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Final report

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1 Acknowledgments

This project was co-ordinated by the Co-operative Research Centre for National Plant Biosecurity (CRCNPB) in collaboration with its partners, including the CSIRO, Department of Employment, Economic Development and Innovation (DEEDI), the Department of Primary Industries Victoria and the NSW Department of Primary Industries.

Thanks also to Dr Ken Walker of the Museum of Victoria who supported training for Thai scientists as well as the development of the Thailand Biosecurity website.

2 Executive summary

Under the WTO there is increasing pressure on countries to comply with agreed sanitary and phytosanitary standards (SPS) to satisfy trading partners and to access markets. Such SPS conditions apply equally to developed and developing countries, however the latter often lack the expertise, infrastructure and organisational processes required to meet such standards. Together, these elements form the Plant Health System of a country and serve both its trading activities and crop protection needs.

Plant pest diagnostics is at the heart of any Plant Health System and countries that do not have the ability to identify its pests cannot satisfy the requisite SPS conditions for trade. Nor can they protect against the incursions of pests or manage the pests that damage crops. The lack of a Plant Health System is then a huge impediment to economic growth for developing countries.

With this understanding, we designed training activities and implemented a workshop program to improve the capability and capacity of plant pest diagnostics in Thailand. The program focussed on current technologies to provide Thailand with fundamental systems and processes that will provide greater efficiencies in diagnostics and which can be expanded across the Thai Plant Health system. The program focussed on providing molecular identification, traditional taxonomy, digital knowledge systems and remote microscopy which led to the following outcomes.

- A Molecular diagnostic laboratory was established in Thailand and staff were embedded in Australian laboratories to learn up-to-date molecular techniques and work practices. The high level of skill attained by Thai scientists was demonstrated in their ability to routinely perform molecular techniques in their own lab, to train their own staff in molecular techniques and to develop diagnostic protocols and optimise existing tests for their own purposes. The focus of this training was on trade sensitive pest groups, such as citrus canker, Huanglongbing, viruses, nematodes, fruitfly and seed-borne bacterial pathogens, to give a good grounding in molecular identification.

The identification of many pests can be performed by traditional taxonomic methods, but taxonomic skills require a high level of training and for logistical reasons, taxonomists can often only focus on becoming expert in a single pest group. While it is important to have taxonomists for definitive identifications, there is much that can be done to improve the general taxonomic skills of all Plant Health staff so that there is less reliance on taxonomists.

- The discovery of new fungal pathogens and the documentation of known pests during the course of this project indicates that staff were able to apply taxonomic principles as well as molecular techniques to verify pest identification. Digital technologies such as advanced image capture techniques and web-based information systems were introduced to Thai scientists so that they could document their diagnostic information and share it over the internet to provide a valuable source of diagnostic information. The Thailand Biosecurity website was established for this purpose (http://padil.gov.au/thai-bio/Search?queryType=all).
- Image capture hardware and software were provided together with intensive training and user guides so that high quality images of pests could be obtained for the website. A special laboratory was established by Plant Quarantine for this equipment and for training staff in image capture techniques and use of the website.

The loss of taxonomic expertise is a global trend and comes at a time when, because of WTO and SPS regulations, there is greater emphasis on high level pest identification. Remote diagnostics can help overcome the scarcity of expertise and the distance between the pest specimen and the expert. Usually, a pest specimen would have to be mailed to an expert, which takes time and possible delays to taking action against the pest. In biosecurity terms this can be costly

- Microscope hardware for remote diagnostics was installed in Bangkok, Chiang Sian, a quarantine port on the northern border with Laos and Myanmar, and at Laim Chabang, the major sea port. A dedicated lab was established in Bangkok for the equipment and remote microscope (RM) operations, as well as for training staff. PQ has plans to establish nine more RM at key border ports to manage the identification of pest interceptions. These systems will be installed according to our specifications.
- An additional use of the RM equipment is that it can be used to remotely train staff in pest identification. RM interactions with experts in Australia were conducted during the course of the project for this purpose.

3 Background

Protecting agricultural production from endemic and invading exotic pests is in the national interest of every country. Under the SPS regulations and agreements of the WTO, a formal process has now been attached to the way in which countries can or should go about protecting its agricultural industries and to ensure food safety for its consumers.

Thailand's farming population accounted for 38% of the country's 65 million population in 2005. Around two-thirds of total farm outputs, which are valued at the current price of 7,104 billion Bahts, were exported as primary and processed farm and food products to final consumers in overseas markets, accounting for around 21% of total export earnings of 4,436 billion bahts in 2005. Undoubtedly, Thai farmers' incomes depend heavily on export markets as important sources of income earnings and the local economy is stimulated through this activity. Thailand also imports agricultural products from many other countries, including Australia. The Thai Australia Free Trade Agreement (TAFTA) sets SPS measures consistent with the WTO. In order to meet these SPS measures and retain market access, Thailand must be able to demonstrate a high level of proficiency in determining and maintaining its pest status.

Plant pests and diseases are responsible for significant losses in agriculture and forestry globally. There are many different types of pests (insects, mites) and diseases (fungi, bacteria, viruses, nematodes and phytoplasmas). The introduction and movement of exotic pests and pathogens are of quarantine concern to many countries and their establishment can cause major disruptions to natural ecosystems and place serious limitations on trade.

