出國報告〈出國類別：出席國際會議〉

出席第7屆國際植物醫學論壇

服務機關：國立嘉義大學
姓名職稱：郭章信 副教授
派赴國家：日本(東京)
出國期間：105年12月1日
至105年12月5日
報告日期：105年12月22日

摘　要

本次會議介紹2005年以來日本植物醫學會之發展與演進，從團隊組成，編撰教材，再經由媒體宣傳以及國際合作，對於學生及人員的訓練非常務實。直至目前為止，日本通過國家考試之植物保護士已有九十二人，如未來達百人以上時，將建議日本政府更名為植物醫師。會中除了介紹尼泊爾、韓國植物醫師之工作及研究外，也討論未來以自動人工智慧機器人來協助病蟲害診斷的可能性，將人力資源能轉移到其他更有價值的地方。日本植物醫學更有一套診斷的標準作業流程與病害預測系統的管理系統。借鏡日本在植物醫學發展成果，期能對台灣植物醫師的教育訓練、人才培育及未來發展有所幫助。此外，日本基因公司也發展了許多病害之快速檢測項目或許也能成為台灣的產業出路之一。會議期間並與各國學者經由交換經驗與心得分享，共同討論而獲取許多寶貴資訊。

目　次

壹、計畫起源與目的

第七屆國際植物醫學論壇參與人員由日本、台灣、韓國、馬來西亞、尼泊爾、越南等亞洲地區植物醫學發展成熟的國家共同的推動植物醫生培訓。本次會議的目的是透過論壇活動來交流各國家最新的臨床植物醫學主題相關訊息，並建立六國之間的聯絡管道，且將擴大到亞洲國家之間，促進各國家臨床植物科學活動。參與國家地理位置相近，了解相關臨床植物醫學情況與討論其前景是非常重要的，希望能藉由此活動提升各國之交流。並參訪東京大學昆蟲多樣性學群、植物醫學群及植物醫院，進行學術交流與討論，期能對本系課程教學及未來研究將有所幫助。

貳、參訪行程

台灣日期	日本日期	活動行程
12/01(四)	12/0 1 (四)	台北(桃園國際機場)14:30→18:30東京成田機場
12/02(五)	12/02(五)	參訪東京大學植物醫學群及植物醫院
12/03(六)	12/03(六)	參加第六屆國際植物醫學論壇
12/04(日)	12/04(日)	參訪東京大學昆蟲多樣性學群
12/05(一)	12/05(一)	東京成田機場17:55→21:30台北(桃園國際機場)

本會議在臨床植物醫學與展望、病害診斷、蟲害鑑定以及防治與管理研討議題如下：

1.10 years of Clinical Plant Science in Japan

2.Development of the sustainable agriculture system for the pest and disease management in Korea.

3.dentification and characterization of *Pseudomonas syringae* pv. Syringae isolated from imported pear scions.

4.Identification of new emerging orchid-infecting viruses.

5.Development of LAMP kit for Rice Stripe Virus detection.

6.Bogia Coconut Syndrome, a devastating phytoplasma disease in Northern papua New Guines.

7.Should the pear psylla, *Cacopsylla pyricola* (Foerster) really occur in East Asia？(Homoptera:Psyllidae)

8.Combination of siagnosis methods for major fruit diseases in Korea: application to a field survey.

9. Plant Clinic Programs in Nepal.

10.Tree protection and brown root rot disease control in the National Taiwan University campus.
11.Organophosphate-based traps reduce control efficiency of OP-resistant tephritid flies.
12.The first private Plant Clinic in Japan: Integration of scientific knowledge and IoT sensor data technology.)

本次議題中，認為有幾項研究值得注意，可以提供我們很好的參考訊息，也對於目前植物病蟲害之診斷防治、檢疫病原微生物及其他作物病害研究有更深層的了解，概述如下:

一、近10年的日本臨床植物醫學

日本法政大學植物醫學系濱本教授，介紹2005年以來日本植物醫學會之發展與演進，從團隊組成，編撰教材，再經由媒體宣傳以及國際合作，學生及人員的確實訓練，直至目前為止，日本通過國家考試之植物保護士已有九十二人，如未來達百人以上，將更名為植物醫師，且會中也討論未來以自動人工智慧機器人來協助病蟲害診斷的可能性，將人力資源能轉移到其他更有價值的地方。

二、開發韓國病蟲害管理之永續農業系統

韓國慶北大學農業與生命科學系李教授報告韓國最近朝向低毒農業(GAP)發展，但因為現存農業低毒病蟲害防治資材不足，所以開發了新的防治資材來提供農民使用，包括使用覆蓋作物草生栽培來改善土壤狀況如菊花、苦木。利用苦楝油灌注土壤防治銀葉粉蝨，且防治率可達60.3%，產量也獲得提升。還有使用矽藻土作防治資材，除可驅蟲外，也可與其它各種有機農產病蟲害防治資材混合使用，且矽藻無毒、懸浮性能好、吸附性能強、容重輕，混合均勻性 好，在土壤中能夠保溫、疏鬆土質、延長藥效、肥效時間、改良土壤，助長農作物生長效果。其它防治資材還有魚尼丁、蜂膠、卵磷脂、天然酪蛋白醋發酵產品、天然蘑菇植物提取物、菸葉提取物（不含純尼古丁），並且利用寄生蜂，捕食性性蟎類作為天敵防治。

三、尼泊爾植物診所計劃。

尼泊爾目前植物醫生有170人，於公共場所使用簡單的設施進行的社區的諮詢服務，每個場所一位植物保護專家和技術輔導隊伍進行訪視，實地現勘觀察與鑑定植物病蟲害，建議農民進行適當的植物保護措施。

四、日本第一家私人植物診所：科學知識和物聯網整合之傳感器數據。

日本ベジタリア株式会社建立病害預測的新技術，www.vegetalia.co.jp提

供多種病害監測與管理，為日本第一家的私人植物醫院，其病蟲害診斷SOP為診斷→訓練教育資訊科技→信息。會中並報告水稻病害監測管理系統BLASTAM。

本會議張貼海報主題如下：

1.Taxonomic revision of the jumping plant-louse genus *Eotrioza* Konovalova (Homoptera: Psylloidea: Triozidae)(參見附件四)

2.Risk Assessment of Genetically Engineered Rice Bt-9, Resistant to Cnaphalocrocis medinalis: Influence on Above-Ground Arthropods in Korea. (參見附件五)

3.First complete genome sequence of an apricot isolate of cherry virus A. (參見附件六)

4.Host preference and the application of using orange jasmine as a trap crop for Diaphorina citri (Himiptera: Liviidae) (參見附件七)

5.Investigation on the Incidence Condition of Ptaya Wet Rot Caused by Gilbertella persicaria after Postharvest. (參見附件八)

6.Pathogen-identification and industrial application Of poinsettia witches' broom. (參見附件九)

