Phytophthora is a group of fungal-like organisms capable of causing enormous economic losses on crops worldwide, as well as environmental damage in natural ecosystems. The name Phytophthora itself literally means “plant destroyer” in Greek. Different Phytophthora species are known to naturally cross and form hybrids. Such hybrids, having DNA from both parents, could have an increased ability to rapidly adapt to new environments and become invasive plant pathogens, causing disease on a wider host range than their parents.

It is difficult to identify the parent species of Phytophthora hybrids using standard DNA sequencing technology. However, this is an important task in order to determine if they pose any significant biosecurity risks (e.g. to provide insights on the origin, virulence and host range of the hybrid).

Through an internal diagnostic capability project, PHEL has started developing the capability of using high-throughput genome sequencing technology for the identification of Phytophthora hybrids. By taking advantage of the massive amount of DNA sequence data generated by this technology, and employing complex algorithms processed by a supercomputer, PHEL scientists have successfully developed a big data diagnostics pipeline for the identification of Phytophthora hybrids. This method is able to reconstruct the key marker genes that can identify each parent of the hybrid, enabling information to be gathered for risk assessment and making informed biosecurity decisions.

- Dr Luciano Rigano

Typical disease symptoms (left) and sexual structures (right) of Phytophthora alni, a hybrid pathogen that is causing destructive alder dieback in Europe. Images: Thomas Jung, Bugwood.org (2110004 and 2110008)
PEST PROFILE: Salvinia (Kariba weed, water fern)

Status: Notifiable organism in New Zealand

Origin: South America, Southeastern Brazil and northern Argentina

Description: Salvinia molesta D.S. Mitch. Salviniiaceae
Salvinia is a free floating fern with tightly overlapping hairy leaves. Highly invasive in still and slow moving freshwater environments in warm areas. Perennial, up to 30cm long and 5cm wide, roots absent, stems irregularly branched and pubescent. Found in the lowland areas of the northern parts of the North Island.

Features and adaptations: horizontal stem; paired leaves above water, brownish-green elliptic to broadly elliptic up to 2.5 x 2 cm, folded in adult form, while flat in juvenile form; modified leaf hairs called trichomes, shaped like egg beaters, and a fine layer of epicuticular waxes enable salvinia to retain an air-film when submerged in water; lower submerged leaves look like feathery roots up to 30 cm long; reproductive structures produced under water (although New Zealand strain is believed to be sterile).

Pathways for spread: Originally introduced in New Zealand as an ornamental aquarium plant. Fragments are spread by water movement, deliberate release (with fish from aquariums/ponds or trade.)

Impact: Spreads rapidly by fragments, producing plantlets from the end of the horizontal stem, and can grow from a single node. It forms dense mats across the water surface blocking out light, outcompeting other water plants and altering the habitats of aquatic life, to the point of killing all other flora and fauna. Large volumes of living matter and rotting vegetation consume oxygen, stagnating the water, and blocking water ways impacting on agriculture, horticulture and amenity, cultural and natural environments.

For more information: NZPCN datasheet Weedbusters Info Sheet NIWA Freshwater Pests
Contact phone number for reporting: 0800 80 99 66

EDITORIAL

In this issue we showcase new methods and technologies that PHEL has integrated into our diagnostic workflow, not only to identify individual organisms, but also to gather additional information about these pests/ diseases. Innovative work currently happening includes; eDNA metabarcoding to accurately detect targeted pests/pathogens which will save time in the lab sorting through large samples, identify the origin of incoming biosecurity risks using geographic origin to aid in improved pathway management, and enhance the speed and accuracy with which identifications can be made with DNA-based diagnostic test development to provide importers and industry with fast and reliable diagnostic services. In addition to protecting New Zealand, PHEL is actively contributing to international projects, for example Euphresco, to help improve international phytosanitary measures by identifying and exploring modern tools, technologies and resources. As an essential service during the COVID-19 lockdown period, PHEL staff numbers were reduced onsite with the rest working from home. This forced us to work remotely whilst keeping teams engaged and implement new systems to communicating within teams.

