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BIOSECURITY CAPACITY BUILDING FOR THE AUSTRALIAN AVOCADO INDUSTRY

L. E. Parkinson, K. Bransgrove, E. K. Dann and A. D. W. Geering

Dr Louisamarie Parkinson BBiotech(Hons), PhD

Centre for Horticultural Science
Queensland Alliance for Agriculture and Food Innovation (QAAFI)
The University of Queensland, Brisbane Qld 4072 Australia
l.parkinson@uq.edu.au | https://www.qaafi.uq.edu.au

THE UNIVERSITY OF QUEENSLAND
St Lucia, Brisbane, Queensland Australia

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L. E. Parkinson, K. Bransgrove, E. K. Dann, A. D. W. Geering

Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Australia

Key words

Ambrosia beetles, Checkpoint™, *Elsinoë perseae*, Fusarium dieback, diagnostic test, qPCR

Summary

The Australian avocado industry is relatively pest and disease-free, due to its geographic isolation and strong quarantine system. However, the threats of exotic pest incursions are increasing due to the globalisation of trade and more frequent movements of people. To cope with these threats, novel diagnostic and surveillance tools are required. In this paper, biosecurity innovations that are being adopted in Australia are discussed, including new real-time PCR assays for avocado sunblotch viroid and *Elsinoë perseae*, as well the roll-out of a digital social networking tool ('Checkpoint') for pest surveillance. Finally, research that is being done on the Tea Shot Hole Borer *Euwallacea fornicatus* and branch dieback is described. This experimental investigation aims to identify the ambrosia beetle and fungal symbiont species associated with branch dieback in Australian avocado and other horticultural and ornamental trees including mango, macadamia and coastal tuckeroo (*Cupaniopsis* sp.); classify and describe any new *Fusarium* or Botryosphaeriaceae fungal species; and confirm the causal agents of dieback in avocado by pathogenicity testing.

The Australian avocado industry

The Australian avocado industry comprises approximately 850 commercial growers with most production from Queensland, followed by Western Australia, New South Wales, Victoria, South Australia and Tasmania (DAWR, 2018a). In 2017–18, Australia produced around 77,000 tonnes of avocado, worth about AUD $958 million, with almost 1,800 tonnes exported overseas, mainly to Malaysia, Singapore and Hong Kong (Avocados Australia, 2019). Australian avocado production has doubled over the past 10 years and is forecasted to reach 115,000 tonnes per annum by 2025 (Avocados Australia, 2019).

In Australia, the cultivar (cv.) Hass represents 78% of production, followed by cv. Shepard at 19%, and other varieties make up the remaining 3% of production (Avocados Australia, 2019). Australian avocados are available all year round, with harvest seasons for Hass being the winter months in Queensland, mainly North Queensland (Atherton Tablelands) and Central Queensland (Bundaberg/Childers), through to spring in the Sunshine Coast and South Queensland and in Northern and Central New South Wales; and in later winter/early spring through to the summer months in the Tristate (New South Wales, Victoria and South Australia along the Murray River) and Western Australia, mainly in the south west of the state (Pemberton/Manjimup) (Avocados Australia, 2019; DAWR, 2018a). The lightest supply period is from January to February, with New Zealand Hass imports supplementing supply from August/September to March (Avocados Australia, 2019; DAWR, 2018a). Harvest of Shepard begins in late summer February, in North Queensland through to mid-autumn April in North and Central Queensland (Avocados Australia, 2019).

Currently, Australia imports avocados only from New Zealand (DAWR, 2018a). Eighty percent of New Zealand's avocado production is imported to Australia (Foulds, 2017). In 2018, Chile was announced to export fresh avocado fruit to Australia, and a report of the biosecurity import requirements and risk analysis by the Australian Government Department of Agriculture and Water Resources (DAWR) was recently published (DAWR, 2018a; Russell, 2018). Potential pests of Chilean avocados of quarantine concern include Mediterranean fruit fly (*Ceratitis capitata*), grape mealybug (*Pseudococcus maritimus*), Chilean flower thrips (*Frankliniella australis*), tamarugo thrips (*Frankliniella gemina*) and western flower thrips (*Frankliniella occidentalis*), avocado brown mite (*Oligonychus punicae*) and avocado red mite (*Oligonychus yothersi*) (DAWR, 2019). However, a preliminary assessment by the DAWR determined that these pests of Chilean avocados did not pose a different biosecurity risk to the same or similar pests associated with other horticultural crops that already have a risk management procedure in place in Australia (DAWR, 2018a, 2019). Chile is one of the world's largest producers of avocados, with around 215,000 tonnes of fruit produced in 2016–2017 (DAWR, 2018b). Almost 70% of Chile's annual harvest (147,000 tonnes) were exported globally in 2016 (DAWR, 2018b). The main avocado cultivar grown in Chile is Hass and the export seasons are between September to March, aligning with Australia's import season (DAWR, 2018a, 2018b).

