Australasian Plant Pathology

First report of Oomycetes associated with the invasive tree Parkinsonia aculeata (Family: Fabaceae)

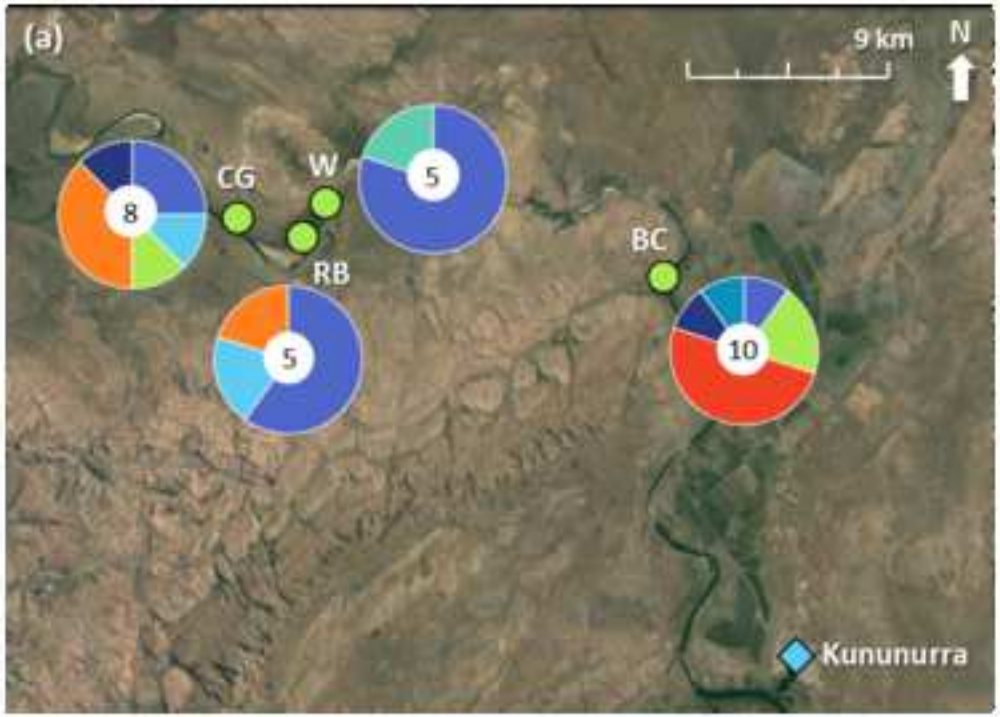
--Manuscript Draft--

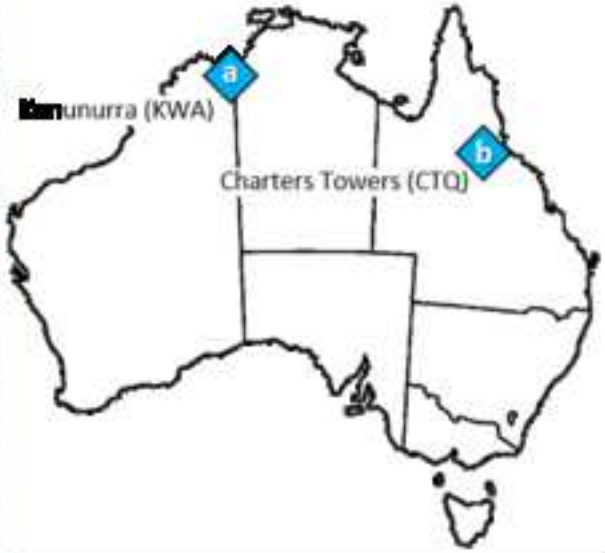
Manuscript Number:	AUPP-D-16-00193R2
Full Title:	First report of Oomycetes associated with the invasive tree Parkinsonia aculeata (Family: Fabaceae)
Article Type:	Original Research Article
Keywords:	Soil pathogen; riparian ecosystem; tree decline; dieback; zoospore baiting; phytophthora
Corresponding Author:	Tracey Steinrucken Western Sydney University Hawkesbury Institute for the Environment AUSTRALIA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Western Sydney University Hawkesbury Institute for the Environment
Corresponding Author's Secondary Institution:	
First Author:	Tracey Steinrucken
First Author Secondary Information:	
Order of Authors:	Tracey Steinrucken
	Sonia Aghighi
	Giles E. St. J Hardy
	Andrew Bissett
	Jeff R Powell
	Rieks D van Klinken
Order of Authors Secondary Information:	
Funding Information:	Meat and Livestock Australia (B.STU.0271) — Ms Tracey Steinrucken
Abstract:	Phytophthora species have caused the decline and dieback of multiple tree species in Australia and around the world. Dieback in invasive trees in Australia has been observed for decades, motivating research into the potential causes of dieback to be used for biological control of these invasive species. Despite wide-ranging and ongoing research into invasive plant dieback, Phytophthora species have been largely ignored as potential causal agents of dieback, with the focus more on latent fungal pathogens living as endophytes. We conducted the first survey of Phytophthora and other oomycetes to determine their association with dieback of the invasive tree, Parkinsonia aculeata L. (Fabaceae). Using zoospore baiting, we recovered 37 oomycete isolates from roots and soil of healthy and dieback-affected P. aculeata in Kununurra, Western Australia and Charters Towers, Queensland. Using molecular taxonomy, we identified ten unique oomycete taxa, predominantly composed of Phytophthora palmivora, Ph. nicotianae and Phytopythium vexans. Parkinsonia dieback occurs across multiple climatic zones including those experiencing severe drought. We recovered fewer oomycete isolates from soil and roots in drought-affected Charters Towers than Kununurra, which had experienced recent rainfall. This may be because oomycetes require soil moisture for the dispersal of zoospores. None of the genotypes identified were consistently isolated from dieback-affected trees suggesting that any association with parkinsonia dieback may be localised. More extensive surveys and pathogenicity screenings of isolated

	oomycetes are required to evaluate their role in the parkinsonia dieback phenomenon.
Response to Reviewers:	To the Editor, Thank-you for accepting our submission "First report of Oomycetes associated with the invasive tree Parkinsonia aculeata (Family: Fabaceae)". I have reviewed and made the changes you suggested in the manuscript, such as changing the abbreviated "QLD" to "Queensland" throughout, and fixing any ambiguity with the "Phytophthora" vs. "Pythium" genera using abbreviations "Ph." and "Py." where appropriate (I.e. not at the start of sentences where I have instead written the genus out in full). I also changed mention of the study plant, "Parkinsonia" to "P. aculeata", removed Figure 2 and have adjusted the orders of Figure 3 and 4. Please contact me should you require anything further via my contact details below. Kind regards, Tracey V Steinrucken Hawkesbury Institute for the Environment CSIRO Health and Biosecurity tracey.steinrucken@csiro.au +61 (0) 409 937 377 +61 (0) 7 3833 5664 Ecoscience Precinct, 41 Boggo Road, Dutton Park QLD 4102

Fig 1

Click here to download Colour figure Fig1.tif





Site		Name	South	East
KWA	BC	Button's Crossing	15°37.255'	128°41.500'
(a)	W	Wetland	15°34.822'	128°31.126'
	RB	Riverbank	15°35.563'	128°30.064'
	CG	Manbi Campground	15°34.613'	128°28.087'
CTQ	BS	Burdekin South	20°03.436'	146°15.368'
(b)	BN	Burdekin North	19°48.694'	146°02.963'
	GC	Groper Creek	19°42.995'	147°31.856'

