Plant Biosecurity Cooperative Research Centre

Final Report

New tools for field grains surveillance and diagnostics of high priority exotic pests

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Contents

1. Executive Summary

INTRODUCTION

Australia has a comprehensive plant biosecurity system which supports its plant production systems. However, increased pressure due to international trade and tourism, geographic shifts in pests, climate change and many other factors has resulted in a high level of biosecurity threat to our industries. Obtaining data on pest and disease status to support market access and to monitor pest and disease incursions in the grains industry is difficult because of the extensive scale of the industry. This project investigated how new and emerging technologies could be used in surveillance of pest and disease biosecurity threats to the grains industry. The aims of this project were to develop and validate better tools for grains surveillance in the field to support market access, and to develop better rapid diagnostic response capability for the key disease threats.

SECTION ONE: INSECT AND SPORE TRAPPING TECHNOLOGY

A: Technology Review and Workshop

A review (Appendix 1) was conducted into new technologies and tools for field surveillance of exotic pests and diseases in the grains industry. This included a workshop in April 2013, involving 26 specialists representing a selected group from research, industry, and technology sectors from across Australia. Prospective technologies were tabled for consideration at the workshop. The review identified four sensing systems targeted for development and evaluation across five target species.

1. INSECT TRAPS

1.1. A high suction frequency insect sampler; target endemic aphids (model system for russian wheat aphid)

Compact high suction (airflow 300–400 m^3/h) frequency traps operating on 240V AC were developed by Burkard Manufacturing Co., UK, for capturing aphids in flight. These traps collect small insects into sampling pots on a carousel mechanism allowing daily sampling over either 4 or 8-days. The devices were successfully deployed in 2016, in South Australia, capturing a wide range of aphid species, including Green Peach Aphid (GPA) and Russian Wheat Aphid (RWA). While manual counts were recorded in the 2016 season, the devices are intended to be used in conjunction with molecular downstream diagnostics. Field testing confirmed these traps caught higher numbers of aphids than traditional yellow pan traps.

1.2. Compact Aphid suction traps: target endemic aphids (model system for russian wheat aphid)

A smaller, more compact version of the above suction traps was developed by the National Centre for Engineering in Agriculture (NCEA) University of Southern Queensland (USQ) partners of this project. Preliminary versions included a miniature virtual impactor or a suction trap with fan blades for vortex airflow. A cyclone type aphid trap was developed next which was more efficient for mixed size particles (tested with sawdust) with recovery rates of over 98%. This prototype has an 8-pot carousel for capture and 'SMART' technology for capturing samples according to temperature, relative humidity, wind speed, wind direction or time. It has a 12V fan for suction, and is designed to be powered by solar panel.

1.3. Insect and Fungal Spore Diagnostics

Molecular analysis of the mixed capture in the insect suction traps is less laborious than manual counting. Collaboration with Molecular Diagnostics (SARDI) found that DNA extraction from mixed capture in insect suction traps had no obvious differences in the amount of DNA extracted from the samples whether stored in ethanol or glycol and whether there was more or less material (0.1g to 0.61g). Most importantly there was no sign of inhibition in both the diluted (1:5) and neat DNA. The lack of inhibition suggests that less extraction buffer could be used, reducing the dilution factor and increasing the chance of detecting a target at low concentration. In addition, qPCR assays for GPA and Western Flower Thrip (WFT) were successfully developed and validated in mixed captures from the insect suction traps. This demonstrates the ability to use molecular assays to quantify known species in the mixed capture samples. Further development of an assay for wheat

stripe rust is still required to identify primers that distinguish between the different rust species such as wheat stem rust, wheat leaf rust and barley stripe rust.

1.4. Smart Insect Trap: target Diamond Back Moth (DBM) (model for exotic Turnip Moth)

The aim was to design an automated insect trap requiring low maintenance, and reduced monitoring hours. A USB microscope camera, mounted on a carousel, was installed in the traps to capture digital images of the trapped DBM moths and images were transmitted to a website (http://www.croppest.com/data/) via the 4G mobile phone network. Automatic sensors, including optical and impedance sensors, were tested for detection of moths, and to trigger the digital capture. The sensors were susceptible to false triggering and clogging from dust. Hence the traps electronic controllers were reprogrammed to examine the capture chamber at predetermined time intervals (every 30min-4h). Image detection algorithms were used to identify if a target was caught and if detected, take and transmit a time-stamped image to the reporting webpage. If no target was detected, a time-stamped test image of the empty chamber was transmitted to confirm the trap was functioning correctly. Traps successfully transmitted images of *Helicoverpa punctigera* (native budworm) but were not suited to the smaller diamond back moth.

Monitoring insect pest populations using pheromone traps can be effective but time consuming. In order to survey for exotic pest insects, biosecurity surveillance programs could potentially piggy-back existing pheromone trap networks with the addition of lures for exotic pests alongside local target pest lures. A pheromone stacking trial was conducted using lure combinations of endemic Native budworm, established Diamond back moth and exotic Turnip moth. The presence of the Turnip moth lure did not affect the attractiveness of the lures for the existing pests. However, Diamondback moth lure was inhibited by Native budworm lure and Native budworm catch was doubled when Diamond back moth lure was present. This evidence of synergism and inhibition indicates the need to test every lure combination before application in a surveillance program. The impact of the local pest lures on the effectiveness of turnip moth lure could not be assessed due to its exotic status.

A commercially available auto-detect pheromone-based insect monitoring system (TrapView®) designed primarily for European orchards and horticultural systems was tested for capture of *H. punctigera* (native budworm) against standard green bucket traps. Some technological issues were observed in the traps, including clogging of insects, and the ability of the program to accurately detect and count moths in the camera images seemed to vary between the two traps. The commercial owners of this trap are improving and developing this system. A number of digital recognition insect traps are available in the commercial market.

2. SPORE SAMPLING

2.1 Mobile jet spore sampler (targets include; wheat stripe rust (model for airborne fungal spores including exotic rust species such as barley stripe rust and lentil rust), and other airborne fungal pathogens including sclerotinia of canola.

The concept of this trap is to allow mobile trapping over a wide-geographical area, ideal for the broad-acre grains industry in Australia. The trap is compatible to downstream diagnostics currently being used e.g. molecular assays. It requires a moderately sized mobile vehicle, such as a motor vehicle or bus, but is not appropriate for UAV applications in this form. Stuart Wili at Burkard Manufacturing Co. UK, was commissioned to develop the Jet Spore Sampler (JSS) prototype. Bench testing and ongoing modifications were conducted at Burkard Manufacturing Co. and also at Rothamsted Research, UK, in collaboration with Prof. Jon West, a recognised world expert in aerial spore trapping. The prototype trap was fitted with 'SMART' capture technology by partners of this project at the National Centre for Engineering in Agriculture (at USQ) in 2016 enabling the device to capture samples according to GPS, temperature, Relative Humidity or time with Bluetooth communications. The complete Mobile Jet Spore Sampler (MJSS) was field tested in South Australia in 2017 and captures were examined with molecular assays in the Molecular Diagnostics Centre, SARDI.

3. AIRBORNE PEST AND DISEASE SURVEILLANCE AND TRAPPING WORKSHOP

A two-day workshop focused on trapping techniques and surveillance strategies for monitoring pest and disease threats to industry was held at Waite Campus 17- 18th May 2016 and presented the new technologies developed in PBCRC Project 2014 and other trapping devices. Prof Jon West from Rothamsted Research (Harpenden, UK) attended this workshop as a keynote speaker. Prof West and Burkard Manufacturing Co.

collaborated with PBCRC Project 2014 in the development of new surveillance tools. Funding for this workshop including international travel and accommodation costs for Prof Jon West was provided by Australasian Plant Pathology Society (APPS) and PBCRC Small Initiative Project SI20060 (with investment from GRDC). The full report for this workshop in Appendix 2.

4. MULTISPECTRAL/HYPERSPECTRAL IMAGING/FIELD ROBOTICS

Multispectral and hyperspectral imaging can be used to detect stress in plants due to pests and diseases, via cameras attached to mobile ground based or aerial platforms. Airborne Research Australia (Flinders University, South Australia) were engaged to conduct aerial imaging using high resolution sensors in manned aircraft in 2014. Four collection dates of aerial imaging were conducted over three field sites. There was no clear correlation observed between disease data and known algorithms defined for vegetative indices applied to image data collected.

Field robots and Unmanned Aerial Vehicles (UAV's) were assessed as new platforms that can capture multispectral images of plant growth and health. SARDI established a pilot study with the collaborative partners the Australian Centre for Field Robotics (ACFR - University of Sydney) and UAV data acquired by FarmingIT (UAV). The ACFR has designed, built and tested an intelligent ground based robotics platform called 'Ladybird'. The UAV platform (Trimble UX-5) from Farming IT provided a rapid and accurate means of capturing NDVI imagery for comparative analysis. As a proof of concept study with ACFR, key objectives were to capture data including NDVI, crop height, disease, chlorosis, necrosis, canopy growth rate and physical characteristics of different cultivars, crop treatments and seed densities. Manual counts were taken for comparison. Results showed that an existing general purpose agriculture research platform (the Ladybird) was highly suited to this application. The UAV was not able to measure differences in heights because of its poor spatial resolution.

5. DIAGNOSTICS OF HIGH PRIORITY PESTS

A review of winter cereal Post Entry Quarantine was completed and submitted to the Department of Agriculture and Water Resources for review. Its titled "Winter Cereal Post-Entry Quarantine: A review of the current post-entry quarantine regulations for imported winter cereal seed for sowing and recommendations to reduce the risks of exotic viruses to the cereal industry". This review is attached as Appendix 5.

Twelve virus species of top priority to the grains industry were identified based on the review on their perceived risks, all of which belong to the following four virus genera: *Bymovirus, Furovirus, Hordeivirus* and *Pecluvirus* (Table 12). All of the virus species identified can be transmitted either by seed and/or by *Polymyxa graminis*, a plasmodiophorid which was recently reported to be present in Australia.

Based on this list, four broad-spectrum diagnostic assays were developed for the detection of these four virus groups. The assays were validated using positive virus controls and synthetic virus constructs and then submitted to the National Plant Biosecurity Diagnostic Network for endorsement. The four National Diagnostic Protocols (NDPs) submitted are attached in Appendix 7. Two of these four assays (for the detection of furoviruses and hordeiviruses) were used in a survey of wheat and barley crops in and around Horsham, Victoria, Australia and the results of the survey were published in the Journal of Virological Methods, titled "Novel genus-specific broad range primers for the detection of furoviruses, hordeiviruses and rymoviruses and their application in field surveys in South-east Australia" (Zheng et al., 2015). This manuscript is attached in Appendix 6c.

Standard Operating Procedures (SOPs) was also developed for individual virus species listed in the High Priority Virus Pest List. Published protocols were used in the SOPs where possible and in the absence of a published protocol, molecular diagnostic assays were developed in this study. A total of 12 SOPs were developed (Appendix 8), for specific testing of *Barley mild mosaic virus* (BMMV), *Barley stripe mosaic virus* (BSMV), *Barley yellow mosaic virus* (BYMV*), Chinese wheat mosaic virus* (CWMV*), Indian peanut clump virus* (IPCV), *Oat golden stripe virus* (OGSV), *Oat mosaic virus* (OMV), *Soil-borne cereal mosaic virus* (SBCMV), *Soil-borne wheat mosaic virus* (SBWMV), *Sorghum mosaic virus* (SrMV), *Wheat spindle streak mosaic virus* (WSSMV) and *Wheat yellow mosaic virus* (WYMV). Two SOPs developed for the detection of BSMV and WSSMV are currently being used by the Post Entry Quarantine station located in Horsham, Victoria for the screening of winter cereal accessions from the Australia Grains Genebank.

The feasibility of using high-throughput DNA sequencing technology (NGS) for the diagnostics of plant viruses was also assessed. Full-length sequences of a number of viruses were obtained, including that of cereal viruses *Barley yellow dwarf virus* (BYDV) and *Maize chlorotic dwarf virus* (MCDV) from a clonal grass sample intercepted by the New South Wales PEQ station. The BYDV isolate was previously thought to be a BYDV-PAV strain, until whole genome sequencing proved it was not and the MCDV isolate was detected using NGS after numerous broad-spectrum tests available failed to detect it.

The feasibility of using Isothermal Loop-mediated Amplification (LAMP) technology for on-site detection of plant pathogens was also assessed. A LAMP assay for the detection of *Barley yellow dwarf virus*-PAV, a strain prevalent in Victorian wheat fields was developed, validated and successfully used in the field in Horsham, Victoria. This is the first time LAMP technology was used to detect a plant pathogen in field conditions in Australia.

2. Introduction

Australia has a comprehensive plant biosecurity system which supports its plant production systems. However, increased pressure due to international trade and tourism, geographic shifts in pests, climate change and many other factors has resulted in a high level of biosecurity threat to our industries.

Obtaining data on pest and disease status to support market access and to monitor pest and disease incursions in the grains industry is difficult because of the extensive scale of the industry. Unlike horticultural and seed production industries, cereal and pulse crops are not intensively monitored by consultants for pest and disease status. Current grains surveillance relies on submission of diagnostic samples to plant health laboratories, routine monitoring of stored grain (not specifically monitored for emergency plant pests [EPPs]), and monitoring of sites such as National Variety Trial, field trial and farming systems sites, by breeders and pathologists. The latter activity is recent, ad hoc, and not supported by rigorous statistics.

The CRCNPB (2005-2012) invested in a number of projects to look at remote sensing technologies for spore trapping and examples include biosensors, hyperspectral imaging, smart traps, aerial and ground based mobile spore traps. The field of sensor technologies is continuing to develop rapidly, e.g. NIR spectroscopy, acoustic detection of insects, biomarker detection with biosensors and laser based gas sensing technologies, as well as monitoring and reporting technologies including field robotics, wireless sensor networks, remote telemetry, digital image transmission and processing systems, web based technologies and Smart phones for alarms, warnings and reporting.

This project endeavoured to review existing technologies, both developed within the CRCNPB and commercially, to determine the potential to utilise sensor technologies for a) cost-effective and robust surveillance and b) better management of endemic pest and diseases in broad acre industries. The first phase of this component project was an identification of the technology limitations to current systems, a review of available and new technologies, consultation between end-users and technology developers and a research plan for development of new sensor technologies for insects and pathogens of grain crops. The second phase was the development of sensor technologies for biosecurity grains surveillance in the field and identification of potential delivery partners for commercialisation.

The second component of this project investigated diagnostic techniques for virus threats to the Australian cereal industry. Current PEQ protocols in Australia for winter cereals are based on grow-out of seed and visual inspection for fungal infections. Virus-testing has not been included, because seed-borne viruses have not been considered a common problem in cereals and the few seed-borne viruses identified have not been considered of economic importance. The yellow dwarf group of viruses (caused by the *Luteoviridae* and not seed-borne) has always been considered the major virus problem in cereals. However, over the last twenty years the virus diseases spread by soil-borne Plasmodiophorid, *Polymyxa graminis*, have become recognized as economically important in cereals and have become increasingly widespread worldwide. These viruses are carried in the tiny resting spores of the vector on the seed. These resting spores can survive for decades and are seen as the most important means of virus spread, as most of the viruses are not seed-borne.

Eleven viruses of winter cereals in three genera (*Bymovirus, Furovirus, Pecluvirus*) that are vectored by *P. graminis* have now been identified. The viruses have been reported in all continents except Australia and although the viruses have not been reported in Australia, the *P. graminis* vector was identified in barley root

samples from a location in southern Queensland in 2009 (Thompson et al., 2011) and in 11 wheat root samples from three widely dispersed locations in southwest Western Australia in 2011 and 2012 (Cox et al., 2012). Cox et al (2012) sequenced all the samples and found *P. graminis* isolates belonging to two different ribotypes in the samples from southwest Australia and a distinct *P. graminis* ribotype in northeast Australia. They suggest that at least three *P. graminis* introductions into Australia have occurred, and that the presence of both *f. sp. temperate* (ribotype I) and *f. sp. tepida* (ribotype II) would mean that soil-borne cereal viruses are likely to become established should they become introduced to the continent in the future. Ziegler et al (2016) investigated the diversity of *P. graminis* ribotypes and their relationships with plant hosts and virus transmission. They concluded that Plasmodiophorids are ubiquitously distributed in agricultural and natural habitats, with a high cryptic diversity, making understanding of the pathogen complexes in which they are involved extremely complex.

The soil-borne viruses represent a group of particularly important pathogens because they can cause severe yield losses in cereal crops. The furoviruses are considered the most widespread and serious of the soil-borne viruses. Furoviruses can cause yield losses of up to 80% of grain production, depending on variety, climatic conditions and time of sowing (Fauquet et al., 2005), with up to 48% grain yield loss in wheat reported in Italy (Vallega et al., 1999)and up to 70% grain yield loss in China (Chen, 1993). Some furoviruses, including *Soil-borne wheat mosaic virus* (SBWMV), are also seed transmitted (Budge et al., 2008). An outbreak of SBWMV was reported in wheat plants in New Zealand in 2002 and the presence of the *P. graminis* vector was also confirmed (Lebas et al., 2009).

Bymoviruses have also been reported as causing severe yield losses, for example, yield losses of 3-87% were reported in wheat infected with *Wheat spindle streak mosaic virus* (WSSMV) in eastern USA (Miller et al., 1992), yield losses of 20-45% have been observed in barley when *Barley mild mosaic virus* (BaMMV) levels are high, though losses can be more severe when the winter has been particularly harsh (Adams, 2002).

The Pecluvirus *Indian peanut clump virus* (IPCV) infects a wide range of crops including peanuts, coarse grains and winter cereals. Delfosse et al (1999) found that wheat and barley crops were susceptible to IPCV under field conditions with wheat yields being reduced on average by 58% and barley plants rarely reaching maturity or developing seed spikes.

P. graminis cannot be controlled by chemicals and the viruses retain their infectivity in the resting spores for many years, therefore, cultivation of resistant plants is the only means of preventing severe losses in areas where soil contains virus-infected spores. The genetic variability of the known viruses and the sporadic reports of newly emerging pathogens, such as *Aubian wheat mosaic virus* (which is possibly vectored by *P. graminis*), make continued resistance breeding and careful monitoring of field crops necessary (Kühne, 2009).

In addition to the eleven known *P. graminis*-transmitted soil-borne viruses, one other exotic seedborne virus poses a threat to the Australian grains industry, namely *Barley stripe mosaic virus* (BSMV-*Hordeivirus*). BSMV is seed-borne in barley and seed infection levels as high as 60% have been found (Jeżewska and Trzmiel, 2009). Malting barley exported from Australia to countries such as China require phytosanitary declarations stating freedom from BSMV. BSMV was included in the EPPO List A2 of quarantine organisms (EPPO 1986).

Despite the potential damage these viruses can pose to the Australian grains industry, there is a serious lack of post-entry quarantine testing for these EPPS. Given that the vector of these viruses is now present in Australia, the risk of entry, establishment and spread of seedborne and seed–carried viruses of cereals due to the large-scale importation of cereal germplasm for breeding programs is paramount and need to be addressed as soon as possible.

Broad-range molecular tests designed to target groups of closely related organisms are best suited for this purpose as the use of generic assays containing degenerate primers can reduce the number of virus-specific tests required. Another advantage of using generic assays containing degenerate primers is its application in the diagnostics of a previously unreported species. When used, it is in the hope that the degeneracy of the primers can accommodate for the genetic variations in the as yet “undiscovered” new virus species. Nevertheless, this method can be hit and miss, since the design of the degenerate primers are dependent on the sequences available at the time of the design and there is no guarantee that such primers can detect previously unreported species. In the case where a previously unreported species is detected, the “reference” genome of this new species is usually generated in the past via genome walking, from which primers can then

be designed for downstream detection of similar isolates. This process, from virus discovery to determination of its genome, can be laborious, time-consuming and expensive.

The use of Next Generation Sequencing (NGS), which is a high-throughput sequencing methodology that generates millions of sequences simultaneously from one sample can be used to determine the full-length genomes of plant viruses in a relatively fast fashion. The main advantage of this method is the simultaneous detection and identification of all plant pathogens present in the sample, relatively free of bias. The feasibility of using NGS for routine plant pathogen diagnostics warranted an in-depth investigation and where feasible, the use of NGS can greatly reduce the number of diagnostic tests required for plant virus detection, especially for previously unreported species.

Lastly, in the case of outbreaks and disease incursions where infectious materials cannot be moved off-site, on-site detection can be extremely useful especially if it is simple and fast. Loop-mediated isothermal amplification (LAMP) is a method that can amplify DNA with high-specificity, efficiency and rapidity under field conditions. Numerous LAMP assays are now available for the on-site detection of plant pathogens such as *Plum pox virus* and *Potato spindle tuber viroid.* The feasibility of using LAMP for the detection of cereal viruses, especially high risk EPPOs was also explored.

Incursions of new pests either through failure of the PEQ system or by other entry pathways are a significant and often long-term cost to industry. It is important to recognise and prioritise key threats to the grains industry, and to have rapid and well-validated diagnostic tools available to the industry. This component of the project aimed to deliver new diagnostic tools for 5 high priority pests and validate them for delivery to the grains industry.

3. Aims

The aims of this project were to develop and validate better tools for grains surveillance in the field to support market access, and to develop better rapid diagnostic response capability for the key disease threats.

4. Materials and Methods

SECTION ONE: INSECT AND SPORE TRAPPING TECHNOLOGY

A: Technology Review and Workshop (Full report in Appendix 1)

A review was conducted into new technologies and tools for field surveillance of exotic pests in the grains industry. As part of this review, SARDI held a stakeholder workshop on 17th April 2013, involving 26 specialists representing a selected group from research, industry, and technology sectors from across Australia. Prospective technologies were identified in a draft review and tabled for consideration by the assembled experts. The workshop provided a focused critique of potential technologies, applications, limitations and proposed several scenarios of surveillance systems that could be considered in detecting exotic pests or disease incursions in grains crops. This was then combined with further scoping activities and discussions with workshop participants and other technology specialists to formulate the final review document (Appendix 1).

This review focused upon four major areas; sensing platforms, sensing technologies, the transmission of sensing data and access to this data.

Various sensing platforms including traps, airborne vehicles, ground based vehicles, fixed installations, crowd sourcing and mobile devices were reviewed. Smart traps have now been developed that are capable of long range telemetry across agricultural landscapes using wireless mesh technologies. These traps are capable of identifying species and transmitting this data to a central repository for visualisation on the web. Advances in the autonomous flight of small airborne vehicles holds promise for low cost broad acre surveillance, as does the availability of ground based vehicles across the grains sector. Fixed installations could be used to collect finer scale environmental data that can be linked to the life cycle of pests. They could also be used as a platform for several complementary technologies. Crowd sourcing and the use of mobile devices have the advantage of reducing the surveillance burden of any one individual by distributing the activity throughout the grains industry, resulting in effective use of these platforms that could achieve greater input into grains surveillance than is currently realised.

A number of different sensors could be attached to these platforms including various devices for recording electromagnetic radiation such as RGB, multispectral and hyperspectral cameras. There is the potential to link hyperspectral signatures to specific groups of crop pests and diseases, although these techniques would most likely require continual monitoring across large areas during a growing season. The sensing of DNA and biomolecules in a field setting poses particular challenges as these applications are best suited to a laboratory environment, but they are likely to be the focus of future field based developments. Other sensors such as impedance sensors, various touch screens, climatic sensors and vibrations through soils and the air are promising candidates for pest detection.

Due to the remote nature of Australian grain fields, telemetry is of particular importance. Technologies exist that are theoretically capable of line of sight data transmission over 10's of kilometres. These technologies are covered by the Low Interference Potential Devices 2000 class licence and do not require special permits to operate within certain power restrictions. Emergent technologies are using low power telemetry that can run off small batteries for a decade. Some access to Telstra's 4G network is available in grain growing regions, though the NBNs satellite data allows for access to data services from anywhere in Australia.

Long term data storage is likely to be an issue for any surveillance program, and the RDSI may be a good choice for data deposition if an open source data approach is taken. Commercial data storage through vendors such as Google and Amazon represent unprecedented value for money though data sovereignty issues remain unclear. Opening up data sets to a broader research audience may provide helpful collaboration for data analytics. The visualisation of data in an intuitive and informative way is key to its utility, and the geospatial and temporal nature of surveillance data lends itself to mapping and charting. Several freely available products are suitable for the presentation of this data including Google maps.

Three sensing technologies considered at the April 17 workshop were considered most feasible.

DNA & Biomolecules - A number of target-specific DNA probes have already been developed but were limited by the laboratory facilities required for their processing. Such techniques are capable of detecting and quantifying targets contained in dirty samples or mixed samples collected from the air. Biosensors promise rapid and portable detection though many are still under early development. Both DNA and biosensors are highly accurate and can be tailored for specific pests.

Digital imaging - Is particularly relevant to insect monitoring, having the potential for cost effective sensing across a range of platforms including portable devices and fixed installations. Challenges exist for the development of completely automated identification systems, since acceptable error margins or false positive rates for biosecurity critical applications are not yet available. However, this technology may be able to offer partial automation, that is the confirmation of pest absence in traps. The Australian Centre for Visual Technologies may assist in automated applications of this technology using digital resources such as the Australian National Insect Collection compiled and maintained by CSIRO.

Impedance and capacitive sensors - Cost effective insect monitoring technology incorporating attractants could be developed for networked monitoring systems. Sensing systems using low cost electronic components may be ideal for exotic pests when simple presence or absence monitoring is required.

Other technologies such as hyperspectral imaging are promising but most compatible with post- incursion surveillance rather than early detection systems, and have limitations related to costs, interpretation of large data sets and available platforms.

Four sensing platforms were considered most feasible:

Trapping - Is an existing and effective method for the surveillance of pathogens and insects. Advances in sensing technologies, such as impedance sensors, could partially or fully automate their function. Mobile traps can offer greater regional surveillance capabilities where smart- traps could be programmed to capture data according to location specific climate triggers. The adaptation of biosensors and DNA technologies to this platform will be essential for the development of full field automation.

Wireless network - Wireless mesh networks of mobile or fixed sensor nodes offer cost effective systems for data collection over large areas. Network nodes can be coupled with simple sensor technologies to feed data rapidly to a centralised hub for data transmission via telemetry. Such systems are adaptable and versatile allowing for the addition or substitution of new sensor technologies as they are developed.

Citizen science - Engaging contributions from the community rather than a limited number of experts or providers can provide systematic collection of data, in particular the utility and mobility of smart phones

represent a potentially expansive surveillance network. There is an opportunity to develop technologies that could enhance the applications of this platform, for example automated diagnosis using digital imaging.

Unmanned Aerial Vehicle - UAVs have utility for fungal spore trapping and detailed aerial multispectral and hyperspectral imaging. In proposed changes to the Civil Aviation Safety Regulations Part 101 the approval process to operate a platform less than 2 kg, within line of sight in rural areas, may require a less onerous online application than heavier platforms. Using these smaller UAVs may provide a strategic platform for single-grid sampling. Larger commercially operated platforms must satisfy additional regulatory and technical requirements, yet they can offer more frequent, diverse and broad scale data collection than ground based methods. Integration of such UAVs into the national airspace is expected as early as 2015, with rapidly growing remote sensing applications in agriculture a driving factor to develop the required technology.

In many instances it will be possible to combine more than one sensing technology with a platform. Cutting edge pest surveillance systems should use an end-to-end approach to inform real- time pest management strategies. These systems have costs associated with them for the development of data access portals and stakeholder engagement. Unfortunately, many promising projects have ceased soon after research funding has been exhausted. By developing technology options that can be maintained and developed by stakeholders and, through integrating these with existing services, the research investment in the current project can be maximised. A durable system must be simple and robust, adaptive to advances in technology, minimise ongoing costs and intuitive in its reporting.

1. INSECT TRAPS

1.1. A high suction frequency insect sampler; target endemic aphids (model system for Russian wheat aphid)

High frequency sampling of airborne insects has been an effective means of studying pest migration and dispersal patterns for decades. The 'gold-standard' of this method of surveillance is internationally recognised by infrastructure and capabilities established by the Rothamsted Insect Survey, a network of stationary insect suction traps established in the United Kingdom. Variations of these traps and approaches are found throughout many countries. In consultation with Dr Rohan Kimber, Burkard Manufacturing Co (UK) sent two prototype high suction insect samplers to SARDI in June, 2015. The suction traps operate on 240V AC and collect small insects into a sampling pot (350ml). The traps operate at a high sampling rate, with approximate average airflow of 300–400 m3/h. A carousel mechanism is incorporated into each trap to allow daily sampling over either 4- (Sampler 1) or 8-days (Sampler 2), with the device automatically indexing sampling pots at the end of a defined sampling period (see images below). Control of sampling periods are currently based on time, but could be further modified to allow meteorological parameters to control sampling periods.

The design is relatively compact, approximately 1 x 2 m, compared to standard insect samplers used around the world (8m towers) to accommodate portability of the device to different sites to sample airborne insects. The device is intended to examine down-stream analysis techniques to identify specific targets within insect populations collected and explore development of more portable and efficient (12v DC) trapping methodology for examination of insect populations migrating during spring in South Australia.

Figure 1: Insect Suction Trap prototypes from Burkard Manufacturing Co (UK) deployed at two field sites during 2016 to monitor for Green Peach Aphid and Russian Wheat Aphid

The two insect suction prototypes from Burkard Manufacturing Co were placed in the field collecting 2-day samples from April 2016. Initially, the 4-pot suction trap (Figure 1 left) was located at the Waite in South Australia, then moved to Balaklava in June in response to the Russian Wheat Aphid incursion that season. The 8-pot suction trap (Figure 1 right) was located at Kapunda in South Australia. The purpose was to evaluate long term sampling of insect targets using these platforms and to monitor the 'model endemic aphid' - Green Peach Aphid (GPA).

Samples collected from the two suction traps were assessed by project entomologist Helen Brodie (SARDI) under a dissecting microscope to identify targets using physical characteristics. The total number of aphids captured were counted (from the mixed-insect population) as well as the total count of GPA in each sample. The number of Russian Wheat Aphids (RWA) was also assessed in direct response to the incursion of this previously exotic insect species. The incursion of RWA was first detected in a commercial crop in South Australia on the 19th May, 2016 and confirmed in the subsequent days at multiple sites in South Australia and Victoria. After the incursion was confirmed, it was found the Kapunda trap had caught one single RWA on May 10th, nine days prior to the detection in the field. Consequently, the suction trap located at the Waite was relocated to a site near the first incursion (Balaklava), in collaboration with Biosecurity SA, and the total number of RWA identified in samples collected from the two suction traps was determined through to October 2016.

