. carambolae* possess significant amounts of endogenous volatiles (e.g. *N*-3-methylbutylacetamide, 6-oxo-1-nonanol, 1,6-nonane- diol) in the rectal glands, and yet 'pharmacophagously' seek the lure source (ME) and sequester it as ECF in substantial quantity; mating advantage by the lure-fed males has been demonstrated[45].

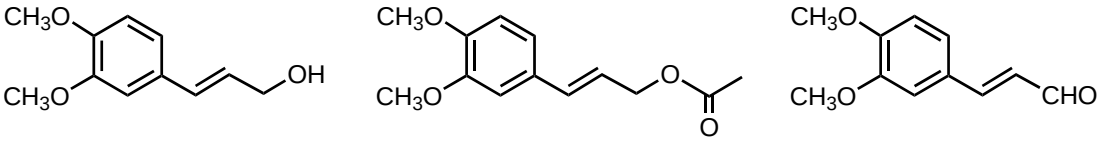
In the case of *Z. cucurbitae*, males fed on CL store its deacetyl compound, raspberry ketone (RK) in the rectal glands together with endogenous volatiles (e.g. *N*-3-methylbutylacetamide, 1,3-nonanediol, ethyl 4-hydroxybenzoate)[25]. *Z. cucurbitae* males fed on either CL or RK were shown to have mating advantage at an early stage after feeding[31].

In the sexual communication system of the olive fruit fly, *B. oleae*, females attract males and the female sex pheromone, 1,7-dioxaspiro[5.5]undecane (olean), has been demonstrated to be useful for bating traps [8]. Although the use of synthetic sex pheromones and other intraspecific chemical communicants is established in lepidopteran species, the use of dipteran pheromones for control or monitoring is not yet common, despite its obvious potential as above. Much detailed work under close scrutiny is required with the dacine fruit fly species to understand the nature of those pheromonal communication systems particularly involving male-specific lure substances.

2. Flowers as lure sources

Males of the lure-responsive fruit fly species frequently visit plants that contain specific chemicals to acquire pheromone materials. Historically, a century ago, ME was found as the powerful male attractant for *Dacus* (*Bactrocera*) species from citronella grass, *Cymbopogon nardus* (Poaceae) [10]. Subsequently, ME was also identified from flowers of golden shower, *Cassia fistula* (Fabaceae), which attracted *B. dorsalis* in Hawaii [13]. The occurrence of ME has been reported for more than 450 species from 80 families spanning many plant orders [38]. Since dacine fruit fly males show strong affinity to some specific floral components, flowers seem to be a good source for the males to acquire these essential chemical components during adult stage.

New attractants for *B. dorsalis* males were isolated from a Hawaiian lei flower, *Fagraea berteriana* (Gentianaceae), and characterized as a mixture of (*E*)-3,4-dimethoxycinnamyl alcohol, its acetate, and (*E*)-3,4-dimethoxycinnamaldehyde [27] (Fig. 3). A similar set of compounds was also found from flowers of *Spathiphyllum cannaefolium* (Araceae) [1]. Since ME is suspected as a human carcinogen [12], these non-allylic compounds might potentially serve as its substitutes in place of ME in the pest management programmes.



3,4-Dimethoxycinnamyl alcohol 3,4-Dimethoxycinnamyl acetate 3,4-Dimethoxycinnamaldehyde

Fig. 3. Attractants for *Bactrocera dorsalis* males from flowers of *Fagraea berteriana*

Above naturally occurring lure chemicals are classified as phenylpropanoids (C6-C3) closely related to ME. By contrast, cue-lure (CL) is a phenylbutanoid (C6-C4) that has never been found from the natural sources (developed as a synthetic attractant; but its deacetyl derivative, raspberry ketone (RK) was found in nature – from the flower of *Dendrobium superbum* (Orchidaceae) as a specific attractant for *Z. cucurbitae* [26].

Our systematic survey on flowers of so-called "fruit fly orchids" in the orchid genus *Bulbophyllum* (Orchidaceae) revealed a series of phenylpropanoids and phenylbutanoids that attract specific fruit fly species. Flowers of *Bu. cheiri* attract males of several *Bactrocera* fruit fly species by floral ME in the rain forest of Southeast Asia [28, 39] (Fig. 4, left). The flower has a specialized hinged see-saw lip structure which temporarily traps the fruit fly between its lip and column, and transfers pollinia to the back of his thorax. The fly is rewarded by the floral attractant to be used as a precursor for sex pheromone and defensive allomone as described in Section 1 (see Fig. 2). In this orchid-fruit fly association, both organisms gain direct reproductive benefits – the orchid flower gets pollinated without having to offer nectar, while the fruit fly boosts its pheromone with the attractant chemical ME. Thus, the floral volatile is defined as "synomone", an interspecific semiochemical that is adaptively advantageous to both the emitting and the receiving organisms. Flowers of another related orchid species, *Bulbo. vinaceum*, produce a complex mixture of phenylpropanoids including DMP and ECF, in addition to ME and attract ME-sensitive fruit flies [40]. The orchid flower seems to endow the male flies not only with precursor attractant ME but also intact pheromone (DMP and ECF), although the complete pollinator spectrum and actual role of such a multi-component system of attractant volatiles is unclear.

Another fruit fly orchid, *Bulbo. apertum* (synonym: *ecornutum*), RK in its fragrance to attract several RK-sensitive *Bactrocera*/*Zeugodacus* species such as *B. albistrigata* and *Z. cucurbitae* (Fig. 4, right) [36]. The pollination takes place when a fly is compulsively feeding on the seesaw lip and momentarily trapped between the lip and column. The attractant chemical can act either as floral synomone (pollinarium-transported, as in *B. albistrigata*) or kairomone (accidental removal of pollinarium leading to total pollen wastage, as in *Z. cucurbitae*) depending on the size of the visiting fruit fly species [36].

Flowers of *Bulbo. patens* attract male of a variety of *Bactrocera* fruit fly species, which show affinity to either ME and RK. The attractant component was identified as zingerone [4-(4-hydroxy-3-methoxyphenyl)-2-butanone] (ZN), a pungent essence of ginger (Fig. 4, middle) [35]. ZN conforms to a chemical structure resembling both ME and RK, and thus shows a potency to attract both ME-sensitive and RK-sensitive fruit fly species (e.g. *B. carambolae*, *B. dorsalis*, *B. umbrosa*, *Z. caudata* and *Z. cucurbitae*), thereby securing effective pollinators in the complex and dense vegetation in the rain forests. It was demonstrated that *B. cucurbitae* males that have fed on ZN sequester the intact compound in the rectal pheromone glands and become more successful in mating than unfed males [14]. Interestingly, males of an Australian fruit fly, *B. jarvisi* (a pest of mango fruit) whose specific attractant was initially unknown, was found to be selectively attracted to ZN in the flowers of *Bulbo. baileyi* that the male flies frequently visit [4, 37]. ZN was also attractive to other species such as *B. aglaiae* and a new species *B. speewahensis* and the rarely trapped *Dacus secamoneae* in the field of northern Australia [4]. This exemplifies a possible use of floral synomones as new monitoring agents for several specific pest species in the orchards.

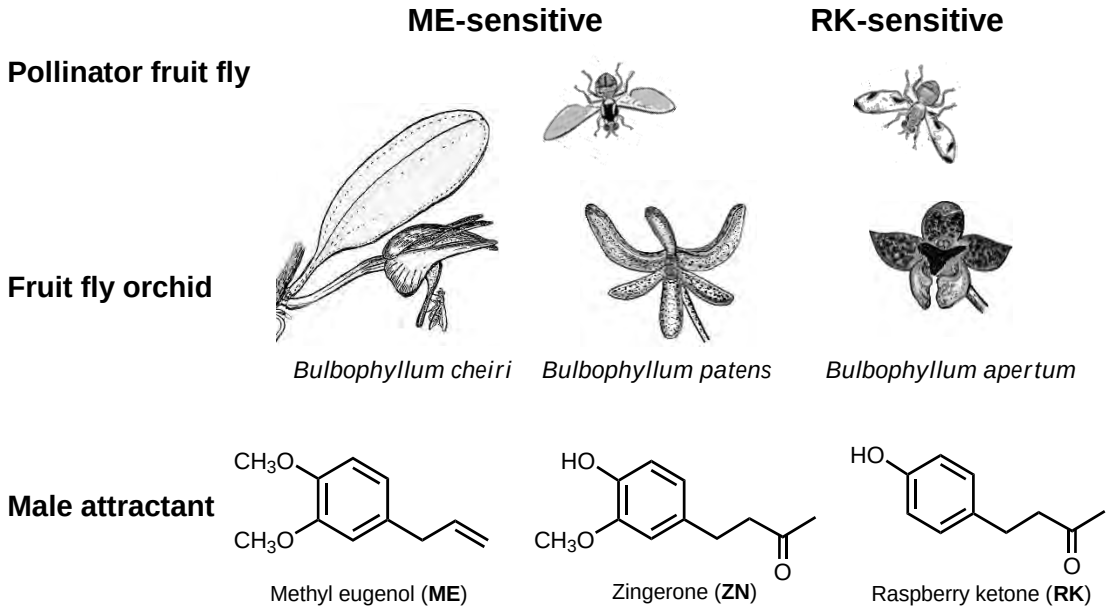


Fig. 4. Floral synomone of fruit fly orchids attracting pollinator fruit fly males.

Note: *Bulbo. vinaceum* emits methyl eugenol (ME); *Bulbo. apertum* produces raspberry ketone (RK); *Bulbo. patens* emits zingerone (ZN) which has a hybrid structure between ME and RK and attracts both ME-sensitive (e.g. oriental fruit fly, *B. dorsalis*) and RK-sensitive species (e.g. melon fly, *Z. cucurbitae*).

3. New attractants derived from male rectal components

3-1. *Bactrocera latifrons*

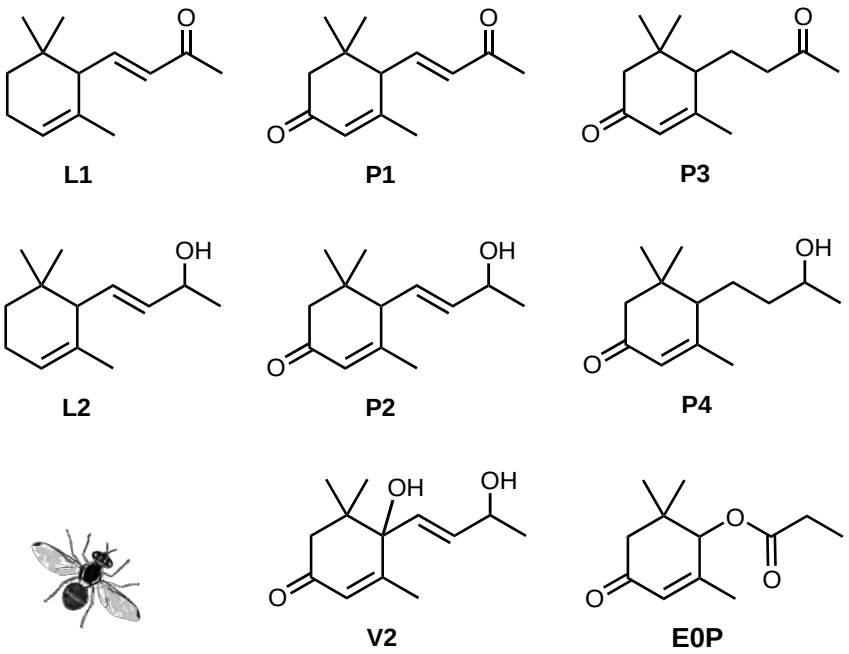
Among the pest fruit fly species with unknown male attractants, α-ionone (L1) and α-ionol (L2) (latilure) were found as specific attractants for the solanaceous fruit flies, *B. latifrons*, during the chemical screening using olfactometry (Fig. 5)[5]. Although the attractiveness of these norsesquiterpenes was weak, the lure activity was significantly enhanced by cade oil[18, 19]. However, the potency of these attractant mixtures appeared not as high as that of ME for *B. dorsalis* or CL for *B. cucurbitae*. Some efforts were put into a survey for potential chemical lures for *B. latifrons* particularly in orchids, but observations showed none of the flies settled on any plant sources.

During the search for solanaceous host fruit volatiles to attract *B. latifrons* females, a variety of eggplants commonly sold in a grocery store in Hawaii was found to strongly attract males of *B. latifrons* rather than females[29]. The males voraciously feed on the eggplant tissues and its extracts, similar to the phenomenon shown by *B. dorsalis* males compulsively licking on ME-containing plants. One of the active principles was identified as 3-hydroxy-α-ionone[29].

Isophorone (3,5,5-trimethylcyclohex-2-enone) and its analogs were also found as moderate attractant for *B. latifrons* males, which significantly enhanced activity of L1 and L2[11]. Based on that knowledge, a series of 3-oxygenated α-ionone analogs (P1, P2, P3 and P4) have been prepared, and their individual activities were evaluated (Fig. 5). Synthetic 3-oxo-α-ionone (P1) showed distinct attractant activity in the laboratory tests; 7,8-dihydro-3-oxo-α-ionone (P3) was shown to be effective in the field tests using Steiner traps[3, 11]. It should be noted that all 3-oxygenated analogs showed potent phagostimulant activity as well as long-range attraction, which appeared typical properties of male lures as in the case of *B. dorsalis* to ME and *Z. cucurbitae* to CL and RK.

P3 has been selected as a possible candidate for use as a monitoring agent for *B. latifrons* in Okinawa (unpubl. data). Further survey also revealed vomifoliol (V2) – isolated from roots of lamb's ear, *Stachys byzantina* (Labiatae), was found to be an extremely potent phagostimulants for the male flies, although long distance attractiveness was very weak, due to its low volatility (unpubl. data) (Fig. 5). Along with the above information, several analogs with an oxygen function on the C-4

position of isophorone have been synthesized to improve the attractiveness. As a result of outdoor cage tests, 4-propionyloxyisophorone (EOP) has been developed as a powerful lure against *B. latifrons*, particularly more effective when used in combination with P3 (unpubl. data) (Fig. 5). EOP is a propionate of 4-hydroxy- isophorone (EO), and the ester evaporates much faster than P3 at ambient temperature, even though they possess almost similar molecular sizes.



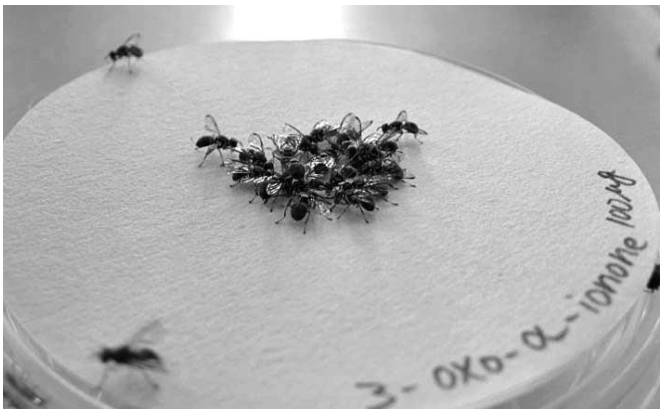
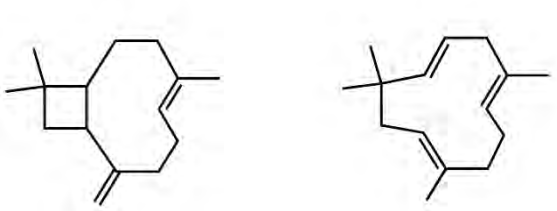


Fig. 5. Structures of attractants/phagostimulants of α-ionone/ionol analogs for the solanaceous fruit fly, *Bactrocera latifron*s. Photo shows a congregation of the male flies attracted and voraciously feeding on 3-oxo-α-ionone (P1).

3-2. *Bactrocera correcta*

B. correcta males are attracted to ME and sequester *Z*-3,4-dimethoxycinnamyl alcohol and ZCF in the rectal glands as mentioned above (Section 1). Besides these two ME-metabolites, wild males were found store extremely large quantities of sesquiterpenic hydrocarbons, such as β-caryophyllene (CP) and α-humulene (HM) in the rectal glands in a survey of local *B. correcta* flies in Thailand. Total amounts of sesquiterpenes often exceeded 250 μg/gland (Fig. 6)[44]. CP is a common plant essential oil component in such as clove (*Syzygium aromaticum*), rosemary (*Rosmarinus officinalis*), lavender (*Lavandula angustifolia*), hops (*Humulus lupulus*), and *Cannabis sativa*.

Field tests were conducted using one of the rectal components – CP, in fruit orchards in Thailand. CP was shown to act as a highly potent male attractant for *B. correcta*, in that its attractiveness appears to be significantly higher than that of ME, whereas the co-inhabiting *B. dorsalis* was never captured by CP-traps[46]. *B. correcta* males respond to CP earlier than their attraction to ME nearing or at sexual maturity[46]. This is highly desirable in a mass-trapping or male annihilation program to reduce mating of feral females. In a monitoring program of both *B. correcta* and *B. dorsalis*, CP can be employed as a useful measure to differentiate the trap catches of each species, or possibly as a highly selective mass trapping agent for *B. correcta*.



β-Caryophyllene (**CP**) α-Humulene (**HM**)

Fig. 6. Sesquiterpene lures specific for *Bactrocera correcta* – identified from the fly male rectal glands

Sequestration of these sesquiterpenes in the pheromonal gland in *B. correcta* males poses a great contrast to that in *B. dorsalis* males, suggesting a potential role of these unique rectal ingredients in the interspecific interactions between these sympatric

species. It has been demonstrated that CP-feeding increased the male mating success in comparison with lure-deprived males, and CP-fed males were equally competitive as ME-fed males when competing for conspecific females in semi-field trials [46].

CONCLUSION

The strong attractiveness to plant allelochemicals is a unique characteristic in *Bactrocera/Zeugodacus* males. We demonstrated the nature of sex pheromone system in conjunction with specific male lure, and interpreted why those male fruit flies are strongly attracted and eagerly feed on the lure chemicals. We successfully found a series of new male specific lures by chemical analyses of flower fragrances in fruit fly attracting plants particularly in 'fruit fly orchids' in tight mutual association with the dacine fruit flies, rectal pheromone gland volatiles from wild males of target fruit fly species, and structural modifications of such molecules through organic syntheses. The strong affinity of fruit flies to these lure chemicals seems to be linked to the flies' pheromone and defensive systems specifically developed within the highly diverged species radiating from central Southeast Asia. We need to pursue further to understand the natural life history of these fruit flies in close association with plants in their natural and agricultural ecosystems.

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Recent advances in the optical control of insect pests using light and color

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ABSTRACT

Technologies employing "light pest control" are becoming an increasingly effective tool in IPM. Until the 1990s, traps using fluorescent tubes and moth-repellent lights using yellow sodium lamps were developed and employed widely. In the 2000s, light-emitting diodes (LEDs) capable of emitting monochromatic light with high luminous efficiency were developed and hence enhanced the potential of light pest control. Simultaneously, numerous studies concerning insect neurophysiology and neuroethology were achieved with new information, such as the spectral sensitivity of insect compound eye and the response behavior to a specific wavelength in many species. These efforts led to the discovery of new biological phenomenon, called the light response reactions. Advances in this area of research in recent years have been astonishing, which motivated the promising study of new light pest control technologies.

Keywords: agriculture, integrated pest management, optical control

INTRODUCTION

Awareness of food safety and security has increased among consumers in recent years. Along with a simultaneous diversification in the demand for agricultural produce, this has generated steady growth in the demand for vegetables grown using reduced amounts of agricultural chemicals and for organic vegetables. In Western countries, organic vegetables have already gained an unassailable position in the market, and the quantities being distributed by major supermarket chains are expanding. Retail stores usually have a section for organic fruits and vegetables, and it is now easier than ever for consumers to purchase organic produce. In Japan, however, while the output of organically grown produce continues to increase each year, the actual market share remains very small (less than 1%). Even with the inclusion of cultivars grown with

reduced quantities of agricultural chemicals, it is still not easy for consumers in Japan to choose organic or reduced-chemical products.

At agricultural production sites, damage to fruits and vegetables caused by insects that are increasingly becoming resistant to pesticides are a growing problem. Large numbers of insect pest species have acquired resistance to pesticides and even to neonicotinoids, which have been used in an increasing number of pesticide formulations since the 1990s. This problem has become more severe by the growing number of insect species that have developed resistance to multiple pesticides, with different modes of action. Consequently, only a limited number of pesticides are effective, and it is becoming ever more difficult to eliminate small insect pests, such as thrips or aphids, using chemical pesticides.

In this situation, the development of insect pest control techniques that can replace or supplement chemical pesticides has become a pressing issue that requires the attention of researchers and technicians involved in pest control.

Why "light pest control" now?

Chemical pesticides have the ability to quickly and completely eliminate insects within the application range; thus, it is not easy to develop alternative pest control technologies with the same level of effectiveness. There is a need for integrated pest management (IPM) to control the density of insect pests through combinations of various available measures, while reducing the use of chemical pesticides. This means using a combination of biological, chemical, cultural, and physical pest control technologies in ways that do not interfere with each other, while taking the natural environment that surrounds agricultural land and the population dynamics of insect pests into consideration. The goal is to keep insect pest damage below the level at which economic consequences become a problem (economic injury level). Reducing the amount of chemical pesticides and the frequency of use is also expected to curb the emergence of pesticide resistance.

It has been over 50 years since IPM was first promoted and a wide range of technologies has been developed. Biological pest controls now established at production sites include the use of the entomopathogenic bacterium *Bacillus thuringiensis* and the introduction of natural insect enemies, such as *Amblyseius swirskii*. Against this background, technologies employing physical pest control,

especially “light pest control”, are expected to become tools for use in IPM. For example, traps using fluorescent tubes (black light) and moth-repelling lamps using yellow sodium lamps were developed as light pest control technologies in the 1990s, but there were few variations and there were limitations on where and how they could be used. However, the 2000s brought enormous changes. Light-emitting diodes (LEDs) capable of emitting monochromatic light with high luminous efficiency were developed, and mass production made them increasingly cheaper; thus increasing their potential for use in pest control. Simultaneously, improvements in techniques for examining insect neurophysiology, such as the spectral sensitivity of the retina, enabled a great deal of research to be conducted on the reactions of different insect species to exposure to light at different wavelengths. This led to the discovery of a new biological phenomenon, the light response reactions (Fig. 1). Advances in this area of research in recent years have been astonishing, resulting in the emergence of new pest control tools.

Basic principles of light pest control : Response reactions of insects to light and color

Light pest control utilizes the response reactions of insects to light and color (light response reactions). The color and shape of objects (or light sources) are detected by the ommatidia, which number from several hundred to several thousand in a compound eye. This sensory information is processed as electrical signal inputs by neural networks in the brain, and the ultimate output is manifested as behavioral or developmental changes. A well-known example of an insect light response reaction is the “phototaxis” of insects that swarm around streetlights. Research to date has identified a variety of other responses (outputs) to light in addition to phototaxis (Fig. 1).

Attraction, also known as “positive phototaxis”, is the response of moving toward a light source. Different species of insects are known to show preferences to different wavelengths and intensities of light, with the term “spectral preference” used to refer to the wavelength that is especially preferred by an insect (Fig. 2). This is an important factor when considering pest control technologies. Many insects have three photoreceptor genes coding for ultra-violet receptors with an absorption maximum in the region of 350 nm, blue photoreceptors in the region of 450 nm, and green photoreceptors in the region of 550 nm. The specific combination of these receptors determines the spectral sensitivity of the compound eye, and the spectral preference is

often in the region of the absorption maximum of ultra-violet or green receptors. Capture devices such as light traps or sticky sheets work on the principle of spectral preference.

Avoidance, also known as “negative phototaxis”, is the response of moving away from a light source. Coleoptera and other species that bore into the stems or fruits of plants have the behavioral trait of avoiding light and seeking hiding places. The wavelength and intensity of the light that produces this avoidance effect appears to differ depending on the species. Installing lights that insect pests avoid at the entrance to a cultivation facility would be expected to prevent invasions, and the direct illumination of crops should reduce the density of pests.

Light adaptation: The compound eyes of nocturnal moths, such as adult tobacco budworms, become accustomed to darkness (dark adaptation), and these insects become more active at night. When lighting is provided at night, light shining on the insect compound eye is reflected as a cat’s eye, but the reflection disappears very quickly. The compound eye changes to its daytime condition and light is no longer reflected. This is called “light adaptation”. Light and dark adaptations occur as a result of the movement of pigment granules within photoreceptor cells, and behaviors in nocturnal insects such as flying, mating, and egg-laying cease completely when the insect is in a state of light adaptation.

Circadian rhythms: Like humans, insects also have internal clocks in the brain, and the physiological status and timing of various behaviors are determined by the light-dark cycle of day and night. The biological clock cells in the brain and other central nervous systems that govern these behaviors are reset if light is shone on the insect during the night, causing the biological clock to be reset. This forward or backward shift is called a “phase shift”, and it causes the timing of activities such as flying or courtship to be thrown into confusion. Shining a light for a certain period of time (~ a few hours) at cultivation facilities can cause disturbances in these activities.

Photoperiodicity: To survive the winter, insects need to suspend their activities and endure the cold by entering a period of dormancy. Many insects take the shortening day length in autumn as a cue to prepare for winter by inducing dormancy. If insects are exposed to light at night for several successive days during this period, the induction of dormancy will be disturbed and the insects will be unable to survive the winter.

Light toxicity: Near-ultraviolet light is known to have a strong cytotoxic effect. It can not only denature proteins, but can also induce mutations by damaging double-stranded DNA in the cell. Some insect species are known to experience a breakdown in the cytoarchitecture of the compound eye upon exposure to ultraviolet or blue light. Light toxicity may also be expressed as a developmental impairment or behavioral disorder. Utilizing the damaging action of light on insects shows promise for use in pest control technologies in plant production sites and food factories.

Masking (or camouflaging): By manipulating the visible components of the light used to illuminate crops, the colors and patterns of objects can become difficult for insects to discriminate. For example, shining a blue light on apples will cause them to appear blackish. Many insects have a high sensitivity to near-ultraviolet rays, and their vision is therefore blocked if they are exposed to light that is lacking the ultraviolet range. Covering the facility with a film that shields ultraviolet rays can prevent the invasion of pests into cultivation facilities; thus, camouflaging the crops inside the facility so that insects cannot easily see them.

Dorsal light reaction: When locusts or dragonflies fly, ambient light (direct light from the sun or scattered light from the atmosphere) impacting the dorsal side of their bodies helps the insects to maintain the correct body angle and to keep level flight, horizontally. Placing a sheet of a material with a high optical reflectance across the ground reflects sunlight upward, which impairs the normal flying behavior of these insects. Mulching sheets for weed control made of a white material with high optical reflectance would likely be effective in preventing the invasion or settling of these insect pests.

Edge reaction: Many insects move toward the boundary region of a dark and light surface due to phototaxis. This is probably the effect of the visual contrast, but an edge can be created not only by a difference in light and dark, which is a difference in light intensity, but also by differences in the wavelength components or in the polarization orientation. Incorporating visual contrast into the design of light traps or sticky sheets is therefore expected to improve capture efficiency.

It can be seen from these examples that there is great diversity in the light response reactions of insects and that these responses are highly species-specific (Fig. 1). The stimuli are dependent not only on the intensity of the light, but are also greatly influenced by factors such as the wavelength, combination of wavelength components

(wavelength distribution), method of illumination (the size of the light source and the angle of illumination), background brightness and color contrast, and the presence or absence of polarization. In addition to the complexity of the stimuli, the insect itself may have inherent reactions that are species-specific, which may differ depending on the developmental stage (larva or adult) and physiological condition (fully fed or fasting state). The individual internal clock is also an important factor, and differences can arise due to diurnal, nocturnal, or crepuscular control cycles. The timing of active behaviors such as flying, courtship, and feeding vary by species. Therefore, to develop effective light pest control technologies, it is first necessary to characterize the reactions of the target insect species, and then full consideration needs to be given to such conditions as the materials used as a light source (bulbs, LEDs, etc.), the shape of the lighting system, and the installation site.

Technological development of light pest control

The main light pest control technologies developed to date utilize the light response reaction (classified in Fig. 3). Here, we review the latest information regarding these control methods.

Among the technologies that exploit the attraction of insects to light, electric insect zappers using black light are widespread. Black light contains near-ultraviolet light and therefore effectively attracts a variety of insect pests. These devices are useful not only in agriculture, but also for eliminating sanitary insect pests. The spectral preferences of various insect species have been determined (Fig. 2), making it possible to target specific species with captures and killing technologies that use LED light sources. Insects that are strongly attracted to near-ultraviolet light (365–385 nm) include the southern green stink bug, which damages wetland rice and vegetables; the brown planthopper, which is a vector of viral diseases in rice; the Ceratopogonidae, which are vectors for the arbovirus that causes damage to livestock; the tobacco beetle, which damages stored dry foods; and the rice leaf bug, which causes spotted rice damage. Similarly, irradiating water basin traps with ultraviolet rays (365 nm) is effective for attracting and killing fungus gnat species. Yellow tea thrips, which damage tea and citrus fruits, are strongly attracted to green light (520 nm). The flower bug (*Orius sauteri*) and mirid bug (*Nesidiocoris tenuis*), which are predatory enemies of thrips, and the tachinid fly (*Exorista japonica*), which is a parasitic enemy of thrips, have been

discovered to have a “unique” spectral preference for purple light (405 nm) (Fig. 4). Because thrips and whiteflies are attracted to ultraviolet and green light, it may be possible to use purple light to selectively attract the predators of pest insects to agricultural areas. Pest control methods using these techniques for field cultivated eggplants and greenhouse cultivated tomatoes have been demonstrated (Fig. 5).

To attract diurnal insect pests, methods using colored sticky sheets (adhesive traps) are more effective than nighttime light exposure. As the daytime use of reflective materials has been developed for different insect species, materials with the necessary properties to reflect specific regions of sunlight to target different insect pest species have also been developed. Melon thrips, a major insect pest of fruit and vegetables, are most effectively captured with blue or cobalt green (spectral reflectivity peak wavelength 503 nm) sticky sheets, while western flower thrips are effectively captured and killed with ultraviolet (355 nm) and green light (525 nm). Trials have recently been conducted in which visual contrasts are applied to sticky sheets to increase insect capture efficiencies through the use of patterns of light and shade using black and white materials.

Many insects were long believed to be unable to see red light (600–700 nm), and it was noticed only recently that brown planthoppers display a slight attraction to red light (660–735 nm). Although the mechanism behind the behavior has not yet been fully clarified, thrip density was reportedly suppressed in the presence of red light (600–660 nm). This has proven to be extremely effective in controlling thrips in eggplants and melons, and considerable progress has been made in its implementation. Based on the response reaction to red, not only red lighting but also red nets, the red light has been effectively used to control thrips species, and the rate of parasitism of outdoor cultivated green onions by onion thrips was decreased with the use of red nets. Manipulating the color of the strands of the nets has been shown to make them more effective for preventing the invasion of thrips and whiteflies into cultivation facilities.

The use of yellow fluorescent lamps to control owlet moths, a type of sucking insect that damages eggplants and peaches, was introduced in the 1990s and has become widespread as a technology for controlling nocturnal moth species. In the cultivation of ornamental flowers, the larvae of the owlet moth species rather than the adults cause damage to leaves and buds. Yellow fluorescent lamps suppress flying and egg-laying activities in adults, and the larvae of species such as the tobacco cutworm in roses and

cotton bollworm in carnations can be similarly controlled. The outdoor cultivation of crops such as lettuce requires a large area, and protection is offered by just a few light sources. High-intensity sodium lamps have been developed to meet this need, and highly efficient high-intensity LEDs are also being considered. The cotton bollworm and the cabbage moth have a high spectral sensitivity to green light (500–550 nm), and this has been shown to effectively control their behavior. Thus, similarly to the use of yellow fluorescent lamps, illumination with green LEDs has also been incorporated into IPM systems for outdoor vegetable cultivation.

Photoirradiation for specific periods of time has been conducted to affect the behaviors controlled by circadian rhythms (Fig. 1), but the observed effects are more delayed than those observed for light adaptation. A few hours of photoirradiation after sunset causes mating behavior to be thrown out of sync in the smaller tea tortrix, a major insect pest of tea. It has been discovered that blue light (around 450 nm) is effective at disrupting the circadian clock, and progress is being made in the development of LED light sources that control mating behavior by disrupting circadian rhythms.

With regard to light toxicity, ultraviolet rays are known to cause cellular injury, while some insect species such as leaf-miner flies have been found to be highly sensitive to blue light (400–470 nm), which inhibits development and causes death. Because this method can kill pests using light in the visible range (without the use of ultraviolet light), it has the potential to be widely used for killing pests in plant production sites and food factories.

The use of films that cut near-ultraviolet rays as pest control methods are based on the principle of masking (Fig. 1). Behavioral analysis using the greenhouse whitefly has clarified the principles of pest control by cutting near-ultraviolet rays. When plastic greenhouses are covered with a film that cuts near-ultraviolet rays, there is no change in the speed of their movement and dispersal within the greenhouse, but invasions from the outside are suppressed, while movement from inside the greenhouse to outside is promoted. Similarly, scattering a fine white calcium carbonate powder on the leaves of citrus trees to disguise their color is likely to be an effective method for masking plants from insects.

The dorsal light and edge reactions are used in methods that disrupt the flight of insect pests (Fig. 1). With regard to the dorsal light reaction, a silvery-white

polyethylene film was introduced as a measure to protect vegetables from the arrival of winged aphids, and its use has spread to the protection of tomatoes and other vegetables from thrips and whiteflies. High-performance, non-woven fabrics made of polyethylene with improved optical reflectance, vapor permeability, insulating properties, and durability have now been developed. The effectiveness of flight disruption by these reflective sheets has been demonstrated not only to control the invasion of small insect pests into cultivation facilities, but also for suppressing the density of pests on crops. With regard to the edge reaction (Fig. 1), studies of the arrival sites on flat LED light sources show that many insect species react to visual contrasts when determining their flight routes. A colored sticky sheet can be used to create a visual contrast using a black and white pattern for shade and light, and this visual contrast can be incorporated into flat light sources not only by controlling the brightness of the light source panels, but also by manipulating the wavelength or polarizing the light to create differences in brightness. A more effective capture can be expected from conventional traps if a visual contrast is incorporated.

Thus, light pest control development has progressed to exploiting insect life cycle phenomena or control methods that no one predicted would be used just 10 years ago. While there are high expectations for these new pest control technologies, the adaptive significance and the active mechanisms of light response reactions are not yet fully understood, and they need to be theoretically proven (Fig. 1). The accumulation of research on topics such as the range over which insect pests are affected by light control measures may lead to the development of practical pest control technologies. In addition, light pest control is likely to be useful for controlling sanitary insects in food factories or retail stores because no chemical products are used. There is interest in developing air cleaners that incorporate mosquito repellant functionality, and light pest control is expected to have applications in domestic insect control devices as well.

Key points when introducing light pest control technology

Light pest control technologies are described in detail in each section of this special feature, but as these are new technologies there are some key points that should be noted prior to introducing them into cultivation systems (Fig. 3). The first key point is that none of these technologies have the same effectiveness as chemical pesticides. For example, experiments in the control of thrips using illumination with red light

(vegetables grown in a greenhouse) have shown a reduction in density of 90–95%, indicating that complete elimination of the insect pests is not achieved. Also, although natural enemies can be attracted with red LEDs, and the density of thrips can then be brought down to fewer than 10%, a density reduction of only 60% is achieved during their peak in summer. This means that light pest control technologies need to be carefully combined with other pest control measures to mitigate the risk of economic damage. The second key point is that even for the same crop, there will be regional differences in cultivation methods, climate conditions, and crop diseases. It is therefore necessary to investigate methods of light pest control that take into account the combination of these conditions. For example, with regard to the use of technology to attract natural enemies of thrips using purple LEDs (Fig. 4), insectary plants first need to be established to attract flower bugs (*O. sauteri*) within the environment in which their predatory effect is needed (Fig. 5). If this preparation is inadequate, the purple LEDs cannot be expected to be effective as a pest control measure. Care needs to be taken to ensure the selection of an insectary plant that suits the particular area and the arrangement of LEDs in the field. Red nets have been highly effective at preventing pest invasions in cultivation facilities, but it is essential that the nets be placed at the invasion entry point at an early stage.

Successful cultivation of crops also requires the management of fertilizer, water, temperature, and various other aspects. When a new technology, such as light pest control, is introduced, it is also necessary to manage the risk of reducing pesticide use, and agricultural producers need to display good judgment developed through years of experience. Furthermore, to reliably realize the effectiveness of light pest control, the cooperation of specialists involved in providing technical guidance and dissemination of information will be essential.

CONCLUSION

Pesticide resistance among insect pests was first reported in the 1950s, shortly after the introduction of chemical pesticides. Given the high selection pressure of chemical pesticides, it is probably fair to say that this problem was bound to occur. Various insect pests have been reported to develop resistance, even to neonicotinoid pesticides, which are comparatively new agents, and there is a never-ending game of cat-and-mouse between the development of new pesticides and the acquisition of tolerance to them.