其中有幾項主題病原之研究論文值得注意，心得如下：

一、採收後紅龍果濕腐病*Gilbertella persicaria*發病情況的調查

紅龍果濕腐病可危害花器、幼果及成熟期果實。採後果實自果梗切處向果肉方向呈現水浸狀腐爛，2~3 天後果實完全呈現水浸狀腐爛，以手輕觸果皮或果實鱗片易剝落，濕度高時，果皮上出現灰色黴狀物，剖開果實可見果肉呈水浸狀腐爛，食用時有異味。田間發生時自果實頂部或傷口處蔓延，幼果或花器受東方果實蠅、瓜實蠅為害時，加重病害蔓延。以連續降雨期間受害最嚴重，造成落花及落果。將紅龍果進行去除雄蕊及花瓣，配合清除落花及落果對紅龍果濕腐病之預防效果，白肉紅龍果在去花蕊及花瓣配合清園處理，第9天後幼果濕腐病罹病率為4.7%，與對照處理7.5%呈顯著性差異，去花蕊及花瓣後於果實採收期第9天後之罹病率為 2.5%，也與對照處理4.8%呈顯著性差異。紅肉紅龍果去花蕊及花瓣後9天(清園)處理及(無清園)處理，幼果罹濕腐病，其罹病率分別為8.8、12.3%，對照無處理為21.3%，呈顯著性差異。果實採收期之濕腐病發病情形，去花蕊及花瓣(清園)處理，其罹病率為6.8%，與對照無處理12.8%呈顯著性差異。有無去花蕊及花瓣，在紅肉及白肉紅龍果幼果或成熟果皆以上層果罹病率最低。下層果罹病率最高，罹病率皆超過6

成。於室內篩選得克利、免得爛、波爾多及枯草桿菌等4種藥劑，可完全抑制紅龍果濕腐病病原菌絲之生長。

圖一、第7屆國際植物醫學論壇全體合照

圖二、日本東京大學濱本教授報告日本植物醫生發展與演進

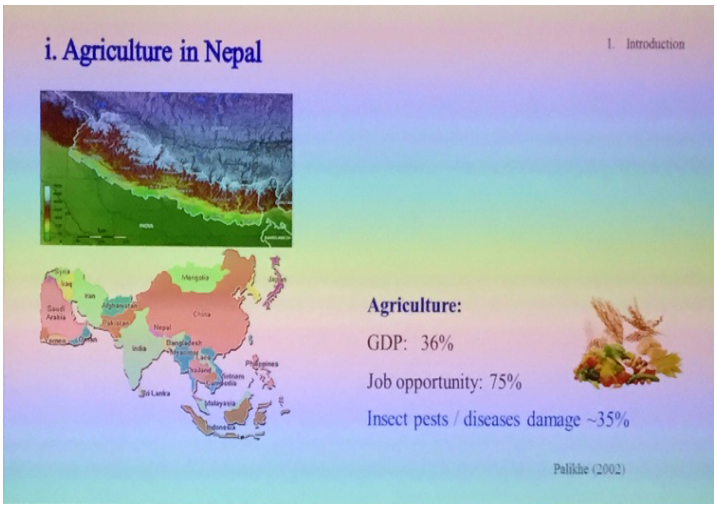
圖三、嘉義大學植醫系感謝日本東京大學難波成任教授協助推動日本植物醫師制度及雙方合作交流

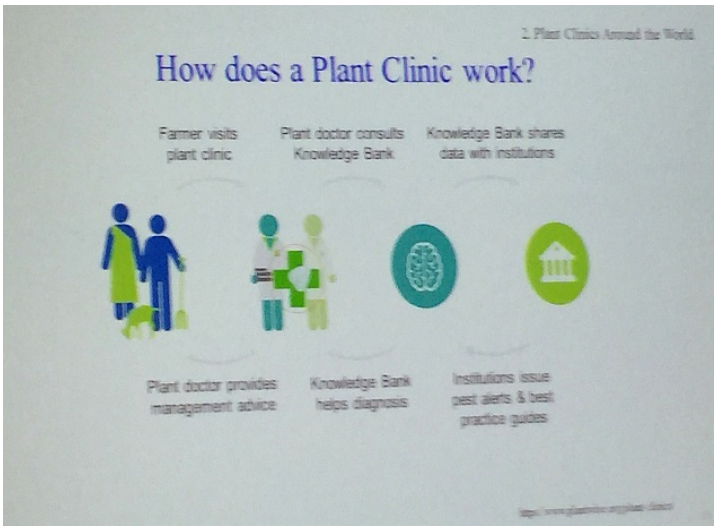
參、會後心得與建議

綜合上述，本屆會議上日本與尼泊爾分享成立植物醫師之歷程，以及日本植物保護士考試之相關制度，值得台灣作為推動植物醫師法之參考借鏡。日本ベジタリア株式会社所開發之水稻田間病蟲害監測管理系統BLASTA，也值得國內作為未來執行水稻流行病監測工作開參考與應用。韓國利用矽藻等資材來作為低毒農業防治管理使用，個人認為相當具有潛力，會議期間經由交換經驗與心得分享，共同討論而獲取許多寶貴資訊。

附件一

尼泊爾農業發展現況與植物醫生診斷體系





附件二

會中報告使用LAMP的等溫擴增反應方法進行李痘瘡病毒檢測與檢測步驟流程

plum pox virus Detection Kit

Product name	Contents	Price (JPY)
plum pox virus Detection Kit	48 tests	47,900
	192 tests	182,000

Abstract

This kit is the product to detect plum pox virus (PPV) by reverse transcription and isothermal amplification reaction using LAMP (Loop-mediated Isothermal Amplification) method. This product can test whether PPV exists in a Japanese apricot (*Prunus mume*). In this kit, a part of PPV genomic RNA is amplified using LAMP method, and PPV infection is judged by the existence of amplification. This kit is effective for the highly-sensitive detection of PPV. It was developed by the Plant Clinic of the University of Tokyo.

The Plant Clinic of the University of Tokyo detected first PPV-D from the Japanese apricot (*Prunus mume*) in Japan March 2009. And then, they developed the LAMP method to detect the PPV from results of the gene analysis of PPV-D which was detected in Japan.

Left: Color bleaking symptom on petal
Right: Chlorotic ringspots on leaf

Overview

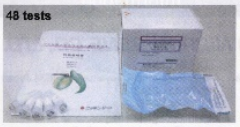
Simple and easy method

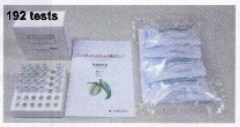
You can get the sample by the toothpick without RNA extraction and purification method.

Clear Judgement

You can observe the result by fluorescence visual detection without electrophoresis.

Component





- PPV Detection Solution
- Fluorescent Detection Reagent
- Enzyme Solution
- Positive Control
- Mineral Oil
- Reaction Tube
- Instruction Manual

Protocol

1 Prepare Test Solution

2 Divide Test Soultion into each Test Tube

3 Prick a ringspot of the leaf with a toothpick

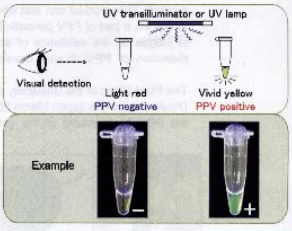
4 Dip the toothpick in Test Solution

5 Add Mineral Oil

6 63°C, 1 hour (Nucleic acids amplification)

7 80°C, 2 minutes (Enzyme inactivation)

8 Judge the result



Products

Product name	Contents	Price (JPY)
TYLCV Detection Kit	10 tests	33,300
	50 tests	33,300
Candidatus Liberibacter Detection Kit	48 tests	42,200
	192 tests	138,800
Bursaphelenchus xylophilus Detection Kit	24 tests	22,200
	96 tests	86,600
Bemisia tabaci Q biotype Detection Kit	12 tests	17,200

[License agreement]
plum pox virus Detection Kit is licensed from Eiken Chemical Co., Ltd.
Nippon Gene Co., Ltd. has been granted the license to development, manufacture and sell the kit for plum pox virus identification.

