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Final report

Project

Enabling Improved Plant Biosecurity Practices in Cambodia, Lao PDR and Thailand

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1 Acknowledgments

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2 Executive summary

Pest surveillance and diagnostics form the basic core of all biosecurity systems, providing fundamental information on pests and diseases that will inform all other plant health activities, including plant protection, pest management, plant quarantine inspection, regulation and certification, biosecurity policy and other trade-related matters such as import and export. When surveillance and diagnostic operations are lacking or inadequate, these higher order operations cannot function, which ultimately impacts agricultural production, and the ability to trade.

The aim of this project then was to strengthen diagnostics and surveillance activities in Laos PDR and Cambodia to establish the necessary capability, diagnostic framework and conditions required to identify plant health issues. The operations of surveillance and diagnostics include many processes, requiring skilled people, laboratory resources, access to diagnostic protocols, data management systems and organisational structures that support these processes. Diagnostic operations then give rise to pest and herbarium collections, information on pest incidence and distribution, knowledge of pest biology and control and ultimately, Pest Lists.

Pest Lists document the pests that are present in a country and are used to support research, pest management, risk analysis, plant quarantine, pest regulation, trade and trade policy. Producing Pest Lists demonstrates a high level of capacity in surveillance and diagnostic operations.

The activities undertaken in this project were therefore designed to improve surveillance and diagnostic processes in Laos and Cambodia to enable the production of Pest Lists. Activities included the introduction of new technologies for capturing and managing pest data, using mobile devices and the Pestpoint® software for pest identification and the establishment of a virtual diagnostic network for Laos, Cambodia and Thailand. This network encouraged cross-country collaboration with the sharing of expertise for solving pest problems while at the same time capturing and storing all pest records in a searchable database. This system was used in surveillance exercises to document and record pest and disease details in the field and to create a permanent, shared record of pest incidence. The record also provides an account of the diagnostic process applied to the pest or disease, which is regarded as a chain of evidence to support Pest Lists.

Molecular techniques are now routinely applied to the identification of pests and diseases and so, selected staff from Laos, Cambodia and Thailand were trained in molecular techniques, analysing many of the pest and disease specimens collected during their own surveys. Basic molecular equipment was installed at the PPC laboratory in Laos and also at the GDA laboratory in Cambodia. Protocols for general and specific analyses were provided as part of the molecular training process.

General surveillance across crops in all three countries showed that plant viruses are widespread and are likely to be a major constraint to production for many crops. Few plant viruses have been previously recorded in the region, possibly because they are difficult to identify without molecular techniques. More than two-hundred plant virus samples were collected and documented from a variety of crops. Many have been tentatively identified and several definitively identified and published as new disease records. These viruses can now be added to country Pest Lists.

Particular attention was given to documenting Banana Bunchy Top Virus (BBTV) in Laos and to the recently introduced Sri Lankan Cassava Mosaic Virus (SLCMV) in Cambodia. Delimiting surveys to gauge the spread of SLMV were conducted in Cambodia, with subsequent verification using molecular techniques by country staff. BBTV is being identified to strain to characterise and map BBTV across Laos and Cambodia. New strains have been tentatively identified from samples collected in Laos.

Chinese commercial banana and cassava production has been increasing in both Laos and Cambodia, with SLCMV first detected on a commercial Chinese operation in Rattani Kiri province in NE Cambodia. SLCMV was previously unknown in Cambodia and is not known to be present in neighbouring Thailand or Laos, and so is of biosecurity concern to these countries. SLCMV surveys in Cambodia were conducted in collaboration with Thai and Laos pathologists as part of the diagnostic network and collaboration developed during this project. Thailand has been instrumental in providing molecular resources and training to Laos and Cambodian staff, for the identification of SLCMV, BBTV and plant viruses generally.

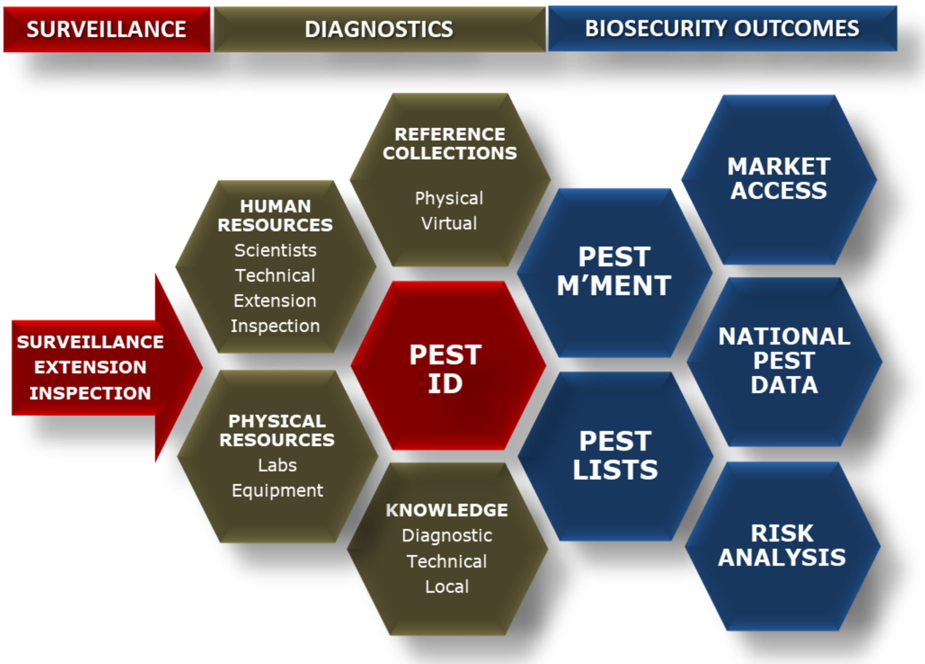
Panama disease Tropical Race 4 (FOC/TR4) was identified from Cavendish (Hom) bananas growing in commercial Chinese plantations in northern and central Laos. It is likely the disease was introduced by Chinese operators and transferred from northern to central Laos by them as contaminated soil. FOC/TR4 is a devastating disease of banana that persists in soil and is transported in water. Banana production cannot persist where it is found. It now poses a risk to further banana production in Laos and across the region unless biosecurity regulations governing planting material are developed and enforced. This record of FOC/TR4 has been published by the project team and Laos, Thai and Cambodian authorities have been notified.

These disease examples have heightened biosecurity awareness in all three countries as a result of this project, because staff were able to conduct surveys for the detection of the diseases and were also able to use molecular techniques for positive identifications. This project has enabled capacity for conducting these biosecurity activities in Laos and Cambodia, such that each of the participating agencies now has the ability to control detection and identification of pests and diseases in their own countries, to document these as a chain of evidence that supports the development of Pest Lists, leading to the development of regulation and trade-related policy.

3 Background

Pest Identification and diagnostics form the core of all biosecurity systems and are pre-requisites for all other plant health activities, including pest surveillance, plant protection and pest management. Capability in diagnostics will therefore underpin plant health. When diagnostic capacity is working at a high level, Pest Lists for crops and commodities can be produced. These lists document the pests that are present in a country and are used to support research, pest management, risk analysis, plant quarantine, pest regulation, trade and trade policy. Diagnostics capability and capacity are then, the “horse” that pulls the “biosecurity cart”.

Capability in diagnostics does however require certain standards, physical and human resources as well as expertise and knowledge. Labs and equipment are of little use without people who know how to use them and without the processes, activities and organisational structures that support surveillance and pest monitoring. It is a complex system of many interacting and interdependent parts. The diagram below shows the relationships between some of the core components of the system, sometimes referred to as the biosecurity continuum.



Currently, the plant health processes and activities operating in Cambodia and Lao PDR are limited. This is largely due to a lack of local expertise, information and physical resources, as well as the organisational processes and communication networks required to form the complex processes that contribute to plant health, trade and protection of the environment. As stated, the plant health system should be built around diagnostics and the resources and knowledge that diagnostics generates, which include pest and herbarium collections, diagnostic protocols, information on pest incidence and distribution, knowledge of pest biology and control and ultimately, Pest Lists, which are verified and supported by these resources. The presence/absence and quality of Pest Lists can then be used as a measure of how effectively diagnostics is operating and contributing to the Plant Health System.

The diagnostic capability required to produce Pest Lists in Laos PDR and Cambodia is weak. The current Pest Lists are not only inadequate, but likely to be inaccurate, having been based mainly on out-dated literature and un-verified identifications. The aim of this project then was to strengthen diagnostics and surveillance activities in Laos PDR and

Cambodia to establish the necessary capability, diagnostic framework and conditions required to identify plant health issues and develop accurate Pest Lists. We hoped to achieve these aims by focussing on particular crops and/or pests for surveillance and diagnostic activities and once established, the same processes and activities can be generally applied to all crops and pests. Laos PDR and Cambodia would then have the foundation for the future development of their diagnostics and surveillance systems and to link these to the growth of their overall Plant Health and Biosecurity Systems.

An essential part of achieving these aims requires the development of a regional knowledge resource base to diagnose and manage plant pests and in the project, we propose to link the staff from Plant Protection Organisations (PPOs) in Thailand, Laos PDR and Cambodia for this purpose. Thailand already has a well-resourced plant health system and will therefore take a leading role in training of staff and supporting diagnostic and surveillance activities conducted during the project. By creating an environment for the development of these regional relationships we hope to create research and knowledge networks for the identification of pests, where information and diagnostic facilities can be shared for mutual benefit.

In order to achieve this, we propose to introduce social networking software and mobile devices that will facilitate communication and sharing of pest problems as they arise. This will require that a stratified process of pest identification and diagnosis is applied, where virtual identification using pest images that are shared within the regional network, becomes the first option in diagnosing a pest problem. We consider this method to be a paradigm shift when it comes to diagnosis and so we anticipate a steep learning curve in relation to the digital technologies employed and the techniques that will be required to convey information in a virtual environment. Nonetheless, given that diagnostic expertise and resources are scarce in the region, this kind of remote diagnostic process can be both effective and efficient in facilitating pest management decisions.

Sanitary and Phytosanitary systems and activities require continual monitoring and development to ensure that processes are meeting the aims of not only the country of origin but also of existing and potential trade partners. In order to achieve this, the WHO and FAO recommend that a system consists of: (i) laws and regulations; (ii) operational control and management; (iii) inspection services; (iv) laboratory services; (v) information, training and other communication. Implementation of these components across the range of crop commodities produced in many countries is a huge task, requiring dedicated personnel to continually locate, diagnose and collate the required information. While the first two components can be achieved generally through consultation and adaptation of existing information, the task of having suitably skilled personnel to implement the remaining three components is difficult, even for developed countries such as Australia. In their report to the Australian government, Beale *et al* (2008) provided nine reasons for the complexity in biosecurity management. Three of these have particular relevance to the partner countries in this project, namely population spread into new habitats and increasingly intensive agriculture; tourism growth and the subsequent increase in passenger and cargo movement, and; a shortage of highly qualified plant and animal pest and disease professionals. If Laos PDR, Cambodia and Thailand to a lesser extent are going to meet these challenges, they will need to develop sophisticated plant health and biosecurity systems which are driven by effective diagnostics and surveillance systems.

4 Objectives

The overall aim of the project was to improve plant biosecurity in the region, where this relates to an increased capacity for and capability to identify and monitor pest activity in trade and production systems. Each country will be enabled to 1. conduct research and preliminary diagnosis of plant pests and diseases, with collaborative research and confirmatory diagnosis being undertaken within the region and 2. Conduct plant pest and disease surveys to establish Pest Lists, and to monitor pest distribution and/or spread. These will be achieved via the following project objectives:

1) To enhance the capacity of crop protection and plant quarantine staff in plant pest and disease diagnosis for plant biosecurity.

Activity 1 - Conduct intensive training courses for key pest/pathogen groups, covering fungal, bacterial, viral and nematode taxonomies using Australian experts.;
Activity 2 - Conduct intensive training workshops demonstrating biosecurity surveillance and crop monitoring programs.
Activity 3 - Conduct training in pest list establishment and pest risk analysis.

2) To establish knowledge-based network capable of sustaining on-going training, diagnostic assistance, and local and regional crop protection / biosecurity information.

Activity 1 - Monitor and evaluate the existing border security demonstration sites at current border posts (international airports).
Activity *2 - Demonstrate* and establish a diagnostic network using Pestpoint® and accessing the existing RMDN, provide training and support, and adapt to local requirements.
Activity 3 - Design and pilot a Smartphone surveillance system for crop protection and local biosecurity assessment
Activity 4 - Establish a network of researchers, border staff, agronomists and farmers to conduct field and commodity surveys.

3) To enhance research capability for analysis of existing and emerging plant health issues

Activity 1 – Implement field surveys and whitefly trapping to establish the presence of Begomoviruses and Potyviruses on target horticultural crops.
Activity 2 – Establish a target list of plant diseases for analysis with country specific focus.
Activity 3 - Establish partner country surveys to assess these diseases.
Activity 4 - Develop expertise for assessing the diversity of plant pests/pathogens within Thailand.
Activity 5 – Provide placement opportunities for partner country staff to visit Australian laboratories and extend their skill set in advanced diagnostics.
Activity 6 - Encourage partners to undertake post-graduate training in specific plant pathogens.

