The role of *Phytophthora* in predisposing *Corymbia calophylla* (marri) to a canker disease

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Title page photo

Corymbia calophylla (marri) with perennial canker on its main trunk.

John Forest National Park, Western Australia

Declaration

I declare that: a) This thesis is my own account of my research, except where other sources are acknowledged, b) All co-authors, where stated and certified by my principal Supervisor, have agreed that the work presented in this thesis represent substantial contributions from myself, and c) The thesis contains as its main content, work that has not been previously submitted for a degree at any other university.

To the best of my knowledge, all work performed by others, published or unpublished, has been acknowledged.

Louise Croeser

August 2021

Abstract

Corymbia calophylla (marri), a keystone tree species in the global biodiversity hot spot of south-western Australia, is suffering decline and mortality due to canker disease caused by the endemic fungus *Quambalaria coyrecup*. *Phytophthora* species, fine root oomycete pathogens, are frequently isolated from the rhizosphere of dying *C. calophylla*, raising the possibility that a *Phytophthora* infection predisposes *C. calophylla* to this endemic canker pathogen by compromising its defence mechanisms. Field surveys conducted across the *C. calophylla* range, found *Phytophthora* to be present in the rhizosphere of *C. calophylla*. Five *Phytophthora* species (*P. cinnamomi*, *P. elongata*, *P. multivora*, *P. pseudocryptogea* and *P. versiformis*) were recovered from healthy and cankered *C. calophylla*. *Phytophthora* incidence was significantly higher in anthropogenically disturbed areas. Pot infestation trials were conducted where the *C. calophylla* plants were inoculated with the recovered *Phytophthora* species. A significant reduction in root volume and even seedling death were observed, demonstrating that *Phytophthora* can adversely affect *C. calophylla* health. In a follow-up trial, *C. calophylla* plants were inoculated with both *P. cinnamomi* and *Q. coyrecup* and subjected to a drought stress treatment. Results indicated that neither *P. cinnamomi* nor the drought stress treatments exacerbated the pathogenic effect of *Q. coyrecup* on the plants. During these trials, weekly reflectance spectroscopic measurements with a portable high-resolution spectroradiometer, were also taken to investigate its potential to track biochemical changes in the *C. calophylla* leaves due to these treatments. Reflectance values displayed differences between treatments, as well as a seasonal trend in the leaves. Bandwidths in the visible and shortwave infrared regions of the electromagnetic spectrum were demonstrated to be important regions for characterising *C. calophylla* response to the *Phytophthora*, *Q. coyrecup*, waterlogging and drought stress treatments. More work is required to identify the optimum wavelengths for *C. calophylla*. Once the optimum bandwidths have been determined, reflectance spectroscopy measurements can be scaled up to canopy level, using unmanned vehicles or fixed-wing aircraft; thus, aiding in the management of this canker disease in *C. calophylla*.

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List of Publications

Journal articles

Chapter 2 published as: Croeser, L., Paap, T., Calver, M.C., Andrew, M.E., Hardy, G.E.St.J., Burgess, T.I. (2018). Field survey, isolation, identification, and pathogenicity of *Phytophthora* species associated with a Mediterranean-type tree species. *Forest Pathology* 48, 3.

Results from Chapter 2 also contributed to the species description paper: Paap, T., Croeser, L., White, D., Aghighi, S., Barber, P., Hardy, G.E.St.J., Burgess, T.I. (2017). *Phytophthora versiformis* sp. nov., a new species from Australia related to *P. quercina*. *Australasian Plant Pathology*, 46, 4, 369 – 378.

Chapter 3 published as: Croeser, L., Admiraal, R., Burgess, T.I., Hardy, G.E.St.J. (2021). Reflectance spectroscopy to characterise the response of a Mediterranean-type tree species to *Phytophthora* root rot and waterlogging stress. *Forestry: An International Journal of Forest Research*.

Conference presentations

Croeser, L., Paap, T., Hardy, G.E.St.J., Burgess, T.I. Is the widespread decline in the health of *Corymbia calophylla* (marri) driven by *Phytophthora* root disease? 2013 Australian Plant Pathological Society Student Conference, 17 September 2013, Perth, Australia. Oral presentation.

Croeser, L., Burgess, T.I., Hardy, G.E.St.J., Paap, T., Andrew, M.E. Spectral measurements for detecting *Phytophthora*-related stress in *Corymbia calophylla* (marri). 2014 IUFRO Conference, 14 November 2014, Esquel, Argentina. Oral presentation.