- Ben Boyd

PHEL’s participation in European research network - Euphresco

Euphresco (European Network for Phytosanitary Research Coordination) is an international network of 70 organisations from more than 50 countries worldwide, contributing to phytosanitary research. This collaborative research initiative aims to enhance coordination and cooperation on plant health research to support policy, science capability, and collaborations across the globe. In January 2020, MPI joined the Euphresco Network as a member. MPI is also the national point of contact for Euphresco. We facilitate the participation of other New Zealand scientists in Euphresco research projects and ensure effective communication of research outputs to New Zealand organisations, in line with Euphresco’s operating principles.

Currently, PHEL scientists are actively contributing to ten Euphresco projects in plant health diagnostics. The topics range from the application of standard DNA barcoding to innovative molecular approaches, including big data diagnostics using high-throughput genomic technologies to investigate and characterise plant pests and diseases. Participation of our scientists in such a large international phytosanitary research network will help to identify and explore modern tools, technologies and resources, develop new diagnostic approaches, and/or optimise and share existing protocols and procedures. It will also assist us to define standard best practices in plant health diagnostics, surveillance, and risk assessment in regulatory settings. For more information about the network, please visit https://www.euphresco.net/.

- Dr Chandan Pal
FAREWELL
MICHAEL SURREY

Michael has been a Senior Technician in the Mycology & Bacteriology team for nearly 10 years. He joined the team to help produce a NZAID manual for pathogens on imported produce for Pacific island nations and assisted with carrying out the training in Tonga and Vanuatu. Over the last years, his daily role has involved working closely with diagnosticians to isolate and identify fungal and bacterial pathogens and plant-parasitic nematodes. This requires skills in microbial culturing and storage, DNA extraction, PCR technology and molecular analysis. He also has successfully managed a recently completed MPI Operational Research Programme funded project on nematode isolation and diagnostics from large soil samples (see ISSUE 3).

Michael holds a B.Sc. in zoology (UoA) and a postgraduate Dip. Sci. in biotechnology (University of Otago). During his 43 year career Michael has worked as a science technician, technical officer and scientist for government departments, universities, CRIs and in private industry, including his own company. His skills and interests also include entomology, insect pathology, nematology, and fermentation/bioprocess technology.

At the end of July, Michael is going to retire to have more time for family, to get more involved with his church activities and to pursue some science projects of interest. He has greatly contributed to the team over the years and brought along many new skills and ideas. The whole PHEL staff will surely miss his cheerful presence and ability to always find the right words. Many thanks, Mike!

Big data and high-throughput sequencing in modern biosecurity diagnostics

High-throughput sequencing (HTS), also known as next-generation sequencing, is a modern and powerful genomic technology for identifying and characterising pests and pathogens using DNA sequence. It has the potential to detect all types of pathogens, such as viruses, bacteria, and fungi, in a single diagnostic test and therefore in future could replace many conventional techniques such as microbial culturing and PCR. Using HTS, it is now possible to sequence the DNA of all organisms in a sample and identify the risk-pathogens and their genetic potential. At PHEL, in parallel with the routine diagnostic techniques, such as morphological identification, PCR, DNA barcoding, serology, and biological indexing, HTS is being used in biosecurity diagnostics, investigations and responses to support biosecurity, regulatory and trade objectives.

We are currently investing in an in-house HTS suite with supercomputers that will strengthen the capability to explore and integrate HTS into routine plant diagnostics. We are continuously exploring and building new HTS capabilities via in-house or collaborative research work to deploy them into our diagnostic capacity, including developing eDNA (environmental DNA) approaches for the detection of emerging pests, tracing the geographical origin of brown marmorated stink bugs, determining the dead/alive state of insects and approximate time of death, and developing protocols for screening plant germplasm/varieties imported into New Zealand for various pathogens.

As the national plant health reference laboratory, PHEL is using HTS to identify and/or validate suspected exotic, new and emerging pathogens alongside existing methods to support their identification. This includes the use of HTS to resolve the taxonomy of novel or emerging viruses affecting New Zealand’s native plants, ornamental and crop species. Other relevant examples include the application of HTS to resolve the taxonomy of fungal hybrids (e.g. Phytophthora); to detect and characterise high impact slow-growing bacterial pathogens, such as Xylella fastidiosa, directly from plant samples; and examine insect guts for potential plant host ranges that insects often feed on. To be at the forefront of biosecurity diagnostics, our scientists are also working collaboratively with various National Plant Protection Organisations (NPPOs), European research partners (Euphresco) and other reputed research organisations (i.e. Hort Innovation Australia) for the transition of HTS-based big data diagnostic capabilities from the research laboratory into our diagnostic activities.

