Building biosecurity capacity

Louisa Parkinson and Andrew Geering, Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland

The Australian avocado industry is relatively free from exotic pests and diseases, due to its geographic isolation and strong quarantine system. However, there are threats of exotic pest incursions that require novel diagnostic and surveillance tools to provide a capacity to respond to incursions.

This R&D update will summarise the biosecurity innovations that are being adopted in the Australian avocado industry, including the roll-out of a digital social networking tool (Checkpoint™) for pest surveillance; new molecular tests for detecting avocado scab fungus, *Elsinoë perseae* (syn. *Sphaceloma perseae*); an investigation of the fungal pathogens vectored by the Tea Shot Hole Borer (TSHB) *Euwallacea fornicatus*; and a new survey project investigating avocado sunblotch viroid (ASBVd).

Monitoring pest and disease threats with Checkpoint™

Checkpoint™ is an online social networking tool for on-farm recording of plant health data. The tool enables growers and crop protection consultants to instantly make an enquiry about a symptomatic tree and have direct access to diagnosticians in the laboratory. Checkpoint can be accessed via *www.checkpoint.tools* and can be used on smartphones and tablets for instant image uploading and record keeping. Pest and disease surveillance activities are recorded in real time with photographs, GPS coordinates, chat logs and steps in the diagnostic chain instantly saved to an online database via smart devices (*Figure 1*). Expertise can be drawn upon from anywhere in the country and scientists in a different capital city can be invited to contribute to the conversation to accelerate the diagnosis.

The aim of this project activity was to establish a network of researchers, extension officers, agronomists and growers who work in the industry that can report on current pest or disease issues in their region. A network, *Australian Avocado Plant Health*, comprising 39 industry stakeholders across all growing regions has been established through use of the Checkpoint tool. This project activity resulted in the adoption of private use of Checkpoint by two major avocado producing organisations in Australia for monitoring health of individual trees. Outcomes of the project include contributions and collaboration with software developers to improve the Checkpoint tool to suit industry needs; and building connections with avocado producers and agronomists through site visits for sample collection and providing plant pathology services.

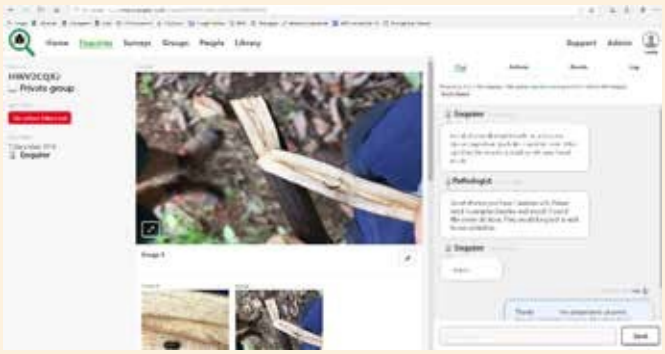

Figure 1. *Use of Checkpoint for recording branch dieback of avocado trees associated with ambrosia beetles.*

qPCR detection of the high risk biosecurity threat, avocado scab fungus

The avocado scab fungus, *Elsinoë perseae* (syn. *Sphaceloma perseae*), is a high priority biosecurity threat for avocados in Australia. Avocado scab results in premature fruit drop and reduced fruit quality, which heavily impacts marketability and can restrict market access to pest-free countries. Scab symptoms begin with scattered corky, raised brown to purplish-brown lesions or 'scabs' which coalesce as the disease progresses, causing deep brown fissures covering the fruit surface.

Although scab symptoms are recognisable, confirmation of presence of *E. perseae* can be difficult, as the fungus is slow growing and can take up to two months for the fungal colony to grow on culture media in a lab. There is a need for a rapid, real-time molecular detection test for *E. perseae* to enable rapid responses to potential incursions.

A real-time quantitative Polymerase Chain Reaction (qPCR) diagnostic test has been developed for the rapid detection of *E. perseae*. PCR-based molecular tests amplifies nucleic acid sequences (such as DNA) of target pathogens by making millions of copies of the target sequence using primers which bind to the target sequences and an enzyme which catalyses the reaction to make the sequence copies. The *E. perseae* qPCR test incorporates fluorescent probes which specifically bind to target DNA sequences, are cleaved by the enzyme and release fluorescent molecules for detection in the qPCR machine. Detection can be visualised in real time (*Figure 2*) as more fluorescent molecules are released during the DNA sequence amplification process. The molecular test also checks for potential user error in sample loading by including an avocado endogenous gene which is simultaneously detected; samples should always give a positive result for avocado DNA regardless of pathogen presence in the plant sample.