The PBCRC2014 project team also collaborated with a scoping project on RWA conducted by Dr Maarten Van Helden (SARDI) and Tom Heddle (University of Adelaide – Honours student). They utilised yellow pan traps (YPTs), commonly used in the study of insect populations and flight dynamics, to monitor the total number of RWA in various trapping locations around SA. Their data was compared to RWA counts collected by the suction trap (ST). Only nearby sites could be examined, since these project activities were not originally intended to be coordinated.

Figure 2: Two high frequency suction trap prototypes (left) obtained from Burkard Manufacturing Co. (UK) fitted with capture pots on time-sequence carousel systems for the evaluation of mixed-population insect sampling captured into liquid (right).

The addition of near-UV light (UV-B) on the Burkard insect suction trap was also evaluated. The reason for this modification was justified by data from field testing of the SMART insect suction trap which showed very few targets were caught at night. The addition of light to insect suction traps increase its surveillance functionality to include moth targets during the night, which otherwise would only be monitored using pheromone based traps.

Modifications to the suction trap to include UV-B light used 12V LED-strip lights with a small solar power system. The strip was wrapped around the top of the intake port and a sensor would only activate the light during night time (Figure 3). This light attraction system has been used in other insect monitoring projects at SARDI, primarily for the study of Diamond Back Moth.

The performance of the high frequency suction trap from Burkard Manufacturing Co (UK) was also compared to the SMART insect suction trap from USQ. This was to directly compare their capture frequencies at a single site at the Waite Campus and assess whether performance differences of captured targets or identify whether each trap is suited to a specific insect monitoring purpose.

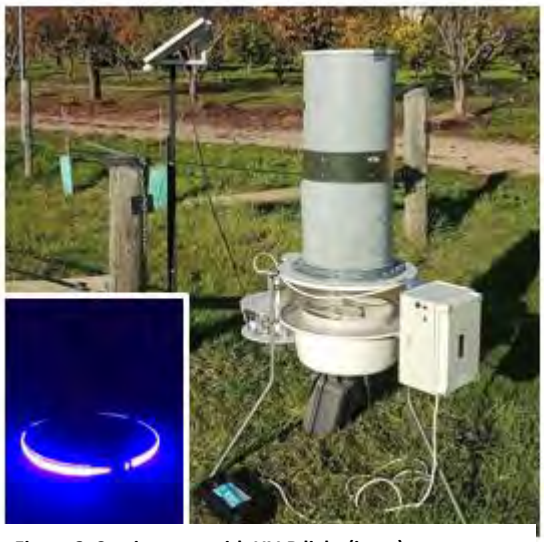


Figure 3: Suction trap with UV-B light (inset)

1.2. Compact Aphid suction traps: target endemic aphids (model system for russian wheat aphid)

The base of an unused 9m suction trap was transferred to QDAF/USQ from University Tasmania to develop a smaller version of the suction trap which could operate from 12 V with solar power. The smaller version should increase deployment capabilities by reducing the transport and power requirements. The traps could be attached to fixed installations, particularly at height to capture aphids in flight.

Initially a miniature virtual impactor (MVI) was incorporated into the trap to address issues of contamination and provide filtering to eliminate larger non-target species. The suction tower system was designed to remove larger insects and debris, and channel aphids into collection vials. Experimentation showed that by adjusting

the distance between the capture chamber and the funnel section the insects were removed from the airflow but it was not effective at filtering on size as some smaller insects were trapped with the larger insects. Various MVI modifications proved to work for removing larger insects but was not effective for passing small insects. It appears that it depends on where in the air stream the insect is when sampling occurs e.g. close to the edge seems to work but in the centre does not. This probably caused the variable results, as it reflects the position of the insect in the airflow relative to the MVI structure. Since the initial plan was to use multiple MVI sections within this prototype to sort by size, this concept was not continued.

A 12-volt version of a suction trap with fan blades producing a vortex to capture insects was built (Figure 4) and tested using sawdust of differing sizes (85 – 89 % capture efficiency) and small wood shavings to model the physical characteristics of winged aphids (98 % capture efficiency). The system uses a 12-volt fan to pump 235 litres per minute and draws about 3 amps e.g. 36 watts.

This prototype did not progress past this stage of development. Preliminary testing was positive but the engineering group chose to redevelop the prototype from a vortex to a cyclone sampler type. This was primarily to achieve a high frequency of sampling but conserve power requirements to operate the device.

A cyclone type aphid trap was developed next which was more efficient for mixed size particles of sawdust with recovery rates of over 98%. This prototype (Figure 5) has a 10-pot carousel for capture and 'SMART' technology for capturing samples according to temperature, relative humidity, wind speed, wind direction or time. It has a 12V fan for suction, and designed to be powered by solar panel. This was deployed in 2016 and 2017 in South Australia.

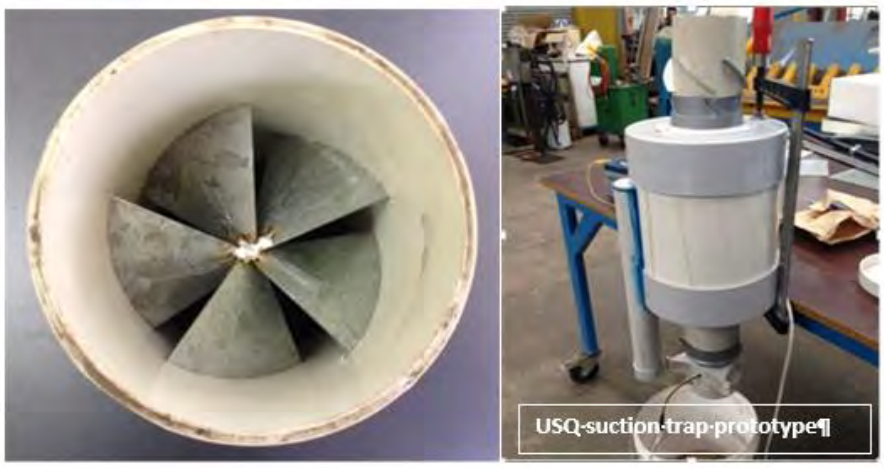


Figure 4. Partial prototype of a high frequency vortex 12-Volt aphid suction trap designed and built by Les Zeller at University of Southern Queensland's National Centre for Engineering in Agriculture. This device was subsequently superseded by a high frequency cyclone sampler developed after preliminary testing.

Figure 5. Component breakdown of the finalised high frequency cyclone 12-Volt aphid suction trap designed and built by Les Zeller at University of Southern Queensland's National Centre for Engineering in Agriculture. This device incorporates an 8-pot carousel fitted with SMART technology to allow capture of airborne pests according to daily time cycle, temperature, RH, or wind speed and direction.

1.3. Insect and Fungal Spore Diagnostics

Collaboration between PBCRC 2014 project staff and SARDI Molecular Diagnostic Centre (MDC) was instrumental in developing downstream diagnostic methods eg. Molecular assays for target pest monitoring.

DNA extraction from captures of insect suction traps: DNA was extracted from insects collected in the suction traps and stored in Ethyl Acohol (EtOH) or polyethylene glycol. Samples were dried then weighed before extraction. All samples were extracted using the same conditions (same volume of extraction and elution buffer). The internal control was added to the extraction buffer to check for the presence of any potential inhibitors after extraction. DNA concentration in the extract from each sample was estimated and DNA was tested in PCR for the internal control neat or diluted 1:5.

Development of diagnostic primers: DNA specific primers were designed for Green Peach Aphid (Russian Wheat Aphid model pest) and Western Flower Thrip (Hessian Fly model pest) by Dr Kelly Hill (SARDI), through collaborative linkages with PBCRC project 2112. The primers were optimised for qPCR conditions used in assays by the MDC at SARDI. They were then evaluated using two tests:

1. To assess efficiency and sensitivity of both assays. This was done in two sample treatments (a) buffer solution (no background insects) and (b) non-target mixed-pest samples (background insects). The latter treatment was representative of samples collected by the Burkard insect suction trap. Single (1) or multiple (10) GPA and WFT, collected by hand and stored in EtOH, were placed into each sample (spiked treatments). Multiple replicates were included.

2. To assess potential inhibition factors and cross-reactivity of the GPA assay only. The WFT assay was not included as it is a published assay that had already included this evaluation. Detection of presence or absence of a single GPA collected in EtOH (hand-picked) or Glycol (suction trapped) was evaluated as an isolated target in solution (no background insects) or mixed-sample (background insects). This also included mixed-samples collected by the Burkard insect suction trap that were never exposed to GPA or had GPA removed to assess GPA residue detection.

Multiple replicates were conducted for both experiments. The aim of these experiments was to ascertain the detection thresholds and sensitivity of the assays.

Development of qPCR assay for wheat stripe rust: A DNA specific primer for Wheat Stripe Rust (model pathogen for exotic barley stripe rust) was designed by Dr Kelly Hill (SARDI), through collaborative linkages with PBCRC project 2112. The wheat stripe rust assay was examined for efficiency and sensitivity using qPCR conditions within assays developed by the MDC at SARDI. This assay is a proof of concept for detection of the exotic barley stripe rust, as the primer design cannot distinguish between the exotic pathogen and its endemic model pathogen.

Wheat rust spore collections took place in the Waite Campus cereal disease screening birdcage trial during October and November 2015 using three sampling devices; a conventional Hirst trap, a rotorod sampler and an MVI (all Burkard Manufacturing Co.). Sampling by the Hirst trap was continuous on a 7-day cycle before replacement of adhesive tape, and the MVI was set to sample over a 3-hour window (10am-1pm) daily on an 8-day cycle before new tubes were required. The rotorod sampler was placed on-site on 4 randomly selected days and operated for a 3-hour collection time, aligned with the MVI sampling period. The target pathogen was wheat stem rust, but several barley and wheat leaf diseases were active in the field site, to examine sensitivity and specificity limits of the molecular detection techniques applied to collected samples. Collected samples were tested using the developed assay.

1.4. Smart Insect Trap: target Diamond Back Moth (DBM) (model for exotic Turnip Moth)

1.4.1 Trap development

Moth traps based on standard green funnel traps were modified by Les Zeller and Paul Kamel (and originally Craig Lobsey) of USQ (formerly of QDAF). The aim was to design a trap which would be automated, require low maintenance, and reduce monitoring hours. A USB microscope camera was installed in each of the traps,

mounted on a carousel, to capture digital images of the trapped DBM moths and images were transmitted to a website (http://www.croppest.com/data/) via the 4G mobile phone network.

Automatic sensors, including two different optical sensors (reflectance and beam interruption sensors) and two different impedance (piezo) sensors, to detect presence of moths, were initially tested to trigger the carousel and position the camera for the digital capture. These modified traps, fitted with lures for DBM, were field tested in South Australia and Queensland in 2014 to 2017.

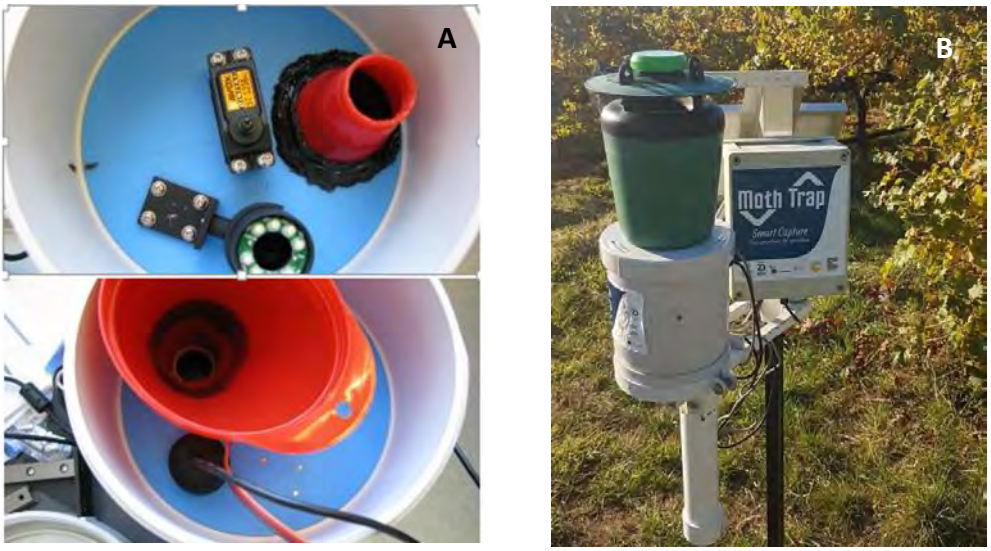


Figure 6. Smart Insect Trap (Moths) designed and built by Les Zeller at USQ National Centre for Engineering in Agriculture. This 12V prototype is fitted with a solar panel and smart capture adapted to a standard bucket trap which uses species specific pheromones to attract a target. Successful capture is detected using on-board analysis of its digital image system (A) that transmits a time-stamped image of the target via the 4G Telstra network. The target is progressed into a collection chamber at the base of the trap (B).

A series of bench and field tests were undertaken to evaluate several prototypes fitted with different sensor systems. This dictated which engineering solution proceeded to development the final form of the device:

- Three traps with reflectance optical sensors were deployed in South Australia on 24 June 2014. Site installation of Moth Trap 0, 1 & 2 was on the northern boundary of a canola crop field located between Bute and Port Broughton; GPS coordinates for the centre trap were 33°43'35.23"South, 137°58'22.46"East. Lures for DBM were fitted. Trap 0 was setup as the coordinator and web access was provided through a Telstra NextG USB dongle. Traps 1 & 2 were configured as satellite units which would periodically (4 hourly) contact the coordinator and upload data by radio link. The traps were spaced approximately 50 metres apart with the coordinator (Trap 0) located in the centre position. Two Delta traps were placed within 20 m of the Traps 0 and 1 as controls.
- Four traps with optical sensors were deployed at Cecil Plains Queensland between 4 and 12 September 2014. GPS coordinates for the south west corner of the field 27°35'23.0''South, 151°13'58.9''East. The sensor tube was modified with a smaller diameter to minimise light that could enter the trap and cause false triggering. Traps were positioned to minimise interference. Both reflectance and beam interruption sensors were tested.
- Trap 1 was a steel framed modified black trap with an extended rain cover and a white light reflectance sensor. The lure was placed in a custom built 'cage' part way down the opening of the funnel. Trap was painted black to minimise false triggering of the optical sensor from light interference.
- Control Trap No.2 was a standard green plastic funnel trap with a pheromone square suspended by wire a short distance from the top.
- Trap 4 consisted of an aluminium frame, an infrared light transmittance sensor, modified with an extended rain cover. The lure was placed in custom built 'cage' part way down the opening of the funnel. Trap was painted black to minimise false triggering of the optical sensor from light interference
- Control Trap 1 was a standard green funnel trap with a slightly different design to Control Trap No.2 such that the pheromone lure is suspended in a 'cage' part way down the opening of the funnel.
- Peizo film and ceramic piezo impedance sensors were tested at the base of traps to detect the impact of moths once dropped through the trap funnel. The ceramic piezo sensor was more robust as it was constructed of two metal disks (brass and silver) separated by a ceramic piezo crystal. Additionally, this sensor outputs very little electrical noise when at rest. The lower portion (instrumented section) of a trap

was constructed and fitted with an offset funnel designed to guide the moth into a receptacle in a rotating disk. Circuitry was constructed to amplify the output from the piezo sensor and connected to a small micro-controller board. This arrangement should detect the impact of moths dropping down the funnel. When an impact was detected the disk rotated to place the moth under the USB microscope camera. This system was tested in the field on a GRDC research site at Pampas-Horrane Rd Pampas Queensland from the 3/09/15 to 13/10/15.

Time determined image capture: A finalised form of the Smart Insect Trap is shown in Figure 6. Subsequent to testing the automatic sensors, in 2016, the traps electronic controllers were reprogrammed to examine the capture chamber at predetermined time intervals (every 30min-4h). Image detection algorithms were used to identify if a target was caught and if detected, take and transmit a time-stamped image to the reporting webpage. If no target is detected, a time-stamped test image of the empty chamber is transmitted to confirm the trap is functioning correctly. This strategy was deemed most suitable for exotic target monitoring, where it is assumed zero to rare influxes would be reported. Field testing was carried out as follows –

- Smart insect trap 1 was placed at the Hart Field Site, South Australia, in September 2016 with a conventional green bucket trap as the control. Both traps were loaded with a lure specific to model endemic target *Helicoverpa punctigera* (native budworm).
- In October 25 to 11 November 2016, traps were placed at Hart Field Site, one with the lure for *H. punctigera*, the second trap with a lure for *Plutella xylostella* (DBM) and a third with both lures. Control traps (bucket and Delta) were also included for both targets.
- Three of the modified traps were placed in a vineyard near Hahndorf, South Australia, to assess the target light brown apple moth (LBAM), in summer and autumn 2017, alongside a TrapView® insect trap.
- Two of the Smart Traps were further modified (#2 & #3) in March-June 2017 at USQ's NCEA. Both traps experienced faults in image capture and quality as well as transmission of the images to the website. The digital microscope camera was replaced in both traps and optimised for high image quality while only increasing the image file size from 25KB to 35KB.
- Smart Moth Trap #1 and #3 were evaluated in the field during September-October, and at the Waite Orchard in November 2017. Trap #2 was not returned from NCEA due to operational failures.

1.4.2 Pheromone stacking

In agricultural systems, monitoring of insect pest populations, both locally established and migratory, provides early warning to growers and enables a more strategic application of controls to protect crops. The concept of early warning systems is the focus of biosecurity surveillance whereby early detection and quick response affords the best chance of eradication and containment of new exotic pests.

Insect pheromones are extensively used in agricultural systems as lures for population monitoring and for mating disruption. In many cases, the lures consist of sex pheromones which enable the capture of one sex of the insect and can provide an indication of the presence of a target insect in the area and some idea of the population size. Pheromones are commercially available for many groups of insects (predominantly strong flyers) including moths (Lepidoptera), flies (Diptera) and beetles (Coleoptera), and may in some cases be utilized for mating disruption programs.

Trap design and incorporation of technology is an active research space and current commercially available traps include inbuilt auto-sensors, digital cameras and sim cards to remotely alert users when an insect has been trapped. The use of automatic traps is gaining momentum in Australia and overseas, especially in intensive horticulture systems. These traps have considerable potential to reduce biosecurity surveillance labour costs associated with manually accessing and assessing traps. Another way to reduce costs of a surveillance program would be to target multiple species in the same trap using multiple lures (Lopez et al 1990). By piggybacking existing monitoring networks (e.g. private grower traps, grower group surveillance networks), the chance of early detection and response could be improved.

Pheromone stacking to target multiple species is an attractive idea, however the applications may be limited depending on the compatibilities of the pheromone lures. Artificial pheromones are prepared using a mixture of chemicals in precise ratios mimicking those naturally released from a species. Synergism (enhancement) or inhibition of pheromones placed in combination has been recorded in previous research with Noctuid moths (Lopez et al 1990).

In Australian agriculture, two significant pest moth species Native budworm (*Helicoverpa punctigera* (Wallengren, 1860) LEPIDOPTERA: NOCTUIDAE) and Diamondback moth (*Plutella xylostella* (Linnaeus, 1758)

LEPIDOPTERA: PLUTELLIDAE) are monitored during the growing season to enable better pest management decisions. The turnip moth (*Agrotis segetum* Denis & Schiffermüller 1775 LEPIDOPTERA: NOCTUIDAE) is an exotic pest threat to the Australian grains industry and if it were to establish in Australia would be yet another species of cutworm that attacks and feeds on a wide range of grain crops.

Here we have explored the potential of incorporating turnip moth (AS) lures alongside those of native budworm (HP) and diamond back moth (DBM) and also lure combinations of these two existing pest species.

Methods and materials

A field site at Waterloo, South Australia (GPS: -34.009326, 138.870358) was selected based on its historical and current experiences with HP flights and moderate DBM activity. The experimental area was established within a canola crop cv. Crusher, at early flowering stage with buffers of >35m. The northern border of the field (approx. 100m from the experimental area) contained scrub and eucalyptus whilst all other field borders consisted of neighbouring canola fields.

Red "delta" pheromone traps (Figure 7) were positioned 30cm above crop height (approx. 140cm above ground) using wooden stakes at 30m spacings in a 7 x 7 Latin square arrangement. Treatments consisted of single, paired and trio combinations of all three pheromones (see Table 1). Lures were placed in the traps using separate nitrile gloves per species lure to prevent cross-contamination. Lures were stored in the freezer in either individual commercial foil packs (*A. segetum*), or wrapped in foil within a ziplock bag, and transported to the field site in insulated coolers.

From 16th Sept 2015, traps were assessed weekly for 6 weeks. Depending on the numbers of trapped insects and the condition of the sticky base, either the base was replaced (same lure transferred to new base) or the moths were removed individually. Lures were not refreshed during the trial.

On 8th October 2015, following some unusual observations, eight additional traps were set up along the western side of the grid at 30m spacings with alternating treatments of blank (no lure) and "Suterra DBM" lures (see Table 2 for lure details). These traps were assessed weekly for the last 3 weeks of the experiment

Analysis: Data was transformed for ANOVA (square root transformation for Poisson distribution where means are equal to variance) and orthogonal contrasts (AOV Contrasts of square root by treatment) conducted for certain lure combinations.

Figure 7: Delta pheromone trap deployed in a canola crop at Waterloo, South Australia

Table 1: Pheromone lures placed in Delta traps deployed in canola field

Treatment	Abbreviation
A. segetum	As
H. punctigera	Hp
P. xylostella	DBM
A. segetum & H. punctigera	As/Hp
A. segetum & P. xylostella	As/DBM
H. punctigera & P. xylostella	Hp/DBM
A. segetum, H. punctigera & P. xylostella	As/Hp/DBM

Table 2: Description and source of lures

Species (abbreviation)	Pheromone blend	Lure style	Source
Agrotis segetum **(As)**	N/A	Rubber septa	Insect Science (Pty) Ltd, Tzaneen, 0850 South Africa
Helicoverpa punctigera **(Hp)**	Z11 – 16 Ald (48.78%) Z11 – 16 OAc (48.78%) Z9 – 14 Ald (2.44%)	Rubber septa	Richard Vickers, Hawker, ACT, 2614 Australia
Plutella xylostella **(DBM)**	Z11 – 16 Ald (45.45%) Z11 – 16 OAc (45.45%) Z11 – 16 OH (9.10%)	Rubber septa	Richard Vickers, Hawker, ACT, 2614 Australia
Plutella xylostella **(Suterra DBM)**	N/A	Rubber septa	AgriSense BCS Ltd (Suterra), Mid Glamorgan, CF37 5SU, United Kingdom

1.4.3 Evaluation of TrapVIew® pest monitoring system

Trapview® is a commercially available auto-detect pheromone-based insect monitoring system designed primarily for European orchards and horticultural systems. The devices are distributed in Australia by ADAMA, and were assessed in 2015 in broadacre crops to monitor native budworm flights. This testing complemented an existing budworm (*Helicoverpa* sp.) moth monitoring program supported by the National Pest Information Service (NPIS), funded by GRDC and run in New South Wales, Victoria and South Australia. Usually this monitoring network consists of growers, agronomists and researchers maintaining traditional bucket traps and relaying moth counts to the monitoring coordinator in each state. Moth count data is collated and distributed regularly to growers and agronomists in order to assist with regional predictions of crop damage by budworm larvae and possible need for control measures.

Four Trapview® devices were provided for comparison with existing devices in New South Wales, Victoria and South Australia (2). Bucket traps designed to lure moths with pheromone into a collecting vessel containing insecticide (Figure 8a) were checked in person weekly. The Trapview® devices, which are based on the delta design using pheromone to lure moths onto a sticky base, (Figure 8b) uploaded one image per day for online viewing and data access. The TrapView® "front end" web page allows users to access the images and the system also uses algorithms to count the moths in the image, report the values and if necessary send text alerts to the user when moth numbers exceed a preset limit.

Figure 8. a) Green bucket trap. b) TrapView® device (green) attached to solar panel, high gain antenna and weather data logger.

2. SPORE SAMPLING

A number of spore sampling devices were tested for sensitivity and application to detect target pathogens. In the initial workshop and the research plan the Syield Autosampler and the Electrostatic spore trap were recognized as potential systems but problems were identified with both. The SYield Autosampler is a fungal airborne spore capture system developed by a United Kingdom consortium including Rothamsted Research, Syngenta and Burkard Manufacturing Co. based around a biosensor for sclerotinia of canola. Dr Rohan Kimber visited Rothamsted Research and Burkard Manufacturing Co. in UK during May-June 2014 to investigate this technology (see CRCPB Report Rohan Kimber SI20004 Internship at Rothamsted Research 2014). While the Autosampler platform was promising, the biosensor technology would require major scientific and financial investment to develop individual biosensors for each target pest/pathogen and hence the biosensor concept was replaced with DNA assays or aptamer technology for diagnostics. DS Scientific (USA) developed a fungal airborne electrostatic spore trap (Ionic Spore Trap) that ionises particles and captures them onto a small plate (stub) of the opposite charge. The electrostatic spore trap is no longer being manufactured, is difficult to automate compared to the other platforms and was considered to be a fire risk. Hence this trap was not included in our testing. Alternative spore trapping systems were tested as detailed in this report.

2.1 Mobile jet spore sampler, targets include; wheat stripe rust (model for airborne fungal spores including exotic rust species such as barley stripe rust and lentil rust), and other airborne fungal pathogens including sclerotinia of canola

The concept of this trap is to allow mobile trapping over a wide-geographical area, ideal for the broad-acre grains industry in Australia. The trap would be compatible to downstream diagnostics currently being used e.g. molecular assays. It would require a moderate mobile vehicle, such as a motor vehicle or bus, but is not appropriate for UAV applications in this form.

Stuart Wili at Burkard Manufacturing Co. was commissioned to develop a Jet Spore Sampler (JSS) prototype, after discussions with him during a visit to Rothamsted Research by Dr Rohan Kimber in 2014. Burkard Manufacturing Co. have the commercial & research capacity in engineering spore trapping technology and ownership of most patents included in the JSS device. SARDI has been advised that the CRCPB are unlikely to seek an IP share or royalty, but will expect acknowledgement of their contribution (e.g. via acknowledgement and inclusion of logo in promotional material, co-branding) to the development of the trap.

Dr Kimber visited Burkard Manufacturing Co. and Prof Jon West of Rothamsted Research in May 2015 when the JSS prototype was approximately at 60% completion. An operational version had been developed for assessment during this visit.

The primary objectives were to evaluate the operation of the JSS prototype and identify improvements and modifications for the next stage of development. This objective was met, and provided vital improvements for Burkard Manufacturing Co. to incorporate into the new version of the prototype. This was possible with the invaluable expertise of Prof Jon West who was involved in all visits to Burkard.

Information provided by Stuart Wili and Jon West prior to the visit indicated that there were some problems with the sampling efficiency, with greater losses of air particles within the suction system rather than into the collection pot. Resolving these issues was the main priority, so a series of testing protocols was established to improve the efficiency. To do this, Lycopodium spores, used routinely in air sampling evaluation by Burkard, were used to visually assess the flow of particles within the system, as they are bright yellow and visible by eye. The components within the JSS that could affect efficiency were tested:

1. Assess different suction rates controlling the device.
2. Try different nozzle sizes that deliver the particles into the settling chamber.
3. Evaluate various increments in the distances between the nozzle into the settling chamber, which in the initial prototype included a Miniature Virtual Impactor (MVI) which was a secondary sampling component to invert the air flow and slow the particle injection into the settling chamber.

This testing took approximately 3 days, with several combinations conducted. Some improvements were made, but efficiency was still below 70%. While this is adequate for most air samplers monitoring endemic pathogens, it is not ideal for air samplers targeting surveillance systems for biosecurity threats, where early detection at very low incursion levels is preferred.

The fourth day of bench tests revealed a critical finding. During a round of tests to optimise flow rates controlling the MVI, the flow was inadvertently close off to near zero. The result was a dramatic improvement in the efficiency of the JSS observed by significantly improved collections of Lycopodium spores into the sampling tube. It was determined that the initial design of the JSS was the problem. The assumption had been made that an MVI device must be included to slow particles down, as they would enter the JSS at approximately 800 L/min. However, the MVI was found to conflict with the JSS, as both systems require suction and this caused negative pressure leading to disruptions to the inversion of the entering air to each component. It was revealed that the original design of the JSS did in fact, slow particles sufficiently so that they can enter the settling chamber passively, and that additional easing of their momentum seems redundant. It was therefore, decided by the evaluation team that the MVI should be removed from the design, and that the prototype be redeveloped based on the inversion physics of the JSS chamber.

Several other modifications were identified during the testing and included in the new specifications of the revised prototype:

1. Without the MVI, power for auxiliary suction was not required. Therefore, a high suction rate 12V DC fan was confirmed as the main power requirement by the device.

2. A larger carousel with more sampling tubes was incorporated for greater flexibility of trapping control parameters.

3. The simple evaluation, using Lycopodium spores, showed collection efficiency was high with the modified device (no MVI), but losses might occur within the JSS chamber that are not visible at high sampling rates. Further testing was required to verify this, particularly at exhaust ports and main inversion chamber.

4. An auxiliary connector on the device was to be included to plug an alternative control box to operate the device, and override the built in control box. The Burkard control box was to be retained not removed, and PBCRC project 2014 engineers could use the auxiliary input to incorporate an electronic board to control sampling by GPS etc.

5. The actual flow rate (sampling intake) was difficult to measure. While 600-800 L/min was estimated, it needed to be verified. A sampling port could be included in the next step of fabrication to use a flowmeter for accurate measurements.

As determined in testing during May 2015, the MVI component was removed from the JSS. Assessments were then made on the base components of the modified prototype, conducted on October 30 2015 by Professor Jon West and Mr Stuart Wili at the Burkard Manufacturing Co. (UK). A number of settings for diameter (D), length (L) in the settling chamber and separation gap (A) from inversion chamber were examined during this testing to determine optimal specifications for each component comprising the prototype design and flow system. The test particle material was, again, Lycopodium spores (approx. 35 µm ø).

A smaller diameter (D) was thought to be prone to getting blocked by small insects or other debris, so the larger diameter was assessed. Keeping the settling chamber straight to a final diameter D of 10mm was not ideal. It still caused deposition of spores inside the final third of the settling chamber. The collection was very good at around 80% but the spores in the collection tube were mostly down the sides. By comparison, a taper to a smaller D showed the spores did not adhere to the sides of the tube as much and formed a convex ‘pile’ at the bottom of the tube.