The geographic isolation of Australia paired with strict quarantine and biosecurity procedures has facilitated protection from exotic pests and diseases that are threats to a range of horticultural industries. Biosecurity planning and monitoring, risk analyses, identifying pest status, and establishing diagnostic capacity for rapid response to incursions are vital in protecting the Australian avocado industry.

This paper provides an overview of some of the current avocado biosecurity capacity building strategies for the Australian avocado industry, which includes i) monitoring emerging biosecurity threats and current pest and disease issues on farm in all Australian avocado growing regions through the use of a web-based tool, 'Checkpoint'; ii) maintaining existing diagnostic protocols for quarantinable pests and pathogens and developing new protocols for high risk threats such as avocado scab fungus, *Elsinoë perseae* (syn. *Sphaceloma perseae*); and iii) investigating the phylogeny and pathogenicity of *Fusarium* spp. associated with ambrosia beetle-mediated branch dieback of avocado and other hosts in Australia.

Monitoring pest and disease threats with Checkpoint™

Checkpoint™ (formerly known as Pestpoint), is a web-based social networking tool for remotely recording disease incidence and plant health data on-farm, to map and identify avocado health issues in each region. This software was developed by the Plant Biosecurity Cooperative Research Centre in Australia (www.pbcrc.com.au). Checkpoint can be accessed via the website: https://www.checkpoint.tools/ and can be used on smartphones and tablets for instant image uploading and record keeping.

Checkpoint enables a farmer or crop protection consultant to instantly make an enquiry about a symptomatic tree and link, using the internet, with diagnosticians in the laboratory. Photographs, GPS coordinates, chat logs and steps in the diagnostic chain are instantly recorded and saved to a database in the Cloud. Thus, pest and disease surveillance activities are recorded in real time, and expertise can be drawn upon from anywhere in the country. Scientists in a different capital city can be invited to contribute to the conversation to accelerate the diagnosis. For individuals, Checkpoint is most useful for record keeping as a complete communication log is created from identifying symptoms on the farm, to laboratory results and recommendations. The aim is 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' of industry members and experts has been established through use of the Checkpoint tool. The network consists of 39 agronomists, extension officers, plant pathologists, technical officers, researchers and avocado industry members from all major growing regions.

Avocado tree health issues across Queensland have been recorded and monitored using the tool and feedback from users on use of the tool has contributed positively towards industry engagement. This project activity resulted in the adoption of private use of Checkpoint by two major avocado producing organizations 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 use and needs; and building connections with avocado producers and agronomists through site visits for sample collection and providing plant pathology services.

qPCR detection of the high risk biosecurity threat *Elsinoë perseae* (syn. *Sphaceloma perseae*)

The avocado scab fungus, *Elsinoë perseae* (previously known as *Sphaceloma perseae*) is a high priority biosecurity threat for avocados in Australia. The pathogen is considered to be host specific (Everett & Siebert, 2018) but closely related *Elsinoë* spp. cause similar scab diseases of other economically important crops, such as *E. fawcettii* and *E. australis* on citrus (Fan et al., 2017).

Avocado scab results in premature fruit drop and reduced fruit quality, which heavily impacts marketability (Everett & Siebert, 2018; Fan et al., 2017). An indirect but nevertheless major impact of this disease is that it restricts market access to pest-free countries such as Australia, Europe and California in the USA (Everett & Siebert, 2018).

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

(Everett et al., 2011). Symptoms in leaves includes red lesions which sometimes results in holes in the centre of the lesion (Everett et al., 2011), and brown abrasions (Newett et al., 2013) which can resemble physical damage caused by wind rub (Everett & Siebert, 2018).