附件三

會中報告使用LAMP的等溫擴增反應方法進行柑桔黃龍病檢測與檢測步驟流程

Citrus greening (Liberibacter asiaticus) Detection Kit

Product name	Contents	Price (JPY)
Citrus greening (Liberibacter asiaticus) Detection Kit	48 tests	42,200
	192 tests	138,000

Abstract

This is the kit which detects *Candidatus* Liberibacter in the citrus leaf by DNA amplification reaction using LAMP (Loop-mediated Isothermal Amplification) method.
Candidatus Liberibacter is uncultured bacteria causing Citrus greening disease.
This kit includes DNA extraction buffer and positive control DNA. With this Buffer, genomic DNA of *Candidatus* Liberibacter is extracted from a leaf.
In this kit, a part of *Candidatus* Liberibacter genomic DNA is amplified using LAMP method, and *Candidatus* Liberibacter infection is judged by the existence of amplification.
This kit is effective for the highly-sensitive diagnosis of Citrus greening disease.
LAMP (Loop-mediated Isothermal Amplification) method allows the whole reaction process, including denaturating, proceeds at a constant temperature in an incubator. Thermal cycling machine is not needed for this kit.
Look at the homepage of Eiken Chemical Co., Ltd. about the detailed principle of LAMP method.
Eiken GENOME SITE; http://loopamp.eiken.co.jp/e/

Citrus greening disease

Diaphorina citri

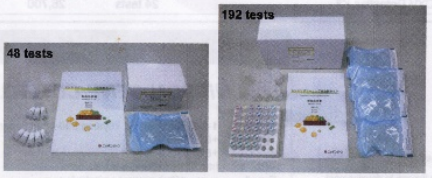
Overview

Simple and easy method
You can get the sample with DNA extraction and detect a bacteria with fluorescence.

Highly sensitive detection
When there is a little sample, it can be detected.

Clear judgement
You can observe the result by fluorescence visual detection without electrophoresis.

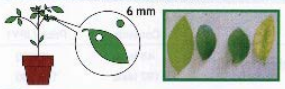
Component



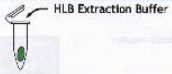
- HLB Detection Solution
- Fluorescent Detection Reagent
- Enzyme Solution
- HLB Positive Control
- Mineral Oil
- HLB Extraction Solution
- HLB Neutralization Solution
- Water (Nucleic acid free)
- Reaction Tube
- Instruction Manual

Preparation

1 Collect the midribs from citrus leaf



2 A leaf is soaked in HLB Extraction Buffer

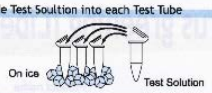


3 95°C, 5 minutes (DNA extraction)
4 Collect water phase
5 Add HLB Neutralization Buffer and 2-propanol
6 Centrifugation
7 Precipitate is rinsed by ethanol and dried
8 Suspended in the Water (Nucleic acid free)

Protocol

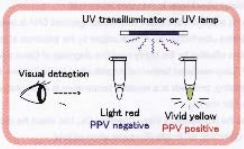
1 Prepare Test Solution

2 Divide Test Soultion into each Test Tube



3 Add DNA Sample

4 Add Mineral Oil
5 65°C, 1 hour (Nucleic acids amplification)
6 80°C, 2 minutes (Enzyme inactivation)
7 Judge the result



Result

Products

Product name	Contents	Price (JPY)
TYLCV Detection Kit	10 tests	33,300
	50 tests	33,300
plum pox virus Detection Kit	48 tests	47,900
	192 tests	182,000
Bursaphelenchus xylophilus Detection Kit for Pine Wilt Disease diagnosis	24 tests	22,200
	96 tests	86,600
Bemisia tabaci Q biotype Detection Kit	12 tests	17,200
CCYV Detection Kit	24 tests	26,700

附件四

The 7th International Conference of Clinical Plant Science 2016, Tokyo

Taxonomic revision of the jumping plant-louse genus *Eotrioza* Konovalova (Homoptera: Psylloidea: Triozidae)

Jin Hyung KWON, Sang Jae SUH, and Yong Jung KWON
School of Applied Biosciences, Kyungpook National University, Daegu, Korea.

Abstract

The monotypic genus *Eotrioza* Konovalova, 1987 was established based on the single species, *Eotrioza ussuriensis* Konovalova, 1987 from Russian Far East. This genus can be separated from other related taxa by having characteristic structure on the third antennal segment and forewing with deep roundly produced anterior margin. After then, the genus *Trachotrioza* Li, 2011 was erected based on the two constituent species, *T. beijingensis* Li, 2011 and *T. apicinigra* Li, 2011 from China. In this survey, we have noticed that these two genera are identical each other morphologically. Thus, the latter genus is placed here to the junior synonymy of *Eotrioza* Konovalova, 1987 (= *Trachotrioza* Li, 2011 syn. nov.). The following new combinations are made here: *Eotrioza beijingensis* (Li, 2011) comb. nov., and *E. apicinigra* (Li, 2011) comb. nov.

Keyword: Homoptera, Triozidae, *Eotrioza*, *Trachotrioza*, East Asia

Introduction

Previously, the monotypic genus *Eotrioza* Konovalova, 1987 was established based on the single species, *Eotrioza ussuriensis* Konovalova, 1987 from Russian Far East. This genus can be separated from other related taxa by having characteristic structure on the third antennal segment peculiarly thickened, and forewing with deep roundly produced anterior margin. In particular, Konovalova (1987) recognized the present genus being closely related to *Trichochermes* Kirkaldy, 1904. However, there have been no records neither on the observation of the host plants nor morphology of the immature stages.

After then, the genus *Trachotrioza* Li, 2011 was erected based on the two constituent species, *T. beijingensis* Li, 2011 and *T. apicinigra* Li, 2011 from China. In the course of the taxonomic survey on these jumping plant-lice from East Asia, we have noticed that these two genera are identical each other morphologically. Thus, the latter genus is placed here to the junior synonymy of *Eotrioza* Konovalova, 1987 which incorporates the above three species known only in East Asia, so far. The following generic synonymy is made here: *Eotrioza* Konovalova, 1987 (= *Trachotrioza* Li, 2011 syn. nov.). The type species, *Eotrioza ussuriensis* Konovalova, 1987 is recorded for the first time in Korea. It is the second faunal record for this taxon since the original description. The biogeographical distribution range of the latter species, previously known only in Russian Maritime Territory, is extended to Korean peninsula southerly. Morphological characteristic features discriminating the constituent species are provided here.

Systematics

Genus *Eotrioza* Konovalova, 1987

Type-species: *Eotrioza ussuriensis* Konovalova, 1987

Type-locality: Russia (Maritime Territory)

Trachotrioza Li, 2011 syn. nov.

Type-species: *Trachotrioza beijingensis* Li, 2011

Type-locality: China (Beijing)

Distribution: Continental region of East Asia

Diagnosis: Head with genal cones well developed, longer than wide, divergent apically; antennae with 3rd segment strongly thickened, covered with numerous setae. Forewings rather stout and oval, widest near middle, costal margin even deeply rounded; Vein Rs strikingly thickened; basal portion of veins furnished with setae. Male genitalia with proctiger deep roundly extended at ventral margin.

