PROJECT REPORT

BOOSTING DIAGNOSTIC CAPACITY FOR PLANT PRODUCTION INDUSTRIES

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

JULY 2024

AN AUSTRALIAN GOVERNMENT DEPARTMENT OF AGRICULTURE, FISHERIES AND FORESTRY RURAL RESEARCH AND DEVELOPMENT FOR PROFIT PROJECT

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Title: Final Project Report
Boosting Diagnostic Capacity for Plant Production Industries

Project Details: This report provides a summary of activities and achievements by project partners, at the completion of the Boosting Diagnostic Capacity for Plant Production Industries project, funded through the Australian Government Department of Agriculture, Fisheries and Forestry Rural Research & Development for Profit program.

Project: PROC-9176065

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Contents

Introduction

Boosting diagnostic capacity for plant production industries was an Australian Government Rural R&D For Profit project running from April 2019 to June 2023. (Project code PROC-9176065.)

A 2018 review commissioned by the Department of Agriculture and Water Resources (DAWR, now DAFF) found the adequacy of Australia's diagnostic resources across diagnostic protocols, reference specimens, image libraries, human capability and in-field procedures varied considerably.

The overarching objective of this project was to ensure Australia's biosecurity services maintain a state of the art and fit-for-purpose diagnostics regime and in-field management capability.

Led by the Grains Research and Development Corporation (GRDC) as principal grantee, a consortium of research and development corporations, industry bodies and government pursued a range of research, capacity building and extension projects aimed at:

- **Enhanced capacity** (Building diagnostic testing skills and capability)
- **Enhanced diagnostic tests** (Increasing options for rapid detection of new biosecurity threats)
- **Enhanced operations** (Training and in-field testing with new diagnostics techniques)

Activity was focused on high-priority plant pests (HPPPs) as identified by Australia's Plant Health Committee, along with established or exotic pests of significant economic importance.

The development of new, rapid diagnostic tests for the presence of these pests was supported by extension activities that provided awareness training to industry and boosted laboratory preparedness for new testing procedures.

This report provides an overview of the project and its outcomes.

For more detail, please visit see the Further Reading section at the end of this publication.

Photo: Plant Health Australia.

Project Partners

Organisation	Industry focus	Participation
Victoria Dept. of Jobs, Precincts and Regions (DJPR) *Now Dept. of Energy, Environment & Climate Action (DEECA)* DJP2003-001SAX	Grains, cotton, horticulture, wine	Service provider Cash and in-kind contributor
AgriFutures	Rice	In-kind contributor
AUSVEG AVL2104-001SAX	Cotton, grains, sugar, horticulture, forestry & wood, wine	Service provider In-kind contributor
BioProtection Research, Lincoln University, NZ LUN2004-001SAX	Grains, Horticulture, Wine	Service provider In-kind contributor
Cesar Australia CES2004-002SAX	All industries	Service provider In-kind contributor
Commonwealth Scientific & Industrial Research Organisation (CSIRO) CSP2004-009SAX	Cereals, vegetables, grains, sugar, forestry, grapes, horticulture	Service provider In-kind contributor
Cotton Research & Development Corporation (CRDC)	Cotton	Cash and in-kind contributor
Queensland Dept. of Agriculture and Fisheries (QDAF) DAQ2004-005SAX	Cotton, grains, horticulture, forestry	Service provider Cash and in-kind contributor
Western Australian Agriculture Authority (WAAA/WA DPIRD) DAW2004-005SAX	Wine, horticulture, grains, cotton	Service provider In-kind contributor
Dept. of Primary Industries, NSW (NSW DPI) DPI2004-012SAX	Cotton, citrus, mango	Service provider In-kind contributor
Tasmania Dept. of Primary Industries, Parks, Water and Environment (DPIPWE) *Now Natural Resources & Environment Tasmania (NRET)* BIO2004-001RTX	Grapes, vegetables, fruit, forestry, nurseries	Service provider In-kind contributor
Northern Territory Dept. of Primary Industries and Resources (NT DPIR) *Now Dept. of Industry, Tourism and Trade (NT DITT)* NTG2004-001SAX	Forestry, horticulture, cotton	Service provider In-kind contributor
Forestry & Wood Products Australia (FWPA)	Forestry	Cash and in-kind contributor
Grains Research & Development Corporation (GRDC)	Grains	Cash and in-kind contributor
Horticulture Innovation Limited (Hort Innovation)	Horticulture	Cash and in-kind contributor
Plant & Food Research New Zealand (PFR NZ) PFR2004-001SAX	Cotton, citrus, mango	Service provider In-kind contributor
Plant Health Australia (PHA) PHA1911-002SAX	All sectors	Service provider In-kind contributor
South Australian Research & Development Institute (SARDI) DAW2004-009SAX	Grains, horticulture, citrus	Service Provider Cash and in-kind contributor
Sugar Research Australia (SRA) SRA2004-001SAX	Sugar	Service provider Cash and in-kind contributor
Wine Australia (Wine)	Wine	Cash and in-kind contributor

Photo: GRDC.

Photo: GRDC.

1. The need for new diagnostics

No one can afford to take biosecurity for granted. Australia's primary production sector is worth $93billion[1] and intrinsically connected to our food and economic security. Continual vigilance and improvement in biosecurity measures is essential at all levels, from our national borders to every farm gate.