The “light pest control” technologies presented here exploit the instinctive nature of insects (Fig. 1; Fig. 3), and while selective pressure (pest control efficiency) is not strong, resistance to light pest control measures is unlikely to appear. In addition, light pest control technologies can easily be combined with other pest control measures, such as the use of natural predators (Fig. 4; Fig. 5), allowing a variety of use patterns. Our investigations will continue, with the cooperation of other parties involved in light pest control, to achieve our goal of ensuring that new light pest control technologies will play a role in low-pesticide-use cultivation systems, and thus reduce the environmental burden of pesticide residues.

ACKNOWLEDGEMENTS

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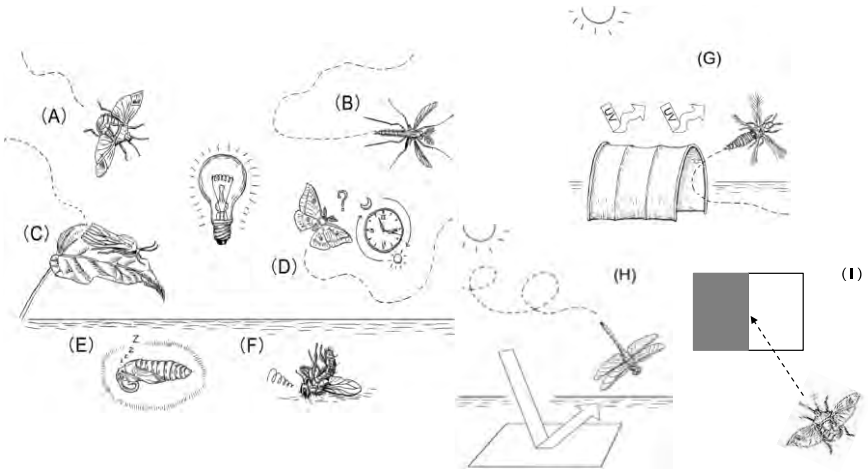


Fig. 1. Various light response reactions of insects (A) Attraction, (B) avoidance, (C) light adaptation, (D) circadian rhythms, (E) photoperiodicity, (F) light toxicity, (G) masking, (H) dorsal light reaction, and (I) edge reaction. Adapted with modifications from Shimoda and Honda (2013). Many aspects of the mechanisms of light response reactions have not yet been determined and the conceptual understanding of these responses may change in the future.

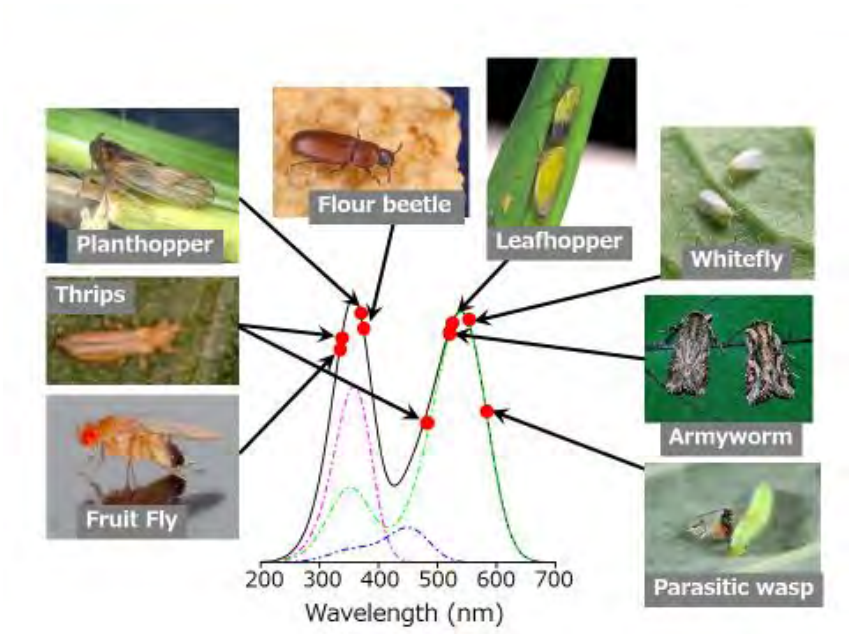


Fig. 2. Light wavelengths that attract insects (spectral preferences). Light of different wavelengths with the same intensity (brightness) was used to determine the spectral preferences of each insect species tested. The sensitivity spectrum of the insect compound eye has peaks in the ultraviolet region (around 350 nm), the green region (around 550 nm), or in both, and many insects exhibit a preference for these regions. This figure shows an example of a bimodal mean sensitivity spectrum, with peaks in both the ultraviolet and green regions. Most insects are attracted to UV or green light. These correspond to the peaks of photosensitivity, determined by an electroretinogram (ERG).

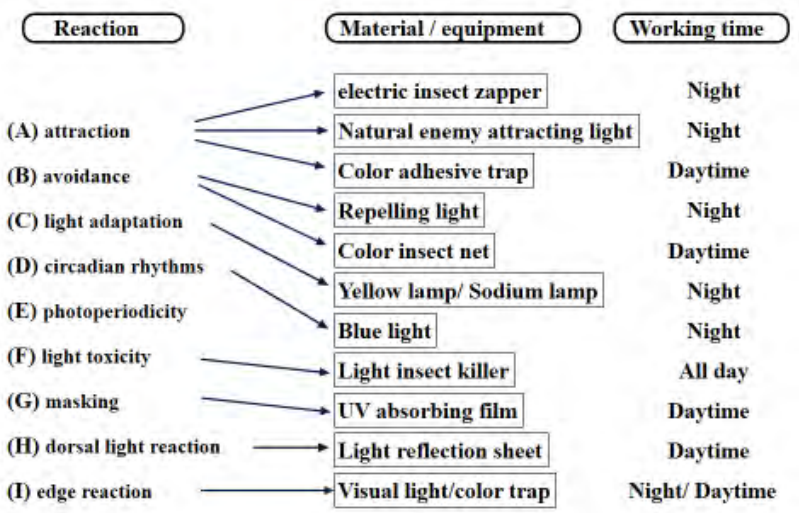


Fig. 3. Insect pest control technologies using light response reactions. The arrows show the relationship between the main pest control materials that use light response reactions and their underlying principles. Pest control materials based on entirely new concepts may well be developed in the future as a result of research findings regarding light response reactions and technological developments.

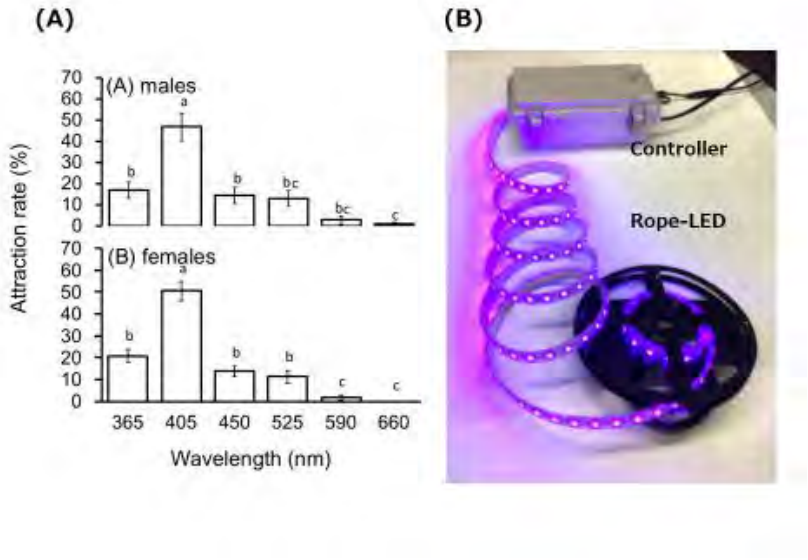


Fig. 4. Predatory bug, *Orius sauteri*, shows a unique spectral preference for violet light. (A) Adult *O. sauteri* were most attracted to violet light of 405 nm, although this wavelength is just at the bottom of the photosensitivity curve. This was the first finding of an insect being predominantly attracted to violet light. (B) An LED-lighting device attracting *O. sauteri* was developed.

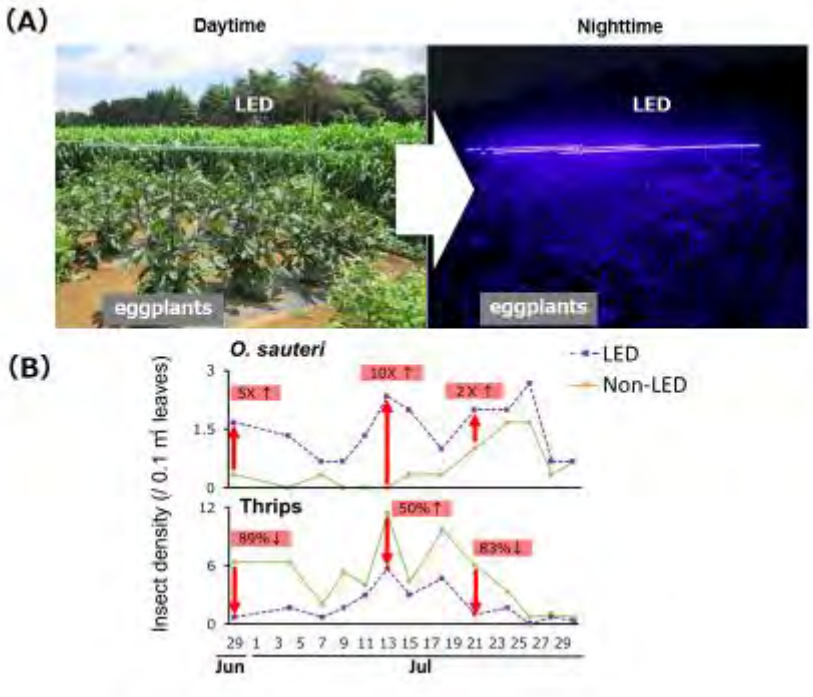


Fig. 5. Introduction of violet-LED lighting into open-field eggplant cultivation

(A) A rope-type violet-LED was installed above the eggplants. The eggplants were cultivated according to conventional farming methods in central Japan, but no pesticides were used so that the effectiveness of LED-lighting alone could be assessed. The intensity of the LED light was very low (1/4,000 of sunshine intensity). Lighting was limited to 3 h in the evening when *O. sauteri* are active. (B) The insect density on eggplants was compared between LED illuminated and non-LED illuminated plots. In the LED plots, the *O. sauteri* density immediately increased when lighting commenced and remained higher than in the non-LED plots. The thrips density was always lower than in the non-LED plots. The thrips density was suppressed to an almost economically acceptable level. The effective control of thrips using violet-LED lighting was confirmed.

Migration analyses and predictions for migratory insect pests toward Japan

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ABSTRACT

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), complex was eradicated in 1986 in Japan, and the state of eradication has been maintained until present. However, male adults of *B. dorsalis* complex were occasionally caught in the surveillance network in southwestern islands of Okinawa and Kagoshima Prefectures. Assuming if these catches were wind-borne migrations, possible source could be estimated with trajectory analysis. The results indicated one third of all the catches from 1986 to 2012 in Okinawa were found to be due to possible wind-borne migration. In 72 percent of those cases, Taiwan was estimated as possible source. In Taiwan, southeastern part was often estimated as source area. On the other hand, several catch cases occurred in 2015, 2017 and 2018 in Kagoshima Prefecture, northwestern part of Taiwan was estimated as source area. Southwesterly winds in the *Bai-u* rainy season were key factor for these estimated migrations. The migration prediction model for the white-backed planthopper, *Sogatella furcifera* (Horváth), was modified by both fining horizontal resolution and implementing assumed landing processes. The prediction was evaluated with net trap data at five sites in three years in Miyazaki Prefecture. The results indicated the prediction hitting ratio has been improved to be 83.8%, demonstrating that the simulation method is one possible approach to unknown landing process. The common cutworm, *Spodoptera litura*, is highly dispersible insect pest and attacks soybean leaves in summer in western Japan. Its dispersion was monitored with searchlight trap and entomological radar. At the same time, soybean leaf damages were investigated. The two-year survey results indicated that the catch peak of the searchlight trap preceded the leaf damage caused by *S. litura* larvae by 3-7 days, potentially suggesting that a short-term forecasting of soybean damage based on the monitoring with the searchlight trap might be feasible. To get firm conclusions, repetition of the survey for one more year will be necessary.

Keywords: migration, prediction, *Bactrocera dorsalis* complex, *Nilaparvata lugens*, *Sogatella furcifera, Laodelphax striatellus*, *Spodoptera litura*, entomological radar

INTRODUCTION

There are many migratory insects [4] or highly dispersible insects. Among them, our study targets are six agricultural insect pest species that immigrate from overseas into Japan: the oriental fruit fly, *Bactrocera dorsalis* complex, the brown planthopper, *Nilaparvata lugens* (Stål), the white-backed planthopper, *Sogatella furcifera*, the small brown planthopper, *Laodelphax striatellus* (Fallén), the common cutworm, *Spodoptera litura* and the oriental armyworm, *Mythimna separata* (Walker).

B. dorsalis complex has been eradicated in Japan in 1986 [10]. Although the state of eradication has been kept since then, male adults were occasionally trapped in the surveillance trap on Japanese southwestern islands every year [15, 22]. Wind-borne immigration source was estimated for these catches [21, 22], and the results are discussed in this paper.

Rice planthoppers are important migratory pests in rice production in Asia. Basically, the East Asian population of *N. lugens* and *S. furcifera* overwinter in the middle to northern Vietnam and Hainan Province of China, they emigrate to southern China in spring, multiply for a few generations there, and migrate northeastward again into Taiwan, Japan, Korea and northern mainland of China [20]. On the other hand, *L. striatellus* can overwinter in mid-latitude region and is thought to be indigenous. However, it was found that a population in eastern China occasionally disperse overseas to Korea and Japan [18, 20]. For these overseas movements toward Japan, prediction systems were developed for users in plant protection stations nationwide [16, 19]. Recently, migration prediction method for *N. lugens* and *S. furcifera* has been improved to present a prediction in a fine spatial resolution. The related research activities are summarized in this paper.

S. litura is also a migratory pest and it is suggested that *S. litura* immigrates from overseas into western Japan from May to mid-July when windy weather conditions occurred around Japan [24]. The species is highly dispersible and polyphagous insect pest that attacks a variety of crops, including vegetables, maize, soybean, flowers, and fruit trees. Of these, soybean seeded in mid-to-late July in western Japan usually

experiences attack by *S. litura* larvae from mid-August to September. To develop a method to forecast soybean leaf damage caused by the larvae, insect dispersion was monitored with a x-band entomological radar and a searchlight trap during the summer soybean season in 2016 and 2017 in western Japan. The results so far are introduced and discussed.

A migratory insect pest other than the above-mentioned ones, the oriental armyworm, *M. separata* is also an important migratory pest [(7)]. The insect immigrates often into northern Japan, or on rare occasions into other regions, mainly in late May to early June (Fig. 2) [(11)]. In the latter case, many larvae suddenly occurred in western Japan in late June 2017 [(25)]. This incident triggered an international research between Japan and China on migration of *M. separata*. This project is not presented in this paper but will be briefly introduced in my conference presentation.

(1) *B. dorsalis* complex

The accomplishment of the eradication of *B. dorsalis* complex in 1986 in Japan never eliminated the insect's problem. The reality has been the opposite. A few to tens of *B. dorsalis* complex has been caught in the surveillance traps every year in Okinawa Prefecture [(15)], and lately this insect has been occasionally captured on southern islands of Kagoshima Prefecture [(22)]. In general, possible arrival routes of the trapped insects are two: accidental importation of infested fruits, or wind-borne immigration from the source regions.

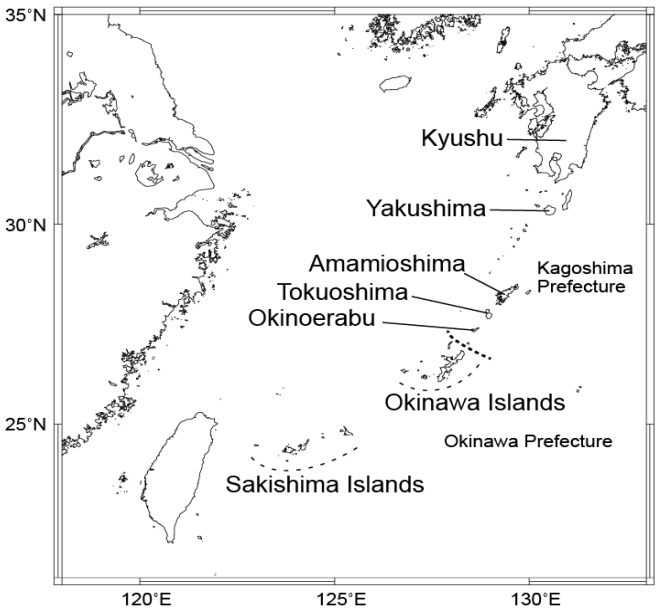


Fig. 1 Study area

Source estimation for Okinawa Prefecture

The latter route for catch cases from 1986 to 2012 in Okinawa was analyzed with trajectory analysis [21]. The surveillance data showed that more insects were trapped on southern Sakishima Islands than northern Okinawa Islands [21] (Fig. 1). Sakishima Islands are closer to Taiwan.

Possible source was estimated by investigating whether there was any air-current connecting between possible source areas and a trapped site before trapping. For that purpose, backward trajectories starting at 500 m above the ground over the trapped site in Okinawa Prefecture were calculated. Twenty-four trajectories for each day started at an interval of one hour, and their trajectory duration was set to be 24 h. Since the time interval of sample collection was mainly set to be 14 days, the analysis period for each catch was set to 14 days. Therefore, totally 336 trajectories for one trapped case were calculated.

The results indicated that in 66 percent, or two thirds, of all the trapped cases, the trajectories were found to reach over any of the possible sources: Taiwan, the Philippines or southern China [21], suggesting wind-borne immigration (Fig. 2). Taiwan was estimated as the source in 71.8 % of the wind-borne dispersion cases, following the Philippines (24.3 %) and southern China (3.9 %) [21]. A southeastern part of Taiwan, mainly Taitung area, was often estimated as the source area, because southwesterly winds often brew in the dispersion events. The peak period of the dispersion was found to occur in June, the *Bai-u* rainy season. Since Taiwan is very close to Sakishima Islands (Fig. 1), and the insect density in Taiwan increases in summer [13], where environmental and entomological conditions as the source seem to be sufficient. In other occasion, southerly winds occurred around a high-pressure system over the Pacific Ocean or at times of typhoon approach. In such cases, the Philippines was estimated as the source.

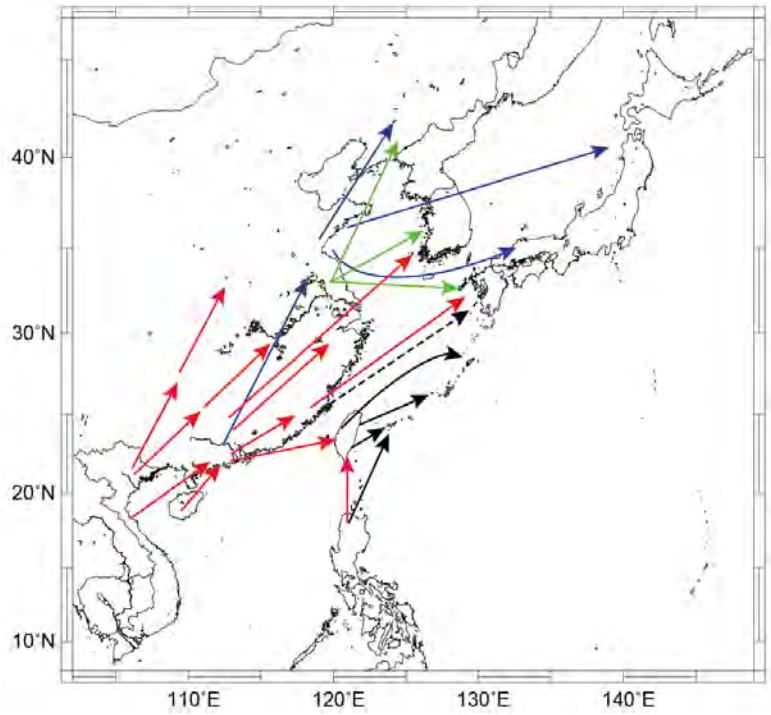


Fig. 2. Schematic of major migration routes of *S. dorsalis* complex (red), *S. furcifera* and *N. lugens* (red), *L. striatellus* (green), and *M. separata* (blue).

Source estimation for Kagoshima Prefecture

In 2015, many *B. dorsalis* complex were trapped and many infested fruits were found on Amamioshima, Yakushima and Tokunoshima Islands in Kagoshima Prefecture [(22)]. At the same time, several *B. dorsalis* males were also found on some islands of Okinawa. A trapped insect on Amamioshima Island was firstly found on June 30, and a trapped insect was found also on Kume Island in Okinawa on July 3 [(22)]. To find the source for these trapped cases, trajectory analysis was performed. The method was the same as described above for Okinawa. The results indicated that northwestern and eastern part of Taiwan were found to be the source for the Amamioshima and Kume Island, respectively. Inversely, forward trajectories from a northwestern point (Taoyuan: 24.90ºN, 121.22ºE) or a west-central point (Yuanlin: 23.96ºN, 120.59ºE) on Taiwan reached over Amamioshima Island for 5 days (June 25-29), or 3 days (June 25-27), respectively. This result suggested western parts of Taiwan were the source for early immigrants on Amamioshima Island in 2015 [(22)].

Moreover, *B. dorsalis* males are also trapped on several islands in Kagoshima Prefecture in 2017 and 2018 (Table 1, Fig. 1). The backward trajectory analysis for

these trapping cases indicated that the northwestern part of Taiwan was estimated as the source.

Table 1 Monitoring data and the trajectory analysis in 2017 and 2018 in Kagoshima Prefecture

Date of collection	Island name (Starting location for trajectories)	Number of trapped *B. dorsalis* males	Date and height for backward trajectories that reached Taiwan	Estimated source
June 21, 2017	Okinoerabu	1	June 12, 13	Northwestern
June 22, 2017	Okinoerabu	1	500m	Taiwan (NWT)
June 27, 2017	Okinoerabu	1		
July 5, 2017	Okinoerabu	2		
July 5, 2017	Tokunoshima	1	June 26, 27, 1000m	NWT
July 5, 2017	Yakushima	4	June 24	NWT
July 10, 2017	Yakushima	3	1000m	
August 1, 2017	Yakushima	1		
August 22, 2017	Yakushima	1	Augsut 9, 10, 11	NWT
August 24, 2017	Yakushima	2	500m	
August 28, 2017	Yakushima	1		
June 5, 2018	Okinoerabu	1	May 27,500m	NWT
June 5, 2018	Tokunoshima	2	May 27	NWT
June 14, 2018	Tokunoshima	1	500m	
June 7, 2018	Amamioshima	1	May 27	NWT
June 14, 2018	Amamioshima	1	500m	

Discussion

The southwestern islands of Japan are distributed long from the southwest to the northeast. There are mainly southwesterly winds in the rainy season. Therefore, it is suggested that the source areas in Taiwan for dispersions to Okinawa and Kagoshima are different (Fig. 2). So far, it is estimated that *B. dorsalis* complex arrived on Yakushima Island (Table 1), which is a farther point in Kagoshima Prefecture from Taiwan (Fig. 1). It is unknown whether the species could arrive in the mainland of Kyushu, or not. However, if the insect could arrive in Kyushu, the possible source area would be Fujian Province, like the migration case of rice planthoppers, whose source was estimated to be Fujian Province [(17)], because Kyushu Island is located in the north of the southwestern islands, southern China is located in the southwestern direction (southwesterly wind direction) from Kyushu. It might be possible that climate change

could affect the distribution and density of *B. dorsalis* complex in southern China. Therefore, it is important to survey the *B. dorsalis* complex in all western Japan.

(2) Rice planthoppers

There are two types of predictions for rice planthoppers in Japanese real-time prediction systems: one for tropical species, *S. furcifera* and *N. lugens*, the other for *L. striatellus*. This is mainly because take-off areas and migration seasons are different between the two groups. *S. furcifera* and *N. lugens* migrating towards Japan mainly take off in southern China in late June to early July, the *Bai-u* rainy season (Fig. 2) [16,20]. These planthoppers emigrate from fields of the early rice crop harvested in July there. In contrast, *L. striatellus* migrates from eastern China, mainly Jiangsu Province, to Korea, northeastern China or occasionally Japan, from late May to early June (Fig. 2) [18, 20]. *L. striatellus* emigrates from wheat fields during harvesting season [23].

However, there are many common features that are listed as follows: the basic prediction model is the same; the horizontal resolution is 33 km; modelled planthoppers take off in the source regions and fly up for some time to make use of upper air-currents; take-off occurs every day during prediction period; fly at the same velocity as winds, taking vertical diffusion into account; relative aerial density of the planthoppers below 100 m above the ground over Japan are used to know the arrival of the insect; modelled planthoppers never land on the surface, so that the prediction of immigration amount is not feasible; email notification is available when prediction is positive for prefectures previously registered by a user; and the database of past predictions is available [16,19]. Using these systems, the insects' arrival time and area is known in real time. The prediction information as well as the monitoring data of light and net traps or the density survey in rice fields are used by prefectural plant protection offices to determine the dates of major immigrations. Based on the immigration dates, the plant protection offices predict the insect's growth stage and release information on insect's density in fields and the right time for chemical control.

Although the current prediction system works well, there are some rooms for improvement to fulfill the users' requests. One was the horizontal resolution of the prediction. It is set at 33 km now that is not enough to distinguish local difference of the immigration within a prefecture of about 100-km size. A prediction in a fine mesh was desired. Another area that the users demanded for improvement was on the prediction of immigration density, or the arriving insect number. The current systems

present an arrival area only. Therefore, this study was set to improve the horizontal resolution of the model calculation and to implement the landing processes to approach the prediction of density

Materials and methods

1. Survey area and species

Since the number of trapped planthoppers is larger in western Japan, Miyazaki Prefecture on Kyushu Island was selected as the survey area. Five net traps with a 1-m ring and tow net mounted at 10 m above the ground were newly set up at five different points; they located in Nobeoka (northern), Miyazaki and Kunitomi (middle), Miyakonojo (southern), and Ebino (southwestern) in the prefecture. The traps were operated from June to July each for the following three years, 2015, 2016, and 2017. Samples were collected at around 09:00 hours local time every morning. *S. furcifera* was selected as a study target, because its trapped number was much larger than that of *N. lugens*.

2. Modification of the migration model

The horizontal resolution of the model was changed to 9 km. The value was determined considering both the distance between local regions in a prefecture (about a few 10-km scale), and calculation cost that increases rapidly as the resolution becomes finer.

No direct observation of rice planthopper's landing process has been reported. Therefore, two types of assumed landing processes were implemented based on two facts. The one fact is that immigration density decreases as migration distance from the source increases (e.g. Fig.3 of Lu et al. [12]), or that the trap catch number is larger in western part of Japan than that in eastern part (Fig.5 of Kisimoto [8], Fig. 8 of Nasu [14]). The other fact is that landing is associated with rain belts of front, suggesting rain falls make planthoppers land [6,9,12,26].

A landing process that planthoppers land at a temporally constant rate, dry landing process, was implemented as follows:

$dC = -\alpha C dt;\ \alpha = const.$ ie. $C = exp(-\alpha t)$

where C, dC, t and dt are aerial density of planthopper, change of aerial density, time, and time step of calculation, respectively. These means that the aerial density will exponentially decrease with flight duration. The value of the constant factor is unknown

and was assumed to be 0.001 with a time step of 40 sec. Fig. 2 indicates how aerial density decreases as flight duration increases. This process was turned on at 15 hours after each take off, when planthoppers flying toward Japan on the East China Sea.

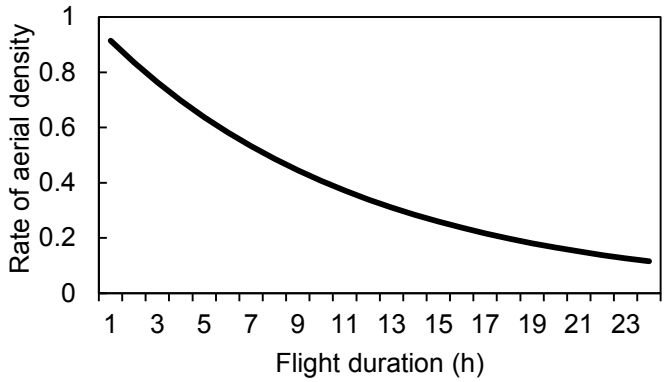


Fig. 2 Decrease of aerial density in dry landing process

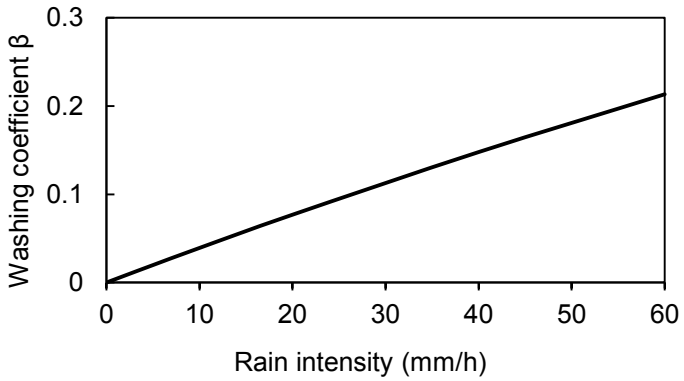


Fig. 3 Washing coefficient depending on rain intensity

When it rains, planthoppers are assumed to land depending on rain intensity. Since there is no direct observation of such a wet landing process, change of aerial density by rain fall was assumed referring to wet deposition process in meteorology as follows:

$dC = -\beta C dt;\ \beta = 1-exp(-aRdt)$

where β, a, and R are washing coefficient (s^{-1}), constant factor (0.0001), rain intensity (mm/s), respectively. The washing coefficient β changes with rain intensity as shown in Fig. 3.

The constant factors, α and a were determined by trial and error, or repeating prediction simulations with changes of values.

A square takeoff area, 3º in latitude by 2.5º in longitude, was set on Fujian Province that is a main possible source for planthoppers immigrating into western Japan. Takeoff time was set at dusk, 19:00 hours Chinese local time.

In evaluation of the new prediction, relative density of *S. furcifera* that landed on the ground in Japan was calculated, and immigration at a monitoring site was determined when relative density was larger than a threshold. Prediction data were compared with daily net trap monitoring data at 5 sites in Miyazaki Prefecture from 2015 to 2017. When no insect was captured, no immigration was regarded to have occurred, and when more than one was captured, immigration was regarded to have occurred.

Results

The monitoring data for three years indicated that there was difference in the number of caught *S. furcifera* within the prefecture; the numbers in southern and southwestern areas were larger than those in eastern and northern areas. The prediction evaluation result indicated that hitting ratio averaged in three years at the five sites was found to be 83.8 % (Table 2). For comparison, evaluation of a prediction model of 9-km resolution without the landing processes was also performed. The result showed the hitting ratio of 77.1 %.

Table 2 Evaluation of the new prediction method in Miyazaki Prefecture

Year	Hitting ratio (%)					
	Ebino	Miyakonojo	Kunitomi	Miyazaki	Nobeoka	Average
2015	85.0	77.5	80.0	70.0	87.5	80.0
2016	82.9	77.5	80.0	77.5	70.0	77.6
2017	96.5	87.9	94.8	93.1	96.5	93.8
Average	88.1	81.0	84.9	80.2	84.7	83.8

Summary

The results showed the prediction accuracy of the high-resolution model was improved with the landing processes. The hitting ratio was over 80 % that is good enough for actual use in a next step. However, the evaluation was performed only in one prefecture in this study. Therefore, an evaluation in other prefectures is desired as well. Nevertheless, it is rather difficult to perform such an evaluation with dense net trap monitoring in a wide area, due to its high evaluation cost. Therefore, it is practical that a prototype migration prediction system with the new model is operated, while the user's feedback is collected for future improvements.

Our knowledge on the landing process of rice planthoppers is very limited. Direct observation with an entomological radar, for example, is impossible because the insects arrive mostly when it rains and radar observation is prevented from rain drops. However, this study presented a different approach to the landing process, an approach with simulation. At least, the results suggested that some landing processes affected the number of immigrants. The fine horizontal resolution should have affected the predicted number of immigrants, because topography in the model became finer when the horizontal resolution was changed, and simulated winds over the mountains could also be changed. The contribution of each of the three factors (rain, distance, and resolution) to the number of migrants is not clear at this moment. Further study is necessary.

(3) *Spodoptera litura*

S. litura is a polyphagous insect pest that attacks a variety of crops. Of these, soybean seeded in mid-to-late July in western Japan usually experiences attack by larvae from mid-August to September. Usually, Japanese farmers perform chemical control based on the severity of actual damage in their soybean fields. However, the number of fields managed by a farmer or an agricultural company is growing recently, it is getting harder to observe every field frequently. The damage could get worse if an initial occurrence was missed. Therefore, this study aims to develop a method to forecast soybean damage. If such forecasting could be realized, chemical control could be effectively timed to target the larvae in its early stages. In this study, therefore, dispersion of the common cutworm is investigated by using entomological radar and a searchlight trap to find the correlation with the soybean damage.

Materials and methods

1. Survey area

The area located in the Saga Plains, Saga Prefecture in western Japan, where rice and soybean are cultivated in summer, was used. The monitoring of the insect occurrence and soybean damage attacked by larvae of *S. litura* was conducted in 2016 and 2017. An entomological radar and a searchlight trap were located at the same place (33.3055°N, 130.3299°E). The soybean field used in 2016 was located about 300-m west of the monitoring site, and that used in 2017 was about 550-m north of the site.

2. Survey of soybean leaf damage

The number of soybean leaves damaged by *S. litura* was counted by checking all the leaves of 1,000 soybean plants in an experimental field to see if there were small white food mark dots on green leaves and newly hatched green 1st-instar larvae on the back of the leaf. The survey was conducted every Monday, Wednesday and Friday from early August to early October.

3. Vertically-emitting visible light trap

The searchlight trap was used to collect flying insects in the night sky. It consisted of a 1,000-watt metal halide lamp vertically emitting a strong narrow visible-light beam in the dark sky, with a large metal funnel set above an insect collection box. It was turned on at 19:00 hours local time and off at 24:00 hours. Samples were collected in the next morning. The species and sex of *S. litura* were later identified based on the morphology of genitalia.

4. Monitoring with entomological radar

A type of vertical-looking radar [2,3] was used to retrieve flight direction, height, speed, body alignment, body size, and shape parameters of individual insects passing over the radar continuously. These flight parameters were retrieved from radar signals with a method by Harman and Drake [5]. Unfortunately, because noise was on a rotary encoder signal, flight direction and body alignment could not be estimated in 2016. Therefore, surface wind direction at a weather station about 10-km southwest of the monitoring site was referenced for that year.

The radar receives various echoes from different species of insects and other animals in the air. To select only *S. litura*-type targets among all the observed echoes, body size and shape parameters of radar cross sections were used. The data were previously measured with live male samples in the laboratory. The measurement method was similar to that in Aldhous [1]. The radar cross section has a form of the equation: $a_0 + a_2*\cos 2ø + a_4*\cos 4ø$ (cm^2), where ø is an angle between the body axis and the polarization of microwave. The conditions found to select *S. litura*-like targets were as follows:

$$0.4 \leq a_0 \leq 1.0 \ (cm^2);\ 1 \leq a_2/a_0 \leq 1.35;\ 0.2 \leq a_2/a_0 \leq 0.5$$

Results

Several catch peaks of *S. litura* including female adults were found in late August and late September in the searchlight trap in 2016. Large catch peaks occurred on 20

Aug and 20-23 Sep 2016. The flight parameters of these *S. litura*-like targets were estimated by the radar. Although no flight direction data were available this year due to the noise on the radar signal, surface wind direction at the weather station closest to the site was northerly during outstanding peaks on 20 Aug and 20-23 Sep. There are other peaks on 26, 30 Aug, and 26 Sep. Wind directions on 30 Aug were southwesterly, and on the other peak days was northerly. The average flight height on the northerly days was 700–800 m. Meanwhile, the average flight speed ranged from 7.6–13.6 m/s.

The results of the 2016 survey also showed that soybean leaf damage appeared some days after corresponding catch by the searchlight trap. The time difference between the catch peaks and the corresponding peaks of the number of newly damaged leaves was found to be 3 to 5 days.

The catch numbers in 2017 were smaller than those in the previous year. The number of newly damaged leaves was also lower than that in 2016. Therefore, pairing of the two peaks was rather difficult for this year. The time difference between the two peaks seemed to be 3–7 days. Among six catch peaks on 27, 29 Aug, 12, 22, 29 Sep, and 3 Oct, only three on 12, 29 Sep and 3 Oct 2017 had many *S. litura*-like targets in the radar observation. It was estimated that the targets moved toward the south or southeast. The estimated flight heights were 700–800 m, which was similar to the previous year. The average of flight speed ranged from 6.3–12.3 m/s.