4) To develop a stratified set of diagnostic protocols for key plant pests

Activity 1 - Assess the utility of pathogen detection methodology, such as Loop-mediated amplification suitable for under resourced laboratories and adaptation to field-based application.
Activity 2. –Evaluate advanced molecular taxonomy for whitefly and applications to detect viruses from vectors in Thailand.
Activity 3 - Enhance the existing skills in RT-PCR within Thailand
Activity 4 - Assess the utility of card technology to capture and transport and process pathogen DNA for research and diagnostic purposes.

5 Methodology

General Strategy: This project has been shaped to deliver project milestones around taxonomic training activities and disease surveys as core activities. Instead of being delivered in a strictly linear fashion, we have integrated project activities so that milestones are delivered concurrently around these core activities. This enables us to repeat activities in different contexts thereby reinforcing the processes and operations in each of the participating countries.

For example, in conducting disease surveys we combine milestones for surveillance, diagnostic training, implementation of diagnostic protocols, the establishment of diagnostic networks, the use of mobile devices for surveillance, electronic data capture, specimen collection and storage and also touch on aspects of other milestones related to Pest Lists and Risk Analysis. By taking this approach we felt we could deliver activities, processes and concepts in a holistic manner that is more appropriate to the continuum of processes that make up diagnostic systems. Figure 1 shows the general pathway of activities and processes that have been applied to meet outputs and milestones.

This scheme contains a series of activities and processes that can be applied generically to other pests and diseases. By repeating these processes in the context of targeted crops and pests, we expect that staff in participating countries will develop the diagnostic practices needed produce a better diagnostic system that informs plant health and biosecurity.

A tool that is central to this approach is the software communication program called Pestpoint® which links the processes shown in Figure 1. Pestpoint® was developed by the Plant Biosecurity CRC and aims to provide a mechanism for the development of diagnostic communication networks where pest identification is a shared and collective process and where pest records and specimen details are stored to provide the chain of evidence required for higher level policy issues such as Pest Lists, Pest Risk Analysis, Area Freedom etc.

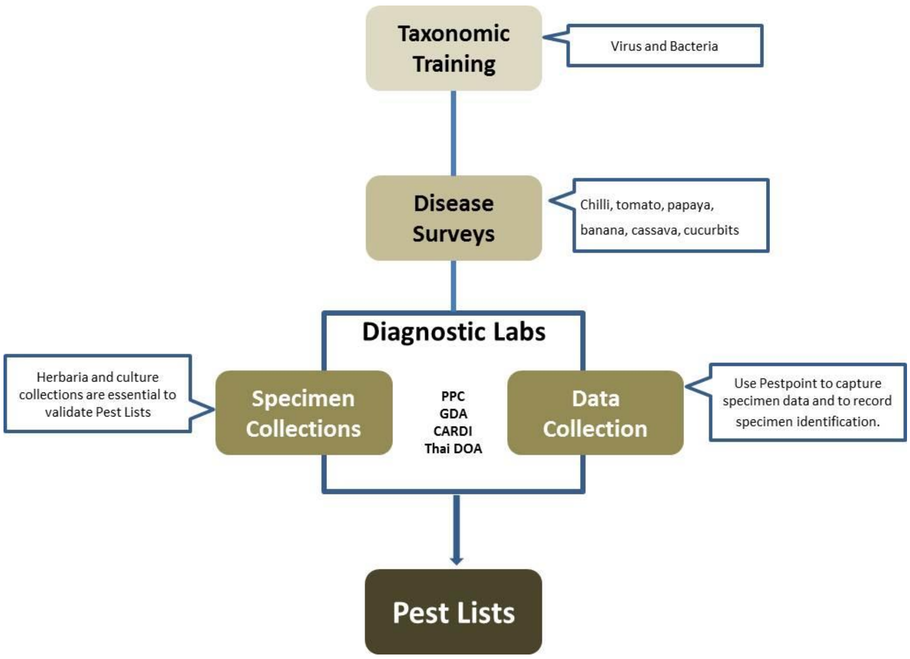


Figure 1. *Shows the sequence of operational activities undertaken in the project to achieve milestones and outputs.*

Mobile Devices and Networking Software: Pestpoint®® is used in core surveillance and diagnostic activities in the project. It provides a tool for recording pest information in the field using mobile devices and provides a communication platform for sharing pest information amongst the project groups in all three countries as well as Australia. Figure 2 shows how mobile devices are used to capture pest images and details in the field and using the Pestpoint® software to share with the project groups to obtain advice on identification and management.

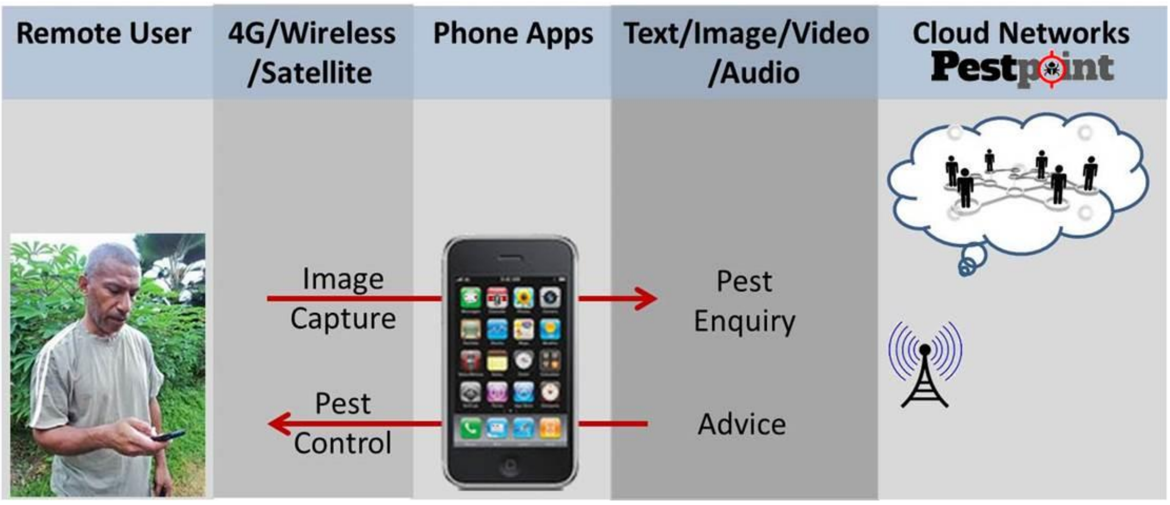


Figure 2. *Mobile devices use Pestpoint® software to capture and share pest information with the project group.*

At the beginning of the project, several workshops were conducted in each country to train staff in the use of mobile devices and the Pestpoint® software. We started the project with this training because these were the tools that would be required for recording surveillance and diagnostic information throughout the project.

The concept of remote diagnostics is a new way of doing things and requires that pest images and other crop information are captured and shared in a social network. This means that good diagnostic images need to be provided so that experts and group members can see the symptoms and the pest clearly. This kind of detail is essential for diagnosticians to assess the problem and propose the cause. However, diagnostics is often an iterative process where possible causes are eliminated according to the information at hand and so it is important that as much detail as possible is presented to the group in the Pestpoint® program. These iterations may occur during discussions within the group and as more information is requested and provided to the group. It should be pointed out, that this approach is focussed on making a pest management decision as quickly and as accurately as possible. It is not always necessary to complete a species, genus or even family identification in order to make a decision about pest control. Although preliminary identifications may be made at these levels in Pestpoint® for some pests and diseases, it is recognised that higher order definitive and confirmatory identifications will almost always require a specimen.

As I have indicated, the process of remote diagnostics is more complex than simply taking a photo of a pest and sharing it with a social network. Staff involved in the project needed to understand the underlying principles of diagnostics in order to provide information that is suitable for pest and disease identification. At the same time staff needed to know how to use mobile devices and wireless microscopes for capture images, because images are the primary source of information used in remote diagnostics. So these initial workshops focussed on teaching a lot of technical skills related to digital equipment, symptoms and diagnosis, photographic techniques, image capture and how to interact in a diagnostic social network. Skills and ability in these processes would be essential for staff to perform their pest and disease surveys, to capture pest and disease information, to record and manage their surveys, to complete the diagnostic processes and to make decisions about managing observed pests and diseases. By using the Pestpoint® program, staff were able to keep a continuous record for each pest and diseases collected, including images, comments from the group, specimen identifiers, diagnostic tests and procedures that were applied as well as recommendations for control - a complete chain of evidence for each pest in a single record.

By using Pestpoint®, staff would be able to replace notepads and transferring written information to computer, they could use a single device to capture GPS, images, host, pest and field details, share with colleagues and obtain advice on identification and control. They would be able to search collective records, exported records as a spreadsheet or express incidence/distribution of pests in a spatial map. Such host-pest records form the primary element in building towards documenting the presence and absence of pests and diseases within a country, of creating Pest Lists and providing the fundamental information required for pest management and trade policy.

Taxonomic Workshops and General Surveys: Following the implementation of mobile devices and the establishment of Pestpoint® networks, a series of workshops were conducted in which some taxonomic training was provided for two major disease groups – Bacteria and Viruses. These workshops provided general background about these groups including classification, symptoms and method of collection, preservation and identification. A number of general and specific protocols for diagnosis were developed and/or provided during the course of the project. In many cases, techniques and procedures that could be performed with existing laboratory equipment were taught and practised during workshops, however these often resulted in low level identifications, since bacteria and viruses require biochemical or DNA/RNA procedures for specific identification. Nonetheless, simple chemical tests, ELISA and disposable dipstick tests were introduced as preliminary steps in the identification process, enabling certain genera/species to be eliminated or confirmed.

As part of the taxonomic workshops, some general surveys were conducted to enable staff to improve their skills with the mobile technology and the Pestpoint® software, before moving to specific and more targeted surveys. During these workshops a number of local crops – chilli, tomato, eggplant, cucurbits and brassicas - were surveyed for pests and diseases. Data was posted to the Pestpoint® app from the field and specimens were collected for further diagnostic evaluation. Staff were encouraged to continue using the Pestpoint® app to document pests and diseases for their general day-to-day use, and to become accustomed to adopting this process. Australian project staff were then able to interact with PC staff between country visits by assisting with pest and disease identifications that PC staff posted to Pestpoint®.

Specific Surveys and Molecular Diagnostic Training: In recognising the need for molecular identification for plant viruses, selected staff were trained in molecular techniques in foreign labs, including Thailand and Australia (See Appendix I). Very few virus diseases have been officially recorded in Laos and Cambodia despite there being a widespread prevalence of viruses on most of the major production vegetable and fruit crops. In fact, it is likely that viruses in general impose the greatest constraints on production of any pest or disease, with many viruses being transmitted and spread by insect vectors. Therefore, improving capability in the molecular identification of viruses and insects generally would be of great benefit to PPO staff in participating countries. Techniques for molecular identification were taught by focussing on specific insect groups as well as viruses of specific crops.

Banana and Cassava became specific target crops for surveys for different reasons. In the case of cassava, Sri Lankan Cassava Mosaic Virus (SLCMV) emerged as an exotic pest in Cambodia during the course of the project, raising biosecurity concerns. In response to the immediate diagnostic needs for SLCMV, a GDA staff member was trained in Australia on both ELISA and PCR techniques so that the disease could be monitored locally by the

Sanitary and Phytosanitary Division in Cambodia and without the reliance on foreign agencies to conduct surveys and process samples. Disused ELISA facilities in the GDA lab were restored and re-commissioned to identify SLCMV from field samples collected during delimiting surveys of the disease. Mini PCI equipment was later added to the lab to extend its diagnostic capability. Additional PCR/RT-PCR training was provided to a number of PC project staff in Thailand for SLCMV, BBTV and a range of vegetable viruses. Thai staff from the Plant Quarantine Research Group (PQRG) assisted with virus surveys and training in molecular techniques in their labs in Bangkok.

Banana surveys were conducted in Laos in response to the need for a Pest List for banana and due to concerns relating to Chinese commercial banana production in Laos. No baseline data existed for the pests and diseases of banana so surveys were conducted in the production areas of the north and central provinces of Laos. All pest and disease samples were recorded in Pestpoint® and returned to the lab for identification, preservation and storage. Fungal and virus samples collected were prioritised for identification. Viruses samples were shipped to both Australia and Thailand where they were identified by staff from PC countries during training workshops for molecular techniques.

Molecular identification using barcoding techniques has now become common practice for many insect groups where taxonomic identification is difficult and/or rapid identification on a large scale is required. Given the difficulty of using traditional taxonomic techniques for the identification of both whitefly and mealybug, taxonomists from Thailand were trained in barcoding techniques in Australia. This training was done in conjunction with the Thai establishment of a specific lab for molecular taxonomy for insects. Whitefly taxonomist Sunadda Chaovalit spent one month learning barcoding techniques at the University of Western Australia with Dr Laura Boykin, whose team is conducting a study of the global characterisation of whitefly, funded by the Gates Foundation. Mealybug taxonomist, Chamaiporn Buamas, studied barcoding for mealybug and scale insects with expert, Dr Lyn Cook, at the University of Queensland. Molecular capability for identifying these two insect groups will be of benefit to the region and particularly to further study of viruses and their vectors in the region. The barcoding techniques that were learned have general application and so can be easily extended to other insect groups.

