Issue 3
DECEMBER 2019

PHELosophies

News from the Plant Health and Environment Laboratory, MPI NZ

Finding that elusive worm

Plant-parasitic nematodes are microscopic worms which can cause major crop loss to pasture and crops worldwide. Currently, nematode diagnostics requires a lengthy manual extraction process, followed by microscopic screening and identification. This process takes up to 2 weeks, and needs to be performed by specifically trained staff. DNA based identification is also used but the current methods still require the isolation of individual nematodes.

For the past two years, PHEL has managed a research project "Rapid Detection and Quantification of Soil-borne Nematodes", funded by MPI Operational Research. The aim of the project is to develop new methodology to process large soil samples during responses and investigations and reduce the time required to obtain results.

For this project, MPI has contracted FERA Science Ltd, York, in UK who have designed and built a bespoke nematode isolation (flocculation) device to quickly and efficiently extract nematodes from large (5kg) soil samples. They have also developed methods to extract all nematode DNA from these samples, which can be followed by fast and sensitive DNA based PCR testing, allowing us to determine if the nematode of interest was present in the sample or not.
To validate this methodology, Michael Surrey from our Mycology and Bacteriology team, who is also the Technical Lead for the MPI project team, visited FERA in October. Michael worked alongside the researchers as they refined the operation of the prototype flocculation device, and the molecular testing protocols. Based on his feedback, further testing and validation will be completed at FERA to improve the protocols to better meet MPI requirements.

Once established at PHEL, these new techniques will greatly improve our diagnostic capability, enabling rapid identification of target nematodes from large soil samples in only 2 days. The significantly reduced timeframe for obtaining diagnostic results will assist with completing delimiting surveys and taking timely actions if new and unwanted soil-borne nematodes were to be found in New Zealand.
The project will be completed in April 2020 with the technology available to MPI by May 2020.

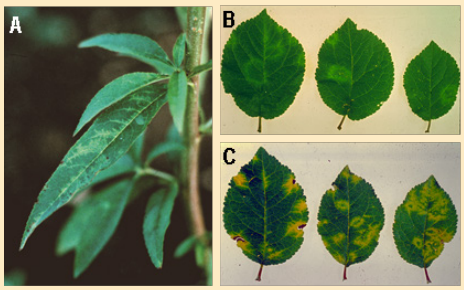
- Michael Surrey

PATHOGEN PROFILE: Plum Pox Virus (PPV)

Status: Exotic, Unwanted Organism

Distribution: Europe, North America, South America, North Africa, Asia and the Middle-East.

Description: PPV infects many species of the *Prunus* genus and is one of the most destructive virus diseases to the stonefruit industry, particularly in apricot, peach, and plum species.



Leaf symptoms of Plum pox virus on A. GF305 peach seedling indicator; B. plum 'Brompton'; C. plum 'Ackermann'.

Symptoms include fruit deformation, discolouration and lesions as well as rings and lines. In severe cases, premature drop of diseased fruit occurs.

The virus is transmitted in the field by several aphid species, as well as mechanically and by grafting. Spread over long distances occurs by movement of infected propagative plant material.

Impact: Horticulture, ornamental and stonefruit industry as well as gardeners/ home owners would be affected if PPV were to establish in NZ. Presence of PPV in a country can create trade barriers with countries that are free from the virus.

Entry Pathways: Nursery stock, undeclared plant material by Air Passengers or in the Mail.

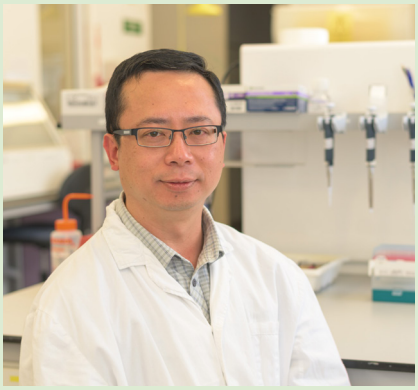
D. Petal discoloration caused by PPV-M on flowers of peach cv. Baby Gold.