Croeser, L., Paap, T., Andrew, M.E., Hardy, G.E.St.J., Burgess, T.I. Does *Phytophthora* predispose marri (*Corymbia calophylla*) to *Quambalaria coyrecup* canker? 2015 Australian Plant Pathological Society, 14-16 September 2015, Fremantle, Perth, Australia. Oral presentation.

Croeser, L., Paap, T., Burgess, T., Hardy, G.E.St.J., Andrew, M.E. Can spectral measurements be used to detect *Phytophthora*-related stress in *Corymbia calophylla* (marri)?, 2015 Australian Plant Pathological Society, 14-16 September 2015, Fremantle, Perth, Australia. Poster.

Croeser, L., Paap, T., Burgess, T., Hardy, G.E.St.J., Andrew, M.E. Spectral measurements as an indicator of stress caused by *Phytophthora* species in *Corymbia calophylla* (marri)?

2015 Conference for the Ecological Society of Australia (ESA), 25 November 2015, Adelaide, Australia. Poster.

Croeser, L., Paap, T., Andrew, M.E., Hardy, G.E.St.J., Burgess, T.I. The role of *Phytophthora* in *Corymbia calophylla* (marri) canker disease. 2016 Dieback Working Group (DIG) Conference, 22 July 2016, Murdoch University, Perth, Australia. Oral presentation.

Croeser, L., Paap, T., Calver, M., Andrew, M.E., Hardy, G.E.St.J., Burgess, T.I. The role of drought stress and *Phytophthora* in *Corymbia calophylla* (marri) canker disease. 2016 Conference for the Ecological Society of Australia (ESA), 28 November 2016, Fremantle, Perth, Australia. Oral presentation.

Croeser, L., Andrew, M.E., Hardy, G.E.St.J., Burgess, T.I. Assessing *Corymbia calophylla* (marri) canker disease using hyperspectral imagery. 2018 Dieback Working Group (DIG) Conference, 29 August 2018, Perth, Australia. Oral presentation.

Croeser, L., Admiraal, R., Burgess, T.I., Hardy, G.E.St.J. The use of reflectance spectroscopy for detecting pathogen infection in *Corymbia calophylla* (marri). 2020 Dieback Working Group (DIG) Conference, 9 September 2020, Perth, Australia. Oral presentation.

Statement of Contributors

This thesis was co-supervised, mainly by Professor Treena Burgess and Professor Giles Hardy. Treena and Giles contributed to the form of ideas, experimental design, and editorial assistance. Co-supervisor Dr. Trudy Paap contributed to the field surveys and advice on the isolation and inoculation of the *Phytophthora* and *Q. coyrecup* isolates. Co-supervisor Dr. Margaret Andrew contributed by supplying the ASD field spectrometer for use in the glasshouse, as well as advise on the initial processing of the spectral data and editing the conference posters. Professors Mike Calver and Dr. Ryan Admiraal provided advice on the statistical analysis and interpretation of the data, and Adjunct Professor Paul Barber advised on the interpretation and presentation of the hyperspectral data. Volunteers (listed by name in the acknowledgements) provided field and glasshouse assistance throughout the project. Chapter 2 was published in collaboration with Treena, Giles, Mike, Trudy, and Meg. Chapter 3 was published in collaboration with Treena, Giles, Ryan, and Paul, and Chapter 4 will also be published with Treena, Giles, Ryan, and Paul.

Chapter 2 presents results of research published in the following journal articles:

Croeser, L., Paap, T., Calver, M.C., Andrew, M.E., Hardy, G.E.St.J., Burgess, T.I. (2018). Field survey, isolation, identification and pathogenicity of *Phytophthora* species associated with a Mediterranean-type tree species. *Forest Pathology*, 48, 3.

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Look deep into nature, and then you will understand everything better. Albert Einstein

Chapter 1: Introduction

Tree decline concepts

In a natural ecosystem, the impact of indigenous pathogens is minimised by the genetic diversity of the native tree population (Manion, 1991). However, when an exotic pathogen is introduced, often the trees have no resistance to the introduced pathogen and its introduction could cause the elimination of an entire species and permanent changes to the species composition in the affected ecosystem (Burgess and Wingfield, 2002). The Chestnut Blight Disease in North America was caused by the introduction of the fungal pathogen *Cryphonectria parasitica* (Anagnostakis, 1987), and it is estimated that this disease killed an estimated four billion trees in the first half of the 20th century, practically decimating chestnut tree populations in North America and Canada.