Subsequent to her training in Australia, Sunadda Chaovalit conducted a whitefly workshop for PC project staff. The workshop was held in Bangkok with the assistance of Dr Laura Boykin and Dr Kris Wyckhuys (CIAT) and comprised surveillance, traditional taxonomy and barcoding techniques for whitefly identification.

Molecular Diagnostics Capacity. Molecular techniques are now broadly used for the identification of most pathogen and pest groups. Some pathogens, such as viruses and bacteria are very difficult to identify using traditional techniques and require molecular identification. As a result of this difficulty and the lack of capacity for molecular diagnostics in Laos and Cambodia, these groups have been largely ignored in these countries, and the capacity to identify them almost non-existent. Whilst the project did not have a budget allocation for molecular equipment it seemed increasingly more important that both Laos and Cambodia should have some capacity for molecular identification. After discussions with the project team in Laos it was agreed that some of the country budget could be diverted to provide some basic PCR equipment. In Cambodia, basic PCR equipment was provided from the collaborating country budget.

Remote Microscopy: The use of remote microscopy (RM) for the identification of pests was trialled in a previous ACIAR project, however technical difficulties with internet and setup of the equipment were barriers to uptake of the technology. In addition, the rise of mobile devices and social networking applications provided an alternative means of performing remote identifications using still images. Nonetheless, RM can provide a secondary method for performing a remote identification when still images are not adequate. With the introduction of Cloud computing, we were able to overcome technical difficulties experienced with the initial RM systems, by developing a software application (RMPy) for live streaming directly to a Cloud server. This system greatly reduces the cost of RM as well as the technical difficulty. The concept of RMPy and Cloud integration for RM is shown in Figure 3 below. The image stream can be accessed in either the Pestpoint® software or from a dedicated website (www.Pestpoint®.asia)

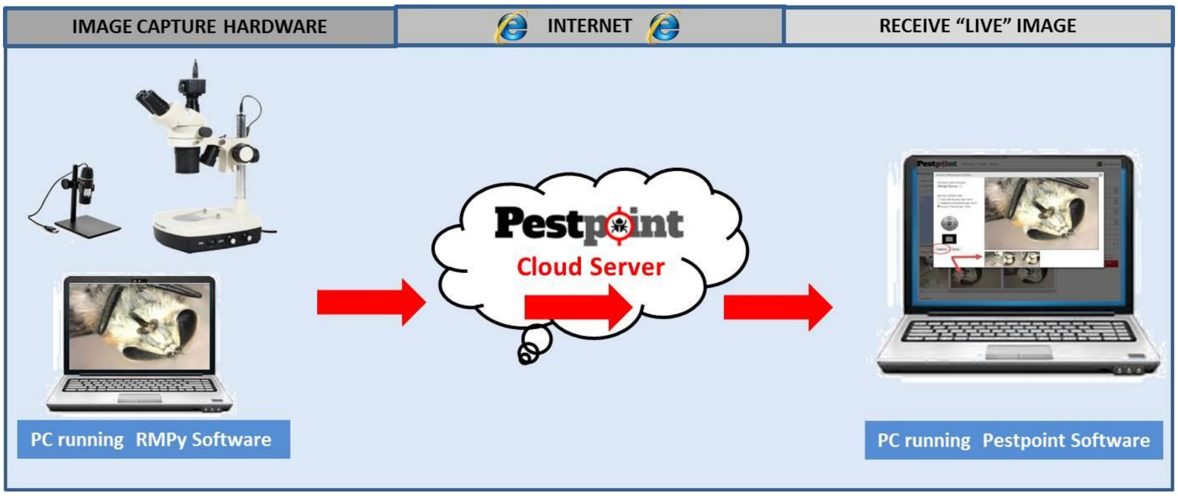


Figure 3. *This cheap and simple RM system uses RMPy software and a Cloud Server to share microscope images in real time over the internet.*

The RMPy system was installed in three major PQ ports in northern Thailand adjacent to Myanmar (Mae sai), China (Chaing saen) and Laos (Chiang Khong) as well as the two major southern ports of Laem Chabang and Bangkok. Plant quarantine officers were trained in the use of the technology and an RM network was established with taxonomic experts in Bangkok. Pestpoint® and Whatsapp were used for communication and recording of RM interactions between PQ stations and taxonomic experts in Bangkok.

Planning Meetings: Meetings for planning general project activities and strategies were held throughout the course of the project, between country project leaders, organisational managers and PC staff. Details of specific activities were regularly discussed by country project leaders to develop actions, roles and responsibilities and outcomes. Table 1 below shows an extract from an Activities- Action plan developed by staff during a project planning meeting. These plans were used to track completed activities and identify incomplete and future activities as well as assign responsibilities to those activities.

Table 1. *An example of the Activities-Action Plan developed by staff during project planning meetings. Extract from 2016 planning Meeting.*

THEME	ACTIVITY	ACTION	STAFF	LOCATION & TIME	RESPONSIBLE PERSONS	COMMENT
1. DIAGNOSTIC CAPACITY	**1.1 Taxonomic Workshops:**	**ACTION 1:** Concentrate on identification of virus and bacterial pathogens **ACTION 2:** Investigate CBS as a repository for mycological specimens http://www.cbs.knaw.nl/Collections/DefaultInfo.aspx?Page=Deposits Thailand to consider storage of bacterial specimens on behalf of Laos PDR and Cambodia. Australia to consider storage of virus specimens.	PPC;GDA;CARDI; RUA;PQRG	2016-2017	KC in Laos; SoS in Cambodia; PP in Thailand; GK & BS	Continue collection of virus samples and document in Pestpoint®. Virus identification activities planned. See1.2 and 3.3. Workshop techniques for collection and storage of whitefly and conduct rearing and transmission experiment.
		ACTION3. Conduct workshop for collection, rearing, virus transmission and preparation of whitefly specimens for molecular identification	PPC, PQRG, GDA, RUA, CARDI	2017	SS in Laos; SoS Cambodia;PP Thailand; GK Australia	Workshop techniques for collection and storage of whitefly and conduct rearing and virus transmission experiments.
	1.2 Disease Surveys:	**ACTION:** Conduct targeted surveys of specific pests in specific crops. For Cambodia a survey of Cassava crops to record the incidence and distribution of cassava mosaic virus. For Laos, possibly to survey the incidence and distribution of banana bunchy top virus (BBTV).	PPC;GDA;CARDI; RUA;PQRG	1. Cassava Survey Cambodia July 2016-2017 2. Banana Survey Laos Jan 2017	1. US and SoS in Cambodia; 2. KC in Laos	1. Cassava mosaic survey samples to be analysed using ELISA; Follow up surveys to be planned. 2.John Thomas/Kathy Crew to cnoduct survey of banana with KC and staff from PPC early in 2017.
	1.3 Pest Lists and PRA:	**ACTION:** Staff for PRA training to be identified and study on-line PRA material on the IPPC website. https://www.ippc.int/en/core-activities/capacity-development/training-material-pest-risk-analysis-based-ippc-standards/	To be selected	To be negotiated	TV Laos; US Cambodia; TBA Thailand	

6 Achievements against activities and outputs/milestones

Objective 1: To enhance the capacity of crop protection and plant quarantine staff in plant disease diagnosis and plant biosecurity

No.	Activity	Outputs/ milestones	Completion date	Comments
1.1	Conduct workshops for key pathogen taxonomies	1.Mycology workshop conducted X 2 2.Virology workshop conducted X 2 3. Bacteriology workshop conducted X 2 4. Nematology workshop conducted. X 2 PC & A	1&2 31/12/13 & 30/04/15 3&4 31/12/13 & 31/07/15	Workshops on virology and bacteriology only were conducted due to relevance of these pathogen groups to local production and the fact that training in Mycology and Nematology was already delivered by other projects managed by Dr Ian Naumann, DAWR. For a full list of taxonomic workshops that were delivered over the course of the project see Appendix I.
1.2	Training in and demonstration of biosecurity surveillance	1.Protocols established and documented. A 2. Aspects of biosecurity surveillance delivered during 1.1 A 3. Techniques utilised during the establishment of 3.1, 3.2 and 3.3 PC	30/06/14 30/09/16 & 28/02/17 31/01/17	Surveillance theory and practice delivered during training workshop. Template for documenting and planning surveillance activities developed for staff members. See Appendix II. Biosecurity surveillance delimiting surveys conducted in Cambodia in relation to SLCMV incursion on Cassava and in Laos in relation to FOC TR4 Incursion on banana. Techniques for planning, collecting, storing, documenting, identifying specimens were taught and applied by staff during all survey workshops involving field surveys with reference to specific pests and crops.
1.3	Conduct workshops in pest list establishment and pest risk analysis	Workshop delivered to PC collaborators	31/03/2017	Training for Pest List establishment is incorporated in all other workshops as part of the sample/data collection and pest identification processes. Publication of pest identified during the project will be the final stage in developing pest lists. Pest Risk Analysis was removed from the program because of delivery of training from other projects (DAWR) and from Thailand independently to this project.

PC = partner country, A = Australia

Objective 2: To establish knowledge based network capable of providing on-going training, diagnostic assistance, and local and regional crop protection / biosecurity information

No.	Activity	Outputs/ milestones	Completion date	Comments
2.1	Monitor and evaluate existing border security demonstration sites	1. Evaluation workshops scheduled with respective personnel. PC 2. Data accessed on usage, screening and captures. PC 3. Site visits by Government and private sector parties conducted. PC & A	30/06/2014 Partial completion Partial completion	Although PQ staff were trained in the use of equipment and Pestpoint® software to report intercepted pests to PPO staff for identification, there has been little activity from the PQ groups in Laos and Cambodia because of staff changes and a lack of cohesion from within the groups. PPOs are aware of the problems and has plans to re-train and re-establish the networks, however, PQ is administered by a different department to PPO so there are command difficulties. In contrast, a Pestpoint®® network was established for Plant Quarantine and PPO in Thailand and our new remote microscopy processes introduced to key border stations at Mai sai, Chiang Saen and Chiang Khong in northern Thailand as well as southern ports of Bangkok and Laem Chabang. Thai PPO is committed to using the network for communicating expertise with PQ officers.
2.2	Demonstrate and establish, local networks of Pestpoint® in each PC	1.Pestpoint® training completed. A & PC 2. Pestpoint® network established. PC 3. Survey data entered to Pestpoint® networks. PC	30/07/2014 30/07/2014 31/03/2017	Staff from Laos PPC, Cambodia GDA, RUA, CARDI and Thai DOA received training in the use of Pestpoint® to document report and share pest information. Networks for each country and one collective for all countries were established in Pestpoint®. General as well as specific pest and disease surveys were conducted throughout the project on horticultural crops, with a focus on virus diseases of banana and cassava.

2.3	Design and pilot a Smartphone surveillance system in each PC and A	1. Survey application reviewed and selected. A 2. Handsets reviewed and selected. A 3. Training in app use and data collection delivered. A 4. Data collection completed. PC 5. System reviewed. A & PC	30/09/14 30/09/14 31/10/14 31/01/15 & 31/03/17 30/06/16	Pestpoint® software was selected for use in the project to document and record all pest and disease survey data. Ipads were provided to run the software and for use in the field to capture pest data. Training in the use of software and hardware for data capture and network sharing was provided to all PC staff. See 2.2.1. Data collection has been ongoing in the project since July 2014 with all records of pests and diseases now saved to Pestpoint® and available as CSV files and spatial maps.
2.4	Establish a network of researcher, border staff, agronomists and farmers to conduct field and commodity surveys	1.Consultation process and selection of participants completed PC 2. Training delivered in survey system in conjunction with 2.3.3 PC & A 3. Field surveys conducted and results collated PC		Established formal diagnostic networks both within and between partner countries and institutions and documented in the Pestpoint® software. Direct inclusion of farmers in network was not practical because of technology and language challenges. Instead PPO staff provided the link with farmers through the use of Pestpoint®. Changes in the software application will remove the reliance on particular hardware, making deployment easier and more far reaching In conjunction with 1.1, data has been collected on viral pathogens in both Laos PDR and Cambodia on a range of horticultural crops and specifically on banana and cassava and chilli. Thailand have conducted independent surveys to detect specific viruses in-country.

PC = partner country, A = Australia

Objective 3: To enhance research capability for analysis of existing and emerging plant health issues

No.	Activity	Outputs/ milestones	Completion date	Comments
3.1	Establish the presence of begomoviruses & Potyviruses on target crops over two growing seasons	1.Survey approach designed in consultation with PC 2. Surveys of crops conducted by PC 3. Collected samples analysed in Australia	30/06/2017	A template for planning and conducting pest and disease surveys was developed for PC staff to use . See Appendix II General surveys of vegetable crops conducted in all PCs with specific surveys being conducted on Banana in both Laos and Cambodia with addition of cassava in Cambodia due to outbreak of SLCM.