***Eotrioza. ussuriensis* Konovalova, 1987**

Eotrioza ussuriensis Konovalova, 1987

Description: General coloration dirty yellow to yellowish brown, with brown to dark brown markings on dorsum, antennal segments 1st – 3rd, 9-10th, and femora; abdominal tergites and antennal sockets pinkish in tint. Forewings semitransparent, light brownish in tint; veins with dark brown patches on the branching portions of C+R and M+Cu, apices of R and C strikingly thickened and darkened; marginal spinules present in cells m1, m2, and cu2.

Male genitalia: Proctiger with ventral margin deep roundly extended mesally. Paramere elongate, about 2.5 times longer than wide, with a tip short and sharply pointed. Aedeagus with distal segment as long as paramere, with apex roundly swollen and gently curved and pointed dorsally. Female proctiger with apex gently narrowed in lateral aspect; length of anus as half as the remainder of proctiger. Subgenital plate slightly shorter than proctiger.

Length: Body male 2.2 - 2.3mm, female 2.3 – 2.4mm; to tip of folded wings male 4.1-4.2mm, female 4.2-4.3mm.

Distribution: Korea (new record: Central), Russia (Maritime Territory).

Host-plant: unknown, so far.

***Eotrioza beijingensis* (Li, 2011) comb. nov.**

Trachotrioza apicinigra Li, 2011, Psyllid. China 2: 1350-1352.

Distribution: China (Beijing).

Host-plant: unknown, so far.

***Eotrioza. apicinigra* (Li, 2011) comb. nov.**

Trachotrioza apicinigra Li, 2011, Psyllid. China 2: 1352-1353

Distribution: China (Yunnan).

Host-plant: unknown, so far.

Fig. *Eotrioza ussuriensis* Konovalova, 1987. A. Head with vertex; B. Forewing; C. Hindwing; D, E. Female genitalia; F. 3rd segment of antenna.

References

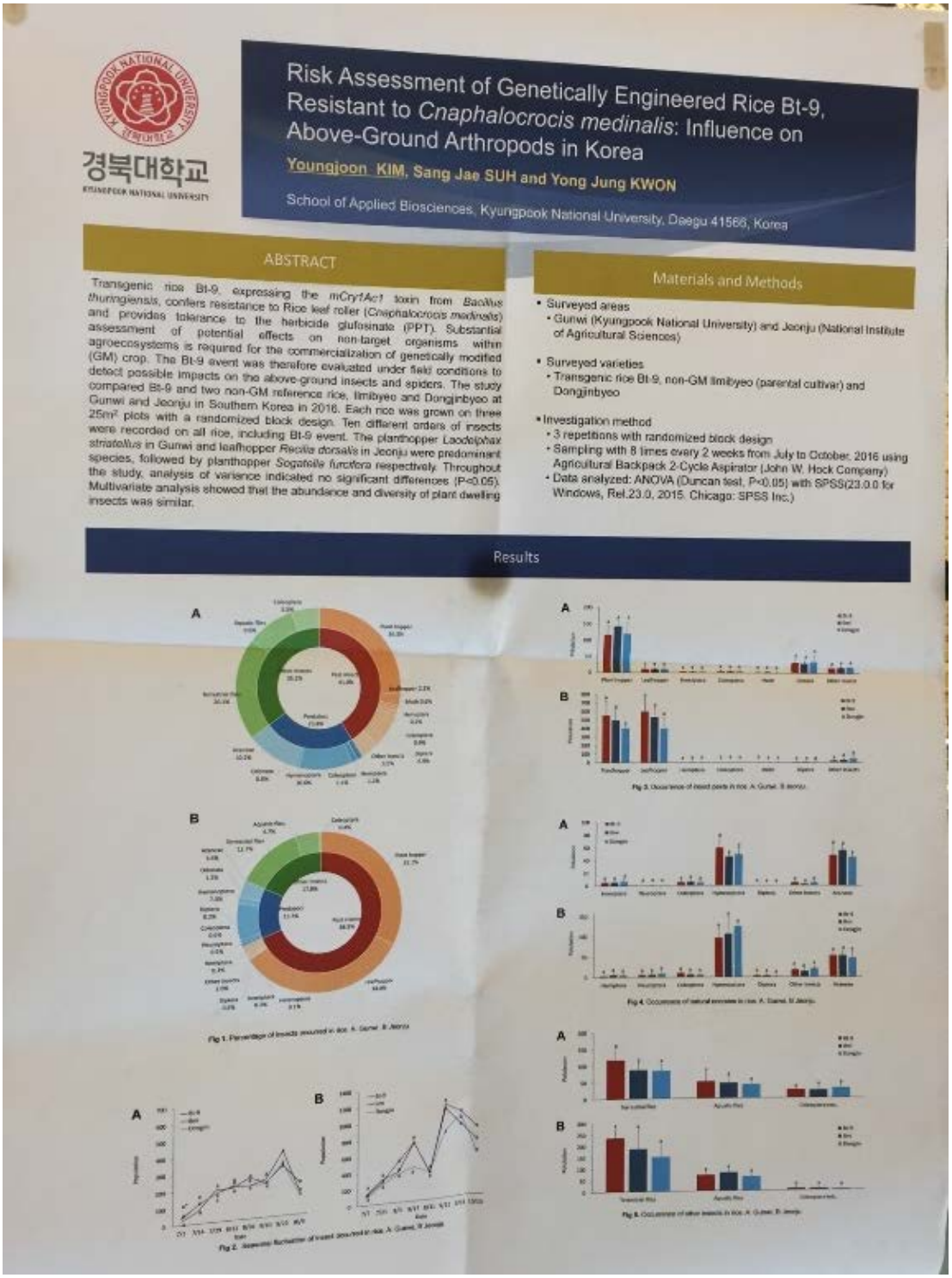
Konovalova Z.A., 1987. A new genus and species from the family Triozidae (Homoptera, Psyllinea) from Primorsky Kray. In: Ler P.A. & Kanyukova E.V., Taksonomiia nasekomykh Sibiri i Dal'nego Vostoka SSSR [Taxonomy of the insects of Siberia and Soviet Far East], Dal nevostochnyi nauchnyi tsentr AN SSSR, Vladivostok (Russia): 34-36.

Konovalova Z.A., 1988. Suborder Psyllinea - Jumping plant-lice. In: In: Ler P.A. et al., Keys to the insects of the Far East of the USSR. 2. Homoptera and Hemiptera: 493-540.

Li, F.S., 2011. Psyllidomorpha of China (Insecta: Hemiptera) 2: 993-1976.

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by Graduate Program for the Undiscovered Taxa of Korea (NIBR201524202).