Often-changing climate conditions favour foreign pathogen germination and growth, but at the same time stresses potential hosts too, thus leaving the hosts vulnerable to infection. Pests and diseases may migrate to new climate-suitable locations faster than their hosts can adapt to the new conditions, thereby causing greater disease outbreaks and disturbances (Sturrock et al., 2011). This, together with land-use change and increased global connectivity, result in the higher risk of biological (pathogen or pest) invasions (Pauchard et al., 2015).

Disease within a tree is reliant on three components, i.e., the presence of a susceptible host, a virulent pathogen, and favourable environmental conditions conducive to the interaction of these three components over time. This interaction is described as the 'disease triangle', and all three these components need to be in place for disease to occur.

The factors contributing to tree decline are described in Manion (1991) as a decline disease spiral model (Fig. 1.1), where the factors are categorised as predisposing, inciting and contributing, which may include:

- Predisposing factors, i.e., climate, site, age, genetic predisposition that can predispose trees to the inciting factors
- Inciting factors, which are typically short-term and may be abiotic (i.e., drought) or biotic (i.e., insect defoliation); trees may recover quickly, but

predisposed trees can go into rapid decline, making them vulnerable to the contributing factors

- Contributing factors, which usually are opportunistic organisms such as leaf parasites, stem borers, root pathogens and canker fungi

When all three types of factors are present, trees appear unable to recover and eventually die.

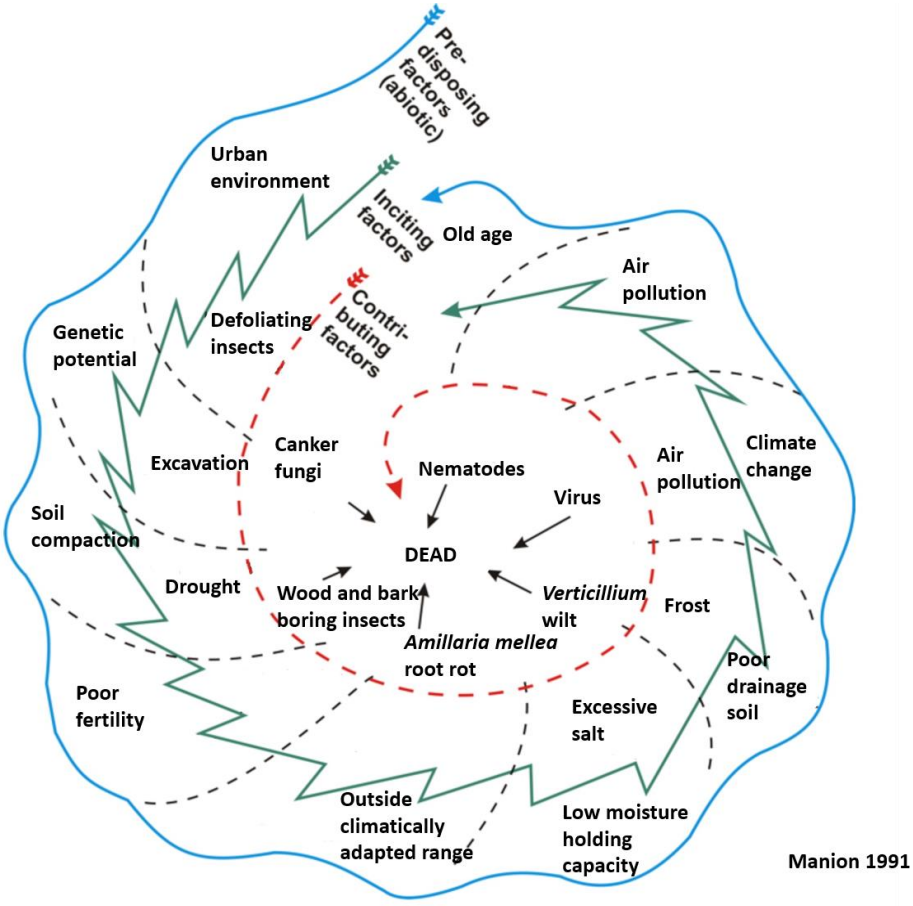

Figure 1.1. Decline disease spiral model, with predisposing, inciting and contributing factors, leading to tree decline and ill health

Role of *Phytophthora* in tree decline

Phytophthora, a genus of plant pathogenic oomycetes from the order *Peronosporales*, contains a group of well-known agricultural crop pathogens, causing a variety of diseases on many hosts, affecting the roots, stems, leaves and fruit of seedlings, annual plants and older trees alike (Erwin and Ribeiro, 1996). It has a long

history; one of the earliest reports lists *P. infestans* as the cause of the devastating potato late blight epidemic in Ireland in 1845 (De Bary, 1876).