As an island continent, Australia enjoys natural protection from many of the world's most destructive plant pests. Within the country, large deserts also provide a degree of protection from species that exist in some regions and not others.

However, biosecurity is not a static problem. Climate change is expanding the habitats of existing pests, aiding the potential spread and survival of invasive tropical species, and driving weather that can support more explosive pest and disease breeding events.

Human activity is another major risk factor. Trade, migration, transport and tourism enable the entry and spread of biosecurity threats. Pests can travel on ships, containers and trucks, on-farm equipment, with transient farm workers, or simply in fruit being carried in a car or caravan. Human movement enables pests to rapidly bridge international and internal boundaries, to infest new areas where they may be unconstrained by predators or plant resistance.

While transport and labour logistics have changed markedly over recent decades, so too have the technologies that can be applied to biosecurity testing. Remote communication, automation, molecular biology and more have all evolved to the point where advanced laboratory technologies can be accessed rapidly and cost-effectively, or even deployed in the field as tools or kits.

The *Boosting diagnostic capacity for plant production industries* project focused on plant production sectors including grain, cotton, sugar, horticulture, viticulture and forestry. The project oriented around high-priority exotic pest species — generally invertebrates and bacteria that are not yet present in Australia but have a high risk of arriving here and posing a significant danger to our crops.

As a rule, the speed with which such a pest or disease incursion could be detected, contained and eradicated is directly related to how quickly the threat can be identified.

Leveraging the latest technology to improve the speed and precision of biosecurity activity has a direct effect on the industry's ability to identify, trace and manage outbreaks. Extension work that ensures diagnosticians are familiar with the available tools, tests and information resources is critical to advancing biosecurity capacity.

Bringing these capabilities to life has taken a multi-faceted approach, ranging from genomic sequencing so lab staff can accurately identify bacteria, to producing fact sheets that will help growers identify and respond to insect pests in their crops.

[1] Source: ABARES Snapshot of Australian Agriculture 2023

2. Plant production threats

The high priority plant pests (HPPPs) at the focus of the *Boosting diagnostic capacity for plant production industries* project are as diverse as the sectors they threaten. Some of the significant biosecurity threats being addressed by the research, development and extension activities are detailed here.

Khapra beetle (*Trogoderma granarium*)

Photo: PaDIL.

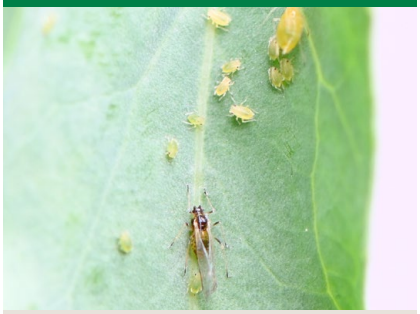
Khapra beetle (*Trogoderma granarium*) is Australia's number one national priority plant pest for grains (and number two national plant pest overall). A native of India, it has spread to northern Africa, the Middle East and south Asia. The beetle feeds on stored grain and dried foodstuffs, causing damage and contamination.

Khapra beetle can survive for long periods without feeding and multiply rapidly when conditions are suitable. It can travel in cracks or crevices inside shipping containers and will inhabit a wide range of cargo types from foods to cardboard packaging, wooden doors and even plastic beads or nuts and bolts.

The pest is difficult to detect and remove, meaning its establishment in Australia could be devastating for grain exports and market access.

Aphids/Mealybugs

Photo: Piotr Trebicki.



Aphids and mealybugs are both worldwide pests of cropping plants, and are widespread in Australia. Populations can build up very rapidly and they feed by inserting mouthparts into a plant to suck its sap. Both aphids and mealybugs can act as vectors for viruses, while mealy bugs also exude a sticky 'honeydew' that can promote fungal growth.

Begomoviruses

Photo: QDAF.

Begomoviruses are known worldwide, including in Asia, the Americas, Africa and Europe, with relatively few examples currently found in Australia. The main vector is the silverleaf whitefly and a wide range of important crops can be infected, including potatoes, carrots, capsicums and cotton.

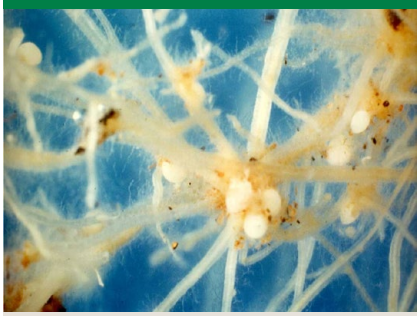
Poleroviruses (*Luteoviridae*)

Photo: Department of Primary Industries and Regional Development, WA.

Poleroviruses are known to cause 26 highly damaging diseases, including potato leaf roll virus, turnip yellows virus, cotton leafroll virus and more. Infection can cause significant plant damage and yield loss.

Cyst nematodes (*Heterodera*)

Photo: John Fisher, UofA.



This large group of microscopic soil-borne invertebrates affects a diverse range of crops, infesting and damaging the roots. While several species are present in Australia, there are many problem species that exist in Europe, the Americas, Africa and Asia which could impact on Australian cropping. Nematodes are easily spread by transporting infested soil on plants, equipment, vehicles or footwear.

Pine wilt nematode and Red ring nemotode (*Bursaphelenchus*)

Photo: PaDIL.