- Dr Chandan Pal

MiSeq machine - a sequencing platform that allows rapid and cost-effective DNA analysis in the lab.
NEW STAFF AT PHEL

Dr Michael Gemmell  
SENIOR SCIENTIST  
BOTANY

Mike brings experience from a wide and varied career, having studied plant systematics, population genetics of lancewood trees and the evolution of marine snails, as well as having worked in positions at DOC, Zealandia, Te Papa and most recently Horizons Regional Council. Mike has adapted well to his new position, despite starting with PHEL in the week NZ went into Alert Level 4 and having to start work during lockdown.

Lisa Lawrence  
LIMS ADMINISTRATOR

Prior to starting with MPI, Lisa worked as a Senior Research Technician at the University of Auckland, using Zebrafish as a model organism for human disease. She has extensive experience with molecular biology techniques, as well as a speciality in Zebrafish development and husbandry. She is very excited to be working for MPI in her new role as a LIMS Workflow Administrator.

PHOTO OF THE ISSUE

Have you seen these kind of yellow spots on hollyhock plant leaves? A closer look of the underside might reveal brown cushiony pustules of the rust fungus *Puccinia malvacearum*. The pustules are formed by beautiful two-celled teliospores (on the right). Photographed by Merje Toome (Senior Scientist, Mycology)

RESEARCH IN THE LAB

eDNA-based smart surveillance for early detection of exotic pests

Biosecurity surveillance, such as MPI's national saltmarsh mosquito surveillance and arbovirus surveillance, is vital for the early detection of insect vectors and vector-borne diseases. However, such surveillance requires labour-intensive taxonomic expertise and resources to examine and identify each individual caught in surveillance samples. Thus, via an in-house operational research programme, PHEL is developing the capability to use environmental DNA (eDNA)-based smart surveillance approaches for the screening of exotic insect pests, especially, mosquitoes (Family: Culicidae) and Culicoides (biting midges; Family: Ceratopogonidae) in large bulk samples collected from surveillance programmes.

The project started in September 2018, and to date, eDNA collection and extraction protocols from surveillance samples have been optimised. Additionally, the eDNA metabarcoding approach has been validated computationally as well as via laboratory tests using DNA extracts from insects in artificial (mock) mixed biological samples. We are currently conducting pilot experiments using the actual environmental samples to assess the effectiveness of the eDNA approach for the early detection of mosquitoes and Culicoides.

Preliminary eDNA experiments successfully detected mosquitoes from national saltmarsh mosquito surveillance, as well as Ceratopogonidae species in the Culicoides trap samples from arbovirus surveillance programmes. However, some inconsistencies in the detection of targeted species were observed in certain scenarios, thus, further tests are ongoing to assess the potential limitations when the eDNA approach is applied to surveillance samples. Overall, our current findings from pilot experiments show the potential of eDNA-based smart surveillance approaches to be applied in the MPI’s biosecurity surveillance programmes in the future.

- Drs Chandan Pal and Jieyun Wu
**Tracing the geographical origin of Brown Marmorated Stink Bug**

Tracing the geographical origin of an invasive pest is crucial to assist in making sound biosecurity decisions during an investigation or response. Knowing the origin(s) of an incursion ensures that timely decisions can be made and the recommended response options are effective.

Since September 2018, through an operational research project, PHEL has been conducting pilot experiments to develop the capability to trace potential geographical origins of the brown marmorated stink bug (BMSB). The project used the high-throughput sequencing-based method called ddRADseq (Double Digest Restriction-site Associated DNA Sequencing) as well as haplotype analysis (mitochondrial cytochrome oxidase genes – Mt-COI/COII) to ascertain the genetic diversity of BMSB populations.