附件五



경북대학교
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Risk Assessment of Genetically Engineered Rice Bt-9, Resistant to *Cnaphalocrocis medinalis*: Influence on Above-Ground Arthropods in Korea

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ABSTRACT

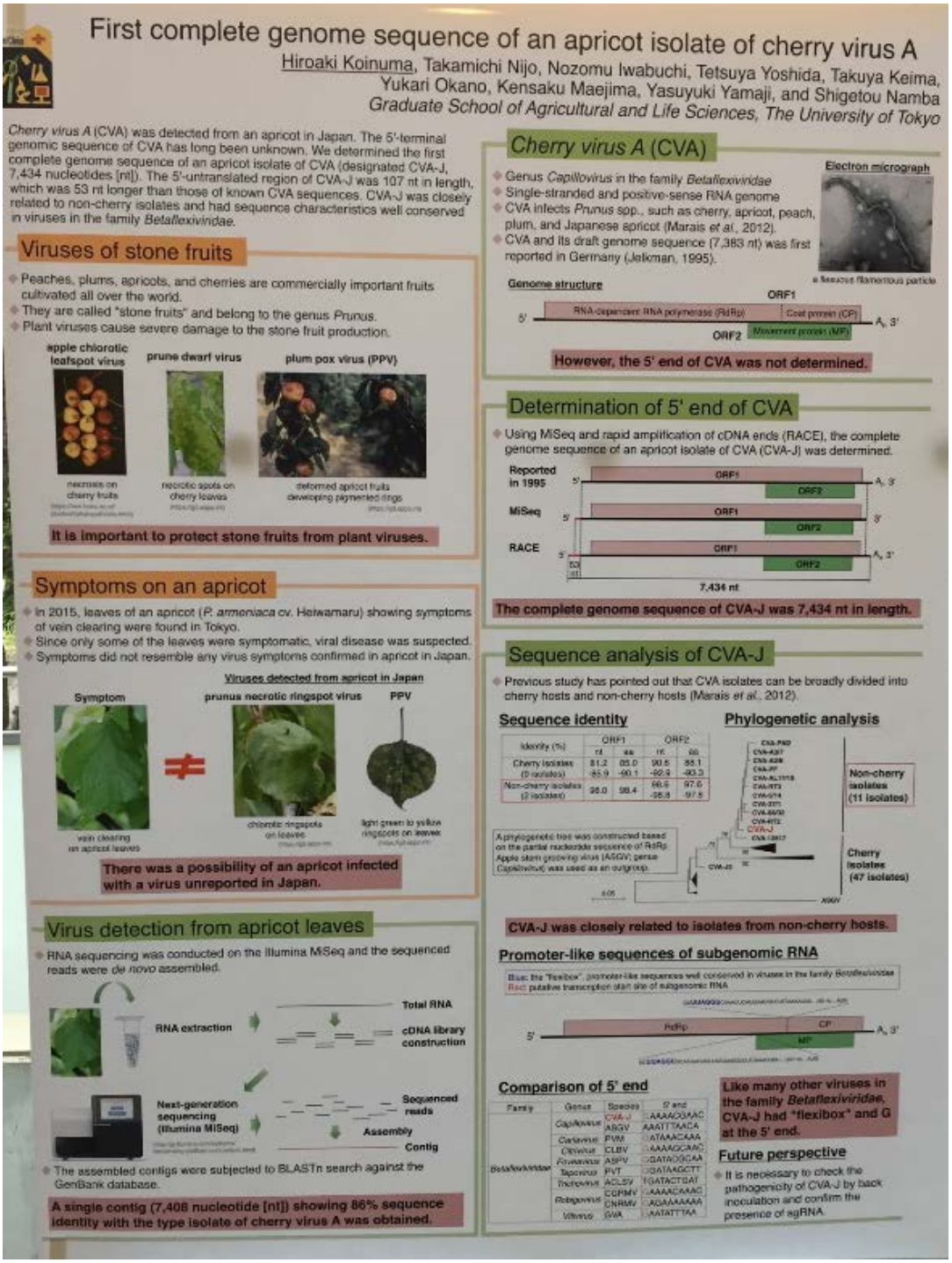
Transgenic rice Bt-9, expressing the *mCry1Ac1* toxin from *Bacillus thuringiensis*, confers resistance to Rice leaf roller (*Cnaphalocrocis medinalis*) and provides tolerance to the herbicide glufosinate (PPT). Substantial assessment of potential effects on non-target organisms within agroecosystems is required for the commercialization of genetically modified (GM) crop. The Bt-9 event was therefore evaluated under field conditions to detect possible impacts on the above-ground insects and spiders. The study compared Bt-9 and two non-GM reference rice, Ilmibyeo and Dongjinbyeo at Gunwi and Jeonju in Southern Korea in 2016. Each rice was grown on three 25m² plots with a randomized block design. Ten different orders of insects were recorded on all rice, including Bt-9 event. The planthopper *Laodelphax striatellus* in Gunwi and leafhopper *Recilia dorsalis* in Jeonju were predominant species, followed by planthopper *Sogatella furcifera* respectively. Throughout the study, analysis of variance indicated no significant differences (P<0.05). Multivariate analysis showed that the abundance and diversity of plant dwelling insects was similar.

Materials and Methods

- Surveyed areas
  - Gunwi (Kyungpook National University) and Jeonju (National Institute of Agricultural Sciences)
- Surveyed varieties
  - Transgenic rice Bt-9, non-GM Ilmibyeo (parental cultivar) and Dongjinbyeo
- Investigation method
  - 3 repetitions with randomized block design
  - Sampling with 8 times every 2 weeks from July to October, 2016 using Agricultural Backpack 2-Cycle Aspirator (John W. Hock Company)
  - Data analyzed: ANOVA (Duncan test, P<0.05) with SPSS(23.0.0 for Windows, Rel.23.0, 2015. Chicago: SPSS Inc.)

Results

附件六


First complete genome sequence of an apricot isolate of cherry virus A
Hiroaki Koinuma, Takamichi Nijo, Nozomu Iwabuchi, Tetsuya Yoshida, Takuya Keima, Yukari Okano, Kensaku Maejima, Yasuyuki Yamaji, and Shigetou Namba
Graduate School of Agricultural and Life Sciences, The University of Tokyo
Cherry virus A (CVA) was detected from an apricot in Japan. The 5'-terminal genomic sequence of CVA has long been unknown. We determined the first complete genome sequence of an apricot isolate of CVA (designated CVA-J, 7,434 nucleotides [nt]). The 5'-untranslated region of CVA-J was 107 nt in length, which was 53 nt longer than those of known CVA sequences. CVA-J was closely related to non-cherry isolates and had sequence characteristics well conserved in viruses in the family Betaflexiviridae.
Viruses of stone fruits
Peaches, plums, apricots, and cherries are commercially important fruits cultivated all over the world.
They are called "stone fruits" and belong to the genus Prunus.
Plant viruses cause severe damage to the stone fruit production.
apple chlorotic leafspot virus
prune dwarf virus
plum pox virus (PPV)
necrosis on cherry fruits
necrotic spots on cherry leaves
deformed apricot fruits developing pigmented rings
It is important to protect stone fruits from plant viruses.
Symptoms on an apricot
In 2015, leaves of an apricot (P. armeniaca cv. Heiwamaru) showing symptoms of vein clearing were found in Tokyo.
Since only some of the leaves were symptomatic, viral disease was suspected.
Symptoms did not resemble any virus symptoms confirmed in apricot in Japan.
Viruses detected from apricot in Japan
Symptom
prunus necrotic ringspot virus
PPV
vein clearing on apricot leaves
chlorotic ringspots on leaves
light green to yellow ringspots on leaves
There was a possibility of an apricot infected with a virus unreported in Japan.
Virus detection from apricot leaves
RNA sequencing was conducted on the Illumina MiSeq and the sequenced reads were de novo assembled.
RNA extraction
Total RNA
cDNA library construction
Next-generation sequencing (Illumina MiSeq)
Sequenced reads
Assembly
Contig
The assembled contigs were subjected to BLASTn search against the GenBank database.
A single contig (7,408 nucleotide [nt]) showing 86% sequence identity with the type isolate of cherry virus A was obtained.
Cherry virus A (CVA)
Genus Capillovirus in the family Betaflexiviridae
Single-stranded and positive-sense RNA genome
CVA infects Prunus spp., such as cherry, apricot, peach, plum, and Japanese apricot (Marais et al., 2012).
CVA and its draft genome sequence (7,383 nt) was first reported in Germany (Jelkman, 1995).
Electron micrograph
a flexuous filamentous particle
Genome structure
ORF1
RNA-dependent RNA polymerase (RdRp)
Coat protein (CP)
ORF2
Movement protein (MP)
However, the 5' end of CVA was not determined.
Determination of 5' end of CVA
Using MiSeq and rapid amplification of cDNA ends (RACE), the complete genome sequence of an apricot isolate of CVA (CVA-J) was determined.
Reported in 1995
MiSeq
RACE
7,434 nt
The complete genome sequence of CVA-J was 7,434 nt in length.
Sequence analysis of CVA-J
Previous study has pointed out that CVA isolates can be broadly divided into cherry hosts and non-cherry hosts (Marais et al., 2012).
Sequence identity
Phylogenetic analysis
Non-cherry isolates (11 isolates)
Cherry isolates (47 isolates)
A phylogenetic tree was constructed based on the partial nucleotide sequence of RdRp. Apple stem grooving virus (ASGV; genus Capillovirus) was used as an outgroup.
CVA-J was closely related to isolates from non-cherry hosts.
Promoter-like sequences of subgenomic RNA
Blue: the "flexibox", promoter-like sequences well conserved in viruses in the family Betaflexiviridae
Red: putative transcription start site of subgenomic RNA
RdRp
CP
MP
Comparison of 5' end
Like many other viruses in the family Betaflexiviridae, CVA-J had "flexibox" and G at the 5' end.
Future perspective
It is necessary to check the pathogenicity of CVA-J by back inoculation and confirm the presence of sgRNA.