Testing showed that the flow of Lycopodium spores through the settling chamber was entirely passive i.e. falling under gravity only with no pressure behind the particles. This is a good result as no disturbance of particles will be experienced within the collection tube. There was no suction upwards when the pump was turned off, which indicates that cross contamination between sampling vials will not be an issue or that loss of collected particles will not occur.

At the end of testing, the optimal set-up was determined to be L= 45mm, D = 4mm, A = 1 to 1.5mm (very little difference was found between zero to 2mm but worse efficiency collection was found at 4mm gap). Collection under the optimal set-up was estimated at 80-90%. Losses were primarily due to deposition in the final third of the settling chamber but reducing L to 30mm did not improve the collection. This was an excellent result as it places this sampler equal or better to the sampling efficiency of the Hirst Trap, an internationally recognised standard for capturing plant pathogens. It also required a sampling control system to be incorporated to ensure sampling is at a consistent velocity (suction rate) in this grid. Therefore, power requirements to automate the electronic and carousel would certainly be based on 12 V DC, most simply via the appropriate adaptor on the motor vehicle.

The modifications to efficiency and operation identified in October 2015 were incorporated into design and fabrication of new components of the JSS. This also include the fabrication and assembly of a new carousel, which can accommodate 15 collection tubes. Changes to the belt drive and stepper motor to drive the larger carousel was also made. In January 2016, all parts were manufactured and the sampler was fully assembled and tested for operation. Those tests were successful, so the sampler was disassembled and all the necessary parts were painted and anodised. Changes were also made to software for the electronic control box, after a fault was detected after earlier testing of the MVI units here in Australia, which share the same control system. The JSS was completed in the first week of March, 2016, and promotional photographs organised by Burkard Manufacturing were sent (Figure 9). The device was shipped to SARDI on March 8th, 2016.

Figure 9. The Jet Spore Sampler developed by Burkard Manufacturing Co. (UK) designed to be fitted to the roof a vehicle. The intake head is specifically suited to sampling speed of approximately 100 km/h and the device is fitted with an automated carousel system to capture spores into 2 ml extraction tubes.

Optimisation of JSS – 2016: The JSS underwent further assessment and optimisation in a specialised wind tunnel (Figure 11) at Rothamsted Research during July 2016, as part of a visit by Dr Rohan Kimber. The testing was conducted on prototype 2 – the fixed (static) version of the sampler (non-mobile) to assess its collection efficiencies compared to current air samplers, and identify further improvements and retrofitted modifications to both prototypes. Important improvements were identified at Rothamsted Research and incorporated at Burkard Manufacturing Co. This was only possible with the expertise of Prof Jon West who was involved in all visits to Burkard and extensive assessment of the device at Rothamsted Research. These modifications have subsequently been incorporated into the prototype 1 at SARDI – the Mobile version of the JSS – in September 2016.

The wind tunnel tests were to make comparisons of the device to the Burkard volumetric spore trap (most international recognised air sampler) as well as other established samplers such as rotorod and cyclone samplers. A series of testing protocols were established to examine their efficiency, side by side, within the Rothamsted Research wind tunnel. This tunnel is a 1 x 1 x 20m tunnel built in the 1960s, and is one of very few operational units in the world accessible for this type of evaluation.

Testing protocols included using two different spore sizes, *Lycopodium clavatum* (Figure 10 top), commonly used spores approx 35um diam) and Giant puffball spores (Figure 10 bottom, *L. giganteum*, approx. 3.5um diam). These spores are used routinely in air sampling evaluation and in for our assessments to assess the collection efficiency of the device in capturing small particles using simulated air flow within the wind tunnel system. Two different wind speeds were evaluated, 3 & 6 m/s, which simulates gentle and moderate wind that is optimal for air sampling.

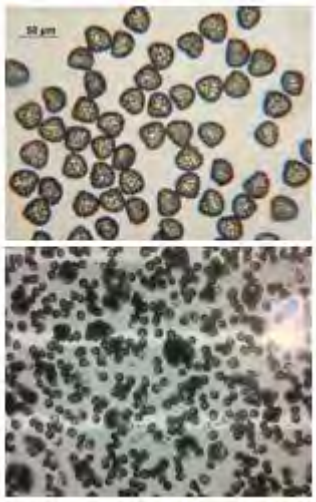


Figure 10. Spores of *Lycopodium clavatum* (top) and *L. giganteum* (bottom)

Wind tunnel testing protocols were conducting systematically, with the JSS placed adjacent to two other devices and their position rotated every 3 tests. Wind speed, spore type, run time, and sample number were recorded each test. When correct wind speed was reached, an aliquot of spores was deposited down an 8m rain-tower located at the head of the wind tunnel. Spores then entered the air stream and to the samplers being evaluated.

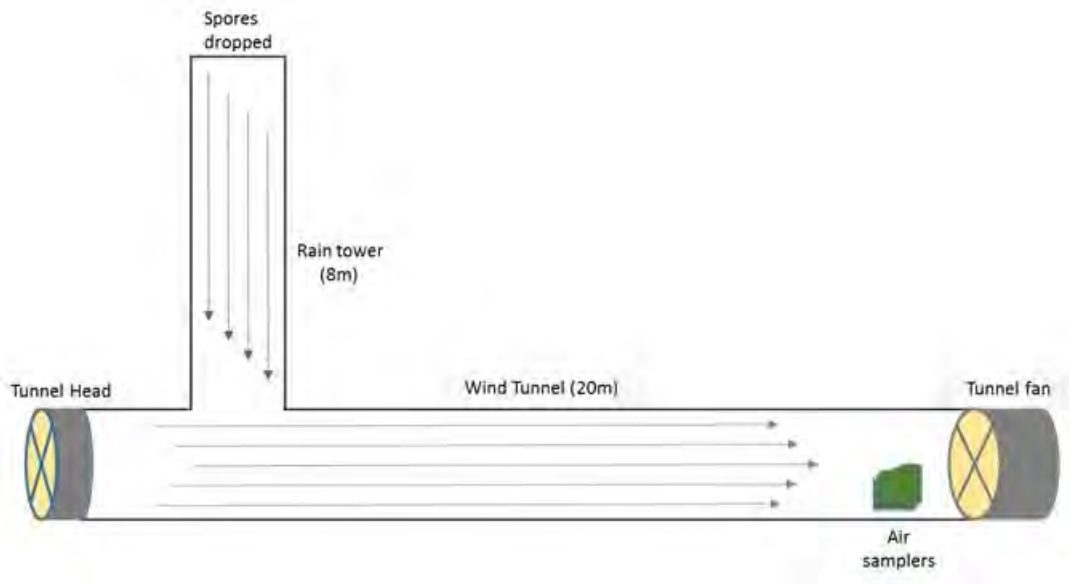


Figure 11. Schematic diagram of wind tunnel at Rothamsted Research.

Initially, 15 test runs were conducted that evaluated the JSS against the Burkard volumentric (impactor) trap and the Burkard rotorod (impactor) trap. Both latter samplers are commonly used in plant pathology. Spores collected by the JSS were in DNA-extraction tubes (virtual impaction rather than impactor sampler) and spores from the Burkard volumetric trap were on adhesive tape. Initial tests during the visit showed the collection efficiency (capture of available spores) was approximately 20% that of the Burkard volumetric trap, which is recognised as having collection efficiency above 90%. As the Lycopodium spores are bright yellow and visible by eye, this also allowed initial assessment of captured spores within the collection tubes in the JSS. This further indicated sampling efficiency losses around the nozzle and settling chamber within the device.

The JSS was taken to Burkard Manufacturing Co, as well as the original 1970's JSS from Prof Jon West's collection of air samplers. Side by side comparisons were made using the suction fan of the new JSS to identify the problems in the virtual impaction system in the new JSS.

The following components within the new JSS were evaluated, problem identified and modified:

1. Collection tube – when the device indexed to a new tube, back pressure was created by the suction fan. A 24 second pause was programmed before the next tube indexed to allow the fan to completely stop and prevent pressure.

2. Sampling head – the omnidirectional head on the JSS prototype 2 was inhibited adequate air intake. A temporary forward facing intake was fitted, to simulate a wind-vane head similar to other air samplers (e.g. MVI). Burkard fabricated a new intake at a later stage to allow the intake head to face prevailing winds.

3. Sampling nozzle - the nozzle size within the JSS was 8 mm, this was reduced to 5 mm.

4. Settling chamber - The collection of spores (bright yellow Lycopodium) was high in the collection tube, with some remaining in the settling chamber. The settling chamber was refabricated from 40 mm length to 20 mm length. The internal dimensions of the settling chamber were also changed. A slight taper from the 5 mm intake to a 7 mm outlet. These changes duplicated the nozzle from the original 1970's.

5. Carousel – the optical sensor was repositioned and a 20 mm spacer ring was fabricated for the carousel stand to compensate for the above changes.

After modifications, the JSS was bench tested at Burkard Manfacturing Co. Significant improvements were apparent, with estimated collection efficiency of Lycopodium spores above 80%. Additionally, the sampling rate (suction frequency) of the JSS was established during this visit, which required fabrication of assessment equipment and the use of a digital hot-wire anemometer. It was found the suction rate of the JSS was 450 l/min. This is a significantly higher sampling rate than other current available samplers, for instance the Burkard volumetric sampler at 10 l/min, the multi-vial cylone sampler at 16 l/min, the Miniature Virtual Impactor sampler at 26 l/min and the Burkard rotorod sampler at 100 l/min. The high rate of the JSS is particularly ideal for air samplers targeting surveillance systems for biosecurity threats, where early detection at very low abundance (rare influxes) levels is preferred.

The JSS was reassembled and taken back to Rothamsted for further evaluation in the wind tunnel. Approximately 40 tests were conducted in the wind tunnel included tests that compared a multi-vial cyclone sampler in place of the rotorod sampler. Repeated tests (3) were made for each different position of the samplers in the tunnel, spore size and the wind speed described previously. Samples from devices that collected into tube, from the JSS and multi-via cyclone samplers, referenced to each test were processed by suspending collected spores into 100ul of water and spore counts made using a haemocytometer under compound microscope. Samples from the impactor traps, volumetric or rotorod samplers, were examined directly under microscope fitted with a graduated grid-referenced eyepiece. The collection efficiency for each sampler and test was determined using the test parameters and the amount of available spores collected.

Analysis of processed samples (partial) are summarised in the following dot-points, which refers to the sampling efficiency compare to the Burkard volumetric sampler:

- 35 um size spores in wind speed 3 m/s the JSS was on average 70%.
- 35 um size spores in wind speed 6 m/s the JSS was on average 98%.
- 3.5 um size spores in wind speed 3 m/s the JSS was on average <1%.
- 3.5 um size spores in wind speed 6 m/s the JSS was on average <1%.

These results identify the following about the sampling effectiveness of the Jet Spore Sampler prototype:

1. In light winds (3m/s) the sampling efficiency was 70% of a Burkard volumetric.
2. In higher wind speed (6 m/s) the sampling rate is equally efficient (98%) to a Burkard volumetric trap. The JSS is likely to be more efficient at greater wind speeds.
3. The sampling rate of the JSS was verified as 450 l/min, which is 45 times greater (more air) sampled in the equivalent time compared to a Burkard Volumetric sampler (10 l/min).
4. The physical mass (weight) of small fungal spores (less than approx. 5um) is not enough to allow separation from the inverted air column within the JSS. Therefore, the device is not recommended for surveillance of very small particles in air.
5. Samples are collected in tubes, which make it more compatible to rapid downstream diagnostics such as molecular assays.

Overall, important aspects of the JSS demonstrated in tests at Rothamsted Research were:

1. The JSS is well suited to moderate to high wind speed sampling. This is optimal since the modified JSS at SARDI will be evaluated as a Mobile Jet Spore Sampler, which samples air while fixed to a moving vehicle, travelling approximately 100 km/h.
2. The JSS captures air particles of approx. 35 um diam. very efficiently. This is most promising, since many airborne fungal spores identified for targeted surveillance for the grains industry between 20-100um (including cereal rust spores).

During April – August 2016, the 'SMART' technology was incorporated into the JSS (located at SARDI) by Les Zeller and Paul Kamel the National Centre for Engineering in Agriculture (NCEA) University of Southern Queensland (USQ) as engineering partners in this project. This was to produce the Mobile Jet Spore Sampler (MJSS). The carousel unit delivered to USQ was found to be too slow for a mobile unit and a new controller board, motor, drive and feedback system has been developed and tested in winter 2016). The component breakdown of this modified sampler is detailed below in Figure 12. This inclusion of the 'SMART' capture technology enabled the device to capture samples according to GPS, temp, RH or time with Bluetooth communications. These parameters can be preloaded into the device using a computer interface prior to sampling. Further modifications were then included so the device can be mounted on the roof of a car/bus.

In May 2017 the device commenced fully automated trapping as a mobile sampler. Several field testing runs were conducted as a means of collecting samples for analysis using qPCR assays for specific targets and compare the results to fixed sampler (static traps) sites in SA and Victoria. The qPCR assays were developed by SARDI's Molecular Diagnostics Centre as part of other project funded by the GRDC (DAS000139). The collection efficiency was also evaluated using a comparison to a modified Burkard impactor trap (with Smart capture control) mounted on the roof of the vehicle alongside the Jet Spore Sampler.

Dr Rohan Kimber visited Rothamsted in mid-2017 to undertake ongoing improvements of the JSS to clarify sampling efficiencies of the devices for 10-20um sized-particles at low to high wind speeds, and identify the cut-off threshold for smaller-sized particles.

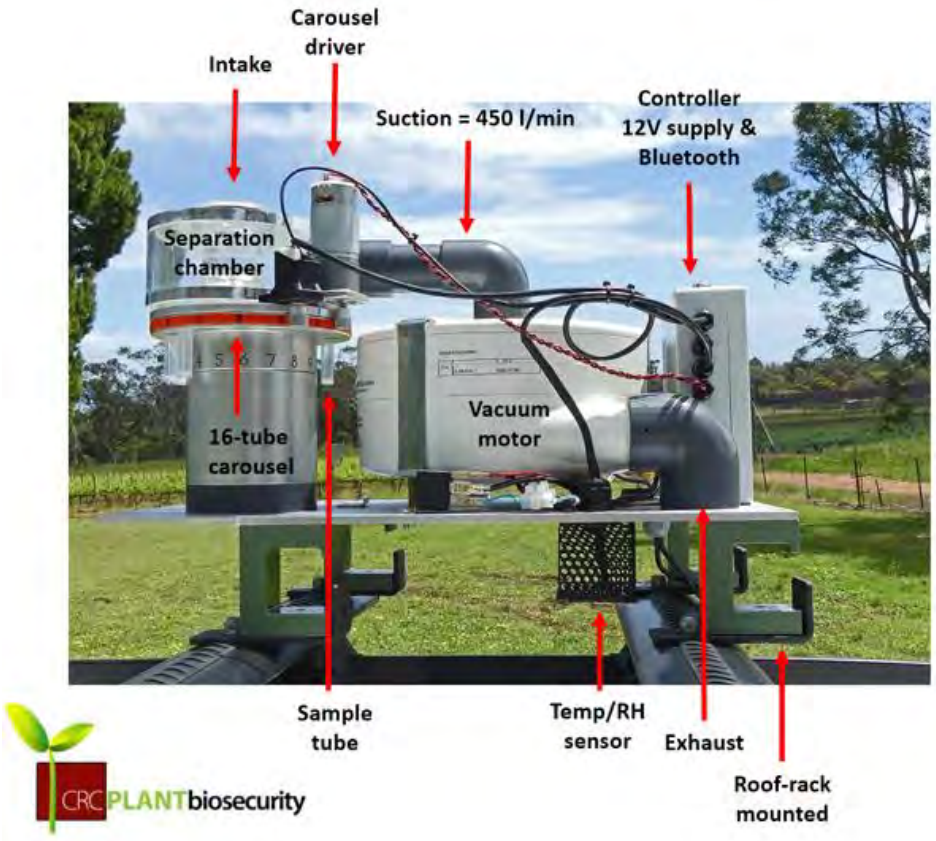


Figure 12. The completed prototype of the Mobile Jet Spore Sampler (with protective cover removed), developed by Burkard Manufacturing Co. (UK) and University of Southern Queensland's National Centre for Engineering in Agriculture, fitted to the roof a vehicle. The main components of the device are labelled including the 2 ml sample tubes that capture airborne particles when the vehicle is travelling at approximately 100 km/h within pre-defined GPS polygons loaded via Bluetooth onto the device.

Operational standards and protocols were developed for the final prototype of the Mobile Jet Spore Sampler in collaboration with NCEA to develop a relatively simple operating procedure. Pre-define polygons (using 'click locations' generating GPS coordinates) are drawn in Google Earth® and uploaded via Bluetooth onto the device as HTML files (Figure 13A) using dedicated software developed by NCEA. While the MJSS (Figure 13B) is designed to operate as an automated sampler mounted to the roof of a vehicle (Figure 13C) passing through the pre-defined polygons, an 'App' was also developed by NCEA for an Android tablet (Figure 13D) to allow the driver to monitor its activity and measured parameters (eg. position, temp, fan operation etc.) and to pause sampling should an event arise that requires shut-temporary down (dust, smoke etc.).

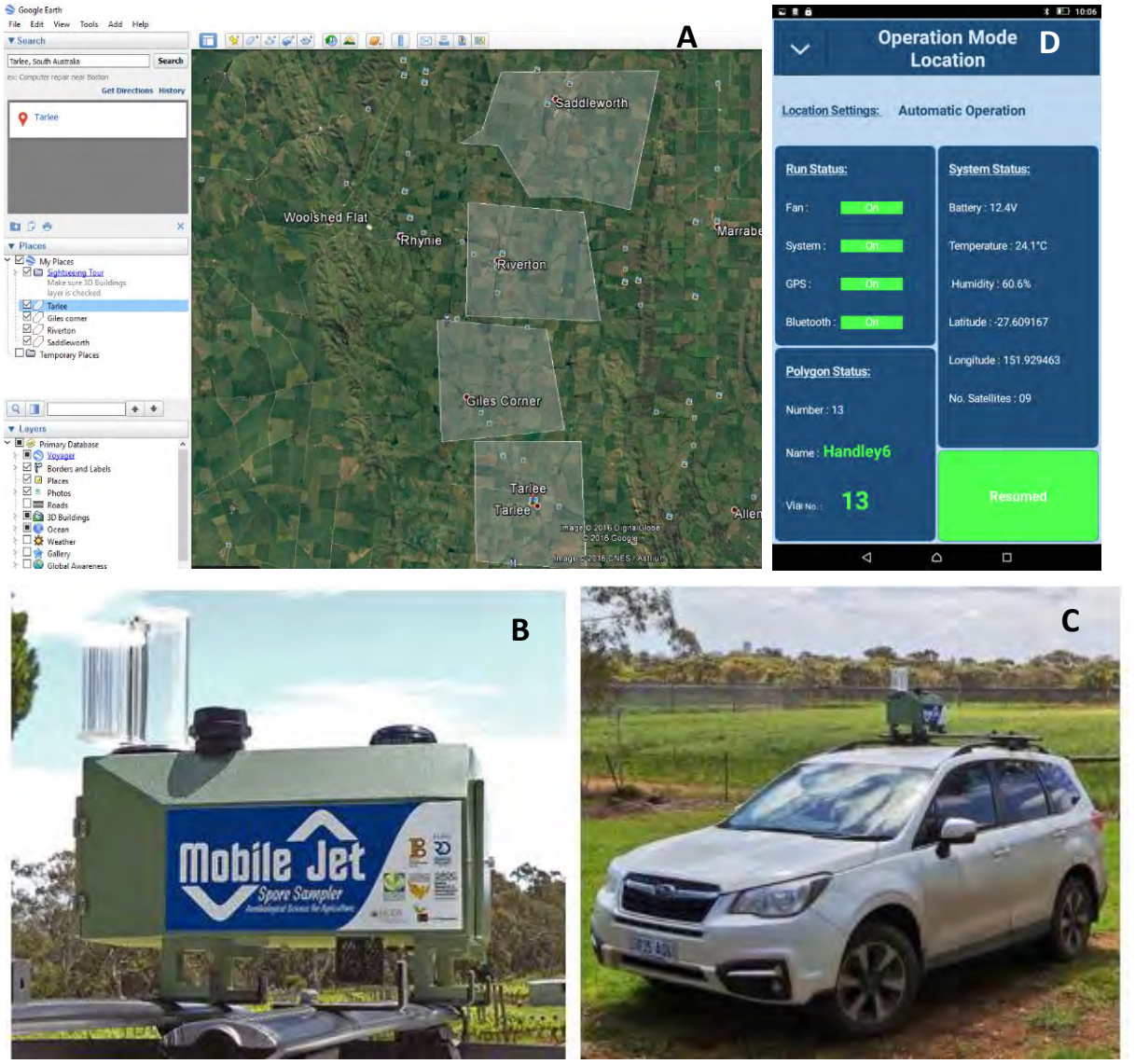


Figure 13. An operational schematic prototype of the Mobile Jet Spore Sampler developed in collaboration with Burkard Manufacturing Co. (UK) and University of Southern Queensland's National Centre for Engineering in Agriculture. Pre-defined GPS polygons are drawn in Google Earth (A) and loaded via Bluetooth onto the Mobile Jet Spore Sampler with specialised intake head (B) fitted to the roof-rack of a vehicle (C) whilst operation can be viewed and controlled using a dedicated App developed for an Android tablet (D) within the vehicle.

3. AIRBORNE PEST AND DISEASE SURVEILLANCE AND TRAPPING WORKSHOP

A two-day workshop focused on trapping techniques and surveillance strategies for monitoring pest and disease threats to industry was held at Waite Campus 17- 18th May 2016 and presented the new technologies developed in PBCRC Project 2014. Attendees included researchers from across the country with interests in pest and disease surveillance using air-sampling platforms and diagnostic techniques, focusing on both endemic and exotic threats to grains, viticulture and horticultural industries. Advances in technology were presented and discussed with an aim to establish surveillance networks and data exchange relevant to local, regional and national threats. SARDI has developed a leadership role in this field and has open relationships to coordinate ideas and methods with research groups in other states.

International collaboration: Prof Jon West from Rothamsted Research (Harpenden, UK) attended this workshop as a keynote speaker. Prof West is an internationally prominent scientist in the field of pathogen dispersal and epidemiology, with extensive expertise in spore trapping, having published numerous papers and reviews on this topic including various plant and human pathogens. In addition to his expertise in this field, he is closely associated with Burkard Manufacturing Co. (UK), the main manufacturer of spore traps used around the world. He had a lead role in a UK consortium in the development of an in-field fully automated spore trapping system and reporting device for a model pathogen, sclerotinia of canola. Prof West and Burkard Manufacturing Co. collaborated with PBCRC Project 2014 in the development of new surveillance tools.

Funding for workshop: Funding for this workshop including international travel and accommodation costs for Prof Jon West was provided by Australasian Plant Pathology Society (APPS) and PBCRC Small Initiative Project SI20060 (with investment from GRDC). The full report for this workshop in in Appendix 2.

4. MULTISPECTRAL/HYPERSPECTRAL IMAGING

4.1 Multispectral/Hyperspectral imaging of crop symptoms from pests and diseases: target endemic rusts, endemic aphids (models for exotic rusts, Russian wheat aphid, Hessian fly)

Multispectral and hyperspectral imaging can be used to detect stress in plants due to pests and diseases, via cameras attached to mobile ground based or aerial platforms. Airborne Research Australia (Flinders University, South Australia) were engaged to conduct aerial imaging using high resolution sensors in manned aircraft during winter and spring 2014 over three field sites (1 virus monitoring and 2 fungal monitoring sites) in South Australia. Sensors fitted to the specialised aircraft collected hyperspectral (SPECIM HAWK and SPECIM EAGLE) data between 400 nm – 2500 nm, thermal and digital RGB data. Four collection dates of aerial imaging were conducted at August 20, 26, October 2 and 30. Disease spread at the field sites was ground truthed by visual assessment of fungal disease (Ascochyta fabae) or laboratory testing of virus infection as described below.

Target pathogens

- *Ascochyta fabae* (representative of foliar fungal pathogens) in a faba bean breeding trial at Saddleworth and Freeling, South Australia. This trial was visually assessed for ascochyta blight in each plot on August 20 and October 31, 2014.

- Beet Western Yellows Virus (synonym: Turnip Yellows Virus) in a commercial faba bean paddock adjacent to a known infected canola paddock. Leaves from faba bean plants were sampled in two x 200 m long transects, every 50 m, leading away from the joining edge with the canola crop. Sampling occurred on 7 August and on 16 October, 2014. Leaves were blotted onto nitrocellulose membrane and submitted to Dr Mohammad Aftab of VIC DEPI at Horsham for virus testing.

Abiotic indicators: Data of abiotic stress factors including 'brackling' (stem breakage) and stem colour (frost damage) were also in a faba bean breeding trial at Pinery, South Australia. This trial was visually assessed for these symptoms in each plot on October 1 2014. Thermal and digital RGB data was collected by ARA to examine the interaction of abiotic and biotic stress factors using aerial imaging indices.

Figure 14: Airborne Research Australia aircraft used for aerial imaging collection

Data analysis was conducted at SARDI for fungal disease severity using established vegetative indices algorithms extracted from hyperspectral data. More detailed analyses were conducted by Dr John Weiss (Senior Research Scientist - BioSciences Research, Agriculture Victoria). This collaborative linkage was established due to complementary objectives between our PBCRC project 2014 and Dr Weiss' role in PBCRC project 2135 - 'Optimizing surveillance protocols using unmanned aerial systems'. This linkage was again exploited during field activities in 2015 to evaluate field and UAV robotic platforms and their potential in pest and disease surveillance.

4.2 Field robotics and Unmanned Aerial Vehicles (UAV's)

Field robots and Unmanned Aerial Vehicles (UAV's) were assessed as new platforms that can capture multispectral images of plant growth and health. SARDI established a pilot study with collaborative partner the Australian Centre for Field Robotics (ACFR - University of Sydney) and UAV data acquired by FarmingIT (UAV).

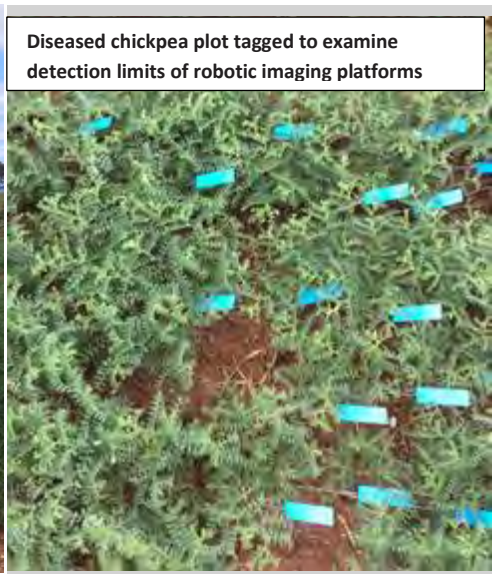
This study provided R & D activities using these technologies applied to field-based systems. The ACFR has designed, built and tested an intelligent ground based robotics platform called 'Ladybird' . The Ladybird represents a $1,000,000 investment, and is equipped with more than $100,000 of sensor technology, including stereo visual, thermal, panospheric and hyperspectral cameras, lidar and a highly accurate inertial global positioning system. The UAV platform (Trimble UX-5) from Farming IT provided a rapid and accurate means of capturing NDVI imagery for comparative analysis.

Field activities associated with this study were conducted in two field campaigns by the ACFR on Aug 17-21 and Sep 23-25th 2015 at 3 field research sites located at 3 different growing districts in South Australia. The activities of the ACFR and FarmingIT was coordinated with routine field based data collected by SARDI staff in the Pulse & Cereal Pathology group (Waite) and the Crop Evaluation group (Clare). This collaborative study also included John Weiss (Vic DEDJTR), who has PBCRC project 2135 to optimise the use of UAV platforms for plant biosecurity surveillance, when detailed disease assessments were collected in August and September during field scanning by the Ladybird and UAV platforms.

ACFR Ladybird – Field scan Aug 25 2015, Lentil trial

Ladybird sensors and guidance

ACFR Ladybird – Field scan Sep 24 2015, Chickpea trial



Diseased chickpea plot tagged to examine detection limits of robotic imaging platforms

SECTION TWO: DIAGNOSTICS OF HIGH PRIORITY PESTS

5.1 Broad-spectrum diagnostic assay development

Group-specific primer design

All group-specific primer designs were based on alignments generated using sequences from the most comprehensive datasets for each virus-groups. Sequences were aligned using the multiple sequence alignment program MUSCLE (Edgar, 2004) and Clustal Omega (Sievers et al., 2011) through the European Bioinformatics Institute (EMBL-EBI) website (McWilliam et al., 2013). Conserved sites within the alignment were identified either by using the nucleotide conserved site finder (NCSF) program (Zheng et al., 2008) and/or by eye. When using NCSF, conserved sites within the alignment were identified using five different measures: average nucleotide variants, entropy, minimum redundancy, minimum variants and maximum count with the site length parameter set to 20. The top 5 sites chosen by NCSF were Conserved sites identified by eye were equal or greater than 17bp in length, with two highly conserved positions followed by a wobble base position. The top 5 sites chosen by each measure, along with sites that were identified by eye were analysed for their rate of consensus decay using methods previously published (Zheng et al., 2008). The resultant "average nucleotide (N) score" of the sites were used to rank the sites. The best ranked sites were assessed for their suitability as primer targets based on their sequence information, such as the possibility of self-hybridisation and primer dimer formation using the program Oligo Analyzer 1.5 (Gene-Link software) (Javed et al., 2010) and OligoCalc (http://www.basic.northwestern.edu/biotools/oligocalc.html).

All primers were designed based on full-length sequences available at the time. Specifically, 54 full-length sequences of the bymovirus RNA 1, representing all 6 member species of the genus *Bymovirus*; nine full-length sequences of the hordeivirus RNAβ, representing three of the four member species of the genus *Hordeivirus*; 13 full-length sequences of the furovirus RNA 1, representing all six member species of the genus *Furovirus* and 7 full-length sequences of the pecluvirus RNA 2, representing all 2 member species of the genus *Pecluvirus* were used (Table 3).

Suitable sites must yield primers with little to no secondary RNA structure (i.e. internal loops) and have a minimum chance of self-hybridisation. Sites with sequences that might self-hybridise at the 3´ end as predicated by the program Oligo Analyzer were deemed unsuitable for universal primer design. Sites were also selected for their proximity to each other (<1kb apart) to allow for efficient amplification of target sequences; sites that were located more than 1kb apart were not considered for universal primer design.