These microscopic worm species are a highly invasive exotic threats to tree crops. Both are too small to see but their insect vectors and dead or dying trees are both signs of possible infestation. Pine wilt nematode is spread by pine sawyer beetles (sp. *Monochamus*) and can affect pine, fir, cedar, larch and spruce. Red ring nematode is spread by exotic palm weevils (sp. Rhynchophorus) which feeds on date, coconut and oil palms.

Fusarium oxysporum spp.

Photo: PaDIL.

Fusarium oxysporum is a large family of soil borne fungi, with both pathogenic and non-pathogenic forms. These affect more than 100 different plant species, including many important crops in Australia.

Xanthomonas citri subsp *malvacearum*

Photo: K.Kirkby, NSW DPI.

This is an exotic bacterial blight that infects the leaves of cotton plants causing long, black lesions. While several strains exist in Australia, local cotton varieties have effective resistance. However, there is a danger of new strains evolving, or of virulent new strains being introduced on green plant matter from overseas. Hypervirulent strains of the bacteria are known to exist in North Africa.

Asian and African citrus psyllid (*Diaphorina citri* and *Trioza erytreae*)

Photo: PaDIL.

Asian and African citrus psyllids are sap-sucking insects that feed on young citrus trees, stunting stem and leaf formation. Both species can be vectors for the devastating huanglongbing (citrus leaf greening) virus. Psyllids can only fly short distances but are easily transported on plant material.

Grapevine red blotch virus (GRBV)

Photo: Plant Health Australia.

GRBV was first detected on vines in California's Napa Valley in 2008 and has since been found on vines throughout the Americas and in Europe. The disease causes blotching on leaves and prevents sugar accumulation in the fruit. It has been detected in Western Australia, South Australia and Victoria without full symptoms developing. It is thought to be carried on new plantings and several sap sucking insects may be disease vectors.

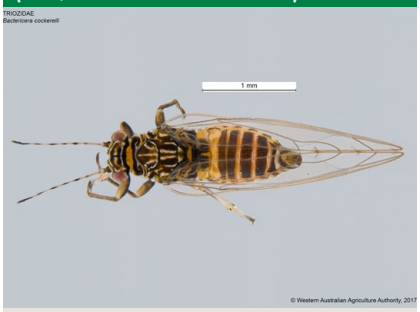
Xanthomas *axonopodis pv allii*

Photo: Howard F Schwarz, Colorado State University, Bugwood.org.

Xanthomonas leaf blight is a bacterial blight infecting onion, garlic, shallot, leek and similar crops. It can be spread by the movement of infested soil, plant material and seed, and can cause yield losses ranging from reduced bulb size to total crop failure. Infection causes tan leaf lesions, which impact photosynthesis.

Tomato potato psyllid (TPP, *Bactericera cockerelli*)

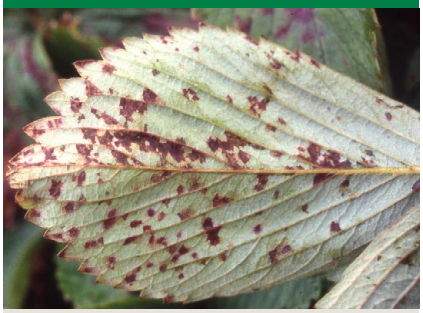
Photo: PaDIL.



TPP is a small, winged sap-sucking insect that feeds on a range of plants including tomato, potato, capsicum, chilli, goji berry, tamarillo, eggplant and sweet potato. It has become established in Western Australia since 2017, with management practices and restrictions on moving susceptible plants and plant material in place to stop its spread to other states.

Xanthomonas fragariae

Photo: PaDIL.



This bacterial infection of strawberry leaves was first reported in Minnesota, USA, in 1960 and has now spread around the world. It is still not well understood but appears to be more virulent under humid conditions.

Spotted winged drosophila (*Drosophila suzukii*)

Photo: PaDIL.

A fruit fly originating in southeast Asia, spotted wing drosophila is now becoming a major pest in both Europe and North America. It infests fruit during early ripening. Fruit fly are easily transported and readily colonise new habitats. Establishment of *D. sukukii* in Australia would pose a grave threat to our fruit production and market access.

Yellow canopy syndrome (YCS)

Photo: QDAF.

YCS is a relatively new and unknown condition affecting sugar that has spread southwards since it first affected Far North Queensland cane fields in 2012. Its cause and origins remain unclear and there is no known management solution. Early indications are that insects may be associated with YCS.

3. The building blocks of our biosecurity

Australia's biosecurity infrastructure comprises a network of federal, state and industry-specific bodies providing surveillance, diagnostic and information services.

Surveillance and screening at international borders is the most obvious way to protect Australian agriculture from invasive threats. However, the task is enormous, with millions of containers, packages, travellers and their luggage entering the country every year. Furthermore, many High Priority Plant Pests (HPPPs) are elusive, highly mobile and/or microscopic.

As a result, the lion's share of biosecurity lies with industry, through high levels of participant awareness and a rapid, targeted response to any outbreak.

The *Boosting Diagnostic Capacity for Plant Production Industries* project aimed to support this fundamental infrastructure by investing in diagnostics research, capacity building and enablement.