		4. Diagnostic protocols deployed in Thailand	30/04/2017	Banana, Cassava and vegetable crop virus samples from Laos surveys analysed in Australia. Begomovirus, Tospovirus, Potyvirus etc protocols deployed in Thailand during analysis of various crop samples.
3.2	Establish target lists of plant diseases for each PC	1. Target crops identified 2. Pests of concern identified. 3. Target lists distributed	31/07/2014 31/07/2015 31/07/2015	PC nominated crop lists & identified key pests. These have been used to initiate survey lists within Pestpoint®. Viruses and bacteria identified generally as pests of concern across all crops. Whitefly also recognised as important vector of virus. Target crops for general surveys and diagnostic training include vegetables; Specific targets include banana in both Laos and Cambodia with Cassava becoming a target in all PCs due to the outbreak of SLCMV in Cambodia.
3.3	Establish field surveys for target pests and diseases for each PC	1. Field surveys designed in conjunction with PC 2. Disease assessment keys for pests of concern designed. 3. Diagnostic protocols and procedures established. 4. Surveys of target crops conducted and samples collected appropriately. 5. Diagnostic procedures to verify pest identity completed	30/03/2017 30/06/2017 28/02/2016 31/07/2017 20/09/2016 30/04/2017 30/09/2017	Specific surveys for banana and cassava were designed and conducted by PCs and conducted in specified locations. Data captured in Pestpoint®. No applicable due to the nature of surveys conducted. Eg severity not measured; incidence/distribution surveys only were conducted. Specific surveys to target SLCMV of cassava in Cambodia and BBTV and FOC TR4 conducted over two seasons Various protocols used to identify specific viruses affecting a number of crop species. Including ELISA, Specific Primer, RT-PCR, LAMP, RCA.
3.4	Develop diversity assessment skills in Thailand	1.Samples from 1.3 identified for further assessment PC 2. Procedures and protocols for assessment identified. A & PC 3. Training in protocol application provided. A & PC 4. Independent assessment of samples conducted in Thailand and Australia A & PC	31/03/15 31/07/16 31/03/15 31/07/16 30/04/2017 30/09/15 31/07/16 30/04/2017 30/09/2017 30/09/2016 30/04/17 30/092017	Specific samples were selected from hundreds of virus samples collected during crop surveys using symptom x host x probablilty criteria in consultation with specialist virologists from Australia . Specialist virologists and project staff contributed to selection of protocols for general and specific virus and bacteria identifications. Selected staff were trained in multiple workshops in bacterial and virus strain identification using various protocols and molecular methods. Selected samples were assessed in workshops conducted in Thailand and Australia molecular labs involving specialist virologists as well as PC staff from each country.
3.5	Provide placement in	1.Collaborators from each PC	30/17/2016 30/10/2016	Placement of Staff from Cambodia, Laos and Thailand in Australian labs for

	Australian laboratories for skills development	placed in Australian laboratories 2. Reports by PC collaborators completed. 3. Future research needs identified	30/06/2017 30/09/2017 30/09/2017	molecular taxonomic training to date includes training for ELISA, PCR techniques for pathogens and barcoding for insects including whitefly, mealybug and scale insects. Competence in molecular identification achieved for general detection and specific detection of some pathogens and whitefly vectors. Need to continue developing molecular skills and building on equipment for molecular analysis in each PC and continued support relationship with Thailand for diagnosis and molecular analysis.
3.6	Encourage post-graduate training in specific plant pathogens	Applications for Masters and PhD scholarships submitted by at least 2 PC collaborators each year.	30/09/14 & 30/09/15 & 30/09/16	Chamaiporn Buamas (Meaylbug /scale taxonomist) from Thailand received JAL but failed to pass IELTS. Khonesavanh Chittarath applied for JAL but unsuccessful, but she received a Crawford Fund scholarship to train in Australia. We have difficulty in persuading PC staff to apply for scholarships. Generally due to their belief that English skills are not sufficient, are not confident of their release from the workplace and are reluctant to leave family.

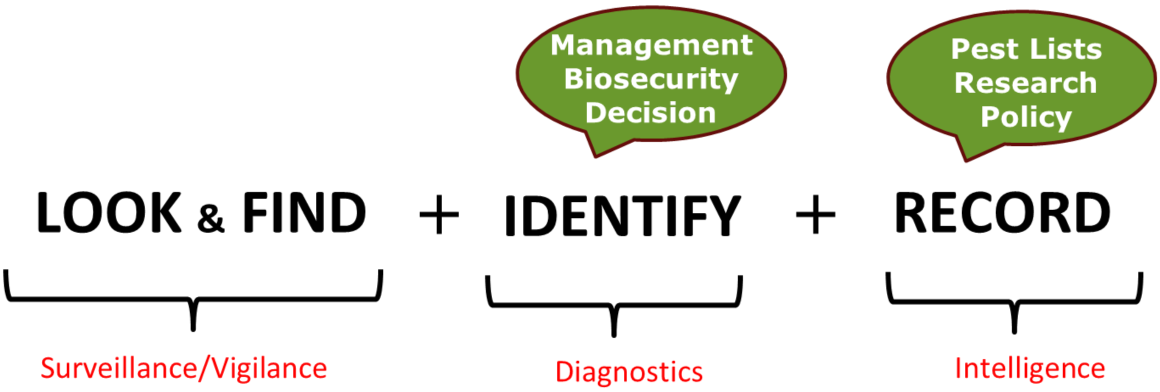
Objective 4: To develop a stratified set of diagnostic protocols for key plant pests.

No.	Activity	Outputs/ milestones	Completion date	Comments
4.1.4	Assess suitable pathogen detection systems for under resourced laboratories PC & A	1. Review of literature completed and recommended protocols selected. A 2. Selected protocols tested and adjusted as required. A 3. Protocols documented and training program established. A 4. Protocols deployed and tested. PC	30/07/2015-30/09/2017 30/07/2016-30/09/2017 30/07/2016-30/09/2017 30/06/2017	Existing protocols from CSU include Bact-ID for bacterial pathogens; LAMP detection assays for viral pathogens; PCR and RT-PCR acquired from Thai collaborators; ELISA and viral PCR and cloning techniques provided by Australian virologists. Protocol testing and adjusting was conducted during the analysis of specific samples and during training workshops when identification fails or is obscure. Protocol adjustment was part of the training process in helping understand the fundamental techniques. All protocols available in digital format r to all PC staff involved in training. Basic PCR lab established in Laos and Cambodia. ELISA for SLCMV established in Cambodia. Specific protocols to detect SLCMV and BBTV deployed together with a suit of additional gene targets for a range of viruses.
4.2.2	Enhance the skills in RT-PCR within Thailand	1. Selection of target organisms made from those identified in 3.3. PC & A	30/07/2016-30/09/2017	A selection of virus samples from various vegetable crops including Cassava and banana were analysed in various training workshops where various techniques were applied to identify pathogens.

		2. Training delivered in sequence analysis, primer design and RT-PCR protocols in conjunction with 3.5. PC & A	30/04/2017	A workshop was conducted in Thailand in April 2017 in the application of RT-PCR for virus samples collected in surveys from Laos, Cambodia and Thailand during the previous two seasons. Sequences were obtained from commercial service provider for analysis against type specimens.
4.3	Assess card technologies for capture, transport and processing of pathogen DNA for R&D activities, PC A	1. Consultation with AQIS PBCRC and PHA completed for importation of card samples. A 2. Sample collection completed in two growing seasons (see 3.3. PC 3. Diagnostic protocols completed and documented for deployment. A	31/07/2014	Approval for importation of genomic DNA on Whatman FTA cards achieved. Consideration given to storage that avoids irradiation treatment as this method disrupts DNA of viral circular structures so they cannot be analysed using PCR techniques. Card technologies found suitable for purified organisms. Some difficulties encountered with direct plant application. The techniques seems to be variable and depends on the type and quality of the sample. However some success with samples returned to CSU laboratories in Australia. Attempts also to use card technologies with bacterial samples and ethanol based preservation of viral samples are underway. Protocols to be documented as soon as possible and catalogued with molecular protocols in digital form.

7 Key results and discussion

The aim of this project was to *enable* improved biosecurity practices in Cambodia, Laos and Thailand. As stated in the background to the project, biosecurity is a continuum that starts with surveillance activities whereby pests are found, observed and intercepted for the purposes of identification. In an agricultural production setting, the outcome of these activities might be to apply a control measure to minimise damage and crop losses. In a plant quarantine setting, it might be to facilitate trade and protect against the introduction of exotic pests. In both instances, the purpose of surveillance and diagnosis is to identify the pest or disease and make a decision about what action to take. The identification and diagnosis are critical to the decision, because the type of pest or disease identified will determine what action should be taken in relation to the pest. Recording the details of the pests and diseases that are found, provides evidence of their presence or absence which can be used to verify Pest Lists, inform research and formulate biosecurity policy. Figure 4 below summarises these processes and shows the progression of activities that support biosecurity practices.



This project therefore set out to build capability (skills) and capacity (skills x resources/ processes) for the key biosecurity practices of surveillance and diagnosis - the LOOK and FIND part of the equation. Training workshops were designed to improve capability in surveillance and diagnostics over the period of the project. These involved workshops in which basic taxonomic training was coupled with field surveys, sample/specimen collection and preservation, diagnostic testing and the process of recording/documenting each of these steps (see Appendix I). Evidence that PC staff advanced their skills in these diagnostic processes can be found in the pest and disease records that have accumulated throughout the project. These records can be found in the Pestpoint® software used to document the pests and diseases that were found during surveys. They show when, how and where surveys were conducted and link individual pest and disease records to the physical specimens that were collected. Each record is linked to an individual within a group and so can reveal the level of competence individuals show in presenting diagnostic information and, where there is group interaction, their capability in working through the diagnostic process to identify the problem.

By using Pestpoint® as a tool to capture and store pest and disease information, PC staff were able to keep track of their specimens as a single record, providing a continuous process for documenting the diagnostic path for each specimen. In biosecurity terms, this would be regarded as keeping a chain of evidence for each pest or disease. Completed Pestpoint® records can then be used as reference documentation for physical specimens, diagnostic processes and protocols, diagnostic verification, pests and disease incidence data and contribute to the establishment of Pest Lists within each participating country. These represent the RECORD part of the equation which provides information and knowledge that is vital to research, management and biosecurity policy. The panel below shows an example of a Pestpoint® record of a disease of coffee that was collected and identified by a network member after completing a laboratory isolation and examination of the pathogen. Note this image shows the Pestpoint® summary of the record only. The complete record can be expanded to show all the specimen details. The record was initiated in the field using a mobile device and the Pestpoint® software. The convenience of being able to start recording specimen details in the field and to immediately share the record with a group of people provides PC staff with a powerful communication and recoding tool and a process that is more efficient and more effective than previous practices.

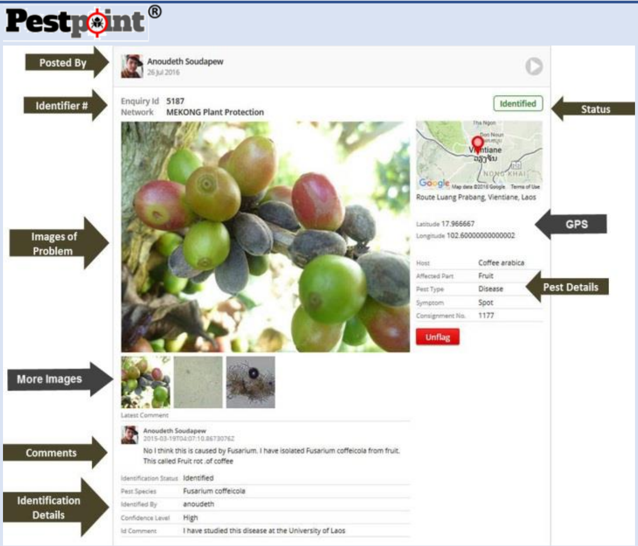
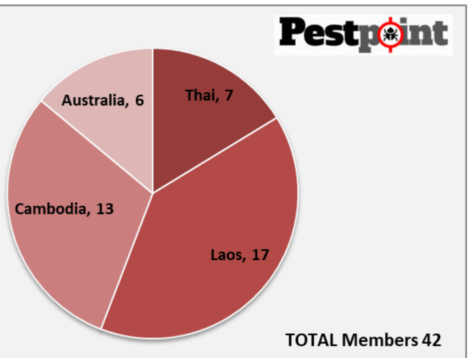


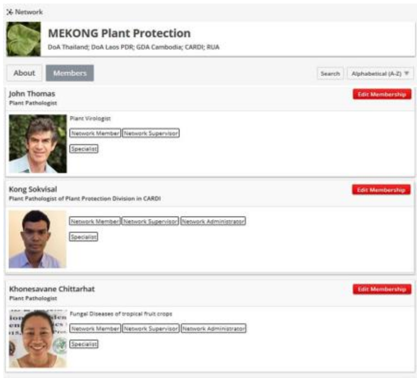
Figure 4. *Where record keeping was previously sporadic or non-existent, Pestpoint® now provides a system for documenting the identification process and the incidence of pest specimens and provides evidence of pest status to inform biosecurity and plant protection. Staff are using mobile devices and software to capture pest data in the field.*

Left: *Shows a summary of a Pest Enquiry record from Pestpopint. Details in the summary include the name of the person who posted the enquiry, images of the pest, its identification status, the location GPS and pest details including host, symptoms, pest type and consignment or sample number.*

PC staff needed to learn many new skills before records such as the one above could be produced. Training in the use of mobile devices, image capture for diagnostics, identifying and evaluating symptoms, performing a causal investigation, using mobile devices and software, were all steps along the way to engaging staff in the remote diagnostic process. Integral to this was the establishment of a virtual network in the Pestpoint®® software, where PC staff could document pest problems. The Mekong Plant Protection Network was established as a shared digital space where PC staff could collectively solve pest identification problems. Figure 5 shows the number of members in this network from each PC. The Pestpoint® members directory details the expertise, role and permissions status for each member.