This study obtained ddRAD sequence data from 389 BMSB specimens obtained from 12 countries in four different continents, including their native and representative invaded countries. Using the 3.6 billion Illumina HiSeq derived reads, we established a BMSB population level DNA sequence database and unravelled high genomic diversities among the BMSB populations. We identified at least three BMSB genetic populations in the invaded countries and two of these in their native countries. Novel BMSB haplotypes, including 47 novel COI and 23 novel COII, have also been identified.

This first comprehensive study on BMSB population genetics using ddRADseq and Mt-COI/COII haplotypes has provided a baseline for further molecular research on BMSB origins and to develop rapid and reliable ways to identify their origins to inform response decision-making.

- Drs Juncong Yan (John) and Dongmei Li

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**RESEARCH IN THE LAB**

**Suspect Fruit Piercing Moth detection**

*Eudocima materna*

PHEL received a notification from a member of the general public, about a fruit-piercing moth found in Auckland. Preliminary identification was made based on photos submitted and later the identification was confirmed when the specimen was received. Subsequently two more sightings of this species were reported from Christchurch. The fruit-piercing moth, *Eudocima materna* (Lepidoptera: Erebidae) has a tropical distribution in Africa, South Palearctic, Indo-Australian region up to Central Pacific. Adults of this species feed on fruits of various plants while larvae feed exclusively on plants of the family Menispermaceae. Menispermaceae is not known to occur in New Zealand. The fruit-piercing moth has a long history of occasional immigration to New Zealand, dating back to 1906, but it has never established so far.

- Dr Prasad Doddala

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**A FASCINATING FIND...**

PHEL entomologists received a number of public enquiries in March about the beautifully coloured Australian Bag Moth *Cebysa leucotelus* (LEPIDOPTERA: Psychidae). The female of the species (top) is unusual in its metallic blue and orange colouration and small wings, which mean they cannot fly efficiently and are often mistaken for beetles. In contrast, the males appear like relatively ‘normal’ moths (left). *C. leucotelus* is native to Australia and was first discovered in Auckland in 1981. The moth is not considered a pest in New Zealand.

For more information see: [CitSciHub](#) and Landcare Research
New diagnostic tests to protect our border and enhance response readiness

Over the past few years, PHEL’s Mycology & Bacteriology team has successfully secured funding through MPI’s Operational Research Programme to improve our diagnostic capability and enhance our readiness for biosecurity responses through conducting proactive research. Currently, we are working on two projects which focus on developing fast and sensitive DNA-based diagnostic tests for exotic plant pathogens (bacteria and fungi), and a pest that could impact the forestry and horticulture sectors in New Zealand.

Since late 2018, we have successfully developed new molecular assays as well as validated existing assays for the detection of bacteria and fungi that are unwanted in New Zealand, and a few more will be developed over the next months (see Table for details).

The method of choice to detect these unwanted organisms is real-time PCR (polymerase chain reaction). To create a new real-time PCR test, we look for short DNA sequences that are unique to each of these organisms. We run the tests with a range of target and non-target organisms to ensure that the tests are specific, giving positive results only for the organisms we are targeting. Thereafter, we conduct more tests with a series of different DNA concentrations to make sure the tests are sensitive enough to detect the presence of very small amounts of the organism, for example, during early stage of infection or even before disease symptoms can be seen on infected plants.

Since these DNA-based diagnostic tests can be scaled up for high throughput testing, they will be useful for processing high sample volumes in the event of a biosecurity incursion as well as for pre-clearance testing for large consignments at the border; or used in surveillance programmes to screen samples for the presence of plant pathogens or pests. The new tests will allow us to provide fast and reliable diagnostic services to protect New Zealand and also contribute to biosecurity beyond our borders, by providing new methods for our international colleagues.

- Drs Hui Wen Lee and Karthik Dharmaraj

Dr Karthikeyan Dharmaraj (left) and Dr Hui Wen Lee (right) discussing progress on developing molecular diagnostic tests for targeted unwanted organisms.