Deoxyinosine bases (dI) were incorporated into the universal primers to reduce the degeneracy of the primers as required, usually at positions of greater than 3-fold degeneracy. As a general rule, no more than 4 dIs were incorporated into any primer and all dIs were placed more than positions away from the 3´ end of the primer.

Positive virus controls

Twenty-one positive virus controls from the genera *Bymovirus, Furovirus, Hordeivirus* and *Pecluvirus* were obtained from various sources. Of the 21 positive virus controls, three isolates represented three out of six distinct members of the genus *Bymovirus,* 11 isolates represented three out of six distinct members of the genus *Furovirus,* six isolates represented three out of four distinct members of the genus *Hordeivirus* and one isolate from the genus *Pecluvirus* (Table 4).

Table 3. Reference sequences used in the design of group-specific degenerate primers

Virus species	GenBank accessions
Bymovirus	
Barley mild mosaic virus	AF536942, AJ242725, AJ544266, AJ544267, AJ544268, D83408, L49381, Y10973, Y10974
Barley yellow mosaic virus	AB430765, AB430767, AB430769, AB500948, AB920780, AF536958, AJ132268, AJ515479, AJ515480, AJ515481, AJ515482, AJ515483, AJ515484, AJ515485, D01091, X69757
Oat mosaic virus	AJ306718
Rice necrosis mosaic virus	U95205
Wheat spindle streak mosaic virus	X73883
Wheat yellow mosaic virus	AB627806, AB627807, AB627808, AB627809, AB627810, AB627811, AB627812, AB627813, AB627814, AB627815, AB627816, AB627817, AB627818, AB627819, AB910329, AB910331, AB910332, AB948222, AF067124, AJ131981, AJ237924, AJ239039, D86634, FJ361764, FJ361765, FJ361766
Furovirus	
Chinese wheat mosaic virus	AJ012005; AJ271838; AB299271
Japanese soil-borne wheat mosaic virus	AB033689
Oat golden stripe virus	AJ132578
Soil-borne cereal mosaic virus	AJ132576; AJ252151; AF146278; AF146279; AF146280
Soil-borne wheat mosaic virus	L07937; AJ298068
Sorghum chlorotic spot virus	AB033691
Hordeivirus	
Anthoxanthum latent blanching virus	None available
Barley stripe mosaic virus	U35769; U35770; U35771; U35772; AY789694; X03854; JF803284
Lychnis ringspot virus	Z46351
Poa semilatent virus	M81486
Pecluvirus	
Indian peanut clump virus	AF239729, AF447396, AF447397
Peanut clump virus	AF447398, AF447399, AF447400, AF447401, L07269

Table 4. Positive virus controls used for diagnostic assay validation

Virus species	Source	Type of material
Bymovirus		
Barley mild mosaic virus	DSMZ[1] (PV-0329)	Dry tissue
Barley yellow mosaic virus	DSMZ (PV-0634)	Dry tissue
Wheat spindle streak mosaic virus	DSMZ (PV-0541)	Dry tissue
Furovirus		
Chinese wheat mosaic virus	Y Shirako – Japan	Fresh tissue
Chinese wheat mosaic virus	J-P Chen – China	Dry tissue
Soil-borne cereal mosaic virus	DSMZ (PV-0552)	Dry powder, nucleic acid
Soil-borne cereal mosaic virus	Loewe[2] (07173-PC)	Dry powder
Soil-borne cereal mosaic virus	Loewe (07127-PC)	Dry tissue
Soil-borne cereal mosaic virus	BioRad[3] (3551984)	Dry powder
Soil-borne wheat mosaic virus	DSMZ (PV-0748)	Dry powder, nucleic acid
Soil-borne wheat mosaic virus	Agdia[4] (LPC-42001)	Dry powder
Soil-borne wheat mosaic virus	Loewe (07174-PC)	Dry powder
Soil-borne wheat mosaic virus	INRA[5], France	Dry tissue
Soil-borne wheat mosaic virus	PHEL-MPI[6] (03-2003-2886)	Dry tissue
Hordeivirus		
Barley stripe mosaic virus	DSMZ (PV-0330)	Dry tissue, nucleic acid
Barley stripe mosaic virus	Agdia (LPC-19500)	Dry powder
Barley stripe mosaic virus	Loewe (07004-PC)	Dry powder
Barley stripe mosaic virus	BioRad (3551870)	Dry powder
Lychnis ringspot virus	ATCC[7] (PV-62)	Dry tissue
Poa semilatent virus	ATCC (PV-162)	Dry tissue
Pecluvirus		
Peanut clump virus	DSMZ (PV-0291)	Dry tissue

[1] Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).
[2] Loewe Plant Diagnostics (Loewe Biochemica GmbH; Sauerlach, Germany).
[3] Bio-Rad (Berkeley, United States of America).
[4] Agdia (Elkhart, United States of America).
[5] National Institute for Agriculture Research, France.
[6] Plant Health & Environment Laboratory, Ministry for Primary Industries, New Zealand.
[7] American Type Culture Collection (Manassas, United States of America).

RT-PCR and cycling conditions

All nucleic acid extracts were tested by RT-PCR for the presence of amplifiable host RNA using primers targeting the plant gene NAD with primers NAD2.1a and NAD2.2b (Thompson et al., 2003). For validation of the primer pairs, one-step RT-PCR tests were done using the SuperScript®III Platinum TaqOne-step RT-PCR kit (Thermo Fisher Scientific, Epsom, UK). Reactions were done in a 20 µL volume as per the manufacturer's instructions. One µL of total RNA isolated from the reference virus positive controls was used. PCR cycling conditions, extension time and expected amplicons size are described in Table 13.

For survey samples, one-step RT-PCR was done using the SuperScript®III Platinum Taq One-step RT-PCR kit in a 20 µL volume reaction as per the manufacturer's instructions and 4 µL of total RNA. All PCR products were separated by agarose gel electrophoresis and visualised using SYBR®safe DNA gel stain (ThermoScientific, Waltham, MA, USA). When the assay produced amplicons of the expected sizes, the assay was assumed to have successfully detected the virus isolate.

Cloning and sequencing

To confirm positive PCR results, selected amplicons were purified using the QIAquick PCR Purification Kit (Qiagen) and then cloned into the pGEMT Easy Vector 2 (Promega, Madison,USA) following the manufacturers' instructions. Plasmid DNA was extracted from white colonies using the small scale alkaline lysis method (Sambrook et al., 1989b) and inserts were detected by restriction enzyme analysis. Three clones were sequenced from each cloned amplicon in both directions using the SP6 and T7promoter primers and an ABI BigDye Terminator Version 3.1kit on an AB3730xl sequencing machine (Applied Biosystems; Australian Genome Research Facility, Australia). A consensus sequence for each amplicon was compiled using ClustalX (Thompson et al., 1997) and the GenBank non-redundant nucleotide database was searched using BLASTN to identify the sequences that matched most closely the consensus sequences (Altschul et al., 1997). A positive detection is considered to be successful if the consensus sequence matched sequences (from their perspective genera) in GenBank in the expected region of the genome.

Sensitivity test

Sensitivity assays were done using selected positive virus control of targeted viruses and synthetic positive constructs to determine the detection limit of the RT-PCR assays. Specifically, ten-fold serial dilutions of the total RNA extracts from selected isolates and/or the synthetic positive constructs were used as templates in one-step RT-PCR. The concentration of the neat template was measured using a Nanodrop spectrophotometer (ND-1000; ThermoFisher Scientific); then serially diluted in nuclease-free water in a 10-fold serial dilution series up to 10^{-5} and 1 µL of the diluted nucleic acid was used as template for each assay. The concentration of the neat total RNA extracts from the selected positive virus controls ranged from 10.36 ng/µL to 378.98 ng/ µL with varied purity based on their ratio of absorbance at wavelengths 260 nm and 280 nm (Table 5). The neat total RNA concentrations were not adjusted, to mimic real life diagnostic samples which often have a wide range of starting concentrations.

Table 5. Concentration and purity of the neat nucleic acid used as the templates in the sensitivity test

Positive control virus	Neat concentration (ng/µL)	Purity (260/280 ratio)*
CWMV	378.98	2.05
SBCMV	74.20	1.88
SBWMV	161.99	2.02
BSMV	10.36	1.96
LRSV	151.60	2.14
PSLV	50.75	2.10
Bymovirus synthetic construct	2.0	N/A
Pecluvirus synthetic construct	2.0	N/A

5.2 Next Generation Sequencing for Plant Virus Diagnostics

Virus samples

A Victorian isolate of *Barley yellow dwarf virus* (BYDV) maintained in *Triticum aestivum* (wheat) – previously confirmed by serology and PCR. A clonal grass sample "A" (*Pennisetum*) with an unknown virus infection, intercepted by New South Wales quarantine officers. This sample was screened for a number of viruses using generic assays designed to target potyviruses, hordeiviruses, furoviruses, rymoviruses and tritimoviruses with all negative results (data not shown).

RNA extraction

Double-stranded (ds)RNA was extracted from the clonal grass sample using Morris and Dodd's extraction method 1 (Morris, 1979) as double-stranded (ds) RNA was the standard method for extraction plant virus RNA. The dsRNA was eluted in 200 µL of TE-1 buffer and stored at -20°C until use.

To investigate the difference between using total RNA (TRNA) and dsRNA as templates for NGS, both type of RNA were extracted from the BYDV isolate currently maintained in wheat. All TRNA were extracted using RNeasy Plant Mini Kit (Qiagen) and a modified lysis buffer as previously described.

Quantity and quality of the RNA extracted was measured spectrophotometrically using the Nanodrop 1000 (Thermo Fisher Scientific) and fluorometrically using the Qubit RNA HS Assay kit on the Qubit quantification system (Thermo Fisher Scientific).

Library preparation and sequencing

Genomic host DNA (gDNA) was depleted from the extracted RNA using the Turbo DNA-free™ kit (Thermo Fisher Scientific) following the manufacturer's instructions. For dsRNA samples, an incubation at 95°C for 5 mins followed by snap chilling was to obtain single-stranded RNA (ssRNA) after the gDNA depletion.

The gDNA depleted (and denatured in the case of the dsRNA extracts) RNA was then used as templates for library preparation using the NEBNext® Ultra™ RNA Library Prep kit for Illumina® (New England Biolabs; Massachusetts, Unite States). All nucleic acid (RNA, cDNA and amplicons) generated during the library preparation stage were quantified using the Qubit and the Agilent Technologies 4200 TapeStation System (Intergrated Sciences; Australia). Libraries were pooled in equimolar concentrations with a final concentration of 4nM and then sequenced at 16 pM using the Miseq® Reagent Kit v3 with 600 cycles on a MiSeq® system (Illumina; San Diego, United States).

Sequence data analysis

All reads were quality checked and trimmed to remove adapter sequences and sequences with a quality score less than 25 using Trim Galore V0.4.0 and Cutadapt V1.4.1. *De novo* assemblies were done using SPAdes Genome Assembler V3.6.0 (Nurk et al., 2013) with the "-careful" parameter and the resulting contigs were searched against the non-redundant nucleotide database at GenBank using BLAST (Altschul et al., 1997).

All map to references alignments were done using Bowtie 2 using the "-very-relax-local" parameter in order to capture more dissimilar sequences to the reference genome (Langmead and Salzberg, 2012) and visualised using Tablet V1.14.04.10 (Milne et al., 2013). Reference sequences/genomes used were dependent on the BLAST results of the contigs. Where no hits were generated by BLAST, a customised dataset containing only plant virus sequences available at the time were used as reference genomes. Where possible, a consensus sequence is obtained from the alignments and used for subsequent analysis.

All contigs of viral origin generated by the *De novo* assembly were cross checked with the consensus sequences generated via mapping to references when possible. Congruous sequences were retained, curated and searched against the GenBank database to determine its identity.

Phylogenetic analysis

Once identified, sequences from closely related species of the sequenced species were then used for subsequent phylogenetic analysis. Sequence alignments were generated using Clustal Omega (Sievers et al., 2011) through the European Bioinformatics Institute (EMBL-EBI) website (McWilliam et al., 2013). Based on the alignments generated, a phylogenetic tree was constructed by the Maximum Likelihood method integrated in MEGA 7 (Kumar et al., 2016).

5.3 Development of RT-LAMP for Barley yellow dwarf virus

BYDV positive controls and sample collection

BYDV-PAV positive controls were kindly supplied by Narelle Nancarrow (AgriBio, DEDJTR) in the form of finely ground whole plant tissues and by Rebecca Vandegeer (AgriBio, DEDJTR) as fresh leaf tissue from BYDV-PAV positive wheat plants kept in growth chamber at AgriBio, Victoria, Australia. Field samples were collected from symptomatic wheat plants suspected of BYDV infection from the fields of Horsham, Victoria, Australia.

Total RNA extraction

Total RNA was extracted from BYDV-infected plant tissue using Mackenzie buffer (Mackenzie et al., 1997) and the RNeasy® Plant Mini Kit (QIAGEN) as previously described.

Crude RNA extraction

As a field-based nucleic acid extraction protocol was needed for on-site testing of BYDV using RT-LAMP, published protocols were tested, with modifications so that these methods utilised minimal laboratory-based equipment making them more feasible for adaptation to field testing.

Prick (water) and prick (sucrose) extraction methods were modifications of the 'Touch-and-Go' method described by Berendzen et al (2005). In both methods, leaf tissue (approximately 2 mg) was punctured inside a tube containing either 10 µL water (prick in water) or sucrose solution (prick in sucrose [50 mM Tris-HCl pH 7.5, 300 mM NaCl and 300 mM sucrose]) using a pipette tip before adding the pipette tip into the RT-LAMP reaction mix and pipetting up and down a few times. The sucrose solution omits the inhibitory effect of EDTA which is component of most lysis buffers for nucleic acid extraction (Mackenzie et al., 1997).

The sucrose prep method previously described by Berendzen et al (2005) was modified so that minimal equipment was required for the RNA extraction. Around 2.5 mg leaf tissue was added to a tube containing 50 µL of sucrose solution and was ground on ice using a pipette tip. The sample was incubated at 100°C for 10 min and centrifuged at 3000 g for 5 sec to pellet the tissue debris that could inhibit the PCR. One µL of the supernatant was then added to the RT-LAMP reaction mix. The sucrose prep method was further modified to investigate if RNA can be extracted without the use of a heat block and a centrifuge. So the method henceforth referred to as Sucrose prep (-inc) was performed without the incubation and the centrifugation steps. The leaf tissue was ground in the sucrose solution. To assist homogenisation, leaf tissue (approximately 10 mg) was placed into a grinding bag containing 1 mL of the sucrose solution and was ground using a hammer to rule out if grinding the tissue well for extraction was the limitation of the RNA isolation method. One µL of the homogenate was added directly to the RT-LAMP reaction mix.

FTA® classic cards (Whatman®) have been reported for efficient sampling and retrieval of viral pathogens from infected leaf tissues (Natarajan et al., 2000; Ndunguru et al., 2005). The extraction method using FTA® card is illustrated in Figure 15. For sample application, FTA® card was placed on a clean, dry surface and leaf tissue was applied either by direct press, hereafter referred to as FTA® Direct press (FTA-DP) method or by spotting plant homogenate, hereafter referred to as FTA® Plant homogenate (FTA-PH) method. In the FTA-DP method, parafilm was placed on the leaf kept on the FTA® card and leaf tissue was ground with hammer until the sap penetrated through the card. For FTA-PH method, leaf tissue (8 mg – 10 mg) was placed in a grinding bag containing 1 mL of MacKenzie buffer, ground with hammer and the plant homogenate (20 µL – 50 µL) was applied onto the card. Samples on the FTA-card were allowed to air dry for 30 min – 60 min and using the Harris Micro Punch™, 2 mm discs from the dried sample spot were taken (1 disc per sample). The sample spot disc was then added to a 1.5mL microfuge tube containing 50 µL of freshly made RNA Processing buffer (10

mM Tris-HCl, pH 8.0, 0.1 mM EDTA, 800 U/mL RNase Out™ (Invitrogen), 2 mM DTT) and incubated at room temperature for 15 min for RNA elution. The samples were briefly agitated by flicking at 5 min interval whilst incubating to facilitate RNA recovery. One µL of eluted RNA was used in subsequent RT-LAMP assays.

RT-PCR and RT-qPCR for BYDV-PAV

To confirm the presence of amplifiable RNA in all RNA extracted, the AtropaNad primer pair designed to amplify a 188 bp region of a highly conserved plant mRNA encoding the subunit 2 of the NADH dehydrogenase gene (Thompson et al., 2003) was used a house-keeping test. To confirm the presence of BYDV-PAV in the samples, two assays were used: a RT-PCR test developed by Rastgou (2005) and a RT-qPCR developed by Nancarrow (2014) (Table 5).

For both the NADH and BYDV-PAV RT-PCR, amplification was performed in 25 µL reaction volume containing 12.5 µL of 2× Reaction mix, 0.5 µL of SuperScript® III RT/ Platinum® Taq Mix of SuperScript® III One-Step RT-PCR System with Platinum® Taq DNA Polymerase kit (Invitrogen), 0.4 µM of each primer, and 1 µL of template RNA. RT-PCR cycling conditions for the NADH and BYDV-PAV assay are listed in Table 5.

For results analysis, 10 µL of the PCR product with the addition of 2 µL of 6× loading dye was resolved on a 2% (w/v) agarose gel stained with SYBR Safe DNA using 0.5× TBE running buffer at 100 V for 45 min and visualised on an UV transilluminator.

For the BYDV-PAV RT-qPCR, each reaction was done in a total volume of 25 µL using 5 µL of 5× Reaction mix and 1.25 µL of enzyme mix (RNA UltraSense™ One-Step Quantitative RT-PCR System; Invitrogen), 0.3 µM of each primer and 0.1 µM of probe and 1 µL of total RNA template. The cycling conditions are listed in Table 5.

Zhao RT-LAMP reaction

The BYDV-PAV RT-LAMP test developed by Zhao et al (2010), hereafter referred to as the Zhao RT-LAMP was performed on four isolates of BYDV-PAV strain. This assay was tested using the originally described primer concentrations (Zhao et al, 2010) with some modifications – reactions were performed in the total volume of 25 µL with 12.5 µL of Isothermal Master Mix (OptiGene). For detection of LAMP product, amplification was performed at single temperature of 65°C in Genie® II (OptiGene) instead of examining the presence or absence of pyrophosphate precipitate as originally described by the Zhao et al (2010) (Table 5).

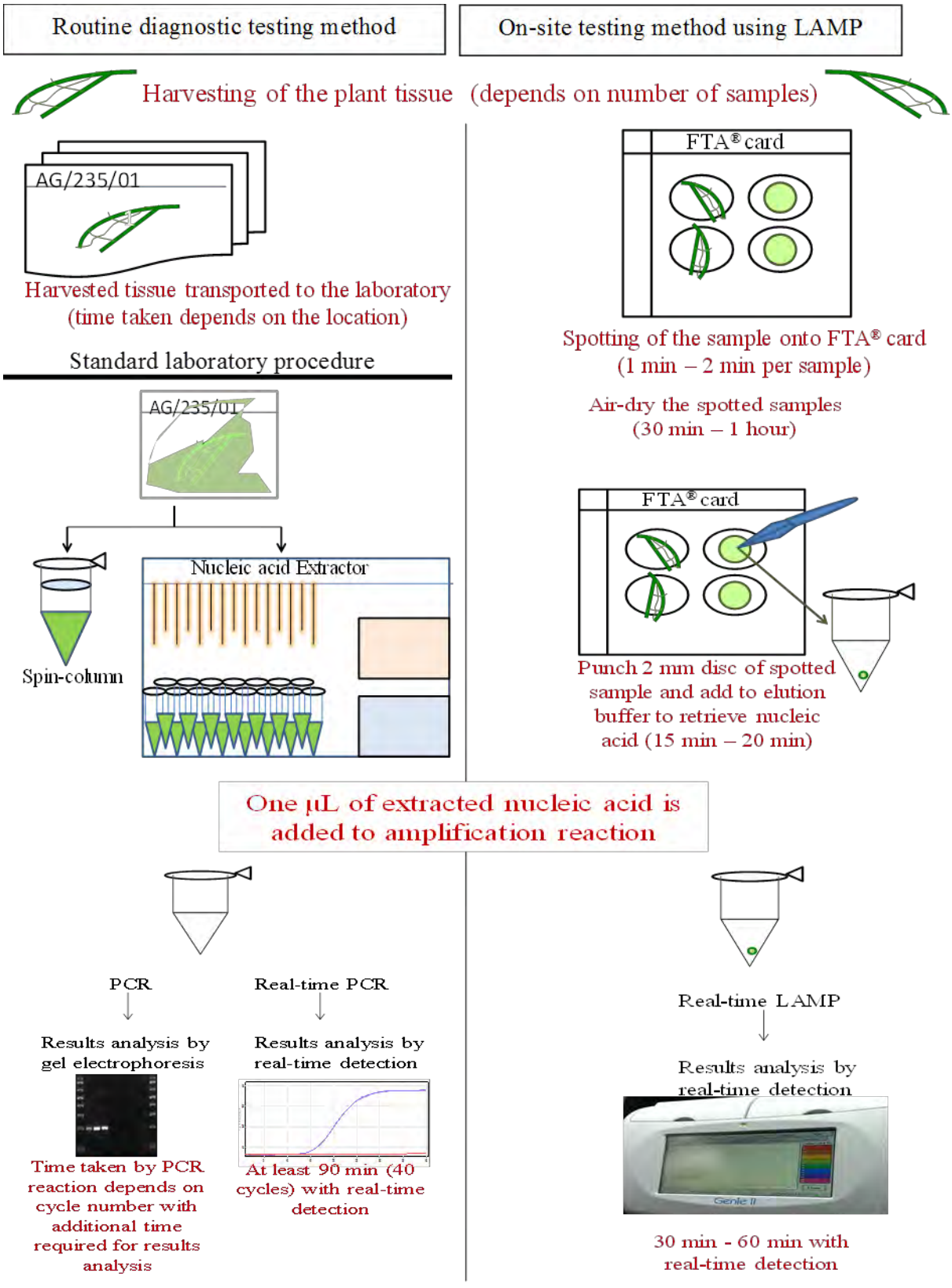


Figure 15. Flow diagram comparisons between the routine diagnostic testing and the developed on-site testing.

Table 5. Previously published RT-PCR, RT-qPCR and RT-LAMP assays and their reaction conditions used for the detection of BYDV-PAV isolates

Assay	Primer ID	Primer sequence (5' - 3')	Region amplified	RT-PCR cycling conditions	Expected product size (bp)
NADH (Thompson et al., 2003)	AtropaNad2.1a AtropaNad2.2b	GGACTCCTGACGTATACGAAGGATC AGCAATGAGATTCCCCAATATCAT	NADH dehydrogenase ND2 subunit mRNA	1 cycle [48°C 30 min], 1 cycle [94°C 2 min], 35 cycles [94°C 30 sec, 55°C 30 sec, 72°C 15 sec], 1 cycle [72°C 5 min]	188
BYDV RT-PCR (Rastgou et al., 2005)	BYDV-1 BYDV-2	GTTCTGCCTCAACATCGGAT AATAGGTAGACTCCTCAACA	Coat protein (CP) gene	1 cycle [48°C 45 min], 1 cycle [94°C 2 min], 40 cycles [94°C 30 sec, 60°C 45 sec, 72°C 60 sec], 1 cycle [72°C 5 min]	744
BYDV RT-qPCR (Nancarrow et al., 2014)	BYDV-PAV left BYDV-PAV right BYDV-PAV probe	CCCTTTCAACTATTTGTCCATCTG GTGGAAACGAAGATGGCTATTG [6FAM] CAGGTCTCCAAGTGTTGTTGCCAA	Coat protein (CP) gene	1 cycle [50°C 15 min], 1 cycle [95°C 5 min], 40 cycles [94°C 30 sec, 53°C 30 sec, 72°C 30 sec]*, 1 cycle [72°C 5 min]	158
BYDV-PAV RT-LAMP (Zhao et al., 2010)	F3-PAV B3-PAV FIP-PAV BIP-PAV	CAACTATGTTGTCTCGTATGGA TGGTCAGTTATGGTGGAAAG CAACATCGGATTCATCACATGTCGGTACACGAACAA GAGAATGGA CACTCCGCATGACTCGGCACAGGCTGACAAAATCC CTCATC	Read-through protein (RTP)	65°C for 40 mins, Anneal-curve analysis from 98°C to 80°C at 0.5°C/sec	Variable sizes

*Fluorescence captured after each cycle

Novel BYDV-PAV RT-LAMP primer design

The complete nucleotide sequence of the Barley yellow dwarf virus-PAV genome (GenBank accession number D85783) was retrieved from NCBI database (http://www.ncbi.nlm.nih.gov/nuccore/1395150) and aligned with 24 cloned sequences of BYDV-PAV (data provided by Narelle Nancarrow) using BioEdit Sequence Alignment Editor (www.mbio.ncsu.edu/bioedit/bioedit.html). From the alignment, a consensus sequence was determined and submitted to Primer Explorer V4 (https://primerexplorer.jp/elamp4.0.0/index.html) for LAMP primer designing using "Common" primer option. Possible primer sets were compared on the basis of melting temperature (Tm) that should be approximately 65°C for F1c and B1c and about 60°C for F2, B2 and F3, B3; the free energy should be -4 kcal/ mol or less for the 3' ends of primers F2, B2, F3, B3, LF, LB and the 5' ends of primers F1c and B1c, and the GC content to be 50% - 60% (Notomi et al., 2000; Nagamine et al., 2002).

Novel BYDV-PAV RT-LAMP assay optimisation

LAMP primers were added to the reaction mixture at the following final concentrations: external primers at 3 pmol and internal primers at 12 pmol. RT-LAMP reactions were performed in total reaction volume of 25 µL and two different LAMP reaction mixes were used during assay development. The first reaction mix was OptiGene's Isothermal Master Mix containing the Bst DNA polymerase where fifteen µL of the reaction mix was used with 0.5 µL of AMV Reverse Transcriptase (RT) enzyme added separately. The second reaction mix was the rapid Isothermal Master Mix ISO-001 containing a novel GspSSD (Geobacillus species) DNA polymerase (OptiGene) with no separate addition of RT enzyme. One µL of RNA template was used for all reactions.

RT-LAMP reactions were performed at 65°C for 35 minutes, with fluorescence detection at the FAM channel and a post-amplification melt-curve analysis step (melting temperature (Tm) or temperature of dissociation determination of the amplicon from 98°C to 80°C at 0.5°C/s) using Genie® II /Genie® III (OptiGene).

Novel BYDV-PAV RT-LAMP assay validation and field testing

The novel RT-LAMP assay's performance was compared to that of the Zhao RT-LAMP assay and the two previously published RT-PCR and RT-qPCR assays. Specifically, the sensitivity of the newly developed BYDV-PAV RT-LAMP assay was compared to that of the RT-PCR, RT-qPCR for BYDV-PAV using 10-fold serial dilutions of the plant total RNA infected by BYDV-PAV in nuclease free water. To compare the results of different PCRs, RT-qPCR was analysed on the basis of quantification cycle (Cq, at which positive detection can be assured) determined by Rotor-Gene Q Series Software by using default settings and RT-LAMP was assessed on the basis of positivity value generated by Genie® II/III. Test results are considered negative when: 1) no products can be visualised via electrophoresis for the RT-PCR; 2) no amplification is observed for the RT-qPCR above the threshold and 3) no amplification is observed for the Zhao RT-LAMP as visualised by the Genie® machine.

For on-site detection, symptomatic leaves (yellow leaf tips) were harvested from the base of wheat plants suspected of BYDV infection and total RNA was extracted using both the FTA-DP and FTA-PH methods. The diagrammatic sketch highlighting the difference between routine procedure and applied method of on-site testing for BYDV detected is as represented in Figure 15.

All results obtained in the field test were confirmed in the laboratory using RT-qPCR.

5. Results

SECTION ONE: INSECT AND SPORE TRAPPING TECHNOLOGY

A: Technology Review and Workshop - see Appendix 1

1. INSECT TRAPS

1.1. A high suction frequency insect sampler; target endemic aphids (model system for Russian wheat aphid)

Aphid numbers (total aphids, Green Peach Aphid (GPA) and Russian Wheat Aphid (RWA)) detected in the suction traps deployed at three different sites in SA during 2016 & 2017 are shown in the graphs below (Figures 16-20). Peak aphid flights were recorded during autumn and spring in 2016 but no significant autumn flights were detected in 2017. Distinct later spring/early summer flights of RWA was recorded, indicating a preference for warmer temperatures by this pest although low numbers were observed at the two sites in 2016.

The high frequency suction trap from Burkard Manufacturing Co. was an effective platform for the collection and monitoring of aphid flights and good operational and functional reliability was experienced over the 2 years of testing in a range of weather conditions.

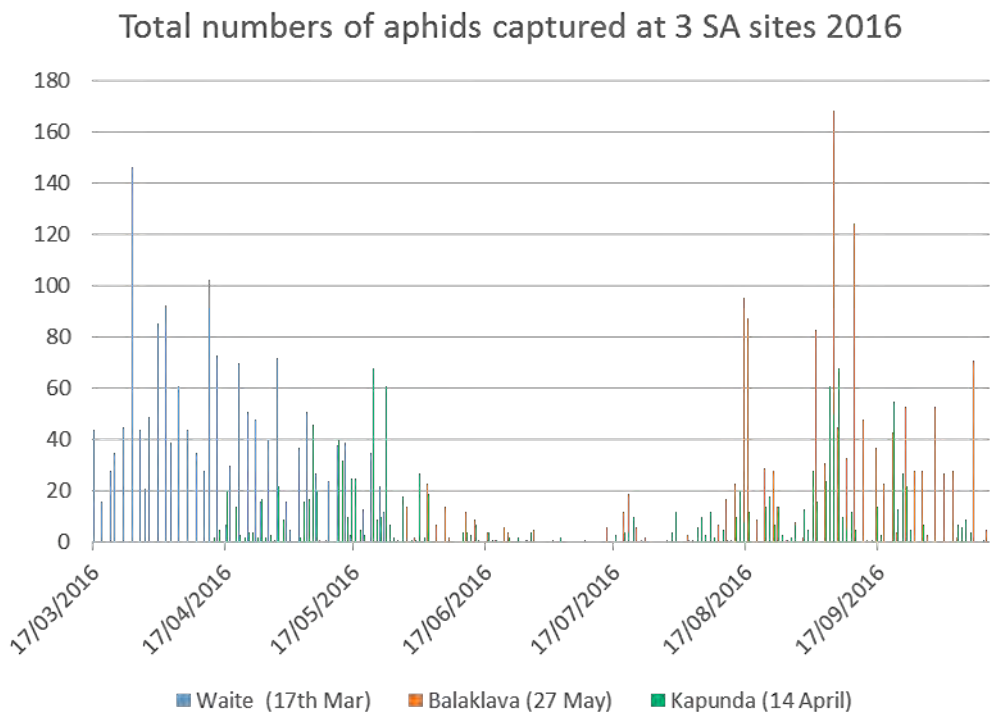


Figure 16: Total number of aphids captured in 2-day sampling periods at 3 testing sites during 2016 using the insect suction traps from Burkard Manufacturing Co (UK).