Biosecurity

1. **Diagnostic capacity building** through diagnostic training; exotic pest identification workshops; building surge capacity for high-priority pests; and enabling faster diagnosis by reducing bottlenecks and boosting capacity. This ensures Australia's biosecurity system is fully enabled and fit for purpose.
2. **Enhanced diagnostic tools** including 'early indicators' for HPPPs; improvements to critical reference collections; improving national diagnostic protocols for HPPPs; and determining the geographical origin of pests in incursions to better inform trade decisions. These steps will enable faster and more accurate identification of potential pests, using the latest diagnostic technologies.

Adoption of R&D

3. **In-field detection, sampling and diagnostic 'blitz' exercises** for primary producers and on-ground personnel to increase awareness of selected HPPPs and testing protocols and to drive nationwide adoption of improved sampling and diagnostic techniques. This will help ensure biosecurity staff are familiar with the processes they will use to manage biosecurity incidents and helps identify potential bottlenecks in the new processes.

Any introduced pest could cause enormous economic and reputational damage to our primary production industries, with severe ramifications for farmers and consumers, food security and the wider economy.

Having the right capabilities in the right hands – and in the right place – is the key to detecting, containing and eradicating any exotic high-priority plant pest.

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OBJECTIVE 1 – Building industry skills and capability (industry capability)

This will be achieved by lifting capability through:-

OBJECTIVE 2 – Increasi

This will be achieved by enha

Industry Network Training

- Review & staff training of Tasmanian NPPPs+HPPPs - TAS DPIPWE
- ASSCT Presentation - SRA
- NT Farmers Federation - NT DPIR
- Articles in Project Partner Publications presentations at Aust. Plant Pathology Symposium, Annual Diagnosticians Workshop, Australian Biosecurity Symposium & Industry Partner workshops/field days - AUSVEG

Workshops & ID Resources

- Nematode Taxonomist trained - CSIRO
- In-field technology for Grapevine Red Blotch virus & Xanthomonas fragariae AGVIC
- iSPY - SARDI
- 20 x HPPP IDs - CESAR
- Tasmanian HPPPs - TAS DPIPWE
- Asian citrus psyllid & HLB workshop - NT DPIR
- On-farm Blitz Workshops + Webinars AUSVEG

Early Detection Tools

- Generic virus+rapid vector ID lab protocols + amplicon sequencing - WAAA
- SWD trap design + endemic cereal cyst nematodes LAMP diagnostic protocol - AGVIC
- HPPP eDNA+genetic variant protocol - CESAR
- Xanthomonas MinION protocol - PFR NZ
- Xanthomonas citri cg/wgMLST - NSW DPI
- Xanthomonas exotic bacteria assay + lab sample SOPs for NFPSP - QDAF

Assay Development

- Aphid/Mealy bug LAMP protocols
- Begomovirus field + Polerovirus rapid protocols - QDAF
- Cyst nematode genome sequenced & LAMP + Grapevine high throughput + Fusarium oxysporum LAMP + Xanthomonas fragariae high throughput + LAMP protocols - AGVIC
- Fusarium mangiferae LAMP protocol - NT DPIR
- Xanthomonas minION protocol - PFR NZ
- Yellow canopy syndrome spectral signature protocol - SRA
- Cyst nematode TaqMan MGB protocol - SARDI

NDPs Developed/Revised

- Cereal+Potato cyst nematodes & Grapevine red blotch & Xanthomonas fragariae & SWD - AGVIC
- Xanthomonas citri subsp malvacearum - NSW DPI & PFR NZ
- Cotton leaf roll dwarf & Begomovirus - cotton & Xanthomonas axonpopodis pu allii & Cotton leaf curl - QDAF
- Cryptotermes (drywood termite) - NT DPIR
- Bursaphelenchus (pine-wood & red ring nematodes) & cyst nematodes - CSIRO
- Exotic cyst nematodes (surveillance) - SARDI

Evaluate Diagnostic Testing Programs

- Lab capacity review - QDAF
- Literature reviews - AGVIC
- Tasmanian HPPPs diagnostics training - TAS DPIPWE
- Sampling feedback + simulated scenario - SARDI

m map

program funded project)

ing diagnostic options for rapid detection (**diagnostic tools**)

nced tools through:-

Reference Collections

- National Insect (Nematode) Collection - CSIRO
- National Collection of Fungi - AGVIC
- WINC + Emu database - SARDI
- Tasmanian Agricultural Insect Collection Holdings - TAS DPIPWE
- Aust. Plant Pest Database - NSW DPI

Geographical Origin Protocols

- Cross sectoral HPPPs - Lincoln NZ

Submitted for SPHD Adoption/Endorsement

- Cereal+Potato cyst nematodes & Grapevine red blotch & Xanthomonas fragariae & SWD - AGVIC
- Xanthomonas citri subsp malvacearum - NSW DPI & PFR NZ
- Cotton leaf roll dwarf & Begomovirus - cotton & Xanthomonas axonpopodis pu allii - QDAF
- Cryptotermes (drywood termite) - NT DPIR
- Bursaphelenchus (pine-wood & red ring nematodes) & exotic cyst nematodes - CSIRO
- Exotic cyst nematodes - SARDI
- Apids/Mealy Bug - WAAA

OBJECTIVE 3 – Pressure testing diagnostic capabilities & improving datasets (**biosecurity operations**)

