MPI Plant Health and Environment Laboratory (PHEL): Jan-Dec 2019 Annual Report

Introduction

The Plant Health and Environment Laboratory (PHEL) of the Ministry for Primary Industries (MPI) is New Zealand's national plant health reference laboratory responsible for identifying or confirming all suspect new plant pests and diseases, and invasive plants found in New Zealand. We help to protect New Zealand's primary industries and the environment from exotic organisms and reassure our trading partners that New Zealand is free of unwanted pests and diseases.

PHEL has two well-equipped laboratory facilities located in Auckland and in Christchurch, including New Zealand's only Level 3B post entry quarantine (PEQ) greenhouse. PHEL is accredited to the ISO 17025 standard (General requirements for the competence of testing and calibration laboratories) and has expertise in Bacteriology, Botany, Entomology, Mycology and Virology. We have Physical Containment Level 2 (PC2) and Level 3 (PC3 – Entomology invertebrate rearing room) laboratories, Level 3B PEQ greenhouse and tissue culture facilities, and a Transmission Electron Microscope (TEM). PHEL also holds numerous reference collections, including historic invertebrate and virus collections.

Our services

Diagnostics:

PHEL provides a range of diagnostic services to identify pests and diseases including bacteria, fungi, insects, mites, nematodes, phytoplasmas, liberibacters, viroids, viruses and unwanted plants. In 2019, PHEL processed 7,951 accessions (each usually containing multiple samples) and completed a total of 57,174 tests. We use a range of diagnostic techniques including microscopy (stereo, compound and TEM), molecular testing (e.g. end-point and real-time Polymerase Chain Reaction [PCR], Sanger and High-Throughput Sequencing [HTS], LAMP etc.), as well as serological (e.g. ELISA) and biochemical tests.

Fig. 1: PHEL scientists using a range of diagnostic techniques (from left to right: a-c): a. examining reference specimens, b. setting up a DNA extraction; c. using transmission electron microscope

Table 1. Summary of the diagnostic testing carried out at PHEL in 2019.

Purpose of testing	Description	Number of tests
Border diagnostics	Samples of suspected exotic pests and diseases found during the inspection of imported goods and produce at border and pre-border (including Post Entry Quarantine)	22,933
Domestic testing	A tailored plant disease testing service to support industry needs for clean planting material (e.g. avocado industry high health scheme programme)	2,095
Export preclearance	Testing to ensuring that products leaving New Zealand meet the import requirements of our trading partners	301
Post border diagnostics	Samples from recently imported material, for determining if the pest or disease could have been introduced from overseas	649
Response diagnostics	Providing diagnostic services during a response to an incursion. In 2019 these included e.g. Queensland fruit fly, Pea weevil, Granulated Ambrosia Beetle	11,051
Surveillance and investigation testing	Samples from various surveillance programmes like General Surveillance, High Risk Site Surveillance (HRSS), Fruit Fly Surveillance, Arbovirus Surveillance, Bee Mite Surveillance, BMSB trapping and Stink Bug Public Awareness	14,170
Other	Diagnostics for Pacific island partners, proficiency testing programmes, tissue culture operations etc.	5,975

Pre-determined testing to support trade:

Pre-determined testing (PDT) is a specifically designed service to screen imported seed, rootstock or other plant material, for regulated pests and pathogens. PHEL's Mycology & Bacteriology team and Virology team test for the presence of high-impact pathogens, for example *Xylella fastidiosa* and *Plum pox virus* (PPV) in nursery stock, or *Cucumber green mottle mosaic virus* (CGMMV) and *Tilletia* spp. (smut fungi) in seeds for sowing, to name a few. Through PDT, we contribute to ensuring that only healthy and clean plant material is imported into New Zealand.

Pest and Disease notifications:

PHEL processed around 3,500 notifications of suspected exotic pests and diseases. These notifications were mainly from the general public and were reported through MPI's Exotic Pest and Disease hotline 0800 80 99 66. Spring and summer are usually the busiest times of the year (Fig. 2). While most of the notifications resulted in native or established organism finds, a few have identified exotic organisms, for example eight notifications resulted in BMSB (Brown Marmorated Stink Bug, an unwanted notifiable pest) positive finds.