Discussion

The study showed that the catch peak of the searchlight trap preceded the leaf damage caused by *S. litura* larvae by 3–7 days. The egg period of the insect is reported to be four days at air temperatures of 25 °C. Therefore, this result indicated that the catch of the searchlight trap potentially indicated the insect's arrival in the survey area. Thus, the study suggests that a short-term forecasting of soybean damage based on the monitoring results with the searchlight trap might be feasible. However, since the numbers of the 2017 survey were too small for firm conclusions, repetition of the survey for one more year will be necessary.

The flight height estimated by the radar was high and close to the height of the mountains located to the north of the monitoring site. The closest distance between the site and the mountains was only 3 km. This suggests that the insects dispersed from the

mountains, or that they came flying over the mountains from somewhere beyond. It is necessary to identify the insect source.

Large target numbers in the northerly winds in late September and October in the two-year study should indicate the likely returning migrations toward the south in autumn. The numbers must include not only echoes from *S. litura*, but also other insect species with similar body size and shape to *S. litura*, because the radar technique is unable to discriminate them. Therefore, the combination of entomological radar and the searchlight trap is necessary to investigate the dispersion of a specific insect target.

SUMMARY

Japan is located to the east of China, which is a big source of migratory insects in mid-latitudes of the north sphere of the rotating Earth, and from there the basic direction of air-currents is southwesterly or westerly. In addition, a distance from the source area to Japan ranges between a few hundreds to one thousand km, which migratory insects can easily fly over. In other words, Japan seems to be an ideal destiny for the migratory insects to land. Taiwan seems to be under a similar environmental condition, too. Therefore, sharing the knowledge and technologies shown in this study should benefit the researchers and growers of both countries. In fact, scientists from NARO and TARI are conducting an international joint research on monitoring and management of *B. dorsalis* complex since 2016. In the research, the Japanese scientists always thank the kind and effective supports provided by Taiwanese scientists. The author hopes that our collaborations continue to not only improve plant protection research, but to expand to other agricultural researches for even deeper understanding in the future.

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Recent regulations on safe plant protection materials in Taiwan

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ABSTRACT

Plant protection materials are very important resources to ensure agricultural production. After huge chemical pesticides were synthesized and used, it in no doubt has increased food and fiber production but it certainly has caused adverse effect to environment and health. Once the plant protection agents were applied to crops, the residues would most likely get into our food chain. This undoubtedly would expose us to the risky residues and make people aware more of the importance of food safety. The trends in recent decades are to develop safe pesticides with low toxicity, high efficiency and high specificity, and to encourage organic and environmentally friendly farming without using excess of chemical pesticides. But a safe plant protection material or its formulation is still regarded as a pesticide and should be regulated by authority under the management acts. This implies a needed complicate registration procedure, which would jack up the costs for commercialization. According to the regulation act of pesticide in Taiwan, no matter whether it is classified as chemical pesticides or biopesticides, data required for registration include studies of physicochemical properties, toxicities, and field trials of efficacy, residue, and phytotoxicity. All of these studies should in compliance with good laboratory practice (GLP) guideline. The recent policy guidelines set for registration and the reduction of pesticide are in conflicts for biopesticide. Most biopesticides are general regarded as safe, but they are still considered at low techniques readiness level (TRL), especially those used as botanic pesticides. Due to the above-mentioned unsettled guidelines and conflicts, it would be a long way to commercialize safe products that in turn can't speed up chemical pesticides reduction and/or replacement. The authority in charge of the regulation acts has been considerate in trying to modify the reclassification of risk assessment.

Deregulation on highly safe products will apply with registration exemption or waive some data requirement.

Keywords: regulation, deregulation, safe pesticide, data requirement, exemption

Potential Application of Immunoassay for the Detection of Pesticide Residues in Agriculture

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ABSTRACT

In Taiwan, the Agricultural and Forestry Agency (AFA) and Food and Drug Administration (FDA) routinely monitor the pesticide residues in agricultural products; samples were collected from production districts, packing sites and all kinds of markets. Currently the multi-residue testing methods of pesticides promulgated by the Ministry of Health and Welfare can determine the residues of 373 pesticides or their metabolites. However, expenses for the installation of needed analytic instruments, labor, and other cost are too expensive for most people, besides it cannot prevent the risky shipments in time from further trade. A rapid bioassay of pesticide residues (RBPR) method targeting on the neurotoxic orgranophosphorus and carbamate insecticides was developed by Taiwan Agricultural Research Institute in 1985 using the acetylcholinesterases extracted from a susceptible housefly strain. In 2017, 360 RBPR stations inspected 1.26 million shipments and a total of 37 tons of agricultural products with toxicity risk were destroyed. Since many of these toxic insecticides were banned in the past two decades, research on developing immunoassay, aiming at 30 insecticides and fungicides that were frequently found in violated fruits and vegetables by FDA, was launched in 2011 to strengthen the RBPR stations with enhanced residue control capability. In a 4-year program, highly specific and sensitive antibodies against 18 pesticides as well as direct and indirect competitive ELISA protocols have been developed with the capability of reaching the detection limits ranging from 0.01 to 2.24 ppb. Commercial test strips and ELISA kits have been under development through technical transfer of the antibodies

and the assay protocols to biotechnology companies since 2016. The first product is a test strip that can detect the residue of dimethomorph in 10 min with the cut-off value of 1 ppm. By making adequate dilutions during the extraction process, the rapid test is able to obtain the result that meets the tolerance standard of various crops, e.g., 0.6 ppm and 2.5 ppm for onion and small leafy vegetables, respectively.

Keywords: immunoassay of pesticide residues, enzyme-linked immunosorbent assay (ELISA), colloidal-gold based lateral-flow immunoassay

INTRODUCTION

To ensure the high yield, many pesticides are mixed and frequently used during crop production to combat the damage cause by pests, diseases and weeds. Located in the sub-tropical region, the year-round cropping system in Taiwan facilitates the propagation of all pests and has accelerated the pest resistance problem; hence the farmers tend to use more pesticides to guarantee effective control.

In order to monitor and understand general situation of pesticide residues in fruits and vegetables, Taiwan has been conducting residues monitoring program since early 1960s. The earliest residue control method in Taiwan used alive housefly adults as testing organism, which was developed by Taiwan Agricultural Research Institute (TARI) during 1959-1962. This 4-hr bioassay method was implemented in 18 wholesale markets around Taiwan, and had been replaced in 1985 by the rapid bioassay for pesticide residues (RBPR) technology that takes only 10 min per assay.

The RBPR method uses the neuroenzyme, acetylcholoinesterases (AChE), extracted from the heads of susceptible housefly colony to detect the cumulative toxicity of organophosphorus and carbamate insecticides [5, 2]. The theory has been echoed by the Food Quality Protection Act (FQPA) of US EPA in late 1990s, which emphasizing the importance of cumulative toxicity of these two groups of insecticides in its report on "*The Use of Data on Cholinesterase Inhibition for Risk Assessment of Organophosphorus and Carbamate Pesticides* [12].

Currently, RBPR network supervised by TARI includes users from farmer's groups, wholesale markets, food suppliers, supermarket chain stores, fast food companies, etc. In 2017, 360 RBPR stations inspected 1.26 million shipments that resulted in demolished 37 tons agricultural products with higher residue risk before consumption. For instance, the RBPR station at Taipei Agricultural Produce Market

has been conducting inspection since 1985; they randomly checks 150-180 shipments every day during midnight hours. All testing results, including double check, need to be finished before auction starts at 3:00 AM, and any shipments with higher residue risk will be withheld on-site [3].

Chemical analysis was not prevailing before 1990s in Taiwan due to the technology and instruments were still new for most countries, and could only detect the residues of 79 pesticides in early 2000s. However, great progress has been made in the analytical methodology, reagents, apparatus, instruments, etc. in the past decade, particularly when new models of GC/MS/MS and LC/MS/MS become more efficient and the detection limits rise to ppb levels. The number of pesticides monitored by multi-pesticide analysis method has thus increased from 136 pesticides in 2006 to 373 pesticides in 2017 [7].

In 2017, the Food and Drug Administration (FDA) inspected a total of 3,341 shipments, and 363 shipments (10.9%) were confirmed to violate the food safety standards [4]. However, the cost at 200 US dollars per sample and time spent is still too high for most growers. Hence, a test method with low cost and fast screening is definitely helpful.

Due to more and more pesticides were registered and a lot of neurotoxic insecticides were banned in the past ten years, RBPR that targeting only on organophosphorus and carbamates insecticides can no longer fulfill current needs. In this regard, TARI decides to initiate the research on immunoassay method aiming at pesticides commonly detected by chemical analysis in 2011.

Development of Immunoassay Technology

The immunoassay technology is based on the principle of specific binding of antibody and antigen. By using antibody as a biosensor, techniques for qualitative and quantitative analysis of target compounds, enzymes or proteins are then developed. Currently, immunoassay methods such as radioimmunoassay (RIA), luminescent immunoassay (LIA), fluorescence immunoassay (FIA), enzyme-linked immunosorbent assay (ELISA), and colloidal-gold based lateral-flow immunoassay are widely used in medicine, plant pathology or quality control in various fields. ELISA technique was first published by Engvall and Perlman in 1971, and has been widely used as diagnostic tools. Due to the highly specific, sensitive as well as the qualitative and quantitative

features, the amount of antibody or antigen can be estimated and is very adequate to be used as rapid pesticide residue assay method. The ELISA method uses enzymes such as horseradish peroxidase (HRP) or alkaline phosphatase (AP) to label antibodies or antigens, after adding substrate of the enzyme, product obtained in the enzyme reaction can be detected by spectrophotometer and calculate the level of color change. For development of immunoassay for pesticide residues, the individual pesticide analogs (hapten) were conjugated with carrier proteins before being inoculated into animals to generate antibodies against the coupled compound from animals, for pesticide itself is too small to induce immunity of the animal.

In the past two decades, immunoassay technology becomes more and more important particularly in human medical diagnosis for its specific and sensitive characteristics, and related test eagents, apparatus and instruments with good quality are readily available. A number of ELISA methods are also derived to meet various needs, for instance, direct competitive enzyme-linked immunosorbent assay (dc-ELISA) and indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) are commonly used to detect pesticide residues.

In the dc-ELISA, pesticide antibody is immobilized on the microplate. The pesticide and pesticide hapten-enzyme conjugate are then added and will compete with each other for binding to the pesticide antibody. The pesticide hapten-enzyme conjugate refers to the pesticide hapten with labeled enzyme such as HRP or AP. After washing, the unbound pesticide hapten-enzyme conjugates are removed; only the binding complex of the pesticide hapten-enzyme conjugates with pesticide antibody is left in the microplate. Then, the substrate was added for the color reaction that were measured by ELISA reader to quantify the pesticide residues; the higher the residual pesticide concentration, the lighter the final coloration.

As to the ic-ELISA, the control antigen, an antigen prepared by linking a pesticide hapten to a protein of a non-immune antigen carrier, is adhered to the microplate first, and then adds pesticide and pesticide antibody (primary antibody). The control antigen will compete with pesticide to conjugate with the pesticide antibody. After washing and removing the free portion, only the binding complex of the control antigen and the pesticide antibody is left. Then add the enzyme-labeled secondary antibody that can recognize the primary antibody and bind with it. After washing and removing the free part, only the conjugate of control antigen, pesticide antibody and the secondary

antibody is left in the microplate. By adding the substrate, qualitative calculation of the pesticide concentration done by the color expression was recorded by the ELISA reader to quantify the pesticide residue. In the ic-ELISA, the higher the concentration of the pesticide, the lighter the final coloration.

In comparison, the procedure of ic-ELISA is more complicated because of the multiple binding of primary antibody and secondary antibody. However, the signal amplification and sensitivity are usually higher, and is commonly used in academic research. On the other hand, procedure of the dc-ELISA is simpler to the users, and is suitable for routine and large-scale testing.

Another rapid detection immunoassay method in medicine is colloidal-gold based lateral-flow immunoassay, and the competition approach is widely used to detect toxin [17] and drug residue in animal foodstuffs [1]. The mechanism of competition approach is crosslink the antibody of drug to colloidal gold (colored material) below sample pad first, after antibody-colloidal gold complex combine with the antigen (drug) in sample solution, the reference antigen in T line of two stealth lines on the test paper can not capture the antibody-colloidal gold-drug, T line will be extreme light or invisible. If there is no drug in sample solution, the reference antigen in T line will capture most of the antibody-colloidal gold complex, and T line will shows up. This technique does not require expensive equipment and easy to operate that may have great potential on pesticide residue detection.

Develop immunoassay kits for pesticide residues screening at TARI

In 2014, aiming at the high-risk pesticides that were frequently detected in the unqualified products in the market, TARI applied the immunological principle of specific binding of antigen and antibody to develop the rapid screening technology for pesticide residues. Curretly, polyclonal antibodies against 18 pesticides have been developed, which include 8 insecticides (acetamiprid, fipronil, thiamethosam, chlorfenapyr, pyriporxyfen, profenophos, deltamethrin, and bromopropylate), 9 fungicides (dimethomorph, difenoconazole, tebuconazole, chlorothalonil, pencycuron, hexaconazole, oxycarboxin, pyraclostrobin, and azoxystrobin) and 1 herbicide (2,4-D). The detection limit (10% inhibition rate of ic-ELISA) of the best antibodies obtained ranging from 0.01 to 2.24 ppb, and the cross-reactivity to 88 pesticides from 50 chemical categories is less than 0.1%.

1. Dimethomorph Rapid Test Kit

Dimethomorph antibody and the test protocol has been transferred to the Taiwan Advance Bio-Pharm., Inc. to develop dimethomorph rapid test kit in late 2016. The cut-off value of the test strip is 1.0 ppm, and the assay can be complete in 10 min. Two invisible lines are bound on the test paper, the upper part is the C line (Control-line), and the lower part is the T-line (Test-line: containing the hapten (dimethomorph)-carrier protein complex). When the concentration of dimethomorph in the sample is less than the cut-off value, 1.0 ppm, T-line will capture most of the dimethomorph antibody-colloidal gold complex and T line will shows up; while if the concentration of dimethomorph in the sample is greater than or equal to 1.0 ppm, the T line will be extreme light or invisible.

The cut-off value of the dimethomorph rapid test kit is set at 1.0 ppm. For practical purposes, the tolerance level for various crops might vary, hence adequate dilutions should be made during the extraction process (Figure 1). For example, the tolerance levels for small leafy vegetable and cabbage lettuce are 2.5 and 10.0 ppm in Taiwan, respectively [(7)]. Sample extracts are obtained by weighing 0.5 g sample first, and then add 1.25 ml and 5 ml extraction solution for leafy vegetable and cabbage lettuce, respectively. As to the grape, strawberry, tomato, cucumber and onion, the tolerance levels are 3, 2, 1.5, 1 and 0.6 ppm, respectively. Extracts are prepared by weighing 1 g of sample for individual crop, then add 3, 2, 1.5, 1 and 0.6 ml extraction solution, respectively and stir thoroughly. Then, follow the normal assay procedure to estimate the residue status.

2. ELISA kit designs for multi-pesticide assay

Some pesticides are commonly used in certain crops e.g., tomato and bell pepper, hence to develop an assay kit that can detect the residues of 4 or 8 pesticides at one time with qualitative and quantitative results might be feasible. In this regard, four pesticides are chosen to develop multi-pesticide assay kit, and they are acetamiprid, fipronil, dimethomorph and tebuconazole. Two concentrations for each pesticide were chosen that gave the inhibition rates of 20-60%, and the detection limit of the above-mentioned four pesticides can be obtained by the regression between the logarithm of the pesticide concentrations and the reaction inhibition rates; they are 0.68, 1.14, 0.23, and 0.61 ppb, respectively. Less than 0.1% cross-reactivity with other 88 non-target pesticides from 50 chemical groups indicated their specificity and sensitivity. Each ELISA plate is pre-

coated with 4 pesticides antibody, and the ELISA kit has the ability to analyze 4 pesticides concentration with 9 samples simultaneously (Figure 2).

To confirm the influence of sample matrix on ELISA, detailed study on the assay protocol of komatsuna (*Brassica rapa* var. perviridis) against these four pesticides were conducted, and the tested concentration was set at 1.0 ppm for all pesticides. Average recoveries of acetamiprid, fipronil, dimethomorph and tebuconazole from komatsuna were 74.0%, 105.3%, 92.8%, and 92.1%, respectively. The pesticide recoveries were between 60-140%, which is comparable to the current inspection method announced by the Food and Drug Administration [(9)]. The CV values were 3.3%, 3.8%, 4.1%, and 6.9%, respectively, which also indicated the stability of this analytical method. The entire operation took only 1.5 hours from sample extraction, dc-ELISA reaction, and pesticide concentration interpretation.

DISCUSSION

Pesticide immunoassay technology has been developed in many countries, and commercial products are available from Japan, the United States, Canada, Norway, etc. The HORIBA Company in Japan develops the Smart Assay series, including 21 ELISA kits for the detection of the residues of insecticides and fungicides by using direct competitive ELISA (HORIBA website). Other ELISA-based pesticide residue analysis products such as ic-ELISA or dc-ELISA kits are produced from Abraxis, EnviroLogix and Bio Scientific companies. The colloidal gold lateral flow immunoassay is a very handy method for on-site residue screening since the technique does not require expensive equipment and easy to operate; however, it requires highly sensitive antibody and is more challenge on wider detection spectrum. Although Wang *et al*. (2015) had developed chlorpyrifos colloidal gold lateral flow immunoassay and the minimum detection limit is 4.0 ppm and Hua *et. al*. (2012) screened a single antibody supplemented with colloidal gold lateral flow immunoassay to identify eight organophosphorus pesticides, their commercialized pesticide test strip is still uncommon.

There are more studies on pesticide residue analysis by ELISA. Watanabe *et al.* (2013) had compiled several reports on the thiamethoxam pesticide ELISA test. The detection limit of the ELISA is 0.05 ppb, which is even more sensitive as compared to that by the HPLC/MS/MS analysis, 0.14 ppb. After addition of thiamethoxam on

cucumbers and tomatoes, the samples quantified by ELISA only diluted with methanol, further diluted and subjected to assay. However, the samples quantified by HPLC needed to be extracted with several organic solvents and SPE first, and then concentrated and adjusted to proper concentration before HPLC analysis. The results showed that the pesticide recovery and accuracy of the two methods were equivalent.

According to the multi-residue analytic method for foods announced by the Food and Drug Administration, the procedures of sample cleanup are very complicated including the necessary use of a lot of highly toxic organic solvents and the long preparation time. After the tedious solvent extraction, purification, drying, re-dissolution and filtration processes, the sample extracts can then be injected into LC/MS/MS and GC/MS/MS [7]. In contrast, the ELISA method use a much more simple extraction process and does not require the use of highly toxic organic solvents. Watanabe *et al.* (2005) added the pesticide chlorfenapyr to apple and peach juice, extracted with methanol for 3 minutes, and the extract was filtered through a filter and diluted 25 times with buffer. The recovery of the obtained pesticide was 88.0-100.0%. Watanabe *et al*. (2007) also applied ELISA to determine residual imidacloprid in fruit juices. The recovery rate of imidacloprid in apple, grape and orange juice were 82.0-128.7%. Watanabe and Miyake (2013) added the thiamethoxam to homogenates of cucumber and tomato, and the recovery rates of thiamethoxam were 88.4-110.2%. Qian *et al*. (2009) added the pesticide chlopyrifos-methyl to cabbage and grapes, and tested it with ELISA; and the recoveries of chlopyrifos-methyl ranged from 82.4 to 94.6%. The above test results indicated that the pesticide ELISA detection method could be simple and highly efficient.

CONCLUSION

The year-round production and small-scale farming system have contributed to very complicated pesticide residue problems in Taiwan. The R&D of immunoassay methods for pesticides with higher residue risk and the commercialization of rapid test kit can provide an effective tool for growers who are willing to understand the residue status of the fruit and vegetable they produced before harvest. In the 4-year research program, antibodies of 18 pesticides are successfully achieved with detection limits ranging from 0.01 to 2.24 ppb, and the run time to complete an assay for test strip and ELISA kit are 10 min and 1.5 hours, respectively. Standard sampling and assay

procedure for more than 30 crop species are also established. Currently, local biotechnology companies have already transferred specific antibodies and the test protocol of individual pesticide to produce commercial kits, and the potential users might include consumers, farmers or the RBPR stations in the production districts, wholesale markets, supermarket chain stores, etc. In the future, test strip and ELISA kit for testing one pesticide, or an ELISA kit designed for the detection of 4 or 8 pesticides simultaneously will be possible, with the hope that consumers can have more confidence on the safety of harvested agricultural products.

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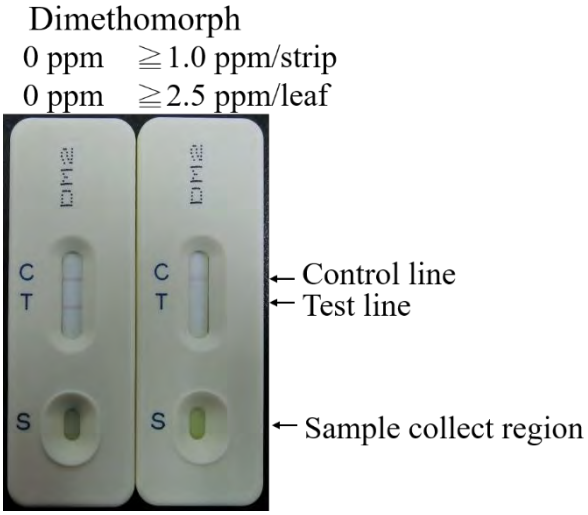


Fig. 1. Dimethomorph in *Brassica rapa* subsp. *chinensis* detected by dimethomorph rapid test strip.

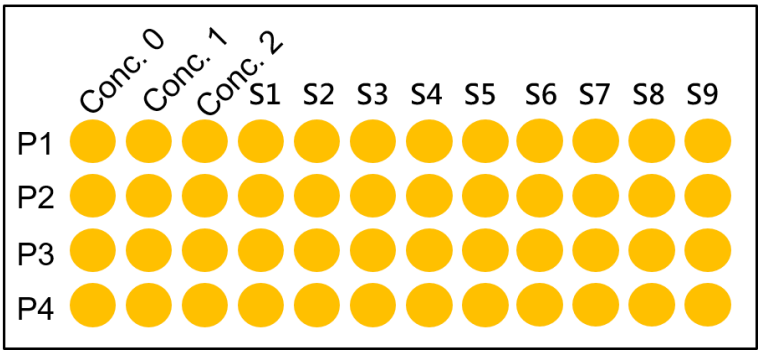


Fig. 2. An ELISA plate is pre-coated separately with 4 pesticides antibodies (P1-P4); and one single plate can estimate the residues of 9 samples (S1-S9) against 4 pesticides simultaneously.

Development and utilization of plant vaccines in Japan

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ABSTRACT

Pepper mild mottle virus (PMMoV), which belongs to the genus *Tobamovirus*, causes serious damage on green pepper (*Capsicum annuum* L.) in Japan. Until 2012, disease caused by PMMoV in Japanese fields has been controlled by the soil fumigant methyl bromide. However, methyl bromide has not been used since, except in critical or quarantine cases in developed countries. Therefore, we developed an attenuated strain to be used as an alternative strategy to control PMMoV infection of cultivated green peppers. The vaccine, L3-163 was obtained by heat treatment of the plant infected with PMMoV-wild type. The vaccine was able to completely protect green peppers from infection by PMMoV-wild type. In addition, large-scale inoculation technique was developed to apply the vaccine. This report described the development of the vaccine and for its use on control of viral diseases, such as PMMoV.

Keywords: *Pepper mild mottle virus*, attenuated strain, plant vaccine, cross protection, bio-control

INTRODUCTION

Cross-protection was first demonstrated by McKinney (1929) [(14)], who observed that in tobacco plants systemically infected with *Tobacco mosaic virus* (TMV)-light green strain, the appearance of yellow symptoms caused by TMV-yellow mosaic strain was repressed. On the other hand, TMV-mild dark green strain did not repress the yellow symptoms. Ever since that observation, cross-protection have been demonstrated in several viruses such as *Potato virus* X, *Potato leafroll virus* and *Citrus tristeza virus* (Gal-On and Shiboleth 2006, Grant and Costa 1951, Salaman 1933, Webb

et al. 1952) [4, 6, 12, 22]. The phenomenon of cross protection is similar to that for vaccination: once a plant was inoculated and infected by an attenuated strain, that plant could not be re-infected by the same wild-type strain or a related viral species. Thus, the attenuated virus acts as a form of biological control. To date, many attenuated viruses have been developed for practical use worldwide (Fulton 1986, Lecoq, 1998, Ziegreen and Carr 2010) [3, 13, 24].

In Japan, many studies about cross-protection have been reported. For example, *Tomato mosaic virus* (ToMV)-L_{11}A isolate is one of such attenuated virus developed in Japan, which provides a high degree of protection against the wild-type ToMV in commercial tomato crops (*Solanum lycopersicum* L., Goto and Nemoto 1971) [5]. This isolate has been widely used throughout Japan. Around 20% of all tomato farmers in the Chiba Prefecture have introduced ToMV-L_{11}A before new tobamovirus-resistant tomato cultivars were developed (Nagai 1984) [15]. Recently, *Zucchini yellow mosaic virus*-2002 attenuated isolate was developed and registered as biotic pesticide. The isolate produced very mild or no symptoms on cucurbit plants. In addition, inoculated cucumber plants had very similar fruit productivity to healthy control plants under field conditions. In field experiments, when other viruses were also present, protected plants significantly suppressed infection with ZYMV, progression of disease severity, and reduction of fruit yield and quality (Kosaka et al. 2006) [11]. As described above, many researches about attenuated plant viruses have been conducted and the isolates have been used in Japan.

Green pepper is one of the important crops in Japan. The total amount of yield of green pepper is near 140,000 tons in a year, and the cultivated areas covered about 3000 hectares in Japan. The main production regions are Ibaraki and Miyazaki prefectures. There are many viral diseases, such as mosaic and necrosis diseases, have been reported on Green pepper in Japan (Phytopathological Society of Japan 2000) [19]. Of these, mosaic disease caused by *Pepper mild mottle virus* (PMMoV), which belongs to the genus *Tobamovirus*, lead to serious damage on green pepper (*Capsicum annuum* L.). PMMoV has a positive-strand RNA genome consisting of 6,357 nucleotides. The virus encodes at least four proteins: a 130 kDa replicase, a 180 kDa replicase, a movement protein, and a coat protein (Kirita et al. 1997) [10]. PMMoV infection reduces plant growth and induces leaf mosaic symptoms and fruit malformation (Wetter and Conti, 1988) [23]. This is of relevance as the shape of pepper fruits affects their salability in

Japan, where they are classified into the following three categories: class A (excellent), class B (good), and unsalable (not marketable).

Until 2012, disease caused by PMMoV in Japanese fields has been controlled by the soil fumigant methyl bromide (Ikegashira et al. 2004) [9]. However, in 1992 at the Fourth Meeting of the Parties to the Montreal Protocol on Substances that Deplete the Ozone Layer, methyl bromide was considered an ozone-depleting substance. In developed countries, therefore, methyl bromide has not been used since 2005, except in critical or quarantine cases. Although Japan has continued to use it for soil fumigation, the government has agreed to phase it out by the end of 2012 (Report of the 28th meeting of the Open-ended Working Group of the Parties to the Montreal Protocol 2008) [25]. Consequently, alternative strategies to control PMMoV infection of cultivated green peppers are urgently required. The use of an attenuated virus was investigated to provide cross protection from PMMoV-wild type.

Some wild species of *Capsicum* carry the hypersensitive response (HR)-associated tobamoviral resistance gene *L*, which exists as four different alleles, L^1, L^2, L^3, and L^4, inherited in a Mendelian fashion (Boukema 1984) [1]. The P_1, $P_{1,2}$, and $P_{1,2,3}$ pathotypes of PMMoV overcome L^1 alleles, L^1-L^2 alleles, and L^1-L^3 alleles, respectively (Rast 1988) [20]. In Japan, many pepper varieties now grown for market carry a resistance gene such as L^3 ,which also confers resistance to these attenuated isolates. In addition, some wild-type isolates of PMMoV ($P_{1,2,3}$) have been found in cultivated Japanese peppers that can overcome the L^3 resistance (Tsuda et al., 1998, Hamada et al., 2007) [7,21]. Therefore, the attenuated isolate was developed for use in green peppers carrying the L^3 resistance gene throughout Japan. This report would be used for the development of the integrated management strategy for control of viral disease.

Development and characterization of the vaccine

Traditionally, attenuated strains were isolated from naturally occurring variants or by mutagenesis of wild type strains using nitrous acid or ultraviolet (UV) irradiation. Nitrous acid is a well-known mutagen that deaminates cytosine and adenine to produce uracil and hypoxanthine, respectively. UV irradiation has been used for isolation of attenuated strain, which causes the formation of pyrimidine dimers in DNA as well as in RNA (Bre´geon and Sarasin 2005, Nishiguchi and Kobayashi 2011) [2, 16]. In addition, some attenuated strains have been isolated after cultivation at higher or lower

temperatures. In this study, the attenuated strains of PMMoV were isolated by cultivation at higher temperature. Firstly, the sap of the leaves infecting PMMoV-wild type was inoculated to the stem of green peppers. The inoculated plants were kept at 40 ℃ for three weeks in a growth chamber. The sap of the stems was inoculated to the leaves of *Nicotiana tabacum* cv. Xanthi-nc and from which single local lesion isolation was conducted. After several weeks, four plants, which did not show any symptoms, were selected as candidates for vaccine production.

Northern blot analysis and Double Antibody Sandwich-Enzymed liked immnosorbent assay (DAS-ELISA) (Fig. 1) were used to analyze the accumulation levels of the vaccine both in cells and the whole plant. Accumulation levels of a vaccine, L3-163 in cells was lower than that of other strains. Similar result was obtained from the analysis of accumulation levels in whole plant. Then the distribution of the vaccines in a plant was analyzed by hammer blot analysis. Distribution of the vaccines in a plant often correlated with cross protection effect against PMMoV-wild type. Therefore, a vaccine, L3-163, which showed a wide distribution in a plant was selected. The nucleotide sequence of the L3-163 was then determined and compared to that of PMMoV-wild type. Several mutations were observed in the genome of the L3-163. Of these, we confirmed that three mutations in 130kDa/180kDa protein genes play an important role for attenuation by the reverse genetic technique using infectious clone of PMMoV-wild type. Finally, an attenuated strain, L3-163, with pathotype $P_{1, 2, 3}$, was commercially registered as a biological control agent in 2012.

Cross protection effects of the vaccine

The effectiveness of the vaccine, L3-163, for the cross protection of peppers from the infection by wild-type PMMoV was evaluated in the greenhouse and in the field (Ogai *et al*. 2011, 2013) [17, 18].

In the first experiment, green peppers cv. Miogi L^3 inoculated with the attenuated isolate were completely protected from infection by wild-type PMMoV, whereas non-treated plants began to show symptoms of infection with the wild-type virus after starting agricultural work (Fig. 2). By the end of the cultivation period, 71.4% of the plants were infected. In the second experiment, diseased plants were identified after starting agricultural work and 76.2% of the plants were infected by the end of the cultivation period. In this experiment, some plants inoculated with the vaccine showed

mosaic symptoms on their upper leaves, but sequence analysis revealed that this was caused by PMMoV-wild type.

We examined effectiveness of the attenuated isolate for other cultivars of green pepper grown in a commercial field situation: TM suzunami, Kyosuzu, and Mihata 2-go. The wild-type PMMoV failed to infect all three vaccine-inoculated cultivars at the end of the cultivation period (Table 1). When growth yields were compared between vaccine-treated and non-treated plants, the yields of both class A and B vaccine-treated plants were significantly higher than those in non-treated plants in all four cultivars. Some plants of Mihata 2-go and Miogi L^3 showed mosaic symptoms that led to loss of fruits. The viral sequence isolated from these plants was shown to be identical to that of PMMoV-wild type.

Accordingly, we examined the levels of protection on the commercial production of pepper fruits provided by the attenuated isolate. Four plants each with the following four levels of infection with the attenuated isolates, e.g., 0, 50%, 75% and 100% were grown in the greenhouse. Mosaic symptoms initially appeared one month after starting agricultural work in all plots, except the 100% plot. The plants in 0% and 50% plots showed a significant yield loss in fruit weights and numbers due to severe mosaic symptoms and fruit malformation. There was no significant difference in numbers, yields, and salable and unsalable peppers among plants grown in 75% and 100% plots. Therefore, these findings suggest that the yields are statistically equal between pepper seedlings vaccinated with attenuated isolate and the healthy seedlings when greater than 75% of the seedlings were successfully vaccinated.

Lecoq (1998) [13] reported the following characteristics of attenuated isolate for cross protection in the field. 1) It can cause milder symptoms on crops in the field, but the crops still produce sufficiently marketable yield and quality. 2) It possesses high histocompatibility to inoculated plants. 3) It is genetically stable. 4) It should retain limited non-intentional spread. 5) It should ideally possess the ability to protect plants from wide range of severe challenging isolates. 6) It should be well control and easy to apply. In Japan, an attenuated isolate for cross protection in commercial fields must also be officially registered as a biological control agent (biotic pesticide). Currently, an attenuated isolate of *Zucchini yellow mosaic virus* (ZYMV), designated ZYMV-2002, is widely used in *Cucumis sativus* L.) cultivation for cross protection against ZYMV wild type (Kosaka et al., 2006) [11], which is the first attenuated virus registered

in Japan as a trade name “Cubio ZY-02” (MAFF Reg. No. 22152; Kyoto Biken Laboratories, Inc., Kyoto, Japan). Based on our results, the vaccine of PMMoV will be registered as a biological control agent to protect varieties of pepper plants grown in Japan. In order to help our farmers familiarize with the vaccine, we also made a video for them.

Inoculation technique of the vaccine

For inoculation of the vaccine, sap of the inoculum was prepared from leaves of *Nicotiana benthamiana* inoculated with the L3-163. The sap was mechanically inoculated with carborundum onto the cotyledons of 10-day-old green pepper seedlings. Mechanically inoculation is useful for the small number of the seedlings. However, it is not convenient for farmers, because farmers generally cultivate more than thousands of seedlings in Japan. To date, large-scale inoculation, which utilizes a high-pressure spray gun, was developed for inoculating tobacco plants with the *Tomato spotted wilt virus* (Hull, 2014) [8]. Therefore, the large-scale inoculation technique was improved for practical applications wherein thousands of green pepper seedlings need to be simultaneously inoculated.

The seedling fixator stand (commercially available from Kyoto Biken Laboratories, Inc., Kyoto, Japan) was developed for large-scale inoculation of vaccine. Place the plug tray planting of pepper seedlings on the seedling fixator and then sticks of comb-shaped structure are placed underneath the cotyledon leaves for support during inoculation. Place netting over the seedlings. Spray the inoculum to the seedlings using the spray gun and air compressor. In this technique, at least 0.3 ml of inoculum solution is required for inoculation of one seedling. More than 98% of seedlings were successfully infected with the vaccine using this method. This inoculation method and the apparatus have been patented to Kyoto Biken Laboratories, Inc. (Japanese Patent No. 5938798).

Future prospects

Plant viruses are intracellular parasites that cannot replicate themselves without a host plant. To date, no antiviral compounds are available for cure of plants with viral diseases. Instead, cross protection has [been used as one of the effective tools for control of viral diseases worldwide. Although it is relatively classical method, it is drawing

great attention again in Japan because it is considered as an environmentally friendly technique.

This study could be useful for effective control of plant viral diseases because the L3-163 strain was shown to protect pepper plants with a high degree of cross protection efficacy against the PMMoV-wild type infection, which resulted in a better yield in greenhouse settings. In the future, new strategies such as developing multiple vaccines that combine several attenuated viruses or exploiting a single attenuated virus that can effectively protect against multiple viruses warrant further investigation.

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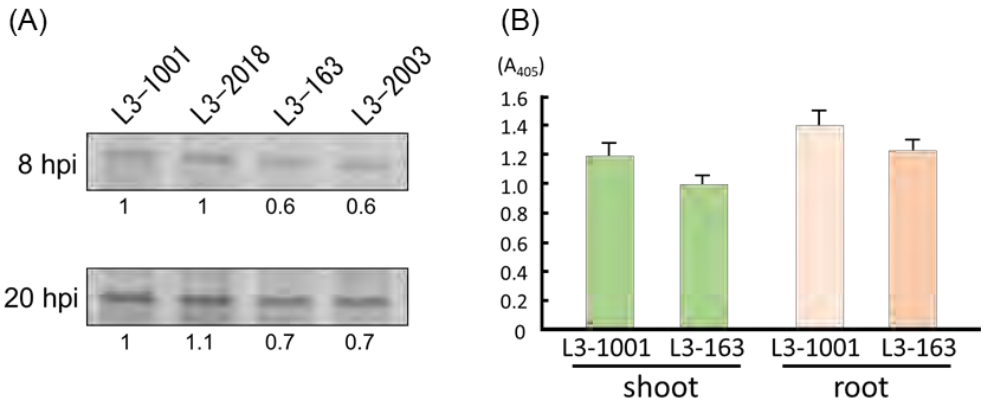


Fig. 1. Accumulation levels of the candidate vaccines, L3-1001, L3-2018, L3-163, L3-2003 in cells (A) and in whole plant (B). Accumulation levels in cells and whole plant were analyzed by northern blot analysis and double antibody sandwich enzyme-linked immunosorbent assay, respectively.