This project activity has resulted in having the diagnostic capacity to quickly respond to potential *E. perseae* incursions if it were to happen to the Australian avocado industry.

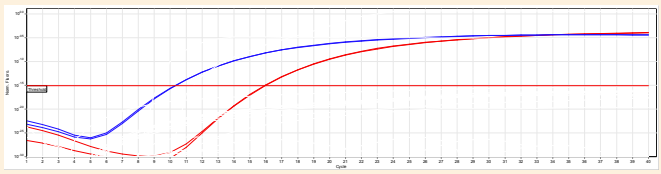

Figure 2 *Real time qPCR amplification of Elsinoë perseae. The red and blue lines represent amplification of two E. perseae cloned DNA samples (target pathogen DNA was artificially synthesized and cloned for use in validating the diagnostic).*

Fusarium spp.

Fusarium dieback of avocado trees in Australia is vectored by ambrosia (scolytid) beetles in the *Euwallacea fornicatus* cryptic species complex (a group of closely related organisms). In Australia, the beetle species is known as the Tea Shot Hot Borer (TSHB), and it is found in South East Asia, Australasia and parts of the USA. The beetles carry symbiotic fungi in their mouthparts, bore into tree branches and deposit the fungus onto xylem tissue within the brood gallery for beetles and larvae to feed. Symptoms of ambrosia beetle related branch dieback include pin holes on branches with leaf wilt and localised branch death due to the vascular damage caused by the fungus.

During the last decade, there have been sporadic infestations of TSHB in avocado orchards in Australia and it appears to be the most severe on the Atherton Tableland in Far North Queensland. This research is investigating the phylogenetic diversity and pathogenicity of *Fusarium* species associated with branch dieback of avocado and other woody tree hosts in Australia.

The project activities to date include ambrosia beetle and branch dieback surveys across growing regions in Queensland, in Far North and Central Queensland and the Sunshine Coast region. Fungal isolates were obtained from symptomatic heartwood of borer-beetle affected branches of avocado trees and other hosts (including macadamia, mango, blueberry, Acacia, Cupaniopsis, Cyclophyllym and Alectryon), and from *Euwallacea* sp. beetle specimens collected from tree branches and traps. Fungal isolates were then identified and the fungal isolate collection contains now 142 isolates and the genera found associated with branch dieback of multiple tree hosts including *Fusarium*, *Bionectria*, *Colletotrichum*, *Lasiodiplodia*, *Phomopsis*, *Nectria* and *Nigrospora*. The preliminary work on the Fusarium genus has identified a possible new species of Fusaria associated with ambrosia beetles. Formal classification and descriptions of the new fungal species will be carried out in the next phase of the project, along with pathogenicity testing of selected fungal isolates on avocado and alternate hosts to identify the causal agents of beetle-mediated branch dieback.

Avocado sunblotch viroid – a new project

In 2018, a review of the status of avocado sunblotch viroid (ASBVd) in Australia was published and strategies for achieving pest-freedom status provided (Geering 2018). It was concluded that ASBVd is uncommon in Australia and is likely to be eradicated through continuation of disease management programs such as the Australian Avocado Nursery Accreditation Scheme (ANVAS). To date, the only records of ASBVd are from the Tristate region, northern NSW, south-eastern Queensland, and the Atherton Tableland. An ASBVd survey project (AV18007) has started, to provide updated data on the national distribution of the viroid, and will continue through to December 2021.

More information

If you would like to be a part of the Australian Avocado Plant Health network and/or trial the use of Checkpoint for your business, or if you suspect any of the biosecurity pest or diseases in your orchard or nursery, please email Louisa Parkinson (*l.parkinson@uq.edu.au*).

If you suspect you've found a new pest or disease, call the Exotic Plant Pest Hotline (Plant Health Australia) on 1800 084 881.

Further reading

Geering, A. D. (2018). A review of the status of Avocado sunblotch viroid in Australia. Australasian Plant Pathology, 47(6), 555-559. *https://doi.org/10.1007/s13313-018-0592-6*

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