Accurate identification of pests is essential for practically all aspects of agricultural development and is critical to the operations of biosecurity that safeguard agricultural production and facilitate trade. Diagnostic capability is at the forefront of activities such as border protection, incursion management, surveillance and pest and disease certification. The efficiency of a biosecurity system therefore depends largely on the feedback between these activities and diagnostics.

Diagnosis of pests and diseases draws on a range of related but complementary disciplines, including those that use traditional taxonomic methods and a range of others that rely on molecular (DNA –based) and biochemical (non-DNA-based) techniques. DNA-based diagnostics can provide new tools that are more reliable and faster than conventional detection methods. Technological advances in DNA-based methods, such as real-time PCR, allow fast, accurate detection and quantification of plant pathogens and can be applied to practical problems. These highly specific tests can provide definitive diagnosis. Biochemical test kits are available for many plant pathogens and can be used for rapid presumptive identification.

The aim of this project was to increase the diagnostic capability of Plant Quarantine diagnostic services in Thailand and to introduce new technologies and techniques for the detection of exotic pests. This was achieved through intensive training and testing, and through the establishment of new facilities and improved work practices in relation to pest diagnosis.

4 Objectives

Objective 1) To enhance the general and specific skills and knowledge of research workers and scientists in Thailand and Australia in plant pest and disease diagnostics using both molecular and traditional diagnostics techniques.

Activity 1: Training in general and specific insect and pathogen identification.

Activity 2: Selected Thai DOA staff were placed in diagnostic laboratories in Australia (Vic DPI – Knoxfield; EMAII – NSW Ag) were they could gain first-hand experience in using molecular diagnostics to detect pests and pathogens.

Activity 3: Establish a molecular diagnostic capability at Thai DOA Plant Quarantine labs in Bangkok.

Objective 2) To develop diagnostic protocols and simple manuals for molecular diagnostics of selected plant pathogens.

Activity 1: Train Thai DOA staff to use the Australian diagnostic protocols for the detection of Potato Spindle Tuber Viroid (PSTVd), citrus canker and a range of fruit flies of quarantine significance.

Activity 2: Engage Thai DOA staff to develop diagnostic protocols for 2-3 pest species of Thai significance (i.e. PMTV, Powdery Scab, PepMV).

Activity 3: Validate diagnostic protocols for pest species in Thailand.

Activity 4: Validate survey protocols in Thailand for the targeted pest species.

Objective 3) To introduce and train staff in the use of advanced knowledge systems that can be used for on-going training, diagnostic information and the establishment of informal networks.

*Activitiy1.*Conduct workshops to introduce and instruct staff on the access and operation of PaDIL (Pest and Diseases Image Library) and its use as a diagnostic tool.

Activity 2. Establish a process for Thai-specific pest and disease data to be entered into the PaDIL database.

Activity 3. Conduct a workshop to demonstrate and create a Thai node of the CRC NPB Remote Microscopes project for the purposes of training, diagnostics and expert networking. Note: These activities include the installation of microscopes and photographic equipment as well as training in the use of the equipment

5 Methodology

The CRC for National Plant Biosecurity (CRC NPB) provided both general and specific training through its participant's network in relevant areas of detection and identification of pests and pathogens. Activities included trialling new detection methodologies, molecular and traditional identification methods, sampling, development and validation of diagnostic protocols, interpretation of test results, maintaining a collection of type specimens and developing a knowledge database for important pest species.

These activities were designed to relate to pests of particular interest to Thailand and where possible, to those pests that are of interest to Australia as well (eg citrus canker and greening, exotic fruit flies). The following strategies were followed for each of the major project areas of 1. Molecular diagnostics, 2. General diagnostics and 3. Digital knowledge systems

1. **Molecular Diagnostics:** Specific training in molecular diagnostics for PSTv, citrus canker and exotic fruit flies was provided by Australian molecular diagnostics and research laboratories located at Knoxfield, Victoria and EMAI located in New South Wales. DOA scientists were located in these labs for a three-month period for each year of the project. They received general and specific instruction in molecular procedures and diagnostic testing, including protocols and processes required to organise and run a diagnostic laboratory. Following each training period, DOA scientists returned to Thailand to complete a specific task related to instruction received in Australia. For example, staff were asked to implement and put into practice, processes and procedures learned in Australia. DOA staff were given a short period of time to initiate this process, then Australian scientists visited DOA labs to assess progress, assist in implementation and refinement of processes and to help DOA staff conduct a training workshop for other DOA scientists (a "train the trainer" process).

   The above strategy attempted to provide intense training by embedding DOA staff in Australian labs then reinforcing the training by setting tasks and following up training in Thailand. This process gave DOA staff ownership through planning and development of their own molecular diagnostics lab. In addition, repetition of this process with the same DOA staff in subsequent years helped re-enforce skills and develop relationships with Australian scientists which it was hoped would extend beyond the life of the project.

2. **General Diagnostics:** Workshops were conducted each year dealing with general groups of pests and diseases, such as fungi, bacteria, viruses, nematodes and various insect groups. These workshops dealt with symptomology and identification in accordance with the principles of plant pathology and entomology. These workshops catered to needs as specified by DOA scientists and were directed to both diagnostic and field staff.

   Further diagnostic training was provided through the use of a Remote Microscope system developed by the CRC NPB. Through this system, DOA staff were able to communicate with a remote expert (eg Australian taxonomist) and view a common specimen located under a microscope at either the remote or base location. This system has provided DOA staff with the opportunity to obtain taxonomic training by linking with experts at various locations in Australia. The system also provides a professional and training link with Australian scientists following workshops in Thailand and will help to re-enforce that training. The system will also provide a remote identification and taxonomic training service within the Thai PQ, linking Thai experts with their colleagues in regional labs.