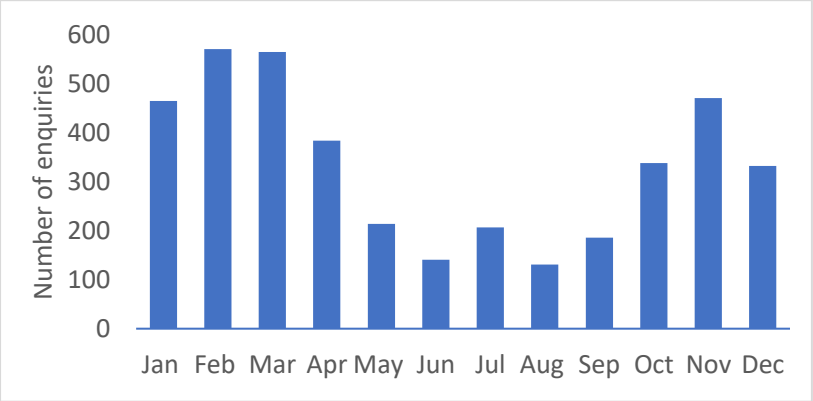

Fig. 2: The number of processed notifications of suspect exotic pests and diseases in 2019.

Post Entry Quarantine:

PHEL is home to New Zealand's only Level 3B post entry quarantine (PEQ) greenhouse, the most stringent quarantine facility for the importation of high-value, high-risk horticultural crops. Plants are held in a highly specialised, fully contained greenhouse and undergo a series of pre-determined tests to ensure they are free of unwanted pests and diseases before they are released. These include molecular based tests such as PCR, graft and herbaceous indexing and microscopic inspections.

In 2019, 61 cultivars (225 total plants tested) of *Fragaria, Malus, Rubus, Vaccinium* and *Vitis* underwent PEQ Level 3B (Fig. 3). Berry fruit were most prevalent accounting for 90% of all cultivars. Twenty-two cultivars were released destined for commercialisation or into local breeding programs, with the remaining set to be released in 2020-2021. Plants were imported from Australia, Italy, Japan, Netherlands, UK and USA.

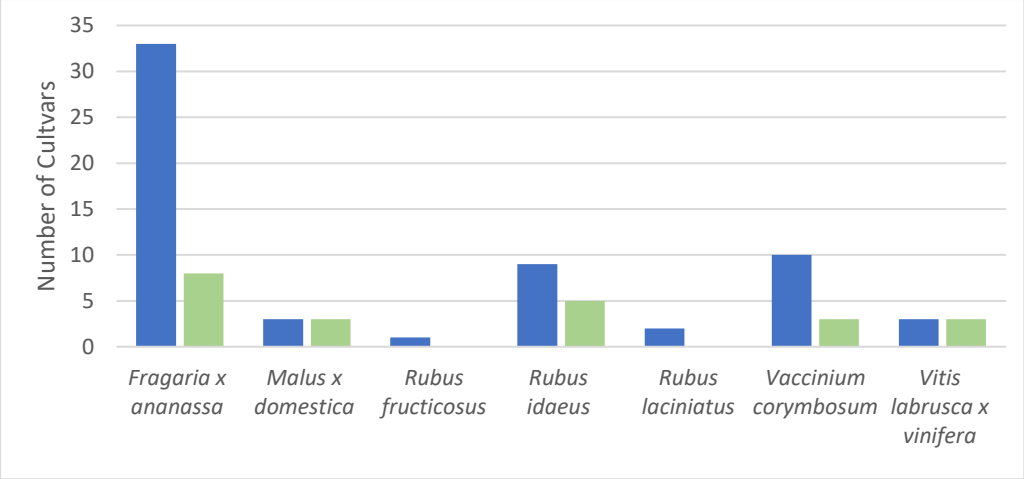

Fig. 3: Total number of cultivars (blue bars) held in PEQ Level 3B greenhouse and the number of released cultivars (green bars) per species in 2019.