For more information:
CABI datasheet

Contact phone number for reporting: **0800 80 99 66**

A. Reproduced by permission from Hadidi, A., Barba, M., Candresse, T., and Jelkmann, W. 2011. Virus and Virus-like Diseases of Pome and Stone Fruits, APS, St Paul, MN, USA.; B-C. Courtesy Biologische Bundesanstalt für Land- und Forstwirtschaft , Bugwood.org D. Courtesy of J.C. Desvignes, CTIFL, FR

INTRODUCING DR WELLCOME HO



Wellcome joined the Plant Health and Environment Laboratory in 2006 as a Senior Scientist and became a Principal Scientist in 2015. His work involves plant disease diagnostics, validation of exotic pathogens, auditing laboratories, and training and mentoring staff. Examples of interesting responses that he has been involved in include: myrtle rust, Psa disease on kiwifruit, and rebutting USDA report of avocado scab disease on New Zealand avocadoes, maintaining country freedom status for New Zealand and allowed trade resume promptly. He is a member of the international Quads Working Groups on DNA barcoding and managing regulatory issues arising from new diagnostic technologies, as well as MPI Science Forum. Over the years, he has implemented a DNA barcoding platform for effective routine diagnosis of fungi and bacteria, and managed a number of diagnostic capability projects.

Prior to this, he was a lecturer, post-doctoral fellow, and honorary associate professor in the University of Hong Kong. He was an editor of two international journals and a curator of a culture collection and fungal herbarium. Some of the memorable moments were organising several fungal expeditions in South East Asia and Australia in collaboration with pharmaceutical companies, and discovered many new to science fungi. He has published over 50 peer-reviewed papers and a number of books, book chapters and popular articles.

Apiculture Surveillance Programme at PHEL

New Zealand is free from many of the most significant pests and diseases of honey bees (*Apis mellifera*); this provides New Zealand beekeepers with export opportunities for live bees and bee products. MPI conducts an annual apiculture surveillance programme to provide assurance of our ongoing country freedom from these pests and diseases and to provide an early warning system, should an incursion take place.

The field component of the surveillance programme is delivered by AsureQuality New Zealand, and samples are forwarded to the Plant Health and Environment Laboratory (PHEL) for processing, inspection and identification. The PHEL bee laboratory runs for six months of the year from April to September.

Three different categories of samples are collected for the programme:

- **Suspect exotics**—these are samples collected and submitted by a beekeeper who has noticed bee mortality in a hive and suspects a new organism may be responsible.
- **High risk sites**—these are samples which have been collected from locations considered as having the greatest potential for entry of an exotic pest or disease e.g. ports, transitional facilities, areas of concentrated beekeeping activity etc.
- **Export consignments**—these are samples which have been collected from populations of bees that are being exported to another country.

The organisms specifically targeted for detection in the surveillance programme are: *Acarapis woodi* (tracheal mite), *Tropilaelaps* spp. (Asian mite), *Euvarroa* spp. (Euvarroa mite), *Varroa* spp., excluding *V. destructor*, *Aethina tumida* (small hive beetle), *Braula coeca* (bee louse) and other exotic bee species. The samples are composed of adult bees and sticky boards, which are processed and examined for the targeted pests.

The New Zealand honey and beekeeping industry is growing rapidly with the number of registered beehives increasing by 94% over the past five years. This highlights the importance of educating the beekeeping community about the risks posed by unwanted honeybee pests and diseases. These organisms are spread either through bee products, used beekeeping equipment, or on bees.

If you suspect one of your hives has an exotic disease or pest, or if you know of a swarm on a ship or in a shipping container or bees being imported illegally, then contact your nearest AsureQuality Apicultural Officer Freephone 0508 00 11 22 or call the MPI exotic pest and disease hotline on 0800 80 99 66.

- Qing Hai Fan and Stacey Lamont

The exotic *Varroa jacobsoni* is one of the mites targeted in the surveillance.

Editorial

It's been a productive and busy year for PHEL. Staff have been involved in fruit fly responses, collaborating with colleagues on *Xylella fastidiosa* diagnostics, as well as sharing diagnostic skills on *Ceratocystis*, funding and validating new technology to rapidly extract and identify nematodes in soil samples, and reached the milestone of finishing the 2016 - 2020 NZAID project.