Fig 2

Click here to download Colour figure Fig2.tif

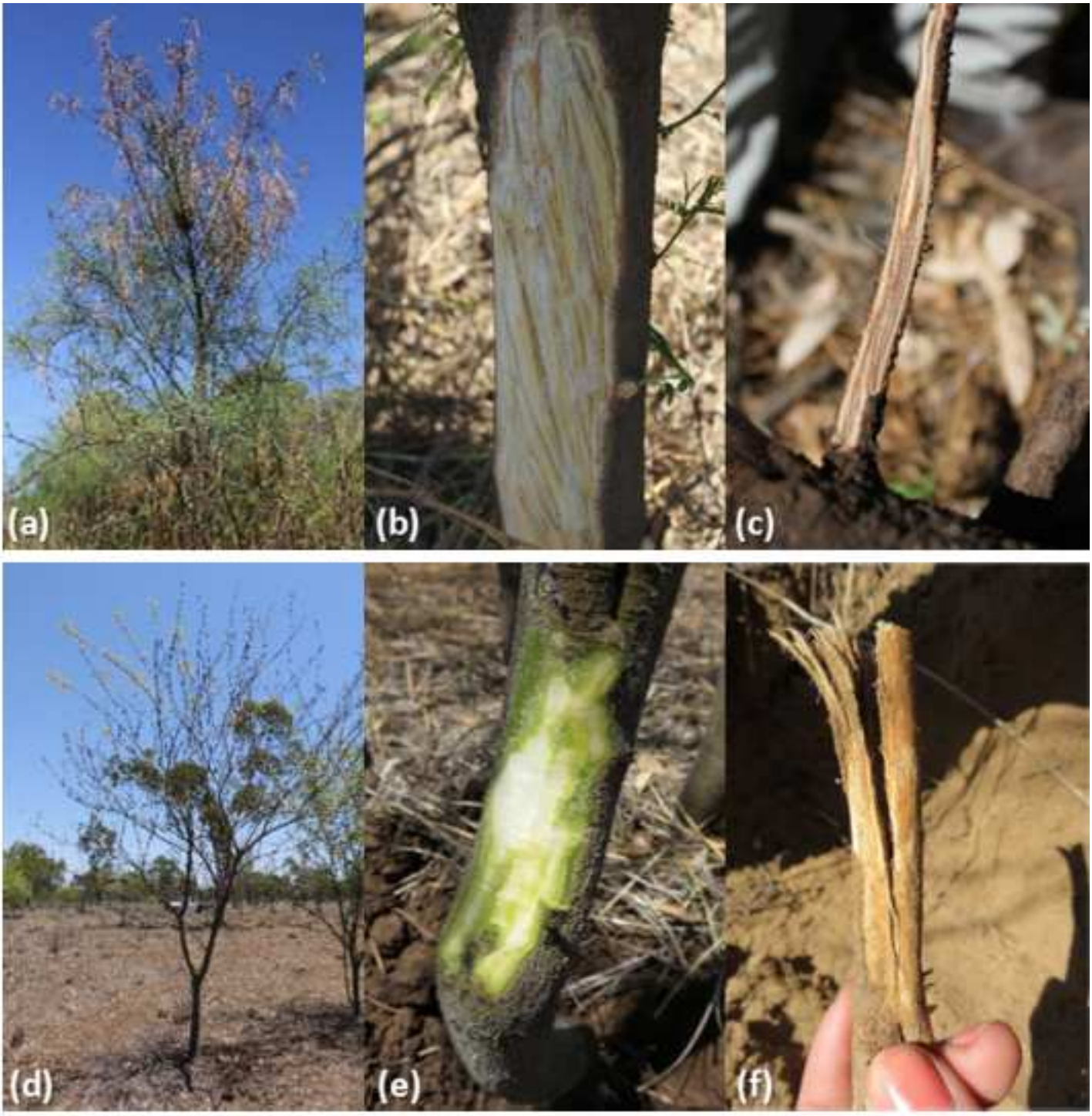
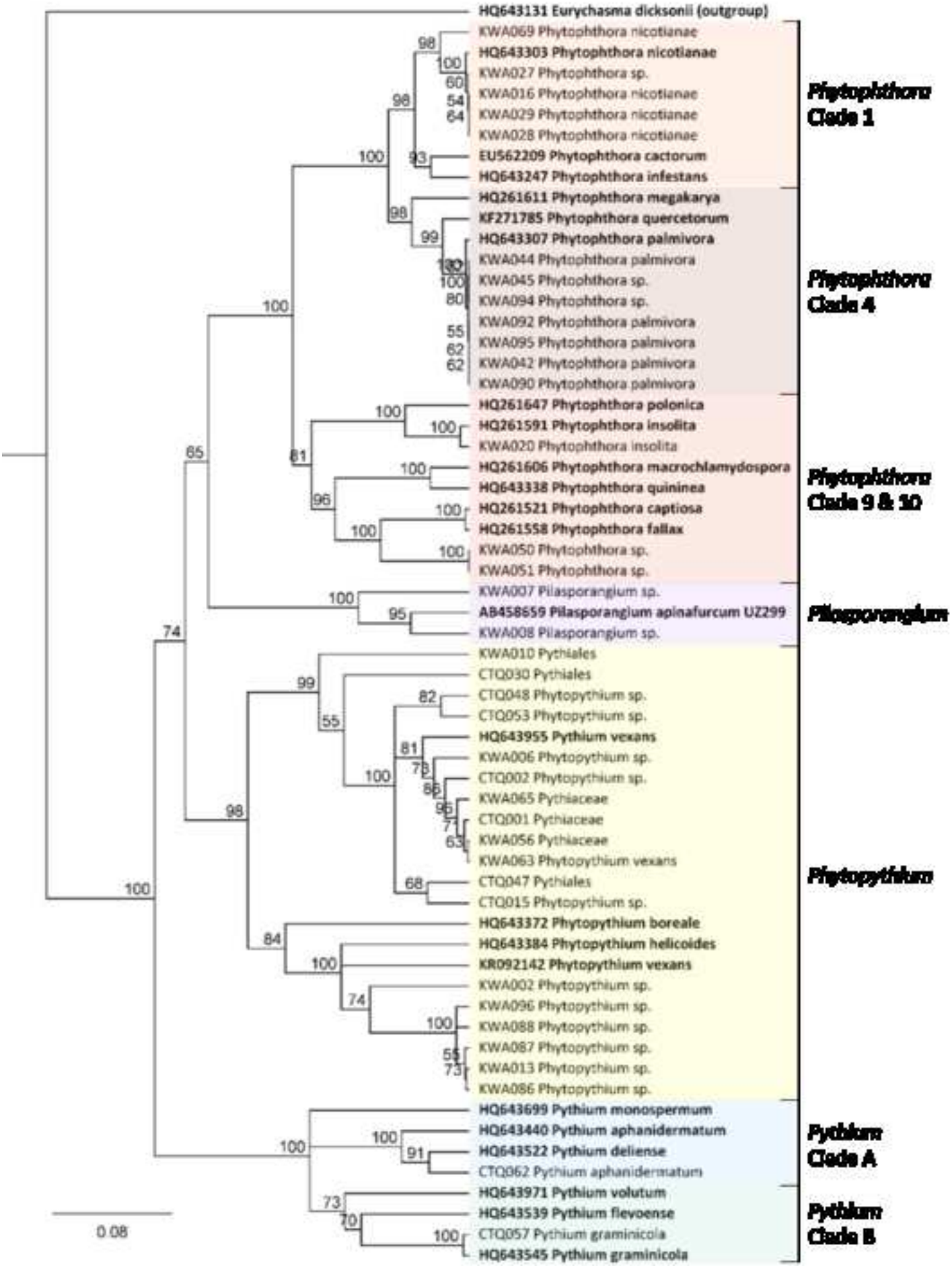


Fig 3

Click here to download Colour figure Fig3.tif



First report of Oomycetes associated with the invasive tree *Parkinsonia aculeata* (Family: Fabaceae)

T.V. Steinrucken[1,2]*, S. Aghighi[3,4], G.E.St.J. Hardy[4], A. Bissett[5], J.R. Powell[1] and R.D. van Klinken[2]

[1] Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, New South Wales 2751, Australia

[2] CSIRO, GPO Box 2583, Brisbane, Queensland 4001, Australia

[3] Research and Technology Institute of Plant Production, Shahid Bahonar University of Kerman, Kerman, Iran

[4] Centre for Phytophthora Science and Management, Murdoch University, 90 South Street, Murdoch, Western Australia 6150, Australia

[5] CSIRO, GPO Box 1538, Hobart, Tasmania, 7001, Australia

* Corresponding author: t.steinrucken@westernsydney.edu.au

Short title: Oomycetes associated with invasive *Parkinsonia aculeata*

Abstract

Phytophthora species have caused the decline and dieback of multiple tree species in Australia and around the world. Dieback in invasive trees in Australia has been observed for decades, motivating research into the potential causes of dieback to be used for biological control of these invasive species. Despite wide-ranging and ongoing research into invasive plant dieback, *Phytophthora* species have been largely ignored as potential causal agents of dieback, with the focus more on latent fungal pathogens living as endophytes.

We conducted the first survey of *Phytophthora* and other oomycetes to determine their association with dieback of the invasive tree, *Parkinsonia aculeata* L. (Fabaceae). Using zoospore baiting, we recovered 37 oomycete isolates from roots and soil of healthy and dieback-affected *P. aculeata* in Kununurra, Western Australia and Charters Towers, Queensland. Using molecular taxonomy, we identified ten unique oomycete taxa, predominantly composed of *Phytophthora palmivora*, *Ph. nicotianae* and *Phytopythium vexans*.