附件七

Host preference and the application of using orange jasmine as a trap crop for Diaphorina citri (Hemiptera: Liviidae)

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Abstract

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a very important vector of Candidatus Liberibacter asiaticus that causes citrus huanglongbing (HLB), a destructive disease of citrus without any efficient chemical cure. Although "healthy seeding system" can successfully prevent the HLB transmission by grafting, it is difficult to prevent the vector transmitting HLB pathogen in the field. Consequently, to realize more about the host preference behavior of the vectors is helpful for setting up new strategies in controlling them. Orange jasmine (*Murraya paniculata* (L.) Jack.) is a common preferable host for ACP. We are trying to test the preference among the orange jasmine and other 4 kinds of citrus, then test using orange jasmine as a trap crop to know whether it could prevent the ACPs' invasion or not. In the preference test of orange jasmine with 4 different citrus, ACPs have higher preference on Ponkan and orange. In those two experiments, ACPs all preferred feeding on orange jasmine. It means orange jasmine has the potential to be a trap crop. As a result, we choose Ponkan to test the effects when trap crops were applied surrounding the citrus. The results revealed that using same number of orange jasmine with the citrus can prevent about the 80% psyllids' invasion to Ponkan during the experimental period of 24h. Using double number of orange jasmine can prevent almost up to 90% of psylid invasion. We also found the trap crop number and species can affect ACPs' invasion. In the two field preference tests, the first field ACP release, we found the psyllids might prefer to choose citrus in spite of the orange jasmine have more young flushing leaves. However, in the second release, the psyllids chose citrus during the first two weeks, but when their offspring appeared in the 4th week, more psyllids chose orange jasmine instead. We suspect that probably the season and host plant condition could change the feeding and oviposition behavior of ACPs and thus make the two different results we observed in the field.

Objective

- To test the psyllid preference between orange jasmine and citrus
- To apply orange jasmine as a trap crop for citrus protection

Materials and Methods

1. Indoor host preference tests between orange jasmine and citrus
One plot of each citrus and orange jasmine were placed in a 60x60x60 cm insect cage, four kinds of healthy citrus: Ponkan mandarin (*Citrus reticulata*), lemon (*C. limon*), orange (*C. sinensis*) and pomelo (*C. grandis*) were used as 4 treatments. 50 ACP adults were released to choose their preferred hosts in every test. The plants were chosen in similar size and flush condition and the distances from the release point to the citrus and orange jasmine are the same. The distribution of ACP were recorded after 24h and 48h. Each treatment was repeated 9 times. All experiments were performed at 27 °C, 60 % RH, and 14:10 L:D photoperiod (Fig.1a).

2. Greenhouse trap crop tests
Four Ponkan plots were set in the middle of a greenhouse, and we used orange jasmine plot as traps and divided into 2 surrounding types, 4 traps and 8 traps respectively. ACPs were released from 4 different directions outside the traps. 25 ACP adults (100/group) were released from each direction, and the ACPs' distribution were recorded after 24h. Two surrounding treatments of no trap and 8 Ponkan were tested as the control group. Each treatment was repeated 3 times (Fig.1b).

3. Field host preference and spatial distribution experiments
Two times field experiments with 40 citrus plants were performed at Chiayi Agriculture Experiment Branch, Chiayi, Taiwan. Another 40 jasmine orange pots were placed 1m beside the 40 citrus plants. Then 1000 ACPs were released from the central point of the field. The number of adult psyllids, plants with nymphs and plants with flush on each plant were continuously recorded for 10 weeks (Fig.1c).

Fig. 1. Experiment illustration pictures. (a) Indoor host preference tests (b) Greenhouse trap crop tests (c) Field host preference and spatial distribution experiments

Results

1. Indoor Host preference between jasmine orange and healthy citrus

Fig. 2. The proportion of ACPs choosing an orange jasmine. Vertical axis is the value which means ACPs chose an orange jasmine in all ACPs which made choice. Dotted line means no significant preference (p≤ 0.1 in Letter L, Q, P and R represented lemon, orange, Ponkan, and pumelo, respectively. The symbol *, **, *** and ns represented significant level of 5%, 1% and no significance, respectively.

Three groups have significant differences at 24h, which are lemon, orange and pumelo group. Another three groups also have significant difference at 48h, which are orange, Ponkan, and pumelo respectively

2. Greenhouse trap crop tests
Using 4 plots of orange jasmine with the citrus can prevent about the 80% psyllids' invasion to Ponkan during the experimental period of 24h. Using double number of orange jasmine can prevent almost up to 90% of psylids' invasion (Fig. 3). According to Chi-square analysis, we also found the trap crop number and species can affect ACPs' invasion (Table 2).

Table 1 Distribution of trap crop tests

treatment	trap (out)	ponk (in)	out/in
mc1	146	38	3.84
mc2	99	11	8.73
cc	85	25	3.40
n		135	

Table 2. Chi-square analysis data of treatments

group	Chi-squared	df	p-value
mc1,mc2	4.4832	1	0.03423
cc,mc2	5.2065	1	0.0225
cc,mc1	0.074359	1	0.7851

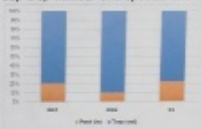


Fig. 3. Proportion of ACPs which made choice. mc1 means 4 trap treatment, mc2 means 8 trap treatment, cc means 8 Ponkan control treatment, and n means no trap control treatment

3. Field host preference and spatial distribution experiments

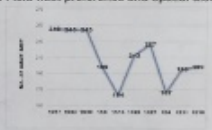


Fig. 4. Population fluctuation of adult ACPs on plants (citrus and jasmine orange) in the first field release.