Figure 5. *The number of staff from participating countries who belong to the Mekong Plant protection Network in Pestpoint. Far Right. A sample from the Members Dirrectory*

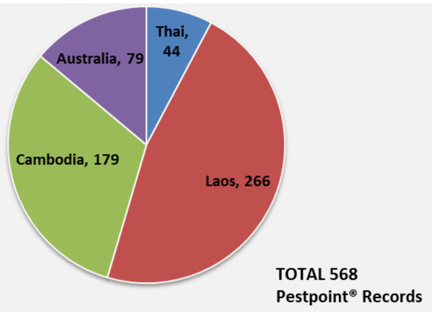




In developing the skills required for performing remote diagnostics, PC staff used Pestpoint® and digital devices to conduct a number of general pest and disease surveys. During these surveys, 568 pests and diseases were recorded on a range of crops. Figures 6 and 7 show the numbers of pest and disease enquiries posted to the Mekong Plant Protection Network until July 2016.

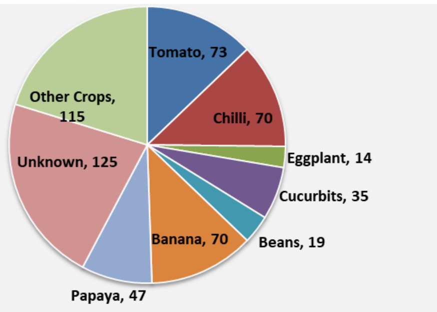
MEKONG Plant Protection
DoA Thailand; DoA Laos PDR; GDA Cambodia; CARDI; RUA

Figure 6. *Shows the relative number of pest enquiries submitted to the Mekong Plant Protection network by country over the past year*



MEKONG Plant Protection
DoA Thailand; DoA Laos PDR; GDA Cambodia; CARDI; RUA

Figure 7. *Shows the number of pest enquiries submitted to the Mekong Plant Protection network by crop over the past year.*



These general surveys allowed staff to become comfortable with the process of conducting surveys, using Pestpoint® to capture, share and discuss pest problems while at the same time identifying and documenting local pests and diseases. As staff became more competent, they applied the same processes to more targeted surveillance. These included biosecurity issues in their respective countries - banana in Laos and cassava in Cambodia. The results of these surveys and the diagnostic activities associated with them give an indication of the abilities of PC staff in conducting biosecurity activities related to pest and disease detection.

Pests of Banana in Laos: Surveys were conducted in northern Laos to determine a baseline for pests and diseases associated with commercial plantation bananas (Cavendish or Hom varieties) and local wild and village bananas (Nam varieties). The purpose of the survey was to identify pests and diseases already present in Laos which could serve as a basis for developing a Pest List to inform trade and Plant Quarantine agencies. Pest surveys of banana had not been previously conducted in Laos and so no Pest list was available. The map below shows the area surveyed and the diseases that were identified from field symptoms. Samples were collected from all sites and returned to the laboratory for confirmatory identifications. Very few insect pests were found during the survey, largely because of the heavy insecticide use in commercial plantations (monocultures). (Overuse of chemicals in these plantations has been the subject of a recent government inquiry.)

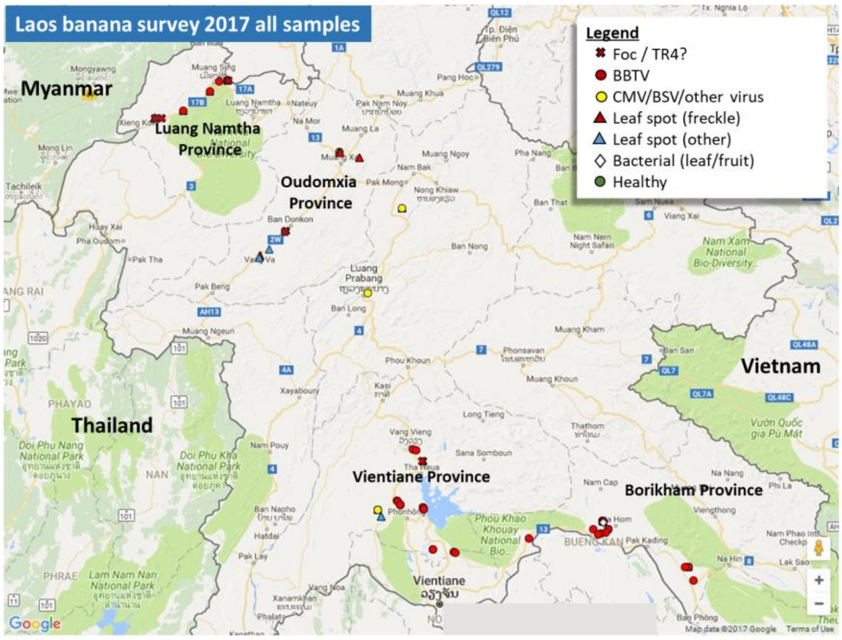


Figure 8. *Map showing suspected diseases and their locations in northern Laos provinces. (map by Dr Kathy Crew, QDAF)*

Banana Bunchy Top Virus (BBTV) was the most common disease found among Hom and Nam varieties, followed by other viruses and a range of leaf spots. Fifty-two suspected BBTV samples were collected from Northern and central Laos. These were identified using molecular techniques during molecular training workshops held in Thailand and in Australia when Laos and Cambodian scientists were embedded in Dr John Thomas' lab at the the EcoSciences Precinct in Brisbane.

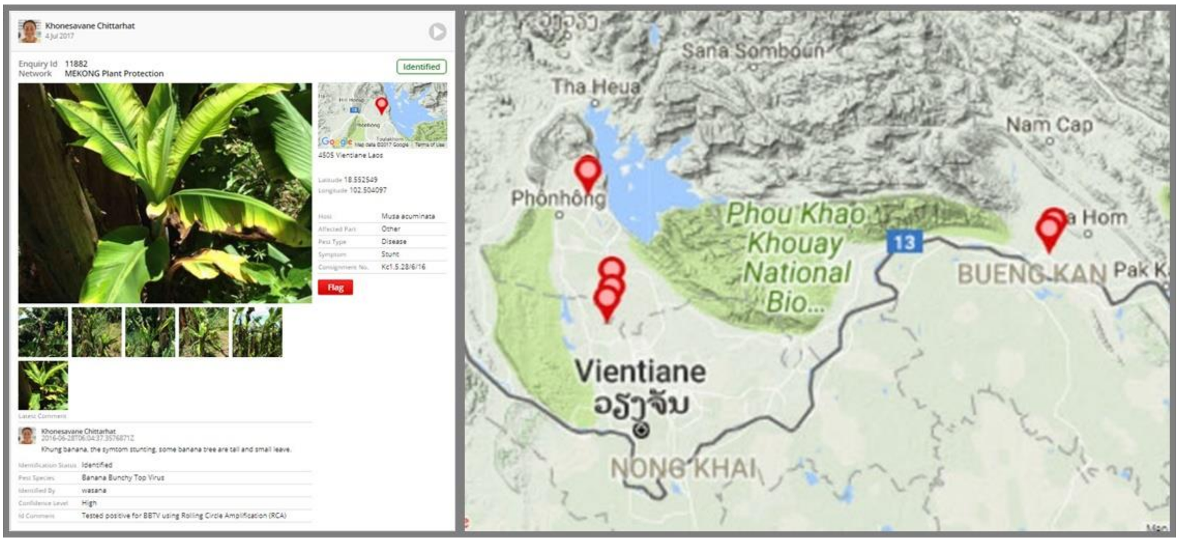


Figure 9. *Left. Pestpoint® BBTV record #11882. Right. Locations of BBTV samples confirmed using molecular techniques.*

BBTV is the most important disease of banana worldwide. Its centre of origin is in SE Asia, however two distinct groups are recognised - the SE Asian Group of strains found in China, Indonesia, the Philippines, Vietnam, Cambodia and Thailand, and the Pacific Group of strains found in parts of the Pacific, India, Pakistan and Africa. Strains in Myanmar appear to be intermediary to the two groups. Given the global separation of these groups it is important to monitor their spread and to ensure that the groups do not cross into new areas where they can cause concern to banana production. It is possible that the different strains can recombine to produce new strains, however the impact of this, if any, is largely unknown. It is therefore important that authorities in the region have the capability to monitor and evaluate BBTV and to share information that could be of national and regional importance.

The presence of Fusarium Wilt (FOC) was of interest in cavendish (Hom) varieties in Laos because Cavendish is resistant to the common Race 1 of FOC but susceptible to the devastating Tropical Race 4 of FOC. Samples collected from Chinese plantations near the border with Myanmar and also near Vang Vieng, Vientiane Province, have been confirmed as FOC/TR4. This finding was reported to the appropriate Laos authorities and subsequently published in Plant Disease as a first report.

The disease is quite widespread in the Chinese plantations and is causing severe damage in some areas of the plantations. Farm managers did not know the cause of the wilt and therefore had taken no steps to ameliorate the problem. As a consequence irrigation water was being recycled and machinery moved between infected and non-infected areas, thus spreading the disease. In addition, these plantations are located close to a major water course which could serve as a means to quickly transport the disease downstream. Upon detecting TR4 in plantations hundreds of kilometres further south in Vientiane Province, we learned from plantation staff that tissue culture plantlets had been used as propagation material but that these had been potted into soil from the northern plantations and transported to the plantations near Vang Vieng.

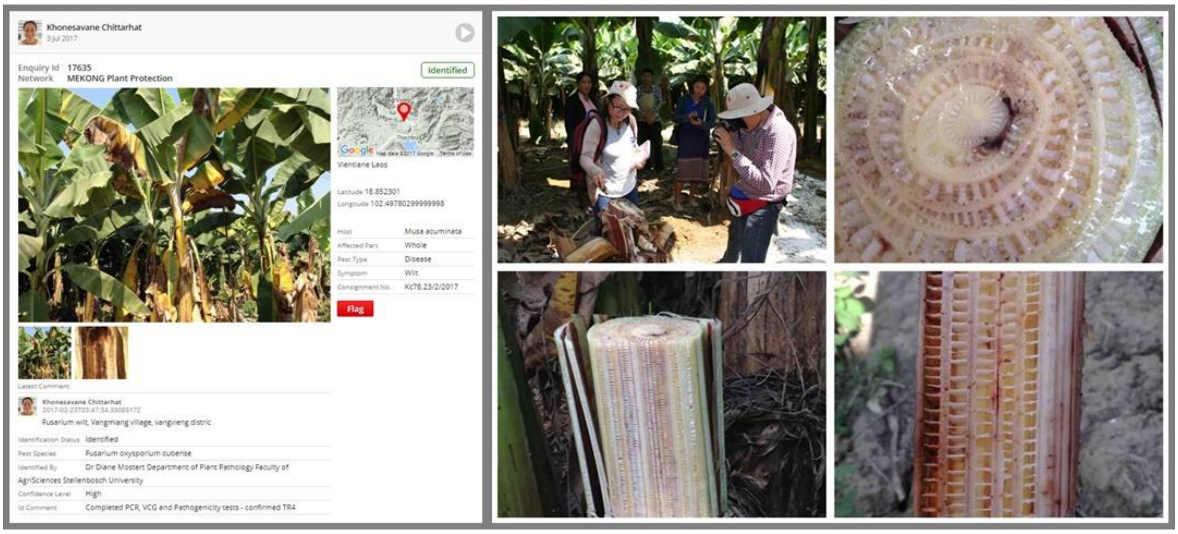


Figure 10. *Left. Pestpoint® record # 17635 of FOC affected plants in a Chinese plantation near vang Vieng, Vientiane Province. Right. Survey team inspect and expose vascular symptoms in wilted banana plants.*

It is likely that the unregulated introduction of banana planting material by Chinese operators into Laos may have resulted in the introduction of TR4, however without previous baseline surveys, it is difficult to know this for sure. Subsequent unregulated movement of planting material may be responsible for the rapid spread of the disease, but again this is difficult to prove due to a lack of prior information. Regardless of their being proof, FOC TR4 is a newly identified disease in Laos as a result of this work. Neighbouring countries should now be informed of the presence of FOC TR4, particularly where Chinese or other foreigners have established agricultural operations and may not be aware of the pest risks or local regulations.

SLCMV Surveys: SLCMV surveys were conducted by PC staff in Cambodia and Thailand. The disease was first detected in Cambodia at the commercial farm run by Chinese company, Holley Farms. Based on symptoms, staff showed that the disease has spread only a short distance from the original point of detection and was not detected in plants from Pursat province in western Cambodia and nor was it detected in surveys conducted in Thailand. The maps in Figure 10 were generated form pest records in Pestpoint®® and show the spatial distribution of SLCMV determined from delimiting surveys. These were tentative records that were later verified using PCR analysis. Figure 11 shows a the spatial distribution and incidence of SLCMV in Cambodia near its point of origin.