Parkinsonia dieback occurs across multiple climatic zones including those experiencing severe drought. We recovered fewer oomycete isolates from soil and roots in drought-affected Charters Towers than Kununurra, which had experienced recent rainfall. This may be because oomycetes require soil moisture for the dispersal of zoospores. None of the genotypes identified were consistently isolated from dieback-affected trees suggesting that any association with parkinsonia dieback may be localised. More extensive surveys and pathogenicity screenings of isolated oomycetes are required to evaluate their role in the parkinsonia dieback phenomenon.

Additional keywords: Soil pathogen, riparian ecosystem, tree decline, dieback, zoospore baiting

Introduction

Dieback is defined in perennial plants as a progressive reduction in plant health resulting in the death of plant parts and often outright death of the whole plant, potentially resulting in local population extinctions, either as a gradual process over many years or as a more sudden occurrence (Manion & Lachance 1992; Ciesla & Donaubauer 1994; Pautasso et al. 2013; Steinrucken et al. 2016). Dieback is not age-related or explained by a specific stress such as fire or prolonged flooding (Mueller-Dombois 1987). Symptoms may include defoliation, browning of stems, and staining of stem tissue.

Phytophthora species are implicated in dieback of multiple tree species in Australia and around the world. *Phytophthora ramorum*, for example, is the cause of Sudden Oak Death (Rizzo & Garbelotto 2003; Garbelotto & Hayden 2012) and is also responsible for dieback of many other tree species including larch (*Larix* spp.; Brasier & Webber 2010), beech and chestnut (*Fagus* spp. and *Castanea* spp.; Brasier et al. 2004). *Phytophthora cinnamomi* causes oak decline in Iberia (Brasier et al. 1993) and little leaf disease in pine (*Pinus* spp.; Otrosina & Marx 1975). In Australia, *Ph. cinnamomi* has resulted in extensive dieback of native forests and bushland (Pratt & Heather 1973), with extensive damage to jarrah (*Eucalyptus marginata*) forest, as well as *Prunus*, *Camellia*, *Erica*, *Rhododendron* and *Jacaranda* species (Newhook & Podger 1972; Dell & Malajczuk 1989). *Phytophthora*, like other oomycetes, disperse via flagellated zoospores that swim towards potential hosts, often via soil moisture or water flow (Scott et al. 2013). Many species might also remain viable in the soil for years due to

environmentally-resistant structures such as oospores and chlamydospores (Judelson & Blanco 2005). *Phytophthora* often have a wide host range making them one of the most important groups of plant pathogens globally (Scott et al. 2013).

Dieback in several important woody weedy trees and shrubs in arid, semi-arid and wet-dry tropical habitats is a widely-researched phenomenon due to the potential for the biotic causal agent(s) of dieback to be used in biological control of these invasive trees (Raghavendra et al. 2017). However, most invasive plant dieback research has focused on putative pathogens derived from stem tissue, in the form of endophytic higher fungi (Haque 2015; Sacdalan 2015; Diplock 2016), and few have considered the involvement of *Phytophthora* species. An exception is the research on European blackberry (*Rubus anglocandicans*) decline in Western Australia (WA; Aghighi et al. 2015), associated with *Ph. cryptogea* and *Ph. bilorbang*. Above- and below-ground biomass of blackberry decreased during regular flooding events when infected with these species, which is consistent with the common method of *Phytophthora* dispersal (Aghighi et al. 2015). In other research on woody weed dieback in arid zones, *Phytophthora* species were likely assumed to be unimportant since many dieback-affected invasive tree species are found in very dry climatic conditions (Raghavendra et al. 2017) with little evidence of root disease, and few studies focused on sampling roots (Haque 2015; Sacdalan 2015; Diplock 2016). If dieback is observed in regions with intermittent flooding or extended wet seasons, it is more likely that *Phytophthora* species are involved. We believe it is therefore important to consider the role of *Phytophthora* in dieback of other weed species, particularly in more mesic habitats.

Parkinsonia aculeata L. (commonly referred to as parkinsonia) is a weed of national significance in Australia (Thorp & Lynch 2000) adversely affecting livestock management, water access and biodiversity, with populations found across northern Queensland (QLD),

northern Western Australia (WA) and the Northern Territory (van Klinken et al. 2009). Introduced from meso-America in the 19th century, this thorny, leguminous tree has become a target species for biological control due to its vast range, presence in remote locations and large populations (van Klinken et al. 2006). Dieback has been observed across its invasive range for two decades with some anecdotal reporting of dieback from the 1950s (van Klinken et al. 2009). Despite extensive research (Toh 2009; Diplock 2016; Steinrucken et al. 2016; Raghavendra et al. 2017; Steinrucken et al. 2017), the cause of parkinsonia dieback remains unknown.

In a molecular study using terminal-fragment length polymorphisms (T-RFLP) analysis, endophytic higher fungi community composition was shown to be related to dieback occurrence in *P. aculeata* (Steinrucken et al. 2016). However, due to the methodology, taxonomic classifications could not be attached to the operational taxonomic units (OTUs). Subsequently, pathogenicity screening of selected endophytic fungal pathogens isolated from *P. aculeata* tissue did not result in dieback-like symptoms or systemic infection in one-year old *P. aculeata* plants, despite the addition of water-stress treatments which reduced plant health (Steinrucken et al. 2017). It is possible that parkinsonia dieback may be due to a combination of environmental stress factors and/or a disease complex involving multiple pathogenic species. Oomycetes have not yet been explored as potential dieback-causing pathogens in this system, but are putative pathogens in other dieback systems in Australian weeds (Aghighi et al. 2015). No environmental surveys have been conducted to determine whether flooding is important for the onset or development of dieback in *P. aculeata*. However, since *P. aculeata* can survive for up to 9 months with the lower portion of their trunks under water, conditions which favour oomycete dispersal and infection, we have conducted the following survey of

oomycetes associated with *P. aculeata* to determine their involvement, or lack thereof, in dieback of this invasive tree.

Materials and Methods

Field survey and sampling

Four sites near Kununurra, Western Australia (WA), and three sites near Charters Towers, Queensland (QLD; Fig 1) were sampled in May and October 2014, respectively. Sampling sites from both regions were alongside water bodies or in areas where flooding occurs. Only *P. aculeata* trees that were unambiguously healthy were classified as healthy, and trees with symptoms consistent with parkinsonia dieback, as described in the introduction, were sampled as such. We collected between five and seven roots (5-20 cm by 0.5-1.5 cm) and approximately 500 g of rhizosphere soil material from five healthy and five dieback-affected *P. aculeata* trees at each site. The health of trees was assessed by scoring percentage canopy and foliage cover (Steinrucken et al., 2016), and by inspecting the tree for signs of dieback or disease such as defoliation, lesions, browning of the stems and signs of root infection. Tools were surface-sterilized between trees with 50% NaHCl for 3 min, and samples were placed in individual zip lock bags, sealed and stored at 5°C for up to 48 h before further processing.

Isolation of *Phytophthora* species

Zoospores from *P. aculeata* soil and root samples (pooled by plant) were baited in a cool, naturally-lit room, using a modified version of the method of Rea et al. (2010). Soil and root

138 samples from individual trees were placed in 1 L plastic take-away containers and covered with
139 distilled water (1:2 volume to volume sample/water). Containers were placed on a heating mat
140 set at 28°C, and left undisturbed for 2 hours to allow sediment to settle. Approximately 15
141 "baits" in the form of youngest, fully emerged leaves, picked fresh from multiple plants
142 (*Leucadendron* sp., *Pittosporum tobira*, *Scholtzia* sp., *Lantana camara*, *Melaleuca scabia*,
143 *P. aculeata* and *Origanum vulgare*) were floated on the water, lower surface down, and left
144 undisturbed for 24 h. Baits were then checked twice a day for 5 days and any baits with
145 brownish or water-soaked lesions were plated onto NARPH, a *Phytophthora* selective medium
146 containing Nilstat, Ampicillin, Rifadin, Hymexazol and cornmeal agar (Hüberli et al. 2000)
147 and incubated in the dark at room temperature. Once aseptate hyphae were observed growing
148 from the plated lesion section, a random selection of isolates were examined with a light
149 microscope to confirm isolation of oomycete species. They were then subcultured onto fresh
150 NARPH medium and repeatedly subcultured until a pure culture was obtained, before being
151 transferred onto half-strength PDA (potato dextrose agar; 19.25 gL^{-1}) amended with 35 mg L^{-1}
152 streptomycin and incubated in the dark at room temperature. Water was carefully poured off
153 the samples, which were then left to air-dry for 6 weeks in the bait containers. The baiting
154 procedure was repeated, followed by isolation and culturing of recovered isolates using the
155 methods above.
156
157 DNA isolation, PCR and sequencing of isolated species
158
159 Mycelia were scraped from pure 7-day old cultures using a sterile blade and added to 400 µL
160 sterile H_2O. DNA was extracted using the MO BIO Powersoil® Isolation kit (Qiagen, Carlsbad,
161 California), PCR-amplified with MyTaq® (Bioline, London UK) according to the

manufacturer with 0.2 µM forward primer DC6 and reverse primer ITS4 (Table 1). PCR conditions consisted of 96°C 2 min; 10 cycles of 95°C for 30 s, 54°C for 30 s and 72°C for 1 min; 25 cycles of 95°C for 30 s, 56°C for 30 s and 72°C for 1 min; and a final round of 72°C for 7 min. Amplicons were then purified with the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, Wisconsin), and were Sanger sequenced with the BigDye Terminator Kit v3.1 (Applied Biosystems, Foster City, California) at the Centre for *Phytophthora* Science & Management at Murdoch University, Perth.