PEQ Level 3B capacity is limited and there is currently a five year wait period to bring plants into the facility. To combat this, three portable greenhouse units have been installed at PHEL and planning is underway for the development of a new site utilising portable greenhouses that will double the size of the current facility.

Fig. 4: PHEL scientist examining plants in PEQ Level 3B greenhouse for any pest or disease symptoms

Technical Advice:

In 2019, PHEL received nearly 400 scientific advice requests. These came from different teams within MPI, as well as from external stakeholders like Government Industry Agreement partners, growers and importers. Some of the examples of the types of requests we receive include country freedom reports, import health standard reviews, diagnostic protocol reviews, Official Information Act requests, interception information, media queries, manuscript reviews and risk assessment advice.

A 130 million NZD Pea industry saved with a world-first successful eradication:

Dave Voice, Principal Scientist from PHEL Entomology, had been at the forefront of the successful eradication of the unwanted pest, pea weevil from Wairarapa and New Zealand, by providing key technical advice on life cycle, biology, treatment options and eradication strategy.

Rapid diagnostics to support regulatory decision:

PHEL diagnostic work is crucial for protecting NZ's primary industries. In response to the detection of the unwanted *Cucumber green mottle mosaic virus* (CGMMV) in watermelon fruit imported in 2019, PHEL carried out rapid testing of potentially affected consignment at the border (840 samples processed in 3 days) to enable rapid decision making by MPI's Imports Team. CGMMV is responsible for significant economic losses worldwide, and it can easily establish in new areas. Its prompt detection at the border enabled MPI to immediately halt affected imports and audit the process for 'pest free area' status of the exporting country. PHEL continues to provide technical advice as the two governments work toward improved systems to safeguard of our cucurbit industry.

National and International networks

PHEL has an extensive national and international network of contacts that allows us to access reference material, technical advice, and scientific collaboration. PHEL scientists are well connected with New Zealand's Universities (i.e. co-supervision, guided visits for students, invited presentations) as well as with Crown Research Institutes (CRIs).

PHEL represents MPI in the Better Border Biosecurity (B3) programme. B3 acts as the pre-eminent research provider for science-based plant border biosecurity solutions in NZ and provides a single point of access to the NZ science system (CRI's and universities) for plant biosecurity research encompassing threats to the pastoral, horticultural, arable and forestry productive sectors as well as to natural ecosystems. PHEL representatives provide input into several programme themes but especially to the 'Diagnostic Theme', being actively involved in six diagnostic projects in 2019.

PHEL staff collaborate with many overseas quarantine laboratories involved in national plant protection work (e.g. United States Department of Agriculture [USDA]; French Agency for Food, Environmental and Occupational Health & Safety [ANSES]; United Kingdom Department for Environment, Food and Rural Affairs [DEFRA]; Australian Government Department of Agriculture and Water Resources [DAWR] etc.) and represent New Zealand on international plant protection bodies providing leadership in biosecurity diagnostics. Technical delegations from our overseas trading partners also visit PHEL to learn about our quality management systems, enabling them to establish ISO 17025 accredited plant pest laboratories and testing capability. Below are examples of some of the PHEL's international collaborations.

International Plant Protection Convention (IPPC): The IPPC is a plant treaty overseen by the Food & Agricultural Organisation (FAO) that is signed by over 180 countries working together to reduce the spread of plant pests and diseases, and helping protect biodiversity and the environment without setting up unnecessary barriers to transport and trade. PHEL makes a significant contribution to the IPPC and in particular to the development of diagnostic protocols.

International Phytoplasmologist Working Group (IPWG) – The main goal of the group is to bring together researchers from entomology, molecular biology, and plant pathology to increase and expand knowledge about phytoplasma diseases worldwide. PHEL continues making significant contributions to the group, and in 2019 was part of the scientific committee for the 4th IPWG meeting which was attended by over 140 people from 43 countries.