Although scab symptoms are recognisable, morphological identification of the causal fungus by isolation from symptomatic material can be difficult as the fungus is slow growing and there is risk of overgrowth by contaminants and secondary microorganisms (Fan et al., 2017). There is therefore a need for a rapid, real-time molecular detection assay for *E. perseae in planta*. A recent taxonomic study reclassified the avocado scab fungus (*Sphaceloma perseae*) as *E. perseae*, based on a multigene phylogenetic analysis (Fan et al., 2017). This sequence dataset has been utilised for assay design.

A real-time qPCR duplex assay has been developed for the rapid detection of *Elsinoë perseae* (syn. *Sphaceloma perseae*) and simultaneously, an avocado endogenous gene as a means for checking potential user error in sample loading or PCR inhibition; samples should always give a positive result for avocado DNA irrespective of the pathogen status. The assay has been optimized with cloned DNA (Fig. 1) and soon will be validated using DNA extracts from pure fungal cultures and field samples.

Fig. 1 Real time qPCR amplification of cloned *Elsinoë perseae*. The red and blue lines represent amplification of two separately cloned *E. perseae* sequences which the primers and probe target and anneal to.

Fusarium spp. associated with ambrosia beetle-mediated branch dieback of avocado

Fusarium dieback of avocado trees in Australia is vectored by ambrosia (scolytid) beetles in the *Euwallacea fornicatus* cryptic species complex. Members of this species-complex include the Tea Shot Hole Borer (TSHB), which originated in Sri Lanka (Danthanarayana, 1968), and is found in South East Asia and Australasia, including Southern India, Southern Thailand, Singapore, Malaysia, Papua New Guinea, Australia (Stouthamer et al., 2017) and Florida in the USA (Eskalen et al., 2012; Mendel et al., 2012); the Polyphagous Shot Hole Borer (PSHB) is found in the USA (Southern California) (Freeman et al., 2013), Israel (Freeman et al., 2013), South Africa (Paap et al., 2018) Northern Thailand, Vietnam, China, Taiwan and Okinawa, Japan (Stouthamer et al., 2017); and the Kuroshio Shot Hole Borer (KSHB) is found in Taiwan and Okinawa and more recently in California, USA (Na et al., 2017; Stouthamer et al., 2017).

Female ambrosia beetles carry symbiotic fungi in mycangial sacs in their mouthparts and deposit the fungus on xylem gallery walls for the larvae and beetles to feed upon (Freeman et al., 2013; Paap et al., 2018). Heavy infestations of the beetle lead to branch dieback, leaf wilt and symptoms of frass and sugary exudates (persitol) (Freeman et al., 2013) at the pin-hole entry sites along the affected branch. Male beetles are haploid, do not have mycangia and are flightless, contrary to the diploid females (Freeman et al., 2013; O'Donnell et al., 2016). An unmated female beetle will produce males, which then mate with their mother to produce the next generation of females to mate with the males, producing a clonal lineage (Freeman et al., 2013). The male beetles remain in the brood galleries, while the females make short flights to colonize neighbouring trees, thus spreading disease (Freeman et al., 2013).

In Australia, Fusarium dieback was identified in suburban coastal tuckeroo trees (*Cupaniopsis anacardioides*) in South East Queensland in late 2017 (Parkinson et al., unpublished). The beetle vector detected in tuckeroo was identified as TSHB but the *Fusarium* symbiont is an undescribed species. There have been sporadic infestations of TSHB in avocado plantations in Australia for the last decade and appear most severe on the Atherton Tableland in Far North Queensland. The phylogenetic diversity and pathogenicity of *Fusarium* species associated with branch dieback of avocado and other woody tree hosts in Australia has not been investigated.

To address this gap in knowledge, ambrosia beetle and branch dieback surveys have been done in Queensland. Plant species examined included avocado in orchards in Far North Queensland including Marbeeba and the Atherton Tableland, and in Central Queensland viz. Childers, Isis Central, and Bundaberg; tuckeroo in residential Sunshine Coast; and *Macadamia* and *Acacia* spp. growing adjacent to an avocado orchard at Childers. *Euwallacea* sp. beetle specimens were collected from brood galleries in symptomatic tree branches and in traps, and were identified morphologically. Fungal isolates were obtained from symptomatic heartwood of borer-beetle affected branches and from *Euwallacea* sp. beetle specimens. Fungal isolates were identified to genus level with PCR, sequencing and multigene phylogenetic analyses.