Phytophthora survives unfavourable conditions by forming thick-walled chlamydospores (asexually reproduced), oospores (which are sexually produced) or even mycelium in infected roots, stems or soil. When conditions become favourable again, i.e., when free water is present in the soil or on aerial plant surfaces, zoospores are released in large numbers from sporangia formed by the oospores or chlamydospores. The zoospores of most *Phytophthora* species are motile but require free water for dispersal to susceptible hosts. The zoospores move around by either by propelling itself forward or by passive movement of the water. The zoospores of root-inhabiting *Phytophthora* species are attracted to root tips due to secretions of some plants. When reaching the roots of a suitable host, the zoospores penetrate the roots, germinate, and grow within the host.

Phytophthora species appear to be widespread and are found in very diverse ecosystems, far beyond agriculture (Hansen et al., 2012). Recent studies have uncovered new *Phytophthora* species from more complex forest ecosystems too, in forests soils, streams and even canopies. *Phytophthora* species associated with forest trees can be classified as aquatic opportunists, foliar pathogens, soil-borne fine-root and canker pathogens (Hansen et al., 2012). In each of these groups, there are aggressive, invasive species.

Phytophthora has been linked to tree decline globally too. It has the ability to cause disease in all the life stages of a forest tree, from root to crown, to causing trunk cankers and foliar blight (Erwin and Ribeiro, 1996). *Phytophthora* is especially invasive in areas where they were introduced recently; European and North American chestnuts (*Castanea* spp.) dramatically declined when the root rot species, *P. cinnamomi* was introduced (Anagnostakis, 1987). *Phytophthora ramorum*, another introduced pathogen, was the cause of trunk cankers and leaf disease with oaks (*Quercus* spp.) and tanoak (*Lithocarpus densiflorus*) in North America (Rizzo et al., 2002), resulting in the devastating die-off of these trees.

In Australia, *Phytophthora*, and especially *P. cinnamomi* is also recognised as a significant contributor of dieback in native vegetation and many forest trees (Cahill et al., 2008, Davison and Shearer, 1989). In 1992, the Commonwealth of Australia declared *P. cinnamomi* as one of five major threats to plant biodiversity in Australia. In Western Australia (WA), an area declared as one of the world's biodiversity hotspots for having an exceptionally high plant diversity, *Phytophthora* is also considered a significant pathogen to its native shrubs and tree species. Approximately, 40% (2 284) of the 5 710 native species in WA tested in pot infestation trials are considered susceptible to *P. cinnamomi* , and another 14% (800) highly susceptible (Shearer et al., 2004). Other *Phytophthora* species are also considered pathogenic to WA's native forest trees and vegetation. *Phytophthora multivora* was isolated from the rhizosphere soils of declining *Eucalytus gomphocephala*, *E. marginata*, *Agonis flexuosa* and 13 other plant species (Scott et al., 2009). In surveys of dying vegetation within remnant bushland, parks, gardens and streetscapes throughout the urban forest of Perth and south-west Western Australia, nine different *Phytophthora* species were recovered (Barber et al., 2013). Many of these recovered *Phytophthora* species, i.e., *P. alticola*, *P. multivora*, *P. litoralis*, *P. inundata*, *P. nicotianae* and *P. palmivora*, *P. aff. arenaria*, *P. aff. humicola* and *P. sp. ohioensis* were recovered from the rhizosphere of native trees.

Corymbia calophylla (marri)

Corymbia calophylla belongs to the Myrtaceae family. Some other distinct native trees belong to this group too, e.g., ghost gum (*C. apparrerinja*), spotted gum (*C. maculata*), lemon-scented gum (*C. citriodora*) and the red flowering gum (*C. ficifolia*). All the species in this family are woody, evergreen and have essential oils. They are also known as *bloodwoods*, as their trunks exude a dark red gum (kino) when injured. *Corymbia calophylla* has leathery leaves and clusters of white, cream, or light pink flowers in late summer/early autumn, making it popular with apiarists (https://perthhoneycompany.com.au/marri-honey/).