Subcommittee on Plant Health Diagnostics (SPHD): SPHD is a subcommittee of Plant Health Committee (PHC) and includes representation from the Australian's state and territory governments, Plant Health Australia, CSIRO and the New Zealand's MPI. There are five working groups in the SPHD and PHEL have been contributing to a number of these over the years. In 2019, PHEL contributed to the SPHD's Surge Capacity Working Group in order to develop a process simulator for *Xylella fastidiosa* for two Australian pilot laboratories, Department of Primary Industries Parks, Water and Environment (DPIPWE), and the Tasmania and Elizabeth Macarthur Agricultural Institute (EMAI), New South Wales. We also contribute through validating and reviewing their national diagnostic protocols.

Horticulture Innovation Australia (HIA): HIA is a grower-owned, not-for-profit research and development corporation for Australia's horticulture industry tasked with investing horticulture levies and Australian Government contributions into initiatives to help the industry. PHEL is a New Zealand partner on two HIA-funded 3-year research projects started in 2019:

- A collaborative project with four diagnostic laboratories in Australia on 'Improving diagnostic preparedness of the horticultural sector to the threat potentially posed by *Xylella fastidiosa*'. This project will review and adopt world's best practice diagnostic methods for the detection and identification of *Xylella fastidiosa*, including field-deployable diagnostic tools and HTS methodologies. This capability will be essential during a biosecurity response with the ability to call on several key laboratories in both New Zealand and Australia to handle the surge in sample numbers for testing.
- A collaborative project with quarantine agencies in Australia on 'Improving plant industry access to new genetic material through faster and more accurate diagnostics using HTS'. The overall aim of this study is to develop an end-to-end quality assurance program and standard operating procedures, which are harmonised with international best practices, to enable the adoption of HTS technology for routine phytosanitary screening of exotic pests in PEQ facilities.

Pacific Horticultural and Agricultural Market Access (PHAMA) Programme: The PHAMA Plus Programme is an aid for trade programme to assist the Pacific Island countries and territories to address market access issues. PHEL has been contributing to the PHAMA Plus Programme, and in 2019 assisted with the development of a field guide for pests and diseases of pacific crops.

Quadrilateral (Quads) Scientific Collaboration in Plant Biosecurity: This initiative was established in 2006 through the Plant Health Quadrilateral agreement between Australia, Canada, New Zealand and the United States of America. This initiative provides a framework for scientific cooperation regarding phytosanitary issues and for identifying and sharing plant protection-based tools and technologies. PHEL members represent NZ and MPI in several working groups that are part of the Quads, including:

- **Quads Molecular Diagnostics Working Group:** This group identifies and collaborates in the development of diagnostic protocols and associated laboratory requirements and processes. In 2019, the group produced a guidance document on proficiency testing, and

updated the compendium of test methods used by the Quads countries – the latter is a living document yearly reviewed.

- **Quads 'Managing regulatory issues arising from new diagnostic technology' Working Group:** This group works towards establishing a well-informed and aligned network within the four countries to exchange information on regulatory issues arising from the use of new molecular diagnostic technologies towards identifying organisms of biosecurity/quarantine interest. During 2019, the group has been focusing on developing a paper that outlines several challenges involved in making regulatory decisions when plant pests are detected with HTS; and provides recommendations and considerations which should be considered when managing these regulatory issues.

- **Quads Seed Health (QSH) Working Group:** The QSH aims to identify areas for cooperation and collaboration on seed health between the Quads countries and explore options for implementing joint or harmonized approaches for managing seed health. In 2019, the group focussed on *Tomato brown rugose fruit virus* (ToBRFV), particularly on diagnostic method development and test harmonization. The group also exchanged information on other emerging risk associated with the global seed trade, and development of pest risk analyses for seeds.
- **Quads Digital Identification Tool (DIT**) **Working Group:** The DIT team works collaboratively on information sharing initiatives. PHEL has been contributing to the preparation of a chapter on "Digital Identification Tools for Biosecurity" to be included in the forthcoming Handbook for Biosecurity (Springer).