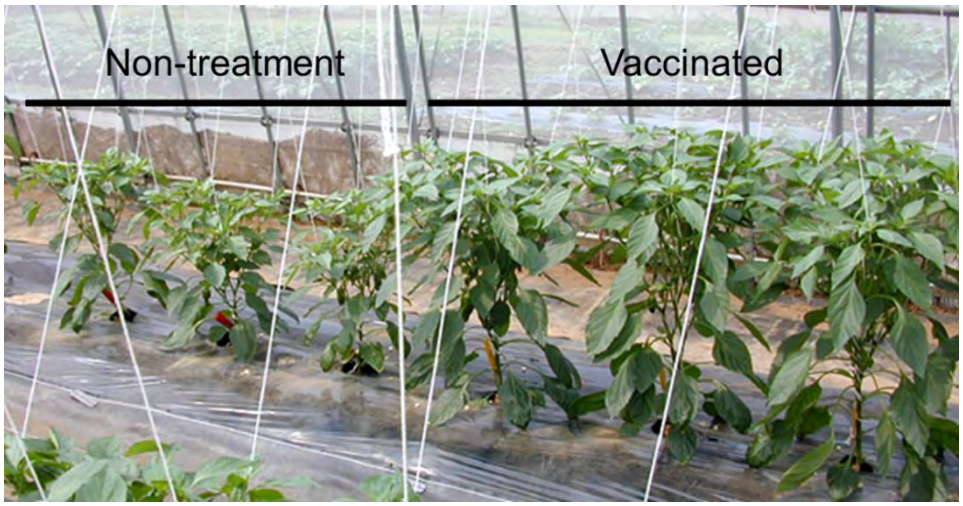


Fig. 2. Symptom comparison between the non-vaccinated and vaccinated plants in field experiments. Vaccinated plants remained asymptomatic throughout the study.

Table 1 Percentages of diseased plants of four green peppers cultivars inoculated with L3-163 and non-inoculated in the field.

Cultivar	Treatment	Diseased plant (%)
TM suzunami	Vaccinated	0.0±0.0
	Non-treatment	100.0±0.0
Kyosuzu	Vaccinated	0.0±0.0
	Non-treatment	100.0±0.0
Mihara 2-go	Vaccinated	3.7±3.0
	Non-treatment	96.3±3.0
Miogi *L*[3]	Vaccinated	3.7±3.0
	Non-treatment	100.0±0.0

The data was modified from Ogai et al. (2013).

Application of control-release formulation of biochemical reagent in the integrated pest control

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ABSTRACT

In order to prolong the releasing period of pest pheromone and stabilize the active components, a paste formulation containing microencapsulated insect sex pheromone was initiated. The microcapsule was formed through the polymerization between the oil phase and aqueous phase. The pheromone molecules were encapsulated inside the hollow space of the capsule as core materials that were surrounded by the polymeric shell. The suspension liquid was then mixed with xanthan gum to form a paste that could be applied in the trap of the target species for monitoring and male annihilation. The microcapsule paste formulation of sex pheromones were prepared for tobacco cutworm (*Spodoptera litura* Fab.), diamond-back moth (*Plutella xylostella* L.), and rice stem borer (*Chilo suppressalis* Walker), respectively. Results of the field tests on the controlled-releasing rates showed that the male pest attraction of the microcapsule paste formulation is effective over 60 days for tobacco cutworm and over 45 days for diamond-back moth and rice stem borer. The head-space GC-MS analyses showed the all the active pheromone molecules are stable during the releasing period in the field with the introduction of anti-oxidant reagent. The most appropriate distance between two traps for male annihilation was evaluated. For diamond-back moth, a modified trap integrated the light luring with pheromone attraction was designed. The best parameters of this trap toward attracting the moth were evaluated using Taguchi methods. Our results suggest that the proposed microcapsule paste formulation of sex pheromones could prolong the releasing period of pheromone lure and maintain the stability of the active compounds in it. Moreover, the microcapsule paste formulation is compatible with other methods in pest control. All these characteristics suggest that microcapsule

paste formulation has its potential to facilitate the application of integrated pest control strategy.

Keywords: pheromone, control-release formulation, integrated pest control

Insecticide horizontal transfer from tephritid male lures containing reduced risk pesticides

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ABSTRACT

Insecticide horizontal transfer extends pesticide efficacy from individual level to population-wide control through insect food sharing behavior. Social insects and non-social insects with food sharing behavior were most studied subjects for the effects of insecticide horizontal transfer. Very little was known about the food sharing behavior among tephritid fruit fly species, a group of serious pests of fruit commodities in tropical and subtropical area worldwide. Male oriental fruit fly (*Bactrocera dorsalis* (Hendel)) and melon fly (*Zeugodacus cucurbitae* (Coquillett)) are known to feed compulsively on methyl eugenol (ME) and cue lure (CL), respectively. These two semiochemicals in combination with broad-spectrum pesticides, i.e. organophosphate and carbamate, were the standard control tactics in the past decades. With the increasing environmental concerns for the use of broad-spectrum pesticides and that in turn cause the escalation of pesticide resistance indeed promoted the introduction of reduced-risk pesticides in recent years. This type of pesticide with strong bioactivity can cause fruit flies to regurgitate soon after ingesting insecticide-laced attractant. Insecticide horizontal transfer via fruit flies' regurgitated droplets that contained insecticide active ingredient of chlorantraniliprole, acetamiprid, thiamethoxam, spinosad and fipronil were demonstrated in this paper. Field trials conducted in Hawaii demonstrated that not only male but also female *Z. cucurbitae* population density reduced significantly in the area treated with CL-fipronil. Laboratory studies further confirmed that droplets of

regurgitated CL-fipronil caused high mortality in both male and female flies that received the regurgitated CL-fipronil within 24 h. Laboratory studies conducted in Taiwan demonstrated that exposing *B. dorsalis* males to individuals fed on ME-spinosad and ME-thiamethoxam caused significantly higher mortality in male flies than that received ME-chlorantraniliprole and ME-acetamiprid treatments. Significantly higher female fly mortality was recorded in contacting flies that fed on ME-spinosad. Data from cage studies demonstrated that male flies did not show preference among ME-spinosad, ME-thiamethoxam and ME-acetamiprid regurgitants. The results from this study demonstrated that male annihilation technique using reduced-risk pesticides are suitable alternatives for organophosphates. The potential of employing insecticide horizontal transfer in fruit fly management was also discussed.

Keywords: Tephritidae, insecticide horizontal transfer, fipronil, spinosad, thiamethoxam, chlorantraniliprole

INTRODUCTION

Tephritid fruit flies (Diptera: Tephritidae) are important economic and quarantine pests of fruits and vegetables in many parts of the world. Current options for managing fruit fly pests in agriculture environment include sanitation, cover spray, proteinaceous baits, and male lures. Cover spray provides temporary control but has little long-term effect. Repeat applications of cover spray are not only costly and labor intensive, but also place significant risks to non-target species in the environment and increase the risk of pesticide resistance escalation [4, 10]. In recent years, the concept of area-wide management approach has gradually shifting growers' practice from single tactic cover spray to multiple tactics integrated pest management [18].

Male annihilation technique using semiochemicals

Attract-and-kill method using insecticide-laced male lures can reduce fruit fly populations by removing large number of males that reduce their mating potential that subsequently decrease crop damage. Successful control of fruit fly populations with attract-and-kill male mass trapping was demonstrated in oriental fruit flies (*Bactrocera dorsalis* (Hendel)), melon flies (*Zugodacus cucurbitae* (Coquillett)), and Mediterranean fruit flies (*Ceratitis capitata* (Wiedemann)) [5, 17]. Methyl eugenol, cue lure, and biolure are semiochemicals that attract male oriental fruit fly, melon fly and Mediterranean fruit

fly, respectively. Male annihilation using organophosphate-laced attractant was widely applied to control insect pests in agricultural production area in the past decades. With the increasing environmental concerns for using broad-spectrum pesticides and that in turn causes the buildup of pesticide resistance indeed promoted the introduction of reduced-risk pesticides in recent years. This type of pesticide with strong bioactivity cause fruit flies to regurgitate soon after ingesting insecticide-laced attractant. Insecticide horizontal transfer via fruit flies regurgitated droplets was first observed in Queensland fruit fly (*Bactrocera tryoni* (Froggatt)) and later documented in *Z. cucurbitae* and *B. dorsalis* [3, 16].

Pathways of insecticide horizontal transfer

Toxic baits that exploit the recruitment and food-sharing behaviors of social insects are effective control measures with the benefits of reducing the amount of insecticide required and the risk of affecting other organisms during pest control application. Insecticides are transferred horizontally through food sharing to pest colonies and hence expand the control from individual to population level. Foraging members of an insect population can transfer insecticide horizontally to the rest of the colony through feeding on regurgitated food, grooming and contacting poisoned individuals [13]. Insecticides with strong bioactivity which cause insect to regurgitate or excrete shortly after ingesting toxin are good candidates for horizontal transfer to facilitate the transfer of toxicants in pest population [9,15]. The toxic regurgitant and feces are attractive to the conspecifics and provide secondary mortality occurred away from application site. It increases the effect of insecticide to area where the insects aggregate. Insecticide horizontal transfer is also recorded in German cockroach (*Blattella germanica* (L.)), grasshopper (*Melanoplus sanguinipes* (Fabricius)), and yellowjackets (*Vespula pensylvanica* Rohwer) [2, 12, 14]. In these studies, horizontal transmission of insecticides occurred through cannibalism, coprophagy (ingestion of feces) and necrophagy (ingestion of dead conspecifics) in both laboratory and field settings.

Regurgitation and insecticide horizontal transfer in tephritids

Little is known about the mechanism of regurgitation and the effect of insecticide horizontal transfer in tephritid fruit flies. It is believed that such behavior condenses the nutrient content by evaporation of excess water. Observations show that *B. tryon*i often

imbibe droplets of regurgitants from feeding surface [6, 7]. *C. capitata* evaporates excess water content in the liquid food source by repeatedly re-ingesting droplets of regurgitated liquid by holding droplet between labellar lobes [8]. Regurgitation has been associated with pheromone-marking in Caribbean fruit fly (*Anastrepha suspensa* (Loew)) and its male offers mating to trophallaxis substance in *Anastrepha striata* [1, 11].

Bull (2010) documented the first case of insecticide horizontal transfer in tephritid fruit flies. Male flies of *B. dorsalis* and *Z. cucurbitae* are known to feed compulsively on methyl eugenol (ME) and cue lure (CL), respectively. Field trials conducted in Hawaii demonstrated that not only male but also female *Z. cucurbitae* population density reduced significantly in the area treated with CL-fipronil. Laboratory studies further confirmed that droplets of regurgitated CL-fipronil caused high mortality in both male and female flies that received regurgitated CL-fipronil within 24 h. Laboratory studies conducted in Taiwan demonstrated that exposing *B. dorsalis* males to individuals fed on ME-spinosad and ME-thiamethoxam caused significantly higher mortality in male flies than those received ME-chlorantraniliprole and ME-acetamiprid treatments. Significantly higher female fly mortality was recorded in contacting ME-spinosad-fed flies. Data from cage studies demonstrated that male flies did not show preference among ME-spinosad, ME-thiamethoxam and ME-acetamiprid regurgitants. The results from this study demonstrated male annihilation technique using reduced-risk pesticides are suitable alternatives for organophosphates. The potential of employing insecticide horizontal transfer in male annihilation program expands the effect of insecticide to the population level.

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Control strategy for rice stripe virus transmitted by small brown plant hopper (*Laodelphax striatellus*)

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ABSTRACT

Rice stripe virus (RSV) causes chlorotic stripes, mottling, and necrotic streaks on rice leaves (*Oryza sativa*), resulting in a loss of production. RSV disease mainly occurs in Asian countries, including Taiwan, Japan, Korea, and China. In Japan, the disease devastated rice production from 1960 to 1985, and subsided thereafter owing to the introduction of new varieties resistant to RSV. However, its incidence has begun to increase again since 2004. Its vector, small brown plant hopper (*Laodelphax striatellus*, SBPH) persistently transmit RSV, and transmitted it from female adults to their progeny via egg. In this presentation, the molecular characteristics of transovarial transmission of RSV in SBPH are introduced. Countermeasures including adjusted timing of pesticide application and use of resistant varieties are discussed.

Keywords: rice stripe virus, small brown plant hopper, resistant cultivars, insecticides, control measures

INTRODUCTION

Rice stripe virus (RSV) causes chlorotic stripes, mottling, and necrotic streaks on rice leaves (*Oriza sativa*). Rice plants severely infected with RSV often show panicle sterility, resulting in lower yield. RSV mainly occurs in Asian countries, including Taiwan, Japan, Korea, and China. Although many Gramineae crops, such as wheat, barley, and corn can be infected with RSV, the damages are usually less severe in the fields. The rice disease caused by RSV was first identified around 1897 in Japan. Its damage on rice production was disastrous between 1960 and 1985. Owing to the introduction of varieties resistant to RSV, the disease gradually subsided and became less significant until 2004. However, the incidence has increased again since 2005.

According to the statistics reported by Japan Plant Protection Association, RSV affected more than 137,000 hectares of rice fields, which account for approximately 9% of the total cultivated area in Japan in 2015. Therefore, effective control measures are urgently needed.

According to the current taxonomy of International Committee on Taxonomy of Viruses, *Rice stripe virus* belongs to the *Tenuivirus* genus, the *Phenuiviridae* family. RSV genome consists of four single-stranded RNA molecules (RNA1 through RNA4), and seven genes are encoded in negative or ambisense orientation. RSV is persistently transmitted by the small brown plant hopper (*Laodelphax striatellus*, SBPH) and other planthoppers[2]. The virus propagates in the insect vectors and is transmitted from female adults to their progeny at the egg stage (transovarial transmission). Transovarial transmission of RSV is considered the main reason for the high viruliferous rate in nature, which makes the control of rice stripe disease more difficult. Understanding how RSV is maintained in SBPH and how RSV is transmitted to rice plants is crucial to developing effective control measures against this disease.

In our presentation, we would like to introduce the characteristics of transovarial transmission of RSV in SBPH, and control measures of RSV using resistant cultivars and the appropriate timing for the application of insecticides.

Molecular characteristics of transovarial transmission of RSV in *SBPH*

Under experimental conditions, the percentage of eggs exhibiting transovarial transmission was estimated to be greater than 90%[5]. The amount of RSV maintained through transovarial transmission was analyzed during the course of development and reproduction of SBPH. The expression of the RSV coat protein (CP) gene was measured by RT-qPCR as an estimate of RSV content in nymphs and adults of SBPH at various developmental stages. The 18S ribosome RNA gene of SBPH was chosen as the reference for relative quantification. Based on the relative CP expression levels, the amount of RSV did not differ significantly during the nymphal stage or between adult females of different ages although the amount of RSV tended to increase slightly as males became older. The average RSV content in males was 1.30 to 2.49 times that in females. These results indicated that RSV multiplies in synchrony with the multiplication of host cells during the nymphal stage, and the increase of RSV stopped or slowed after the insects reached the adult stage. Then, the amount of RSV in SBPH

female adults was compared between generations. The amount of RSV in three viruliferous females and their progenies was analyzed by RT-qPCR. The result indicated that RSV content did not differ significantly between parents and their progenies. (For full article of this study, see Okuda et al. 2017[3])

The viruliferous population of SBPH were grown on a susceptible rice cultivar Koshihikari and its near isogenic lines harboring RSV resistant gene(s), and examined whether these resistance traits affect the retainability of RSV in SBPH. The result showed the percentage of viruliferous insects was not significantly different among susceptible and resistant rice plants. Then, the transovarial transmission efficiency of SBPH grown on different hosts was examined for four generations. The percentages of viruliferous insects in the first generation grown on rice cv. Koshihikari, Asahino-yume (resistant to RSV), and wheat cv. Norin 60 were 50.0%, 46.8%, and 51.3%, respectively. The percentages decreased as generations progressed; however, the transovarial transmission efficiency was not significantly different between susceptible and resistant rice cultivars or between rice and wheat. These results indicated that the resistant properties of rice cultivars have no direct effect on the retainability of RSV in SBPH.

Control using resistant varieties

Rice cultivars resistant to viruses are widely used to minimize the damage due to virus infection[1]. Screening of RSV resistant rice varieties was initiated in the early 1960s, and the results showed a high level of resistance in some Indica type rice varieties and Japanese upland rice varieties[7]. A resistance gene (*Stvb-i*) was identified in an Indica-type rice cultivar Modan. Through breeding programs, *Stvb-i* gene was successfully introduced to develop resistant cultivars, which effectively control RSV disease in some areas in Japan. Currently, molecular marker-assisted selective breeding for *Stvb-i* made it possible to accelerate the development of new varieties. Introduction of *Stvb-i* into susceptible rice cultivars, Aichinokaori and Hatsushimo (designated as Aichinokaori SBL and Hatsushimo SBL), which replaced the original cultivars contributed to the reduction of pesticide usage and provide stable productions.

However, owing to the preference of Japanese consumers, some old varieties, which are not resistant to RSV, are still in use. Particularly Koshihikari, which was registered in 1956, still accounts for more than 36% of the total rice production for 2016 in Japan. A near-isogenic cultivar of Koshihikari was developed by introducing RSV resistant

genes, Stva and Stvb, originated from Japanese upland rice, Kanto72 and registered as Koshihikari Kinchushi SBL1 (SBL1) in 2012. In 2016 and 2017, SBL1 was grown in a district heavily affected with RSV in Ibaraki Prefecture to examine its practical readiness in the field. The result showed the RSV infection rate of SBL1 was less than 1% while that of Koshihikari grown in an adjacent field reached 100% at the end of the cultivation period. Now we are soliciting farmers who can engage on a large-scale trial of SBL1 cultivation.

Control using chemical insecticides

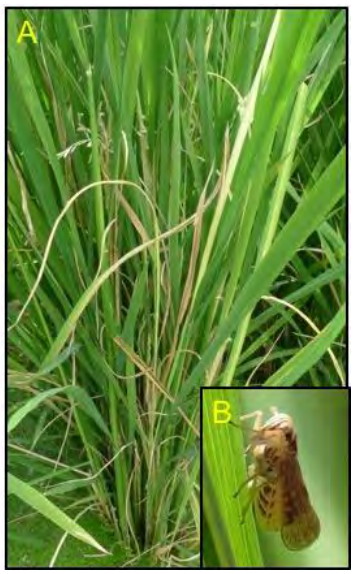
It has been reported that diseases caused by insect-borne viruses can be effectively decreased by the identification of the emergence of their vectors and timely application of insecticides. As for RSV, it would be possible to reduce the damage by elucidating multiple factors based on virus transmission and the occurrence ecology of vectors to control them at the optimal timing. We have studied how the RSV causes damage and spreads within paddy fields. Our investigations revealed that in a rice producing area in Ibaraki Prefecture, in central Japan, diseased plants start to appear in mid-June to early July, and from which the disease spreads from affected plants to adjacent plants throughout the season. This suggests that RSV viruliferous SBPH enters paddy fields, where they infect plants and lay eggs. Subsequently, hatched viruliferous nymphs transmit RSV to surrounding plants, thereby rapidly spreading the disease. Our analysis of the damage caused by RSV showed that the earlier the onset of disease, the more extensive damage cause due to a reduced number of healthy panicles. This suggests that to reduce damage caused by RSV, it is necessary to ensure the growth of a sufficient number of healthy panicles by controlling the vector insect during the crop's early growth stage. To be most effective, pest control efforts should be timed to target either the first-generation adults that colonize the paddy fields or the second-generation nymphs and adults that cause the rapid increase in the number of diseased plants within a field. (For full article of this study, see Shiba et al. 2017[4])

The timing of SBPH emergence can be precisely estimated using a prediction program based on the total effective temperature for SBPH, which is provided by Japan Plant Protection Association. Insecticide application such as silafluofen at 100 ppm timed for the emergence of the second-generation nymph resulted in effective suppression of the disease[6]. To make the prediction more precise, we are developing a

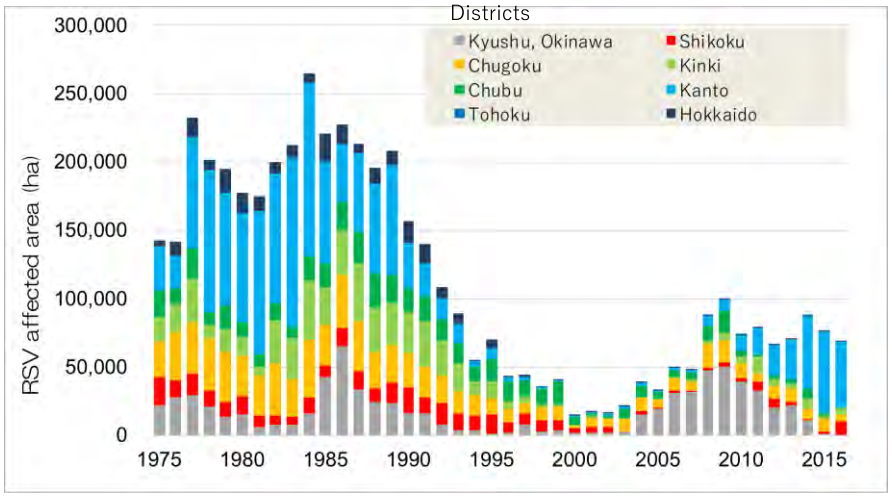
new program based on the total effective temperature and the Agro-Meteorological Grid Square Data System to predict the emergence of SBPH in a one square km spatial resolution. We hope this program will help farmers to obtain the optimal control measure of RSV diseases.

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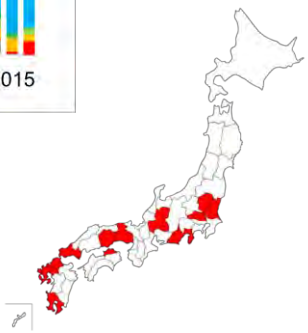
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A
B

Fig


Districts
Kyushu, Okinawa
Shikoku
Chugoku
Kinki
Chubu
Kanto
Tohoku
Hokkaido
RSV affected area (ha)
300,000
250,000
200,000
150,000
100,000
50,000
0
1975
1980
1985
1990
1995
2000
2005
2010
2015

2 0



Winter climate and cultivar effects on severity of Pierce's Disease in table grapes

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ABSTRACT

Grapevines infected with *Xylella fastidiosa* can recover from infection following exposure to cold temperatures. The rate at which vines recover is dependent on duration of cold exposure, cultivar susceptibility, and time of inoculation. Previous studies primarily focused on climate conditions experienced in the northern California wine grape growing regions, and only a small number of cultivars. This study aims to increase available information on probability of vine recovery in warmer regions. Cold treatment representative of southern California climate conditions led to very low rates of recovery in susceptible grapevines. However, even at low chill hour treatments, rate of recovery varied based on cultivar and time of inoculation. Comparison of *Xf* strains isolated from northern and southern California locations did not show any significant difference in curing under the same conditions. Evaluating the rate at which *Xf* infected grapevines can be cured by cold temperatures is important for developing region-specific management strategies, and for understanding pathogen spread in a variety of locations.

Keywords: *Xylella fastidiosa*, climate, Pierce's Disease, cultivar, winter-curing

INTRODUCTION

In California, epidemiology of Pierce's disease (PD) of grapes, caused by *Xylella fastidiosa*, is dependent on regional climate variation and insect vector species. Disease develops more quickly, and symptoms are more severe when temperatures are high during the summer, and the pathogens range is generally limited to warmer climates [1]. PD strains of *X. fastidiosa* are inherently sensitive to low temperatures and cold treatment can effectively eliminate *X. fastidiosa* from infected plants [2, 3]. In the

field, winter conditions can eradicate infections in grapevine and almond, particularly when the plants become infected late in the growing season [4-6]. This phenomenon, termed "overwinter curing" is an important consideration for disease management and epidemiology. However, current knowledge of overwinter curing is limited to studies conducted in the northern California wine grape growing regions, using a limited number of wine grape cultivars [7]. In northern California, vector control early in the growing season is used to manage blue-green sharpshooter populations and to prevent early season infections with *Xf* [8]. Late season infections are not problematic in northern regions because it is likely that they will be cured over the winter. In southern California regions such as the lower San Joaquin Valley and the Temecula region, Glassy-winged sharpshooter is the primary vector [9]. In these areas, secondary spread is more of a concern, and winter conditions may not be cold enough to prevent infection reoccurrence [10, 11]. Glassy-winged sharpshooter populations can be high late into the summer [12], and decisions about when to implement vector control measures need to consider the possibility that late season infections may persist . Additionally, as some cultivar variation has been observed in rates of overwinter curing [7], it is important to evaluate a wider variety of wine and table grape cultivars so that specialized recommendations can be made for areas with high disease pressure. The purpose of this study is to better understand the frequency of overwinter curing under Southern California climate conditions so that region-specific management strategies can be developed. This information also will be useful in evaluating spread of PD in other locations around the world that have very warm winter climates.

Southern California chill hours have minimal curing effect on *X. fastidiosa* infected grapevines. Different grape-growing regions within California have varied winter temperatures. Chill hours, measured as cumulative number of hours below 45 degrees (Fahrenheit), can range from 0 to greater than 2000 within the state of California in each year. In the Temecula region and the southern San Joaquin Valley where PD is problematic, number of chill hours is significantly lower than the North Coast and Napa regions where overwinter curing is known to occur (Table 1). Grapevines (*Vitis vinifera* Chardonnay) were artificially inoculated with *X. fastidiosa* [13] and grown for 12 weeks for infections to become established. Then infected plants were subjected to treatment in a cold chamber at 40°F for different lengths of time to

replicate chill hours experienced in different regions of California (Fig. 1). Following cold treatment, plants were grown back from dormancy, and evaluated for continued infection by PCR after 16 weeks of re-growth. At lower chill hours, vine recovery was minimal, but increased with longer duration of cold treatment (Fig. 1). This suggests that under conditions representative of climate in southern California regions, curing is less likely once plants become systemically infected.

Time of inoculation is still important at low chill hours. Because time of inoculation can affect rate of curing, grapevines were artificially inoculated at multiple timepoints throughout the summer. These vines were subjected to cold treatment at 40°F for 800 hours to represent the colder end of the southern California temperature range. Plants that were treated after 8 weeks post-inoculation had a higher curing rate than plants that were treated at 18 weeks post-inoculation (Table 2). This shows that even at low chill hours, time of inoculation prior to cold exposure can still influence vine recovery.

Northern and southern California *X. fastidiosa* isolates are cured at the same rate. To investigate whether different isolates of *X. fastidiosa* strains in California have adapted to different climate regions, rates of overwinter curing were compared in plants inoculated with *X. fastidiosa* Stag's Leap, a northern California isolate, and *X. fastidiosa* Bakersfield-1, an isolate obtained from the southern San Joaquin Valley in 2016 (Table 2). These strains are characterized as subspecies *fastidiosa*, and are closely related based on multi-locus sequence typing. Grapevines inoculated with Stag's Leap and Bakersfield-1 had very similar rates of curing when treated after 8 or 18 weeks post-inoculation. It is likely that at least within California, strains of *X. fastidiosa* causing PD, have a similar response to cold temperatures.

Grapevine cultivar influences rate of curing. As previous studies of overwinter curing focused on a limited number of wine grape varieties, we evaluated twelve different table grape cultivars for rate of overwinter curing. Varieties screened included Autumn Royal, Autumn King, Crimson Seedless, Red Globe, Flame Seedless, Princess, Scarlet Royal, Summer Royal, SugarOne, Thomson Seedless, Valley Pearl, and PD tolerant rootstock variety Tampa. Cabernet Sauvignon was included for comparison as this cultivar has been used extensively in previous studies of overwinter curing. Vines

were inoculated with *X. fastidiosa* Stag's Leap as described above, and infections were allowed to progress for 16 weeks. Infection status was determined using qPCR prior to cold treatment at 40°F for 800 hours. Following cold treatment, vines were grown back from dormancy for 16 weeks, and tested with qPCR and visual symptom evaluation for reoccurrence of infections. Number of plants infected before and after cold treatment is shown in Figure 2. Some highly susceptible cultivars such as Red Globe are difficult to evaluate because of severe PD symptoms leading to high vine mortality. However, Cabernet Sauvignon had a higher rate of curing compared with some susceptible table grape varieties such as Scarlet Royal. This highlights the importance of evaluating the different cultivars for rate of overwinter curing under different climate conditions.

Future prospects

It is likely that overwinter curing of grapevines infected with *X. fastidiosa* does not occur consistently in southern California grape-growing regions. To the extent that overwinter-curing does occur in southern regions, time of inoculation is still important for predicting the amount of vine recovery. A wider range of cultivars, including both wine and table grapes need to be evaluated for overwinter curing under a variety of climate conditions. Additionally, field studies using mechanically inoculated grapevines are necessary to fully understand the extent to which overwinter curing can be predicted across different regions, and in different cultivars.

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Table 1. Chill hours in California (2017-2018)

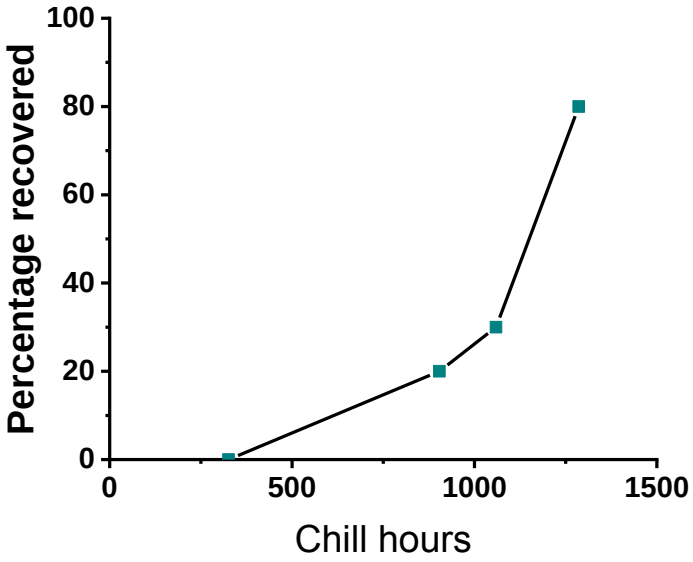
Location	**Hours <45F**
Temecula	171
UC Riverside	191
Arvin (S. San Joaquin Valley)	851
Parlier	977
Napa	1084
Mendocino (North Coast)	1194

Data compiled by the University of California - Davis Fruit & Nut Research & Information Center (http://fruitsandnuts.ucdavis.edu/Weather_Services/chilling_accumulation_models/Chill_Calculators).

Table 2. Effect of *X. fastidiosa* strain and time of inoculation.

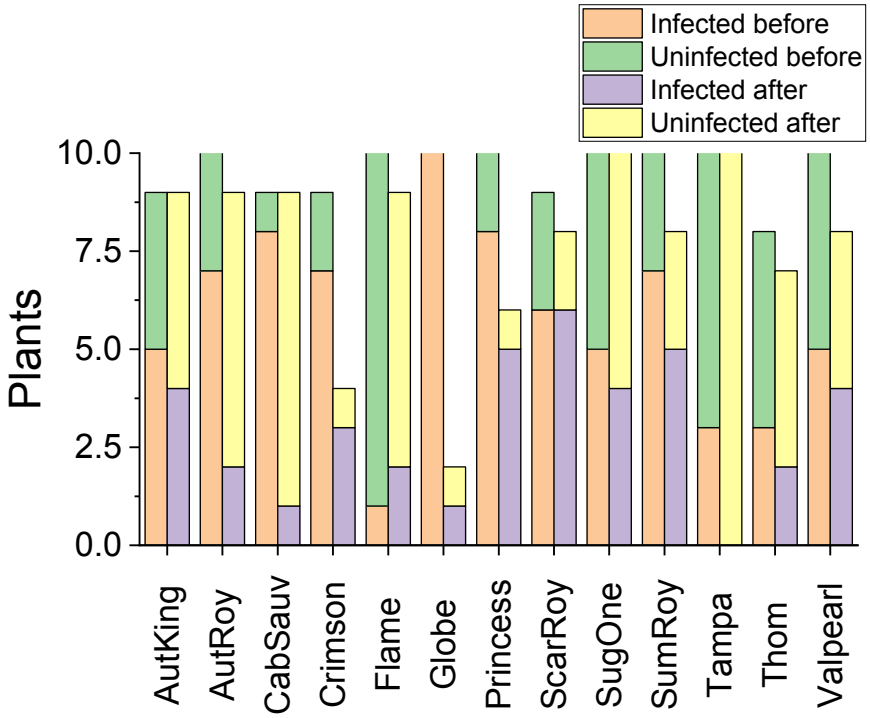
Weeks post-inoculation	**Strain**	**Plants infected after treatment**
8	Bakersfield 1	12/20
8	Stag's Leap	10/20
18	Bakersfield 1	18/20
18	Stag's Leap	17/20

Fig. 1. Effect of number of chill hours on vine recovery.



Susceptible grapevines (*Vitis vinifera* Chardonnay) were inoculated with *X. fastidiosa* Stag's Leap and then after 16 weeks post-inoculation, subjected to cold treatment at 40°F for varied lengths of time. Vines were tested before and after cold treatment to confirm infection status using qPCR. Recovered vine were determined as those that tested positive or *X. fastidiosa* prior to treatment and negative for *X. fastidiosa* 16 weeks after treatment. A total of 20 vines was included in each cold treatment.

Fig. 2. Effect of cultivar on overwinter curing.



Vines inoculated with *X. fastidiosa* Stag's Leap were subjected to cold treatment at 40°F for 800 hours. Infection status was determined before and 16 weeks after treatment using qPCR.

Occurrence and management of basal stalk rot of water bamboo in Taiwan

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ABSTRACT

In 1999 a severe basal stalk rot disease of water bamboo (*Zizaniae latifolia*) occurred in Nanto County in the summer, and as of today Nanto is still the only county manifested with the disease. The causal agent of the disease was considered to be *Pythiogeton zizaniae*. The local varieties 'Green Shell Early' and 'Red Shell' were both susceptible, with the former being more susceptible. For the variety 'Green Shell Early', the edible swelling culms (water bamboo shoots) could be harvested in April–June and July–October, designated as the first and the second harvest period, respectively. The disease appeared from late April to October, but in July the disease severity scaled up to 43.8% in the individual field. The disease could cause severe yield loss of the second harvest period. For the isolation of this pathogen, the more distant from the cutting wounds the less frequent the pathogen could be isolated. By draining the field for 30 days near the end of first harvest period (around May and June), the disease severity could be reduced. Although the yield of the first harvest period was mildly decreased, that of the second harvest period was greatly increased, amounting to higher yield than the conventional farming. Planting water bamboo one month in advance and in combination with lighting at night could accelerate the harvest time, and accordingly draining was advised to be conducted earlier to reduce the disease severity and prevent the yield loss due to the drainage. Furthermore, the method treated with drainage and cut-off all the above ground plants post the first season would assist new tillers growth and significantly reduce basal stalk rot disease. The disease severity after treatment using the above-mentioned method was down to 1.4%, which was significantly lower than that of merely drainage treatment or non-drainage.

Keywords: *Zizaniae latifolia*, *Pythiogeton*, basal stalk rot, drainage, harvest period regulation

INTRODUCTION

Water bamboo (*Zizania latifolia* (Griseb.) Turcz. ex Stapf., syn. *Zizania caduciflora* Turcz.), also named as water shoots, water oat, Manchurian wild rice, and Coba, is a kind of perennial herbaceous aquatic plant. It has been cultivated in China for more than 2,000 years and in Taiwan for about 200 years. The plant is usually infected by a smut fungus, (*Ustilago esculenta* Henn.), which causes swelling of its basal culms [2, 3, 7, 11, 12, 14, 15]. The swelling culms, which usually grow under water, are consisted of plant tissue and fungal mycelia and have traditionally been used as a famous aquatic vegetable, named as water bamboo shoots, also called as *jiao-bai-sung*, *gau-sun* or *kah-peh-sung* in Taiwan [4, 8, 9, 13].

The largest water bamboo cultivation area in Taiwan is in Nantou County, especially in Puli Township. According to the Agriculture and Food Agency of the Council of Agriculture, there were 1600 hectares of fields in Puli planted with water bamboo in 2016. Those Puli fields represented 83% of all land in Taiwan planted with the crop, and they produced about 40,000 metric tons—92% of the nation's total output of water bamboo shoots.

There are two types of cultivars planted in Taiwan. The first one is a two-season crop plant, which is planted in the early spring and then can be harvested twice in the late spring to early summer (first harvest period) as well as in the fall (second harvest period); the other is the one-season plant which is planted in the late spring and then can be harvested once each year, in the fall. The first type is more popular than the second type because of high yield and quality, and 'Green Shell Early' is the most popular cultivar in Taiwan, which is also the main cultivar in Puli. 'Red Shell' is the major example cultivar of the second type of water bamboo in Taiwan. All plants grow in paddy fields with 45-60 cm deep of water in the growing season and the crops are renewed for the next planting season [9, 10].