The initial identification of all sequences was obtained via BLAST search (http://www.ncbi.nlm.nih.gov/BLAST). Further identification of isolates was derived by interpreting a combination of the first 100 BLAST matches. Using an approach similar to Vega et al. (2010), we were cautious in identifying isolates at the genus level or above, given the occurrence of misidentified sequences in GenBank (Vilgalys 2003). The cut-off point for assigning species names was 99% identity; genus names was 95–99% identity, family name 90–95% identity; sequences with lower identity with members of several families were identified only at the ordinal level. Once taxa were assigned, those classified as oomycetes were MUSCLE aligned to 796 bp, and a Jukes-Cantor UPGMA phylogenetic tree (TreeBASE Submission # 20917) was built using bootstrap resampling and 1000 replicates using Geneious® v8.1.6 (Biomatters, Auckland, New Zealand). We used an *Eurychasma dicksonii* voucher sequence (HQ643131) as the outgroup and voucher specimen sequences downloaded from GenBank were included (when available) for each identified taxon.

Results

The symptoms of parkinsonia dieback in WA (leaf death, minimal defoliation, red streaking of the trunk and roots under the bark; Fig 2 a-c) were different to those observed in Queensland (leaf loss, no red streaking of the trunk or roots under bark; Fig 2 d-f). In both cases, these symptoms were consistent across dieback-affected *P. aculeata* trees in each area. Locally-occurring species in Queensland included bellyache bush (*Jatropha gossypiifolia*), *Melaleuca* spp., Noogoora burr (*Xanthium occidentale*) and *Lysiphyllum cunninghamii*. In WA, bellyache bush, *Melaleuca* spp., buffel grass (*Cenchrus ciliaris*), caltrop (*Tribulus terrestris*) and Noogoora burr were most common. None of these species showed signs of dieback similar to parkinsonia dieback. At all sites, the ground beneath *P. aculeata* was usually bare soil and the trees were generally 1 - 3 m apart.

During zoospore baiting, the most effective baits were leaves from *Scholtzia* sp., *Leucadendron* sp., *Pittosporum tobira*, and leaflets from *P. aculeate*. No additional taxa were isolated in the second round of baiting.

From a total of 141 isolates recovered from *P. aculeata*, sequence data resulted in their taxonomic classification to two zygomycetes (*Rhizopus* spp.), one basidiomycete (*Rhizoctonia* sp.) and 22 ascomycetes (mostly *Epicoccum* sp. and *Fusarium* spp.). The 37 isolates classified as oomycetes (Table 2) included fifteen which were classified as *Phytophthora* spp. (including *Ph. nicotianae*, *Ph. insolita* and *Ph. palmivora*), two classified as *Pythium* spp. (*Py. graminicola* and *Py. aphanidermatum*), twelve classified as *Phytopythium* spp. (including one *Phytopythium vexans*) and two *Pilasporangium* spp. Three were classified only to Pythiaceae, and another three only classified to Pythiales (Table 3). The taxonomic classifications of each isolate was supported by the phylogenetic tree (Fig 3).

Diversity of recovered isolates was lower in Queensland compared to WA, with no isolates identified as *Phytophthora* spp. recovered from Queensland (Fig 1). *Phythophthora nicotianae*

was isolated from two sites in WA, and *Phytopythium vexans* was isolated from one site in Queensland. Isolates classified as *Ph. palmivora* were only isolated from dieback-affected *P. aculeata* in WA (Table 3). *Pythium aphanidermatum* was isolated from a dieback-affected tree in WA, *Py. graminicola* was isolated from dieback-affected *P. aculeata* in Queensland, and dieback-affected *P. aculeata* in WA.

Discussion

We isolated multiple species of *Phytophthora* and *Pythium* from the roots and soil of healthy and dieback affected *P. aculeata* in WA, but no isolates identified as *Phytophthora* spp. in Queensland. Diversity of oomycetes in Queensland was therefore lower compared to WA from where *Ph. palmivora*, *Ph. nicotianae,* and *Phytopythium vexans* were isolated. This is also the first time a difference in dieback symptoms for this species has been observed, which suggests that parkinsonia dieback in Kununurra, WA, may be caused by a different agent(s) or stress factors than in Queensland. Future investigations should involve surveying more dieback sites and further comparisons of symptoms in the populations as well as testing of putative pathogens. Although *Ph. palmivora* was isolated only from samples collected in association with dieback-affected trees, this was only at one site in WA. Therefore the results from this preliminary survey did not allow us to determine if there was a significant association between *Ph. palmivora* or other oomycetes and *P. aculeata*, or parkinsonia dieback.

The most commonly isolated oomycetes were *Ph. palmivora*, *Ph. nicotianae* and *Phytopythium vexans*. In order to determine the likelihood of these taxa being associated with parkinsonia dieback, and their potential for use as biological control agents, we describe them in more detail below.

Phytophthora palmivora has a wide host range. Most notably it has resulted in 20-30% reduction of the global cocoa crop (Erwin & Ribeiro 1996). Pathogenicity trials implicated *Ph. palmivora* in dieback of durian (*Durio zibethinus*) in multiple orchards in far north Queensland, causing significant root rot (Vawdrey et al. 2005). *Phytophthora palmivora* has been used as a mycoherbicide for the biological control of the invasive plant, milkweed vine (*Morrenia ororata*), a pest in citrus orchards in Florida, USA (Templeton & Greaves 1984). However, since *Ph. palmivora* is not host-specific and can persist in the soil for several seasons, the use of this mycoherbicide was restricted (Templeton & Greaves 1984). There is evidence that both *Ph. palmivora* and *Ph. nicotianae* infect and cause rot in citrus (Widmer et al. 1998). *Phytophthora nicotianae* also has a very wide host range with a cosmopolitan distribution and is a major pathogen of tobacco, ornamentals, tomato and some *Banksia* and eucalypt species (Erwin & Ribeiro 1996; Cline et al. 2008).

Phytopythium vexans (formally *Pythium vexans*, de Cock et al. 2015) has been reported to cause root and collar rot of kiwifruit in Turkey (Polat et al. 2017), is pathogenic to apple trees (Tewoldemedhin et al. 2011) and grapevines, and has been isolated from several other woody hosts (Spies et al. 2011). It has also been implicated alongside *Ph. palmivora* in dieback of durian in northern QLD (Vawdrey et al. 2005).

Differences in the number of isolates recovered from QLD compared to WA may be accounted for by average rainfall in the time leading up to sampling (Bureau of Meteorology, 2016). Kununurra, WA, experienced 1113.6 mm of rain in the 5 months leading up to the sampling date in May, whereas Charters Towers QLD was at the end of the dry season and had received only 41.8 mm of rain for the same period of time before sampling in Octoberand the dry spell was preceded by a long drought (Bureau of Meteorology, 2016). Zoospores produced by oomycetes swim in water to infect new plant tissue (Scott et al. 2013), a feature which

zoospore baiting takes advantage of (Rea et al. 2010). Diseases caused by *Phytophthora* species are therefore often correlated with increased soil moisture or rainfall, as in the case of blackberry decline (Aghighi et al. 2015). Plants are known to become more susceptible to pathogen infection when stressed (Agrios 2005) and in the case of *P. aculeata*, plant vigour deteriorates significantly more under drought stress compared to inundation stress (Steinrucken et al. 2017). This is especially the case with *Phytophthora* diseases, which frequently kill the lateral and finer roots during infection, and then during drought periods (e.g. the long, hot, dry summers in the Mediterranean climate of WA), infected plants do not have sufficient roots to extract water from soil, so they succumb (Erwin & Ribeiro 1996; Judelson & Blanco 2005). While we did observe red streaking of the roots (Fig 2c), there was no evidence of dead or dying lateral or fine roots in dieback-affected *P. aculeata*. Despite relatively low abundance, oomycetes were isolated even in the drought-affected sites in Queensland, so it is likely that these species are able to remain dormant but viable during drought.