This will be achieved by biosecurity training through:-

Train against NDPs

- HPPP workshops - SARDI
- Tasmanian HPPP factsheets & presentations - TAS DPIPWE

Incursion Scenario

- 5 day intensive workshop - PHA

Blitzes

- Surveillance + response - NT DPIR
- Surveys - QDAF
- Bioblitzes & sample collection kits - TAS DPIPWE
- Khapra beetle surveillance - SARDI

Proficiency Testing

- Ring test assays/NDPs - QDAF + NSW DPI + AGVIC + PFR NZ + NT DPIR
- 2 labs - TAS DPIPWE

4. Protecting Australia's farms

Boosting Diagnostic Capacity for Plant Production Industries involved multiple partners and a wide range of individual project elements comprising diagnostics research, capacity building and extension.

Projects were undertaken under the three main pillars of building diagnostic capacity, advancing diagnostic tests and informing hands-on stakeholders from producers to biosecurity field-staff.

The specific project activities are detailed below.

Building diagnostic capacity

PARTNER	Cesar Australia
INDUSTRIES	All sectors
PROJECT	Diagnostic tools and extension resources

Photos: EnviroDNA Pty Ltd.

Spray aggregation on a raspberry plant (left) and tree rolling (right) to collect water samples for eDNA testing.

OUTLINE

Cesar Australia took primary responsibility for the development of pest identification resources for industry stakeholders and exploring use of environmental DNA (eDNA) diagnostics to identify and track High Priority Plant Pests (HPPPs) in the field.

Specifically, Cesar undertook:

- Development of pest identification collateral.
- Establishing the suitability of HPPP species for eDNA diagnostics and development of probes for high risk biotypes.
- Identifying and prioritising the risks posed by HPPP species priorities and developing diagnostics protocols for high risk biotypes.

OUTCOMES

This project resulted in the development of:

- Twenty new HPPP factsheets.
- Three eDNA molecular assays using both qPCR and metabarcoding detection methods developed to level 4 detection.
- Identification and prioritisation of the risks posed by genetic variants of 20 HPPPs.

PARTNER	Northern Territory Department of Primary Industries and Resources (NT DPIR) *Now Department of Industry, Tourism and Trade (NT DITT)*
INDUSTRIES	Forestry, horticulture, cotton
PROJECT	Biosecurity capacity and succession building

Photo: NT DITT.

NT DITT-supported training session on Asian citrus psyllid and citrus greening disease.

OUTLINE

The Northern Territory's position and climate places its primary producers at high risk from exotic pest incursions. The NT Government recognises this risk and the importance of having capable, suitably trained biosecurity officers now and into the future.

The threats to many of the NT's crops have national implications, including for cucurbits and tree crops such as mangoes and citrus.

OUTCOMES

This project enabled the commencement of two National Diagnostic Protocols – one for West Indian Drywood Termite (*Cryptotermes brevis*) and Drywood Termite (*Cryptotermes dudleyi*), and one for Western Drywood Termite (*Incisitermes minor*).

The project also:

- Validated *Xanthomonas citri* subsp. *malvacearum* and *Fusarium* sp. assays developed by project partners NSW DPI and Agriculture Victoria.
- Provided two training workshops for biosecurity personnel and mango growers.

PARTNER	Plant Health Australia (PHA)
INDUSTRIES	All sectors
PROJECT	Extension provider

Photo: Plant Health Australia.

Diagnosticians inspect greenhouse-grown tomato leaves during PHA field blitz training.

OUTLINE

PHA designed a five-day 'Pest Blitz workshop for early to mid-career diagnosticians, aimed at supporting specimen diagnosis, protocol development and knowledge building through networking, to improve biosecurity preparedness.

OUTCOMES

This project delivered a five-day 'Pest Blitz' workshop with simulation exercises that attracted 20 participants. The workshop included in-field training for lab-based diagnosticians, training in sample collection techniques and identification, and advice on pest surveillance. The workshop enhanced the diagnosticians' multi-disciplinary knowledge while promoting more effective inter-lab networking and collaboration.

PARTNER	Western Australia Agriculture Authority (WAAA)
INDUSTRIES	Wine, horticulture, grains, cotton
PROJECT	Nanopore diagnostics for plant pathology

Photo: WA DPIRD.

DPIRD lab scientist Tonny Kinene conducting nanopore DNA sequencing in the field.

OUTLINE

Nanopore sequencing is a new DNA sequencing technology that can be used in the field to extract genomic information within hours, rather than weeks or months. WAAA and DPIRD researchers worked to develop rapid turnaround tests for HPPPs in a laboratory setting and the protocols for their in-field application. This includes processes for exotic and endemic plant pests where diagnostic protocols are currently limited, out of date or non-existent.

OUTCOMES

As a result of this project:

- Ten new applications for nanopore sequencing were developed, including five rapid species identification protocols for aphids and mealybugs – three of which have an in-field focus.
- Proof-of-concept LAMP PCR protocols were developed for four grapevine viruses.

PARTNER	Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE) *Now Natural Resources and Environment Tasmania (NRET)*
INDUSTRIES	Grapes, vegetables, fruit, forestry, nursery
PROJECT	Boosting awareness and diagnostic capacity

Photo: NRE TAS.