In May of 2000, Puli experienced its first outbreak of basal stalk rot of water bamboo. Several hundred hectares of water bamboo were infected with the disease, and farmers' losses were heavy. Several researchers were sent to Puli to try to combat the outbreak. With the unsolved stem rot problem in hand, the researchers were confronted with an

additional issue the following year: The growth of the water bamboo was being stunted [(5)]. Fortunately, the researcher discovered the causal agents of the stem rot disease and the stunting disease of water bamboo and then developed several techniques to solve the basal stalk rot disease by utilizing the management methods of these two diseases without having to use chemicals[(6)].

Disease investigation and pathogenicity test of basal stalk rot disease

The disease occurred in summer of 1999 and continued to damage the water bamboo crop in the summers of the consequent years in Nanto County. The symptoms of the disease exhibited yellowing new leaves, basal stalk rot, stripe rot of the swelling culms, and even plant death (Figure 1A-J). In the summer seasons of 2000 to 2005, the disease spread from about 500 hectares to about 1000 hectares, mainly occurred in the intersection area of Mei River and Nankan River. Whereas, water bamboos grown in other counties have not shown any symptom of the basal stalk rot disease.

A detailed disease investigation was conducted in 2001 in two bamboo fields, which were nearby and owned by a farmer in Puli Township. One field (field A) showed the disease symptoms but the other (field B) didn't. Mild basal stalk rot disease occurred in field A since April one month after harvest. The disease reached the peak in July, with the highest disease incidence of 84.5% and disease severity 43.8%, and the disease incidence was down to 59.5% and the disease severity to 20.1% in October. No disease symptom showed in Field B in this year's observation. The yields of the first and second harvest period of field A were 11.2 and 11.8 km/ha, respectively, while those of field B 12.7 and 19.4 km/ha, respectively. 'Red Shell' water bamboo also exhibited mild basal stalk rot disease in near field in Puli Township during the disease investigation (Figure 2). *Pythiogeton zizaniae*, a new member of Oomycetes, was consistently isolated from the the diseased plants of the infected area. The pathogenicity was confirmed by artificial inoculation of water bamboo plants with zoospore suspension of the microorganism [(1)]. The pathogen caused disease symptom of the new tillers 1.5 month after artificial inoculation at the cut wounds generated during harvest.

Effect of irrigation water and cut wounds generated during harvest on the disease

In further field observation, necrosis usually started from the cut wounds generated during harvest and then expanded down to the healthy tissue (Figure 1K, L). The result

of the pathogen isolation confirmed the observation that the isolation ratios of *P. zizaniae* is 72, 78, 50 and 35% from the wound, sections 1st, 2nd, and 3rd, respectively. Other than that, the results of field trials showed that no matter whether water bamboo was planted in monoculture field soil or virgin soil, basal stalk rot will occur as long as the irrigation water used was from the upstream fields where the crops had the disease. Furthermore, the non-harvest plants as well as the plants in the fields with low-leveled irrigation water showed no or much lower disease incidence when compared with the harvested plants, which had cut wounds in the same test field.

Because the pathogen could produce movable zoospores that in turn could be easily spread to the whole field or other fields via irrigation water. The above results showed that the main source of the pathogen was from irrigation water in the fields, and the cut wounds generated during harvest were the main infection sites.

Draining irrigation water for disease management

Because the pathogens were spread through irrigation water and infected water bamboo through the cut wounds, drainage during the growing season could reduce the disease. However, drainage would affect the yield of water bamboo shoots because water bamboo grew poorly in paddy field without deep water. When draining the irrigation water near the end of the first harvest period for one month (about in May), it could reduce the disease incidence from 84.5% to 54.5% and disease severity from 38.8% to 24.3% in July, compared to the conventional field, which was irrigated the whole season. Although the drainage treatment reduced the yield of the first harvest period of water bamboo shoots from 12.8 ton/ha to 11.1 ton/ha, it prevented the yield loss of the second harvest period from 17.8 ton/ha to 13.4 ton/ha when compared to the conventional farming with irrigating during the whole growing season (Figure 3).

In further study, one-month-long drainage periods were respectively conducted in May, June, or July to estimate the effect of drainage on reducing disease severity of basal stalk rot. The result showed that earlier drainage (in May) could reduce disease severity and had a good yield of the second harvest period, but the yield of the first harvest period decreased. In contrast, late drainage (in July) had the best yield of the first harvest period, but disease index and yield of the second harvest period were the worst (Table1).

Combination of drainage, harvest period regulation and cutting off the shoots after harvest for disease management

Although earlier drainage (in May) could reduce disease severity and had a good yield of the second harvest period, it reduced the yield during the drainage period while the price of water bamboo shoots was higher than that in the second harvest period. Thus, farmers tried to plant water bamboo earlier in order to harvest earlier to prevent the yield loss during drainage in May. However, earlier planting led to a stunting disease, which was a physiological disorder (Figure 4A). Fortunately, another technique (night-lighting for 8 hours during the seedling stage) was developed to prevent the stunting disorder (Figure 4B). So earlier planting (with night-lighting) and drainage of irrigation water for 30 days in May could not only reduce the disease severity but also reduce the yield loss during drainage (Table 2).

Because water bamboo shoots were harvested underwater, the pathogen could be easily spread by irrigation water and then penetrate the crops from the cut wounds generated from harvest. The pathogen still colonized in the stem even if drainage executed after harvest period and would cause disease symptom of the new tillers. Further study showed that during the drainage period after the first harvest period, cutting off the stem and shoots above the ground and letting new tillers grow from the old stumps would significantly reduce the disease incidence and severity. The disease severity was 1.4%, significantly lower than that by drainage only (8.1%) or non-drainage treatment (13.2%). The yield was significantly higher than that of regular cultivation but only similar to that by drainage only (Table 3).

DISCUSSION

Because basal stalk rot pathogen, *P. zizaniae*, was mainly spread by irrigation water and infected the crops from the cut wounds generated during harvest, it could be easily understood that most of the water bamboo fields exhibited severe disease due to the irrigation water from the adjacent field. The pathogen might reach to the isolated or upstream fields by contaminated boots, machines, and equipment, and then spread downstream to other fields. That might be the reason why the disease spread to about 1,000 hectares in several years.

The pathogen would cause disease symptom of the new tillers 1.5 months after infection, so the crop showed severe symptom in June and July, 1-2 months after the peak of harvest while not many shoots left at that time. In July, the number of tillers was most severely reduced, which seriously affected the yield of the second harvest period.

Drainage in May (late harvest period) could reduce disease severity, but it reduced the yield in the drainage period. Planting water bamboo earlier in order to harvest earlier could prevent the yield loss due to early drainage in May and prevent the yield loss caused by the disease. However, earlier planting resulted in stunting growth, but nighttime illumination during the growth of tillers can avoid this problem.

Drainage and cutting off the shoot after harvest could avoid the basal stalk rot disease; regulating planting season with night-lighting at seedling stage in the short-day season could shift the harvest period to avoid yield loss during drainage. Utilizing the management methods for these two diseases, the researcher developed a unique system for regulation of water bamboo shoots during harvest period that made the growth of water bamboo shoots a year-round industry in Taiwan.

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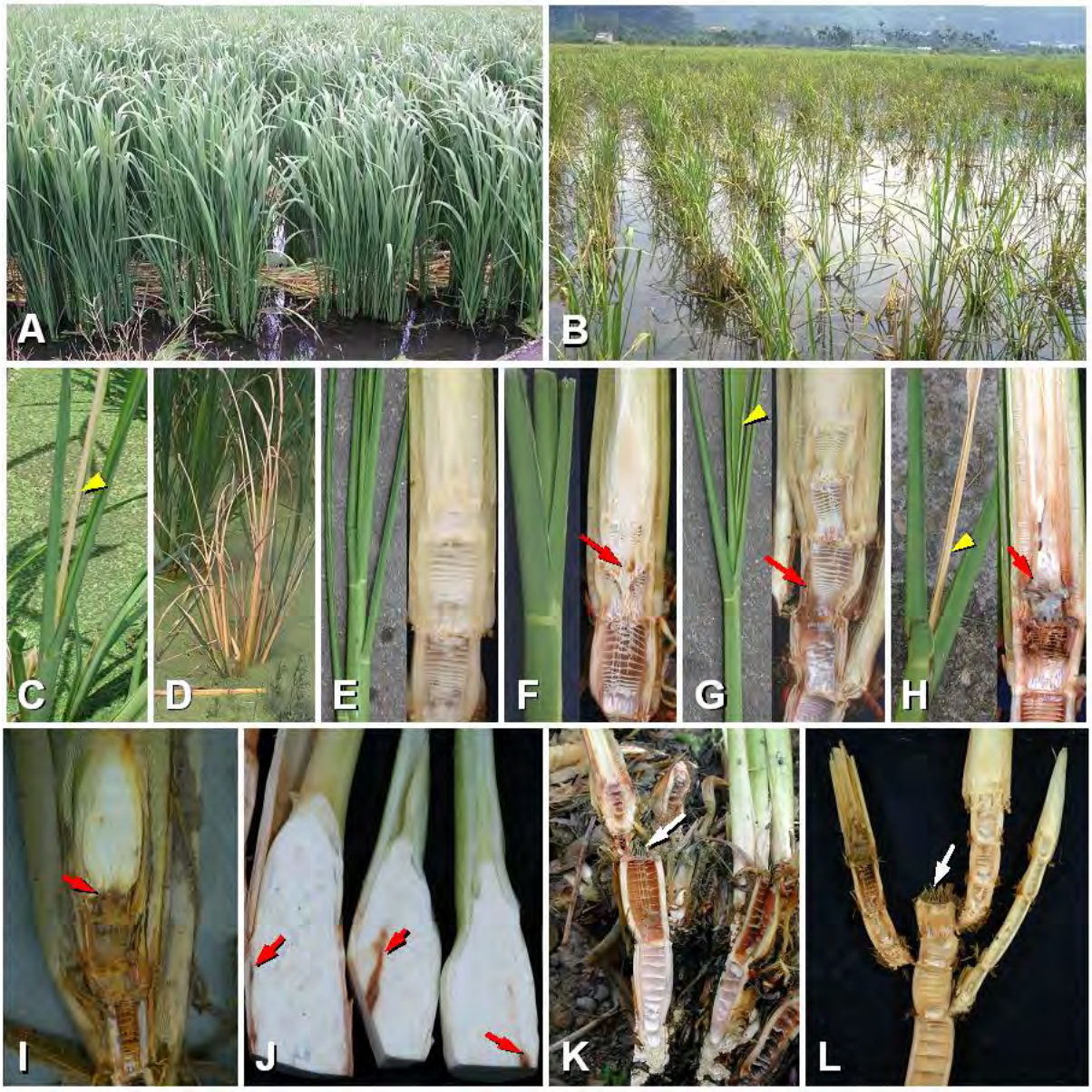


Fig. 1. Symptoms of basal stalk rot of water bamboo plants, healthy (A) and diseased plants (B) in the field. Basal stalk rot causing yellowing of new leaves (C) and death (D) of water bamboo. Leaves and basal stalk symptoms of healthy (E), mildly diseased (F), and severely diseased (G) water bamboos. Symptoms of basal stalk rot on eatable galls of water bamboo. Wilt or yellowing of new leaves (arrowheads) (H & I) whereas brown necrosis tissues (arrows) (J) caused by the disease. Cut wounds generated during harvest (arrows) and lateral stalk with basal stalk rot (K, L).

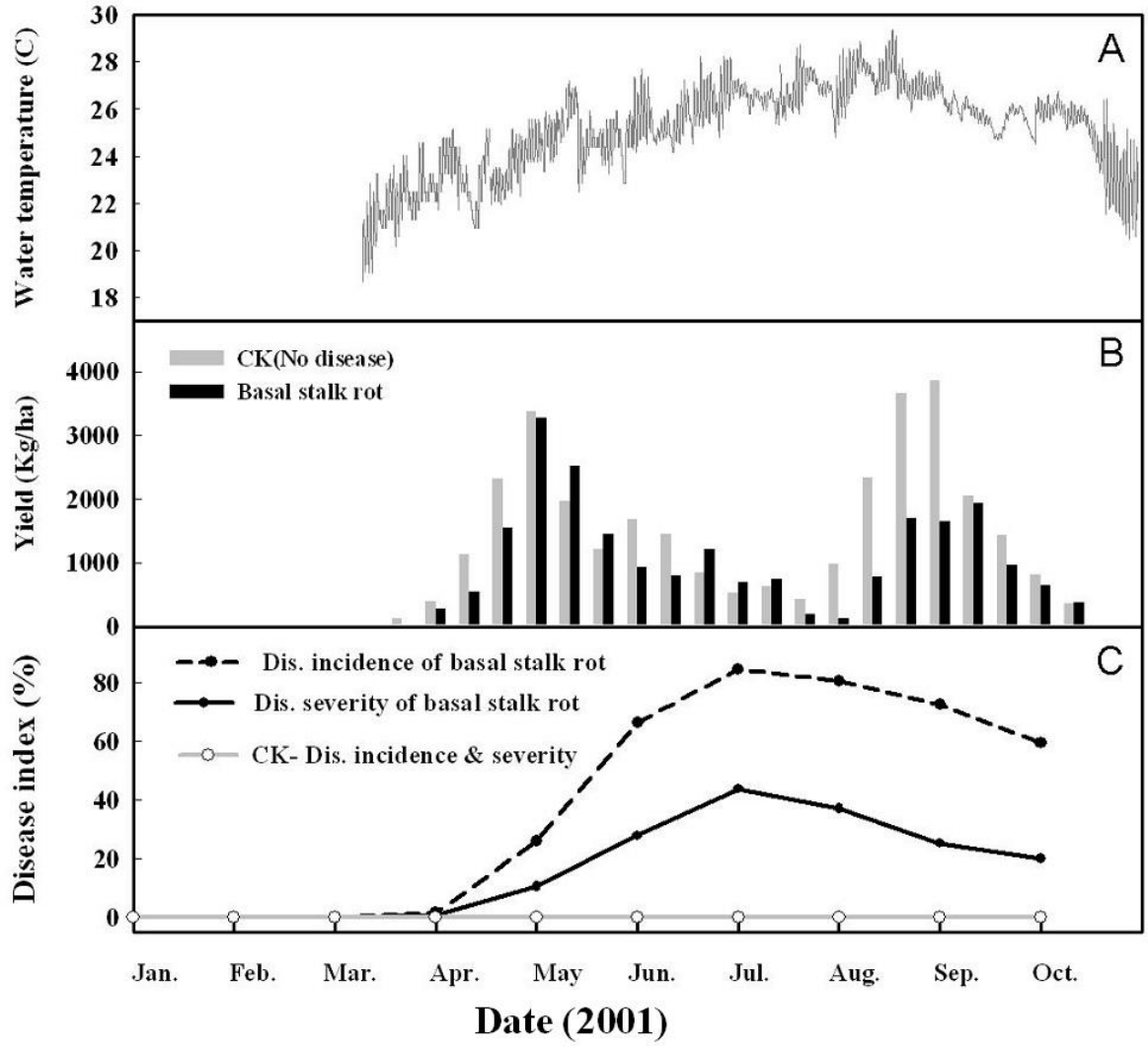


Fig. 2. Field water temperature (A), yields (B) and disease survey (C) of basal stalk rot of water bamboo at Puli. (Huang 2013)

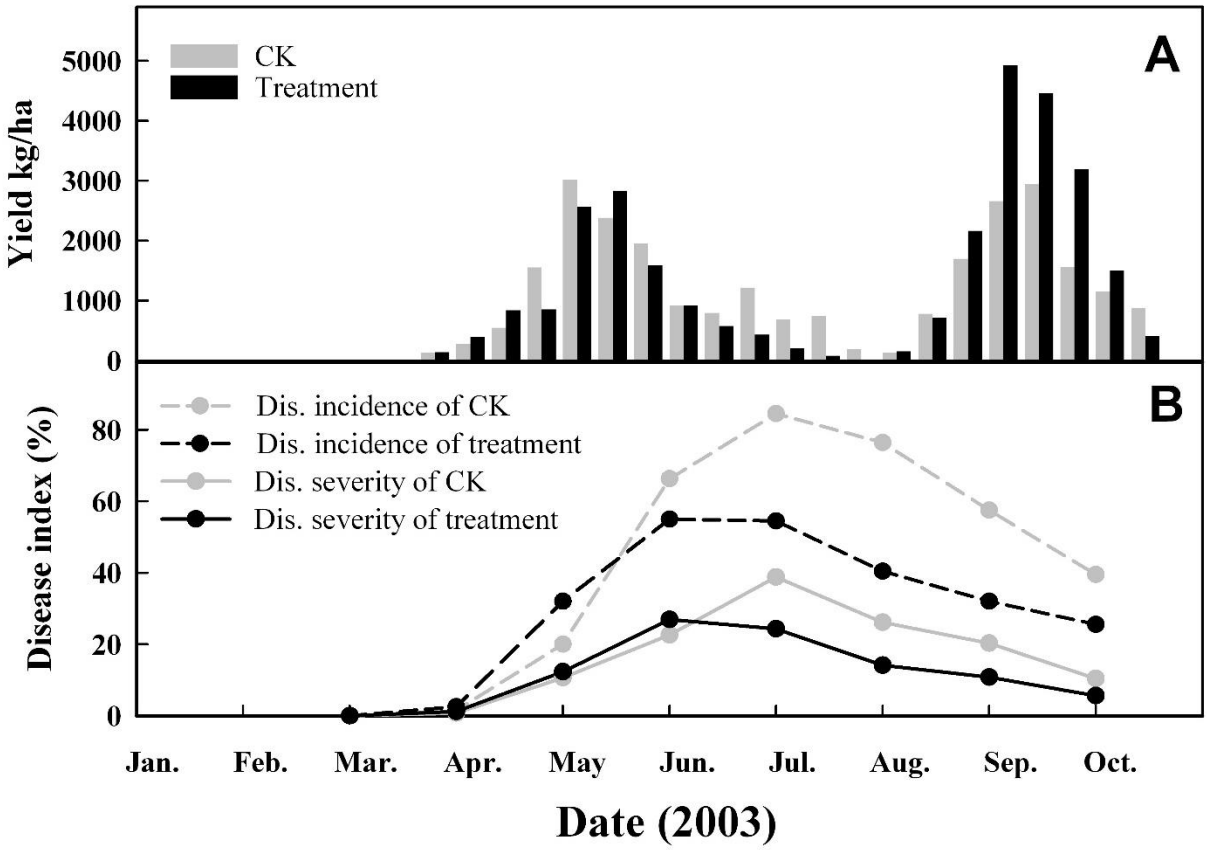


Fig. 3. Effect of drainage on water bamboo yield (A) and basal stalk rot disease (B) of water bamboo. CK: irrigated fields in whole season; treatment: drainage for one month from May 1st. (Huang 2013)

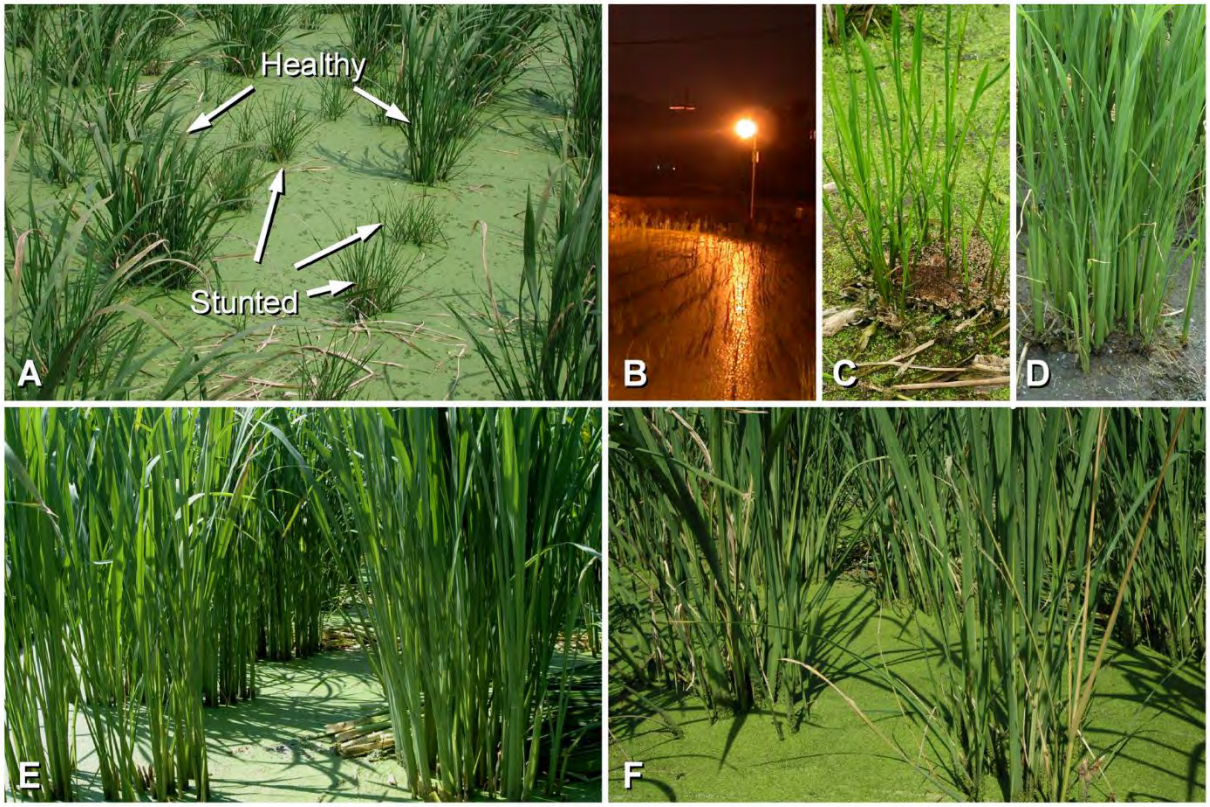


Fig. 4. Harvest period regulation with drainage after harvest for management of basal stalk rot of water bamboo. The stunted plants of water bamboo due to earlier planting in November (A), early planting accepted night lighting to prevent plants from stunting (B). Drainage after harvest and cutting old shoots off could promote the new shoots grow (C, D), and effectively decrease the basal stalk rot disease of water bamboo (E) as compared to the common cultivation process where the fields were irrigated whole season long (F).

Table 1. Effect of drainage period on the yield and basal stalk rot of water bamboo

Drainage period [1)]	*Dis. incidence % (Dis. severity %)*				*Yield (t/ha)* [1)]		
	May		*July*		*1st season*	*2nd season*	*Total*
CK	*21.5 a* [2)]	*(6.5 a)*	*89.0 a*	*(29.4 a)*	*9.7 a*	*13.5 c*	*23.2 b*
May	*19.0 a*	*(6.2 a)*	*57.0 b*	*(12.6 b)*	*6.3 b*	*21.4 a*	*27.7 a*
June	*22.0 a*	*(7.9 a)*	*71.0 ab*	*(17.4 b)*	*7.2 ab*	*19.8 a*	*27.0 a*
July	*18.0 a*	*(6.1 a)*	*81.0 a*	*(26.6 a)*	*9.1 a*	*16.2 b*	*24.3 b*

[1)]: First or second harvest periods mean the days before or after July 1.

[2)]: Means followed by the same letters within each column are not significantly different at p=0.05 according to the LSD Test.

Table 2. Effect of early planting with night lighting and drainage after first harvest period on the basal stalk rot of water bamboo.

Planting [1)]	*Drainage date* [2)]	*Dis. incidence % (Dis. severity %)*				Yield (t/ha) [3)]		
		May		*July*		*1st season*	*2nd season*	*Total*
Conventional	*No*	*12.0 a* [4)]	*(4.0 a)*	*89.5 a*	*(20.3 a)*	11.1 a	16.0 c	27.0 d
	Yes	*12.5 a*	*(3.3 a)*	*65.0 bc*	*(10.7 b)*	8.1 b	22.5 ab	30.6 c
Early	*No*	*15.5 a*	*(2.8 a)*	*77.5 ab*	*(16.8 a)*	12.3 a	19.6 b	32.9 b
	Yes	*9.5 a*	*(2.5 a)*	*61.0 c*	*(8.4 b)*	12.0 a	23.7 a	35.7 a

[1)]: Conventional: planting (nursery) at regular date (Dec. 1st, 2005) without night lighting. Early: earlier planting 45days earlier with night lighting for 60 days (8 hr/day).

[2)]: Drained the field water for 30 days from May 1st.

[3)]: First or second harvest periods mean the days before or after July 1

[4)]: Means followed by the same letters within each column are not significantly different at p=0.05 according to the LSD Test.

Table 3. Effect of drainage alone and drainage in combination with severed shoots after the first harvest period on the basal stalk rot of water bamboo.

Treatment [1)]	*Dis. incidence % (Dis. severity %)*		*Yield (t/ha)* [2)]		
	May	*July*	*1st season*	*2nd season*	*Total*
CK	*10.3 a* [3)] *(1.5 a)*	*65.0 a (13.2 a)*	10.5 a	20.5 b	*31.0 b*
A	*7.7 a (0.9 a)*	*47.3 b (8.1 b)*	11.7 a	23.4 a	*35.1 a*
B	*8.7 a (1.1 a)*	*10.3 c (1.4 c)*	11.2 a	22.2 ab	*33.4 ab*

1): All seedlings were planted in November 1st. CK: No drainage; A: drainage from May 1 to May30, but shoots were not severed; B: drainage in combination with all severed shoots.

2): First or second harvest periods mean the days before or after July 1.

3): Means followed by the same letters within each column are not significantly different at p=0.05 according to the LSD Test.

Application of a bio-control agent for controlling strawberry anthracnose in Taiwan

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ABSTRACT

Strawberry (*Fragaria* × *ananassa* Duchesne) is an economically important crop in Taiwan, however because of the outbreak of strawberry anthracnose in early 2010, the short supply of strawberry seedlings became a serious problem for strawberry production. The isolates of anthracnose causing organisms were collected from major strawberry cultivation areas in Miaoli County, and mainly identified as *Colletotrichum siamense*, these isolates were proved to be pathogenic to the leaves, crown and roots of strawberry. The bio-control agent of *Bacillus amyloliquefaciens* P-2-2, was isolated from rice paddy field and expressed effectively on inhibition of many fungal pathogens of strawberry. The strawberry seedlings used in this study were treated with 400-fold dilution of *B. amyloliquefaciens* P-2-2 fermentation broth once for every two weeks to protect the crown of the seedlings from anthracnose infection before transplanting. Sterilization of soil by biological soil disinfestation method was done during field preparation. After transplanted in the production field, the strawberry transplants were continuously treated with 400-fold dilution of *B. amyloliquefaciens* P-2-2 once for every two weeks until the end of the growing season. The results of field test, shown that the survival rate of the untreated group in demonstration area of Dahu Township was 83.9%, whereas the survival rate of seedlings treated with *B. amyloliquefaciens* P-2-2 increased to 95.1%. The replanting rate of strawberry in the untreated group area was 37.6%, compared to the *B. amyloliquefaciens* P-2-2 treated area which was reduced

significantly to 10.7%. Upon those results indicated that application of bio-control agents with other practices were effective for controlling strawberry anthracnose.

Keywords: Taiwan, strawberry, anthracnose, bio-control agent, biological soil disinfestation

INTRODUCTION

Strawberry is an important economic crop in Taiwan. Although the total domestic production area is only about 550 hectares, of which 90% is located in Miaoli County, the total value of fresh and processed fruits exceeds 1.8 billion of New Taiwan Dollar (NTD). If the strawberry seedlings needed for transplanting at 45,000 plants per hectare, the total annual demand will reach 25 million plants. However, in recent years, due to the occurrence of strawberry anthracnose, the supply of strawberry seedlings is insufficient. In addition to the abnormal climate, the temperature is still very high (above 25^{o}C) right after transplanting of strawberry seedlings in September through October and November, resulting in transplanted strawberry seedlings damaged by anthracnose with gangrenous crown and withering and dying leaves. The replanting rate is as high as 30 to 40%, which causes a big crisis in the strawberry industry. It is urgent to solve the problem of strawberry anthracnose.

This study intends to use the biocontrol agent and soil disinfection methods to prevent the occurrence of anthracnose disease in strawberry seedling stage and field cultivation period. Because strawberries suffer from many pests and diseases during the seedling stage and field cultivation period, farmers often applied chemical pesticides to control these problems. Countries such as the United States, Japan, etc., cannot completely achieve the economic production of strawberries without application of chemical pesticides. Through the biocontrol agent and health management during this study, we might have the opportunity to lead the world in the production of organic, pesticide-free strawberries.

The techniques for integrated pest management of strawberry

The techniques for integrated pest management of strawberry during vegetative and flowering stage developed by TARI describe as following.

1. Pre-treatment of strawberry seedlings before transplanting: Before transplanting, strawberry seedlings were soaked at 1000-fold dilutions of "Anthracnose no trace", "Black Asura" and 200-fold dilution of vegetable oil mixture to reduce the contaminated primary inoculum of pathogens and insects into the field.
2. Pest management in strawberry vegetative and fruiting stages:

a. Disease management: The main diseases of strawberry are fruit rot caused by *Phytophthora* spp., powdery mildew disease by *Sphaerotheca aphanis* and gray mold disease by *Botrytis cinerea*. Before the rainy season, the naturalized phosphorous acid (1000-fold dilution) is applied once a week for three times to prevent the occurrence of fruit rot. Emulsified sunflower oil (200 to 500-fold dilution) is applied at the beginning of powdery mildew and gray mold. The "vigor energy" (1000-fold dilution) is applied for controlling anthracnose disease.
b. Pest management: Pests include *Tetranychus urticae*, *Aphis ichigocola*, *Spodoptera litura*, *Scirtothrips dorsalis*, and *Frankliniella intonsa*. Among them, *Frankliniella intonsa* mainly causes harm at flower organs during flowering stage. The other four pest species may occur year-round. It is necessary to follow the actual monitoring data of pests and diseases in order to master the appropriate methods and timing of control measures. Samples of the old and middle leaves, and young shoots were monitored. At the nursery and before flowering in the field, strawberries were sprayed with the vegetable oil mixture to inhibit the populations of spider mites. At the beginning of flowering stage, the biological natural enemies "*Mallada basalis*" were released to control the two-spotted spider mites and strawberry aphid, and the "*Orius* spp." was released to control the thrips (*Scirtothrips dorsalis* and *Frankliniella intonsa*). Each plant received about 10 eggs or 5 larvae of the first instar of "*Mallada basalis*", once every 7 to 10 days until the late harvest. The invasion of *Spodoptera litura* can be controlled by spraying *Bacillus thuringiensis* if needed. If the insect pest is found to be serious and the control efficacy of natural enemies and vegetable oil mixture are not sufficient, then the recommended chemical pesticides in the plant protection manual will be used for prevention and control.

3. Field sanitation: Weeds in and around the strawberry filed must be removed. The debris of unwanted branches, leaves, flowers, and fruits should not be left in the field, for they may serve as the habitat for the growth and proliferation of pathogens and pests. The unwanted strawberry leaves should be removed and placed in the bag and taken away from the field. They should be immediately burned or buried under the ground for at least 30cm deep, and should not be disposed in the field or in the drainage ditch.

Identification of strawberry anthracnose

Strawberry anthracnose is an important disease in the cultivation of strawberry. The pathogen could infect the leaves, fruits, petioles and stolon and cause dark brown sunken lesions on strawberry. The pathogen can also damage the crown of strawberry, causing symptoms like browning of the vascular bundle and withering of the strawberry

plants. Strawberry anthracnose pathogen often infects strawberry at the seedling stage and causes signs of withering between the late seedling stage and the early stage after transplanting. It can even cause the death of strawberry seedlings, resulting in the lack of seedlings as transplanting supplies. The anthracnose not only increases the cost of strawberry cultivation, but also affects the quality of the fruit, so the detection of pathogens and the establishment of a healthy seedling system are crucial [8]. The purpose of this study was to identify the pathogens of strawberry anthracnose to facilitate and monitor both in nursery and in filed diseases for better management. The anthracnose isolates were collected from major strawberry cultivation areas such as Dahu Township, Miaoli County and Guoxing Township, Nantou County. A total of 20 isolates collected from 2010 to 2018 were identified by using specific primers designed for *Colletotrichum acutatum*, *C. gloeosporioides* and *C. boninense* species complex [7, 9, 12, 14]. The *C. gloeosporioides* species complex was further identified by using specific markers [3] that could discriminate between *C. fructicola*, *C. aenigma*, and *C. siamense* within *C. gloeosporioides* species complex. The results showed that 18 isolates were *C. siamense*, one isolate was *C. fructicola*, and another one belonged to *C. gloeosporioides* species complex, but specific species could not be identified. According to the primary result, *C. siamense* was the main species that caused strawberry anthracnose in Dahu Township and Guoxing Township (Fig. 1).

The pathogenicity test of C. fructicola and C. siamense

C. fructicola and *C. siamense* were originally isolated from the diseased crown and leaf of strawberry, respectively. The cultures of *C. fructicola* and *C. siamense* were grown on PSYA medium for 5-7 d, the cultures were then flooded with distilled water to wash off the spores; this was followed by filtration through a single layer of Miracloth (Calbiochem, San Diego, USA) to remove any mycelial residue from the conidial suspension. The final concentration of the suspension was adjusted to 1 x 10^1, 10^3 and 10^5 spores/mL and was used for the experiments of both inoculation and spore germination. The leaves, crown and roots of strawberry were inoculated with different inoculation methods. The roots were soaked in spore suspension after the root tips were freshly cut; the damaged crowns were drench-inoculated with spore suspension after petioles were removed. The leaves were spray-inoculated with spore suspension. The inoculated strawberry plants were kept in a moisture chamber for one day before they were moved to greenhouse for one week for symptom observation. The results showed that *C. fructicola* was pathogenic to the crown and roots but not to the leaves of strawberry plants (Fig. 2), whereas *C. siamense* was pathogenic to the leaves, crown and roots (Fig. 2). The primary results showing different pathogenicity between two

anthracnose species might indicate that strawberry anthracnose disease in Taiwan was mainly caused by *C. siamense*.

Antagonistic test of biocontrol agent against fungal pathogens

Previous studies indicated that biocontrol agent, *Bacillus amyloliquefaciens* P-2-2, was able to induce disease resistance in vegetable crops. The pathogens (*Colletotrichum siamense*, *Rhizopus* sp., *Sclerotinia sclerotiorum*, *Pythium* sp., *Fusarium* sp. and *Botrytis cinerea*) were isolated from diseased strawberry plants. The inhibitory effect of *B. amyloliquefaciens* P-2-2 on mycelial growth of fungal pathogens of strawberry was conducted by dual culture of the respective pathogens and *B. amyloliquefaciens* P-2-2 on 1/2PDA+1/2NA medium, and the mycelial length were recorded and the inhibition rate was calculated. The inhibition rate of *B. amyloliquefaciens* P-2-2 against *C. siamense*, *Rhizopus* sp., *S. sclerotiorum*, *F. oxysporum* and *B. cinerea* was 45.76, 16.91, 44.75, 8.45, and 42.05%, respectively. The results indicated that application of *B. amyloliquefaciens* P-2-2 to strawberry plants could not only effectively inhibit the anthracnose pathogen and but also to other diseases caused by other tested fungal pathogens (Fig. 3).

Control efficacy of biocontrol agent against strawberry anthracnose in greenhouse

The healthy strawberry seedlings (varieties Toyonoka and Tianlai No.1) provided by the farmers of Dahu, Miaoli county. The beneficial microorganism *B. amyloliquefaciens* P-2-2 was used to test its control efficacy on strawberry anthracnose. Once the strawberry seedlings were established in the greenhouse, their leaves were sprayed with different dilution of *B. amyloliquefaciens* P-2-2 fermentation broth one day before they were inoculated with anthracnose pathogen. A total of four groups, distilled water and 200-, 400-, and 600-fold diluted *B. amyloliquefaciens* P-2-2 fermentation broth were used to test their inhibitory efficacy on the control of anthracnose disease. After being inoculated with spores ($1x10^5$ spores/mL) of anthracnose pathogen, the strawberry seedlings were maintained in the acrylic box for one day before the cover was removed. The strawberry seedlings were then transferred to greenhouse for 7-14 days. The numbers of the anthracnose lesions were recorded. It was found that 200-fold and 400-fold diluted *B. amyloliquefaciens* P-2-2 fermentation broth were able to inhibit the formation of anthracnose lesions on Toyonaka variety. It was found that 400-fold and 600-fold diluted *B. amyloliquefaciens* P-2-2 fermentation broth were able to inhibit the formation of anthracnose lesions on Tianlai No.1 variety.

The best dilution rate of *B. amyloliquefaciens* P-2-2 fermentation broth was 400-fold for both tested strawberry varieties (Fig. 4).