Innovation and Preparedness

PHEL strives to constantly improve our preparedness for emerging biosecurity threats by researching novel diagnostic tools, adopting the best equipment, technology, and methods in biosecurity diagnostics, and continuously working to improve efficiencies in the laboratory and in data management. This is achieved through *in-house* initiatives, external collaborative projects and Operational Research projects.

Evaluation and adoption of innovative technology:

- Implementation of the *FreezerPro* system - a paperless, web-based, freezer management system that allows for easy data tracking and retrieval.
- Trialling the digital droplet PCR (ddPCR) technology.
- Building HTS capability – an area identified by PHEL management as a key strategic area to be developed over the next five years. Some examples of the work in the HTS area in 2019 include:
    - PHEL participation in new HIA projects to improve plant industry access to new genetics through faster and more accurate diagnostics using HTS' and 'development of HTS methodologies for *Xylella fastidiosa* detection and characterization'. The latter project also looks at development of field-deployable diagnostic tools, an important preparedness capability in case of an incursion response.
    - PHEL representation in the Quads working groups looking at development of HTS operational guidelines for diagnostics, and managing regulatory issues associated with the technology.

- Investment in an Oxford Nanopore MinION with the aim of using it for routine virus screening. The team has been working on developing protocols for reliable detection of plant RNA viruses and viroids.

Fig. 5: The MinION, a portable real-time device for sequencing, connected to a laptop.

Operational Research:

<u>eDNA-based smart surveillance for early detection of exotic pests</u>

MPI's national saltmarsh mosquito surveillance and arbovirus surveillance, is vital for early detection of insect vectors and vector-borne diseases. However, it is very labour intensive and requires specific expertise and resources if we were to identify each individual caught in surveillance samples. Therefore, PHEL is developing environmental DNA (eDNA)-based smart surveillance assays to screen for exotic mosquitoes (Diptera: Culicidae) and biting midges - *Culicoides* spp. (Diptera: Ceratopogonidae) in bulk samples. In 2019, eDNA collection and extraction protocols from surveillance samples were optimised and the eDNA metabarcoding approach was validated with promising results.

<u>Tracing the geographical origin of Brown Marmorated Stink Bug (BMSB)</u>

Tracing the geographical origin of an invasive pest is crucial to make sound biosecurity decisions during an investigation or a response. PHEL has been researching HTS-based method called ddRADseq (Double Digest Restriction-site Associated DNA Sequencing) as well as haplotype analyses (mitochondrial cytochrome oxidase genes – Mt-COI/COII) to ascertain the genetic diversity of BMSB populations and to trace potential geographical origins of BMSB. Using 3.6 billion Illumina HiSeq derived reads, a population-level DNA sequence database for BMSB was established and at least three genetically distinct BMSB populations have been identified. Several novel BMSB haplotypes have also been identified.

<u>Lucid™ interactive key for Myrtaceae plants</u>

As part of MPI's preparedness and capability to be able to respond to the damaging plant diseases of myrtles, such as myrtle rust, PHEL worked on developing a Lucid™ interactive key to plant species in the family Myrtaceae. Some of the iconic plants of New Zealand, such as pōhutukawa, manuka, etc.,

belong to the family Myrtaceae. The interactive key would include both introduced (exotic - cultivated and naturalised) and native species to New Zealand and be delivered as web-based and an app for deployment onto mobile devices. A draft key with 21 characters and 90 character-states has been prepared for the 99 priority Myrtaceae species/taxa. More than 2,000 images have been collected and screened for their suitability for further use to assist in species identification. This interactive key will be publicly available in late 2020, enabling biosecurity officers from MPI and regional councils, and 'citizen scientists' to reliably and accurately identify species of Myrtaceae present in New Zealand.