Onion leaves being prepared for testing in The Department of Natural Resources and Environment Tasmania laboratories.

OUTLINE

The Tasmanian Government used the project to build HPPP awareness and diagnostics capacity in the state, from industry stakeholders through to lab processes. Specific activities included:

1. Capacity building: Prioritising HPPP threats in Tasmania and developing training resources on key pests, conducting workshops for industry, educating biosecurity operations personnel and developing sample submission kits.
2. Enhancing diagnostic tools: Improving the Tasmanian Agricultural Insect Collection holdings of HPPP examples, and increasing proficiency in testing for HPPPs.
3. Field 'blitz' exercises to give industry and biosecurity staff hands-on experience of in-field detection and diagnostics for a plant pest outbreak.

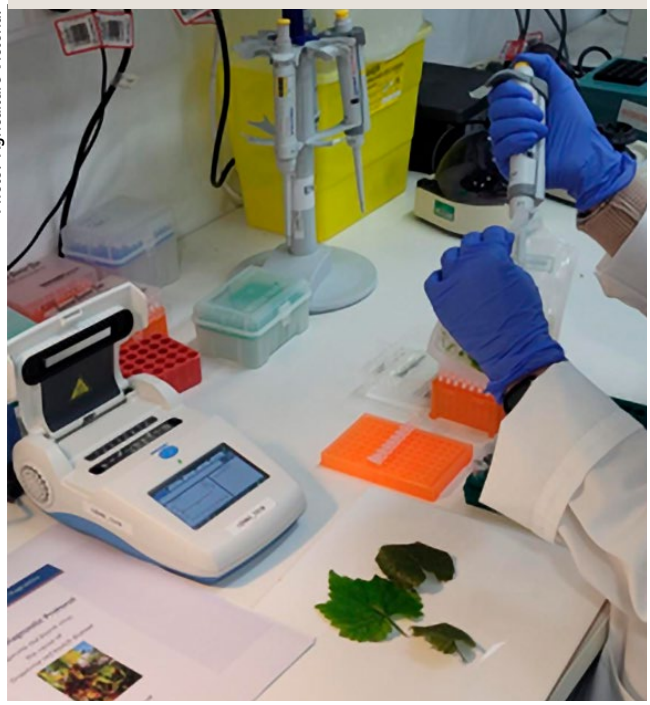
OUTCOMES

As a result of this project:

- Industry and biosecurity operations staff improved their knowledge of, and ability to detect, HPPPs through practice sample submissions and reporting training.
- Biosecurity information and response networks were improved.
- The plant health diagnostics capacity and competency of local laboratories was improved.

PARTNER	Victorian Department of Jobs, Precincts and Regions (DJPR) *Now Department. of Energy, Environment and Climate Change (DEECA)*
INDUSTRIES	Grains, horticulture, cotton, wine
PROJECT	Boosting diagnostics for plant production industries

Photo: Agriculture Victoria.



Using loop mediated isothermal amplification for the detection of grapevine red blotch virus in grapevine material.

OUTLINE

The Victorian DEECA undertook four major lines of research to boost diagnostic capacity for plant production industries:

1. Whole genome sequencing and effector diversity in Australian *Fusarium oxysporum* species to support the development of high throughput diagnostic tests for subspecies (*formae speciales*) of this important soil-borne pathogen. These would provide specific molecular detection in rapid, accurate and efficient tests.
2. Developing expertise in nematode identification, along with new testing methods to reduce reliance on the skills and availability of individual specialists.
3. Building surge capacity for industry surveillance, in-field triage and high-throughput testing during a high priority pest incident, with a specific focus on grapevine red blotch virus and *Xanthomonas fragariae*.
4. Developing a new high-throughput DNA sequencing (metabarcoding) approach for detecting Spotted winged Drosophila in trap samples. Population modelling will also be used to target surveillance activities and the viability of developing a rapid DNA test (LAMP) for in-field detection of eggs and larvae in fruit will also be evaluated.

OUTCOMES

As a result of this project:

- Three National Diagnostic Protocols were developed or updated for Grapevine Red Blotch virus, Spotted Wing Drosophila and *Xanthamonas fragariae*.
- Researchers validated molecular diagnostic technologies for primary diagnosis, in-field detection and high throughput surveillance of *Fusarium oxysporum formae speciales*, *Heterodera* species, *X. fragariae* and Spotted Wing Drosophila.

Improving diagnostic testing

PARTNER	CSIRO
INDUSTRIES	Cereals, vegetables, grains, sugar, forestry, grapes, horticulture
PROJECT	Improving nematode diagnostics

Photos: CSIRO.

Dr Sarah Collins using one of the molecular techniques developed at ANIC for identification of cyst nematode specie

Dan Huston (right) explains the intricacies of morphological identification of cyst nematodes to project participants.

OUTLINE

CSIRO undertook enhancement of nematode diagnostics across all affected industries through capability succession, enhancement of reference collections and testing protocol development.

This included training a high-level taxonomist who can deal with a wide range of species and situations and developing reference collections of exotic pest species and their near relatives with a focus on the priority cyst nematodes.

OUTCOMES

As a result of this project:

- A high-level nematode taxonomist has been trained.
- An extensive collection of morphological specimens and genetic material for both local and exotic cyst nematodes has been created.
- Two National Diagnostic Protocols for cyst nematodes (*genus Heterodera*) and pine wood nematode (*Bursaphelenchus xylophilus*) have been developed.