The methods for disinfection of irrigation water

Many methods are available for disinfection of irrigation water including filtration, heat treatment, oxidizing agents, and ultraviolet radiation. Plant pathogens showed different sensitivity to different disinfection methods [1, 4, 6, 15, 16]. Chlorine, as either calcium hypochlorite or sodium hypochlorite, is commonly used to treat irrigation water because it is inexpensive and easy to apply. When chlorine is introduced to water, it reacts to form free chlorine species of hypochlorous acid (HClO) or hypochlorite (ClO^-) ions, depending on the pH of the water, which oxidize organic materials, including any pathogens present in the water [17]. Hypochlorous acid is the stronger, faster oxidizer, but is more prevalent when the pH of the water is between 6.5 and 7. As the pH of water increases, the hypochlorous acid is converted to hypochlorite, which is a weaker oxidizer and disinfectant. The disinfection efficacy of hypochlorous acid is 80 times higher than that of sodium hypochlorite. The inhibition effect of hypochlorous acid on germination of anthracnose spores was conducted. Aliquots (5 μL) of hypochlorous acid solutions were dropped onto glass slides and mixed with an equal volume of conidial suspensions (2×10^4 spores/mL), to make final concentrations of hypochlorous acid of 0.05, 0.25, 2.5, 5, 25 and 50 ppm, with a final concentration of the conidial suspension set at 1×10^5 spores/mL. DW was used as a control. The germination of conidia was observed and recorded 16 h after incubation. The inhibition rates of 50, 25, 5, 2.5, 0.5, 0.25, and 0.05 ppm hypochlorous acid solution on the spore germination of anthracnose pathogen were 100, 95.07, 85.20, 65.70, 52.47, 61.77, and 59.20%, respectively (Fig. 5).

The methods for disinfection of cultural medium and field soil

Methyl bromide had been rated as the most effective chemical fumigant for many plant diseases and pests [2, 5]. However, it destructively damages the ozone layer which in turn makes the sunlight become more harmful to human health [2]. It has been prohibited for agricultural use under the law; therefore, some soil-borne diseases have become more difficult to control by other available measures. Other soil sterilization methods, such as soil solarization and flooding, required high temperature and a long incubation period [2, 5]. A new method, named as biological soil disinfestation (BSD), developed by Shinmura [10, 11], fortified by soil reduction, which required neither high temperature nor a long incubation period, consisted of following three steps: (1) introduction of easily decomposable organic materials e.g., molasses, rice bran, rice

straw, wheat bran, etc., at a rate of 100-200kg/acre of soil, (2) flooding the soil by irrigation, and (3) covering the soil surface with plastic film to maintain the reduced soil conditions. BSD using molasses or wheat bran proved to be effective against a wide range of soilborne pathogens, such as *Fusarium oxysporum*, *Ralstonia solanacearum*, and *Verticillium dahliae*, as well as the nematodes *Meloidogyne incognita* and *Pratylenchus* sp. [10, 11, 13].

Disinfection of strawberry cultivation medium (such as peat moss and coconut fiber) was accomplished by the steam produced by water continuously heated by three cascaded gas water heaters (Fig. 6). Sterilization of soil by biological soil disinfestation (BSD) (Fig.7) with the introduction of easily decomposable organic materials to soil, flooding the soil by irrigation, and covering the soil surface with plastic film to maintain the reduced soil conditions. Application of *Bacillus amyloliquefaciens* P-2-2 to the cultivation medium and fields after disinfection (Fig. 8) to occupy the niche of pathogens of anthracnose and Fusarium wilt of strawberry was conducted.

Using beneficial microorganism and hypochlorous acid to cultivate health strawberry seedlings

In order to cultivate healthy strawberry seedlings, the tissue-cultured strawberry seedlings that are free of any specific pathogens were provided to the farmers to be used as mother plants. They were treated with the application of 400-fold diluted *B. amyloliquefaciens* P-2-2 fermentation broth to protect the crown of strawberry seedlings from the infection of anthracnose spores. Lastly, they were sprayed with the hypochlorous acid solution daily to kill the anthracnose spores disseminated from the diseased tissues of the seedlings (Fig. 9).

The result of field test

The survival rate of the untreated group in demonstration area of Dahu Township was 83.9%, whereas that of healthy strawberry seedlings treated with *B. amyloliquefaciens* P-2-2 broth increased to 95.1%. The replanting rate of strawberry in the untreated group area was 37.6% (Fig. 10), whereas that of the *B. amyloliquefaciens* P-2-2 treated area was reduced to 10.7% (Fig. 11). The use of *B. amyloliquefaciens* P-2-2 for 6 months increased the cost by no more than NTD 1,800, but it reduced the loss of about NTD 25,300 (including about NTD 20,500 for replanting strawberry seedlings and NTD 4,800 for labor wages). In addition, the use of biocontrol agent to grow healthy strawberry seedlings allows the growers to transplant the seedlings into the field in mid-September (Fig. 12). Other farmers, who do not use these technologies, fear that due to the shortage of strawberry seedlings, or high temperature, could only transplant

the strawberry seedlings until the end of October, which would delay strawberry harvest time and lose the good marketing opportunities.

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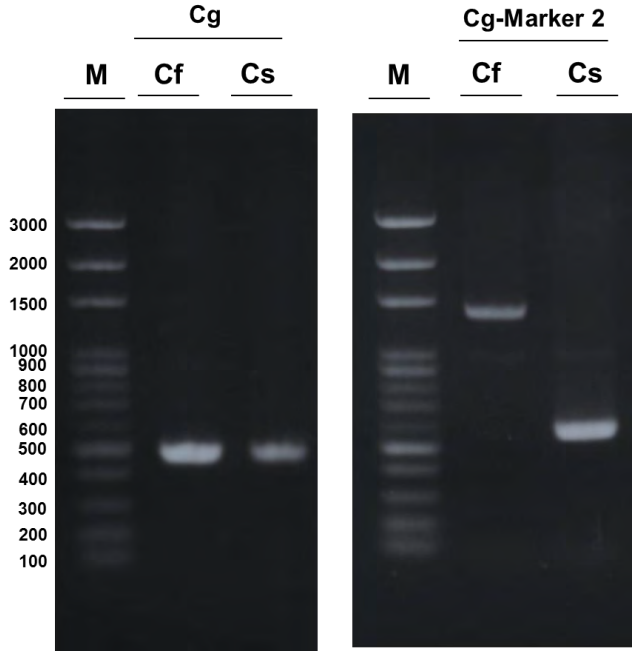


Fig. 1. Identification of strawberry anthracnose with primer for *Colletotrichum gloeosporioides* species complex and specific markers.

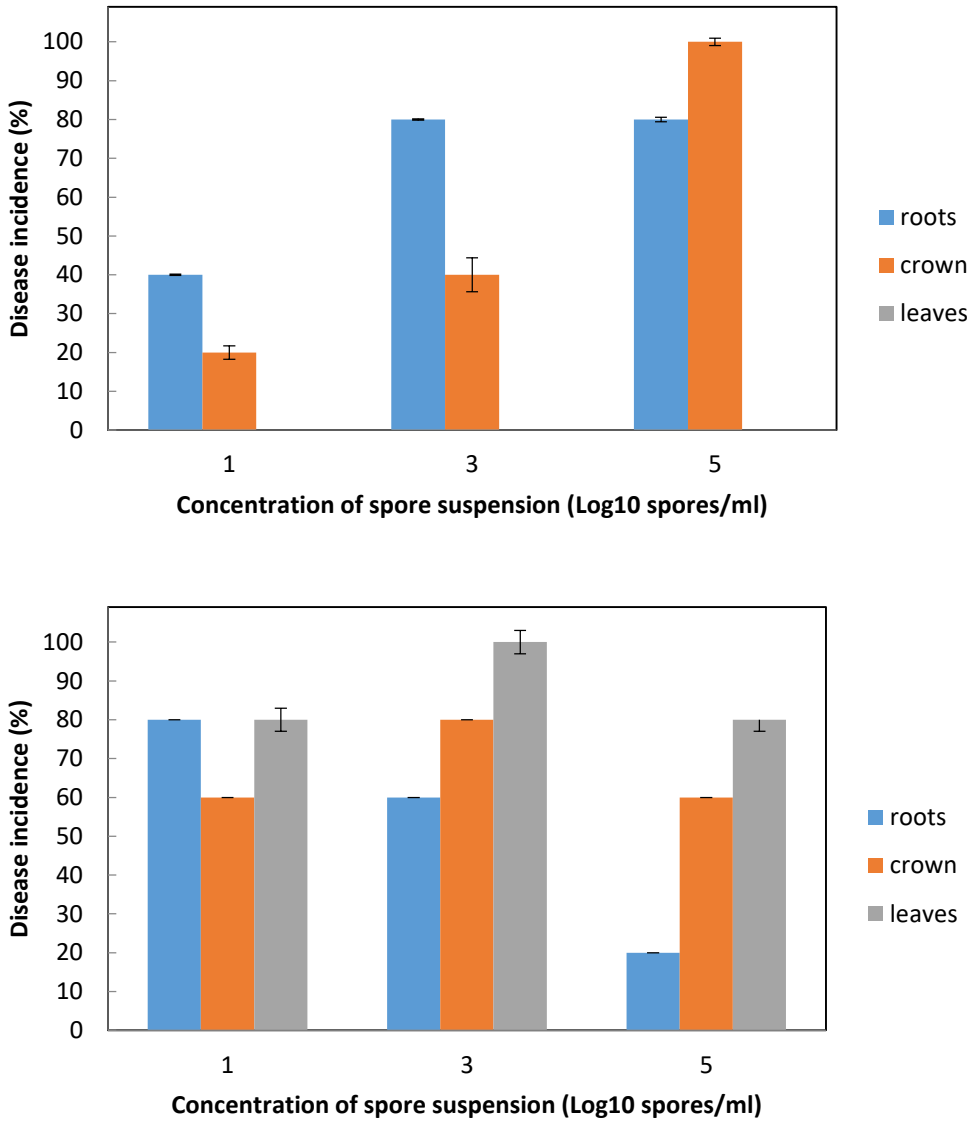


Fig. 2. Pathogenicity test of *C. fructicola* (upper) and *C. siamense* (lower).

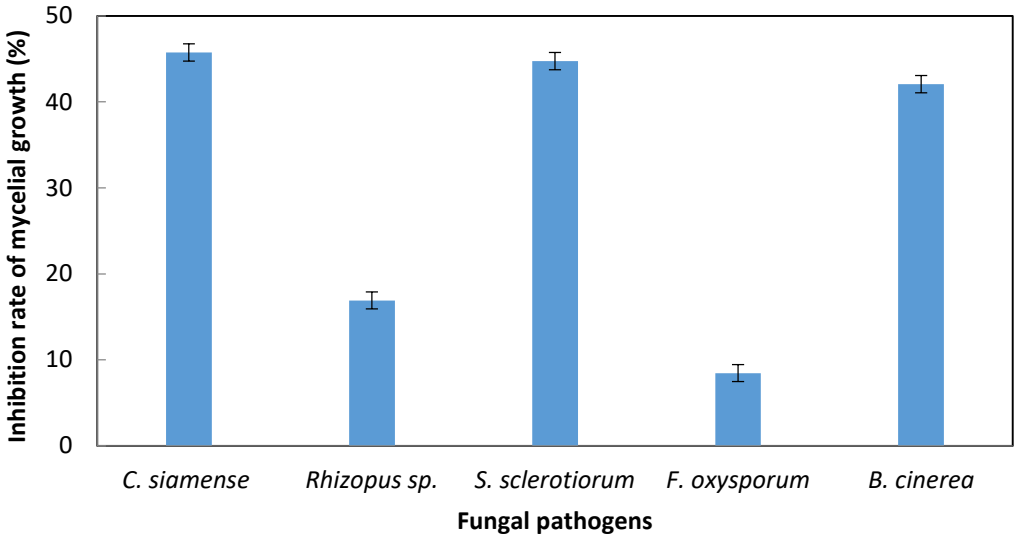


Fig. 3. Antagonistic test of biocontrol agent against fungal pathogens.



Fig. 4. Control efficacy of biocontrol agent against anthracnose on two strawberry varieties in greenhouse.

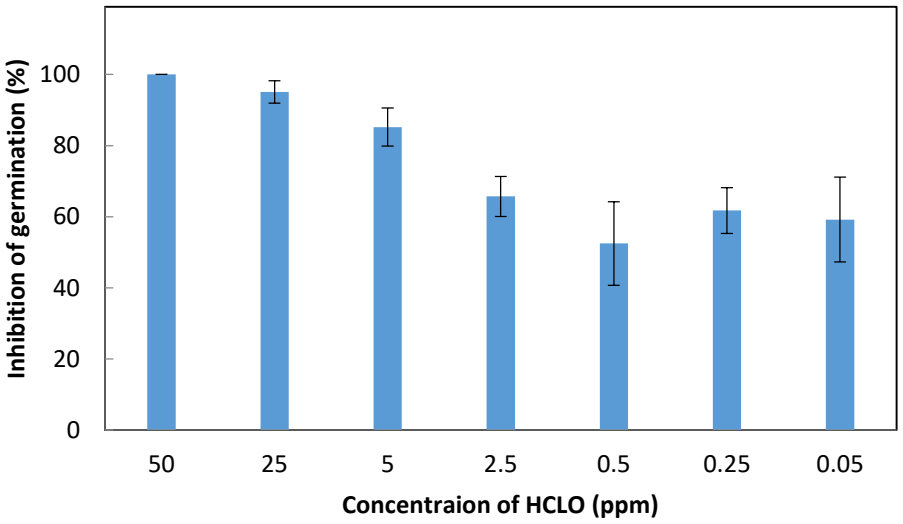


Fig. 5. The inhibitory effect of hypochlorous acid (HClO) on the spore germination of strawberry anthracnose pathogen.



Fig. 6. The gas water heaters used to generate steam for the disinfection of cultivation medium for strawberry.



Fig. 7. Soil covered with plastic film for disinfection; soil was added with organic matter followed by being flooded with water before being covered with plastic film.



Fig. 8. The biocontrol agent was applied to the disinfected soil.



Fig. 9. Propagation of healthy strawberry seedlings treated with biocontrol agent and hypochlorous acid.



Fig. 10. Many dead strawberry seedlings due to anthracnose disease in the untreated field.



Fig. 11. Healthy strawberry seedlings effectively protected from anthracnose disease with the treatment of biocontrol agent and soil disinfection.



Fig. 12. Strawberry seedlings cultivated and protected with the biocontrol agent against the anthracnose disease and were transplanted in mid-September.

An hierarchical method for identifying current and emerging pest threats under climate change uncertainty

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ABSTRACT

Pest threats to agriculture depend primarily on the climate suitability of the pest risk area and the susceptibility of the hosts being grown there. There are a number of efficient and robust techniques available to identify pest threats under historical climatic conditions. It is possible to identify lists of risky pest species, estimate the suitability of the climate in the pest risk area, including the pest phenology, and even estimate the economic risk the species might pose, given climate suitability and knowledge of the hosts' distribution within the pest risk area. The more detailed the information on the risks that is sought, the more expensive and time-consuming the techniques. Hence, a structured, hierarchical approach is generally to be preferred. Considering climate change effects on pest risks is only now gaining traction, some 15 years after the techniques were first pioneered. Some pest risk techniques are inherently more suited to exploring pest risks under climate change than others. Climatic changes due to the greenhouse effect are difficult to predict because the key forcing functions driving changes in the climate are greenhouse gas emissions, and these are driven by socio-economic and geopolitical factors that are inherently uncertain at timescales that are relevant for assessing pest risks. While our ability to forecast weather systems and to hindcast climate patterns continues to improve, our ability to forecast climate changes remains stubbornly constrained by uncertainty regarding future greenhouse gas emission patterns. In turn, this constrains our ability to forecast the future climate suitability patterns for pest organisms and their hosts. While we can confidently generate future climate patterns based on emissions scenarios, we are unsure which scenario is likely to be realised. Logically, managing emerging risks under such intractable uncertainty demands a special approach based on a sensitivity analysis. In this paper we outline a hierarchical method for identifying pest risks and demonstrate some of these pest risk methods in the context of Taiwan, generating insect

pest risk lists and potential distribution maps for two case study pests. We also outline a method for managing emerging pest risks under climate change uncertainty.

Keywords: biosecurity, climate change, CLIMEX, DYMEX, invasive pest assemblages, pest risk assessment, self-organising maps

Integrated management strategy of pests in response to climate change - a case study of oriental fruit fly

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ABSTRACT

Based on the climate change and the fact that pests have been able to adapt to the island's climate and the environment of crops, their biological characteristics passed down by heredity continuously. These pests with their annual or seasonal peak of population fluctuation often resulted as epidemic pests year by year. The epidemic costs growers greatly in order to suppress pest damage. However, most pests will re-establish new population by dispersal, seeking refuge, or media transmission. If one cannot master the traces of pests, which often make effective prevention and control of material efficiency greatly discounted, so it is urgently needed to develop pest-monitoring technology in the field. With the progress of computer information technology, it can now effectively manage the big data from long-term collection. In order to show the pest dynamic information, it is ideal to use the geographic information systems (GIS) and other information networks. To establish the geographical distribution and density monitoring data in Taiwan, we set up 61 stations to collect and show the spatial dynamics of pest population and the risk of crop damage in 1994. In addition to collect the data of population density per ten-day, it provides a common control of fruit flies, masters the effective use of materials, and improves the prevention and cure efficiency. The purpose is to reduce the cost of crop production. Combined with the information of the complete geographic location, the distribution of pest spatial and land cover can be addressed quickly, and so as to assess the risk of pest occurrence and damage, which in turn improves the overall control efficiency. But the climate change has become a major global issue, researchers have started to predict the potential distributions of pest and the areas they cause economic crop damage based on the climatic data and biological models of development. Based on the potential geographical distribution of pest species, ecological niche model postulated the species that can occur in a particular area with the appropriate survival environment, climatic factors, distribution, and other biological factors. In recent years, a tool referred to as CLIMEX (CLIMatic

IndEX) which could integrate with the application of information management platform, including spatial data, geographic analysis, artificial intelligence applications, data mining, and the development degree-day of organism's model, was widely used. The tool is to detect or predict risk analysis of the potential distributions of the pests and vectors. A stable and excellent risk management model can be established based on this analysis. This study is to explore the climatic effect on the potential distribution of pests and compare the difference of present monitoring system in Taiwan. In order to assess the climate change impact on the distribution of pest or other possible risks of invasive pest. The research focuses on population growth of the Oriental fruit fly (*Bactrocera dorsalis* (Hendel)). Two objects are addressed: (1) Comparison of the impact of climate change on different locations, and (2) Climate stress effects on the geographic distribution of pests. The study attempts to effectively forecast the probability of dispersal of pests. Then, a real-time warning and control measures could take action according to probability of occurrence which will be useful for the farmers to manage pest dynamic.

Keywords: monitoring, geographic information systems, CLIMEX.

INTRODUCTION

With perennial high temperature and humidity in Taiwan Island, the weather impact on crop productions, often because of increased population to plant quality during cultivated period, thus affect the interests of farmers.

As climate change is a global issue, most researchers attempt to use the climate model and climatic data to analyze spatial statistics of geographic information systems (GIS), select the climatic zone of crops or the risk pointers of pests, with the GIS operations and modules, the location of crop suitability and pest risk, collected a large number of pests by GIS layer, and then find out the regularity of its distribution, through the operation of ecological niche model, can draw the potential distribution, and then convert this data into map, can show the potential distribution of pests, effectively predict the probability of spread distribution. And then provide real-time warning and prevention measures, to provide the cultivation and pest management for the farmers. Pest management must be integrated into the whole cultivation management system, and properly set up the monitoring and preventive measures, and to practice the best prevention and control efficacy, and timely take appropriate preventive measures to achieve a multiplier effect.

By using GIS management, in addition to monitor the pest density of the island along with the climate change, and to provide the relevant geographical risk location, it can set an effective time to prevent the outbreak of the pests to achieve the desired effect. Based on the climate of the island, the pests have been able to adapt to the climate change and to the environment of the crops, the biological characteristics of the pests pass on by heredity, continuously, so that these pests each year or season will appear the peak of population, resulting in the occurrence of pests, that caused the production workers the greatest loss. However, most of the pests will re-establish new population by dispersal, seeking refuge or media transmission. If we can handle the traces of pests, often make effective prevention and control of material efficiency greatly, so the pest monitoring work on a considerable proportion, focus on the epidemic situation can make effective use of materials. Through geographic information system technology and digital imaging technology, a pest risk distribution can be provided in detail and with the high risk of location of agricultural environment. With the information technology, the data can be effectively managed to their respective geographical location.

Integrated farmer association's personnel can set up monitoring network, such as for oriental fruit fly, that can regularly provide the farmers early and timely implementation of control measure. Rapidly handling on the spatial distribution and host plant location can be used to assess the risk of pest occurrence and damage, and to improve overall control effectiveness. In this paper, the implementation methods of density monitoring and the application in pest management are described separately.

I. Pest Monitoring System:

Pest management rarely relies on a single control practice, usually a variety of tactics are integrated to maintain pests at the economic threshold levels. The goal of the integrated pest management is not to eliminate all population. However, the aim is to reduce pest populations to less than damaging numbers. The control tactics were used by integrating varieties of pest control that include pest resistant or tolerant plants, and cultural, physical, mechanical, biological, and chemical control. Applying multiple control tactics minimizes the pests' population whether it will adapt to any one tactic dependent on the variety crops and economic cost in Taiwan.

A case study on monitoring of oriental fruit fly, the data collected at each time show the relative location, by inputting density data with the arrangement of colors

representing density variations. It can show the position and color difference of population size from the map. Displaying the color difference from the map, the density of the oriental fruit fly in Taiwan Island and that in neighboring areas can easily be identified. Pest density monitoring is an important work for pest control, because it is helpful to determine the timing of prevention and control of materials, to take appropriate preventive measures and to estimate economic loss. The monitoring system of the oriental fruit fly was established in Taiwan in 1994 (including Kinmen and Penghu). There are at least 61 townships (districts) of cultivated areas, each cultivated area is covered with nine traps by using GPS /GIS. The monitoring information was published as newsletters at the website (http://www.tari.gov.tw/form) on a 10-day interval. It is described as follow:

A. Set up the monitoring trap

(1). **How to choose monitoring site:** Choosing major cultivated fruit and vegetable areas or high risk densities areas, township as a unit, where several traps containing male lure agent were set.

(2). **How long should the time interval be set**: The time interval was determined based on the characteristics of pest life history, manually collected data in three minutes per month, regular implementation, and long-term cumulative data.

(3). **How to collect data information**: Collect the specimens by mail or fax quantitation data, geo-statistical analysis and drawing, display with geographic information system.

(4). **How to deliver the pest information**: Publish newsletter every 10 days as a reference for pest management, and send it by mail or e-mail to BAPHIQ (Bureau of Animal and Plant Health Inspection and Quarantine), Country government, and farmer association and, also the information will be placed on TARI web site (http://www.tari.gov.tw/) and the OPEN DATA Platform, in general, for free download or valuable applications.

B. Decision Making

Based on the long-term monitoring data, geographical location, host plant production, and marketing information, the implementation of fruit flies common prevention strategy can be summed up.

(1) Various kinds of control strategies should be implemented in each period, which can make the limited control materials more effective. The critical period of each phase varies slightly depending on the region, crop and climate.

(2) Geographical location and crop diversity: according to the color of the density, the climate change is used as the partition:

(3) In according to the historical data, the population distribution of fruit flies was higher than that of Changhua, Chayi, Tainan, Kaohsiung and Pingtung [(1)]. Based on nearly 20 years of monitoring data, it showed that the population distribution of fruit flies in high-risk areas. According to the climatic type potential geographical distribution and geographical location analysis, respectively, the different color grade risk presents the distribution as shown in Fig. 1.

(4) Through long-term monitoring of pest dynamics, its seasonal fluctuation is clearly illustrated. If combined with crop growth it can also make aware of the epidemic risk distribution area and with that the control measure can be fully implemented in the epidemic site.

This study analyzes the relationship between the situation in high-risk areas based on the cultivation area of fruit and the population data of fruit flies in the past years, and the evaluation of the pattern of the population dynamics for fruit flies. Through long-term collection of regionals population monitoring data, it can provide effective mastering of fruit fly dynamics in recent years, with the prevention of materials, and the acquisition of a strong preventive measures [(3)].

II. AW-IPM Case study in Taiwan:

The Area Wide-Integrated Pest Management (AW-IPM) control program of the oriental fruit fly (OFF, *Bactroceradorsalis* (Hendel)) was established in 2001 in Taiwan. All available OFF control techniques have been utilized (1) Methyl eugenol bait for male annihilation. (2) Protein bait for female fly control. (3) Bagging of the fruits. (4) Field sanitation. During recent years of the AW-IPM control practice, the OFF population decreased below the economic threshold and fruit production was still in excellent quality with a financial gain for the growers.

The area-wide (AW) control of the oriental fruit fly (*Bactroceradorsalis* (Hendel), OFF) was tested at Chiay in Taiwan. Successful control measure by practice area-wide control were obtained in large acreage districts, such as the 15,000 hectares sugar apple plantation in Chyai, as well as a single bitter gourd orchard with acreage less than one

hectare. Control methods were constructed for both districts and the technology has been transferred to local farmer associations for future implementation [3]. Growers in this area show a common interest in production, hence are highly cooperative in managing the fruit fly population. All available OFF control techniques have been utilized. The study mentioned a well organized OFF density monitoring program was established with some monitoring sites, which covered 500 ha cultivated area. So far, by promoting the AW-IPM control friendly environment that can be easily operated by the farmers. In general the OFF population were suppressed at a low density level within two years, and the population has been reduced to less than 2.0 FTD (fly/trap/day). However, the threat of invasion remains at outside areas. Therefore continuing suppression is necessary in Chung-Pu (Fig. 2). The small acreage model in guava is important since there are thousands of similar production areas like that in Chung-Pu, Taiwan, and field demonstration sessions on area-wide OFF control to other guava growers have been planned to start in the near future.

Another demo site in Fenglu. The control program had been started since 2012. We set up 18 monitoring sites to take an action to suppress the population densities to low density level based on the information collected. Through 6-year continuous controlling program, we achieved the outcome that led the population control to a limited area, especially the hot spot area in Fenglu (Fig. 3).

III. The climate change of CLIMEX

Climate change is one of the main factors affecting the geographical distribution and population of biological species. By estimating the geographical distribution and quantity of known species to estimate the climatic conditions required its occurrence, or by directly using biological data of species growth and development, adding climatic parameters and validating the predicted results with known distributions to adjust the climatic parameters of the selection. A stable and excellent risk management model can be established based on this analysis. This study is a basis to explore the climatic effect on the potential distribution of organisms and is compared with present surveys in Taiwan. In order to assess the climate change impact on the distribution of pest or other possible risks of invasive pest, this research focuses on population growth of the Oriental fruit fly. Two objects are addressed: (1) Comparison of climate change impact on different location. (2) Climate stress affects the geographic distribution of pests. To improve the accuracy of the system in predicting the potential geographical distribution

of the species. Based on the conceptual design of the CLIMEX [2, 4], the potential geographical distribution and relative abundance dynamics of the species are predicted by analyzing the climatic conditions in the areas where they have occurred. Early in Australia it was used to predict the potential geographical distribution of exotic plants and agricultural pests in order to manage such species. It has since been used all over the world to analyze the distribution of suitable living areas for agricultural diseases, weeds, and other arthropods.

The theoretical basis of the model of potential geographical distribution of species is based on the ecological niche model, which assumes that when a species appears in a region, the region must have suitable conditions for survival, such as climatic factors, crop distribution and other factors. The biological climate model for predicting species is based on two basic assumptions: (1) the species passes through 2 periods within one years, that is, a period suitable for population growth and unsuitable for survival. This implication is the biological species' birth and death phenomenon (2) climate is the main factor affecting species distribution, using a series of parameters to describe species' different responses to climate, stress index (Stress Indices, SI, cold, hot, dry and wet), as well as the relationship of factors to the survival of population in extreme climatic conditions.

The climatic parameters required to describe the species are mainly composed of three components: Growth index (GI), Stress index (SI), and other limiting conditions. The growth index includes the most appropriate temperature, humidity range and other suitable environment for growth and reproduction, and the adversity index includes climatic factors that limit the extreme temperature and humidity of species survival, including the parameters of other joint effects, such as diapause and effective accumulated temperature, based on these 3 parameters, The species Eco-climate index (EI) is used to synthesize the most appropriate climatic conditions and suitable habitats for species. The brief description is as follows:

1. **Growth index (growth index)**

The growth indices can be divided into weekly growth indices (weekly growth Index, GI_W) and annual growth indices according to the time scale (annual growth Index, GIa), the annual growth index is calculated on the basis of the weekly growth index, in which the growth indices of species are related to temperature, humidity

and illumination, taking into account the diapause of insects and the effects of plant (dormancy) on growth.

Diapause is an adaptive performance of species to extreme environments, low temperature or insufficient plant sunshine (such as winter diapause), or too long sunshine and too high temperature can induce diapause (such as summer diapause). Diapause index indicates the effect of diapause on the growth and development of species, and if the species were found as diapause, the diapause index DI_w=0; otherwise, the weekly Diapause index DI_w=1. In Taiwan, diapause is uncommon in agricultural pests.

2. **Stress Index (Stress index)**

There are 4 commonly used adversity indices: cold stress (CS), thermal stress index (Heat Stress, HS), wet stress index (Wet Stress, WS), dry stress index (Dry Stress, DS). It means that if any one of the indicators of adversity is greater than the extreme environment, the species cannot survive.

3. **Interaction (interaction) Stress index**:

Refers to the interaction between climatic factors, thermal - Wet stress index (Hot-wet Stress, HWS), thermal - Dry Stress index (hot-dry Stress, HDS), cold - Wet stress index (Cold-wet Stress, CWS), cold - Dry Adversity index (cold-dry Stress, CES). For example, the thermal - Wet stress index is used to indicate the effects of poor environment on the growth and development of species, the temperature range of species growing from the base development temperature (DV0)), the appropriate temperature lower threshold , the upper threshold to the highest development temperature (DV3). The humidity range from the minimum humidity (SM0) required for development to the highest growth humidity (SM3), exceeding these scope of adverse environmental conditions can lead to negative population growth (negative population growth).

The indices of Growth, Stress and interaction (interaction) were integrated into the eco-climatic Index, and the range of values 0~100, when the value is close to 0, it indicates that the species is unsuitable, and that the environmental conditions of the region are ideal. The smaller the EI value, the less suitable the region is for species to survive; the greater the EI value, the more suitable the region is for the species to

survive. Normally, when EI is greater than ten, species can survive, and EI is greater than 100, indicating that the area is ideal for species to survive.

In this study, the influence of climate change on the geographical distribution of important pests is studied, and the influence of climatic factors on the potential geographical distribution of pests in Taiwan region is CLIMEX by using the biological climate model, which is consistent with the existing survey geographic data. This can be used to assess the impact of climate on the distribution of such pests or the risk of other possible invasive pests. The Oriental Fruit fly is the target pest, making its potential geographical distribution in the world, exploring in Asia the production and comparison of the growth index and the biological climate index of oriental fruit flies, as illustrated in the following illustration:

A. **Climate change impacts on the potential distribution of pests in different regions and eras:** With yellow (EI<10) suitable for survival to red (EI>60) the most suitable for survival of the regional changes, from three years of climate change in the context of Asian and Southeast Asia in the Oriental fruit flies, the potential geographical distribution of the impact of a greater. It is not obvious to the Taiwan region that the insect is affected by the climate **(Fig. 4).**

B. **Restriction conditions on the geographical distribution of pests**: Extreme temperature or high rainfall will have a significant impact on the geographical distribution of the population. In addition to the central mountains, the probability of distribution is small, and the other oriental fruit flies are suitable for survival. Compared with the geographical distribution of the Oriental fruit flies, which were established in the field for a long time, the situation in the south-central region was almost suitable, and other areas could be adjusted or reinforced by other factors, even the difference in latitude was not obvious. According to potential geographical distribution of fruit flies evaluated by the biological climate model, and it was found that the western region and the east had a very suitable trend for the survival of fruit flies obviously illustrated in the blue and yellow areas.

C. **The EI value of geographical area is estimated by CLIMEX**: The climate is suitable for growth and development, and food available supply is abundant that leads to difficult control of pest damage and outbreaks are the most likely outcome. If the extreme climate affects the growth of crops that leads to poor food quality that

in turn make the agricultural pests need to overcome adverse climatic conditions resulted in an unstable population growth for the pests. In order to analyze the distribution of harmful bio-adaptive growth regions, if combined with the distribution data of host pant, while using various methods to interpret the data, it can be closer to real situation, which will be helpful to the early warning decision of the hot spot of the pest epidemic situation in the future. As climate factors influence crop growth, it also affects the growth of agricultural pests, and the relationship between pest and host crops, which has achieved the spread and control of agricultural pests.

D. **Climate spatial information integration**: a large amount of domestic climate data (Central Weather Bureau, https://www.cwb.gov.tw/) and downloaded global climate change scenario data (CLIMond, https://www.climond.org) are available. Due to the general geo-spatial interpolation mode (such as IDW, Kriging) often fails to fully reflect the effects of factors such as the terrain environment on climate, so it is necessary to grid the spatial data into the climate format developed by using PRISM model, interface with OPEN platform from the Central Weather Bureau. The content of the data provided the identification of the data set project and content, and released by XML data analysis of archives for the climatic data is spatially measured, and the daily real-time climatic data of the weather station in the whole Taiwan region are gridded spatial interpolation by using GIS to obtain the climatic grid data. After that, the data of pest grids site are interchangeable with interface properties, which can be combined with meteorological disaster protection early warning and land cover layer, as the relevant database of land use in Taiwan. Combined with platform is to estimate the degree day of the pest. The purpose is to explore the impact of climate change on pest or food security and risk assessment generation, the potential geographical distribution of pests in Taiwan was established by CLIMEX ecological niche model, which could be used as an assessment of potential geo-spatial distribution of invasive pests or natural enemies, and the basis for negotiation or policy of quarantine units had been provided.

Future prospects

A. According to the regular pest monitoring situation, the annual fluctuation data are provided regularly

(1) To assist in providing local pest outbreaks and distribution for the farmers' associations, township district offices and cooperative sites to. To give advice on the dynamic information and prevention of pest in the island, and to promote the epidemic and response in the event of production and marketing organization.

(2) To provide and activate fallow production area, carry on the notification mechanism of important pest epidemic information and the implementation of plough or pest management data.

(3) To establish the mapping database for the density and spatial distribution of important pests in the whole Taiwan, accumulated from long-term data has been built much more data of major pest density changes in the past years. Regional pest field monitoring data, which provides research subjects for long-term species ecological survey or species diversity. Pest density monitoring data, with the interface to provide complete land cover, climate disaster and pest risk assessment, to promote the protective operation for farmer.

(4) To arrange the best decision making and control calendar formulation. To finish the outcome, we must investigate the dynamics of pests in the field and analyze the growth and decline of different regions in different periods as a density index for the preventive measures. The means of the level of population can be taken as follow:

(a) It should be no need to take preventive (do nothing);

(b) It should be adopted by prevention and control strategy, according to the degree of prevention, suppression, and eradication.

(c) It should use chemical control: the formulation of rational use of pesticide strategy, the use of different types of pesticides with different mechanisms of the agent, to strengthen the effectiveness of prevention and resistance production.

(d) It should be done with control calendar dependent on the population fluctuation.

(e) It should practice the integrated pest management.

(f) It should implement the area-wide control (island-wide control).

CONCLUSION

Getting rapid information can help us to understand and solve many ecological problems such as insects. Due to the rapid development of network applications, the information system will be more intelligent on the network. In future, the system will have announces for growers to practice early pest management action. It will reduce pest outbreaks and the economics losses. Integration of the consultation information platform to connect pest diagnosis system including pest risk early warning query, pest generation change query, pest monitoring data query, climate data query, will be mainly to provide farmers, researchers. So they can quickly master the distribution of pest spatial and hosts, so as to assess the risk of pest occurrence and damage, and improve the overall control efficiency. Meanwhile the information transmission, the relevant units in time to grasp the latest outbreak to take effective means of prevention and treatment can be achieved. The system not only compares the implementing efficiency of each control area to promote the confidence of farmers, it can also objectively assess the fairness, efficiency and maneuverability of the distribution of materials.