<table>
<thead>
<tr>
<th>TARGET ORGANISMS</th>
<th>IMPORTANCE</th>
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<tbody>
<tr>
<td><strong>Ceratocystis species</strong>*</td>
<td>Fungi causing severe wilt and dieback diseases on a wide range of plant hosts</td>
</tr>
<tr>
<td><strong>Clavibacter sepedonicus</strong>**</td>
<td>A bacterium causing ring rot in potato</td>
</tr>
<tr>
<td><strong>Cronartium harknessii</strong></td>
<td>A fungus causing western gall rust of pine trees</td>
</tr>
<tr>
<td><strong>Cryphonectria parasitica</strong>**</td>
<td>A fungus causing chestnut blight</td>
</tr>
<tr>
<td><strong>Diaporthe vaccinii</strong></td>
<td>A fungus causing twig blight of blueberry</td>
</tr>
<tr>
<td><strong>Lissachatina fulica</strong></td>
<td>A pest (Giant African snail) of several horticulture and agricultural crops</td>
</tr>
<tr>
<td><strong>Monilinia fructigena, M. polystoma, M. mumeola and M. yunnanensis</strong>**</td>
<td>Fungi causing brown rot of stone and pome fruits</td>
</tr>
<tr>
<td><strong>Monilinia kusanoi</strong>*</td>
<td>A fungus causing blights and fruit rot of Prunus species</td>
</tr>
<tr>
<td><strong>Monilinia vaccinii-corymbosi</strong>*</td>
<td>A fungus causing mummy berry disease in blueberry</td>
</tr>
<tr>
<td><strong>Phytophthora species</strong>**</td>
<td>Oomycetes causing root rot and dieback of many plants</td>
</tr>
<tr>
<td><strong>Phytophthora helicoides</strong></td>
<td>A fungus-like organism causing root rot of kiwifruit and other horticultural crops</td>
</tr>
<tr>
<td><strong>Verticillium nonalfalfae MLST2 (Multilocus Sequence Type 2)</strong>*</td>
<td>A fungus causing wilt disease of the gold-fleshed kiwifruit (cv. Hort16A)</td>
</tr>
<tr>
<td><strong>Xanthomonas citri subsp. citri</strong>**</td>
<td>A bacterium causing citrus canker</td>
</tr>
</tbody>
</table>

* New assay developed at PHEL
** Previously published assays validated for use at PHEL; Assays for the targets shown in green have already been completed, in yellow are in progress, and in white are planned in the coming months.
A small yet powerful device for plant virus diagnostics

The Oxford Nanopore Technologies MinION is a low-cost, portable sequencing device which plugs directly into the USB port of a computer. The MinION device can easily fit into the palm of a person’s hand, yet is capable of sequencing all the DNA from a complex organism within 24 hours. The sequence data can be analysed in real-time with the first usable data being available 2 minutes after the sequencing run has started. Not constrained to a laboratory environment, the MinION has been used up mountains, in the jungle, in the arctic and on the International Space Station. Nanopore-based sequencing works whereby the sequence of nucleic acids is inferred from changes in the ionic current across a membrane as a single DNA molecule passes through a protein nanopore.

The PHEL Virology team have been trialling this ground-breaking technology for the generic detection of plant virus and virus-like organisms. By sequencing the nucleic acid of a diseased plant, any pathogens present will also be sequenced and subsequently identified by comparing the sequences against reference databases. Initial results look promising in that a range of virus types can be detected. Further validation work is in progress after which the PHEL Virology team will use this technology for screening surveillance and post-entry quarantine samples.

- Dr Lia Liefting

A team of PHEL staff led by our resident videographer, Matthew Leamy, are working on developing a short video to promote the work carried out at the lab as part of the International Year of Plant Health. Some stills from the footage can be seen here.
PHEL PROVIDING AN ESSENTIAL SERVICE DURING COVID-19

As an essential service, PHEL continued to operate throughout the lockdown period. Minimal staffing and social distancing was introduced in the laboratory, while innovative solutions were implemented in our homes to create work stations from rearranged lounge rooms, garages and even back yards.

A new set-up outside the lab for receiving samples. A very empty carpark at PHEL Tamaki lab. Morning tea break – with social distancing.

Some images of our staff working from home during the lockdown

PHELosophies 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

PEST AND DISEASE HOTLINE
Call to report any exotic pests or diseases of plants or animals

0800 80 99 66