Rapid detection and quantification of soil-borne nematodes

Extracting and identifying nematodes from soil with traditional methods is a time-consuming process. This project aims to provide methodology for bulk isolation and high throughput molecular detection of soil-borne plant parasitic nematodes. To facilitate sample processing, a novel nematode extraction device was custom-built, and methodology developed for subsequent DNA-based detection of unwanted nematodes. The new methodology allows to process a 10kg soil sample within 2 days, a process that would have previously required around 2 weeks of processing time. The research is carried out in partnership with the Food and Environment Research Agency (Fera) Science Ltd, UK, and the protocols have been validated by PHEL.

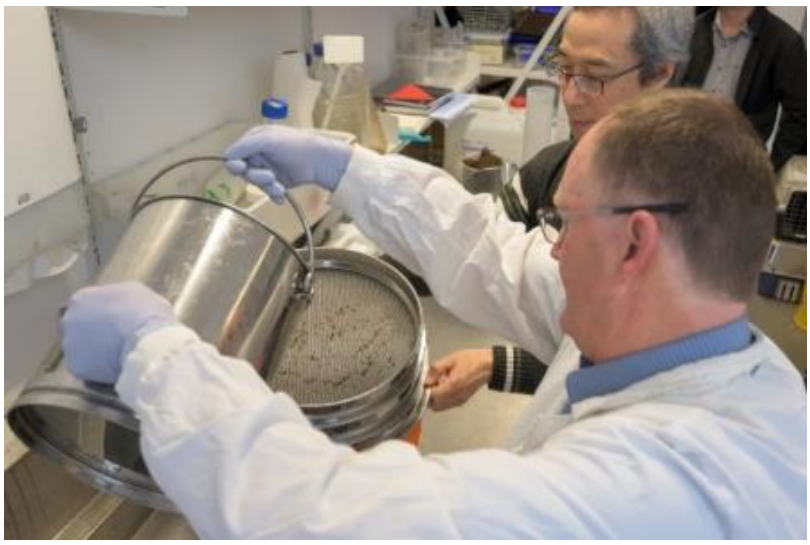

Fig. 6: Extraction of nematodes from a bulk soil sample.

Developing Real-time PCR tests for Forestry and Horticultural Pests

This project aims to develop and/or validate real-time PCR assays for detecting high impact pests of concern to forestry or horticulture crops. In 2019, assays were validated for the detection of citrus canker pathogen, *Xanthomonas citri* subsp. *citri* and the chestnut blight pathogen, *Cryphonectria parasitica*. Pathogen specific assays were also developed into duplex assays by incorporating a plant DNA control targeting the COX gene. Work also started to develop a new real-time PCR assay for the detection of kiwifruit pathogen, *Verticillium nonalfalfae*, particularly the kiwifruit strain.

Real-time PCR for Detection of Plant Pathogens on Germplasm Imports

This project aims to develop and/or validate specific real-time PCR assays for fungal pathogens that are regulated on imported plant germplasm. In 2019, a generic TaqMan PCR assay for rapid and sensitive detection of *Ceratocystis* species was developed. This dual-probe based assay that is also duplexed with plant internal control, has been fully validated and will allow us to detect the presence of any *Ceratocystis* species in symptomatic as well as non-symptomatic plant material. A generic qPCR assay was also validated for the detection of *Phytophthora* species.

Viability PCR

PHEL completed a 3-year viability PCR (vPCR) project that aimed to develop assays for three pathogen-matrices: a bacterial strawberry pathogen (*Xanthomonas fragariae*), a fungal *Brassica* seed pathogen (*Leptosphaeria maculans*), and kiwifruit and stonefruit wilt pathogen *Ceratocystis fimbriata* (*sensu lato*). The vPCR method uses a pre-treatment with nucleic acid intercalating dyes which selectively inhibit the PCR amplification of DNA derived from dead cells. The ability to distinguish between live and dead cells is useful to determine if the DNA detected from samples (e.g. border interceptions) is from living or dead cells, the latter meaning that there is no biosecurity risk. Two assays (*X. fragariae* and *L. maculans*) have been now validated and are available in the lab to use in the diagnostic process as required. Experiments using vPCR could not distinguish between viable and dead spores of *C. fimbriata* spiked in *Prunus* plant tissue.