3. **Knowledge Systems:** DOA staff were trained in the use of PaDIL, a web-based database that provides a library of diagnostic quality images of pests and diseases. The first stages of any diagnosis is based on both symptomatology and morphological features of the pest or pathogen. The PaDIL website, designed and operated by Australia, can provide DOA scientists with a quick and easy-to-use reference for diagnosing pests and diseases. In addition, PaDIL will soon contain full Diagnostic Protocols with National accreditation, for a range of exotic pests and diseases. Adherence to recognised protocols and standards will be a requirement for the acceptance of specific identifications in many countries, so having access to these protocols will be of benefit to Thailand in relation to trade.

4. As part of the reinforcement process, DOA staff will be trained in the use of equipment for capturing high quality images that can be stored in PaDIL. In this way, pests that have specific relevance for Thailand can be stored in the database and publicly accessed, thus providing Thailand with some “ownership” for the process and the information. This process will also help DOA staff prepare a list of exotic threats and accumulate diagnostic information for Risk Analysis.

Staff who were included in the above training were those already involved in research and operational activities with the Thai DOA, Entomology, Plant Pathology and Plant Quarantine units. Benefits to Australian scientists include the opportunity to gain diagnostic experience with pests and pathogens present in Thailand that may be biosecurity threats to Australia.

Generally speaking, many diagnoses consist of a chain of evidence gathered from a variety of interrelated disciplines, including symptomatology and identification through traditional and molecular characteristics. Detection in the first instance relying on recognition of specific symptoms then application of traditional techniques to isolate the pest or pathogen and to determine a possible identification. A final and often definitive step is to verify the identification using a DNA-based test. Emphasis was given to training in all these aspects of diagnostics and in particular to the most up-to-date molecular techniques for the development and application of diagnostic tests. It was expected that those trained would gain the capacity to not only apply tests accurately to validate a diagnoses, but would have the ability to apply molecular techniques to the development of new diagnostic tests, in particular, those relevant to pests and diseases of importance to their own agricultural industries.

The approach specified above was designed to give Thai researchers a basic grounding in diagnostic principles with emphasis on the application of these principles to specific pests and diseases. An effective and enduring learning process was established by connecting Thai researchers with Australian researchers through the CRC NPB to provide access to experts, knowledge and material resources, This was an important aspect to ensure that the training is ongoing at a professional level, through the development of both formal and informal networks.

6 Achievements against activities and outputs/milestones

Objective 1: To enhance the general and specific skills and knowledge of research workers and scientists in Thailand and Australia in plant pest and disease diagnostics using a range of molecular and traditional diagnostics techniques.

no.	activity	outputs/ milestones	completion date	comments
1.1	Training in general and specific insect and pathogen identification.	Staff trained in general principles of identification. Staff able to identify specific pests and pathogens	3x workshops. October 2009 October 2010 March 2011 3x Placements July 2008 Feb 2010 April 2011	Three workshops were held in Bangkok that covered mycology, bacteriology and molecular identification. Staff from the Plant Pathology and Plant Quarantine groups attended the courses which were run by Dr Roger Shivas, Dr Dean Beasley, Dr Anthony Young and Ms Yu Pei Tan. Local pathogens were studied for the training and 10 new pest Factsheets were developed and posted to the PaDIL website. Several new pathogen records and first host records were made during these workshops. Two scientists were placed in labs in Australia for 4-6 weeks for each of three years and trained in traditional and molecular identification of nematodes and bacterial pathogens.
1.2	Place selected Thai DOA staff in diagnostic laboratories in Australia (Vic DPI – Knoxfield; EAMI – NSW Ag)	Staff will gain first hand experience in using molecular diagnostics to detect pathogens in field samples. Staff able to conduct specific PCR assays	3x Placements Sept. 2008 April 2009 Fedruary 2011	Placements of two staff members, in each of two labs, for periods of up to three months, and repeated for three years. A range of molecular techniques and lab processes were taught with specific instruction relating to citrus canker, PSTv and species of exotic fruit fly. Generally, molecular diagnostics for virus, bacteria and nematodes was covered, as well as WHS, record-keeping and processes for developing standard lab practices.
1.3	Establish a molecular diagnostic capability at Thai DOA Plant Quarantine labs in Bangkok.	A molecular diagnostic capability in Thai DOA labs that can be used to detect both endemic and exotic pests and pathogens.	3x Workshops March 2009 March 2009 March 2010	A workshop was held to audit exisiting equipment and lab practices and to determine what equipment would be needed for future molecular diagnostics and how the molecular lab should be organised within the existing workspaces. The equipment list was prioritised accounting for needs and budget and purchased locally. The two rooms used as labs were completely refurbished by DOA and the addition of new equipment has now brought this lab up to a high standard.