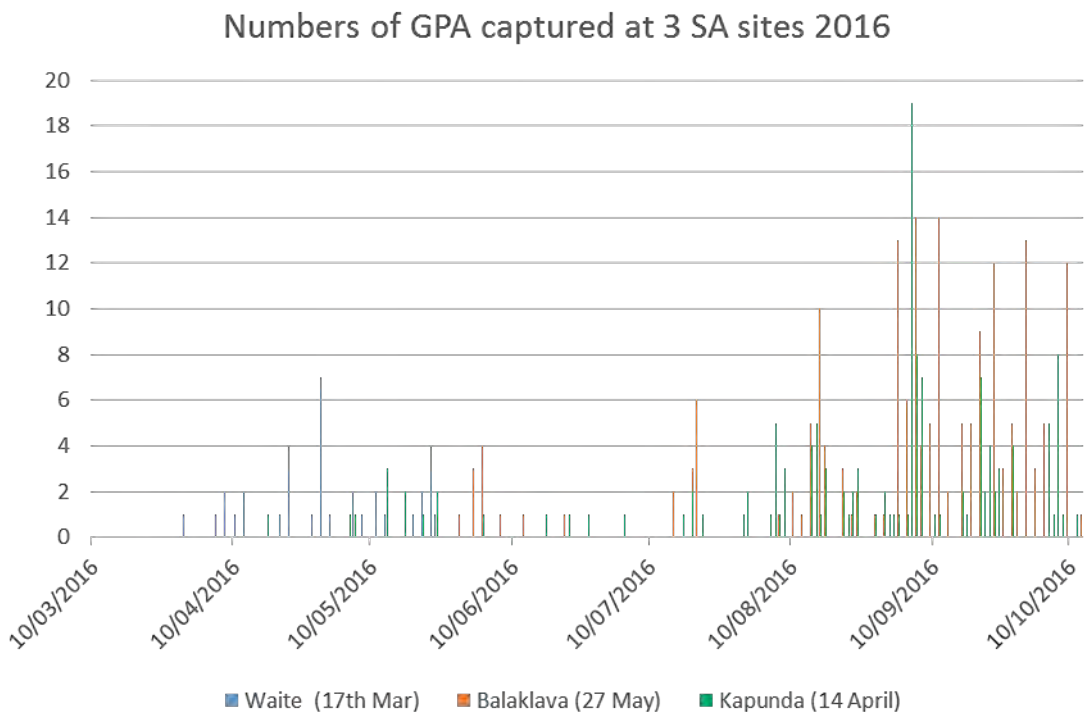


Figure 17: Total number of Green Peach Aphid (GPA) captured in 2-day sampling periods at 3 testing sites during 2016 using the suction traps from Burkard Manufacturing Co (UK).

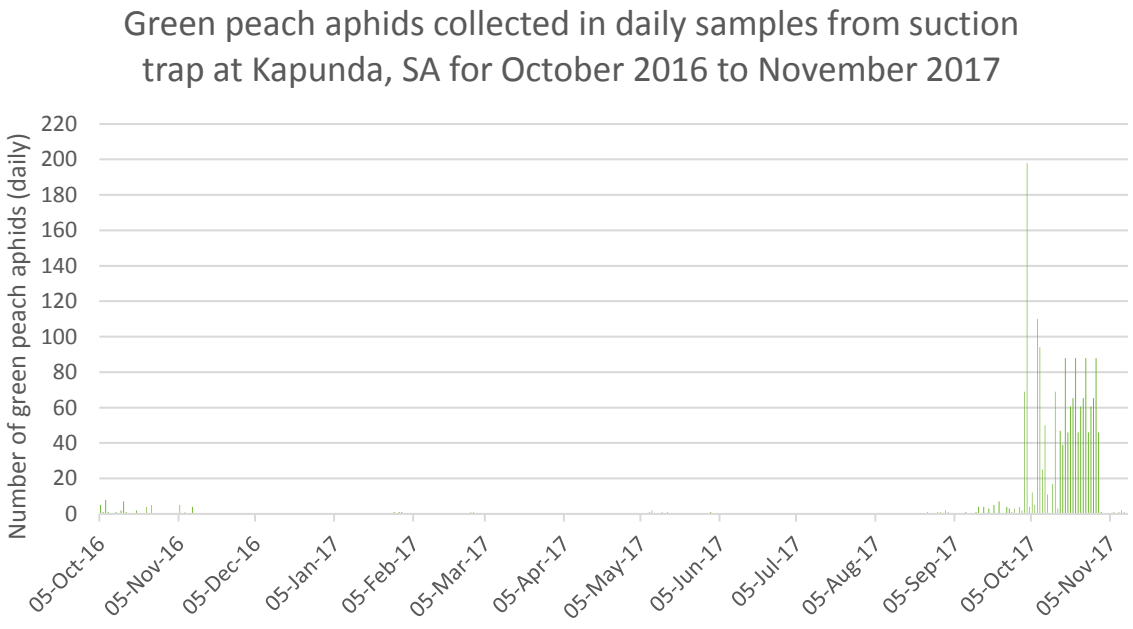


Figure 18: Total number of Green Peach Aphid (GPA) captured in daily samples at a single testing site (Kapunda, SA) from October 2016 to November 2017 using the suction trap from Burkard Manufacturing Co (UK).

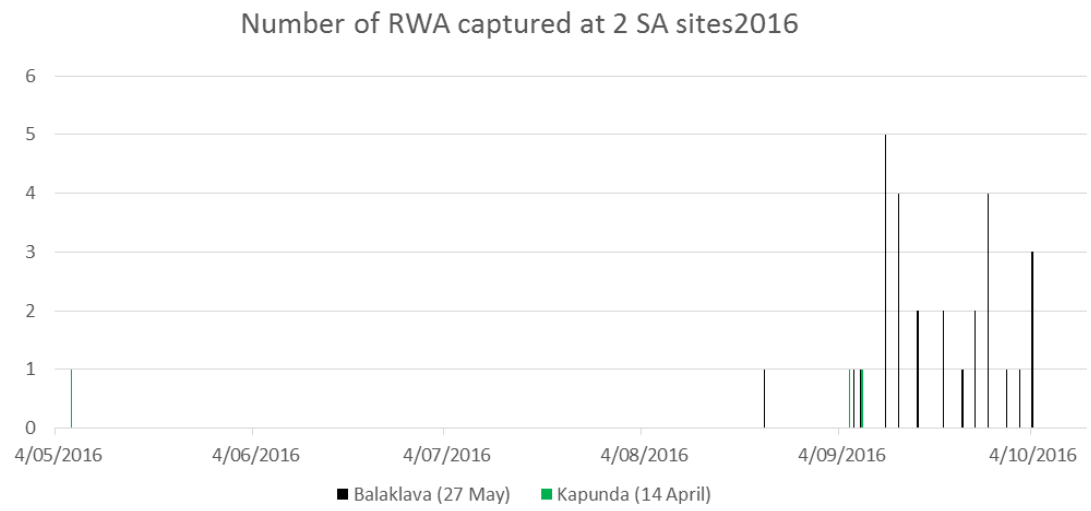


Figure 19: Total number of Russian Wheat Aphid (RWA) captured in 2-day sampling periods at 2 testing sites during 2016 using the suction traps from Burkard Manufacturing Co (UK)

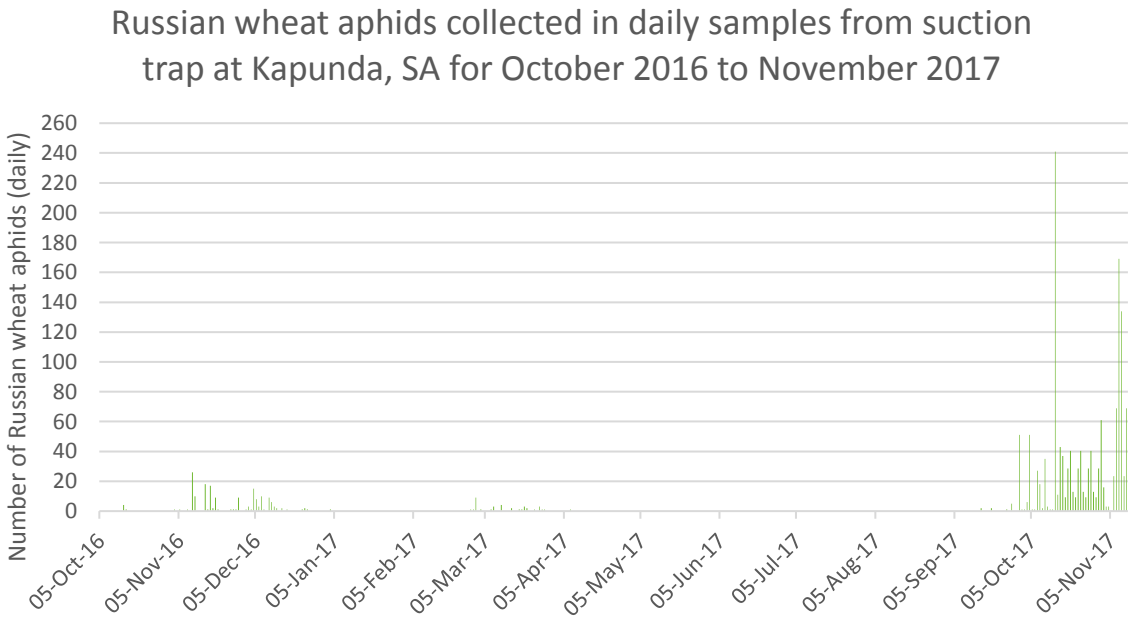


Figure 20: Total number of Russian Wheat Aphid (RWA) captured in daily samples at a single testing site (Kapunda, SA) from October 2016 to November 2017 using the suction trap from Burkard Manufacturing Co (UK).

Table 6 shows that the suction traps (STs) generally resulted in higher counts than those from the yellow pan trap (YPTs). This confirmed that the application of a surveillance platform based on trapping flying insects using suction traps was effective.

Table 6: Evaluation of Yellow Pan Traps (YPTs) compared to Suction Traps (ST) from Burkard Manufacturing Co (UK) to monitor for Russian Wheat Aphid at 2016 testing locations over four monitoring timelines. The sampling locations using the different traps were not the same and these results are for comparisons only.

Start trap	End trap	Days trapping	Sum of 4 YPTs	Kapunda ST	Balaklava ST
24/08/2016	1/09/2016	8	0	0	0
1/09/2016	15/09/2016	14	3	2	11
15/09/2016	5/10/2016	20	2	0	16
5/10/2016	19/10/2016	14	2	5	3

The addition of light (UV-B) to the trap showed that this device could also be used to evaluate targets that would otherwise be monitored using pheromone taps, such as bucket, delta or SMART moth traps. The UV-B strip lighting was activated from dusk until dawn by a light sensor. The suction trap operated during June (2017) for a 2-week period with or without UV-B light and set on a daily time change sequence. Captured targets (image right) were visually identified and counted into pest classes as shown in Figure 21. When UV-B light was activated, significant numbers of flies (includes mosquitoes) and moths were recorded which were not when the device operated without the addition of UV-B light during night-time.

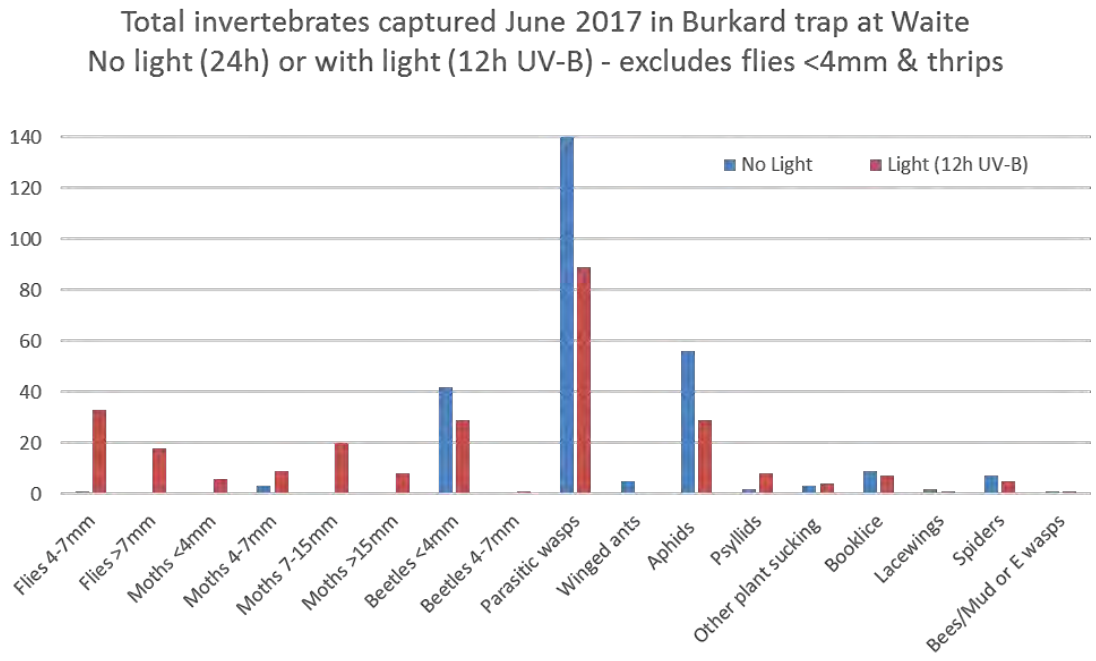


Figure 21: Total number of insects captured, classified into pest groups, using the suction traps from Burkard Manufacturing Co (UK) operating on a daily time sequence fitted with or without 12-h night-time near-ultraviolet light (UV-B) located in an orchard testing site at the Waite Campus during June 2017.

The results of the side-by-side comparison of the high frequency suction trap from Burkard Manufacturing Co (UK) and the SMART insect suction trap from USQ at the Waite Campus in June 2017 showed that both devices were directly comparable for capture frequencies of both insect numbers and insect classification group (Figure

22). However, the high frequency suction trap from Burkard Manufacturing was found to have a higher count of aphids than the SMART insect sampler, which may reflect a more suitable sampling intake port on this device (points upwards).

Figure 22. Comparison of the high frequency cyclone 12-Volt aphid suction trap (foreground) with an 8-pot carousel fitted with SMART capture technology from the University of Southern Queensland's National Centre for Engineering in Agriculture and the high frequency suction trap (background) from Burkard Manufacturing Co. (UK) fitted with 4-pot carousel on time-sequence control. (Note: Smart Moth Trap is seen centre-left).

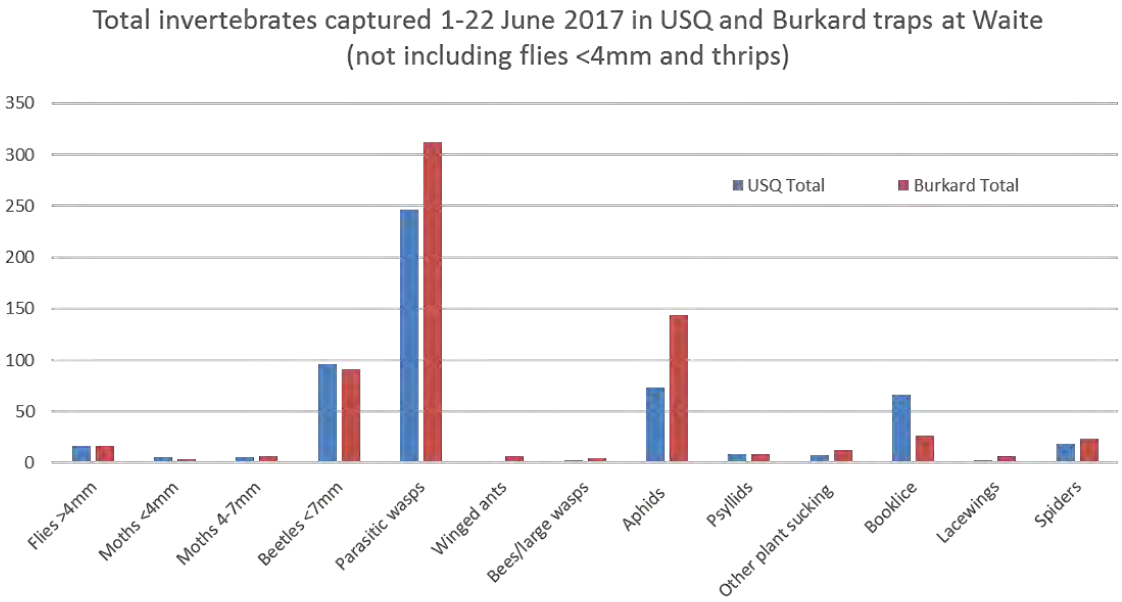


Figure 23: Total number of insects captured, classified into pest groups, using the suction traps from Burkard Manufacturing Co (UK) compared to the SMART insect suction trap from University of Queensland's National Centre for Engineering in Agriculture. Both devices operated on a daily time sequence located in an orchard testing site at the Waite Campus during June 2017.

1.2. Compact Aphid suction traps: target endemic aphids (model system for Russian wheat aphid)

The functionality of the SMART capture system was evaluated over the final months of the project. Different parameter settings were used to obtain data sets which demonstrate how this function may be used to obtain information pest dynamics. Manual identification (visual diagnostics based on morphology) of pest species or classification groups was conducted by SARDI entomologists on the project.

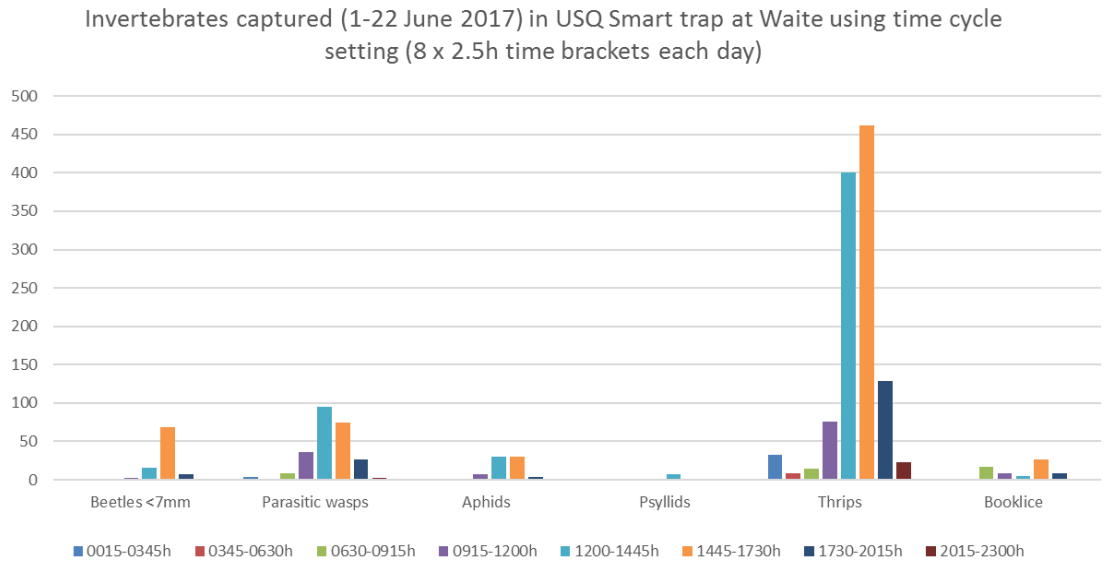


Figure 24: Number of insects captured, classified into six groups, within 8 daily time-cycle segments by the SMART insect suction sampler. The 'Daily Time-cycle' capture setting demonstrates which time-bracket of the day the target was captured the device.

Further evaluation of the SMART cyclone suction trap is required, with capture parameters such as temperature and wind direction. Initial attempts to test temperature as the capture parameter revealed that the temperature sensor on the trap was reported elevated readings compared to ambient readings. This was because the sensor was located within the housing of the trap. Modifications were made to locate the temperature and RH sensors to outside the trap, within a specialised sensor housing attached to the main body of the trap. Attempts to test wind direction as the capture parameter also failed due to a malfunction in the fan motor which was subsequently replaced.

1.3. Insect and Fungal Spore Diagnostics

DNA extraction from insect suction traps: (Table 7) There were no obvious differences in the amount of DNA extracted from the samples, whether stored in ethyl alcohol (ETOH) or glycol (PEG) and whether there was more or less material (0.1g to 0.61g). Most importantly there is no sign of inhibition with both the diluted (1:5) and neat DNA. The lack of inhibition suggests that less extraction buffer can be used, which will reduce the dilution factor and increase the chance of detecting a target at low concentration.

Sample	Weight	Preservation	DNA Concentration		Yeast Ct	
No.	(g)	reagent	Counts/µl	pg/ µl	Neat	1:5
1	0.26	ETOH	202	2481	14.4	16.9
2	0.30	ETOH	210	2615	14.6	16.9
3	0.61	ETOH	214	2686	14.6	16.9
4	0.10	PEG	208	2588	14.5	16.8
5	0.10	PEG	195	2356	14.6	16.9
6	0.34	PEG	207	2560	14.5	16.9
7	No insect + internal control		190	2255	**14.6**	**16.9**
8	No insect – internal control		166	1828	UD	UD

Table 7. DNA concentrations of insect samples from suction traps, stored in ethyl alcohol or glycol and tested as neat samples or diluted 1:5.

Development of diagnostic primers:
Results for experiment 1 to assess efficiency and sensitivity of both GPA and WFT assays-
Both GPA and WFT were detected in the mock mixtures and the trap samples spiked with 1, 5 and 10 insects (Table 8). As expected, lower Ct values were observed with increasing number of target insects added. Detection levels for 1 and 10 insects were similar between the samples with no background and the spiked samples (mock mixtures and trap samples), suggesting that the 'dilution' effect did not affect detection. GPA was not detected in sample 14 containing 10 non targets and spiked with one GPA and one WFT. Given that (1) one WFT was detected in this sample and (2) the level of detection in other samples with only one GPA, this is more likely due to a problem with the spiking itself rather than a problem with the extraction.

Table 8: Evaluation of qPCR assays for detection of Green Peach Aphid (GPA) and Western Flower Thrip (WFT) DNA using spiked treatments of 1, 5 or 10 targets in isolated solution (no other insects) or mixed-sample solution (background insects). UD = Undetected.

Sample #	Test type	Number of GPA	Number of WFT	Number of non-targets	B1-1 (ml)	DNA (C/ul)	DNA (pg/ul)	Yeast	WFT	GPA Ann 50C
1	Quant	1	1	0	7	189	2458	13.6	32.7	28.2
2	Quant	1	1	0	7	186	2390	13.6	34.4	29.3
3	Quant	1	1	0	7	183	2331	13.4	33.5	27.7
4	Quant	1	1	0	7	187	2407	13.6	32.6	26.3
5	Quant	5	5	0	7	188	2437	13.4	27.9	25.1
6	Quant	5	5	0	7	194	2548	13.6	27.5	25.6
7	Quant	5	5	0	7	200	2671	13.4	26.8	26.1
8	Quant	5	5	0	7	196	2583	13.4	25.6	25.5
9	Quant	10	10	0	7	190	2461	13.3	25.6	24.4
10	Quant	10	10	0	7	197	2601	13.6	25.6	24.5
11	Quant	10	10	0	7	191	2486	13.3	25.9	25.0
12	Quant	10	10	0	7	200	2671	13.3	26.6	23.8
13	Mock mixture	1	1	10	7	200	2671	13.3	34.6	27.4
14	Mock mixture	1	1	10	7	191	2482	13.7	32.0	UD
15	Mock mixture	1	1	10	7	203	2717	13.5	32.9	26.2
16	Mock mixture	1	1	10	7	194	2549	13.5	32.1	28.0
17	Mock mixture	1	1	100	10	239	3411	13.3	30.8	27.5
18	Mock mixture	1	1	100	10	207	2797	13.3	32.5	28.8
19	Mock mixture	1	1	100	10	199	2649	13.6	32.1	27.6
20	Mock mixture	1	1	100	10	227	3182	13.3	31.8	27.2
21	Mock mixture	10	10	10	7	210	2866	13.3	28.5	24.0
22	Mock mixture	10	10	10	7	205	2752	13.3	28.7	24.5
23	Mock mixture	10	10	10	7	210	2853	13.3	27.6	25.0
24	Mock mixture	10	10	10	7	212	2903	13.5	27.9	24.4
25	Mock mixture	10	10	100	10	218	3018	13.2	26.2	24.4
26	Mock mixture	10	10	100	10	207	2799	13.2	27.4	24.5
27	Mock mixture	10	10	100	10	200	2663	13.2	27.5	25.2
28	Mock mixture	10	10	100	10	215	2953	13.1	27.2	24.2
29	Trap sample	1	1	0.26 g	10	323	5051	13.3	29.1	27.0
30	Trap sample	1	1	0.46 g	10	285	4310	13.2	28.1	27.7
31	Trap sample	10	10	0.55 g	10	315	4884	13.2	26.0	24.6
32	Trap sample	10	10	0.60 g	10	257	3777	13.3	25.8	25.2
33	NTC DNA					162		UD	UD	UD
34	NTC Lab							UD	UD	UD
35	Positive control @ 200 pg/ul (GPA and WFT) or 2pg/ul (Yeast)							23.0	21.6	24.9

Results for experiment 2 to assess potential inhibition factors and cross-reactivity for the GPA assay (Table 9).

There was good detection and efficiency of the assay with no obvious signs of inhibition from mix-sample treatments by the abundance of non-targets. Higher Ct values (30-32) for 1 GPA was observed than in the previous experiment. In contrast, the last treatment gave Ct values for 1 GPA (27-28) more consistent with the previous experiment even though 1-4 aphids were spiked in this treatment. This could simply be due to the variation between individual aphids. The result within treatments across the two experiments, shows Ct between replicates can vary by more than 1 Ct value. This could be due to a range of factors including the size, life stage, extraction efficiency. There is also one surprising result with treatment 5 (never exposed to GPA) for which one rep had a CT of 27 (i.e. consistent with one individual). The possibilities to explain this are that (1) The assay detected GPA from a predator or from fragments of GPA or (2) a counting error, that there was one GPA in the sample. When comparing to experiment 1, lower Ct values (i.e. more DNA extracted) with single aphids in a mixture compared to single aphids on their own were observed. This may be because the larger amount of material in the samples with mock mixture is more adapted to the extraction protocol than one single aphid. It seems like the DNA from the background sample is acting as a 'carrier'. No cross-reactivity was observed with the non-target aphid, *Macrosiphum rosae*.

Table 9: Evaluation of qPCR assays for detection of Green Peach Aphid (GPA) DNA using presence and absence of the target in different preservation solutions (EtOH or Glycol with no other insects) or within mixed-sample treatments (Glycol with abundant non-target insects). A cross-reactivity check was also included using non-target aphid, *Macrosiphum rosae*. UD = Undetected.

Tube #	Mixed non-targets	Green peach aphid (GPA)	Green peach aphid (GPA)	Fluid in	Rep	Drained**	DNA		Ct Values	
	Trapped and stored in glycol	*Extracted from glycol sample*	*Field collected and stored in EtOH*	tube	No	(Y / N)	C/ul	pg/ul	GPA	Yeast
1			✓ (1 GPA per tube)*	ETOH	1	N	210	2529	30.5	13.8
2					2	N	215	2637	31.2	13.7
3					3	N	218	2698	31.0	13.8
4	✓		✓ (1 GPA per tube)*	Glycol	1	Y	288	4041	31.7	13.8
5					2	Y	269	3685	31.4	13.8
6					3	Y	280	3896	30.6	13.8
7		✓ (1 GPA per tube)		Glycol	1	N	228	2882	32.5	13.8
8					2	N	209	2523	30.1	13.8
9					3	N	212	2570	32.0	13.7
10	✓ (exposed to GPA, but GPA now removed)			Glycol	1	Y	270	3702	UD	13.7
11					2	Y	247	3254	UD	13.7
12					3	Y	249	3299	UD	13.7
13	✓ (never exposed to GPA)^			Glycol	1	Y	241	3125	UD	13.7
14					2	Y	259	3473	27.4	13.8
15					3	Y	295	4182	UD	13.8
16		3 x *Macrosiphum* sp. (probably *rosae* [Rose aphid]) from glycol		Glycol	1	N	197	2282	UD	13.7
17					2	N	216	2653	UD	13.7
18					3	N	233	2972	UD	13.7
19	✓	✓ (number of GPA in brackets) 81(3), 82(4), 83(1)		Glycol	1	Y	235	3012	27.6	13.7
20					2	Y	289	4068	28.0	13.7
21					3	Y	309	4450	27.0	14.0
22	Ntc Lab				1				UD	UD
23	Ntc PCR				1				UD	UD
24	Positive control (GPA DNA @ 200pg/ul) - Eudunda, gDNA1				1				24.4	UD

Samples delivered on the 22.01.16 and stored in the fridge.

** Samples were drained on the 27.01.16 using a buchner funnel on Whatman paper and washed with water. Dried at RT for 30 min and transferred the samples back into its tube.

* Field collected ETOH stored GPA were late stage nymphs 1.4-1.5mm long

^ This non-target sample was never exposed to GPA prior to adding the EtOH GPA.

qPCR assay for wheat stripe rust:

The wheat stripe rust assay was examined for specificity and sensitivity using qPCR conditions within assays developed by the Molecular Diagnostics Centre at SARDI. This experiment showed the wheat stripe rust assay detected 2ng/ul of DNA from the target pathogen (*Puccinia striiformis* f. sp. *tritici*) at a Ct value of 22.7, but some cross-reaction was observed with DNA of two off-targets included in the evaluation (wheat stem rust - *Puccinia graminis* f. sp. *tritici* (Ct 33) and wheat leaf rust *Puccinia triticina* (Ct 38)). Results indicated that using this gene target, some cross-reaction with wheat stem and leaf rust, and barley stripe rust (tested by sequence alignment in silico), will occur and may confound results from mixed samples. The results indicated that the assay was inconsistent in detecting and quantifying wheat stripe rust spores in both field samples and on spiked tapes. The issue is likely to reflect difficultly in disruption of the rust spores during the process, to release DNA and activate binding to the primers. Further development of this assay using these preliminary primers will not proceed. The end-point application of this assay will require additional funds to design new primer sets to distinguish between *Puccinia* species and *forma specialis*. Further development of a more suitable assay is recommended.