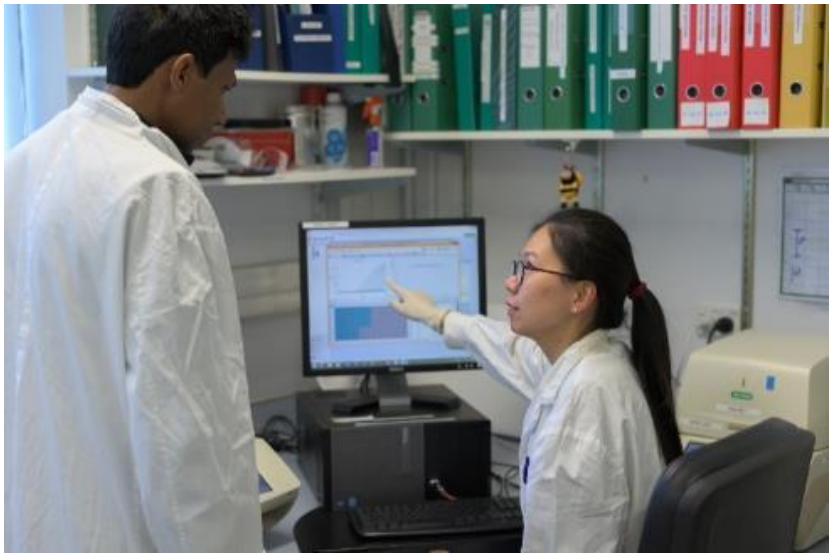

Fig. 7: PHEL scientists in the lab, developing new PCR assays for detecting unwanted pests.

Training

New Zealand Aid:

PHEL will be concluding a three-year project '*Enhancement of biosecurity and market access in the Pacific – phase 2*' in August 2020. The project aimed to enhance Pacific island nations' biosecurity capability including improved surveillance, incursion investigation, response management, diagnostics, pest management and verification. Training workshops in entomology and plant pathology diagnostics, inspection, incursion investigation, and response modules have been delivered to diagnosticians and quarantine inspectors. A molecular laboratory was set up at Biosecurity Authority Fiji (BAF) and assistance was provided to establish a dedicated Plant Health laboratory in Cook Islands. Quality management system was established at BAF laboratory.

Development of diagnostic tools was one of the main deliverables of the project and the following resources were published in 2019: an Entomology diagnostic manual – 'Identification of Insects of biosecurity importance'; an online database PHELdi (Plant Health and Environment Laboratory Diagnostic Image Library) with species information for more than 1000 insects of biosecurity importance to New Zealand and the Pacific.

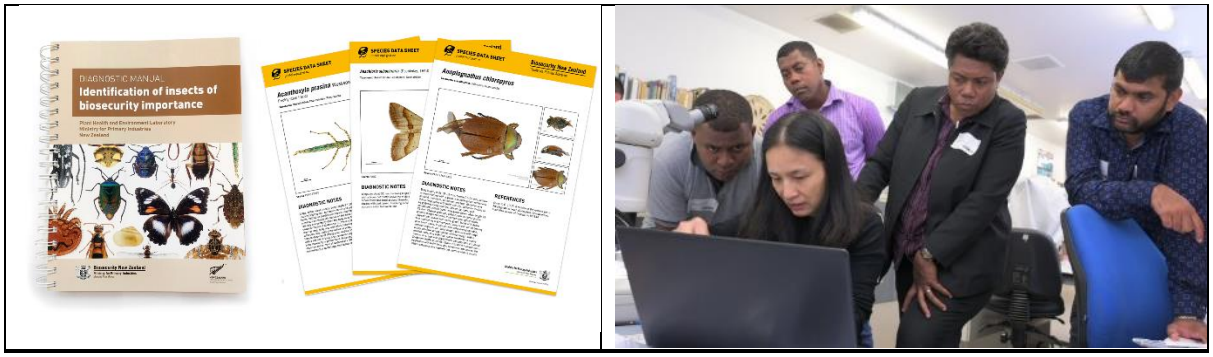

Fig. 8: New Zealand AID programme: Diagnostic manuals and fact sheets designed (left); A training session delivered for Pacific Islands' biosecurity staff (right).