PHEL - at the forefront of the 2019 Fruit fly responses

In the space of a week three separate detections of exotic fruit flies were made in Auckland; 14 February 2019 a male *Bactrocera tryoni* Queensland Fruit Fly (QFF) in Devonport, 18 February 2019 a male *Bactrocera facialis* in Otara, and 20 February 2019 male *Bactrocera tryoni* QFF in Northcote.

In a fruit fly response PHEL Entomology team has the key responsibility to screen all response trap samples for adult fruit flies, and process all fruit collected around sites with positive finds to detect immature fruit fly stages. To be able to work onsite, two mobile laboratories were deployed and based at the Royal New Zealand Navy Devonport Naval Base, then later moved to the response field headquarters in Rosedale. The mobile labs were used to process fruit from all three response zones (Devonport, Northcote and Otara), as well as for screening trap samples and public submissions from the North Shore. Meanwhile, entomologists back at PHEL handled public enquiries related to suspected finds of fruit fly from across New Zealand.

Our entomologists also undertook many site visits, including to the site of every positive fruit fly find, to survey for potential host plants, as well as inspect and collect fruit. The Entomology team is a key contributor of technical knowledge to support many response work streams. PHEL issues a host list to inform trapping (what trees fruit fly traps should be deployed in), fruit monitoring (what plants need to have fruit collected and how much), and movement control. We also contribute

The PHEL mobile lab set up at Devonport Naval Base.

to specifications for surveillance and organism management. We are constantly analysing possible fruit fly life cycle scenarios to help educate and guide our decisions and actions.

In addition to the rapid morphological identification of any fruit fly found we also undertook molecular diagnostics such as real-time PCR to confirm the morphological identifications and sequencing to investigate any possible information on relationships between the flies. We also coordinated selected testing for investigation of point of origin using stable isotope analysis.

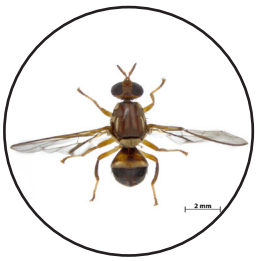
- Shaun Bennett

PHEL Entomology technician Stacey Lamont processing fruit collected in response zones.

QFF RESPONSE BY THE NUMBERS

More than
1,800
trap samples screened*

More than
3,700kg
fruit processed*



Nearly
2,000
photos screened
(from >950 QFF enquiries)

* Numbers for QFF and Facialis FF responses combined

Tour the labs!

Each year, we welcome hundreds of visitors to our Auckland and Christchurch laboratories and show them first-hand what we do on a daily basis by giving an overview of our diagnostics, research and project work, and post entry quarantine processes. Our guests are often fascinated by the complex aspects that we handle and leave with a better understanding of how we contribute to protecting New Zealand.

In 2019, we hosted groups of MPI staff, including new quarantine officers, our colleagues from Border Clearance, Plant Imports, Operational Research, GIA Readiness and Response teams, as well as students from universities. We also welcomed our local customers and growers, to whom we provide diagnostic services, and international visitors from Australia, Japan, Fiji, Cooks and Vanuatu, who were here to learn how biosecurity diagnostics is carried out in New Zealand.
- Merje Toome

PHOTO OF THE ISSUE

A dead ground beetle (*Carabus elysii*), endemic to China, was found by a member of the public in their pillow stuffing.

Collaborating with Australia on *Xylella fastidiosa* diagnostics

Xylella fastidiosa, an insect vectored bacterial pathogen with a wide host range, is one of the highest plant biosecurity threats to both Australia and New Zealand. The impact of *Xylella* overseas has been catastrophic with outbreaks in Europe costing up to a billion dollars in damages.

The detection of this pathogen is difficult as it has several different genotypes, many hosts don't exhibit symptoms, and diagnosis can be very time consuming. However, early detection and fast turn-around time of diagnostic results could be essential for successful eradication and management of this devastating disease.

PHEL Mycology and Bacteriology team is the only New Zealand partner on a new collaborative research project with four diagnostic laboratories in Australia on "Improving diagnostic preparedness of the horticultural sector to the threat potentially posed by *X. fastidiosa*". This project is managed by Horticulture Innovation Australia, and will review and adopt world's best practice diagnostic methods for the detection and identification of *X. fastidiosa*. 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.

- Rob Taylor

Devastating impact of *Xylella fastidiosa* on an olive grove in Italy (Image: D.Gunawardana).