Corymbia calophylla is a much loved and popular tree in WA, often planted as an amenity tree on paddocks, road verges and big parks. The native habitat of this tree

is the jarrah forest (Fig. 1.2), where, together with *Eucalyptus marginata* (jarrah), it forms part of the dominant overstory tree species. The jarrah forest is found mainly on the western edge of the Darling scarp. The area has a Mediterranean climate, with long dry summers and warm easterly winds to mild, wet winters (Shearer and Tippett, 1989b). The jarrah forest is rich in wildlife, hosting several endangered marsupials such as the numbat (*Myrmecobius fasciatus*) and woylie (*Bettongia penicillata*). The immature pods of *C. calophylla* serve as food source for the red-capped parrot (*Purpureicephalus spurius*) as well as Australian ringnecks (*Barnardius zonarius*), and its mature seed is an important food source for the three locally occurring black cockatoo spp., i.e., the endangered Carnaby's black cockatoos (*Calyptorhynchus latirostris*), Baudin's cockatoo (*Calyptorhynchus baudinii*), and the forest red-tailed black cockatoo (*Calyptorhynchus banksii*).

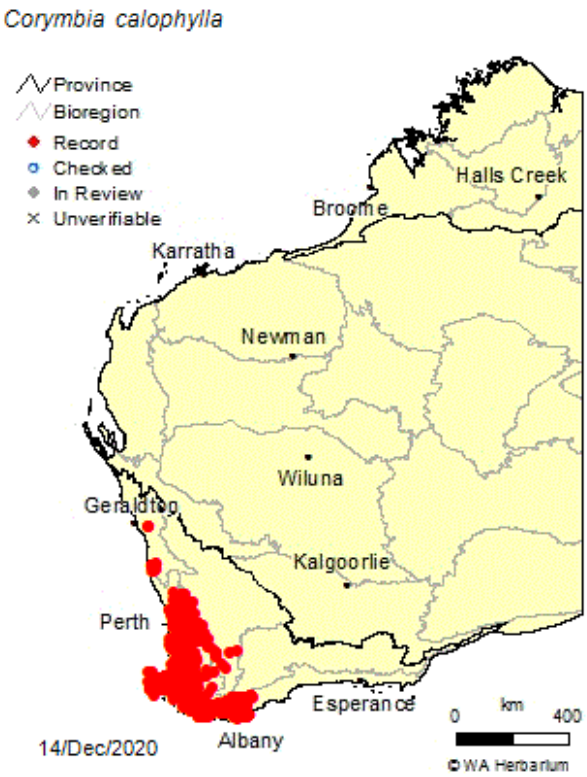

Figure 1.2. Native range of *Corymbia calophylla* (marri) in Western Australia (https://florabase.dpaw.wa.gov.au)

Canker disease of *C. calophylla*

There are reports, dating back as far as the late 1920's, of a canker disease affecting *C. ficifolia* (red flowering gum), a sister tree of *C. calophylla*, which at the time were

planted in Kings Park (Perth, Western Australia) as amenity trees, bordering Fraser Lane in the park (Fig. 1.3) (Smith, 1970), in honour of Queen Victoria's Jubilee.

Figure 1.3. Fraser Lane in Kings Park in the 1930's, with *Corymbia ficifolia* (red flowering gum) flanking the lane. Image. Botanic Gardens and Parks Authority

By 1938, most of these red flowering gums had succumbed to this canker disease, thus they were removed and replaced with the lemon-scented gums (*C. citriodora*). By the late 1930's the canker disease had also been reported on *C. calophylla*, and by the 1970's widespread canker disease among *C. calophylla* was observed (Smith, 1970), raising concern with the community. Paap et al. (2008) identified the pathogen as *Quambalaria coyrecup*. This pathogen is a basidiomycete fungus, and evidence suggest that this pathogen is endemic to SWWA ((Paap et al., 2016). Disease incidence is significantly greater on trees growing on anthropogenically disturbed sites, such as paddocks and along roadsides (Paap et al., 2016). Usually, the pathogen enters the tree via openings caused by injury, where it germinates and grows inside the tree and causes the bark to crack (Fig. 1.4A). The tree responds to the pathogen by producing kino and tries to wall it off by depositing a cellulose barrier layer in the affected area. When conditions become favourable for the pathogen, it grows again, and the tree responds again by depositing another layer of cellulose close to the pathogen; this is typical of a perennial canker and results in concentric rings of wood killed progressively year by year, resembling a target. Often

Q. coyrecup girdles its host and in the process eventually kills it too. Frequently, cankers develop in the smaller branches of the tree first, causing it to die off – this results in 'flagging' (Fig. 1.4B).