PC = partner country, A = Australia

Objective 2: To develop diagnostic protocols and simple manuals for molecular diagnostics of selected plant pathogens.

no.	activity	outputs/ milestones	completion date	comments
2.1	Train Thai DOA staff to use the Australian diagnostic protocols for the detection of PSTVd, citrus canker and selected fruit flies.	A diagnostic capability to detect PSTVd, citrus canker and fruit flies.	3 xPlacements as per Activity 1.2. Sept. 2008 April 2009 Fedruary 2011	Protocols for the detection of a range of potato viruses (PSTVd, PVY, PVA) citrus canker, Huanglongbing, bacterial pathogens of seed (*C. michiganense*), nematodes and various species of fruit fly were learned and or developed during training in Australia and subsequently implemented in the PQ and Plant Pathology labs in Bangkok under supervision of Australian scientists. Thai staff also trained other staff in general molecular techniques with minimal supervision.
2.2	Engage Thai DOA staff to develop diagnostic protocols for 2-3 pests of Thai significance (i.e. PMTV, Powdery Scab, PepMV).	Enhanced diagnostic capability to detect a number of pests of importance to Thailand	3x Workshops as per Activity 1.3. March 2009 March 2009 March 2010	A significant output of the training was the generation of a diagnostic manual for the detection of *Potato spindle tuber viroid* (PSTVd) prepared by Ms Preyapan Pongsapich and Ms Sukhontips Sombat. They also worked with Australian scientists to develop a direct RT-PCR test on infected tubers that is more sensitive than the grow-on ELISA test for the detection of PVY, PLRV, TSWV and PVS.
2.3	Validate diagnostic protocols for pests in Thailand.	Ensure quality and applicability of the diagnostic protocol in Thailand	March 2011	Protocols and techniques for identifying a number of potato viruses, including PSTVd and PVY were performed successfully in the PQ molecular diagnostic lab.
2.4	Validate survey protocols in Thailand for targeted EPPs.	Data generated for the presence/absence of pests in areas surveyed.	March 2011	Civil unrest throughout the project limited the time and extent to which Australian scientists could travel and this activity did not reach its potential. However virus protocols were applied to samples collected from a survey of potato production areas. Potato virus Y and Potato virus S were detected as well as unknown tospoviruses which are currently being characterised by the group.

PC = partner country, A = Australia

Objective 3: To introduce and train staff in the use of advanced knowledge systems that can be used for on-going training, diagnostic information and the establishment of informal networks.

no.	activity	outputs/ milestones	completion date	comments
3.1	Activity 1 Conduct a workshop to introduce and instruct staff on the access and operation of PaDIL (Pest and Diseases Image Library) and its use as a diagnostic tool.	Staff trained in the use of PaDIL.	3x workshops Oct 2009 March 2010 June 2011	Staff from Plant Quarantine, Entomology and Plant Pathology were trained in the use of PaDIL and the Plant Biosecurity Toolbox for pest identification. Staff also trained in techniques for image capture and post processing using the image standards required for PaDIL. All staff now have a personal Dashboard in PaDIL and can save and share their searches with others, create Factsheets and track their own PaDIL usage history.
3.2	Activity 2 Establish a process for Thai-specific pest and disease data to be entered into the PaDIL database.	Thai staff member trained and appointed to liaise with Australian PaDIL co-ordinator for Thai updates. Camera hardware and software purchased for image capture.	Jan 2011	Walaikorn Ratchadenchakul and Chamaiporn Buamas spent three weeks at the Museum of Victoria with Dr Ken Walker learning advanced image capture and how to author species pages and faceted keys in PaDIL. Both were appointed as Administrators of the Thailand Biosecurity Image Library that was created during their training. Walaikorn and Chamaiporn added species pages for mealy bugs and fruit flies of quarantine importance to the Thailand Biosecurity Image Library. Nikon digital microscope camera, Nikon Digital Sight PC interface and image capture and montaging software was purchased and installed in the PQ labs.
3.3	Activity 3 Conduct a workshop to demonstrate and create a Thai node of the CRC NPB Remote Microscopes project for the purposes of training, diagnostics and expert networking.	Thai staff trained in the use of Remote Microscopes. Camera hardware and software purchased to establish a Thai Remote Microscope Node.	4x Workshops Oct 2009 March 2010 March 2011 June 2011	Nikon binocular and compound microscopes, digital cameras with DS-L2 internet server were purchased and installed in a dedicated laboratory at the PQ labs in Bangkok. This lab now has dedicated fast internet and static IP for Remote Microscopy, and is used for Remote Identifications as well as a training facility for staff learning how to use PaDIl, and Remote Microscopy. Groups of staff from PQ, Entomology and Plant Pathology were trained in the use of the equipment for remote identifications during three separate workshops in Bangkok. Equipment for RM was also installed at the PQ border inspection station at Chiang Sien and in the Entomology department in Bangkok. Staff at Chiang Saen were also trained in the use of RM for identification.

7 Key results and discussion

Positive results arising from this project include:

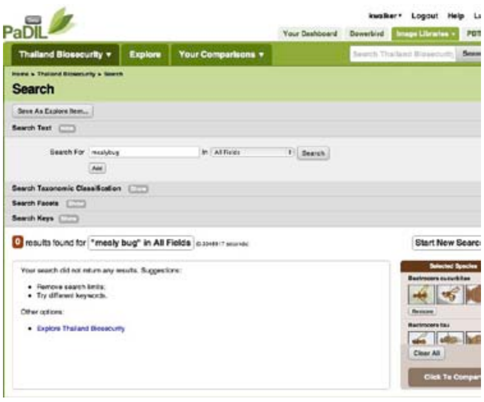
1. Increased *capability* for plant pest diagnostics. A total of eighteen workshops ranging from two weeks to three months in length were conducted during the course of this project. These workshops aimed to raise the skill levels of Thai diagnostic staff in molecular identification, traditional taxonomy, digital diagnostic systems and remote microscopy. We found that in all these areas, the staff had reached a high level of proficiency as evidenced in their abilities to perform diagnostic tasks independently and to convey these skills by teaching them to other staff members. The “train the trainer” concept was tested in our workshops as we gave control of the teaching to those Thai staff who had already been trained. We found this to be highly successful because the training was conducted by Thais for Thais and in the Thai language. We were able to evaluate the success of this method by observing the work that was performed by staff during the workshops and later during subsequent workshops, when we could see a marked improvement in skill levels amongst staff in general.