The project was recently covered by media:

New project will boost capability to detect number one plant biosecurity threat

Success story: 2016-2020 NZAID Project is accomplished!

Dr Katharina Hofer training staff from BAF pathology staff at PHEL.

PHEL had the key role in the delivery of the project 'Enhancement of Biosecurity and Market Access in the Pacific'. The main objective of the project was to enhance the biosecurity capability in the Pacific, focusing specifically on Fiji, Cook Islands, and Niue.

In Fiji, this was achieved by training the Biosecurity Authority Fiji (BAF) staff in pest and disease symptoms recognition and diagnostics, surveillance techniques, incursion investigation, and response management. In addition, Plant and Food Research assisted us in conducting a scoping study for pest management.

In Rarotonga, we have conducted pest identification as well as pest and disease symptom recognition trainings for biosecurity inspectors of the Cook Islands and Niue.

Number of workshops held as part of the PHEL's NZAid Project.

Training Workshops	Fiji	New Zealand	Cook Islands/ Niue
Entomology Diagnostics	6	4	4
Mycology & Bacteriology diagnostics	7	4	-
Virology diagnostics	1	1	-
Surveillance techniques	3	1	-
Investigation & Response Management	2	-	-
Pest & Disease Symptom Recognition	2	-	2

Number of staff trained:

- In morphological and molecular diagnostic techniques: 4 entomology and 4 pathology staff from BAF laboratories
- In pest and disease symptom recognition on fresh produces: 11 Cook Islands', 4 Niue and over 70 BAF inspectors.
- In investigation and response management: 40 BAF staff
- In surveillance techniques: 25 BAF staff

Key achievements of the project:

- Building molecular diagnostic capabilities in entomology and plant pathology at BAF laboratories
- Setting up a molecular laboratory at BAF
- Establishing a quality management system at BAF laboratories
- Establishing a basic entomology laboratory in Rarotonga
- Developing diagnostic tools:
  - » Pest and disease symptoms recognition manual
  - » Entomology Diagnostic manual
  - » Diagnostic Image library – PHELdi, which will be created as an MPI external website
- Gaining a good knowledge of the systems and processes used at BAF during import and export inspections

NZAid training participants at a Symptom Recognition Workshop run in the Cook Islands with PHEL staff scientists Dr's Disna Gunawardana and Asha Thomas.

THE NUMBERS *01 June - 30 November 2019*

Confirmed stinkbug submissions to PHEL*:

96
Brown Marmorated Stink Bug
(*Halyomorpha halys*)

45
Yellow Spotted Stink Bug
(*Erthesina fullo*)

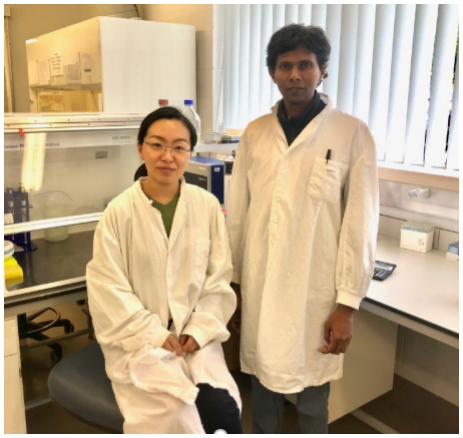
*both species not present in New Zealand

Sharing our diagnostic skills

Grace Sun from the Australian Department of Agriculture, Victoria, visited the PHEL Mycology & Bacteriology team in September. The aim of her visit was to learn about the diagnostic processes we use for *Ceratocystis* when testing imported plant material. *Ceratocystis* species are important fungal pathogens that affect a wide range of hosts, including kiwifruit and stone fruit. The three day training included demonstration of the isolation methods, morphological characters of *Ceratocystis* cultures, and DNA based detection and identification of *Ceratocystis* species. Grace also received an overview of the process we follow for performing routine diagnostics at PHEL.

As part of our diagnostic improvements, PHEL has recently developed a real-time PCR assay to detect *Ceratocystis* species from suspected infected plant material. This methodology was shared with Grace and as a result, the Mickleham Post Entry Quarantine facility in Victoria has shown an interest to use our assay to test plant material imported into Australia. Following Grace's training at PHEL, they have already successfully tested our assay at Mickleham, showing that the methodology is robust and transferrable to other laboratories.