Figure 11. *Pestpoint® spatial maps generated from survey data showing locations of suspected SLCMV in relation to Holley Farms (indicated by the data flag) where the disease was first recorded.*

Figure 11 shows a Pestpoint® record of SLCMV from Holley Farms, showing the symptoms and subsequent PCR analysis that indicates the plants specimens from Pestpoint® Enquiry record # 11536 were infected with SLCMV.

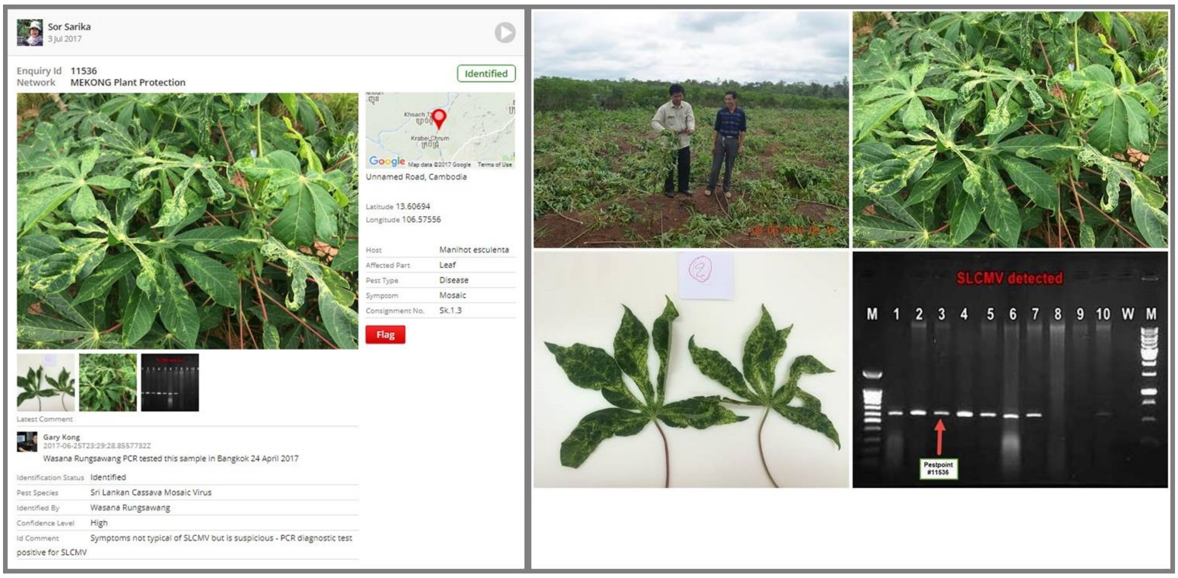


Figure 3. *Left. Pestpoint® Enquiry #11536 Record Summary of a cassava sample from Holley Farms in Ratanak kiri province. Right. Images from the Pestpoint® Enquiry 11536 showing symptoms of SLCMV and the amplification band (arrowed) that indicates a positive PCR reaction for SLCMV.*

SLCMV is a serious pest of cassava and can have a high impact on yield. Management of SLCMV will require that farmers and PPO staff can detect and identify the disease and apply strategies to limit its spread. This will require ongoing surveys to monitor the spread of the disease. Strict regulations should be put in place for the movement of planting material to limit spread. All of these activities require that the local authority has the ability to conduct surveys and identify the disease. The symptoms of SLCMV can be difficult to separate from symptoms caused by other pests and disorders, therefore it is essential that suspected infections are analysed with proper diagnostic tests.

Increasing Diagnostic Capacity: The ongoing identification of many pests and diseases in the PCs will require that staff have skills in molecular identification, access to relevant diagnostic protocols as well as equipment, such as PCR, and ELISA. During the course of the project, selected staff were trained in molecular techniques on multiple occasions, at locations in Australia and Thailand (see Appendix I). In most cases, PC staff applied these techniques for the identification of plant viruses using their own samples, collected during country surveys. Additionally, two Thai taxonomists were trained in molecular barcoding techniques for the identification of whitefly, mealybug and scale insects, again using their own specimens collected in the PCs. Basic PCR and ELISA equipment was installed at the labs of the PPC, Laos and the GDA, Cambodia, so that the PCs could become self-reliant in molecular identification. The protocols used for molecular identification during the training workshops have been made available to relevant PC staff. Selected protocols will be validated by PC staff during the coming months under the project variation that has been granted to Dr Ben Stodart of CSU. This process will complete the training and establishment of basic molecular diagnostics in both Laos and Cambodia. Thailand has advanced molecular capability and will assist Laos and Cambodia in establishing their molecular capacity.

8 Impacts

8.1 Scientific impacts – now and in 5 years

Diagnostic systems are fundamental to biosecurity services and are comprised of many components that are connected in a dependent manner. They are complex and require high levels of organisation, and rely on knowledge, skills, documented processes, technology, and other physical resources. At the heart of the system is the ability to detect and identify pests and diseases. All other parts of the system are dependent on these two processes, commonly known as surveillance and diagnostics.

The skills, resources systems and processes that are required to perform surveillance and diagnostics are weak in Laos and Cambodia and inadequate to comply fully with the SPS required to support trade and plant health. In this project we therefore focussed on strengthening the processes and resources needed to support surveillance and diagnostics to a greater extent, and to direct these processes towards the development of Pest Lists. Having Pest Lists for specific commodities is a key goal for countries wanting to expand trade. These lists are used to support export and import requirements for trade and for plant quarantine and are therefore vital for both protecting and supporting agricultural industries. Developing Pest Lists requires the application of multiple processes and therefore requires a high level organisation, skill and resources. By improving the PCs capacity to perform these processes, we believe their biosecurity system will be strengthened.

A key element of good diagnostic processes is collecting, organising and storing information on pests, starting with the initial field data and working through to the identification and specimen curation stage. Being able to track any single specimen from collection to storage is desirable, however, data collection and management is often limited in developing countries. We have improved the process of data collection and storage by introducing mobile devices that use the Pestpoint®® software. This system not only enables users to enter data anywhere along the diagnostic pathway, but allows users to form communication networks where they can share and discuss pest problems. As a result, staff in PCs can access expertise beyond their own organisations to a regional and even international level - it increases the diagnostic capability within these countries without having to train and employ new people. At the same time this process disseminates knowledge and facilitates learning because of the interactive nature of using a social network for diagnostic purposes. An important output of the system is the automatic documentation of pests and disease which can form the basis of Pest Lists and provide valuable information for the management of pests and diseases at a research context and at the farm level. From a biosecurity perspective, Pestpoint® provides a private and secure environment for sensitive pest information as well as keeping a “chain of evidence’ in relation to every pest and disease problem that is reported and documented.

So in the context of the above, the project had the following scientific impacts:

1. **Remote Diagnostics:** Improved system for capturing surveillance data using mobile devices and wireless microscopes to capture images and record pest and disease details in real time from the field.
2. **Improved Diagnostic Capability:** The use of Pestpoint® improves the “suspect” and “preliminary” identification processes for pests and diseases using remote diagnostics in a virtual environment and accessing a wider range of experts.
3. **Improved Diagnostic Capacity:** Molecular skills development together with the establishment of basic molecular labs in Laos (PPC) and Cambodia (GDA) improve the ability to make “confirmatory” identifications for pests and diseases.

4. **Better surveillance operations.** Improved methods and processes for conducting pest and disease surveys were developed by the PC staff that included guidelines and templates for planning and conducting surveys.
5. **New Baseline Data for Pest Lists:** 1-4 above resulted in the documentation and in many cases, identification of endemic pests and diseases that were not otherwise officially recorded in the PCs.
6. **Greater Biosecurity Awareness:** PC staff investigations of exotic disease incursions in relation to Chinese commercial operations in both Laos and Cambodia raised awareness of procedures required for delimiting surveys and regulatory obligations.
7. **Pathogen Storage:** Agreements were established with Thailand and QDAF Australia for the long-term storage of bacteria and virus specimens respectively.
8. **Remote Microscopy:** Capacity for remote microscope identification has been established at five major Plant Quarantine ports in Thailand. Remote microscopy will provide a secondary form of remote diagnostics at these ports with Pestpoint® being used as the primary method of communicating with taxonomists in Bangkok.

This project has introduced systems, processes and developed patterns of behaviour for surveillance and diagnostics that will be maintained into the long term. We believe this is because the framework that we have proposed during this project has resonated well with both PC staff and their managers. The use of Pestpoint® as the primary tool for surveillance and diagnostics operations beyond this project has been discussed at a management level with the PCs, and we expect uptake to follow as commercial trials get underway early in 2018. This will allow the networks, relationships and co-operation developed for diagnostics in this project to continue into the future as Pestpoint® will offer the means for maintaining these linkages and will facilitate co-operation. With this, PCs will not only maintain and improve their knowledge and expertise in diagnostics, but they will continue to document pests and diseases and contribute to both national and regional data.

8.2 Capacity impacts – now and in 5 years

The ability to monitor, detect and identify pests and diseases has critical implications for protecting against the introduction and spread of new pests and diseases, formulating policy and regulation to manage pests, developing trade and food security. Diagnostics and surveillance capability are prerequisites to establishing a plant health system, underpinning agricultural production systems and economic development.

Pest Surveillance: In completing a number of survey tasks, staff in Laos and Cambodia have shown that they now have the skills and knowledge to plan and conduct surveys for biosecurity purposes and are able to support and validate pest detections with better data capture procedures (Pestpoint®), improved laboratory practices and diagnostic techniques. Selected staff from Cambodia, Laos and Thailand were trained in specific molecular identification techniques in laboratories in Australia and Thailand. During their training candidates worked on specimen samples collected during project surveys, ensuring that both the training and the content of workshops was relevant to both their local pests problems and to achieving project outcomes. Moreover, collaboration within and between the participating countries has benefited from joint workshops, shared surveys and the communication network fostered by the Pestpoint® program. As an integral part of the project, Thailand has taken on a mentoring role in these activities by providing training, expert support and access to their molecular laboratories in Bangkok.

Diagnostics and Data Capture: In addition to planning and completing the pest surveys, staff were trained in sample and data collection, as well as techniques for identifying specific pests of biosecurity interest, including SLCMV, Banana Bunchy Top Virus (BBTV) and *Fusarium oxysporum* fsp *cubense* TR4. Basic ELISA and PCR equipment has been procured and installed in labs at GDA, Cambodia and the PPC, Laos where trained staff can now process SLCMV or BBTV samples collected during surveys. FOC samples were isolated by PPC staff and pathogenicity testing conducted to confirm Koch's Postulates. FOC samples were subsequently identified by Professor Altus Viljoen at Stellenbosch University, who is part of a consortium for FOC TR4 which includes FAO, IITA and Biosecurity International. Joint publication of the FOC identification in the journal, Plant Disease, will foster collaboration with professor Viljoen's group who will provide ongoing support for the identification of FOC TR4. In time it is hoped that preliminary PCR identification of FOC TR4 can be completed in country.

In addition to documenting pests observed during surveys, Pestpoint® was used by PPO staff throughout the year to document general pest problems across a range of crops. Through Pestpoint®, we are able to continuously and remotely engage with staff and networks in the identification of local pests and pest problems. This allows us to maintain engagement when we are not in the country and to gauge the level of diagnostic competency for each staff member and more broadly, for the groups in each country. Overall, we believe that both capability and capacity of staff have improved in relation to many of the surveillance and diagnostic processes previously outlined.

Specimen Storage and curation: Maintaining type specimens to verify the presence of pests and disease in a country, is critical to establishing Pest Lists. However, the process of storing and maintaining collections of some pathogens is extremely difficult, time-consuming and expensive. As a result we negotiated for all bacterial cultures from Laos and Cambodia to be stored in proper facilities in Thailand. Likewise, DNA and RNA from virus samples will be stored in Australia by Professor John Thomas at the QDAF EcoSciences Precinct. These arrangements will help to maintain the vital link required to establish Pest Lists now and into the future.

Remote Microscopy: The remote microscope (RM) system used in previous projects has been upgraded with a much simpler software system developed by the PBCRC. The new system is inexpensive, is technically simple to use and maintain, can use mobile or fixed internet to stream live images and operates at low internet bandwidths. It also couples with the Pestpoint® software to capture live images and store records of RM interactions. This new system overcomes many of the technical and cost issues of the previous system.

This new RM system was recently implemented in Thailand at several Plant Quarantine land and sea ports at the request of the Plant Quarantine Research Group (PQRG). The Plant Quarantine group has used the RM system to provide staff with a link to experts based in Bangkok, so that pest identification can be achieved more quickly. The Pestpoint®® program will be used to document the pests that are intercepted in imported goods which will serve as a chain of evidence that can be used to support trade policy and regulation with trading partners, particularly China.