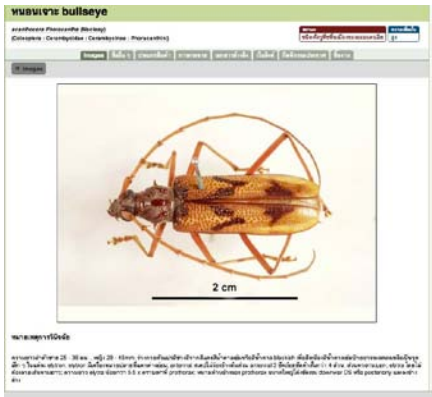
Top left: DOA technical, scientific and managerial staff gather to design and plan their new molecular lab with Australian scientists. **Top Right:** *Parichate Tangkanchanapas and Gary Kong discuss electrophoresis results from a virus assay*. **Above:** Thai scientists in the new moleculr lab**: left**: Running an ELISA assay for virus detection. **Centre:** *Dr Gary Kong watches as Ms Preyapan Pongsapich use a new centrifuge.* **Right:** *Nuttima Kositcharoenkul pipettes DNA samples.*

2. <u>Increased *capacity* to perform plant pest diagnostics.</u> Physical resources in the form of laboratory equipment and access to digital technology were provided by the project to improve the capacity of Thai staff to perform pest identifications. Lab equipment for molecular diagnostics was selected through a process whereby Thai scientists were asked assess their needs in terms of the types of molecular testing that would be performed, then the list of equipment was prioritised against the available budget. Equipment was purchased locally and installed to create a functional molecular laboratory. Additional investment by DOA refurbished and extended the lab space. In addition microscopes (Compound and binocular) plus additional hardware and software for remote microscopy and image capture were purchased and installed in a dedicated Remote Microscopy lab, refurbished by DOA. The following equipment was purchased and installed.

   1. PCR machine
   2. Gel documentation system
   3. Transilluminator
   4. Centrifuge – benchtop
   5. Pipettes
   6. Miscellaneous small items
   7. Fume Hood
   8. Nikon SMZ 1500 Binocular microscope
   9. Nikon 80i light microscope
   10. Nikon DS-L2 web server
   11. Nikon digital sight interface plus video camera.

A new Thailand Biosecurity database and webpage were created in PaDIL to provide Thai staff with their own digital space for uploading and accessing pest information relevant to Thailand. This will allow Thai staff to capture and store pest information that can be shared with all users for the purpose of pest identification.





Above: *Thailand Biosecurity Image Library in PaDIL* (http://padil.gov.au/thai-bio/Search?queryType=all) (

3. <u>New Processes and Systems.</u> This project has helped Thai Plant Quarantine to develop foundation processes and systems that will enhance its ability to build on and improve both its *capacity* and *capability* in plant pest diagnostics. This comes from helping Thai staff to develop an understanding of the requirements for modern plant pest diagnostics and allowing them to design and create processes that suit their needs and their work environment. This was evident in their remodelling and refurbishment of both the molecular and remote microscopy laboratories. The motivation for PQ to improve the facilities indicates a desire to build a system that is both functional and enduring. The PQ proposal to DOA to fund a further nine Remote Microscope installations shows a future commitment to the systems that were introduced in this project and for these systems to be an integral part of Plant Quarantine processes.

Top: *DOA staff work on image capture in the new Remote Microscopy Laboratory, Plant Quarantine , Bangkok.* **Centre left** *A Plant Quarantine officer at Chanthaburi border station with Cambodia uses a USB microscope and microcomputer to capture images of scale on lime fruits.* **Centre right***: PQ Inspectors use a USB microscope to inspect longan fruits from China.* **Above:** Thai scientists using remote microscopy in the new RM laboratory

8 Impacts

8.1 Scientific impacts – now and in 5 years

A good diagnostic system should include a number of principle elements – These are: 1. adequate laboratory facilities with best practice lab processes, 2. Skilled people supported by knowledge systems and 3. Communication systems and networks that connect diagnostic expertise with border stations and remote users. This project has contributed to all three elements and established diagnostic practices and systems that are equal to those used by Australian scientists. These are the foundations of a Plant Health system that determine Thailand's ability to detect and respond to pest threats in a timely and efficient manner and to demonstrate to trading partners that they have a high standard of phytosanitary capability.

The establishment of new technologies, such as molecular diagnostics, digital knowledge systems and remote microscopy puts Thailand at the forefront of how all Plant Health systems will look and operate in the future. There is a strong commitment from the PQ group to continue to grow these elements into the future, with a proposal already submitted to equip nine border stations with remote microscope technology. During the final stages of this project, PQ invested in the construction of a dedicated laboratory for conducting remote microscopy, capturing high resolution images and training staff in these technologies. This indicates the impact that this project has had on the perception of new technologies for the future of Plant Quarantine in Thailand. It also shows that Thailand has the capacity to be a regional leader for Plant Health diagnostics and to assist its neighbours to improve their own diagnostic systems for mutual benefit.