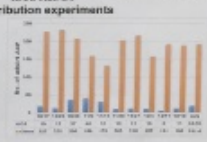


Fig. 5. Distribution of adult ACPs on different plant species in the first field release. Orange bars represented the citrus, and blue bars represented the orange Jasmine.

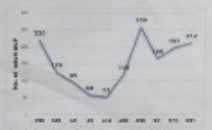


Fig. 6. Population fluctuation of adult ACPs on plants (citrus and jasmine orange) in the second field release.

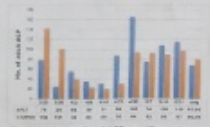


Fig. 7. Distribution of adult ACPs on different plant species in the second field release. Bar colors are the same as in Fig. 4.

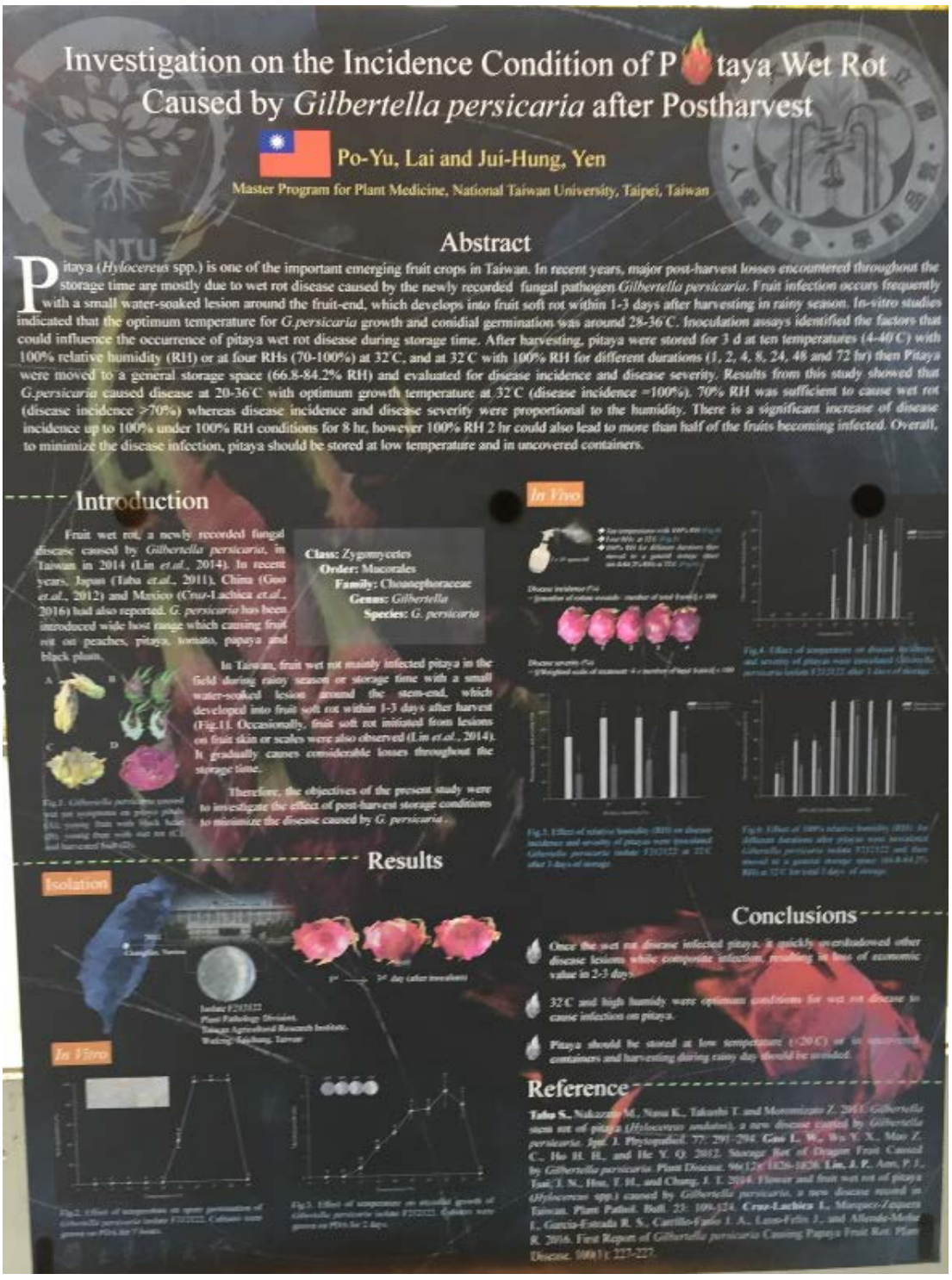
Conclusions

- Indoor host preference tests revealed that psyllids significantly prefer orange jasmine than Ponkan and orange.
- Indoor trap crop tests revealed that numbers of trap crop and host species strongly affect psyllids to invade into Ponkan plantings.
- We suspect that probably the season and host plant condition could change the feeding and oviposition behavior of ACPs in the natural field.

Acknowledgements

I want to thank Dr. Ting-Hsuan Hung and Dr. Shiuh-Feng Shiao for their guidance, Ming-Chung Chu PhD and Pin-Hsin Yu for experiment advice, the people at Chiayi Agriculture Experiment Branch for providing field and help. Finally, this work was supported by the Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Taiwan (105AS-10.6.3-BQ-B1).

附件八


Investigation on the Incidence Condition of Pitaya Wet Rot Caused by Gilbertella persicaria after Postharvest
Po-Yu, Lai and Jui-Hung, Yen
Master Program for Plant Medicine, National Taiwan University, Taipei, Taiwan
Abstract
Pitaya (Hylocereus spp.) is one of the important emerging fruit crops in Taiwan. In recent years, major post-harvest losses encountered throughout the storage time are mostly due to wet rot disease caused by the newly recorded fungal pathogen Gilbertella persicaria. Fruit infection occurs frequently with a small water-soaked lesion around the fruit-end, which develops into fruit soft rot within 1-3 days after harvesting in rainy season. In-vitro studies indicated that the optimum temperature for G.persicaria growth and conidial germination was around 28-36 C. Inoculation assays identified the factors that could influence the occurrence of pitaya wet rot disease during storage time. After harvesting, pitaya were stored for 3 d at ten temperatures (4-40 C) with 100% relative humidity (RH) or at four RHs (70-100%) at 32 C, and at 32 C with 100% RH for different durations (1, 2, 4, 8, 24, 48 and 72 hr) then Pitaya were moved to a general storage space (66.8-84.2% RH) and evaluated for disease incidence and disease severity. Results from this study showed that G.persicaria caused disease at 20-36 C with optimum growth temperature at 32 C (disease incidence =100%). 70% RH was sufficient to cause wet rot (disease incidence >70%) whereas disease incidence and disease severity were proportional to the humidity. There is a significant increase of disease incidence up to 100% under 100% RH conditions for 8 hr, however 100% RH 2 hr could also lead to more than half of the fruits becoming infected. Overall, to minimize the disease infection, pitaya should be stored at low temperature and in uncovered containers.
Introduction
Fruit wet rot, a newly recorded fungal disease caused by Gilbertella persicaria, in Taiwan in 2014 (Lin et.al., 2014). In recent years, Japan (Taba et.al., 2011), China (Guo et.al., 2012) and Mexico (Cruz-Lachica et.al., 2016) had also reported. G. persicaria has been introduced wide host range which causing fruit rot on peaches, pitaya, tomato, papaya and black plum.
Class: Zygomycetes
Order: Mucorales
Family: Choanephoraceae
Genus: Gilbertella
Species: G. persicaria
In Taiwan, fruit wet rot mainly infected pitaya in the field during rainy season or storage time with a small water-soaked lesion around the stem-end, which developed into fruit soft rot within 1-3 days after harvest (Fig.1). Occasionally, fruit soft rot initiated from lesions on fruit skin or scales were also observed (Lin et.al., 2014). It gradually causes considerable losses throughout the storage time.
Therefore, the objectives of the present study were to investigate the effect of post-harvest storage conditions to minimize the disease caused by G. persicaria.
Results
Isolation
In Vitro
In Vivo
Conclusions
Once the wet rot disease infected pitaya, it quickly overshadowed other disease lesions while composite infection, resulting in loss of economic value in 2-3 days.
32 C and high humidity were optimum conditions for wet rot disease to cause infection on pitaya.
Pitaya should be stored at low temperature (<20 C) or in uncovered containers and harvesting during rainy day should be avoided.
Reference
Taba S., Nakazono M., Nasu K., Takeshi T. and Moromizato Z. 2011. Gilbertella sum rot of pitaya (Hylocereus undatus), a new disease caused by Gilbertella persicaria. Jpn. J. Phytopathol. 77: 291-294. Guo L. W., Wu Y. X., Mao Z. C., He H. H., and He Y. Q. 2012. Storage Rot of Dragon Fruit Caused by Gilbertella persicaria. Plant Disease. 96(12): 1826-1830. Lin, J. P., Ann, P. J., Tsai, J. N., Hsu, T. H., and Chung, J. T. 2014. Flower and fruit wet rot of pitaya (Hylocereus spp.) caused by Gilbertella persicaria, a new disease record in Taiwan. Plant Pathol. Bull. 23: 109-124. Cruz-Lachica I., Marquez-Zequera I., Garcia-Estrada R. S., Carrillo-Fasio J. A., Lazo-Felix J., and Allende-Molar R. 2016. First Report of Gilbertella persicaria Causing Papaya Fruit Rot. Plant Disease. 100(1): 227-227.