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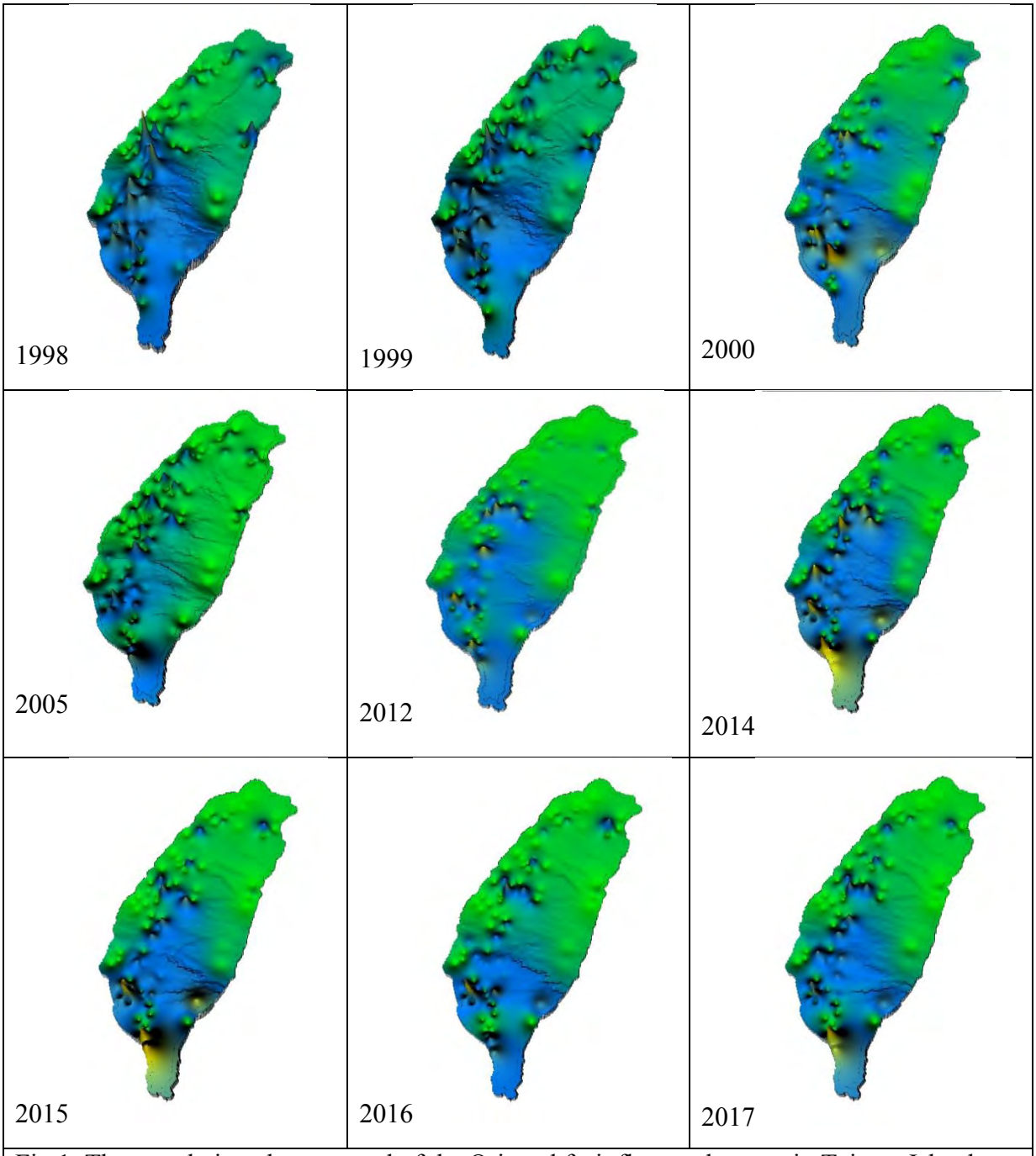


Fig.1. The population change trend of the Oriental fruit fly year by year in Taiwan Island.

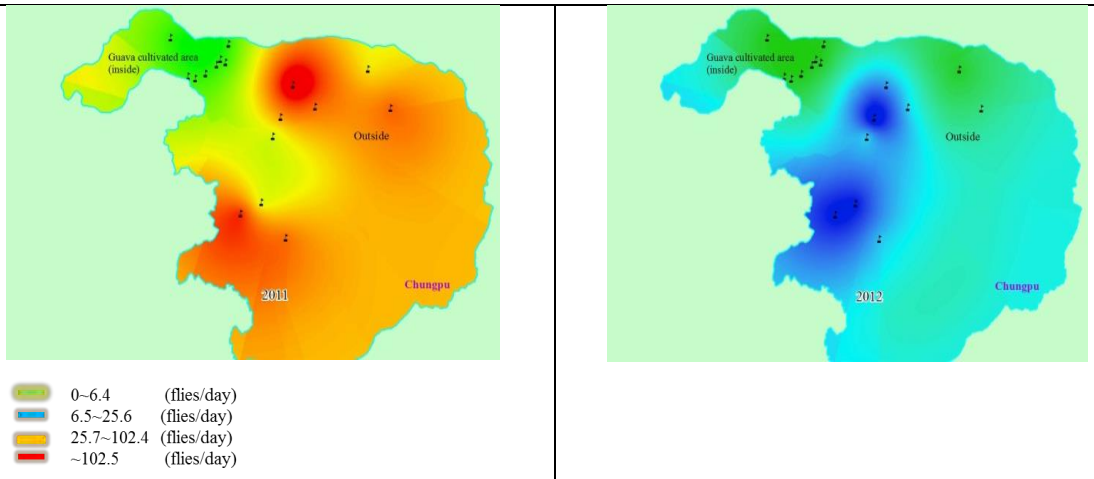


Fig. 2. The population change trend of the Oriental fruit fly at ChungPu from 2011 to 2012.

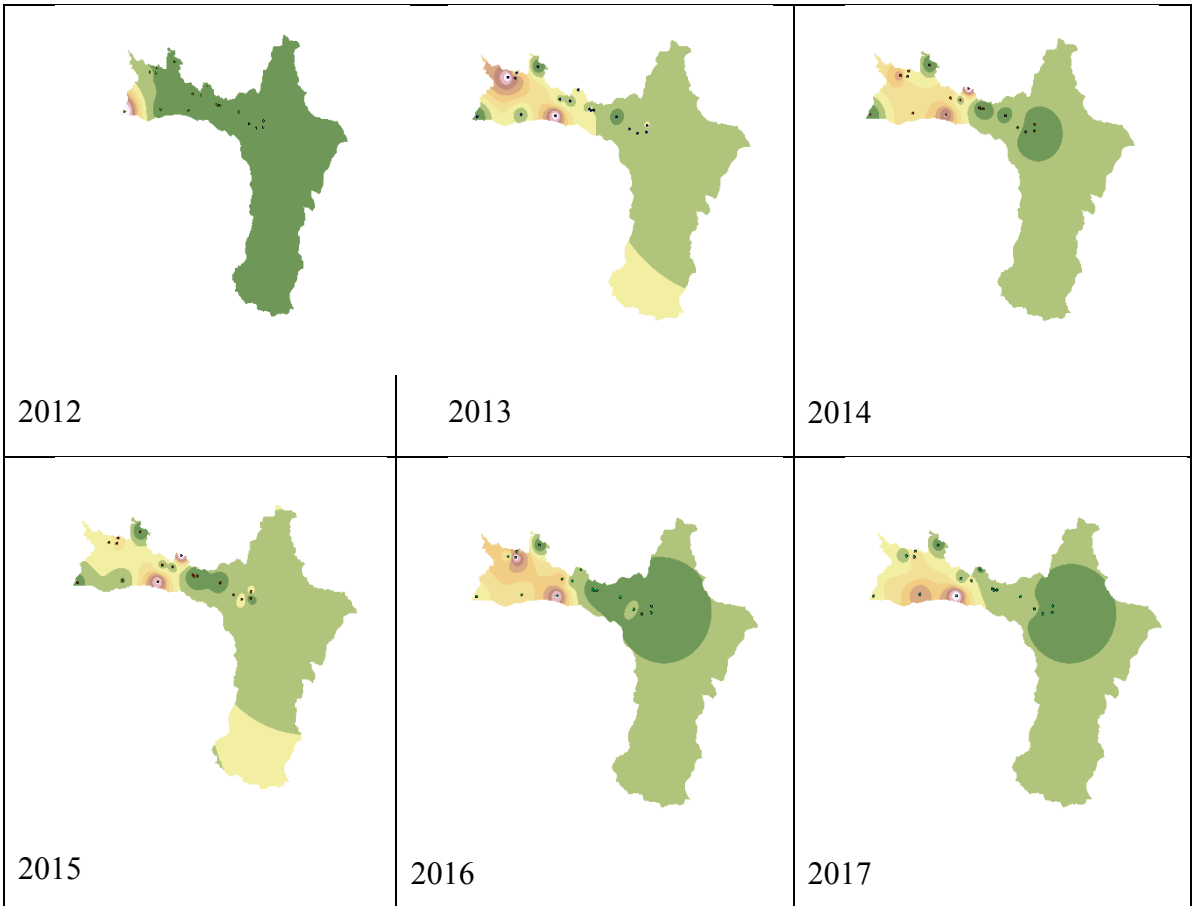


Fig 3. The population change trend of the Oriental fruit fly at FengLu from 2012 to 2017.

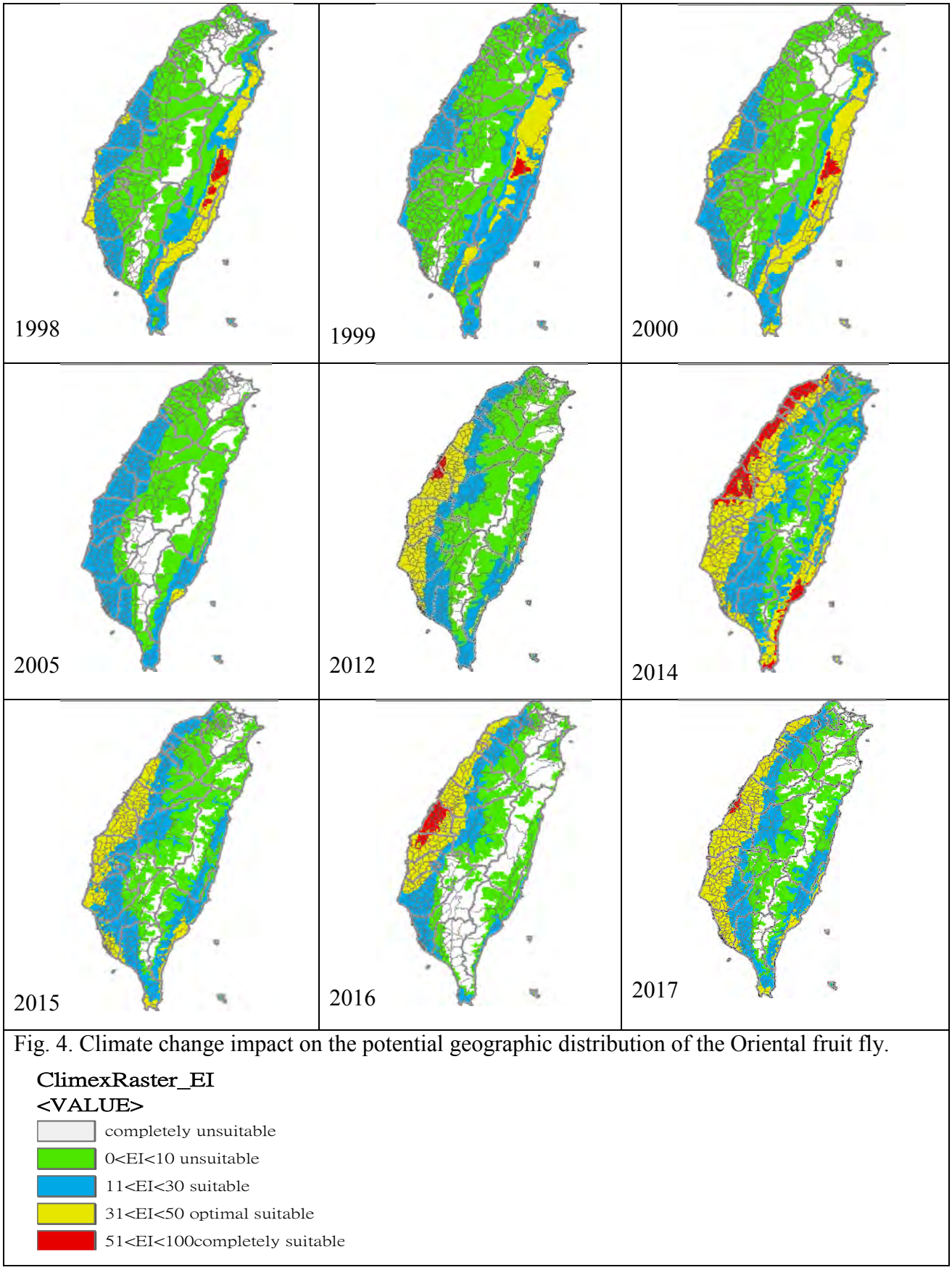


Fig. 4. Climate change impact on the potential geographic distribution of the Oriental fruit fly.

Pest distribution model comparisons in integrated management of key crop pests: empirical studies of Taiwan and Southeastern Asia

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ABSTRACT

Wide ranges of pests affect crop production and cause considerable yield losses. Several conceptual modelling frameworks have been proposed, and the choice of an approach depends largely on the objective of the model and the availability of data. Existing challenges on the modelling of insects are listed. Difficulties reside on the development and adaptation of adequate strategies to predict insect pests for their management to ensure enhanced agricultural production. This paper presents a summary of advances in insect population distribution and pest mapping around Asia. Following the step-wise modelling comparisons innovations, we evaluate the distributions of *Trichogramma ostriniae* and native host, *Ostrinia furnacalis* in southeastern Asia, and target host, *Ostrinia nubilalis* in North America, using a combination of MAXENT and CLIMEX modelling approaches. These correlative and process-based niche models led to similar predictions about the expected distribution of the two species in Asia, and enabled the emphasis of likely mismatches between the host and its natural enemy. Records of successful inundative releases involving the introduction of *T. ostriniae* in North America were modelled and used to adjust parameters in the CLIMEX model. Releases took place outside in the suitable climate areas identified from the native range of this species, suggesting that permanent wasp populations might only establish based on changes in temperature-related life history parameters.

Keywords: biocontrol, model comparison, environmental niche model, adaptation

INTRODUCTION

Global warming caused by the over-exploitation of petroleum and tropical rainforest seemed to get worse and worse in recent decades with the clear evidence of increasing frequency and intensity of the extreme climate events. The extreme weather-related events increase globally, as predicted by the latest IPCC report [(1)], such as massive floods in Pakistan (2010) and Thailand (2012); the ongoing crippling droughts across sub-Saharan Africa, Amazonian Brazil (2005 and 2010) and in the central United States; record heat waves, as in the United States (2012), Russia (2010), western Europe (2003) which took tens of thousands of human lives.

The natural and agricultural ecosystems and the species in them will have to adapt to these changes, which are probably unprecedented in recent evolutionary history. Parmesan and Yohe [(2)] predicted that climate change would be a major factor contributing to a significant increase in extinction rates of all species. Since agricultural productivity depends on a wide array of biotic interaction involving mutualists (e.g. pollinators, natural enemies) and antagonists (e.g. herbivores and pathogens), thus studies exploring the effects of extreme weather related events on multitrophic interactions will shed more light on the consequences of these events on primary production in cropping systems and on interactions with these important mutualists and antagonists [(3)]. Researchers even claim that extreme climate change will facilitate the coextinction rates of endangered species, parasites, and mutualists [(4)], which in turn can affect the production of crop simultaneously. For example, pollination and biocontrol using natural enemies on crops largely depend upon its interactions between different trophic levels that are highly vulnerable to extreme climate events, which may break down the trophic network and lead to food shortages in modern society.

Population interactions between pests and their natural enemies are inevitably complex, involving many components among diverse scales. To reveal issues that are related to the impacts of extreme climate on the interaction level, the most crucial but difficult approach is to build up a long-term and large-scale database that enables further comparisons and analysis focusing on certain species or time series. However, given the variability in how studies are conducted and the often-sparse sampling durations, it is almost impossible to reveal the general pattern and impacts simply by comparing and analyzing [(5)]. While the influence of climate on range boundaries may be mediated through species interactions, species distribution models (SDMs) driven

by bioclimatic data are increasingly being applied to deal with the large scale and long-term issue with distribution shift and population dynamics.

There are two prevalent approaches in modeling the risk establishment and expansion of an insect pest. The "inductive" approach uses pattern-matching functions that chase down the potential exploitation of a non-indigenous invasive species to new areas by comparing the long-term meteorological data for each selected location where the species is absent to the location of origin or locations where the species prevails [6, 7]. To apply this approach, computer-aided tools, such as CLIMEX and MAXENT, have been developed and used to predict species' demography for pest management and potential climate change effects [6]. It uses minimal data sets and simple functions to describe the species' response to temperature and other climatic factors and shows advantages where detailed life cycle parameters for the species are limited; however, it failed to consider the biological characteristics in the modeling framework, hence, could only provide the potential establishment risk maps of the targeted species but not the further information about its population dynamics within a growing season or year in a given region [8].

By contrast, the "deductive" approaches adopt process-based climatic response model for targeting pest species (phenology model): the main advantage is that the rule they use are based upon causation, not correlation. This kind of model packages, e.g., Dymex and Insect Life Cycle Modeling (ILCYM), are based on detailed laboratory assessment for deriving life-table parameters and simulated population dynamics according to long-term temperature data for a given region and time [9]. The development of insects, as in other ectothermic organisms, depends on the ambient temperature. This temperature dependency can be applied in a process-oriented framework; forecasting the potential distribution of insect species is totally independent of observed occurrence, and this approach is therefore referred to as "deductive" [8]. Degree-day (DD) models, employing linear models using accumulation of temperature above the minimum temperature threshold [10] have been established to describe the development of different insect pests. However, due to the non-linearity of the development curve, especially deviation from the intrinsic optimal temperature of the species, these models become poor predictors of insect development. DD models work well for intermediate temperatures, but produce errors when daily temperature fluctuates to extremes [11]. For better evaluation, more sophisticated and progressive

models were developed by using non-linear models of higher biological significance [12], and by including stochastic function for variability in development times within a population among individuals [13].

Simulation models were further developed as a powerful means for representing the dynamic of organisms responding to a series of external factors. Dymex package enables the user to deal with a class of ecological models referred to as mechanistic or process-based models, without the need to be familiar with a computer programming language [14]. ILCYM software calculates the life table population parameters based on within-the-day temperature variability and use these to evaluate the potential effect of certain pest management strategies. Moreover, ILCYM includes a sub-module for the development of a two-species interaction model for linking the parasitoid and its host phenology models through a deterministic simulation procedure under constant and fluctuating temperature conditions. This process uses the predicted temperature-dependent development times and parasitoid fertility rates for simulations of the host population growth and development [8]. To make predictions of species distributions precisely, different types of niche models are increasingly used both in biodiversity conservation and in applied ecology at the same time [15, 16]. The choice of the model type is typically determined by the type of data that is available for the species.

CASE STUDY

The Asian corn borer (ACB), Ostrinia furnacalis Guenée (1854), is a serious pest of corn causing more than 9 million tons of yield loss per year. The geographic range of *O. furnacalis* extends from eastern and southeastern Asia to western Pacific islands [17]. The egg parasitoid Trichogramma ostriniae Pang & Chen (1974), the predominant species, parasitizes *O. furnacalis* across the native range of most corn growing regions of southeastern Asia [18, 19]. In China, using *Trichogramma osatriniae* for biocontrol of Asian corn borer has been conducted since early 1950s. Because of its success in China, T. ostriniae has been introduced to control the closely related European corn borer Ostrinia nubilalis Hübner (1917) in northeastern USA (New York, Massachusetts, Delaware and other locations) and Canada (southern Quebec) since the early 1990s [20, 21]. Early inoculative releases showed promising results, with up to 80% of parasitism achieved in cornfields at those locations [20]. These novel range data offer an

opportunity to test how well our modeled responses trained by native range can capture them.

By using a correlative ENM approach in conjunction with the semi-mechanistic approach (CLIMEX), we aim to identify suitable areas for effective inundative releases of T. ostriniae. The specific aims of the current study are: (1) using complementary modelling methods, to reveal the efficacy of T. ostriniae and ACB in current climates and (2) to understand the climate suitability for T. ostriniae in North America for control of European corn borer.

Distribution data

Occurrence locality data were obtained from published references [22] (n = 111 for Trichogramma ostriniae, n = 149 for Ostrinia furnacalis), field surveys in 2014 and 2015 (n = 9 for T. ostriniae, n = 28 for O. furnacalis) and Global Biodiversity Information Facility (GBIF) database (*n* = 1091 for O. nubilalis) (Fig. 1). The listed North American distribution of T. ostriniae was not used in model training, rather we adopted them to test the validity of our modeled responses, because all these data were obtained from successful inundative releases in biological control programs, and it is not clear if permanent populations have established at these release points [23].

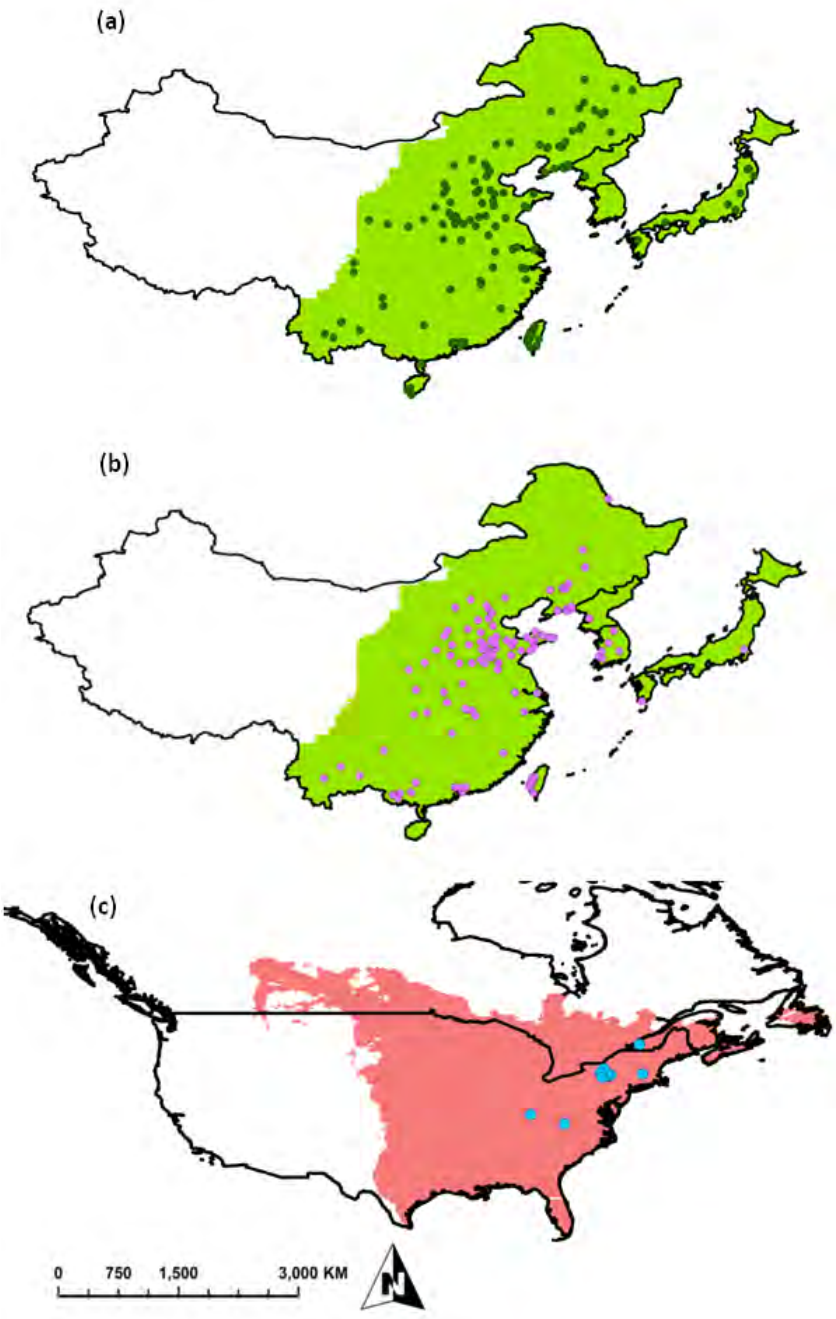


Figure 1 Native distributions of (a) Ostrinia furnacalis and (b) Trichogramma ostriniae. Sources are published references and field surveys were performed in 2014 and 2015 in China, Japan, Korea and Taiwan. (c) Inoculative releases locations of T. ostriniae undertaken in North America were obtained from published references. Colored areas represents backgrounds used in Environmental Niche Model (ENMs) construction based on the modified Köppen-Geiger classifications (Green = Asia-trained model; Pink = America-trained model) [(22)].

Environmental data and model projections

We obtained environmental data from CliMond [24], data formats suitable for both correlative ENMs and CLIMEX models. For ENM model construction, CliMond provides 35 bioclimatic variables describing means, seasonality and trends for temperature, precipitation, solar radiation and soil moisture. We used a grid cell resolution of 10′, which is approximately 20 x 20 km at the equator. Model details were indicated in Wu *et al.* (2018) [22].

To explore relative abundance in distribution under climate change for T. ostriniae and O. furnacalis in southeastern Asia, we adopted the CSIRO Mk. 3.0 models (available from CliMond, [24]) for the current time. American occurrence data surveyed in the current study were all inundative release points, so we only adopted response curves of the North American model to adjust our CLIMEX parameters to modify projections of North American data (Table 1).

Table 1 CLIMEX parameter values for the final model for *Trichogramma ostriniae* determined by iteratively changing parameter values until the performance best fit known native distributions (see Fig. 1)

Index	Parameter	Value[a]	
		T. ostriniae (Asia)	*T. ostriniae* (adjusted for America)
Temperature	DV0 = Lower threshold	13 °C	10 °C
	DV1 = Lower optimum temperature	20 °C	15 °C
	DV2 = Upper optimum temperature	29 °C	25 °C
	DV3 = Upper threshold	35 °C	30 °C
Moisture	SM0 = Lower soil moisture threshold	0.15	
	SM1 = Lower optimum soil moisture	0.35	
	SM2 = Upper optimum soil moisture	0.80	

	SM3 = Upper soil moisture threshold	1.50	
Cold stress	TTCS = Cold stress Temperature threshold	1 °C	0.5 °C
	THCS = Cold Stress accumulation rate	-0.00013 week^{-1}	-0.00005 week^{-1}
Heat stress	TTHS = Heat stress Temperature threshold	35 °C	
	THHS = Heat Stress accumulation rate	0.02 week^{-1}	
Dry stress	SMDS = Dry stress threshold	0.1	
	HDS = Dry stress rate	-0.02 week^{-1}	
Wet stress	SMWS = Wet stress threshold	1.5	
	HWS = Wet stress rate		

Adjusted values are underlined. [a]Values without units are dimensionless proportions.

Distribution comparison

Subtracting the output of *T. ostriniae* from *O. furnacalis* highlights areas likely to be particularly suitable for conducting *T. ostriniae* releases (Figs 2a & 2b). These areas were mostly distributed across Hebei, Shandon, Henan, Anhui and Shananxi provinces of China, and Korea. The maps of preference difference present only relative values for suitable habitat, and these would need to be refined through empirical studies.

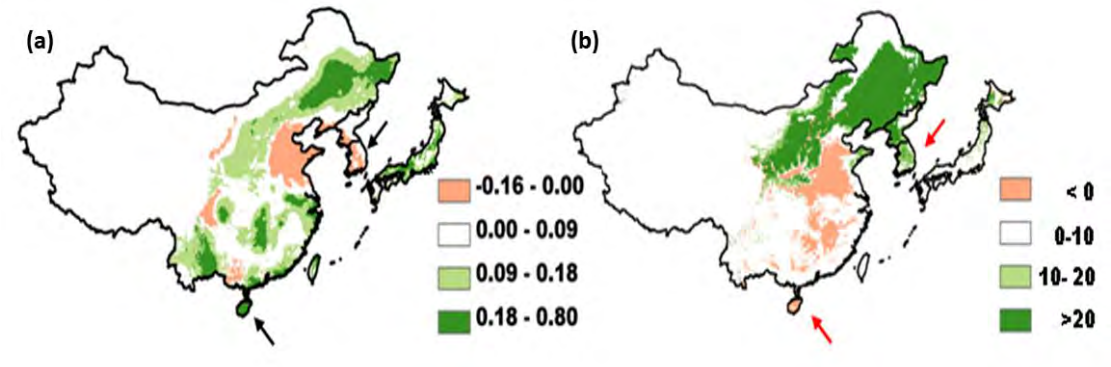


Figure 2 Distribution fitting models between *Ostrinia furnacalis* and *Trichogramma ostriniae* built on CliMond data sets. (a) *T. ostriniae* logistic MAXENT output subtracted from *O. furnacalis* logistic MAXENT output. Pink areas indicate where

more suitable climate space was predicted in the *T. ostriniae* model than the *O. furnacalis* one; (b) *T. ostriniae* CLIMEX output subtracted from *O. furnacalis* CLIMEX output. Pink areas indicate where more suitable climate space was predicted in the *T. ostriniae* model than the *O. furnacalis* one. Green areas indicate where more suitable climate space was predicted in the *O. furnacalis* model than for *T. ostriniae*. Arrows indicate different fitting performances (black: better; red: worse) between MAXENT and CLIMEX models.

Model projections

The variance of climate variables between Asia and North America also lowered the performance of CLIMEX projections (AUC = 0.404, Fig. 3a). To deal with the constraints, response curves of the America-trained MAXENT models were adopted to modify CLIMEX parameters and acquire a new North American projection (Table 1) and massively improved the power of the latest model ($AUC_{adjusted}$ = 0.772, Fig. 3b) to predict where inundative releases occurred.

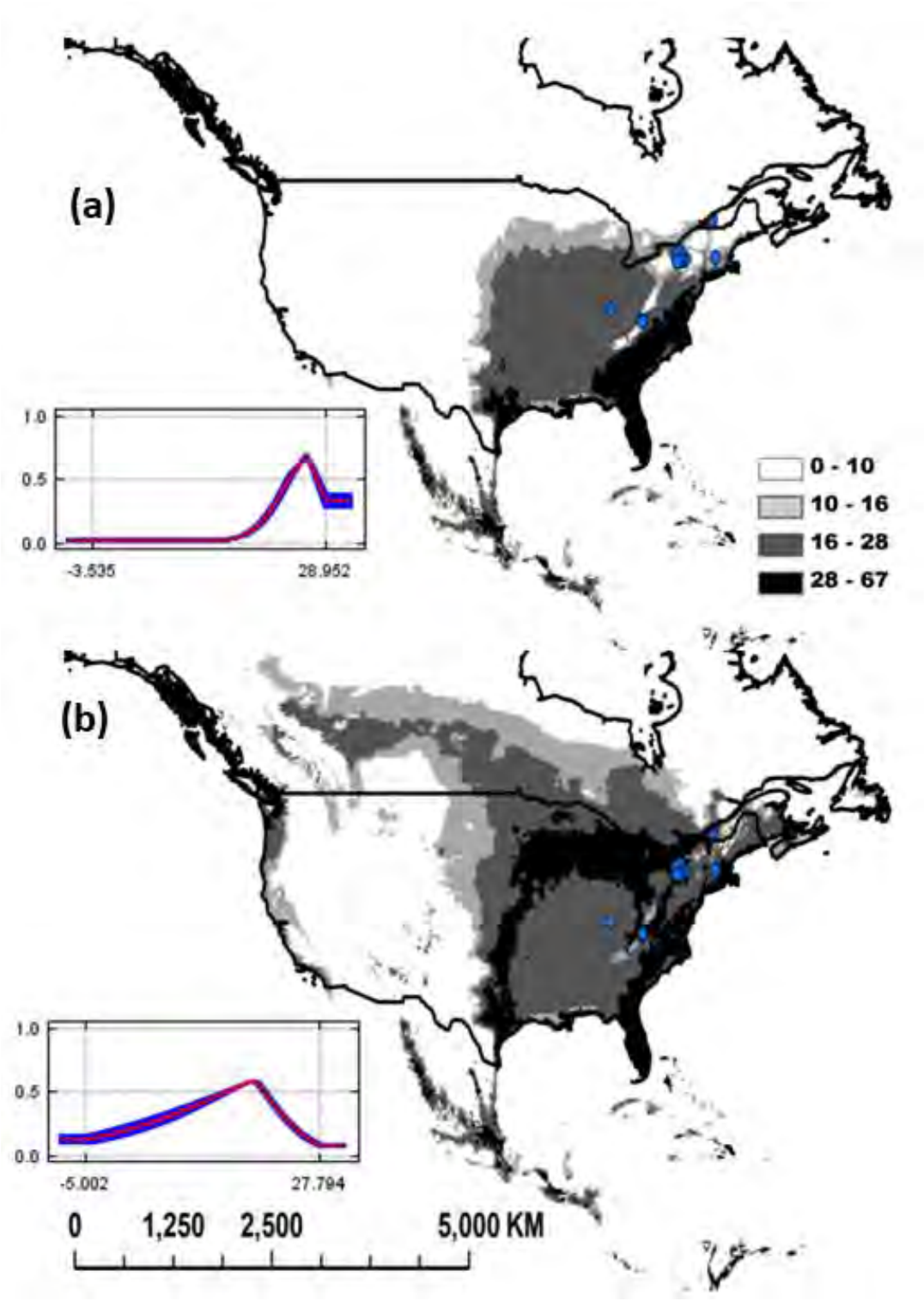


Figure 3 (a) 'original' and (b) 'adjusted' projections (lowered temperature index and cold stress parameters based on response curves of mean temperature of wettest quarter listed). Shading reflects Ecoclimatic Index (EI). Projections for North America are Miroc-H A1B SRES projections.

DISCUSSION

The projection of the Asia-trained model on the North American areas highlights the potential limit of MAXENT (inherent issues of extrapolation) [25]. These projections could be integrated and could supply adjustments for parameter tuning in the CLIMEX model. To sum up, the inundative occurrence data and CLIMEX projections suggested that T. ostriniae presented different temperature-related life history parameters (lowered cold threshold and cold stress accumulation rate). Such differences in life history traits may reflect the fact that T. ostriniae populations have not become permanently established. However evolutionary changes among T. ostriniae populations might also provide an explanation. Wang et al. [23] have previously suggested that individuals from North American populations grow faster than Chinese populations (9 days at 24°C for America; 10 to 11 days at 25°C for China). These possibilities remain to be tested.

It is worth highlighting some limitations in our SDMs fitting process. Distributional data for both T. ostriniae and O. furnacalis were limited due to recent survey efforts being restricted and a lack of precise locality data associated with most published records [26, 27]. Meta-analyses have suggested for competing natural enemy species that antagonism may result in effects on the target being less than additive, and this deleterious outcome is most obvious when the same life stage of a host is attacked [28]. Our model approaches overlook the influence of other Trichogramma species present across the predicted distributions. Therefore, any inference regarding areas suitable for T. ostriniae ought to be made with caution and requires empirical studies that include molecular identification of potential competing Trichogramma species given by the cryptic nature of species in this group [19].

In conclusion, two different modelling comparisons provided insights into factors controlling the distribution of a pest host and a parasitoid that is important for its control. In predicting inundative release areas of T. ostriniae in a novel area, we had to change parameters and this led to an alternative hypothesis. Areas where successful parasitism might be expected were identified, but additional data are required to determine whether T. ostriniae may have become established in its novel range outside these areas, which might reflect adaptation to local climate conditions.

ACKNOWLEDGMENTS

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Introduction of a model simulation program and its application in a demonstrative expert system to predict optimal spraying time for pest: PopModel 1.5 and Pest Forecast System

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ABSTRACT

Three major frequently asked questions from farmers are about identification of the pest, an optimal pesticide spraying schedule, and the most effective pesticide for the targeted pest. Various protection calendars for each major crop have been provided. However, climate changes and the innovative development in the information techniques (IT_s) are uprising and making those protection calendars useless. Recent ITs expand in various field with a name of techniques, e.g., Bid data, Iot, AI, etc in order to automate machine, link information between machines, and help human's decision-making. However, the prediction of the pest occurrence and its damage in a crop is not easily done with these new techniques because the prediction is the new frontier that those techniques are employed to approach and overcome. More than three decades have been passed since the theoretical base of the pest model and algorithm for computing process on it were initiated. There is still no usable pest forecast system available for the growers mainly due to the lack of the techniques that can be easily simulated. I used a new concept for creating models with own calculation system and those are incorporated into a simulation program, Popmodel 1.5., with the simulation engine of PopModel 1.5. An expert system for managing pests are demonstrated with a program system called the Pest Forecast System. The Techniques in PopModel 1.5 and the visualized example of the Pest Forecast System will help open a new era in agriculture.

Keywords: model, simulation, protection, PopModel 1.5, pest forecast system.

INTRODUCTION

For thirteen years working in a local agricultural institute of a branch of government of Korea, I was often asked by farmers on a crop pest with the following three frequently asked questions (FAQs) which could be abbreviated into three words: What, When, and How. 'What' represents the identification of the pest. Most farmers have no interest in the scientific classification of the pest but whether the pest is controllable or not. 'When' refers to the optimal spraying time for effectively controlling one or several pests that occur at the same time. 'How' is usually about an effective control method. Farmers usually asked which pesticide is effective to control the pest and wanted the commercial name of pesticide sold in market. This preference on the commercial name may be one reason why a farmer continues to apply the same pesticide successively or frequently in a crop. They were confident with this particular one because it did not cause the pest's resistance problem based on their experience of using various pesticides. Protection calendars illustrating pests and their occurrence and damage season, a spraying schedule of the pesticides were provided and updated annually by a related agricultural station of the government and pesticide companies. However, such FAQs continued to emerge because the spraying schedules are different from farmer's cultivation methods and locations whereas the pest's occurrence and damage are dependent on the local climate and surrounding environment. Additionally, the identification of a pest and the selection of an effective pesticide for a pest(s) are not easily solved without the help of an expert.