1.4. Smart Insect Trap: target Diamond Back Moth (model for exotic Turnip Moth)

1.4.1 Trap development

Automatic sensors

- The traps modified with optical sensors and tested in South Australia in 2014 found there were issues with weather proofing, false triggering, power supply and target efficacy.
  - False triggering of the optical sensor was due to rainfall and moisture on the electronics. A bead of silicon was applied to rim of traps to prevent water drops blowing into traps, holes were drilled in the base of the collection chamber for drainage, circuit boards were waterproofed, and Vaseline applied to ensure a tighter fit of the funnel. Rust was also affecting parts of the trap including the sensors. Sunlight entering the trap at certain times of the day leading to small changes to the ambient light was also triggering the sensor. This is because the sensitivity is very high in order to detect DBM and the sensor needs to respond to very small changes of light. The sensitivity of the circuit was reduced which affected the repeatability to detect DBM but was applicable for bigger moths. A modified sensor tube (smaller diameter) was made to reduce the amount of light entering the trap and therefore reduce false triggering. This may mean that a different size tube for different target moths would be required.
  - Power supply: The batteries initially failed and the system stopped working. The solar panel was not able to deliver enough power to back up the system, and the master trap lost all communication.
  - Target efficacy: One or two DBM entered the green traps but fewer than in the control Delta traps which captured 6 to 10 DBM, questioning the suitability of the green trap for DBM.

Capture data: South Australia July 4 2014

Green Trap #0 (master);	1 x DBM, 6 x flies (blowfly & midges), 1 x aphid, ~10ml water
Delta trap 20m west of #0,	10 x DBM and numerous insects
Green Trap #1 (constant light)	2 x DBM, ~5ml water
Delta trap 20m west of #1	6 x DBM and numerous insects
Green Trap #2 (intermittent light)	1 x large moth (not DBM), ~10ml water

- Results of the visual capture in the traps with optical sensors tested in Queensland in 2014 are in Table 10 below. However, these did not correlate with the optical sensors. A significantly high number of false sensor counts were registered in the traps during times when the sun was at a low elevation (Trap 1 had 72 counts on 29 Sep in Trap 1 during 8.00 to 9.00 am and 3.00 to 4.00 pm and 64,205 counts on 3 Oct which were found as false triggers not moth capture). The inside and outside surface of the funnel were painted with a matte black paint to mitigate against this effect and while this reduced the proportion of false counts it did not eliminate the problem. This issue was more prevalent in the original reflectance sensor which was the motivation for the design of the second sensor which operated on a beam interruption technique. The second problem was that dust (atmospheric as well as moth scale) would tend to build up on the optical components. This was not a significant problem with the first (reflectance) sensor design because it was designed to cope with long term changes automatically due to its self-adjusting threshold. This issue however began to show up in the second (interruption type) sensor after some weeks of testing. It became progressively less sensitive to objects (moths) dropped through it.

Table 10. Moths captured in smart moth traps and control traps, Cecil Plains, Queensland, Sept-Oct 2014

Date	Trap 1		Control Trap 2		Trap 4		Control Trap 1	
	Live moths	Dead moths	Live moths	Dead moths	Live moths	Dead moths	Live moths	Dead moths
29-9-14	8[a]	6[a]	9	-	2[b]	-	6	12
3- 10-14	30[c]	98[c]	5	29	4[d]	8[d]	15	47
	Replaced with Trap 3							
7-10-14	4	13	-	15	-	5[e]	6	66
10-10-14	5	20	1	17	3[f]	8[f]	8	22

- The piezo film sensor was not physically robust being a thin film which could be easily damaged or pierced. In addition, due to the films' construction it behaved as an antenna and thus outputs an electrically noisy signal even when there are no impacts upon it. The ceramic piezo sensor also had issues with false triggering, from wind or trap disturbance. Some images of captured moths were transmitted via the 4G mobile phone network and saved on the website http://www.croptest.com/data/ (Figure 25); image numbers reached a maximum of 9 on 12 Oct 2015.
-

Figure 25. Images of captured moths from smart moth traps with impedance sensors, Pampas-Horrane Rd, Queensland, October 2015.

Time determined image capture:

- The smart insect trap 1 tested in September to November 2016 at the Hart Field Site recorded no images despite the trap capturing over 100 budworm moths. The control trap, a conventional green bucket trap with the same lure, captured the same number. It was found that a faulty connection prevented the camera from functioning, and subsequently preventing the carousel from processing the captured moths. This was resolved and addressed in the other traps.
- The smart insect trap 1 tested at Hart Field Site in October to November 2016 successfully recorded and uploaded four images of *H. punctigera* to the website. Comparably, the control bucket trap captured 5 moths over the same ten-day period. The trap design and detection algorithms were not optimal for smaller insect targets such as DBM, and tests with the smart traps confirmed this with no capture recorded. The bucket trap loaded with both lures (stacked) captured 18 *H.punctigera*. This again supported results from pheromone stacking research (see below) that these two lures interact, promoting the chemical attractant to *H. punctigera*.

Figure 26. Images of captured targets from Smart Moth Trap #1 (left and centre) reported to the website (http://www.croptest.com/data/) using time-determined image capture and transmission via 4G mobile telemetry every 2 hours at Hart (South Australia) in October-November 2016, and from an upgraded camera in #3 trap (right) during further testing at University of Southern Queensland in May 2017. All images are <35KB.

- Results of Light Brown Apple Moth (LBAM): Significant flights did not occur during the testing period, although a small number of moths were captured in a standard delta trap. The vineyard collaborator also had a TrapView® (see below) operating at the same site and reported the same outcome. No further tests could be conducted to assess the traps performance on LBAM as repairs and upgrades were required.

During testing and evaluation of the Smart Moth Traps in 2017 several technical issues were experienced which highlighted numerous reliability and maintenance problems with their design and operation:

- Battery life – poor performance was often experienced despite changing brands and Ah outputs. This may reflect inadequate solar panel feed but most likely excessive power requirements of the device.

- Weather proofing – moisture penetrated the photographic chamber on some traps more than others despite identical design.
- False triggers – images were sometimes reported to the website with no apparent target captured. This indicated the device detected a capture but this was false.
- 4G coverage – in some instances preferred site selection was not possible due to inadequate coverage of the mobile network to allow image transmission.
- Image quality and position – initial prototypes did not have adequate image quality. An upgrade in the digital camera in later prototypes improved this quality but had poor positioning over the photographic chamber, sometimes resulting in partial image capture of the target.

Unfortunately, due to several technical issues in the three Smart Moth Traps in the final year of testing, no traps were reliably functional by the end of 2017. This highlighted that, while these were prototypes, a significant design improvement will be required before a commercially ready version would be acceptable.

1.4.2 Pheromone stacking

Native budworm moth numbers remained high for the first 4 weeks of the trial period before dwindling rapidly, whereas DBM numbers remained low until the final 2 weeks when the crop began to dry out and newly emerged moths swelled the adult population (Figure 27). Turnip moths were not detected in any traps. Nine specimens of black cutworm (*Agrotis ipsilon*) were trapped, however they were not significantly associated with any treatment.

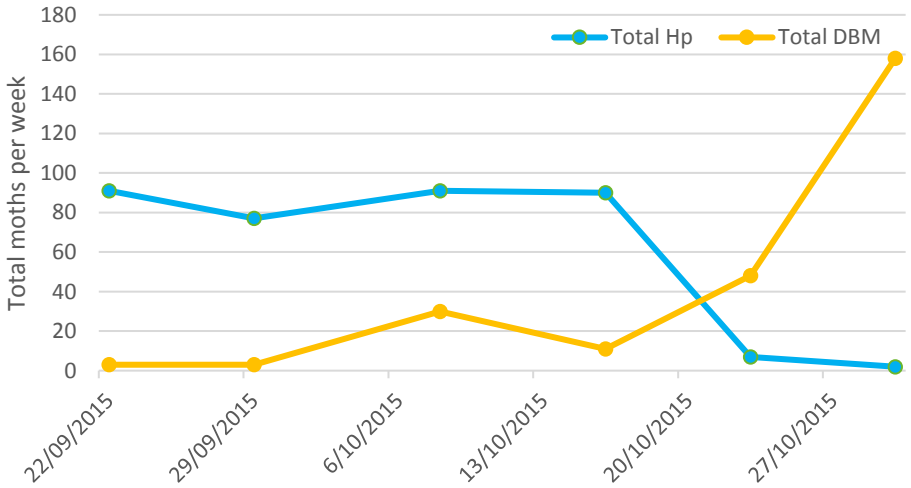


Figure 27. Changes in moth capture across the 6 weeks showing sum of Hp moths in "Hp only" traps, and DBM in "DBM only" traps. Hp = *H. punctigera* (native budworm), DBM = *P. xylostella* (diamondback moth)

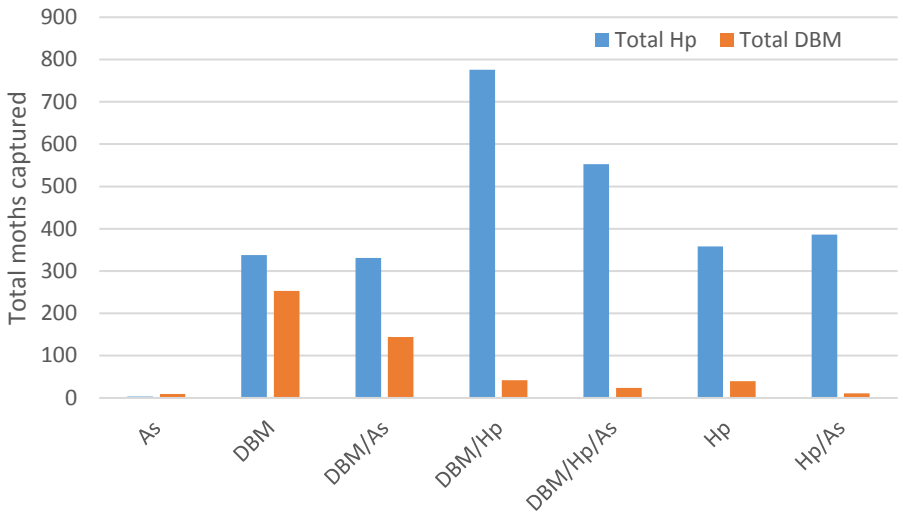


Figure 28: Total budworm and DBM moth catches across all traps and dates from 16 Sept – 30 Oct 2015, where n-7 for each treatment. X-axis is lure combinations where As = *A. segetum* (turnip moth); DBM = *P. xylostella* (diamondback moth); Hp = *H. punctigera* (native budworm)

High numbers of HP and DBM were captured during the experimental period and clear patterns of synergy and inhibition were apparent (Figure 28). The presence of AS lure paired with either HP lure or DBM lure did not affect the catch of HP (P = 0.8277) or DBM (P = 0.7994). (See Appendix 3 for statistical output)

Budworm moth was attracted to traps containing DBM lure (P < 0.0001); to such a degree that almost twice as many HP were caught in the HP/DBM traps compared to HP alone. Additionally, the DBM lure alone attracted the same number of HP as the HP lure alone. The total HP captured from the HP/DBM paired treatments approximated the total HP captured from both the HP single and DBM single treatments. This suggests a strong synergy between these lures for attracting HP.

DBM were significantly less attracted to the DBM lure if HP lure was present (P < 0.0001) indicating an inhibitive effect of HP lure.

During the first three weeks of assessment, it became apparent that HP were attracted to the DBM lures. This unexpected result raised concerns regarding the lure quality or potential contamination. To confirm the authenticity of this result, a new batch of DBM lures were procured from a different supplier (Suterra DBM) and placed along one edge of the trial array alternately placed with blank traps.

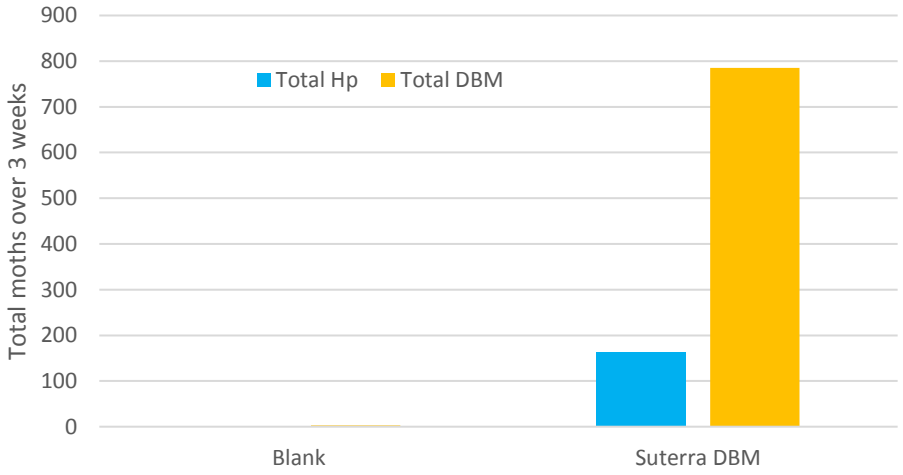


Figure 29: Total budworm and DBM moths captured in additional four "Suterra DBM" lures and 4 "blank" traps over 3 weeks. DBM = *P. xylostella* (diamondback moth); Hp = *H. punctigera* (native budworm)

Budworm moths were also attracted to the new Suterra DBM lure (Figure 29), however comparisons with the pre-existing DBM lures were not possible due to different length of weathering period on the original (3 weeks old) lures and the Suterra DBM lures (fresh). An additional complicating factor was the temporal change in moth populations (Figure 27) changing the ratio of HP and DBM moths active in the environment at the later dates compared to the earlier dates.

An attempt was made to conduct an identical stacking trial in Iran where AS is an established pest, however due to low populations, no useful data could be generated. It is possible or even likely that, as with the DBM/HP combinations were we saw one lure inhibited and one lure enhanced, AS moth catch would be effected by the presence of other lures. Similar inhibition and enhancement patterns have been observed in lure combinations for Noctuidae moths (Lopez et al 1990), Tortricidae moths (Knight et al 2014) and Noctuidae/Crambidae moths (Eizaguirre et al 2007).

1.4.3 Evaluation of TrapView® pest monitoring system

Table 11 presents the comparative weekly moth counts in TrapView® and Bucket Traps at four sites across southern Australia. In general, TrapView® did not capture as many moths as the traditional Bucket Traps.

Table 11: Weekly counts of *H. punctigera* (native budworm) moths in bucket traps (not specified) and Trapview® traps (specified in brackets) in four South Eastern broadacre cropping districts

State	Location	Week ending								
		31/08/15	7/09/15	14/09/15	21/09/15	28/9/15	5/10/15	12/10/15	19/10/15	26/10/15
VIC – Mallee	Ouyen	4	57	0	144	24	0			
	Ouyen (TrapView)	0	10	10	6	7	19			
NSW – Riverina	Wagga	0	0	0	0	0	1	17	24	
	Wagga (TrapView)	0	0	0	3	0	6	3	108	1
SA – Yorke P.	Pt Broughton	176	131	232	154	20	0	14		
	Pt Broughton (TrapV)	0	0	17	3	40	38	5	0	0
SA – Mid North	Waterloo	0	0	131			11	189	118	20
	Waterloo (TrapView)	0	0	7	8	14	0	17	1	0

Some issues were noted with the Trapview® trap design. Primarily the sticky bases were not sufficient to catch and hold the large moths and within several days the bases were no longer sticky due to dust and moth wing scales. At one site (Pt Broughton, SA), the sticky base was replaced with corflute sheets coated with Tanglefoot® (a traditional base used in delta traps) and this improved the retention of moths. Other issues arose with regards to the sheer number of the moths clogging the trap. This was expected to happen at some point but occurred more quickly than anticipated due to intense moth activity, and some suggestions have been made regarding modifications to minimise the frequency of manual maintenance. If these traps were deployed in order to detect an exotic moth, presumably in low and as yet unnoticed density, this would be less of an issue. Some technological issues were observed in some traps and the ability of the program to accurately detect and count moths in the camera images seemed to vary (at least between the two SA traps). A more comprehensive assessment of user experiences with Trap View is in the file uploaded to the PBCRC Imap site named "Trapview® as a surveillance tool for exotic pests in broad acre grain crops." A full report on the TrapView® testing is presented in Appendix 4.

Adama, the company who owns Trapview®, has since developed multiple iterations of the trap's design and functionality. This primarily is to improve its reliability, maintenance and durability to provide a commercial service to industry (https://www.adama.com/australia/en/innovation/trapview.html). No further assessments of the traps were requested and direct comparisons with Smart Moth traps developed in this project were not possible. However, engagement with Adama for commercialisation of the Smart Moth Traps, or fusion of the two traps into one system, could be considered and plausible in future discussions.

The Rapid Aim® trap is now being incorporated into a commercial company under that name (http://rapidaim.io/). No assessment of the trap was possible in this project, though many attempts were explored to compare against other similar systems. Evaluation of the Rapid Aim® trap remained within the company, who has now developed to system to offer effective monitoring of Fruit fly. Further engagement with Rapid Aim® for commercialisation of their trap to grains pests could be considered and plausible in future discussions.

2. SPORE SAMPLING

2.1 Mobile jet spore sampler, targets include; wheat stripe rust (model for airborne fungal spores including exotic rust species such as barley stripe rust and lentil rust), and other airborne fungal pathogens including sclerotinia of canola.

The Mobile JSS commenced fully automated trapping as a mobile sampler in 2017. Fourteen field testing 'runs' were conducted throughout South Australia and Victoria and collected samples (air samples collected in 2ml tubes) were presented to SARDI's Molecular Diagnostics Centre (MDC) for analysis using qPCR assays. These assays are used to detect and quantify species-specific DNA in environmental samples for the purpose of monitoring important endemic fungal pathogens to the grains industry. Four assays are presented in this report to demonstrate the results from mobile surveillance and comparison to a network of fixed samplers (static traps), used routinely to monitor these pathogens in South Australia and Victoria. The results are presented as:

- Figure 30: Showed a high prevalence of DNA detected in samples of *Didymella pinodes/Phoma pinodella,* causal agents of ascochyta blight of field pea, using the Mobile JSS. This pathogen is common in most districts and aerial dispersal is an important part of disease dissemination each season. There was a good correlation between detection between mobile and fixed samplers. Fixed samplers often had higher detection level than the mobile device but could be explained as fixed devices sampled the entire day whilst the mobile JSS sampled for approximately 15 min.
- Figure 31: Showed a high prevalence of DNA detected in samples of the fungus *Leptosphaeria maculans,* causal agent of Blackleg of canola, using the Mobile JSS. While aerial dispersal of this pathogen is common during crop emergence the importance of aerial dispersal during growing season is not well understood. Good correlation was shown between mobile and fixed sampling devices. Fixed samplers often had higher detection level than the mobile device, most likely due to a longer sampling duration.
- Figure 32: Showed a moderate prevalence of DNA detected in samples of the fungus *Pyrenophora teres teres,* causal agent of Net Form Net Blotch of barley, using the Mobile JSS. This pathogen is common in most districts and though aerial dispersal is an important part of disease dissemination there were few reports of localised epidemics in barley crops in the higher rainfall districts of the mid north of South Australia. Excellent correlation was shown between mobile and fixed sampling devices.
- Figure 33: Showed a single 'incursion' event of DNA detected in samples of the fungus *Oculimacula yallundae*, causal agent of eyespot of wheat, using the Mobile JSS. This directly correlated with detection using a fixed sampler located at that site. This clearly demonstrated the mobile JSS was also suited to the surveillance of 'rare influxes' of airborne pathogens.

The preliminary evaluation of the Mobile JSS showed successful operation and functionality of this prototype. However, a few issues will need to be resolved:

- The device showed some sensitivity to rough terrain which sometimes affected the position of the sampling chamber over the collection tube. This will most likely be overcome by a change in the sensor array (hall effect & codex sensor) for determining vial position.
- Improving water resistance of the device. Some issues in heavy rain were experienced.
- The device cannot sample very small particles (<10um). Alternative sampling techniques, such a high volume cyclone technology, may need to be considered in future prototypes.

The MJSS as a device for effective mobile surveillance of airborne pathogens was also shown using the four model endemic pathogens, as well as the potential of the device to monitor rare influx events, using the model pathogen *Oculimacula yallundae.* This showed a viable and promising application for this device to generate valuable information for the grains industry in both integrated disease management and biosecurity decisions.

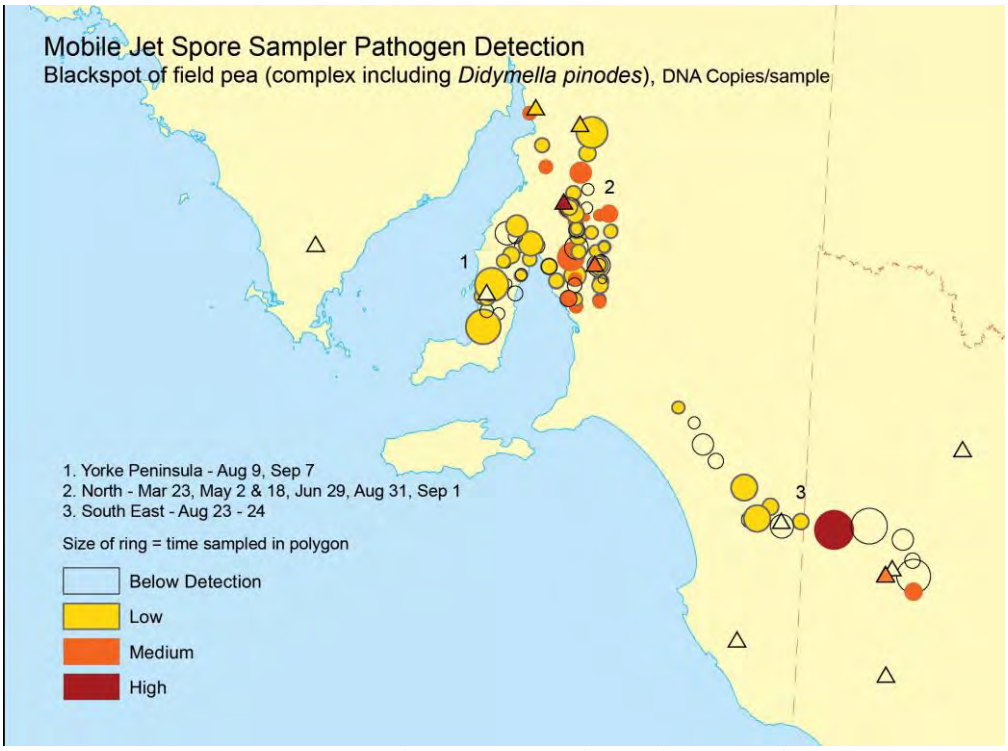


Figure 30. Spatial map showing semi-quantified amounts (low, medium & high) of DNA (copy number) detected by a qPCR assay developed by SARDI's Molecular Diagnostic Centre specific to *Didymella pinodes*/*Phoma pinodella*, causal agents of ascochyta blight of field pea, and applied to air samples from the Mobile Jet Spore Sampler that collected at 450 L/min during multiple field tests in South Australia and Victoria in late autumn to early spring 2017. Circle size represents time (mins) the device sampled in each polygon and triangle symbols represent nearby fixed air samplers (Burkard Volumetric traps) that sampled at 10 L/min used in collaborative projects.

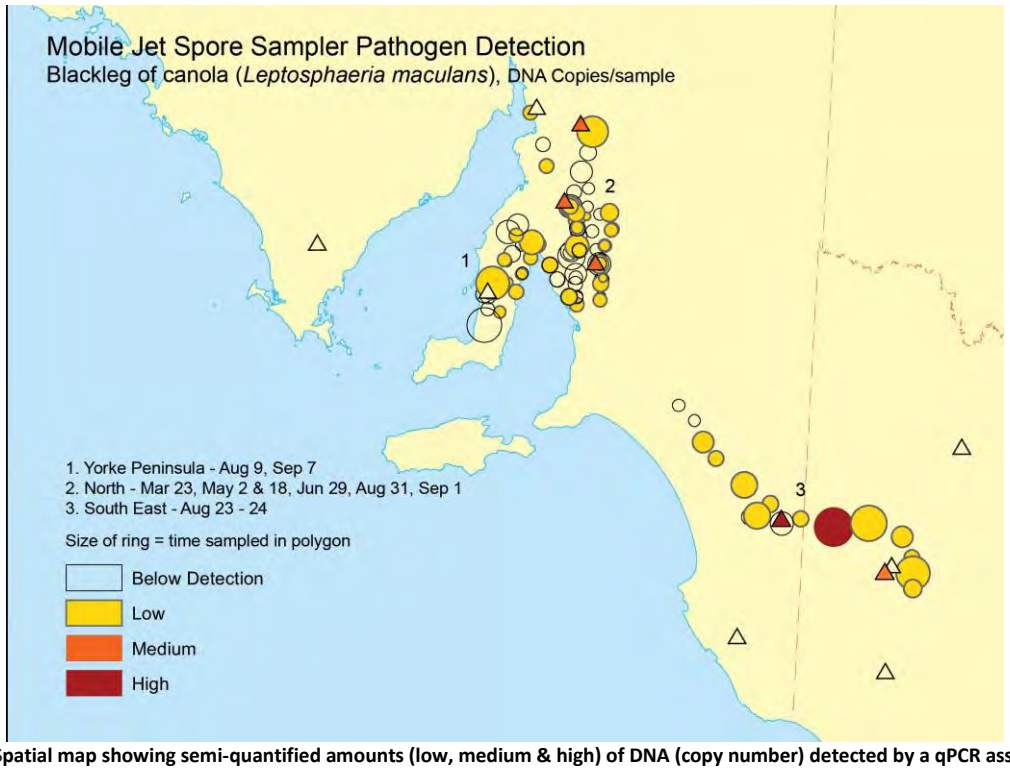


Figure 31. Spatial map showing semi-quantified amounts (low, medium & high) of DNA (copy number) detected by a qPCR assay developed by SARDI's Molecular Diagnostic Centre specific to *Leptosphaeria maculans*, causal agent of blackleg of canola, and applied to air samples from the Mobile Jet Spore Sampler that collected at 450 L/min during multiple field tests in South Australia and Victoria in late autumn to early spring 2017. Circle size represents time (mins) the device sampled in each polygon and triangle symbols represent nearby fixed air samplers (Burkard Volumetric traps) that sampled at 10 L/min used in collaborative projects.

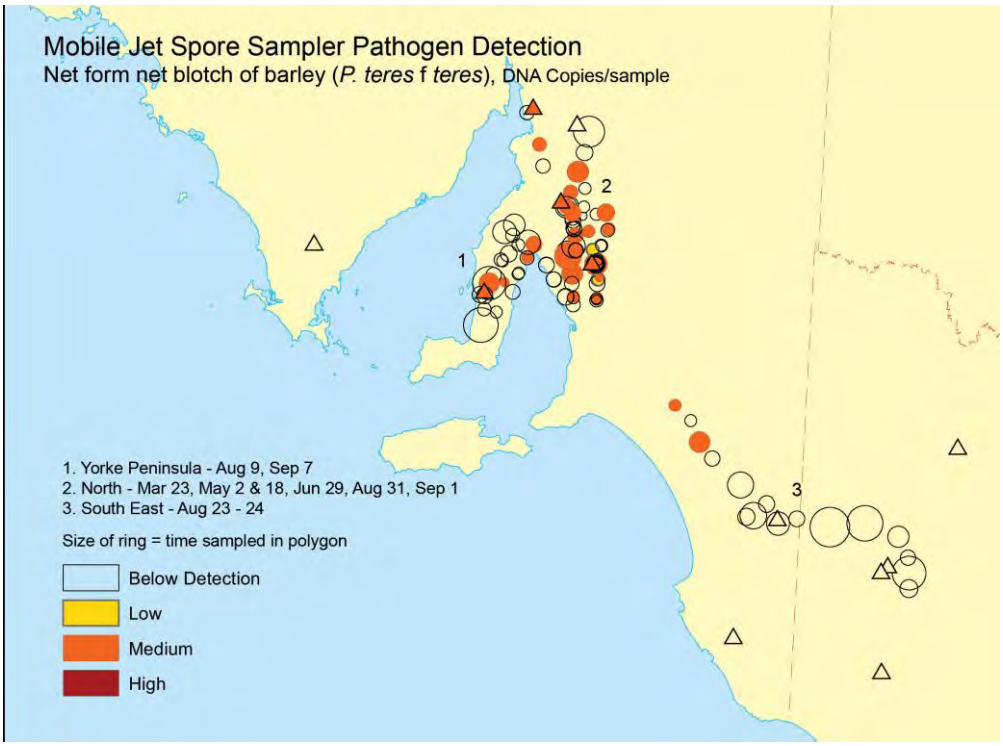


Figure 32. Spatial map showing semi-quantified amounts (low, medium & high) of DNA (copy number) detected by a qPCR assay developed by SARDI's Molecular Diagnostic Centre specific to *Pyrenophora teres teres* , causal agent of Net Form Net Blotch of barley, and applied to air samples from the Mobile Jet Spore Sampler that collected at 450 L/min during multiple field tests in South Australia and Victoria in late autumn to early spring 2017. Circle size represents time (mins) the device sampled in each polygon and triangle symbols represent nearby fixed air samplers (Burkard Volumetric traps) that sampled at 10 L/min used in collaborative projects.