附件九

Pathogen-identification and industrial application of poinsettia witches' broom

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Abstract

Poinsettia (*Euphorbia pulcherrima*) is an important ornamental crop in Taiwan. The commercial cultivars of poinsettia are usually free-branching, which is related to poinsettia witches' broom. As the result, the disease benefits the industry of poinsettia. The pathogen of poinsettia witches' broom is phytoplasma, which is a phloem-limited bacteria. According to the taxonomy, the predominant type of phytoplasma which infects poinsettias belongs to 16SrDNA group III. Few cultivars are infected by 16SrIII and 16SrI. Recently, there is not enough research which demonstrates whether different group of phytoplasma infection can cause different symptoms on poinsettias. As a result, it is difficult to use phytoplasma to produce poinsettia which have stable features. In this research, commercial poinsettia cultivars were collected and their nucleic acids were extracted. The sequences of 16SrDNA fragments were analyzed to realize which group of phytoplasma infects each poinsettia cultivar. Next, the phytoplasma in poinsettia was eliminated by tetracycline to understand the natural feature of each poinsettia cultivar. Finally, each group of phytoplasma was inoculated to poinsettias which were phytoplasma-free to understand the effect of different group of phytoplasma infection. The results of sequence analysis showed that all of the cultivars tested were infected by 16SrIII phytoplasma. Next, the poinsettias treated by tetracycline were significantly taller and the number of branching was significantly fewer than those treated by water. Finally, the tetracycline-treated poinsettias inoculated by periwinkle leaf yellowing phytoplasma (16SrI) were significantly shorter than the uninoculated poinsettias, but the number of branching did not show significance. The poinsettias inoculated by poinsettia branch-inducing phytoplasma (16SrIII) were significantly shorter and the number of branching was significantly larger than the uninoculated poinsettias. The results demonstrate that different group of phytoplasma can cause different symptom on poinsettias. The poinsettias which have more branching are more commercially valuable. As a result, if we want to enhance the industrial value of poinsettias, inoculating 16SrIII phytoplasmas to the poinsettias may be better than inoculating 16SrI phytoplasmas.

Figure 1. The features of poinsettias treated by tetracycline (left) and poinsettias treated by water control (right). (A) Cultivar Red Velveteen (B) Cultivar Dulce Rosa

A

Red Velveteen	Number of branching	
Control	6.5±2.12	a
Tetracycline	0.33±0.57	b

B

Dulce Rosa	Number of branching	
Control	4.33±0.57	a
Tetracycline	0.00±0.00	b

Table 1. The branching numbers of poinsettias. (A) Cultivar Red Velveteen (B) Cultivar Dulce Rosa

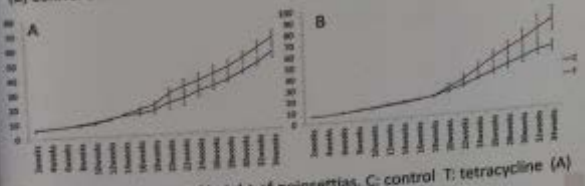

Figure 2. The changes of height of poinsettias. C: control T: tetracycline (A) Cultivar Red Velveteen (B) Cultivar Dulce Rosa

Figure 3. Poinsettia cultivar Red Velveteen inoculated by different group of phytoplasma (right) and uninoculated control (left). (A) Poinsettias inoculated by 16SrI phytoplasma. (B) Poinsettias inoculated by 16SrIII phytoplasma

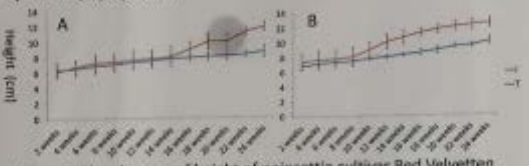

Figure 4. The changes of height of poinsettia cultivar Red Velvetten inoculated by different group of phytoplasma. I: Inoculated by phytoplasma T: Phytoplasma-free control (A) 16SrI (B) 16SrIII

Figure 5. Poinsettia cultivar Dulce Rosa inoculated by different groups of phytoplasma (right) and uninoculated control (left). (A) Poinsettia inoculated by 16SrI phytoplasma. (B) Poinsettia inoculated by 16SrIII phytoplasma

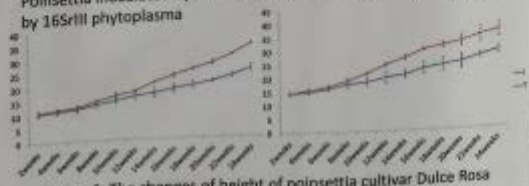

Figure 6. The changes of height of poinsettia cultivar Dulce Rosa inoculated by different group of phytoplasma. I: Inoculated by phytoplasma T: Phytoplasma-free control (A) 16SrI (B) 16SrIII

Red Velveteen	Number		Dulce Rosa	Number	
Control	0.33±0.57	a	Control	0.00±0.00	a
16SrI	0.67±1.15	a	16SrI	0.33±0.57	a
16SrIII	3.67±0.57	b	16SrIII	3.33±0.57	b

Table 2. The branching numbers of poinsettias inoculated by different group of phytoplasma

Reference

1. Lee IM, Klopmeyer M, Bartoszyk IM, Gundersen-Rindal DE, Chou TS, Thomson KL, Eisenreich R. 1997. Phytoplasma induce free-branching in commercial poinsettia cultivars. Nat. Biotechnol. 15:178-182