- Merje Toome



Grace and Karthik (PHEL scientist who developed the DNA based *Ceratocystis* detection assays) getting ready to set up PCR assays in our lab.

Senior Advisor Quality Assurance, Sumathi Murugan, who leads the Quality Management system at PHEL.

DID YOU KNOW?

PHEL is providing internationally recognised assurance for all the testing it carries out with its accreditation to ISO 17025:2017 (General requirements for the competence of testing and calibration laboratories). It is one of the very few plant health laboratories in the world covering a broader testing scope of insects, mites, fungi, bacteria, phytoplasmas, viruses, viroids and botanical identifications. We have **23 authorised Key Technical Personnel** signing out the tests conducted using diverse diagnostic technologies.

Cluster Flies

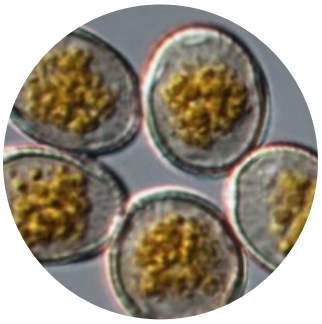
Photo / Tristram Brelstaff

Every few years there are large outbreaks of Cluster fly in NZ. They become a nuisance when thousands of flies seek hibernation sites over the winter inside buildings in rural areas. Reports describe layers of slow moving flies (at least 25mm thick) aggregating in sunny rooms. Once removed, the area is often re-infested days afterwards by more flies. There are two species which share this behaviour, *Pollenia psuedorudis* and *P. rudis* (DIPTERA: Calliphoridae). Both are native to Europe and were found to have been accidentally introduced to New Zealand in the 1980's where they have spread to many parts of the North and South Islands. They are a medium sized fly (10-15mm), dark grey in colour, slow moving, and hairy. In spring after overwintering the adult female flies lay eggs in the soil, and the larvae feed on earthworms, eventually killing the host. They can have up to five generations over one summer if conditions are good. It is in the autumn and winter months when the adults seek shelter to overwinter that they can be found "clustering" around buildings. It has been a few years since this fly has been seen in large numbers, so keep an eye out for it next autumn.

- Ben Boyd

Photo / Liz Brook - Central Districts Farmer

PHEL 2019 Highlights



PHEL developed 16 new diagnostic tests for the detection of high impact pests and diseases

Five of these tests, for Kyuri green mottle mosaic virus, Raspberry ringspot virus, *Dirioxa pornia, Bactrocera (Zeugodacus) cucurbitae* and *Xanthomonas fragariae* are currently undergoing IANZ accreditation.

PHEL scientists presented at 28 meetings

35 oral and poster presentations introducing the work that PHEL does, were given at national and international events this year.

We have two new portable glasshouses

to allow greater capacity for Post Entry Quarantine service that PHEL provides to germplasm importers.

The lab was involved in 10 new responses

including Queensland Fruit Fly, on the North Shore of Auckland, Facialis Fruit Fly in Otara and the detection of Granulated Ambrosia Beetle (GAB), in West Auckland

Nearly 500 exotic insect species were identified

from a total of more than 4,700 identifications this year.

342 suspected exotic organism detections were investigated

from a total number of 1323* notifications that were managed by the incursion investigation team

*for the period 1 July 2018 - 30 June 2019

We closed two responses this year

The Virology team finalised the work on the PMTV (Potato mop top virus) and Post-Entry Quarantine (PEQ2018) responses that began in 2018.

Successful completion of the 2016-2019 NZAid project

This year we have trained 46 Pacific biosecurity staff over 38 workshops organised by the PHEL NZAid Project team, bringing the second phase of the NZAid project '*Enhancement of Biosecurity and Market Access in the Pacific – phase 2'* to a close.

PHEL*osophies* is a biannual newsletter produced by the Plant Health and Environment Laboratory, Ministry for Primary Industries New Zealand.

For further information please contact:
auckland@mpi.govt.nz

Biosecurity New Zealand
Ministry for Primary Industries
Manatū Ahu Matua

PEST AND DISEASE HOTLINE

Call to report any exotic pests or diseases of plants or animals

0800 80 99 66