8.2 Capacity impacts – now and in 5 years

Thai PQ now has a molecular laboratory with highly trained and skilled staff, capable of conducting, developing and interpreting molecular identification tests. Staff also understand the principles and processes required to develop and maintain a molecular facility and so are likely to improve their lab and its capability over time. In this project, staff were generally trained in a range of molecular techniques with reference to specific pests and pest groups – fruit fly, viruses, fungi, bacteria and nematodes. Their abilities were tested by Australian scientists in the Thai PQ molecular lab established during this project. The scientists trained in Australia also conducted their own workshop to train other PQ staff in molecular techniques. This was highly successful and indicates a high level of expertise and again, the potential for Thailand to provide regional molecular diagnostic support. The PQ group invested in a complete refurbishment of the molecular lab during this project which indicates their commitment to molecular diagnostics into the future.

Digital technologies were introduced to PQ, Entomology and Plant Pathology staff that will enable them to access quality diagnostic information (Plant and Disease Image Library [PaDIL] and Plant Biosecurity Toolbox [PBT]) and to create their own digital information for pests relevant to Thailand. A pre-requisite for creating digital content is the ability to capture high quality images. This project provided the equipment necessary for capture images and trained staff who have begun to create their own image library in Thailand Biosecurity within PaDIL (http://padil.gov.au/thai-bio/Search?queryType=all). Now that we have created a website for Thailand Biosecurity and given staff access to image capture equipment there is now a clear pathway for future development of digital pest information for pests relevant to Thailand, providing that there is an ongoing commitment by staff to populate the website.

Thailand now has expertise and the capability to extend its remote microscope network because this technology has been introduced in this project. The establishment of a dedicated lab for RM in Bangkok together with a plan to purchase and install nine more RM units, demonstrates a future commitment to this technology as an important part of the Thai diagnostic system. The dedicated RM lab will serve as a training venue for teaching image capture and remote microscopy so that staff from regions can be trained as required.

8.3 Community impacts – now and in 5 years

The benefits from having an efficient diagnostic capability flow indirectly to communities and is implied through greater pest awareness and often better pest management leading to improved productivity. A vigilant diagnostic system can help protect agricultural industries from the damaging effects of pests by creating alerts for endemic pests or by raising awareness for pests that could enter the country through trade routes. Pest species can spread and cause yield losses quickly and so systems are required that enable farmers to react quickly to control pests, and pest identification is the first stage in this process.

Communities impact is therefore difficult to measure, as is the economic impact (see below) because the only measurable benefit is in preventing exotic pests from damaging crops and trade agreements. There is no way of knowing how much damage would have occurred if a particular pest had not been intercepted or if an alert had been delayed, yet we know that new pests can cause considerable damage to farmers yield and to market access for export products. Under this pretext then, it would be right to assume that improved biosecurity preparedness such as that afforded through this project provides indirect community benefits into the longer term and as Thai diagnostic capability matures.

8.3.1 Economic impacts

This project focussed on building diagnostic capability and establishing the infrastructure required to improve diagnostic efficiency in the Thai Plant Quarantine system. These are important and basic elements required in any Plant Health system that will deliver services to prevent and/or ameliorate the effects of exotic pests on the country’s import and export plant industries. Because of the preventative nature of biosecurity it is difficult to put a dollar value on its benefits, and so economic impact is often based on a projected cost resulting from a specific pest incursions.

The benefit/cost ratio of establishing and maintaining a biosecurity infrastructure could be determined from the ratio of the cost of such an infrastructure to the net loss experienced by agricultural industries in the absence of a biosecurity infrastructure i.e. an inability to intercept and manage an incursion. Whilst it is relatively easy to calculate the cost of maintaining quarantine and biosecurity infrastructure it is problematic to predict the absence of it on agricultural industries, or to predict its ability to prevent incursions from occurring.

Bearing in mind that Thailand is an agriculture - based economy with 41% of the land area used for crop production, it is probably fair to suggest that the impact of incursions will be relative to the size and value of the industry affected. Industries such as rice production, which account for about half of all agricultural production and 20% of agricultural export earnings may be hard hit by an exotic incursion although small industries that supply domestic economies may also suffer greatly from an incursion and have high social and economic impacts at a local level. For example, small land holders, of which there are numerous in Thailand, may not be able to withstand the economic impact resulting from an exotic pest or pathogen or conversely, will benefit greatly from interception and/or early detection of exotic pests and pathogens.

The recent introduction of the powdery scab pathogen into Thailand on imported seed potatoes is an example where surveillance, monitoring and testing may have prevented or curtailed the incursion. Instead, the pathogen has now spread throughout the production areas and poses a significant threat to the local industry. Other pathogens, such as Potato Spindle Tuber Viroid, Leaf Roll Virus, *Phytophthora infestans* and Potato Cyst Nematode could also enter Thailand via infected planting seed and so there is a need for quarantine surveillance and detection capability for these pathogens. Similarly, the export of products from Thailand require certification of freedom from certain pests and pathogens. For example, the export of pumello from Thailand may require a certification for freedom from citrus canker and Thailand would be required to demonstrate a capability and confidence in making such a declaration to an importing country.

The current project has provided both skills and lab infrastructure that improve the ability of Thai PQ to identify and respond to pest threats more efficiently. There is strong support for these new developments from the Thai PQ managers (because of trade implications) and a good prospect that they will continue to develop their expertise and lab infrastructure into the future.