Oospores from species such as *Ph. palmivora*, and *Ph. nicotianaea* are particularly adapted to remaining dormant through drought, avoiding desiccation and microbial degradation due to their thick walls, chemical composition, and ability to reproduce via selfing (Ashby 1928; Kaosiri et al. 1980). In the case of *Ph. palmivora*, and likely other *Phytophthora* species, they may also persist in perennial water bodies (Ko 2003), to then be moved across the landscape during flooding events. Many Australian parkinsonia populations are located in drought-affected areas that also experience seasonal flooding (van Klinken et al. 2009). These conditions are likely to be suited to a number of oomycetes due to their life history, since zoospores require soil moisture to move and infect new hosts (Scott et al. 2013). Consequently, they may take advantage of drought-stressed *P. aculeata* at the first sign of soil moisture sufficient for infection. Alternatively, when *P. aculeata* puts out new roots in more favourable,

wetter conditions, the new roots are attacked by pathogens in the rhizosphere. Lower rates of infection in drier conditions may be because desiccated plants have fewer young roots to infect, however this does not necessarily make them less susceptible to infection (G.E.St.J. Hardy, unpublished data). Pathogenicity screening of oomycetes isolated from dieback-affected *P. aculeata* would be essential as a next step in testing this conjecture.

Although this survey did not allow us to determine whether oomycetes are associated with parkinsonia dieback, we believe it is likely that some *Phytophthora* species (e.g. *Ph. palmivora*) might be associated with *P. aculeata*, at least in WA. Hence, the cause of dieback in *P. aculeata* is, as yet, unknown, but the diversity of species isolated in this study suggest that oomycetes should be considered in future community and pathogenicity work when investigating dieback in *P. aculeata*. All of the oomycetes isolated in this study have relatively broad host ranges, meaning their suitability for use as biological control agents for *P. aculeata* is unlikely. If, after further studies, putative dieback-causing agents are identified via more extensive surveys, pathogenicity testing may determine if dieback can be used for biological control of *P. aculeata* and could open the door to the development of novel technology for improving management and control of widespread weed populations.

Acknowledgements

This research is funded by Meat and Livestock Australia via a technical assistance grant (for TVS; B.STU.0271). TVS is also supported by the Australian Government via an Australian Post-graduate Award and the Hawkesbury Institute for the Environment at Western Sydney University. The authors would like to thank Kay Bailey (WA Agriculture) and Kelli Pukallus (Biosecurity Queensland) for fieldwork, Briony Williams (Murdoch University) for help with

isolations, and Kylie Ireland, Louise Morin (CSIRO) and two anonymous reviewers for their helpful comments which greatly improved this paper.

307 Table 1. Primers used in this study

Primer	Location	Direction	Sequence (5`-3`)	Reference
DC6	18S	Forward	GAGGGACTTTTGGGTAATCA	Cooke et al. (2000)
ITS4	28S	Reverse	TCCTCCGCTTATTGATATGC	Gardes and Bruns (1993)

308
309

Table 2. Isolates classified as oomycetes from soil and roots of *Parkinsonia aculeata*, identified to closest match (CM) on the NCBI database and by phylogenetic analysis using the ITS backbone from Robideau et al. (2011). Site of sampling in Kununurra (KWA) and Charters Towers (CTQ) from Fig 1, and disease status of host plant are also provided.

Isolate	CM Taxon	CM Accession	%PWI[a]	%QC[b]	Assigned Taxonomic ID[c]	Site[d]	H/D[e]
CTQ001	*Pythium vexans*[†]	HQ643954	93.9	100.0	Pythiaceae	BN	H
CTQ002	*Pythium vexans*	GU133601	97.1	99.6	*Phytopythium* sp.	BS	D
CTQ015	*Pythium vexans*	GU133601	97.1	99.6	*Phytopythium* sp.	BS	D
CTQ030	*Pythium vexans*	GU133578	88.8	71.5	Pythiales	BS	D
CTQ047	*Pythium vexans*	GU133601	87.2	100.0	Pythiales	BS	D
CTQ048	*Phytopythium* aff. *vexans*	HQ643371	95.2	95.1	*Phytopythium* sp.	BS	D
CTQ053	*Pythium vexans*	GU133593	98.9	94.5	*Phytopythium* sp.	BS	D
CTQ057	*Pythium graminicola*	HQ643545	100.0	100.0	*Pythium graminicola*	BN	H
CTQ062	*Pythium aphanidermatum*	KF667387	100.0	100.0	*Pythium aphanidermatum*	BS	D
KWA002	*Pythium* sp.	KP183943	96.6	100.0	*Phytopythium* sp.	BC	D
KWA006	*Pythium vexans*	GU133593	96.3	100.0	*Phytopythium* sp.	CG	D
KWA007	*Pilasporangium apinafurcum*	AB458659	84.9	97.2	*Pilasporangium* sp.	CG	D
KWA008	*Pilasporangium apinafurcum*	AB458659	91.9	97.2	*Pilasporangium* sp.	RB	H
KWA010	*Phytopythium* aff. *vexans*	HQ643371	87.1	100.0	Pythiales	RB	H
KWA013	*Phytopythium vexans*	KR092142	91.4	89.9	*Phytopythium* sp.	RB	H
KWA016	*Phytophthora nicotianae*	LT628539	100.0	100.0	*Phytophthora nicotianae*	CG	D
KWA020	*Phytophthora insolita*	GU111612	99.0	100.0	*Phytophthora insolita*	W	D
KWA027	*Phytophthora nicotianae*	LT628539	97.6	100.0	*Phytophthora* sp.	CG	D
KWA028	*Phytophthora nicotianae*	KU248811	99.4	97.5	*Phytophthora nicotianae*	CG	D
KWA029	*Phytophthora nicotianae*	LT628539	99.4	100.0	*Phytophthora nicotianae*	CG	D
KWA042	*Phytophthora palmivora*	KY357521	99.5	100.0	*Phytophthora palmivora*	BC	D
KWA044	*Phytophthora palmivora*	KY357521	99.3	100.0	*Phytophthora palmivora*	BC	D
KWA045	*Phytophthora palmivora*	KY357521	98.3	100.0	*Phytophthora* sp.	BC	D
KWA050	*Phytophthora fallax*	HQ261559	91.3	98.9	*Phytophthora* sp.	CG	H
KWA051	*Phytophthora fallax*	HQ261559	91.0	98.9	*Phytophthora* sp.	W	D
KWA056	*Phytopythium* aff. *vexans*	HQ643371	93.5	100.0	Pythiaceae	BC	D
KWA063	*Phytopythium* aff. *vexans*	HQ643371	99.1	100.0	*Phytopythium vexans*	BC	D
KWA065	*Phytopythium* aff. *vexans*	HQ643371	92.6	93.5	Pythiaceae	CG	D
KWA069	*Phytophthora nicotianae*	KJ506196	99.0	89.7	*Phytophthora nicotianae*	RB	H
KWA086	*Phytopythium vexans*	KR092142	92.5	85.7	*Phytopythium* sp.	W	D
KWA087	*Phytopythium vexans*	KR092142	92.3	85.0	*Phytopythium* sp.	W	D
KWA088	*Phytopythium vexans*	KR092142	92.5	86.0	*Phytopythium* sp.	W	D
KWA090	*Phytophthora palmivora*	KY357521	99.8	100.0	*Phytophthora palmivora*	BC	D
KWA092	*Phytophthora palmivora*	KY357521	99.9	100.0	*Phytophthora palmivora*	BC	D
KWA094	*Phytophthora palmivora*	KY357521	98.8	100.0	*Phytophthora* sp.	BC	D
KWA095	*Phytophthora palmivora*	KY357521	99.8	100.0	*Phytophthora palmivora*	BC	D
KWA096	*Phytopythium vexans*	KR092142	90.7	84.0	*Phytopythium* sp.	RB	H

[a] % PWI: percentage pairwise identity
[b] % QC: percentage query coverage
[c] Sequences deposited in GenBank under accession numbers KY938843 to KY938875
[d] Site key: BC – Button's Crossing KWA, CG – Manbi camp ground KWA, RB – river bank KWA, W – Wetland KWA, BN – Burdekin North CTQ, BS – Burdekin South CTQ, GC – Groper Creek CTQ
[e] Disease status of host plant: H – healthy, D – dieback
[†] Also known as *Phytopythium vexans* (de Cock et al. 2015)

316 Table 3. The number of oomycetes recovered and identified in this study from dieback-
317 affected (D) or healthy (H) *Parkinsonia aculeata* in Queensland (QLD) and Western
318 Australia (WA). For those isolates identified to species or genus, reference to their
319 association with plant disease in the literature is provided.