PHEL Professional Development Opportunities:

The purpose of this in-house training initiative is to provide professional development opportunities at PHEL. Several training opportunities were arranged in 2019, which have helped to improve the staff awareness, promote professional development, increase staff capability and strengthen and support interactions within and between teams. Attending the trainings also aligns with the career development pathway for technical staff.

Responses

2019 Auckland fruit fly responses:

The discovery of a single male Queensland fruit fly, *Bactrocera tryoni* (QFF) on 12 February 2019 in a surveillance trap from the Auckland suburb of Devonport activated a level 1 response. To perform the response diagnostic functions safely, two portable and well-equipped laboratories were set up in Devonport to facilitate inspection of traps and public submissions, and to allow prompt processing of sampled fruit. No further adult flies or immature stages were found in the vicinity of the detection in Devonport.

A second fly was trapped on 20 February in Northcote, over 5 km away and from the Devonport detection, and further 9 flies were individually trapped from the region between the initial detection and 15 July. In addition to providing the diagnostics in this response, PHEL scientists were part of the technical working group (TWG) and the technical advisory group (TAG), providing valuable technical advice during the response and contributing to its success. This included providing valuable intel about the likely origin of the found fruit flies, which was determined through detailed genetic analysis.

During this response, a different species (*Bactrocera facialis*) was also detected in Auckland (Otara, South Auckland) on 18 February. Further flies were detected on 21 February and 5 March. *B. facialis* is unlikely to withstand an Auckland winter and has a limited host range when compared to QFF. However, *B. facialis* was still an unwanted pest and similar commitment was made to eradicate it. No evidence of breeding population was found.

Over the duration of these responses, a total of **2,477 traps** were screened, **167 public submissions** were processed, and **3,894 kg of fruit** was inspected for the presence of any life stages of exotic fruit flies. Following the thorough surveys, PHEL was able to provide proof of absence of the QFF and facialis FF in New Zealand, which was accepted by our trading partners.

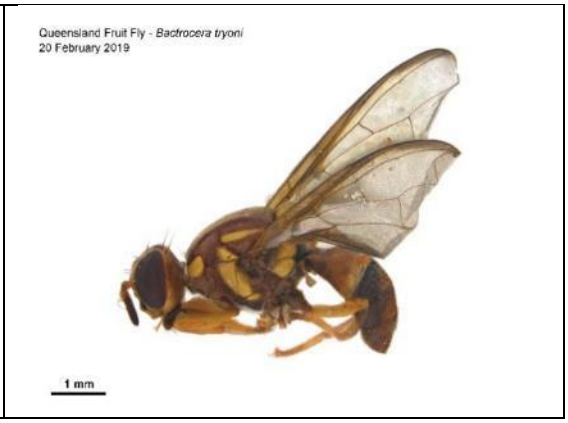

Fig. 9: PHEL entomologist processing fruit for presence of immature stages (left); Male Queensland Fruit Fly (right).

Our People

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Other Staff	
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Senior Laboratory Technical officer	Jane Martin
Specimen Reception	Daniela Ta'ase

*Staff departed MPI in late 2019

Publications

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Rigano, L., Toome-Heller, M., Hofer, K., and Alexander, B.J.R. 2019. Draft genome sequence of *Pseudomonas* sp. strain ICMP22404, isolated from *Solanum lycopersicum* plants with pith necrosis symptoms. *Microbiology Resource Announcements* 22: 8(34).

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PHEL produces a biannual newsletter - *PHELosophies*. Link to the 2019 June and December issues can be found here:
https://www.mpi.govt.nz/protection-and-response/laboratories/plant-health-and-environment-laboratory/publications/

Videos covering some aspects of the work at PHEL:

This is us – PHEL - https://www.youtube.com/embed/lyQcfX3-4BA

Enhancing Biosecurity in the Pacific - https://youtu.be/5E4d58Qxaps

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