8.3.2 Social impacts

In Thailand, where the economy is largely agriculture-based and where rural communities form a dominant group in the structure of Thai society, it is easy to see how any factor that affects agricultural production has a significant economic impact on these communities with resultant social consequences. The magnitude of these consequences will vary but in many ways be disproportional to the benefits of a pest incursion not occurring. For example, by preventing an incursion, production will not be improved, it will only be sustained and social disruption will be prevented. Conversely a failure to demonstrate "Area of Freedom" of an exotic pest could result in the loss of an export market and have significant impact on the profitability of local crops. Again it is in the context of prevention or "non-events" that impacts arise from improved biosecurity and social impacts are generally a long way downstream from the primary impacts and are therefore difficult to measure. Nonetheless, stability or the lack of disruption to agricultural production and markets through improved pest diagnosis must have long-term social benefits

8.3.3 Environmental impacts

The establishment of exotic pests and diseases can have environmental impacts beyond agricultural crops. Many pests and pathogens establish themselves in natural ecosysytems and have profound impacts on native vegetation and indigenous species by competing, displacing habitat and destroying native communities. Ecological "domino" effects usually follow such disruptions to the equilibrium of natural systems and can range from small to large impacts depending on the extent of plant and animal species affected and their relative importance as keystone species in maintaining and supporting the ecosystem. Predicting the extent of such impacts is in most cases not possible and given the wisdom of hindsight (actual invasions by exotic species), it is a common position to prevent incursions from occurring. Viewed in this way, biosecurity processes and systems that minimise the risk of establishment of exotic pests and pathogens have positive impacts on the integrity of environmental, social and economic assets of a country

8.4 Communication and dissemination activities

Press Releases

1. Digital diagnostics expand international surveillance (Catherine Norwood)
2. Thai training targets citrus canker, fruit fly, potato virus (Catherine Norwood)
3. Remote Microscopes – Pest IDs at your Fingertips (Rural Press)

4. Australia extends digital pest-detection network to Asia (Kate Scott)

Video

1. A promotional video of the CRCNPB project which contains reference to the Thai project and contains images from PQ in Thailand is available on YouTube.

   http://www.youtube.com/watch?v=epbhdU6kgH4

2. Two interviews were recorded by Morning Talk Television Program hosted by Dr Valerie McKenzie. The first was a studio interview about the project with Dr Gary Kong in March of 2009, the second was a feature on the project filmed at the PQ labs in Chatuchak on the 21st June 2011. It includes interviews with scientific staff, Senior Expert Mr Udorn Unahawutti, Dr Gary Kong and captures a demonstration of a live remote microscope session with Mr Michael Thompson from his lab at CSIRO in Canberra. Both programs were aired globally to more than 170 networks.

   http://www.thanaburin.co.th/morning-talk-tv-show/

3. The Department of Agriculture of Thailand also produced a feature documentary on the project which was aired on the Government television program.

Above left: Dr Valerie McKenzie introduces a television feature on the ACIAR project for Morning Talk, which ran for 25 minutes and was broadcast by more than 170 networks worldwide. **Above right:** Senior expert Udorn Unahawutti explains the remote microscopy process to the Director General of the DOA, who officially opened the laboratory on the 21st June 2011.

User Manuals Produced for Training Workshops:

1. Digital Microscopy User Manual
2. Remote Microscopy User Manual
3. Plant Biosecurity Toolbox Authors Guide.

9 Conclusions and recommendations

9.1 Conclusions

A key project deliverable at the commencement of this project was for the Thai DOA Plant Quarantine group in Bangkok to have new and improved diagnostic technologies to 1). reliably detect biosecurity threats and 2). to demonstrate area freedom, 3). to gain entry to export markets and 4). to respond to pest incursions. As a result of the one-on-one training sessions of five Thai scientists in the DPI Victoria labs and the four workshops in Thailand, including the successful “train-the-trainer” workshop held in March 2010, the Thai DOA now has a molecular diagnostic capability that can support Plant Quarantine. It is important that the Thai DOA continue to support this capability through the provision of lab consumables that will enable the DOA scientists to use the molecular diagnostic laboratory and to encourage the technical staff to maintain and use validated PCR protocols for testing plant specimens. A molecular diagnostics capability cannot be maintained on an *ad-hoc* basis but rather requires an ongoing investment in staff and resources to facilitate diagnostic and experimental activity in the lab.

We observed a marked increase in the skills of the Thai DOA groups involved in both molecular and traditional taxonomic training over the course of the project. Many of these people also trained in the use of image capture and remote microscopy and became highly proficient in the use of these technologies to capture and store diagnostic information. The staff who we trained, successfully trained their peers in the use of all the technologies they had learned from Australian scientists, indicating that they were able to comprehend and assimilate their knowledge within their own cultural setting. The development of tangible resources by the trainees, such as a competent molecular laboratory, molecular tests, new pest records and descriptions published in PaDIL as well as a Thai Biosecurity website, are the results or outputs arising from detailed and specific training and which demonstrate that staff have achieved a high level of capability.

It might also be mentioned that an intensive mentoring approach was taken for delivering the training for these workshops, where strong relationships and networks were established between scientists, and where learning was reinforced through follow up workshops that tested skills. I believe this has been a highly successful approach in delivering benefits to both Thailand and Australia.

Dr Roger Shivas made the following statement regarding pest records and reports arising from his taxonomic workshops.