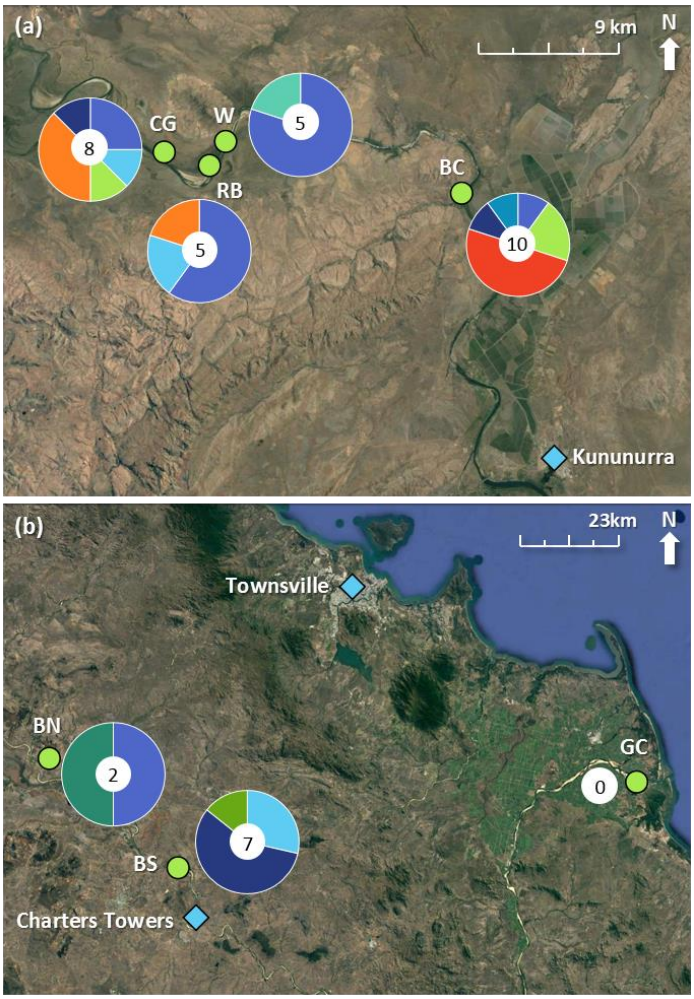
	QLD		WA			
Identified Species	**D**	**H**	**D**	**H**	**Total**	**Susceptible host species**
Phytophthora sp.			4	1	5	Multiple (Erwin & Ribeiro, 1996)
Phytophthora insolita			1		1	*Rhododendron* sp., apple, cucumber (Erwin & Ribeiro, 1996)
Phytophthora nicotianae			3	1	4	Multiple (Widmer et al. 1998; Cline et al. 2008; Valencia et al. 2011)
Phytophthora palmivora			5		5	Multiple (Kaosiri et al. 1980; Erwin & Ribeiro 1996; Vawdrey et al. 2005)
Pythium aphanidermatum	1				1	Bean, sugar beet, cucumber, tobacco (Middleton 1943)
Pythium graminicola		1			1	Grasses, grains (Middleton 1943; Kageyama et al. 2005)
Phytopythium sp.	4		5	2	11	Multiple (Farr et al. 1989)
Phytopythium vexans			1		1	Multiple (Farr et al. 1989; Vawdrey et al. 2005; Spies et al. 2011; Polat et al. 2017)
Pilasporangium sp.			1	1	2	NA – soil saprophyte (Uzuhashi et al. 2010)
Pythiales	2			1	3	NA
Pythiaceae		1	2		3	NA
Total number of isolates	7	2	22	6	**37**	

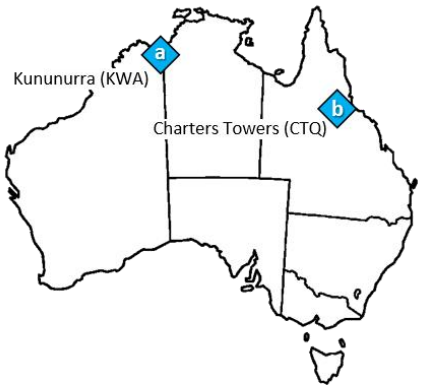
320

Fig 1. Maps of Kununurra, Western Australia (KWA; a) and central-east Queensland (CTQ; b) showing locations of *Parkinsonia aculeata* sampling sites, and proportion of oomycete isolate genotypes identified in this study. The number inside each pie chart is the number of oomycete isolates in total from that site

Fig 2. Symptoms of *Parkinsonia aculeata* dieback in Kununurra WA in May 2014: (a) brown leaves and crown dieback, (b) underbark trunk streaking not associated with a lesion, (c) root streaking; and in Charters Towers, Queensland in October 2014 (d) defoliation from crown, (e) no trunk streaking (f) no root streaking

Fig 3. Jukes-Cantor UPGMA tree derived from an alignment (981 bp) of partial sequences of the internal transcribed spacer (ITS) region of isolates classified as oomycetes from *Parkinsonia aculeata* roots and soil in this study (TreeBASE Submission # 20917). Isolate codes indicate geographic origin, i.e. KWA: Kununurra Western Australia, CTQ: central east Queensland. Sequences in **bold** are reference sequences from GenBank and are from voucher specimens where possible. Apart from *Pilasporangium* spp., taxonomic assignation to clades was as per the ITS backbone in Robideau et al. (2011). Sequences from isolates identified in this study have been submitted to Genbank (Accessions: KY938843 to KY938875)





Site		Name	South	East
KWA	BC	Button's Crossing	15°37.255′	128°41.500′
(a)	W	Wetland	15°34.822′	128°31.126′
	RB	Riverbank	15°35.563′	128°30.064′
	CG	Manbi Campground	15°34.613′	128°28.087′
CTQ	BS	Burdekin South	20°03.436′	146°15.368′
(b)	BN	Burdekin North	19°48.694′	146°02.963′
	GC	Groper Creek	19°42.995′	147°31.856′

Oomycete isolate taxa

- UC Pythiaceae
- UC Pythiales
- *Phytophthora* sp.
- *Phytophthora insolita*
- *Phytophthora nicotianae*
- *Phytophthora palmivora*
- *Phytopythium* sp.
- *Phytopythium* vexans
- *Pythium aphanidermatum*
- *Pythium graminicola*

UC = unclassified

Legend

- Sampling site
- City/Town

340

341 Fig 1.

342

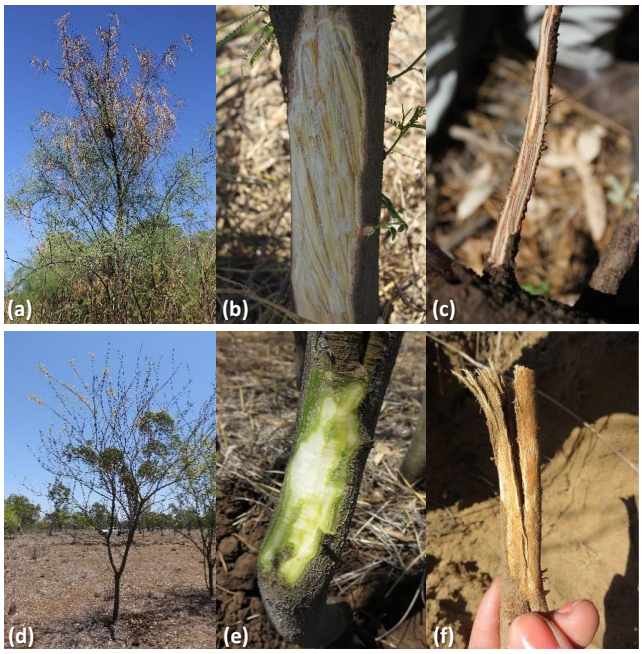


Fig 2.