Currently, the PQ officers do not have a standard format for recording pest interceptions and have no database of interceptions that can be used to inform management, who in turn, have little ability to put pressure on trading agents in relation to intercepted pests. At the moment, PQ rarely takes action in relation to interceptions for fear of retaliation in the form of trade blocks. Imported products found to contain pests are allowed to continue passage into the supply chain unobstructed, unless the port facility has access to fumigation. In reality, the PQ system in Thailand for imported products monitors pests that enter the country but has little capacity to prevent the movement of infested products beyond the border. Management within the PQ Group anticipates that having proper documentation of intercepted pests will provide them with better mechanisms for taking action in relation to pests intercepted in imported products.

With growing emphasis on border protection within Thailand, it is likely that the Thais will continue to strengthen their capacity to monitor and document pest interceptions and so both RM and Pestpoint® will continue to meet their requirements in this regard.

Both Lao and Cambodian partners are now involved in surveillance programs operated through FAO, CIRAD and CIAT. Not only are the partner staff capable of undertaking the field surveillance aspects of the work, the capacity exists to complete, or nearly complete, the diagnostic and reporting process in-country. This improves the data security and ownership issues that have caused complications for the partner countries recently.

8.3 Community impacts – now and in 5 years

Community impacts have not been directly measured. However, the implementation of survey protocols for the detection of cassava SLCMV and for Panama disease would be expected to have flow on benefits in the future. Delimiting surveys for SLCMV have allowed targeted management, and assisted in the containment of the disease. Left unchecked there would have been, and may still be, serious ramifications for the production system and the communities involved. Similarly for Panama disease, delimiting surveys have identified both infected and non-infected areas, and led to official reporting of the disease to biosecurity authorities in Laos. Both cassava and banana are commodities of interest for the investment sector, particularly for foreign backed companies. Successful detection of existing disease constraints, and the identification of disease incursions will provide beneficial community impact in the long term.

8.3.1 Economic impacts

SLCMV is a serious threat to cassava production in Cambodia and the region. Processed cassava is Cambodia’s largest agricultural export, and while prices fluctuate on the world market, the ability to maintain or potentially increase market share will be critical to the economic viability of the sector. More generally though, the project has highlighted to PC staff that plant viruses are widespread and possibly causing more economic damage to crop production than any other pest group. With this knowledge, and greater capacity to identify plant viruses, it is expected that PCs will direct greater focus to their control and in so doing, decrease the economic impact of plant viruses on production. The cooperation between partners within the project provided opportunity to validate testing and inspection protocols. Shared data and training allowed Thai partners to experience SLCMV symptoms first hand, and to obtain positive samples on which to validate their testing procedures. Combined, this provided an avenue to ensure improve the chances of Thailand in protecting the economic viability of cassava, which would not have been as easily achieved outside the project.

Banana production is also experiencing considerable foreign investment in both Lao PDR and Cambodia. Should issues such as Panama disease increase in the region, the viability of the production system will be limited, resulting in short term economic gain. However, due to poor levels of biosecurity compliance and inconsistent disease reporting, a subsequent increase in the distribution of FOC TR4 has been reported, not only by Ms. Khonesavanh Chittarhat, but also by the Bioversity International. Due to the support Ms Chittarhat received, she and the Plant Protection Center were able to undertake an extensive survey of banana plantations for FOC TR4. Her results demonstrated a demarcation of the pathogen, being present in northern and central Lao, but absent from southern Lao. While banana plantations backed by China, and subsequent exports, have been curtailed due to strict environmental pollution regulations being introduced by the government of Lao, the significance of the demarcation zone will provide the opportunity to maintain pest free status in the southern region of the country, and contribute to sustainability of the industry. Ms Chittarhat has reported to MAF regarding the issue, requesting the implementation of strict quarantine to protect the region. As a comparison and gauge of economic impact, according to Bioversity International and the Pilipino Banana Growers and Exporters Association (PBGEA), a break-down of the banana industry in the Philippines would result in approximately 330,000 workers losing their jobs, and an annual loss of AUD1 billion in wages.

Economic impacts are expected in the longer term as a result of continued collaboration brought about by the relationships established during this project. As diagnostic capabilities have improved, and the application of Pestpoint® to biosecurity systems is adopted, it would be expected that verified pest records will be generated which may assist in market/trade negotiations. Thailand has provided quarantine provisions for the acceptance of samples for diagnostic purposes from both Cambodia and Lao, and access to storage facilities for verified samples. Both these play a critical role in trade negotiations involving sanitary and phytosanitary conditions.

8.3.2 Social impacts

Broader social impacts are yet to be realised, however some impacts may occur within 5 years of project completion. Stability in production systems, with reliable output of quality produce for internal and trade markets will benefit society as a whole.

In terms of direct impact for the project partner staff there have been significant observable changes in the role and interaction of individual staff. In Lao PDR, Ms. Khonesavanh Chittarhat has evolved from a laboratory based leader to essentially undertaking the leadership aspects of the project in-country. She is recognised by the Ministry, and at the provincial level as an in-country expert with the capability to address production issues. In Thailand, the project partners were dominated by female experts who have high standing within their department. Dr Preyapan Pongsapich and Dr Nuttima Kositcharoenkul both provided leadership and operational expertise throughout the project. Ms Moojum Rungsawang provided laboratory leadership and was instrumental in maintaining the communicative networks. From Cambodia, the development of Ms Sor Sarika from a research assistant to the taking the lead in the diagnostic laboratory was also evident. In terms of gender equity, from the partner side this was met. However, the balance on the Australian side was far less evident.

The interaction between the partner countries has also undergone significant increase, with communication across Pestpoint® and Facebook, and the more traditional email platforms. Partners are using their social networks to interact and disseminate pest information, and share scientific knowledge and ideas. Discussions are on-going as to how these relationships will be maintained in a professional/working sense. However, socially the interaction is self-driven.

Overall, the project activities have resulted in raising biosecurity awareness among staff and organisations responsible for plant protection, which in turn impact on production systems and on farmers who ultimately feel the impact of pests and diseases. These indirectly affects social systems and are indeed important components of the pathways that bring about changes that ultimately benefit communities. The examples of SLCMV and FOC TR4 have been used to highlight to staff, a set of generic skills that are required to manage plant pests and plant health systems. These skills are what eventually impact social systems for agricultural production.

8.3.3 Environmental impacts

FOC TR4 persists in soil and is disseminated freely in water and overland flows. In tropical environments, these factors assure that FOC will spread quite rapidly. Land that has been affected by FOC TR4 becomes unviable for banana production within a few years. As awareness of FOC TR4 increases, and areas are declared infected or free, strategic management can be implemented rather than the generalised spray and pray approach currently used.

8.4 Communication and dissemination activities

New Pest and Disease Alerts: Publications documenting new virus diseases found in Cambodia and Laos are currently underway (see 10.2 Publications), with the definitive identification testing required for publication being undertaken in Australian laboratories by PC staff. Similarly, definitive testing for FOC TR4 is also underway at Stellenbosch University in South Africa. When completed, the findings will be presented in international journals and the data will be entered into the global database for FOC and viruses.

The Laos Government has been informed of the presence of FOC TR4 in its northern and central provinces, and permission to share this information has been received from government officials. As a potentially new and devastating disease of banana in Laos, we have an obligation to preserve the privacy of this information within the project until the government has been informed and they agree for us to share the information. Given that FOC TR4 can be so easily spread, it is in the interest of the region that the neighbours of Laos be informed so that they can be vigilant and prepared and implement precautions that might limit the biosecurity risks associated with the movement of plants and planting material. Chinese agricultural operations exist in all countries of the Greater Mekong Region (GMR) so each country should be on alert given that Chinese operations may be associated with new pest introductions.

Pest Lists: Detecting, identifying and verifying pests and diseases is the first step towards developing country Pest Lists. Such lists act as evidence of pest presence, informing both the country to whom the list belongs as well as its neighbours and trading partners. The pest information developed during the course of this project will contribute to Pest Lists in Cambodia, Laos and Thailand and will provide valuable information for all PPOs across the region. As far as we are aware, there is currently no mechanism in place that obligates countries in the region to notify their neighbours in the event of a new pest or disease outbreak, or indeed for countries to provide evidence of the pests and diseases that have been detected, as either endemic or introduced. Without this formal mechanism, countries must rely on official and unofficial reports and the dissemination of information casually and in the media. The development and sharing of Pest Lists is an important mechanism for bridging this gap in what can be regarded as vital biosecurity intelligence that informs both Plant Protection, Plant Quarantine and Trade activities.

9 Conclusions and recommendations

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9.1 Conclusions

The developing countries of SE Asia are agricultural based and so depend on increasing their trade in agricultural commodities to grow their economies. As signatories to the WTO, trade-related agricultural production is regulated by the Sanitary and Phytosanitary Standards as specified by the IPPC under the FAO. Being able to comply with SPS is a barrier for developing countries, as more trading nations insist on them meeting the appropriate phytosanitary standards. In order to meet these requirements, a country would need to have a well–developed Plant Health System, which has a skilled workforce, access to technology and the physical resources needed to demonstrate an ability to comply with SPS. In addition it would need to develop appropriate biosecurity policy and be able to implement systems and operational processes that supported it. For poor countries in the region such as Laos PDR and Cambodia, developing this kind of plant health system is extremely difficult, requiring financial and governing support at many levels.

In contrast, capacity-building projects such as this one tend to work on isolated parts of the plant health system, simply because of logistics and therefore, have difficulty in making an impact on the entire system. In addition they face disruption to their programs due to regular changes of PC operational staff, management and organisational structure. As a result, progress can be slow and may diminish with time after the project has ended. The challenge then is to deliver a set of operations, processes, skills that are valued by the PCs, will be adopted and extended by the PCs, and that will enhance agricultural production, either directly or indirectly.

Despite these problems, we believe that the program we implemented during this project will be sustained long after the project has ended. Why? Because we have introduced a systems approach to link together multiple diagnostic operations and multiple sectors in the plant health community, to provide information and knowledge outcomes that benefit biosecurity at multiple levels. By paving the way for mobile devices and software to facilitate data capture and sharing, the PCs now have a defined pathway in a new operational paradigm that overcomes many of the difficulties of the past, where data capture, storage and retrieval were disconnected within and between operational sectors of plant health. At the core of this system is the Pestpoint® application that universally serves surveillance and diagnostic operations, and allows pest data to be aggregated and analysed and applied to research, pest management and biosecurity policy. The importance of Big Data to invention, innovation and better decision making is now a reality and those industries that are able to access and utilise it will prosper into the future.

If developing countries are going to improve their agricultural production systems, they will need to move towards systems that facilitate Big Data outcomes. If they are going to meet SPS requirements for trade and plant protection, they will have to adopt processes that are designed to document their diagnostic activities and validate the data that they collect. In this project we have provided the means, the skills and the practices that Laos PDR, Cambodia and Thailand will need to adopt, as they transition to a Big Data approach to support pest management and biosecurity outcomes.

9.2 Recommendations

A common difficulty in capacity building projects is ensuring that skills, operations and practices are continued after the project ends and the commissioned agencies no longer have a presence in the PCs. Capacity building ultimately requires that change is accepted where new processes are adopted and new behaviours are developed that support and build on the changes that are made during the project. We therefore recommend the following steps towards sustaining the operations that were proposed during this project.

1. Maintaining a system for data capture and documentation should be a priority for the PCs. National adoption of the Pestpoint® application has the potential to gather pest data at a national scale where it can be used to support multiple plant health and biosecurity application.
2. Pestpoint® commercialisation by the PBCRC will provide ongoing support to those PCs wanting to adopt the Pestpoint® system. Negotiations for Pestpoint® licencing with PPOs should be pursued and followed up by the PBCRC.
3. PCs should continue to support the diagnostic network that was developed during the project and to foster inter-country co-operation on biosecurity issues that are of mutual concern. For example the discovery of SLCMV in Cambodia and FOC TR4 in Laos raise biosecurity concerns for neighbouring countries, however there is no formal mechanism whereby a country can notify its neighbours when pests like these have been found.
4. Maintaining specimens of identified pests and diseases is a requirement for validation under SPS, but is challenging for some countries. Sharing culture collections was established during this project as a means of overcoming the difficulty, with Thailand accepting bacterial cultures and QDAF, Australia accepting virus specimens for both Laos and Cambodia. This agreement should be maintained and new agreements for fungal specimens should be found. Eg PCs should approach CBS for storage of mycological specimens.
5. Surveillance activities should be expanded to document pests and diseases of other crops besides those examined during this project. The surveillance and diagnostic processes that were established during the project for the development of Pest Lists should be applied by PCs in this regard.
6. The higher level biosecurity processes that proceed from surveillance and diagnostics operations are poorly developed in the PCs. Strengthening these will require that good linkages are developed so that the plant health system operates and grows as a result of feedback loops between surveillance, diagnostics, plant protection, pest and disease regulation and biosecurity policy. Ultimately this system facilitates trade. There is scope for a project that would help to harmonise the components of the existing systems in order to achieve specified trade outcomes for each PC.
7. Partner countries have independently identified country specific commodities, and several that could form the basis of regional cooperation opportunities. During the extension period of the project, Myanmar was also provided opportunity to attend workshop activities. Therefore, the opportunity exists to develop a multilateral project encompassing biosecurity issues at both a local and regional level, with country specific focal points, but global impact. Thailand has the capacity to act as a regional technology hub, with potential for in-kind investment. China is also a growing investor in the region, with the opportunity to develop public-private partnerships. The adoption of ASEAN-GAP also poses the opportunity to embed sound biosecurity practice and policy in Cambodia, Lao and Myanmar. This will be critical in establishing and building trade opportunity, and for the operation of ASEAN as a trading conglomerate.