Recently the greenhouse gases produced by human activity accelerate global warming (IPCC, 2014). The climate change causes immigration, accelerating occurrence time and shortening pests' overwintering period. Incursion of new pest, out-breaks of the pest, and abundant increase of the native pests will result in the economic loss in crops by destroying the pest management system based on a protection calendar. The information techniques (ITs) based on computer techniques have been developed and are expanding to various field with these new commercial brand names, e. g., Big-Data, IoT (Internet of Things), A.I. (Artificial Intelligence), etc. This kind of technique will help solve the identification of pest, provide recommendation of an optimal protection method, release farmers from labors, and so on by substituting the protection calendar. Therefore, automated machines and a convenient, reliable, and predictable

management system will be the best solution for answering those FAQs as well as coping with the dynamic situation caused by the climate change.

Prediction is a representative field and direction that the new ITs try to pursue and achieve. The effective protection period for the pest (i.e., spraying time) cannot be precisely predicted using the past collected and accumulated information in a database. For example, recent weather forecast has a high accuracy in weather prediction that was not achieved by using previously collected climate data in local weather station but by using simulation of various models. That is, the effective protection time for a pest or a group of pests that can be predicted by simulating the occurrence of the pests' models. Almost three decades have passed since Wagner et al. (1985) developed the programmatic simulation method for the insect model and Curry and Feldman (1987) founded a theory for the insect model. However, there have been a few articles validating their models through a simulation process, even though they are still not practical for farm usage as of today. The simple method to predict the insect occurrence is a Degree-Days-based model. Degree-Days-models have been widely used in disease, insect, and crop models because it is easy to build with a linear equation (Y=aX+b) and simple to apply. However, it couldn't tell when pests occur, but the peak time, so that all efforts failed to develop a sound decision-making system. While the combination of a developmental rate model (reviewed in Wagner et al., 1984a) and a developmental complete distribution model models (reviewed in Wagner et al., 1984b) build by a non-linear equation showed more reliable prediction capability, but this kind of models is hard to be used in practice without a help of a programmed program. These problems are basically caused from the lack of an appropriate program tool or a flat-form system to simulate the various models. Precisely predicable models are so complicated for being used in practice. They are composed of many stages or steps with a complicated linkage and various factors that affect the model. Furthermore, even a model for the same species, the model structure, content, and simulation method can be different from a model developer, the purpose of model, and application way. Although several commercial simulation programs (ie. STELLA (https://www.iseesystems.com), SIMILE (http://www.simulistics.com/), DYMEX (http://climatemodel.net/dymex.htm)) have been used in building and simulating models for crop, disease, and insect, The above-mentioned programs have a limitation in building such a complicated model so that the model built with those programs are mostly suitable for a study in a laboratory

level. There are many published papers for models but most models have no simulation of the model or have only low-grade simulation. Consequently, it is natural that models developed in the past have not been applied or failed in a farm level. In order to realize a future pest management system, techniques for building and simulating various kinds of models in insect, disease, and crop should be developed in advance.

With the goal of creating an omnipotent simulator that can build simply and simulate easily and fast in mind, various kinds models, a new simulation program was developed with the flat-forms, STAGE MODEL and POPULATION MODEL, for models with unique calculation system. PopModel1.5 is a latest version of the simulation program. This simulation engine is incorporated into a demonstrative expert system, Pest Forecast System (PFS),that can visualize a pest occurrence and the damage it causes in a crop and recommend some effective pesticides for the pest as well.

PopModel1.5

1. Program architecture

We used C/C++ to develop the system on function calculation, data management, and model simulation. In order to provide a user-friend interface, Windows API was used in coding PopModel1.5 and PMFList. PMFList is an add-on program to PopModel1.5 and provides a convenient environment for user to create, check, and manage functions that defined freely by user (Fig. 1). User can build and simulate freely the various models in the provided templates, STAGE MODLE and POPULATION MODEL with PopModel1.5 (Fig. 2). The STAGE MODEL is the unique and reliable template to contain any stage-type models that users think and is simulated reliably as standardized simulation (Fig. 2C). The POPULATION MODEL template is for a group of stages, which also shows a comprehensible structure of stages and their simulation procedure (Fig. 3). The data for forms of PopModel 1.5 is designed as a simple structure to be easily used in model simulation for the user's subjective usage. The user's data and the simulation outputs were displayed in a window and also transferred easily to Excel program and vice versa (Fig. 4).

Substantial times of tests were conducted by ensuring that the calculation codes and the simulation procedures in both templates, STAGE MODEL and POPULATION MODEL are error-free.

2. Calculation system

The data used in model simulation are a series of values. Thus, the values used in calculation are delivered or provided by other processes or code procedures, which causes difficulty in programing models and often results in a low efficiency and unexpected errors in calculation. However, our calculation system is based on the array concept. That is, each function can be calculated with the position information on "start", "now", and "end" in an array range and the position information can be used directly in the calculation of a function by using array variables, I, N, and T, respectively (Fig. 5). Therefore, user can access and use any valid position of the data. Also, our calculation system provides such various variables designated as P, X, R, C, Y, S, G for user's convenience. P variables are for the parameters used in a function (Fig. 1C). X and R variables are for user's data (Fig. 2B, 3C). For example, in a temperature-dependent

model, temperatures are used as the input for an X variable in the function. However, other data like density can be used directly or indirectly in that function. So, we provide R variable for those data. C variables are for the unit models composing a stage model because stage models are usually composed of several functions. So, the result of other functions or the formula of the function itself can be used in the simulation of a stage model. Those complicated process are easily done in our calculation system by using C variable (Fig. 2C). Y variable represents a formula and can be used as a variable indicating the output of the formula in a stage model (Fig. 2C). Our stage model can produce multi-outputs by one time simulation of the STAGE MODEL. S variables represent a stage that user sets to a stage in the population model and G variables are used for producing initial data of the stage model in the opulation model.

Our calculation system also provides such various defined functions as mathematical functions, condition functions, array functions for simulating various formulae and models.

3. STAGE MODEL

The stage model is so constructed to compensate different researchers' subjective or abstractive concept on the content and structure. For example, the number of functions and data in a stage model used among researchers are different and the simulation method/process can therefore be changed as for their practical application. In order to solve those problems, we figured out the stage model template to contain various models and to provide a confident simulation method (Fig. 2C). User can load functions in [COMPONENT FUNCTION] in the STAGE MODEL template and makes a procedure formula in [MAIN FUNCTION] using C variables indicating functions in [COMPONENT FUNCTION]. X or R variables used in [MAIN FUNCTION] indicate the data to be loaded in '[INPUT] X or R data' before simulation. The strong point of this stage model is that the original form of the functions loaded in [COMPONENT FUNCTION] are used without modifications. So, user can simulate any stage model with various data in a slight modification, if needed, of the formulae in a part of [MAIN FUNCTION], which helps comprehend model structure and process and also provide compatibility of the models between the model builder and the model user.

We provide three types of simulation method, CALCULATOR, ARRAY, and TRANSITION in [CAL. METHOD], which are a kind of simulation process and related

with the [CAL. RANGE] and [CAL. CONDITION] in the STAGE MODEL template (Fig. 2C). Because our calculation system is based on the array concept, the formulae in [CAL. CONDITION] and [MAIN FUNCTION] and each function in [COMPONENT FUNCTION] are calculated with the array information of "start", "now", and "end". User can also use the array information with the array variables, I, N, and T, respectively. CALCULATOR method produces the results according to the information in [CAL. RANGE]. In ARRAY method, the formulae in [MAIN FUNCTION] are calculated from the "start" to "end" position indicated in [CAL. RANGE] and produce results corresponding to the position in the array (Fig. 5A). [CAL. CONDITION] has an important role in ARRAY and TRANSITION (Fig. 5B, C). In case of the ARRAY with a starting condition, the formula in the 'Start if =' in the in [CAL. CONDITION] is calculated from the start to end indicated at [CAL. RANGE], and then the position value of I to be used in the calculation of the formulae in [MAIN FUNCTION] is set to the position where the result of the formula in 'Start if=' is true (=1) at first, thereafter the formulae in [MAIN FUNCTION] are calculated from the position of I to end producing results (Fig. 5B). TRANSITION method is an expanded form of ARRAY with 'Start if='. The simulation procedure of ARRAY with 'Start if=' is repeated whenever the result of the formula in 'Start if=' is true (= 1) for all the range indicated in [CAL. RANGE]. Finally, all the produced outputs from the formula in [MAIN FUNCTION] are summed according to their position in the array (Fig. 5C). That is, TRANSITION method is useful to simulate for the multi-cohort simulation.

Our stage model has a remarkable characteristic to produce more than one result by one time simulation. For example, stage emergence model produces only the output of the daily number of the group that completes their stage and go to the next stage. In order to know the remaining number of the group in the previous stage, we have to spend times to make a model and simulate it again. In PopModel1.5, user can easily get one or more results by adding one or more formula in [MAIN FUNCTION].

4. POPULATION MODEL

The POPULATION MODEL template of PopModel1.5 is designed for the group of stages with a various linkage and simulation procedure (Fig. 4A). Stages are simulated in the easy and comprehensible order from top to bottom and from left to right following the arrow from the start stage determined by user. Users do not need to

make model again in order to change the starting stage but only move the indication mark of the start stage to other stage that users want to start at first. Also users determine which stage should be re-entered, if needed, by setting a Loop arrow and determine the number of re-entrance. Popmodel1.5 visualized automatically the simulation order of stages from the start stage with arrows. Therefore, any users comprehend easily the whole progress in the simulation of any population models. In a STAGE MODEL in the POPULATION MODEL template, users can use other outputs in the other stage by using S variables after being set to the stage. S0 is a variable reserved for the initial data in each stage and can be used, if it is necessary to simulate the stage model at the first simulation (Fig. 4D). The simulation procedure of the stage model in population is a little difference from the stage model simulation. As well as the data loaded X, R, and S variable itself, a new data produced through a calculation with those data in the simulation of a stage. So, we provide G variable that represents the data produced by calculating the formula described in 'G var' in the STAGE MODEL template. Therefore, the data for G variable was produced at first and then STAGE MODEL is simulated as the procedure mentioned in STAGE MODEL section according to the information that user inputs.

POP ULATION TEMPLATE provides three sections: PRERUN, [MAIN], POSTRUN. PRERUN is for a section when user needs to produce data that is necessary for the [MAIN]. POSTRUN is for a section where user arranges the output that user really needs from the previous sections, PRERUN and [MAIN].

Pest Forecast System

1. Program architecture

The Pest Forecast System comprises the following two programs, PFSmanger and PFSviewer. They are programed with C/C++ and Windows API. PFSmanager is designed for a manager in the level of a Server where all the basic information and models for the crop, insect, disease, and agrochemical could be managed and updated. PFSviewer is a client program for a farmer who can get the predicted an optimal spraying time and best pesticide with the information that farmer selected the crop in and location of a farm. The meteorological date required for simulation of the prediction models for the pest is designed automatically accumulated by a program, CMDBmanager, equipped in the Pest Forecast System. This system is a demonstration program for developing an expert system to be used in agriculture in future.

2. Meteorological data

The CMDBmanger in the Pest Forecast System is designed to start checking for new weather data and collecting them in its own Database whenever the models of the pest that should be simulated according to the setting information by farmer. The CMDB collects the daily weather information of the location by visiting the website of the Korean meteorological administration (KMA, http://www.kma.go.kr/). In this program, the collected weather factors are low temperature, high temperature, average temperature, humidity, precipitation, amount of snowfall, and wind. The normal weather data provided by the KMA are also collected as well as the daily data. The Collected data are accumulated in its own database. In order to predict a future, data like a pest occurrence and crop growth needs basically a future weather information, which is provided by filling the normal weather data after the last accumulated weather data in the database. With this process, the weather data for simulating the pest occurrence is automatically provided in PFSviewer.

3. PFSmanager

Most role of PFSmanager is to manage the five databases named as ‘Refer DB’, ‘Climate DB’, ‘AgroChem DB’, ‘Crop Pestlist’, and ‘Model DB’ (Fig. 8A). The content of each DB is visualized in the right window as for the option (Fig. 8B, 8C). ‘Refer DB’

is for the classification of the crop, disease, insect, and agro-chemicals, which is a basic information used commonly in 'AgroChem DB', 'Crop Pestlist', and 'Model DB'. Even in the same pest, the status and the importance for the control are different among crops. The database for 'Crop Pestlist' is a basic information used for providing the optimal spraying time and recommending the best pesticide. The database for 'Agrochem DB' is for the legal agro-chemicals. This information is basically used in recommending the best pesticide, too. The function of recommending pesticides for the pest in a crop is provided in the option of 'Agrochem DB'. The 'Model DB' is for the model for insect, disease, agro-chemical, and crop, but only insect model is working in this version. This version of the Pest Forecast System is a program for a demonstration to visualize what kinds of Database and information are required and how they are to be managed at least.

4. PFSviewer

PFSveiwer is a client program for a farmer. This kind of program should be simple to use and deliver useful and compacted information to a farmer with a comprehensible form. Farmer only needs to set the location of a farm and the crop in the farm (Fig. 9A). The predicted level of the occurrence and damage of the pest in a crop is visualized with blue and red line, respectively in a year (Fig. 9C). This program provides a diary to record the history of the applied pesticide information in 'Agrochem DB' (Fig. 9D). Those applied information is visualized in Fig. 9C with the pest occurrence and damage information as related to the date with an effective level of a pesticide to each pest. Predicted spraying time and the recommended pesticide is shown in Fig. E. PFSviewer is a demonstration program to show what kinds of information should be delivered for a farmer, how the information are to be visualized.

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PopModel1.5 is an upgraded version from PopModel1.0 and the copyrights were reserved for Choi, Kyung San and Kim, Dong-Soon in 2017 (C-2017-003991). The copyrights of CMDBmanager is reserved by RDA (C-2017-012553) and that of Pest Forecast System is reserved by RDA, Jeju national university, Andong national university, and GyeongSang national university (C-2017-030630). All the above-mentioned programs were developed by Choi, Kyung San. RDA owns the right on two patents (10-1578421 and 10-1589235) for the STAGE MODEL and its calculation system of PopModel1.5 and a Korean company, Jaemi, got an exclusive license by December of 2017.

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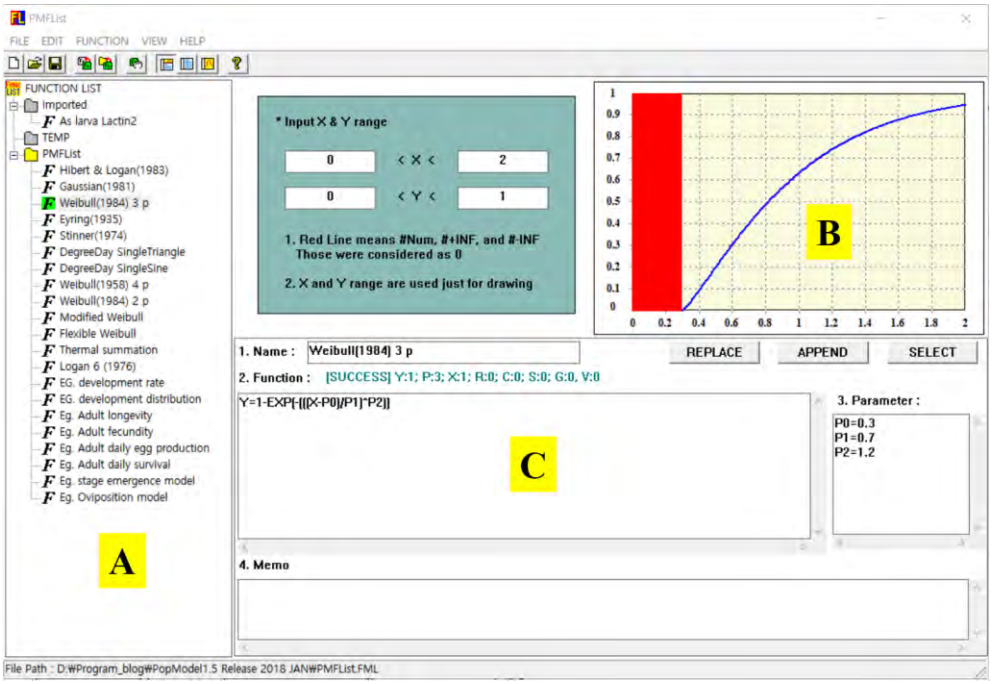


Fig. 1. Screen of the PMFList program. (A) Files containing the functions are displayed, (B) The figure of function selected by user, and (C) Function information is displayed and can be edited by the user.

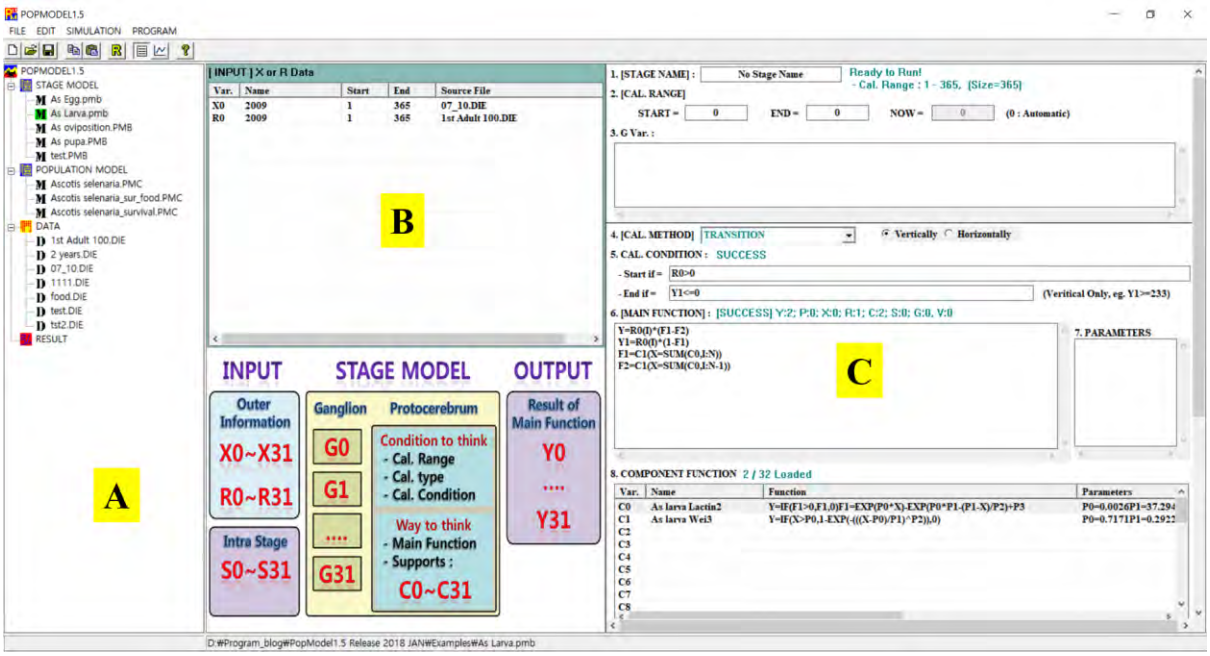


Fig. 2. Stage model. Screen of PopModel1.5 program. (A) Files used in PopModel program, (B) Data loaded on X or R variable are used in the simulation of the stage model, and (C) The information of a stage model.

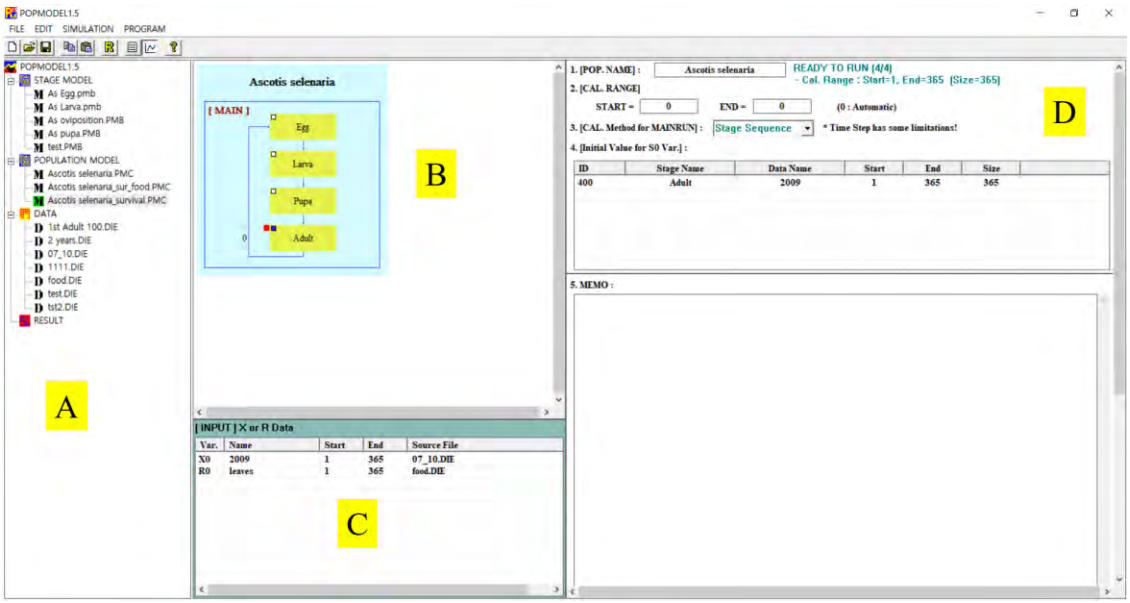


Fig. 3. Population model. Screen of PopModel1.5 program. (A) Files used in PopModel program, (B) GUI Builder for a population model with stages and their linkage, (C) Data loaded on X or R variable are used in the simulation of the population model, and (D) the setting window for the population model.

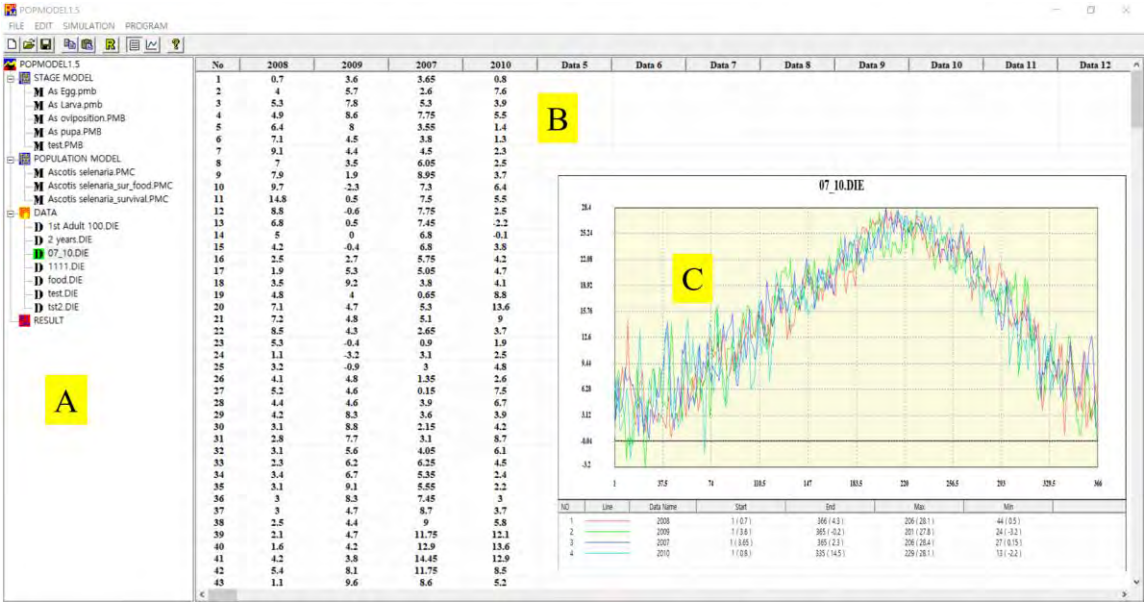


Fig. 4. Data Screen of PopModel1.5 program. (A) Files used in PopModel program, (B) and (C) Data in a file are displayed in text mode or graph mode, respectively.

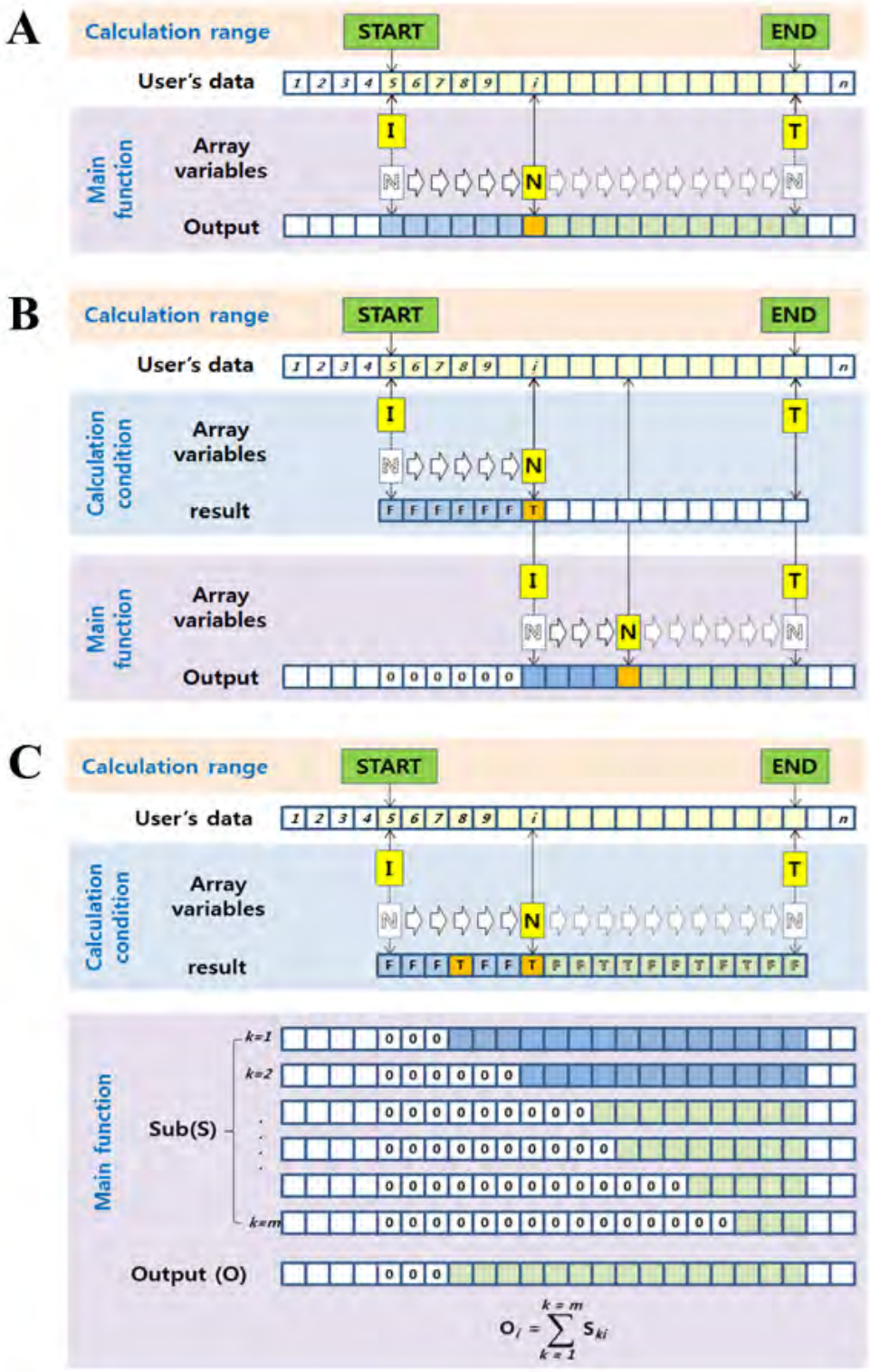


Fig. 5. Scheme of calculation method of the stage model in PopModel1.5 program. (A) Array, (B) Array with a conditioned start, and (C) Transition.

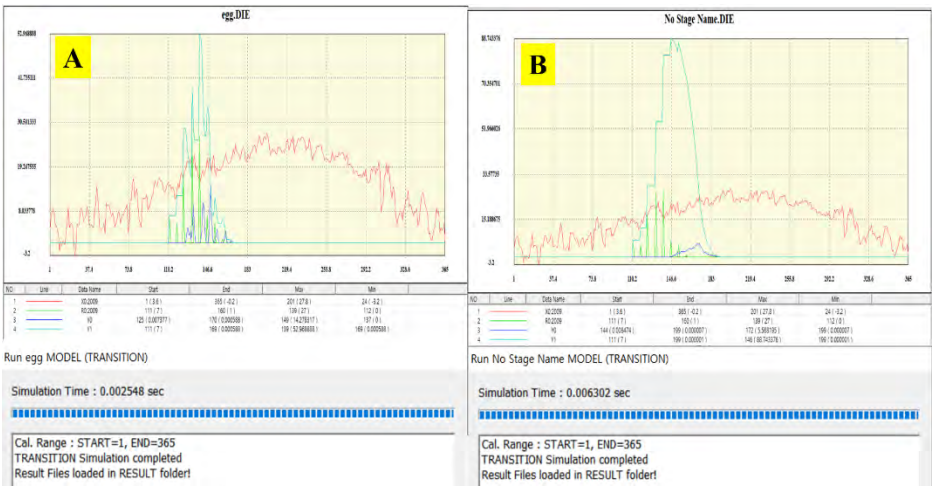


Fig. 6. Outputs of the stage models simulated with PopModel1.5 program according to the daily temperature data (red line). (A) The occurrence of the hatched larva (blue line) and the remaining egg population (light blue line) were predicted from the egg laid data (green line) with the egg emergence stage model (B) The pupal occurrence (blue line) and the remaining larval population (light blue line) were predicted from the predicted hatched larva data (green line) with the larval emergence stage model. Stage emergence models of *Ascotis selenaria* and their simulation method were described in Choi and Kim (2014).

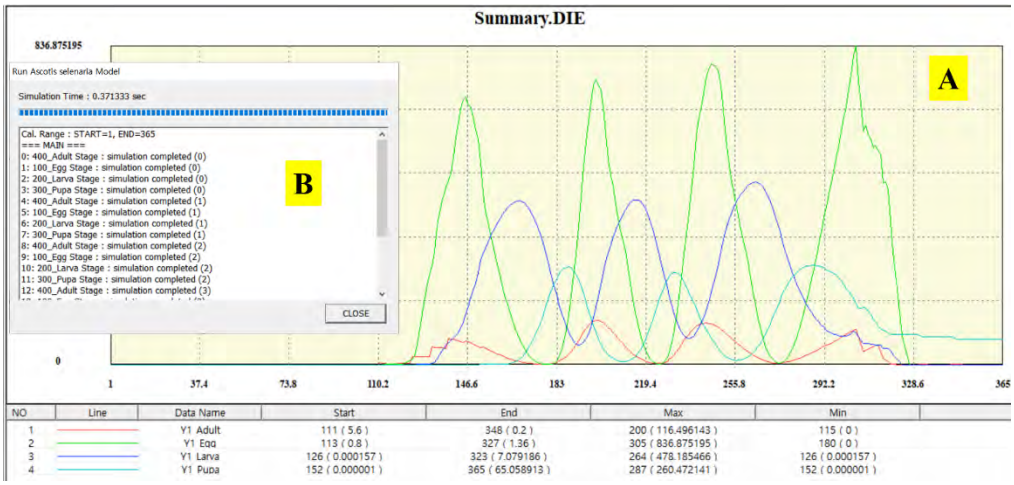


Fig. 7. Outputs of the population model of *Ascotis selenaria* simulated with PopModel1.5 program. (A) 1st to 4th occurrence of the generation at each stage; egg (red line), larva (blue line), pupa (light blue line), and adult (green line) (B) Simulation progress window for the population model. Stage emergence models and adult's oviposition model of *Ascotis selenaria* and their simulation method were described in Choi and Kim (2014, 2016).

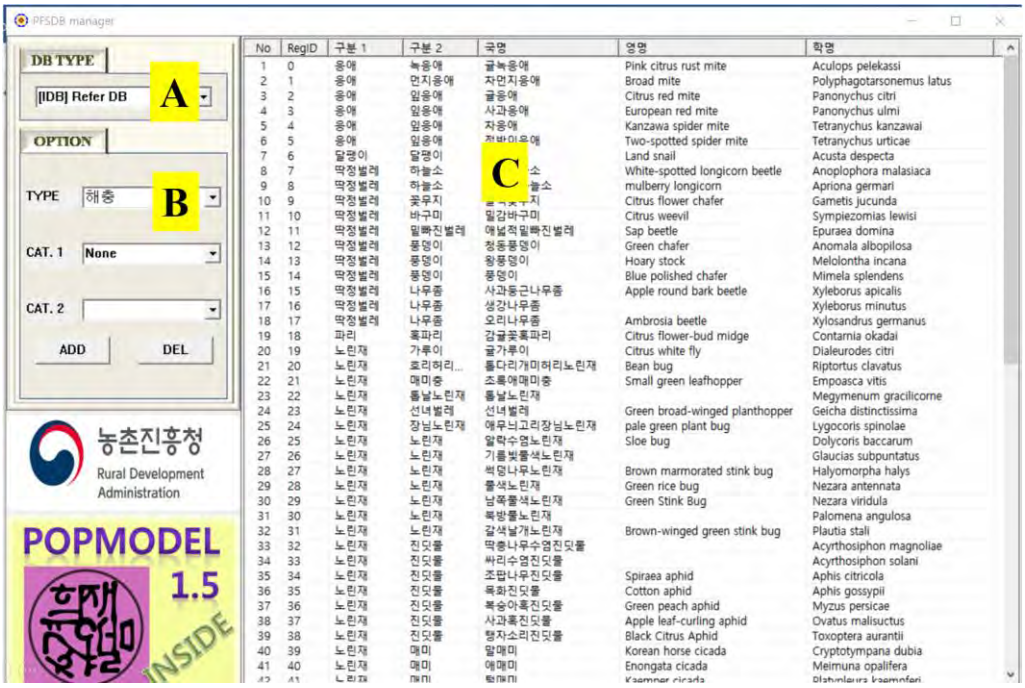


Fig. 8. Screen of PFSmanager of the Pest Forecast System. (A) Database types used in PFSmanager, (B) Options for each database, and (C) Content of the database.

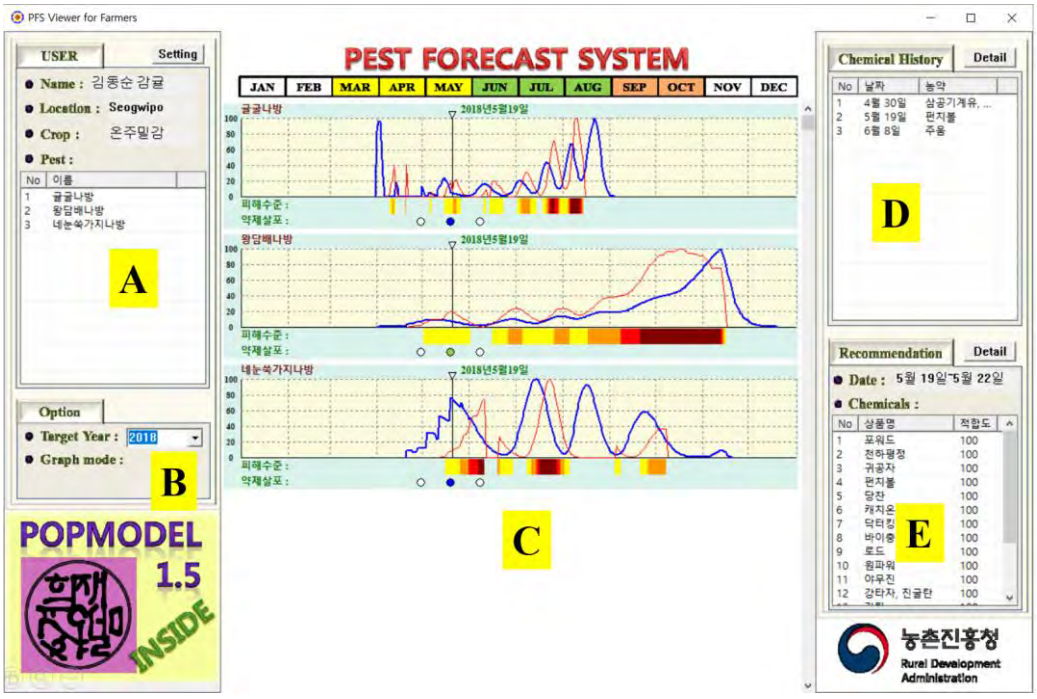


Fig. 9. Screen of PFSviewer of the Pest Forecast System. (A) Setting information of a farmer, (B) Year to be simulated, (C) Visualize the pest occurrence, spraying time, and the effectiveness of pesticide at the sprayed time the selected year, (D) History of applied chemical information at a crop in a location by the farmer, and (E) Optimal spraying period to be controlled and the recommended pesticide with their profitability to the pest to be controlled.

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