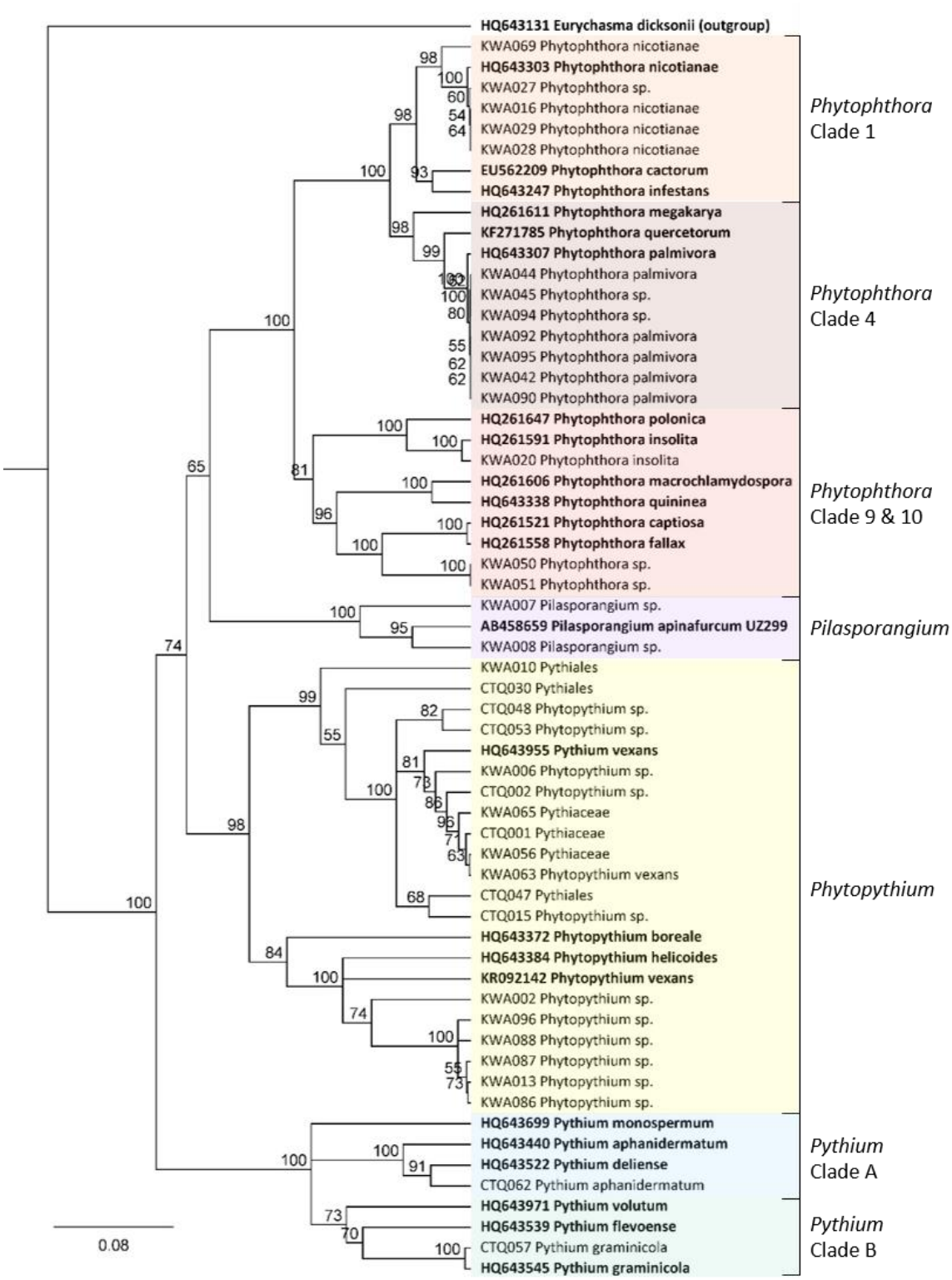

HQ643131 Eurychasma dicksonii (outgroup)
KWA069 Phytophthora nicotianae
HQ643303 Phytophthora nicotianae
KWA027 Phytophthora sp.
KWA016 Phytophthora nicotianae
KWA029 Phytophthora nicotianae
KWA028 Phytophthora nicotianae
EU562209 Phytophthora cactorum
HQ643247 Phytophthora infestans
Phytophthora Clade 1
HQ261611 Phytophthora megakarya
KF271785 Phytophthora quercetorum
HQ643307 Phytophthora palmivora
KWA044 Phytophthora palmivora
KWA045 Phytophthora sp.
KWA094 Phytophthora sp.
KWA092 Phytophthora palmivora
KWA095 Phytophthora palmivora
KWA042 Phytophthora palmivora
KWA090 Phytophthora palmivora
Phytophthora Clade 4
HQ261647 Phytophthora polonica
HQ261591 Phytophthora insolita
KWA020 Phytophthora insolita
HQ261606 Phytophthora macrochlamydospora
HQ643338 Phytophthora quininea
HQ261521 Phytophthora captiosa
HQ261558 Phytophthora fallax
KWA050 Phytophthora sp.
KWA051 Phytophthora sp.
Phytophthora Clade 9 & 10
KWA007 Pilasporangium sp.
AB458659 Pilasporangium apinafurcum UZ299
KWA008 Pilasporangium sp.
Pilasporangium
KWA010 Pythiales
CTQ030 Pythiales
CTQ048 Phytopythium sp.
CTQ053 Phytopythium sp.
HQ643955 Pythium vexans
KWA006 Phytopythium sp.
CTQ002 Phytopythium sp.
KWA065 Pythiaceae
CTQ001 Pythiaceae
KWA056 Pythiaceae
KWA063 Phytopythium vexans
CTQ047 Pythiales
CTQ015 Phytopythium sp.
Phytopythium
HQ643372 Phytopythium boreale
HQ643384 Phytopythium helicoides
KR092142 Phytopythium vexans
KWA002 Phytopythium sp.
KWA096 Phytopythium sp.
KWA088 Phytopythium sp.
KWA087 Phytopythium sp.
KWA013 Phytopythium sp.
KWA086 Phytopythium sp.
HQ643699 Pythium monospermum
HQ643440 Pythium aphanidermatum
HQ643522 Pythium deliense
CTQ062 Pythium aphanidermatum
Pythium Clade A
HQ643971 Pythium volutum
HQ643539 Pythium flevoense
CTQ057 Pythium graminicola
HQ643545 Pythium graminicola
Pythium Clade B
0.08

Fig 3.

References

Aghighi S, Burgess TI, Scott JK, Calver M & Hardy GESJ (2015) Isolation and pathogenicity of *Phytophthora* species from declining *Rubus anglocandicans*. Plant Pathol, 65**,** 451-461. doi: 10.1111/ppa.12436

Agrios GN (2005) Plant pathology, Burlington MA, Elsevier Academic Press.

Ashby SF (1928) The oospores of *Phytophthora nicotianae* Br. De Haan, with notes on the taxonomy of *P. parasitica* Dastur. Trans Br Mycol Soc, 13**,** 86-95. doi: 10.1016/S0007-1536(28)80007-6

Brasier C, Denman S, Brown A & Webber J (2004) Sudden oak death (*Phytophthora ramorum*) discovered on trees in Europe. Mycol Res, 108**,** 1108-1110. doi: 10.1017/S0953756204221244

Brasier C, Robredo F & Ferraz J (1993) Evidence for *Phytophthora cinnamomi* involvement in Iberian oak decline. Plant Pathol, 42**,** 140-145. doi: 10.1111/j.1365-3059.1993.tb01482.x

Brasier C & Webber J (2010) Plant pathology: sudden larch death. Nature, 466**,** 824-825. doi: 10.1038/466824a

Ciesla WM & Donaubauer E (1994) Decline and dieback of trees and forests, Rome, Food and Agriculture Organization of the United Nations.

Cline ET, Farr DF & Rossman AY (2008) A synopsis of *Phytophthora* with accurate scientific names, host range, and geographic distribution. Plant Health Prog. doi: 10.1094/PHP-2008-0318-01-RS

Cooke DEL, Drenth A, Duncan JM, Wagels G & Brasier CM (2000) A molecular phylogeny of *Phytophthora* and related oomycetes. Fungal Genet Biol, 30**,** 17-32. doi: 10.1006/fgbi.2000.1202

de Cock A, Lodhi AM, Rintoul TL, Bala K, Robideau GP, Abad ZG, Coffey MD, Shahzad S & Lévesque CA (2015) *Phytopythium*: molecular phylogeny and systematics. Persoonia, 34**,** 25-39. doi: 10.3767/003158515X685382

Dell B & Malajczuk N (1989) Jarrah dieback — A disease caused by *Phytophthora cinnamomi*. *In:* DELL, B, HAVEL, JJ & MALAJCZUK, N (eds.) The Jarrah Forest: A complex mediterranean ecosystem. Dordrecht: Springer Netherlands.