The recommendations listed above point to an opportunity across the partner countries in which the plant biosecurity continuum could be further developed. Thailand offers a regional example of a developed biosecurity system, with operational and regulatory systems in place. The conclusion of this current project has provided Cambodia, Lao, and to a certain extent Myanmar, a mid-level system in which the operational staff responsible for surveillance and diagnostics are now capable of undertaking these critical tasks. The recognition of capability is evident from the inclusion of these staff in programs now operated by FAO, CIRAD and CIAT. However, the biosecurity continuum now needs to be improved in an upward and downward manner. At the higher operation (regulation) level, incorporation of surveillance data, and resultant reporting, needs to be improved. At the lower operational level, provincial department staff and grower groups need to be exposed further to the filed diagnostic purpose, and included in surveillance networks. This could be achieved with the expanded role out of PestPoint ® and a focus on crops of regional importance. Linkage with ASEAN-GAP policies and procedures would also provide the impetus to improve the production system as a whole.

10 References

10.1 References cited in report

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10.2 List of publications produced by project

Khonesavanh Chittarath, Thavrith So, Sarika Sor, Wasana Rungsawang, Preyapan Pongsapich, Gary Kong, John E Thomas and Andrew DW Geering. (2017) First record of *Papaya ringspot virus* in Cambodia and confirmation of its presence in Laos. Plant Disease – First Look https://doi.org/10.1094/PDIS-08-17-1197-PDN

K. Chittarath, **G. Kong**, **D. Mostert**, **A. Viljoen K.S. Crew**, **J.E. Thomas** (2017). First report of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (VCG 01213/16) associated with Cavendish bananas in Laos. *Australasian Plant Disease Notes*. IN Press.

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Posters produced by Khonesavanh Chittarhat (Lao language)

How to identify *Fusarium* wilt of banana disease.

Fusarium wilt disease of banana.

Come clean, leave clean.

Yellow sigatoka disease of banana.

Black spot disease of banana.

11 Appendixes

11.1 Appendix 1:

Table 1. *List of Training Workshops and Activities completed during the project*.

#	Date	Location	Name Workshop/Training	Attended by	Organisation/ Group	Expertise
1	28 July- 8 August 2014	Laos/Cambodia/ Thailand	Pestpoint® Training- Remote Diagnostics	Project Staff;Thai PQRG; PPC Laos/ Cambodia GDA, CARDI and RUA staff	Thai DoA/Laos MAF/Cambodia GDA, CARDI,RUA	Plant Pathologists and Entomologists
2	13 March 2015	PQRG, Bangkok, Thailand	Project Planning Meeting	Gary Kong; Surapol Yinasawapun; Preyapan Pongsapich	Thai DoA	Project Leader;Director PQRG; PC Project Leader
3	15-21 March, 2015	PPC, Vientiane, Laos	Bacteriology Workshop/Survey	Gary Kong; Ben Stodart; Nuttima Kositcharoenkul; PC staff	Thai DoA/Laos MAF/Cambodia GDA, CARDI,RUA	Bacteriologist (Thai) Plant Pathologists
4	20-30 April 2015	GDA, Phnom Penh, Cambodia	Mango Pest Survey – Collaboration with HORT/2012/003	Gary Kong; GDA, CARDI and RUA staff	GDA, CARDI and RUA	Entomologists
5	6-17 July 2015	PPC, Vientiane, Laos	Virology workshop	Project Staff; PPC Laos/ Cambodia GDA, CARDI and RUA staff		Virologists, ,Plant Pathologists
6	16-17 July 2015	PQRG, Thailand	Project Planning	Gary Kong; PQRG Staff	Thai DOA	Director PQRG, PC Project leader
7	October – November 2015	EcoSciences DAF Qld	Molecular Training	Khonesavanh Chittarath, Laos PPC	MAF Laos PDR	Molecular Virology
8	15-16 February 2016	GDA, Phnom Penh, Cambodia	Project Planning/Midterm Review	Gary Kong; Ben Stodart; Richard Markham; PC Directors and project leaders	Thai DoA/Laos MAF/Cambodia GDA, CARDI,RUA	Directors of PC organization/ Plant Pathologists and Entomologists
9	17-26 February 2016	Phnom Penh Cambodia	Pestpoint® training/Field Surveys	Project Staff; PPC Laos/ Cambodia GDA, CARDI and RUA staff	MAF, GDA, CARDI and RUA	Plant Pathologists and Entomologists
10	23-27 May 2015	Thailand	Remote Microscope Training	Gary Kong; PQ staff Border Stations	Thai DOA and PQ	Plant Quarantine Officers
11	26 June – 9 September 2016	QDAF, Brisbane, QLD	ELISA techniques for identification of SLCMV	SOR Sarika;	GDA, Cambodia	Plant Pathologist
12	Sep-16	Cambodia	SLCMV survey of Ratanak kiri province	GDA, CARDI and RUA staff	GDA, CARDI, RUA	Plant Pathologists and Entomologists
13	October - November 2017	University of Western Australia	Whitefly barcoding	Sunadda Chaovalit	Thai Dept. of Agriculture; Entomology Group	Entomologist/Taxonomist
14	27-28 October 2016	PPC, Vientiane, Laos PDR	Planning Meetings	G. Kong; T. Vongsabouth (deputy director) andStaff from PPC	Ministry of Agriculture and Forestry; Plant Protection Centre	Plant Pathologists

#	Date	Location	Name Workshop/Training	Attended by	Organisation/ Group	Expertise
15	31-Oct-10	Bangkok Dept of Agriculture	Planning Meeting	Gay Kong; Director Plant Quarantine Group and Staff	Thai Dept. of Agriculture	Plant Pathologists and Entomologists
16	1-4 November 2016	Bangkok Port and sea Port Laem Chabang	Remote Microscope new system setup and training	Mike Thompson; Gary Kong ; Plant Quarantine staff	Plant Quarantine	PQ Inspectors
17	15-November-5 December 2016	University of Queensland	Mealybug and scale insect barcoding	Ms Chamaiporn Buamas	Thai Dept. of Agriculture; Entomology Group	Entomologist/Taxonomist
18	Dec-16	Cambodia Pursat Province	SLCMV Survey Pursat Province	Thai DOA, GDA, CARDI and RUA staff	GDA, CARDI and RUA	Plant Pathologist/Entomologist/Agronomist
19	12-24 Feb 2017	Laos PDR	Banana pest survey Northern Provinces	PPC staff; Dr John Thomas; Dr Kathy Crew	Ministry of Agriculture and Forestry; Plant Protection Centre	Plant Pathologists/virologists
20	9-10 March 2017	Bangkok Dept of Agriculture	Planning Meeting	Gay Kong; Director Plant Quarantine Group and Staff	Thai Dept. of Agriculture	Plant Pathologists and Entomologists
21	13-17 March 2017	Laem Chabang/Bangkok Ports	Remote Microscope Workshop	Mike Thompson; PQ Staff; Director PQRG	Thai Dept. of Agriculture	Plant Quarantine Staff
22	13-14 March 2017	Vientiane; PPC	Planning Meeting	G. Kong; T. Vongsabouth (deputy director) andStaff from PPC	Ministry of Agriculture and Forestry; Plant Protection Centre	Plant Pathologists
23	06 April 2017	Phnom Penh	Planning Meeting	B Stodart;Project Staff	GDA;CARDI;RUA	Entomologist/Plant Pathologists
24	27 March – 06 April 2017	Phnom Penh	Banana Survey	B StodartProjects Staff	GDA;CARDI;RUA	Entomologist/Plant Pathologists
25	19-28 April 2017	Bangkok, Plant Quarantine Dagnostic Labs	Training in molecular techniques, including PCR, RT-PCR, RCA, sequencing.	Staff from GDA, PPC, MPPD	Cambodia GDA, Laos PPC and Myanmar PPD	Plant Pathologists
26	4-10 June 2017	Thailand Border Stations	Remote Microscope new system setup and training at Mae sai, Chiang saen and Chiang Khong border Stations northern Thailand	Gary Kong; Director Plant Quarantine Research Group	Thai Dept of Agriculture	Plant Quarantine Inspectors
27	3-7 July 2017	Bangkok Plant Quarantine Dagnostic Labs	Whitefly vector -virus workshop. Surveillance; Taxonomic and moleciular identification; collection and preservation.	Dr Laura Boykin; Dr Kris Wyckhuys Ms Sunadda Chaovalit	GDA, CARDI, RUA, PPC Thai DoA	Molecular Ecologist; Insect Ecologist; Plant Pathologists and Entomologists
28	August 30-September 1	Thai PQRG Bangkok	Pestpoint® training	Mike Thompson; PQRG, Surveillance, Taxonomic staff	Thai Dept of Agriculture	Entomologists, Plant Pathologists, Weed Scientists

#	Date	Location	Name Workshop/Training	Attended by	Organisation/ Group	Expertise
29	4-8/09/2017	Thai PQ Northern Borders	Remote Microscope Training	Mike Thompson; PQRG Director PQ Staff	Thai Dept of Agriculture	Plant Quarantine Officers
30	27/09/2017	Brisbane	Pestpoint® Workshop Planning	Gary Kong; Mike Thompson; Ben Stodart	CSU	Project leader, Co-Leader, sub-contractor
31	28/09/2017	Brisbane	ACIAR Review; Pestpoint® Workshop	Gary Kong; Ben Stodart; PC Project Staff	PPC; GDA; DoA	Country Project Leaders.
32	2 -20/10/2017	EcoSciences DAF Qld	Molecular analysis training	Khonesavanh Chittarath (Laos) ; SOR Sarika (Cambodia)	DAF Qld; UQ	Virologists
Activities Completed During Extension						
#	**Date**	**Location**	**Name Workshop/Training**	**Attended by**	**Organisation/ Group**	**Expertise**
33	9-10 Nov 2017	Pakse, Lao PDR	Pestpoint®, Banana & cassava disease survey	Khonesavanh Chittarath (Laos), ; Mike Thompson; Ben Stodart, PAFO staff, Pakse	PPC, PAFO	Project leaders, plant pathologists
34	13-17 Nov 2017	Cambodia	Pestpoint®, Banana & cassava disease survey	GDA, Mike Thompson; Ben Stodart	GDA	Project leaders, plant pathologists
35	14-16 March 2018	Cambodia	Pestpoint®	GDA, Mike Thompson	GDA	Technical expert
36	26-30 March 2018	Thailand	Pestpoint® & molecular diagnostics	Preyapan Pongsapich & Yuvarin Boontop (Thai), Mumu Thein & Kim Nyunt Lee (Myanmar), Mike Thompson	Thai DoA	Technical expert, virologist, whitefly taxonomist

11.2 Appendix 2

Table 1. *Describes some of the basic diagnostic skills that staff were expected to develop from applied surveillance and diagnostics activities undertaken during the project.*

Basic Skill	*Capability required for basic skill*	*Advanced skills development*
Evaluate a crop and identify symptoms/signs of major pest groups	Capability to capture and record appropriate field data	Field evaluation methodology, including - sampling/monitoring strategies; collection and capture of background infomation (farmer discussion); symptom recognition; imaging; Pestpoint®
	Demonstrate and apply a sound understanding of the typical symptoms associated with major pest groups	Recognition of symptom and signs associated with pest groups; monitoring skills; sample evaluation skills; imaging; data collection and recording via Pestpoint®
	Capacity to collect samples and prepare for transfer to the laboratory	appropriate preparation for field sampling; sampling strategies; identifying appropriate samples to collect; sample storage; imaging; sample tracking
	Ability to provide general management recommendations	Apply an understanding of symptom and signs to deduce the likely pest group; an understanding of general plant health; risk analysis skills to determine likelihood of further damage and or spread; understanding of applying management recommendations in the context of farming system and or appropriateness of recommendation; recording data
Evaluate a collected sample and provide a presumptive identification of a pest group	Appropriately assess a collected sample and provide logic in determining subsequent analytical steps, including the capability to query appropriate resources	Information evaluation to determine missing data; Computing skills enabling appropriate searching of resources; capture and record assessment details; protocol development/operating procedure documentation skills.
	Demonstrate the technical skills required to achieve isolation of a pest where appropriate	Media preparation; aseptic technique; sample preparation; evaluate success of isolation; decision making for further analysis.
	Demonstrate the capability to assess an isolate/specimen & apply appropriate methodology to achieve presumptive ID, utilising available resources	Computer skills allowing searching of appropriate information resources; Planning of additional analytical steps; technical skills to conduct additional analysis; data capture, interpretation and reporting
Specimen Curation	Demonstrate the ability to collect, preserve and store specimens for reference. May include virtual specimens.	Understand techniques required for collection, preservation and storage of specimens for identification and reference
Data Management	Demonstrate ability to record data relevant to specimens and to manage this data in relation to identifications and curation.	Proficiency in recording methods and tools used for recording and managing data.