PARTNER	New South Wales Department of Primary Industries (NSW DPI)
INDUSTRIES	Cotton, citrus, mango
PROJECT	Increasing bacterial diagnostic capacity

OUTLINE

Researchers at NSW DPI used *Xanthomonas* bacteria as a model organism for developing new techniques to identify pest HPPP bacteria species quickly and accurately. Bacterial technology is constantly being revised as new technologies become available and this project took advantage of genome sequencing to improve identification of *Xanthomonas* pathogens. The same genomic techniques can be used to determine the current diversity of *Xanthomonas* in Australia.

OUTCOMES

The project resulted in new molecular tools for identifying *Xanthomonas citri* subsp. *malvacearum, the* causal agent of bacterial blight of cotton, and these tools have been added to the National Diagnostic Protocol for Bacterial Blight of Cotton.

In addition:

- Taxonomy isolates in the NSW state culture collection were listed in the Australian Plant Pest Database.
- A comparative core genome/whole genome Multi Locus Sequence Typing (cg/wg MLST) scheme was developed for identification of *Xanthomonas citri* strains.

PARTNER	Bio-Protection Research (Lincoln University, NZ) *Now Lincoln University (Lincoln NZ)*
INDUSTRIES	Grains, horticulture, wine
PROJECT	Determining the geographic origin of priority insect pest detections

OUTLINE

A study to identify the geographic origin of an exotic pest (or confirm its local origin), thus providing a scientific basis for declarations of continued area freedom and market access, and informing subsequent biosecurity and risk mitigation responses.

Analysing quantities of specific isotopes and biologically inert elements can provide a 'chemical fingerprint' for a pest's past diet and therefore its place of origin.

Researchers sampled plants from two 500-kilometre transects in Australia's south-eastern states to build a reference database of bioavailable-strontium isotope levels and ascertain whether lead isotopes can improve determination of an insect's geographic origin.

OUTCOMES

The study found lead isotopes have potential for provenance marking, which will enable enhanced inter-regional resolution.

The project also generated previously unavailable data on biosecurity-relevant strontium isotopes for the Melbourne area.

PARTNER	Plant and Food Research New Zealand (PFR NZ)
INDUSTRIES	Cotton, citrus, mango
PROJECT	Using *Xanthomonas* as a model organism for increasing bacterial diagnostic capacity

Photo: Sandra Visnovsky, PFR NZ.

Plant and Food Research NZ researchers Sarah Thompson and Fernanda Jacobo complete DNA extraction, ready for sequencing, from the back of a ute.

OUTLINE

Working in parallel with NSW DPI, researchers at PFR NZ explored using 'next generation' nanopore sequencing to develop diagnostic protocols for bacterial plant-infections.

Nanopore devices are small and can be operated via a laptop computer, making them ideal for rapid diagnostics in the field. The long sequences obtained with this technology enable very specific identification of species and subspecies.

OUTCOMES

The researchers developed laboratory-based protocols for detection of *Xanthomonas citri pv malvacearum* and non-laboratory methods for DNA extraction and nanopore sequencing for identifying *Xanthomonas citri pv malvacearum* in the field.

These new methods have been incorporated into the National Diagnostic protocol developed by NSW DPI.

PARTNER	Queensland Department of Agriculture and Fisheries (DAF)
INDUSTRIES	Cotton, grains, horticulture, forestry
PROJECT	Developing biosecurity diagnostics and surveillance

Photo: DAF.

Dept. of Agriculture and Fisheries Queensland diagnosticians process samples during a field trip to Far North Queensland.

OUTLINE

QDAF aimed to maximise the early detection of HPPPs for the cotton, grains, vegetable and forest industries by developing affordable, field deployable screening tests for exotic viruses. Their research focused on poleroviruses and begomoviruses that infect cotton crops as these bacteria groups pose a threat to many other sectors.

The team also worked with other project partners on sequencing Xanthomonas to develop diagnostic tests that can distinguish between possible exotic Xanthomonas and endemic Pseudomonad infections.

Additionally, QDAF reviewed the ability and capacity of the national biosecurity infrastructure to accurately diagnose forest pests and pathogens. This included developing standardised national protocols for assessing forest pest and disease samples.

OUTCOMES

Researchers developed:

- In-field assays for identifying Begomoviruses
- A National Diagnostic Protocol for Begomovirus
- Rapid tests for Cotton Leafroll Dwarf virus and Cotton Bunchy Top virus
- Standard Operating Procedures for identifying the 31 top forest pests

PARTNER	South Australian Research and Development Institute (SARDI)
INDUSTRIES	Grains, horticulture, citrus
PROJECT	Building diagnostic and high throughput surveillance capabilities

Photo: South Australia Dept of Primary Industries and Regions.

Diagnostic work at the South Australian Research and Development Institute (SARDI).

OUTLINE

SARDI undertook development of high-throughput diagnostics for HPPP surveillance in border and field screening. The work focused on developing industry appropriate resources to support unambiguous identification of exotic pests.

OUTCOMES

Researchers developed a high-throughput capability for exotic cyst nematodes, including molecular assays for two species (*Heterodera* filipjevi and *Heterodra* carotae) plus DNA sampling recommendations and surveillance protocols.