Diplock ND (2016) Parkinsonia dieback: investigations into its cause, ecology and potential for biological control. Dissertation, The University of Queensland.

Erwin DC & Ribeiro OK (1996) Phytophthora Diseases Worldwide, St Paul Minnesota, APS Press.

Garbelotto M & Hayden KJ (2012) Sudden oak death: interactions of the exotic oomycete *Phytophthora ramorum* with naïve North American hosts. Eukaryot Cell, 11**,** 1313-1323. doi: 10.1128/EC.00195-12

Gardes M & Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. Mol Ecol, 2**,** 113-118. doi: 10.1111/j.1365-294X.1993.tb00005.x

Haque A (2015) Investigation of the fungi associated with dieback of prickly acacia (*Vachellia nilotica* subsp. *indica*) in Northern Australia Dissertation, The University of Queensland.

Hüberli D, Tommerup IC & Hardy GES (2000) False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi* Australas Plant Pathol, 29**,** 164-169. doi: 10.1071/AP00029

Judelson HS & Blanco FA (2005) The spores of *Phytophthora*: weapons of the plant destroyer. Nat Rev Microbiol, 3**,** 47-58. doi: 10.1038/nrmicro1064

Kaosiri T, Zentmyer GA & Erwin DC (1980) Oospore Morphology and Germination in the *Phytophthora palmivora* Complex from Cacao. Mycologia, 72**,** 888-907. doi: 10.2307/3759732

Ko WH (2003) Long-term storage and survival structure of three species of *Phytophthora* in water. J Gen Plant Pathol, 69**,** 186-188. doi: 10.1007/s10327-003-0033-3

Manion PD & Lachance D (1992) Forest decline concepts: an overview. *In:* MANION, PD & LACHANCE, D (eds.) Forest Decline Concepts. St. Paul Minnesota: The American Phytopathological Society.

Mueller-Dombois D (1987) Natural dieback in forests. Bioscience, 37**,** 575-583. doi: 10.2307/1310668
Newhook F & Podger F (1972) The role of *Phytophthora cinnamomi* in Australian and New Zealand forests. Annu Rev Phytopathol, 10**,** 299-326. doi: 10.1146/annurev.py.10.090172.001503
Otrosina W & Marx D (1975) Populations of *Phytophthora cinnamomi* and *Pythium* spp. under shortleaf and loblolly pines in little leaf disease sites. Phytopathol. doi: 10.1094/Phyto65_1224
Pautasso M, Aas G, Queloz V & Holdenrieder O (2013) European ash (*Fraxinus excelsior*) dieback - A conservation biology challenge. Biol Conserv, 158**,** 37-49. doi: 10.1016/j.biocon.2012.08.026
Polat Z, Awan QN, Hussain M & AkgÜL DS (2017) First Report of *Phytopythium vexans* Causing Root and Collar Rot of Kiwifruit in Turkey. Plant Dis. doi: 10.1094/PDIS-11-16-1554-PDN
Pratt B & Heather W (1973) The origin and distribution of *Phytophthora cinnamomi* Rands in Australian native plant communities and the significance of its association with particular plant species. Aust J Biol Sci, 26**,** 559-574. doi: 10.1071/BI9730559
Raghavendra AKH, Bissett AB, Thrall PH, Morin L, Steinrucken TV, Galea VJ, Goulter KC & van Klinken RD (2017) Characterisation of above-ground endophytic and soil fungal communities associated with dieback-affected and healthy plants in five exotic invasive species. Fungal Ecol, 26**,** 114-124. doi: 10.1016/j.funeco.2017.01.003
Rea AJ, Jung T, Burgess T, Stukely MJC & Hardy GESJ (2010) *Phytophthora elongata* sp. nov., a novel pathogen from the *Eucalyptus marginata* forest of Western Australia. Australas Plant Pathol, 39**,** 477-491. doi: 10.1111/ppa.12436
Rizzo DM & Garbelotto M (2003) Sudden oak death: endangering California and Oregon forest ecosystems. Front Ecol Environ, 1**,** 197-204. doi: 10.2307/3868064
Robideau GP, de Cock AWAM, Coffey MD, Voglmayr H, Brouwer H, Bala K, Chitty DW, Désaulniers N, Eggertson QA, Gachon CMM, Hu C-H, Küpper FC, Rintoul TL, Sarhan E, Verstappen ECP, Zhang Y, Bonants PJM, Ristaino JB & Lévesque CA (2011) DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. 11**,** 1002-1011. doi: 10.1111/j.1755-0998.2011.03041.x
Sacdalan A (2015) *Mimosa pigra* dieback in the Northern Territory, Australia: Investigation into possible causes Dissertation, The University of Queensland.
Scott P, Burgess T & Hardy GESJ (2013) Globalization and *Phytophthora*. *In:* LAMOUR, K (ed.) *Phytophthora*: A Global Perspective. Wallingford, UK: CAB International.
Spies CFJ, Mazzola M & McLeod A (2011) Characterisation and detection of *Pythium* and *Phytophthora* species associated with grapevines in South Africa. Eur J Forest Pathol, 131**,** 103. doi: 10.1007/s10658-011-9791-5
Steinrucken TV, Bissett A, Powell JR, Raghavendra AKH & van Klinken RD (2016) Endophyte community composition is associated with dieback occurrence in an invasive tree. Plant Soil, 405**,** 311-323. doi: 10.1007/s11104-015-2529-y
Steinrucken TV, Raghavendra AKH, Powell JR, Bissett A & van Klinken RD (2017) Triggering dieback in an invasive plant: endophyte diversity and pathogenicity. Australas Plant Pathol, 46**,** 157-170. doi: 10.1007/s13313-017-0472-5
Templeton GE & Greaves MP (1984) Biological control of weeds with fungal pathogens. Trop Pest Manage, 30**,** 333-338. doi: 10.1080/09670878409370907
Tewoldemedhin YT, Mazzola M, Botha WJ, Spies CFJ & McLeod A (2011) Characterization of fungi (*Fusarium* and *Rhizoctonia*) and oomycetes (*Phytophthora* and *Pythium*) associated with apple orchards in South Africa. Eur J Forest Pathol, 130**,** 215-229. doi: 10.1007/s10658-011-9747-9
Thorp JR & Lynch R (2000) The Determination of the Weeds of National Significance. National Weeds Strategy Executive Committee. Launceston.
Toh R (2009) Investigation of fungi pathogenic towards seedlings of *Parkinsonia aculeata* – their potential for use as mycoherbicides. Dissertation, The University of Queensland.

458 Uzuhashi S, Tojo M & Kakishima M (2010) Phylogeny of the genus *Pythium* and description of
459 new genera. Mycoscience, 51**,** 337-365. doi: 10.1007/s10267-010-0046-7
460 van Klinken RD, Campbell SD, Heard TA, McKenzie J & March N (2009) The biology of
461 Australian weeds: *Parkinsonia aculeata* L. Plant Prot Q, 24**,** 100-117. doi:
462 van Klinken RD, Flack LK & Pettit W (2006) Wet-season Dormancy Release in Seed Banks of a
463 Tropical Leguminous Shrub is Determined by Wet Heat. Ann Bot, 98**,** 875-883. doi:
464 10.1093/aob/mcl171
465 Vawdrey LL, Langdon P & Martin T (2005) Incidence and pathogenicity of *Phytophthora*
466 *palmivora* and *Pythium vexans* associated with durian decline in far northern Queensland.
467 Australas Plant Pathol, 34**,** 127-128. doi: 10.1071/ap04093
468 Vega FE, Simpkins A, Aime MC, Posada F, Peterson SW, Rehner SA, Infante F, Castillo A &
469 Arnold AE (2010) Fungal endophyte diversity in coffee plants from Colombia, Hawai'i,
470 Mexico and Puerto Rico. Fungal Ecol, 3**,** 122-138. doi: 10.1016/j.funeco.2009.07.002
471 Vilgalys R (2003) Taxonomic misidentification in public DNA databases. New Phytol, 160:1**,** 4-5.
472 doi: 10.1046/j.1469-8137.2003.00894.x
473 Widmer T, Graham J & Mitchell D (1998) Histological comparison of fibrous root infection of
474 disease-tolerant and susceptible citrus hosts by *Phytophthora nicotianae* and *P. palmivora*.
475 Phytopathol, 88**,** 389-395. doi: 10.1094/Phyto.1998.88.5.389.
476
477