> “…….these reports are not without significance. The first reports two new pathogens and diseases of dragon fruit; the second is a new host genus for soybean rust (never been reported on this genus of legume); the third is *possibly* the first report in south-east Asia of a South American rust on a potentially weedy South American tree (the South Africans were interested in this rust as a biological control agent some years ago).
>
> I think that we have found a very good formula for running a diagnostic workshop i.e. keep it real by using the participants' specimens to explore morphological and molecular methods. It all went very smoothly.”

9.2 Recommendations

Due to the success of the molecular diagnostics training component of this project, future activities for the Thai DOA molecular diagnostics team at the Plant Quarantine facility should focus on advancing their collective skills in:

- Bioinformatics, particularly in the area of primer design for test development and phylogenetic analysis of sequence data.
- Producing Standard Operating Procedures (SOPs) for the detection of significant pests and pathogens such as the PSTVd manual that was produced as a part of this project.
- Developing skills to optimize PCR protocols to better suit their molecular diagnostics facility
- Establishing a reliable and validated collection of positive and negative controls to support the diagnostic tests conducted in the Plant Quarantine lab.

- Actively surveying crops in Thailand to better understand the incidence and distribution of endemic pathogens in Thailand. Active and targeted surveillance programs are also an effective means of validating pest diagnostic protocols. This is particularly important for molecular based tests to ensure that there are no organisms, particularly micro-organisms (i.e. bacteria, fungi) that will generate “false positive” test reactions.

Similarly, the foundations have been laid for digital knowledge capability and a system for using remote microscopy for pest identifications. The following are recommended.

- PQ needs to expand these capabilities, which includes training additional staff, so that these technologies become a routine part of the diagnostic system and contribute to a more efficient quarantine and crop protection system.
- There is scope to add new technologies, such as portable digital microscopes and Smart phone reporting tools to improve the data capture activities from border interception points and to provide more detailed records of pest incidence and occurrence.
- Regional networks for sharing pest information could be developed using digital technologies. This would benefit regional pest management by making better use of expertise and knowledge for pest identification and raise awareness throughout the region of pest presence.
- Thailand has the capability and capacity to act as a regional leader for diagnostics services and has the ability to train its neighbours (Myanmar, Laos and Cambodia) to raise their diagnostics capability and strengthen Plant Quarantine cooperation in the region. Future projects should help to foster this role for the mutual benefit of Thailand and its neighbours.

10 References

10.1 List of publications produced by project

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2. Milinkovic M., Bottcher, C., Pongsapit, P., Sombat, S. and Rodoni, B. (2010). Improved methods for detection and control of *Potato virus Y* (PVY) in potatoes. 9th Australasian Plant Virology Workshop, Melbourne Australia (16-19 November 2010).

3. Molecular Techniques as a Tool for Plant Pest Diagnosis (In Thai and includes RT-PCR, PCR, RNA Extraction, Virus RT-PCR, Agarose Gel Electrophoresis, Gel Doc).

4. Thailand Biosecurity Image Library. http://padil.gov.au/thai-bio/Search?queryType=all

List of fact sheets uploaded to PaDIL

1. Suneerat Seemadua; Alternaria spot (*Alternaria brassicicola*) -. www.padil.gov.au/viewPestDiagnosticImages.aspx?id=4455

2. Wanich Khampanich; Maize leaf blight (*Exserohilum turcicum*) -. www.padil.gov.au/viewPestDiagnosticImages.aspx?id=4457

3. Pornpimon Athipunyakom; Pseudocercospora leaf spot (*Pseudocercospora jatrophae*) –www.padil.gov.au/viewPestDiagnosticImages.aspx?id=4458

4. Downy mildew of Cucurbitaceae – Wanpen Srichart. www.padil.gov.au/viewPestDiagnosticImages.aspx?id=4459

5. Komsorn Seangchinda; Pseudocercospora leaf spot (*Pseudocercospora rhinacanthi*) –www.padil.gov.au/viewPestDiagnosticImages.aspx?id=4460

6. Sopa Pisawongprakarn, S., Warangkana; Cercospora leaf spot (*Cercospora apii*) – Sae-uang www.padil.gov.au/viewPestDiagnosticImages.aspx?id=4461

7. Tharntip Bhasabutra; Canna rust (*Puccinia thaliae*) – www.padil.gov.au/viewPestDiagnosticImages.aspx?id=4462

8. Suneerat Seemadua; Nimbya leaf spot (Nimbya *gomphrenae*) -. www.padil.gov.au/viewPestDiagnosticImages.aspx?id=4463

9. Photchana Trakunsukharat; Rhizopus rot (*Rhizopus stolonifer*) – www.padil.gov.au/viewPestDiagnosticImages.aspx?id=4464

10. Supatra Intavimolsri, Wanpen Srichart; Powdery mildew of Sesame (*Oidium violae*) - www.padil.gov.au/viewPestDiagnosticImages.aspx?id=4465

11. Mr Wanich Khampanich, Ms Warangkana Sae-uang & Ms Sopa Pisawongprakarn Annotated checklist of *Curvularia* in Thailand.

12. Dr Pornpimon Athipunyakom, Translation of Smut Fungi of Thailand Lucid key.

13. Dr Pornpimon Athipunyakom, First record of *Colletotrichum truncatum* and *Neoscytalidium dimidiatum* on *Hylocereus undatus* (dragon fruit) in Thailand. Manuscript.