The fungal isolate collection contains 142 isolates collected from symptomatic tree branches and beetle specimens (Table 1). The genera found associated with branch dieback of multiple tree hosts include *Fusarium*, *Bionectria*, *Colletotrichum*, *Lasiodiplodia*, *Phomopsis*, *Nectria* and *Nigrospora* (Table 1).

Further work includes multigene sequencing and phylogenetic analyses on the *Fusarium* spp. with descriptions of new fungal species expected. Pathogenicity testing of selected fungal isolates will be carried out on avocado and alternate hosts in future experiments.

Table 1 – List of isolates collected to date which have been identified with gene sequencing of ITS, RBP2 and TEF-1α partial gene regions, and approximate identities confirmed with Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Conclusions

Australia is a large country and the avocado industry is dispersed across many, sparsely populated regions. Adoption of tools such as Checkpoint may create digital networks that join industry members together. The development of new diagnostic tests will allow rapid responses to pathogen incursions, if they should occur, and will help generate evidence of pathogen freedom to facilitate trade. Finally, the study on ambrosia beetles will clarify the pest status of this group of insects in Australia, and provide data on alternative hosts.

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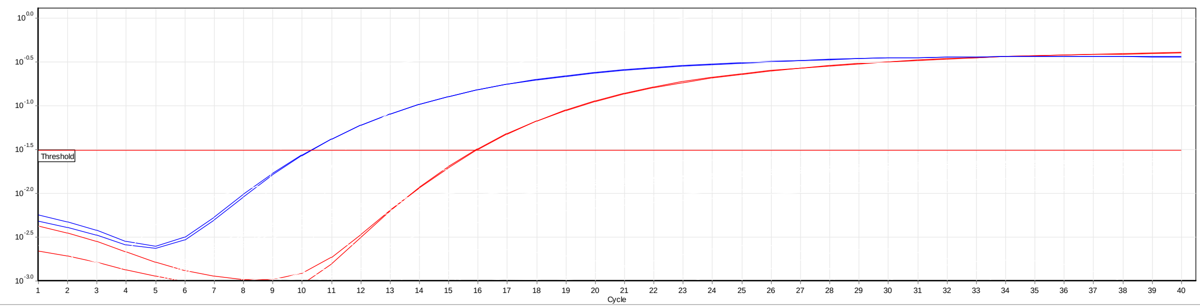

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Fungal isolate identity (number of isolates)	Host	Locality (region in Qld)
Fusarium sp. (38)	Avocado (*Persea americana*)	Atherton, Mareeba, Mutchilba, Tolga, Walkamin (Far North Qld); Bundaberg, Childers, Isis Central (Central Qld)
Bionectria ochroleuca (1)	Avocado (*Persea americana*)	Bundaberg (Central Qld)
Colletotrichum spp. (2)	Avocado (*Persea americana*)	Bundaberg, Childers (Central Qld)
Phomopsis sp. (4)	Avocado (*Persea americana*)	Mareeba (Far North Qld); Bundaberg (Central Qld)
Lasiodiplodia spp. (7)	Avocado (*Persea americana*)	Mareeba (Far North Qld); Bundaberg, Childers (Central Qld)
Nectria pseudotrichia (2)	Avocado (*Persea americana*)	Mareeba (Far North Qld)
Nigrospora sphaerica (1)	Avocado (*Persea americana*)	Bundaberg (Central Queensland)
Fusarium sp. (24)	Tuckeroo (*Cupaniopsis anacardioides*)	Minyama, Maroochydore (Sunshine Coast, South East Queensland)
Fusarium sp. (5)	Mango (*Mangifera indica*)	Tolga, Dimbulah (Far North Qld); Woodstock (North East Qld)
Fusarium sp. (1)	Blueberry (*Vaccinium* sp.)	Tolga (Far North Qld)
Fusarium sp. (9*)	Macadamia (*Macadamia integrifolia*)	Childers (Central Qld)
Fusarium sp. (13*)	Wattle (*Acacia* sp.)	Childers (Central Qld)
Fusarium sp. (15)	Tea Shot Hole Borer (*Euwallacea fornicatus*)	Mareeba (Far North Qld)
Bionectria sp. (1)	Tea Shot Hole Borer (*Euwallacea fornicatus*)	Walkamin (Far North Qld)

*Isolate identities to be confirmed by sequencing.