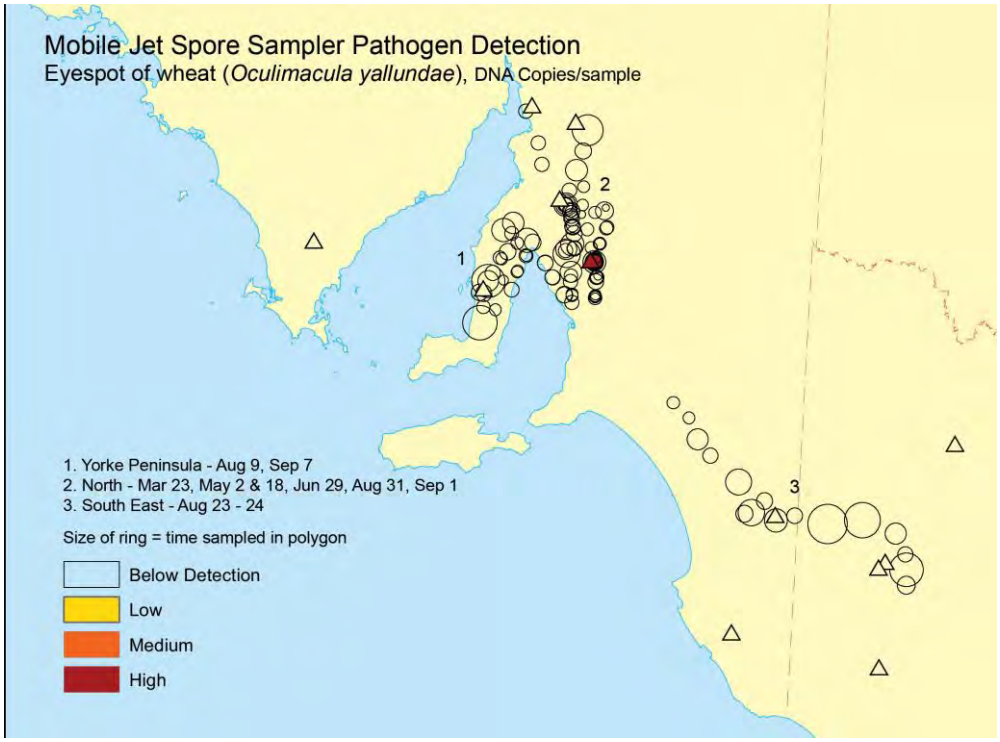


Figure 33. Spatial map showing semi-quantified amounts (low, medium & high) of DNA (copy number) detected by a qPCR assay developed by SARDI's Molecular Diagnostic Centre specific to *Oculimacula yallundae*, causal agent of eyespot of wheat, and applied to air samples from the Mobile Jet Spore Sampler that collected at 450 L/min during multiple field tests in South Australia and Victoria in late Autumn to early spring 2017. Circle size represents time (mins) the device sampled in each polygon and triangle symbols represent nearby fixed air samplers (Burkard Volumetric traps) that sampled at 10 L/min used in collaborative projects.

The collection efficiency of the Mobile JSS, collecting at 450 L/min, was also evaluated in comparison to a modified Burkard impactor trap (with Smart capture control), collecting at 10 L/min, mounted on the roof of the vehicle alongside the Jet Spore Sampler (Figure 34). The purpose of this technique was also to assess isokinetic sampling of device in the airstream, mainly whether over- or under-sampling of particles occurred when travelling at high speed (100 km/h). However, the results revealed that the Burkard impactor Smart trap did not capture any fungal spores (using the same model pathogens where qPCR assays were available) whereas the Mobile JSS showed good levels of detection. This confirmed the correct design features of the JSS for mobile surveillance, particularly the sampling head design.

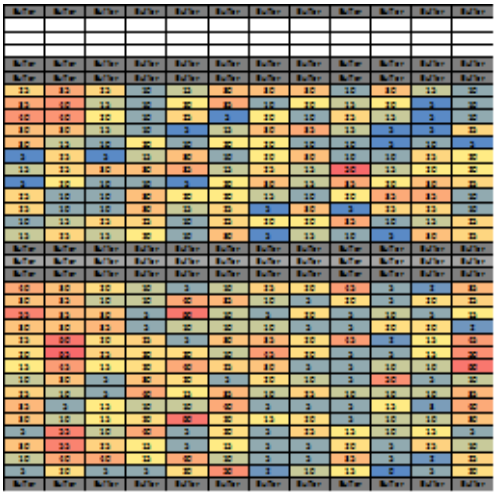
Figure 34. Field evaluation of the Mobile Jet Spore Sampler prototype (left) and alongside a SMART impactor spore trap (right) developed by Les Zeller at University of Southern Queensland's National Centre for Engineering in Agriculture developed as part of a previous Plant Biosecurity CRC project.

3. AIRBORNE PEST AND DISEASE SURVEILLANCE AND TRAPPING WORKSHOP – REPORT IN APPENDIX 2

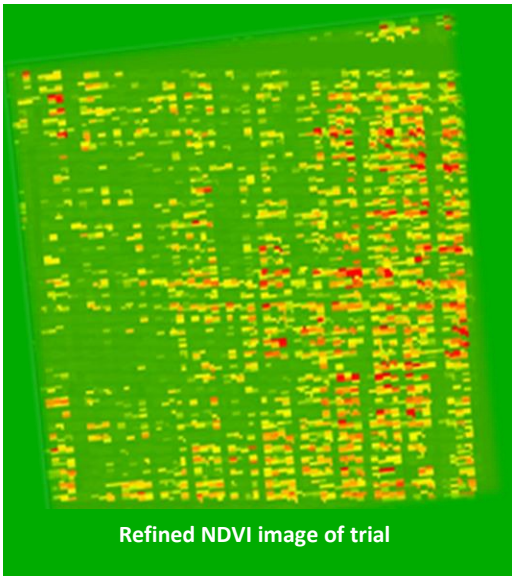
4. MULTISPECTRAL/HYPERSPECTRAL IMAGING

4.1 Multispectral/Hyperspectral imaging of crop symptoms from pests and diseases: target endemic rusts, endemic aphids (models for exotic rusts, Russian wheat aphid, Hessian fly)

Disease severity data in faba beans, both qualitative and quantitative, were compared with refined NDVI collected by ARA aerial imaging (Figure 35 below right). There was no clear correlation observed between disease data and known algorithms defined for vegetative indices applied to image data from two faba bean trial sites in South Australia during 2014. John Weiss compared results from August 20, for quantitative data of ascochyta blight severity in faba bean plots at Saddleworth South Australia (Figure 35 below left) and hyperspectral data collected by ARA aerial imaging of the same trial site. Preliminary analysis showed no significant correlation (R2 = 0.004) was observed between NDVI/EVI or other known indices with visual estimates of ascochyta blight severity in field plots (graph below centre). However, the resolution of the hyperspectral imagery collected by SPECIM HAWK and SPECIM EAGLE is not highly detailed for field plot applications, compared to paddock scale applications. They are line-scanning hyperspectral instruments that do not run at extremely high frame rates, and although the survey aircraft can fly quite slowly (~40m/s), the along-track sampling interval could not be reduced much below 1m. Accordingly, the only way to meet the resolution requirement for this study was to fly in the same direction as the long axis of the subplots, at a height above ground to give ~0.5m cross-track sampling. This means the flight lines were planned in that direction, which is close to east-west. This is not ideal from a solar illumination perspective, since the sun needs to be to one side of the flight track with an expected BRDF effect (cross-track brightness variation).



Conditional formatting (Excel) showing disease severity in faba bean field plots



Refined NDVI image of trial

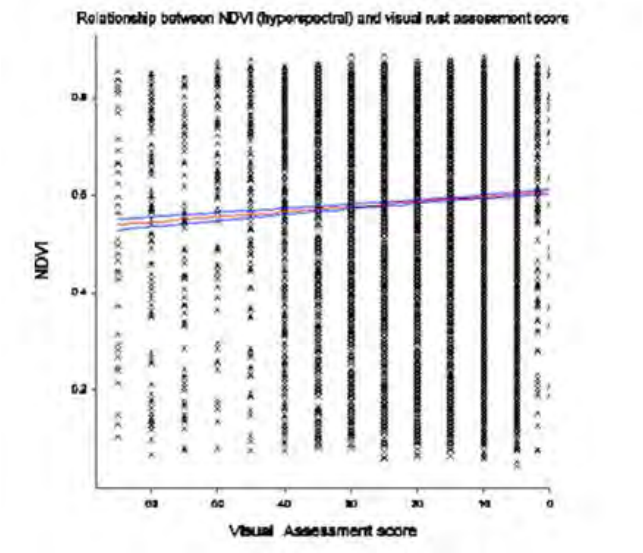


Figure 35. Disease severity data in faba bean plots represented by conditional formatting (above left), NDVI image the faba bean plots (above right) and comparison of NDVI and visual assessments (bottom).

4.2 Field robotics and Unmanned Aerial Vehicles (UAV's)

A report of this field study was provided in February 2016 by James Underwood and Alex Wendel at the Australian Centre for Field Robotics, and another report compiled by Brooke Schofield (SARDI) in March 2016 as part of a broader collaborative project to evaluate field and aerial robotic platforms for the automated capture and processing of plant phenotypic traits. A summary of these reports is provided here, as this pilot study was initiated by PBCRC 2014 to better understand the potential of these technologies for surveillance of pest or diseases in grain crops.

As previously outlined, image data was collected by the Ladybird and UAV over experimental field sites in South Australia to compare with manual in-field measurements. As a proof of concept study with ACFR, key objectives were to capture numerous data, including NDVI, crop height, disease, chlorosis, necrosis, canopy growth rate and physical characteristics of different cultivars, crop treatments and seed densities. These properties are all conventionally measured and assessed on-ground for each plot. Some of these properties, though scientifically important, are often omitted from trials because of the time and labour required to obtain these measurements. Multispectral imagery was collected using a low-cost and low-weight UAV system with the ability to mount different cameras and sensors. Acquired imagery was loaded into ArcGIS software to carry out analyses. The extents of individual plots were identified within the imagery and were statistically evaluated for parameters such as NDVI, crop height and canopy cover.

Processing of the Ladybird data was carried out by researchers at the ACFR (Australian Centre for Field Robotics) to transform the raw sensory data into output data relevant to scientific phenotype assessment. UAV image pre-processing (mosaicking, georeferencing etc.) was performed by the commercial operator. Further analysis of these images (NDVI, height and canopy evaluation) was carried out in ArcMap software by Brooke Schofield at SARDI. The quality of both data sets was evaluated in terms of repeatability and accuracy compared to manual measurements.

Results showed that an existing general purpose agriculture research platform (the Ladybird) was highly suited to this application. Two operators were able to obtain the relevant Ladybird data over a period of four days in August and three days in September, operating at greater rate of coverage when compared to traditional manual field work practices. The accuracy of the Ladybird system was found to be high when compared with conventional measurement practices and was determined to have a high repeatability. A strong linear relationship was observed between Ladybird hyperspectral NDVI estimates and the manual Greenseeker measurements with r2 = 0:83 in August and r2 = 0:72 in September. The relationship between Ladybird lidar heights and in-field measurements was also strong with r2 = 0:90 in August and r2 = 0:95 in September.

A method was developed to quantitatively measure and evaluate the NDVI, plant height and growth rate in different crops using multispectral digital imagery acquired from a low-altitude UAV that would closely approximate the visual scores given by an expert on the ground. The comparison of the UAV and Greenseeker NDVI values show that a moderately strong yet arbitrary relationship exists between the two; they did not exhibit a relationship with a unity gradient and the measurements were on a completely different scale. This could potentially be useful for phenotypic assessment if this study was repeated and the results were somehow calibrated. The UAV was not able to measure differences in heights because of its poor spatial resolution. A greater understanding of the utility of the UAV data from one commercial operator has been gained but the degree to which this is representative of agricultural UAV providers on the whole is uncertain.

This research finding from the Ladybird has since been published in the Journal of Field Robotics entitled 'Efficient in-field plant phenomics for row-crops with an autonomous ground vehicle'.
http://onlinelibrary.wiley.com/doi/10.1002/rob.21728/full

SECTION TWO: DIAGNOSTICS OF HIGH PRIORITY PESTS

Summary

A review of winter cereal Post Entry Quarantine was completed and submitted to the Department of Agriculture and Water Resources for review. Its titled "Winter Cereal Post-Entry Quarantine: A review of the current post-entry quarantine regulations for imported winter cereal seed for sowing and recommendations to reduce the risks of exotic viruses to the cereal industry". This review is attached as Appendix 5.

Twelve virus species of top priority to the grains industry were identified based on the review on their perceived risks, all of which belong to the following four virus genera: *Bymovirus, Furovirus, Hordeivirus* and *Pecluvirus* (Table 12).

Based on this list, four broad-spectrum diagnostic assays were developed for the detection of these four virus groups. The assays were validated using positive virus controls and synthetic virus constructs and then submitted to the National Plant Biosecurity Diagnostic Network for endorsement. The four National Diagnostic Protocols (NDPs) submitted are attached in Appendices 7-10 (B). Two of these four assays (for the detection of furoviruses and hordeiviruses) were published in the Journal of Virological Methods, titled "Novel genus-specific broad range primers for the detection of furoviruses, hordeiviruses and rymoviruses and their application in field surveys in South-east Australia" (Zheng et al., 2015). This manuscript is attached in Appendix 6.

Standard Operating Procedure (SOP) was also developed for individual virus species listed in the High Priority Virus Pest List. Published protocols were used in the SOPs where possible and in the absence of a published protocol, molecular diagnostic assays were developed in this study. A total of 12 SOPs were developed (Appendix 8), four for the group-specific tests and 12 for specific virus testing. Two SOPs developed are currently being used by the Post Entry Quarantine station located in Horsham, Victoria for the screening of accessions from the Australia Grains Genebank.

The feasibility of using high-throughput DNA sequencing technology for the diagnostics of plant viruses was also assessed. Full-length sequences of a number of viruses were obtained, including that of cereal viruses *Barley yellow dwarf virus* (BYDV) and *Maize chlorotic dwarf virus* (MCDV) from a clonal grass sample intercepted by the New South Wales PEQ station.

The feasibility of using Isothermal Loop-mediated Amplification (LAMP) technology for on-site detection of plant pathogens was also assessed. A LAMP assay for the detection of *Barley yellow dwarf virus*-PAV, a strain prevalent in Victorian wheat fields was developed, validated and successfully used in the field in Horsham, Victoria.

Winter cereal PEQ manual

Twelve virus species of top priority to the grains industry were identified based on their perceived risks, all of which belong to the following four virus genera: *Bymovirus, Furovirus, Hordeivirus* and *Pecluvirus* (Table 12). The testing of this top priority virus list is recommended to be included in the Australian Biosecurity Import Conditions (BICON) for the importation of cereal seeds for sowing. This list was included in the review of the winter cereal Post Entry Quarantine, a document titled "Winter Cereal Post-Entry Quarantine: A review of the current post-entry quarantine regulations for imported winter cereal seed for sowing and recommendations to reduce the risks of exotic viruses to the cereal industry" (Appendix 5).

The review also investigated four import risk pathways for cereal viruses through the importation of winter cereals, coarse grains, sugarcane and clonal grasses. The level of risks to the winter cereal industry was assessed for each pathway and recommendations were made for the reduction of risk for each pathway. A flow chart for winter cereal PEQ diagnostic is illustrated in Figure 36 below.

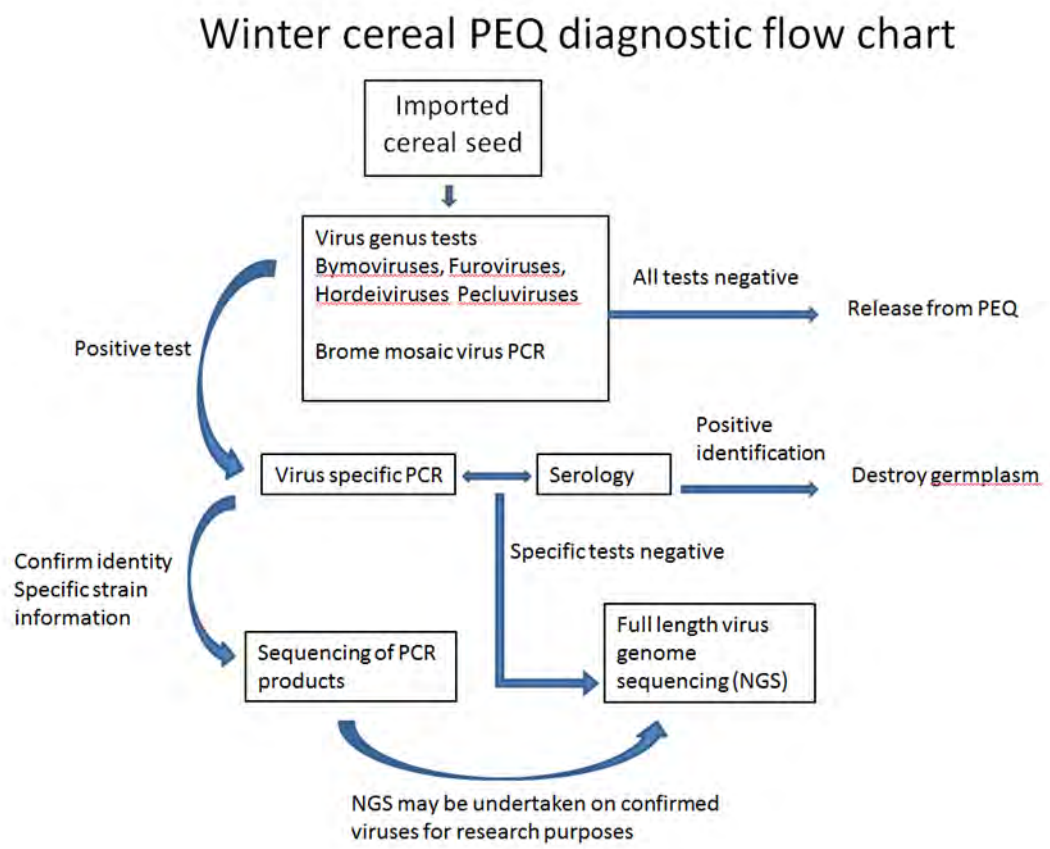


Figure 36 Winter cereal PEQ diagnostic flow chart

Table 12 PHA provisional High Priority Virus Pest list for the grains industry, with additional viruses identified in this review and additional references (from Winter Cereal Post-Entry Quarantine Manual)

Virus species	Virus family	Genus	Synonyms/ strains	Geographic distribution	Transmission				Host range	Risk
PHA risk					Seed-borne	Vector	Vector in Australia	Mechanical transmission		Seedborne, seed-carried, soilborne
Barley yellow mosaic virus Priority EPP (Medium+High Economic Impact)	Potyviridae	bymovirus		UK, Europe, China, Japan, Korea	no	*Polymyxa graminis*	yes	yes	barley	Yield losses in barley 15-50% (Plumb et al 1986) Vector present, soil-borne, seed-carried in *P.graminis* spores.
Barley mild mosaic virus Priority EPP (Medium+High Economic Impact)	Potyviridae	bymovirus		UK, Europe, China, Japan, Korea	no	*Polymyxa graminis*	yes	yes	barley	20-45% yield loss in barley (Adams 2002). Vector present, soil-borne, seed-carried in *P.graminis* spores.
Oat mosaic virus Not in PHA Threat Summary Tables	Potyviridae	bymovirus		Europe, USA	no	*Polymyxa graminis*	yes	yes	oats	Yield losses in oats 25-50% (Clover et al 2002); 66-80% (Catherall et al 1979). Vector present, soil-borne, seed-carried in *P.graminis* spores.
Wheat spindle streak mosaic virus Low	Potyviridae	bymovirus	wheat yellow mosiac virus	USA, Europe, Canada, Asia, China, Japan, Africa	no	*P. graminis*	*yes*	yes	wheat, durum wheat, cereal rye	Yield losses in wheat up to 25-50% in Nth America Slykhuis 1970) and 3-87% in eastern USA (Miller et al 1992) Vector present, soil-borne, seed-carried in *P.graminis* spores.

Wheat yellow mosaic virus Not in PHA Threat Summary Tables	Potyviridae	bymovirus		Japan, China, Korea, France, Germany	no	*Polymyxa graminis*	yes	yes	wheat	Yield losses in wheat in Japan greater than 30% (Ohki et al 2014), in China up to 40–50% (Wang et al (2010). Vector present, soil-borne, seed-carried in *P.graminis* spores.
Chinese wheat mosaic virus Medium-Negligible	Virgaviridae	furovirus		Asia, France	yes	*P. graminis*	yes	yes	wheat - only natural host triticale cereal rye barley	Yield losses in wheat 10-70% (Chen 1993, Diao et al 1999a). High risk because vector present, soil-borne, seed-carried in *P.graminis* spores and seed-borne.
Oat golden stripe virus Not in PHA Threat Summary Tables	Virgaviridae	furovirus		Europe	unknown	*P. graminis*	yes	yes	oats	Yield losses in oats up to 55% (Catherall et al 1979). Vector present, soil-borne, seed-carried in *P.graminis* spores.
Soilborne cereal mosaic virus Priority EPP (Medium+High Economic Impact)	Virgaviridae	furovirus	European wheat mosaic virus, soil borne rye mosaic virus	Europe	yes	*P. graminis*	yes	wheat	rye triticale wheat	Yield losses in wheat up to 50-70% (Ratti et al 2004). High risk because vector present, soil-borne, seed-carried in *P.graminis* spores and seed-borne.
Soilborne wheat mosiac virus Priority EPP (Medium+High Economic Impact)	Virgaviridae	furovirus	wheat mosaic virus soilborne barley mosaic, Chinese wheat mosaic virus	Europe, NZ, China, Japan, Nth and Sth America, Zambia	yes	*P. graminis*	yes	yes	wheat triticale cereal rye barley	Yield losses in wheat 40-50%, durum wheat 70% (Clover et al 2001) High risk because vector present, soil-borne, seed-carried in *P.graminis* spores and seed-borne.

Sorghum chlorotic spot virus Not in PHA Threat Summary Tables	Virgaviridae	furovirus		Kansas only (USA)	unknown	*P. graminis*	yes	yes	Sorghum, maize, wheat, barley	Yield loss unknown (Kendall et al 1988). Vector present, soil-borne, seed-carried in *P.graminis* spores.
Indian peanut clump virus Medium peanut Very Low-cereals- Why???	Virgaviridae	Pecluvirus		India Pakistan	yes in wheat but not barley	*P. graminis*	*yes*	yes	wheat barley	Average yield loss 58% for wheat in India; barley plants rarely reaching maturity or developing seed spikes (Delfosse et al 1999). Seed-borne in wheat (Delfosse et al 1999). Vector present, soil-borne, seed-carried in *P.graminis* spores.
Barley stripe mosaic virus Priority EPP (Medium+High Economic Impact)	Virgaviridae	hordeivirus	barley yellow stripe	Worldwide reported in Aust?	yes	no known vectors	n/a	yes	barley wheat oat maize rye	Yield losses in barley up to 60% (EPPO 1986), 17% (Jeżewska et al 2009), 35-40% (Carroll 1980), 35% (Chiko et al 1978). Yield loss in wheat 40-75% (EPPO 1986). Seed and pollen-transmitted in barley. Seed transmission up to 60% Jeżewska et al 2009a).

5.1 Broad-spectrum diagnostic assays

Based on the list of High Priority Virus Pest list for the grains industry (from Winter Cereal Post-Entry Quarantine Manual), four DNA-based broad-spectrum diagnostic assays were developed for the detection of bymoviruses, furoviruses, hordeiviruses and pecluviruses. All four assays contained degenerate primers designed to target all member species of their respective groups; the primer details and the assay conditions are listed in Table 13. All of these primers were validated using either virus positive controls and/or synthetic positive constructs.

Table 13 Primer details and RT-PCR cycling conditions for the broad-spectrum diagnostic assays for the detection of bymoviruses, furoviruses, hordeiviruses and pecluviruses.

Primers	Primer sequence (5' - 3')	RT-PCR cycling conditions	Amplicon size (bp)
BymoLZF	CICCICAYACIGTIGGHAT*	1 cycle [48°C 30 min], 1 cycle [94°C 2 min], 35 cycles [94°C 30 sec, 48°C 30 sec, 72°C 30 sec], 1 cycle [72°C 5 min]	418*
BymoLZR	AAITTITTITCATCRCCRTTG		
Furo1F	AATGACGGTTTGGGTCGAA	1 cycle [48°C 30 min], 1 cycle [94°C 2 min], 35 cycles [94°C 30 sec, 54°C 30 sec, 72°C 45 sec], 1 cycle [72°C 5 min]	684
Furo3R	CGCTCATTKATCTCCTTCAT		
Hordei1F	TAIACACTIGCYGAIAGYGCKGAA	1 cycle [48°C 30 min], 1 cycle [94°C 2 min], 35 cycles [94°C 30 sec, 50°C 30 sec, 72°C 40 sec], 1 cycle [72°C 5 min]	614
Hordei1R	GIACIATIGGCCAATACTTATTNGG		
PecluF	TCCAGYAATAACACACTCTT	1 cycle [48°C 30 min], 1 cycle [94°C 2 min], 35 cycles [94°C 30 sec, 55°C 30 sec, 72°C 40 sec], 1 cycle [72°C 5 min]	660-665*
PecluR	GTYTACGCCAGCCACTAA		

*IUPAC notation for degenerate bases: N=A+C+G+T, K=G+T, H=A+T+C, R=A+G, Y=C+T, I= deoxyinosine

** Amplicon size dependent on virus strain

All four protocols were submitted to the National Plant Biosecurity Diagnostic Network for endorsement (Appendix 7). Two of these broad-spectrum tests (for the detection of furoviruses and hordeiviruses) were used in a survey of wheat and barley crops in and around Horsham, Victoria, Australia, and the results of the survey were published in the Journal of Virological Methods. The manuscript is titled “Novel genus-specific broad range primers for the detection of furoviruses, hordeiviruses and rymoviruses and their application in field surveys in South-east Australia” and is attached as Appendix 6.

5.2 Next Generation Sequencing for Plant Virus Diagnostics

Case study - Clonal grass sample "A"

Reads and contigs generated

A total of 3,157,305 reads were generated for the clonal grass sample, where the template was DNase treated dsRNA. A total of 900 contigs were generated via *de novo* assembly. When searched against the Genbank, one contig of 4721bp in length matched to that of *Maize dwarf chlorotic virus* strain Severe (MDCV-S; accession: AY362551).

Based on this, the sequence of MCDV-S was used as reference for which all 3,157,305 reads were mapped to. A total of 32,867 reads were mapped to this reference sequence, generating a consensus sequence of 11,772 nucleotides in length, with a genome coverage of 99.5% and 98.7% in nucleotide identity. The depth coverage over the entire sequence is shown below as visualised in Tablet (Figure 27).

Figure 27. Sequence reads of clonal grass sample A mapped to the MCDV-S genome (accession: AY362551) as visualised in Tablet

Phylogenetic analysis of MCDV from clonal grass

The full-length sequence of the MCDV isolate found in clonal grass sample "A" (a *Pennisetum* host) was used in an alignment with three MCDV reference sequences retrieved from GenBank. The MCDV from *Pennisetum* was closely clustered with the MCDV-S strain, with 100% bootstrap support (Figure 38).

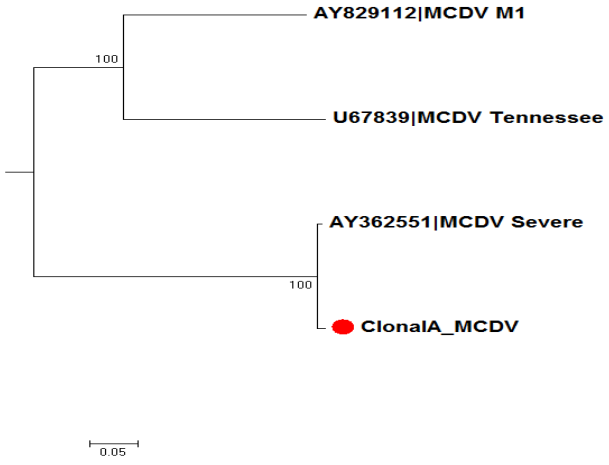


Figure 38 Maximum likelihood tree constructed using an alignment of four full-length sequences from three MCDV references and the MCDV strain found in clonal grass sample "A"

Case study - A Victorian BYDV isolate

Comparison between reads and contigs generated when using different RNA types as templates for NGS

A comparison was done between the sequences generated by using TRNA and dsRNA of the same Victorian BYDV isolate as templates for NGS. The total number of reads for the dsRNA template almost tripled that of the

TRNA template. However, the number of reads had no bearing on the number of contigs that were of viral origin nor their length (Table 14).

When the longest viral contig generated by sequences from the TRNA template reaction was searched against the GenBank database, the closest match was that of BYDV-PAS (accession: AF218798). This sequence was subsequently used as the reference genome, to which the sequence reads were mapped to. The number of mapped reads for the TRNA template was more than double that of the dsRNA template, with slightly more genome coverage (Table 14).

Table 14. A comparison of sequence data generated by NGS using two different RNA templates

Sample template type	Total number of reads	Total number of contigs	Contigs of viral origin	Longest viral contig (bp)	Total number of reads mapped to reference*	Percentage of genome sequenced	Nucleotide identity to the reference
Total RNA (TRNA)	2,203,565	2490	4	5658	17112	95.84%	89.1%
Double-stranded RNA (dsRNA)	9,295,364	33822	6	4069	8611	95.65%	89.2%

* Reference genome used was BYDV-PAS (accession: AF218798)

Phylogenetic analysis of the Victorian BYDV isolate

The full-length sequence of the Victorian BYDV isolate was used in an alignment with two BYDV reference sequences from BYDV-PAS and BYDV-PAV retrieved from GenBank. These two references were chosen because they were the top match for the Victorian isolate of BYDV when it sequence was searched against the GenBank database. However, the Victorian BYDV did not cluster closely with either the BYDV-PAS nor the PAV strain type (Figure 39).

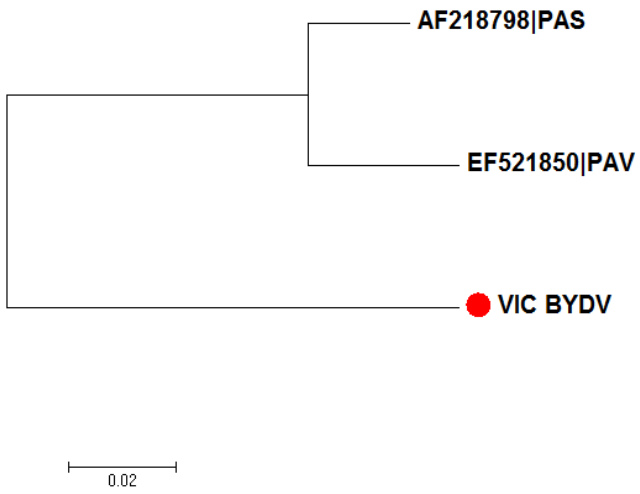


Figure 39. Maximum likelihood tree constructed using an alignment of three full-length sequences from two BYDV references and the Victorian BYDV strain sequenced in this study

NGS pipeline for plant virus diagnostics

A pipeline including the extraction method, the library preparation, the sequencing technology platform and the bioinformatics tools used for sequence analysis was developed for the diagnostics of plant viruses using NGS (Figure 40).