Further to this, the Waite Insect and Nematode Collection (WINC) at the University of Adelaide was updated.

The researchers also developed a database to improve diagnostics for Khapra beetle, Tomato potato psyllid (TPP), and Asian and African citrus psyllid – using images, DNA sequencing and validation of existing molecular assays for Khapra beetle and TPP – to support high-throughput diagnosis of these pests.

Finally, SARDI researchers added their Khapra beetle and TPP expertise to targeted diagnostic testing 'blitzes' and provided extension and training workshops on HPPPs for consultants, growers and on-the-ground biosecurity personnel.

PARTNER	Sugar Research Australia (SRA)
INDUSTRY	Sugar
PROJECT	Advancing diagnosis of yellow canopy syndrome (YCS)

Photo: Sugar Research Australia.

Sugar Research Staff examine leaf samples and sticky traps to identify potential YCS vectors and symptoms.

OUTLINE

SRA has been assessing differences in insect fauna between cane showing the symptoms of YCS and unaffected fields. Fields where YCS appears to have been mitigated by insecticide applications are of particular interest.

Research is also being conducted to allow earlier detection of YCS symptoms using hyperspectral imaging, which will allow the development of YCS within and between cane fields to be analysed in more detail.

OUTCOMES

A PhD student was engaged to determine target species and rapid molecular methods of identifying insect species were developed. Best trapping methods training for industry and staff training.

Enabling in-field personnel

PARTNER	AusVeg
INDUSTRIES	All
PROJECT	Extension provider

OUTLINE

AusVeg undertook extension activities designed to inform and educate trained biosecurity professionals at the state and federal level, as well as peak industry bodies, growers, agronomists and diagnosticians.

Education resources, workshops and in-field activities were developed to support faster and more accurate identification of exotic pests, early detection, disease management, grower engagement and implementing the new diagnostic protocols developed through the project.

OUTCOMES

Five workshops delivered and ten articles published.

5. Improved diagnostic capacity

Faster
and more accurate diagnosis of exotic pests/threats

Support and equipment for all
plant health diagnosticians

10 assays
developed

1
nematode
taxonomist employed (succession planning)

6
early detection
tools developed

25 identification resource
fact sheets

5
reference collections updated

5-day "Pest Blitz"
incursion scenario workshop for diagnosticians

Proficiency
testing
of state labs

Numerous training
workshops/webinars
for biosecurity personnel and industry

11 national diagnostic protocols
(NDPs) developed and/or revised and submitted to the Subcommittee on Plant Health Diagnostics (SPHD) of the National Plant Biosecurity Diagnostic Network for endorsement and adoption

6. Glossary

ADSW	Annual Diagnostics and Surveillance Workshop
ADW	Annual Diagnostics Workshop
AGVIC	Agriculture Victoria
APPS	Australasian Plant Pathology Society
ASSCT	Australian Society of Sugar Cane Technolgists
AUSVEG	AusVeg Limited
BOOSTING DIAGNOSTICS	Boosting Diagnostic Capacity for Plant Production Industries
CESAR	Cesar Australia
CRDC	Cotton Research and Development Corporation
CSIRO	Commonwealth Scientific and Industrial Research Organisation
eDNA	environmental deoxyribonucleic acid
FWPA	Forest and Wood Products Australia
GRDC	Grains Research and Development Corporation
HORT	Horticulture Innovation Australia
HLB	Huanglongbing
HPPPs	high priority plant pests
HPPs	high priority pests
LAMP	loop mediated isothermal
LINCOLN NZ	Lincoln University New Zealand
MinION	A nanopore sequencer. A small, handheld device that can do long read sequencing of samples in real time
NDPs	National Diagnostic Protocols
NFPSP	National Forest Pest Surveilance Program
NPBDN	National Plant Biosecurity Diagnostic Network
NSW DPI	New South Wales's Department of Primary Industries
NT DITT	Northern Territory's Department of Industry, Tourism and Trade
PCR	polymerase chain reaction
PFR NZ	Plant and Food Research New Zealand
PHA	Plant Health Australia
QDAF	Queensland's Department of Agriculture and Fisheries
qPCR	quantative polymerase chain reaction
R&D	Research and Development
RNA	ribonucleic acid
RPA	recombinase polymerase assay
SARDI	South Australia's Research and Development Institute
SOPs	standard operating procedures
SPHD	Subcommittee on Plant Health Diagnostics
SRA	Sugar Research Australia
SWD	Spotted Wing Drosophila
TAS NRE	Tasmania's Natural Resources and Environment
WAAA	Western Australian Agriculture Authority
WINC	Waite Insect and Nematode Collection
WINE	Wine Australia

7. Following through

Follow-up information for growers, advisers and diagnosticians

Useful contacts

State Grains Biosecurity Officers
grainsbiosecurity.com.au/gbo-profiles/

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Resources

National Plant Biosecurity Diagnostic Network (NPBDN)
Includes fact sheets for major horticultural pests.
plantbiosecuritydiagnostics.net.au/resources/

Further reading

Investment in diagnostics pays dividends for grain growers, GRDC Ground Cover, November 2022
groundcover.grdc.com.au/weeds-pests-diseases/biosecurity/investment-in-diagnostics-pays-dividends-for-grain-growers

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