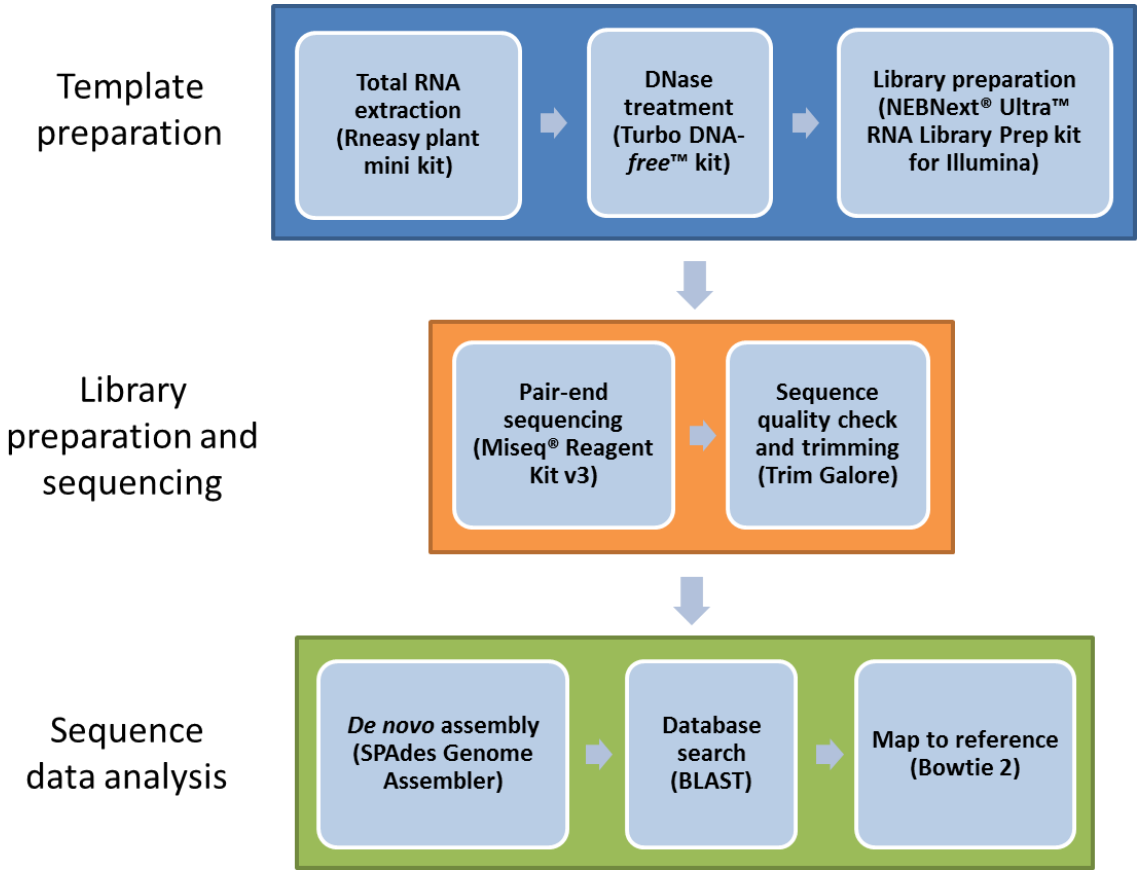


Figure 40 Schematics of the NGS sequencing and sequence data analysis pipeline for plant virus diagnostics

5.3 LAMP

Zhao RT-LAMP assay for BYDV-PAV detection

The Zhao RT-LAMP assay was performed on the four positive isolates of BYDV-PAV strain, the Zhao RT-LAMP was not able to detect any of them (data not shown) and therefore a novel RT-LAMP assay for detecting BYDV-PAV was developed.

Novel BYDV-PAV RT-LAMP assay

A set of four LAMP primers were designed for the detection of an Australian strain of BYDV-PAV. The primer sequence and their optimised concentrations in the final reaction is listed in Table 15.

Table 15. Novel BYDV-PAV RT-LAMP primers and their optimised final concentration in the RT-LAMP reaction

Primers	Primer sequence (5' - 3')	Final concentration (pM)
F3-PAV SB	GGCTTTCAATCCGTTGAC	3
B3-PAV SB	CATCTGTGAATTGGCAATCA	3
FIP-PAV SB	CAGTTGTCGCCTGTGGAGTTGGTGGAAACGAAGATGGC	12
BIP-PAV SB	GAGTGGGCAATTACAAAGGGTGTATTAACTTGAGATCTTTGTGGC	12

The optimised assay annealing condition is 65°C for 35 minutes, with fluorescence detection at the FAM channel and a post-amplification melt-curve analysis step (melting temperature (Tm) or temperature of dissociation determination of the amplicon from 98°C to 80°C at 0.5°C/s) using Genie® II /Genie® III (OptiGene).

The anneal derivative peak temperature of the RT-LAMP products was 87.2°C (± 0.3).

Validation and performance comparisons

The newly developed RT-LAMP assay for the detection of the Australian isolate of BYDV-PAV was as sensitive as the RT-PCR assay tested but 1000-fold times less sensitive than the RT-qPCR assay tested as determined by the amplification curve and melt curve analysis. The RT-qPCR assay was able to detect BYDV-PAV in the lowest concentration tested (1×10^{-5} dilution) whilst both the RT-PCR and the RT-LAMP assay were able to detect a dilution of the total RNA at 1×10^{-2} (Table 16). NTC reactions were negative in all of the PCR tests as expected, ruling out any possibility of contamination (data not shown).

Crude-RNA extraction

The FTA-DP and FTA-PH extraction method produced the most reproducible and reliable results when compared to other previously published methods based on both the time of detection (when the amplification plot has taken off) and the temperature of the anneal analysis after amplification (Table 17). Positive control included in each test had a positive detection for BYDV-PAV (Table 17) and all no template controls (NTC) were negative in all tests (data not shown). Healthy controls that had a time of detection but no corresponding anneal curves were considered negative.

Consequently, both of the FTA-DP and FTA-PH extraction methods were used for in-field validation of the newly developed BYDV RT-LAMP assay in Horsham, Victoria, Australia.

In-field validation of the novel BYDV-PAV RT-LAMP

RNA extractions in the field were carried out using the FTA-DP and FTA-PH methods, which took less than 2 hours in total. The application of the leave tissues onto the FTA cards took approximately 15 minutes; the drying of the spotted FTA® card took approximately 35 minutes; the extraction of RNA from the FTA cards took approximately 15 minutes and the RT-LAMP reaction was completed in less than 35 minutes.

In total, seven plants were sampled, four were leaf tissues (L1, L2, L3, L4) and two were stem tissues (S1, S2), each processed with the FTA-DP and FTA-PH methods, resulting in 14 individual RNA samples.

Of the 14 RNA samples tested, all samples produced amplification curves. All reactions except for one (L3, DP) where amplification curves were observed also had the correct temperature dissociation peaks (87.31°C - 87.71°C) as compared to the positive control used in the reaction (87.46°C). Sample L3 (DP) had an amplification curve but no melt curve was observed, and is therefore considered as negative (Figure 41).

For confirmation, the same samples were processed for total RNA isolation and subjected to RT-qPCR for BYDV-PAV detection. All sample tested were positive for BYDV.

Table 16. Sensitivity test results from the BYDV-specific RT-PCR, RT-qPCR and the novel RT-LAMP assays

Total RNA dilution	RT-PCR	RT-qPCR	RT-LAMP
1×10^{0}	+	+(14.1 ± 0.1)	+(17:05/15:15)
1×10^{-1}	+	+(17.0 ± 0.1)	+(31:00/27:30)
1×10^{-2}	-	+(20.8 ± 0.1)	-?
1×10^{-3}	-	+(24.3 ± 0.1)	-
1×10^{-4}	-	+(28.7 ± 0.0)	-
1×10^{-5}	-	(35.7)*	-

Positive detection is indicated by plus sign (+), negative detection is indicated by minus sign (-), C_q is the quantification cycle on RT-qPCR when positive detection can be ensured and t_p d^1/d^2 is the time of positivity of result in duplicates (d^1 and d^2). Asterisk (*) indicates that positive detection was obtained in first replicate only and question (?) indicates no amplification signal but a peak in the melt curve was observed in first replicate only.

Table 17. Results of different RNA extraction methods used on healthy and BYDV-PAV infected wheat leaf tissues

Method	Samples	Detection	Time of detection (mins)	Anneal temperature (°C)
Prick (water)	Healthy	+/+/+	1:00/0:45/1:15	-
	Infected	+/+/+	1:00/0:45/0:45	-
	Positive control	+	20	86.89
Prick (sucrose)	Healthy	+/+/+	1:15/1:00/1:15	-
	Infected	+/+/+	1:45/1:00/1:30	-
	Positive control	+	19:15	86.79
Sucrose prep	Healthy	-/-/-	-/-/-	-
	Infected	-/-/-	-/-/-	-
	Positive control	+	21:15	86.79
Sucrose prep (-inc)	Healthy	+/+/+	1:00/1:00/0:45	-
	Infected	+/+/+	1:00/1:45/1:00	-
	Positive control	+	21:30	86.89
Grinding in sucrose solution	Healthy	+/+/-	1:15/1:00/-	-
	Infected	+/+/+	1:00/1:15/1:15	-
	Positive control	+	18:30	86.79
FTA-DP	Healthy	+/+/+	3:15/6:00/7:30	-
	Infected	+/+/+	12:15/16:15/19:45	87.89/87.39/87.19
	Positive control	+	18:30	87.09
FTA-PH	Healthy	-/+/+	-/5:40/6:30	-
	Infected	+/+/+	15:30/17:10/19:05	87.61/87.31/87.52
	Positive control	+	20:00	87.56

Positive signal of amplification is indicated by plus sign (+) and negative signal is indicated by minus sign (-). Positive control was the total RNA isolated using MacKenzie buffer and RNeasy® Plant Mini Kit (Qiagen). All assays were tested in triplicates and the results are represented as replicate 1/ replicate 2/ replicate 3.

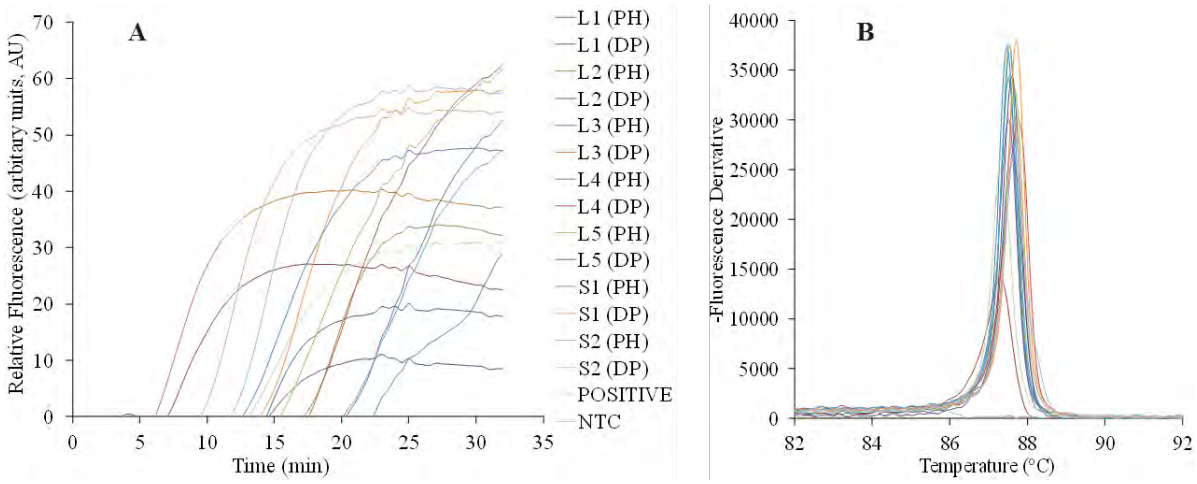


Figure 41. Field testing results of BYDV-PAV detection in wheat plant at Horsham, Victoria.

Panel A is the amplification curve of the tested samples and B is the melt curve analysis results of the RT-LAMP amplified products. The sample application method onto the FTA® classic cards were Plant homogenate (PH) and Direct press (DP) and four leaf tissues (L1, L2, L3, L4) with two stem tissues (S1, S2) were utilised in the method, where NTC stands for no template control.

6. Discussion & Conclusion

SECTION ONE: INSECT AND SPORE TRAPPING TECHNOLOGY

This project has provided new tools to address gaps in monitoring for the presence of exotic and endemic pests in the grains industry, as current surveillance schemes were disparate and had yet to decisively engage new technology and strategies to achieve greater compatibility to rapid diagnostics or coordination as a network. This research has resulted in more advanced trapping platforms e.g. 'Smart' Spore and Insect Trapping systems which collect samples referenced to parameters such as GPS and climate data for molecular downstream diagnosis of captured targets. The project used engineering solutions to include automation and innovation into trapping systems with high sampling frequencies, which will be better suited to detect rare influxes of exotic spores or pests.

Our pheromone trapping work demonstrated that before pheromones can be stacked, extensive investigation must first determine that interactions between the lures will not confound the resulting data. Inhibition effects will be detrimental to any surveillance program, however synergism, whilst unacceptable for population monitoring of established pests (since it will artificially inflate data), may still be acceptable or even preferred in surveillance programs attempting to detect presence of a hitherto undetected species. We demonstrated that AS lure did not interfere with HP lure or DBM lure, however due to its exotic status we could not determine the effect (if any) of HP or DBM lure upon the attractiveness of AS lure to its target. Although this study has demonstrated the limitations of pheromone stacking combination of turnip moth, native budworm and diamondback moth, it has revealed a potential "booster" for detection of native budworm. This enhancement by diamondback moth lure may only reflect the increased quantities of key components but would need to be investigated further.

The reasons behind the interactions observed among the lures are predominantly related to chemistry. In this trial, the HP and DBM lure compounds were very similar; they shared two of their three components and these two components were present in high and equal quantities in each lure. However, Eizaguirre et al (2007) saw interactions between lures even when the lures had no common components. Additionally, the quantities of components in a lure is not necessarily proportional the release rates (Karandinos et al 1977). It should be noted that amongst lure manufacturers there exists some considerable variation in formulations (Williams et al 2013) and different variants may not induce the same interactions. Temperature has also been identified as a significant factor with some moth species only receptive to pheromones during a specific temperature range (Shorey 1974).

The commercial opportunities for the Smart Insect Trap (Moths) are not imminent. The field of wireless data transmission (telemetry) and miniaturisation of electronic engineering changed rapidly during the time of this project. At the commencement of the project there was only one prototype platform that claimed attraction, capture, detect and wirelessly report moth pests (ie. Z-Trap®) and that was found not to be suitable. At the conclusion of this project there are another three systems sharing this space using three different approaches (our Smart Trap, RapidAIM®, Trapview ®). It is difficult to argue a commercial advantage our Smart Moth Trap over these other systems which have been established by market driven forces and investment, particularly RapidAIM® which wisely evolved their system into low-cost traps linked to a Low-Power Wide-Area Network (LPWAN). It is therefore, concluded that commercial competition using these new tools and systems will dictate what opportunities will arise for the grains industry for the surveillance moth pests in future.

Air sampling for the purpose of monitoring insect abundance was found to be an effective means of pest surveillance. Suction traps, both the Vortex suction traps from Burkard Manufacturing Co (UK) and the Smart cyclone trap from USQ, which sampled at 1.7 m heights were found to be particularly suited to small flying insects such as aphids. Smart capture potentially offers greater value to pest surveillance to identify optimal scenarios or conditions in which to use suction traps e.g. optimal temperature, wind direction or speed that specific pests might take to the wing (i.e. insect behaviour). This is a valuable tool to entomologists and a new technology that is more compatible to downstream diagnostics. Evaluation of these devices is ongoing, and will progress with greater collaboration with the Rothamsted Insect Survey group (UK), who utilise the 'gold standard' in insect migration surveillance through the use of a network of tall (8 m) suction traps, and in the potential coordination with technologies such as Vertical Looking Radar and high speed digital analysis.

The results from the preliminary evaluation of the Mobile Jet Spore Sampler confirmed successful operation and function of this device and the value of mobile surveillance for both endemic pathogens and rare influx pathogens. The latter demonstrated the application of this device to biosecurity through surveillance of exotic pathogens threatening the grains industry. The evaluation of the Mobile JSS is ongoing in collaboration with Rothamsted Research and Burkard Manufacturing Co (UK). Additional experiments are still in progress to better understand the spatial variation of pathogen dispersal and how it effects spatial resolution of results reported by analysis of samples collected by the device. Of particular importance is how this spatial variation relates to temporary or long-term placement of a network of static traps so that information generated by mobile surveillance can directly inform management of diseases at the grower, industry or government level.

There is no obvious competitor or technology that is currently comparable to the purpose and function of the Mobile Jet Spore Sampler in the world. Therefore, this has generated a lot of interest and opportunity for further applications of the device for improved surveillance of airborne pathogens across a landscape. Devices mounted on vehicles and UAVs are likely to be used increasingly both in research, to understand locations of sources and dispersal processes, and in practical risk alert and monitoring networks within agricultural industries. Savage et al. (2012) used computational models to simulate a number of dispersal events and showed that sampling strategies based on mobile platforms have a much greater probability of detecting airborne spores than strategies based on stationary traps, and that mobile trap strategies required a far lower number of traps to achieve a reasonable probability of detection. However, mobile sampling strategies should conducted be in conjunction with stationary sampling installations as part of an area-wide sampling strategy. Furthermore, ground-based automated samplers and those designed to facilitate easy analytical steps by non-specialists are also likely to benefit from simpler, cheaper and more rapid diagnostic methods to add precision on disease control decisions at the field, farm and regional scales.

Australia's national plant health surveillance is delivered by Government of States /Territories for industry to benefit from data supporting market access/area freedom of pests and diseases. This project has made a significant step in providing tools for State biosecurity agencies to translate into data flow supporting these responsibilities by using new techniques and automation to feed accurate diagnostics, and ready access to short-term incursion-response equipment or long-term surveillance capacity. In addition, fast decision-making on exotic pest incursions increases the likelihood of pest eradication, which in turn benefits producers in terms of management costs and maintaining market access.

The potential for commercialisation of Smart Trap functionality developed by Les Zeller at University of Southern Queensland's National Centre for Engineering in Agriculture is one of the main outputs of this project that was considered by the PB IP Board. As anticipated, the PB IP agreed that, given the narrow/small research-only market Smart Trap design and purpose, the small number of companies internationally that supply the market for spore/insect trapping and the necessity to incorporate the smart trap features into third party spore/insect traps, commercialisation of the smart trap design was not the preferred route to maximise delivery of impact. Accordingly, the PBIP Board has agreed that the smart trap technology design should be published when appropriate.

Preliminary discussions were undertaken with Stuart Wili at Burkard Manufacturing Co during 2017, without having a confidentiality agreement in place as directed by the PBIP Board, to seek the possibility of adoption of the Smart functionality into their commercially available air samplers going forward. The outcome of those discussions revealed that Burkard (Stuart Wili) was interested but did not envisage a substantial market demand for the novel functionality and additional complexity in engineering requirements to meet that functionality to captured samples, and instead would prefer correlated meteorological data to time-based capture settings.

New opportunities for Smart Capture functionality in new sampling equipment developed within this project have now been progressed through a new cross-sectoral project lead by Horticulture Innovation Australia (HIA) funded by the Australian Federal Department of Agriculture Water and Resources (DAWR). This $21M project, entitled "Improving Plant Pest Management Through Cross Industry Deployment of Smart Sensor, Diagnostics and Forecasting" commenced in July 2017 and is part of the departments 'Rural R & D for Profit scheme'. Funding for this work is over 5-years (2017-2022) with direct contribution and collaboration from six major Research and Development Corporation's (Grains, Horticulture, Viticulture, Sugar, Cotton and Forestry) and several research partners.

An important objective in this new project is a direct outcome of the PBCRC 2014 project, that 'smart capture' will be used in air-sampling equipment being built in that project. SARDI is the lead research partner commissioned to build 'Flexi-Hub' surveillance equipment that incorporates smart automation. Therefore, the background intellectual property for the 'Smart capture system' for pest/spore sampling to be used in "Improving Plant Pest Management Through Cross Industry Deployment of Smart Sensor, Diagnostics and Forecasting" project will be considered to be owned by PB IP Ltd, a PB CRC Ltd subsidiary company, and made available with no restrictions to this project for research purposes and functionality of air-samplers included in the flexi-hub surveillance units.

To fully realise the benefits of these new technologies, area-wide (network) applications of these devices are required to fully appreciate their role in coordinated reporting for point of origin and dispersal dynamics of targets within fields or growing regions. This applies not just to exotic targets in the grains industry, but also endemic threats, and equally applicable to any agricultural industry where airborne pests impact production. For effective pest and disease management that intend to use Smart Traps, fixed or mobile surveillance efforts must merge with robust sampling protocols, rapid detection and data interpretation for accurate interrogation of spatial data generated from a network of samplers. This is particularly important given the size and scale of Australia's agricultural industries to achieve a reduction in the economic impact and overall improvement in the time for an industry to respond to incursions of airborne pests and plant pathogens or the information flow for improved management decisions by the end-user.

SECTION TWO: DIAGNOSTICS OF HIGH PRIORITY PESTS

The lack of virus-testing for seeds being imported into Australia pose a risk to the grains industry. The twelve top priority virus species identified are of particular concern. Some of these viruses, such as furoviruses and bymoviruses, have devastated wheat fields in Europe, Asia and North America, and the only effective control measure is through resistant variety breeding. The risk of entry, establishment and spread is greatly increased with the recent discovery of *P.graminis,* the primary vector for bymoviruses, furoviruses and pecluviruses. The current PEQ procedures relating to the importation of winter cereal seed for sowing are woefully inadequate, and an update is long over-due. This risk will only grow as the large-scale importation of cereal germplasm for breeding programs grows ever larger. The deployment of the four broad-spectrum molecular tests designed to target these four virus groups would greatly enhance quarantine efforts at the PEQ stations by i) improving the diagnostic capability of PEQ stations; ii) significantly reduces the number of individual tests required for virus screening, thereby ii) reduces the turn-over time for releasing the imported accessions.

The application of NGS in plant virus diagnostics has great potential, as demonstrated with the clonal grass case. A *Pennisetum* host, suspected of a virus infection, failed to be detected by numerous group-specific diagnostic assays. The use of NGS not only detected a virus, but also allowed its identity to be confirmed simultaneously. The *Maize chlorotic dwarf virus*(MCDV) belongs to the genus *Waikavirus,* and is one of the two most important viruses of corn. This virus is known to infect *Pennisetum* and a wide-range of clonal grasses, usually imported into Australia as ornamental grasses and often seen in public and private gardens/parks and on the side of many roads/highways. Without the use of NGS, the unknown virus species in the *Pennisetum* would not have been detected in such a quick manner. NGS can also provide a valuable research tool to enable virologists to gain greater understanding of their target organisms, such as the case with the Victorian BYDV isolate. Previously thought to be a strain of BYDV-PAV, it was through whole genome sequencing that it was discovered to be quite different to existing PAV and PAS strains. The genetic diversity of virus species within the BYDV disease complex has always confounded diagnostics, and it is envisioned that the whole genome sequencing afforded by NGS could bring diagnosticians one step closer to getting this problem resolved.

The initial assessment of the LAMP technology is positive and the portability of this technology makes it very appealing in mobile diagnostics. The sensitivity of the BYDV RT-LAMP assay tested ranged from equal to 10 times less than that of the traditional thermal RT-PCR and the specificity is similar to that of the thermal RT-PCR. The major advantage is that the test can be completed within 45 minutes for RNA viruses, and coupled with the nucleic acid extraction method developed for use with LAMP, the entire test can be completed within 1.5 hours with approximately 30 minutes of actual hands-on time. This novel RT-LAMP assay developed for BYDV detection was validated and used in the field at Horsham, Victoria, the first of LAMP to be used under field-conditions in Australia. The shortfall of this RT-LAMP assay is that it is highly specific to the BYDV strain it was designed to detect and might not be able to detect other strains of BYDV. The primer design of a LAMP assay could also be quite complex, requiring prior knowledge of the target organism. Nevertheless, once the assay is developed and validated, LAMP is relatively easy to use, requiring minimum training for laboratory technicians as well equipment. In conclusion, LAMP technology can lead to a faster detection of BYDV in the field, and this technology can easily be applied to the detection of other pathogens.

7. Recommendations

1. Smart Trap technology design to be published when appropriate since commercialisation of the smart trap design in a narrow market is not the preferred route to maximise delivery of impact.

2. New opportunities for Smart Trapping functionality in new sampling equipment to be progressed through a new cross-sectoral project ("Improving Plant Pest Management Through Cross Industry Deployment of Smart Sensor, Diagnostics and Forecasting") lead by Horticulture Innovation Australia (HIA) funded by the Australian Federal Department of Agriculture Water and Resources (DAWR).

3. The background intellectual property for the Smart Trap system for pest/spore sampling to be used in "Improving Plant Pest Management Through Cross Industry Deployment of Smart Sensor, Diagnostics and Forecasting" project will be considered to be owned by PB IP Ltd, a PB CRC Ltd subsidiary company, and made available with no restrictions to this project for research purposes and functionality of air-samplers included in the flexi-hub surveillance units.

4. Ongoing investment in development of diagnostic assays (laboratory based and in-field systems) for endemic and exotic pests to maximise the efficacy of the surveillance systems developed in this project.

5. Further research into pheromone science to determine interactions between lures before pheromone stacking is a feasible option.

6. The proposed new winter cereal PEQ virus-testing processes for 12 exotic seed-borne and soil-borne viruses be adopted by amendment to the BICON import conditions for winter cereal seed for sowing.

7. Coarse grain seed for sowing is tested for Indian peanut clump virus, particularly if the germplasm is from India or Pakistan where the virus is endemic.

8. DAWR support the continued development of robust protocols for identifying viruses using NGS, as this is a new technology which requires research input to ensure that viral sequences identified with NGS represent actual infectious viruses in the plant material.

9. LAMP technology can be used as for on-site detection of cereal virus and its application should be further explored.

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9. List of Appendices

Appendix 1. PBCRC Project 2014 Technology Review and Workshop Report

Appendix 2. Report on Airborne Surveillance and Trapping Workshop

Appendix 3. Data analysis Pheromone stacking

Appendix 4. TrapView® report

Appendix 5. Winter Cereal Post-Entry Quarantine: A review of the current post-entry quarantine regulations for imported winter cereal seed for sowing and recommendations to reduce the risks of exotic viruses to the cereal industry.

Appendix 6a. Underwood J, Wendel A, Schofield B, McMurray L, Kimber R. Efficient in-field plant phenomics for row-crops with an autonomous ground vehicle. *Journal of Field Robotics*. 2017;00:1–23 https://doi.org/10.1002/rob.21728

Appendix 6b. West, J.S. and Kimber, R.B.E. (2015). Innovations in air sampling to detect plant pathogens. *Annals of Applied Biology*, 166: 4–17. doi: 10.1111/aab.12191

Appendix 6c. Zheng, L., Tang, J., Clover, G.R.G., Spackman, M.E., Freeman, A.J. and Rodoni, B.C. (2015) Novel genus-specific broad range primers for the detection of furoviruses, hordeiviruses and rymoviruses and their application in field surveys in South-east Australia. *Journal of Virological Methods* **214:**1-9

Appendix 7. National Diagnostic Protocols for Bymovirus, Furovirus, Hordeivirus and Pecluvirus groups

Appendix 8. Standard Operating Procedures for detection of 12 specific viruses

Appendix 9 Publications and Extension for PBCRC Project 2014

10. Abbreviations/glossary

ABBREVIATION	FULL TITLE
ACFR	Australian Centre for Field Robotics
ANOVA	Analysis of Variance
ARA	Airborne Research Australia
AS	*Agrotis segetum*, turnip moth
BaMMV	Barley mild mosaic virus
BSMV	Barley stripe mosaic virus
BYDV	Barley yellow dwarf virus
BYMV	Barley yellow mosaic virus
CWMV	Chinese wheat mosaic virus
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DBM	Diamond Back Moth, *Plutella xylostella*
DNA	Deoxyribonucleic Acid
EPP	Emergency plant pest
EtOH	Ethyl Alcohol
GRDC	Grains Research and Development Corporation
GPA	Green Peach Aphid
GPS	Global Positioning System
HP	*Helicoverpa punctigera*, Native budworm
IP	Intellectual Property
IPCV	Indian Peanut Clump Virus
JSS	Jet Spore Sampler
LAMP	Loop-mediated isothermal amplification
LBAM	Light Brown Apple Moth
MCDV	Maize chlorotic dwarf virus
MDC	Molecular Diagnostic Centre
MVI	miniature virtual impactor
NBN	National Broadband Network
NCSF	Nucleotide Conserved Site Finder
NDP	National Diagnostic Protocols
NDVI	Normalised Difference Vegetation Index
NGS	Next Generation Sequencing
NIR	Near Infra-Red
NPIS	National Pest Information Service
OGSV	Oat golden stripe virus

OMV	Oat mosaic virus
PBCRC	Plant Biosecurity Cooperative Research Centre
qPCR	quantitave Polymerase Chain Reaction
PEQ	Post Entry Quarantine
QDAF	Queensland Department of Agriculture and Fisheries
RDSI	Research Data Storage Infrastructure
RH	Relative Humidity
RWA	Russian Wheat Aphid
SARDI	South Australian Research and Development Institute
SBCMV	Soil-borne cereal mosaic virus
SBWMV	Soil-borne wheat mosaic virus
SOP	Standard Operating Procedure
SrMV	Sorghum mosaic virus
ST	Suction Trap
UAV	Unmanned Aerial Vehicle
URAF	Unmanned Research Aircraft Facility
USB	Universal Serial Bus
USQ	University of Southern Queensland
USQ NCEA	University of Southern Queensland National Centre for Engineering in Agriculture
VIC DPI	Victorian Department of Primary Industries
WFT	Western Flower Thrip
WSSMV	Wheat spindle streak mosaic virus
WYMV	Wheat yellow mosaic virus
YPT	Yellow Pan Trap

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