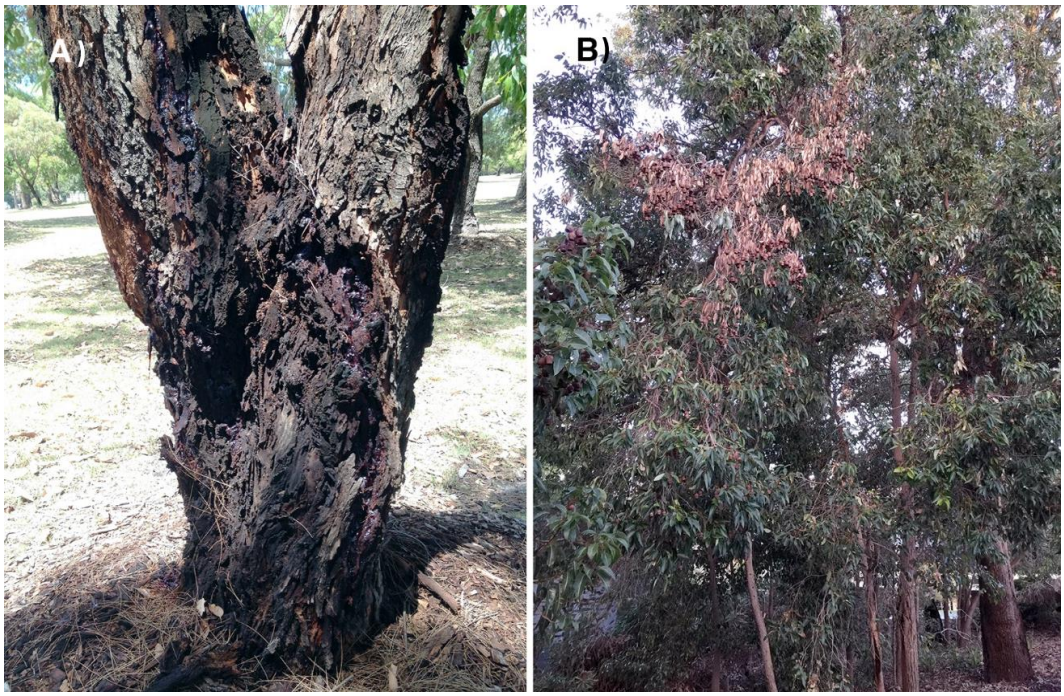

Figure 1.4. A) Cankered *Corymbia calophylla* trunk, with cracked bark and sunken patches visible, B) 'Flagging' in *C. calophylla*; the presence of a dead upper branch due to the canker disease.

Corymbia calophylla cankers are worst in disturbed areas linked to *Phytophthora* introduction, raising the possibility that a *Phytophthora* root infection could play a role in *C. calophylla* canker by affecting its defence mechanisms (Paap et al., 2017a) , thus predisposing it to canker disease. *Corymbia calophylla* is considered field-resistant to *P. cinnamomi*, but a range of *Phytophthora* spp. (including *P. cinnamomi*) are often recovered from the rhizosphere of dying *C. calophylla* (Barber et al., 2013).

Reflectance spectroscopy

Surveying for *C. calophylla* canker and *Phytophthora* is labour some and expensive. There is a need for exploring alternative methods in assessing *C. calophylla* for the presence of *Phytophthora* and/- or cankers. Recent development in the use of reflectance spectroscopy as a tool for assessing tree health is often reported in the literature. It has long been used in agriculture and ecology to measure biotic and abiotic stress with plants (Table 1.1). Reflectance spectroscopy is a quick and non-

invasive technique, whereby the amount of light reflected from leaf pigments into the atmosphere is measured and used to assess vegetation health.

Reflectance values in the different areas of the electromagnetic spectrum are related to different traits in the leaves (Fig. 1.5). In the red and blue regions of the visible range (390–750 nm) of the electromagnetic spectrum, chlorophyll absorbs light strongly, with maximum absorption between 660 and 680 nm. In the red edge region (680–780 nm) an abrupt change in leaf reflectance takes place, from strong absorption of visible light by the chlorophyll pigments, to the high reflection thereof (Cho and Skidmore, 2006). This is an important region in determining stress with vegetation (Vogelmann et al., 1993). In the near-infrared region (NIR) (750–1 400 nm) multiple scattering of light due to the internal leaf structure and canopy architecture takes place, while liquid water (1 400–1 850 nm) and foliar dry matter (1 850–2 500 nm) absorption takes place in the shortwave infrared region (SWIR) (1 400–2 500 nm) (Croft et al., 2014).

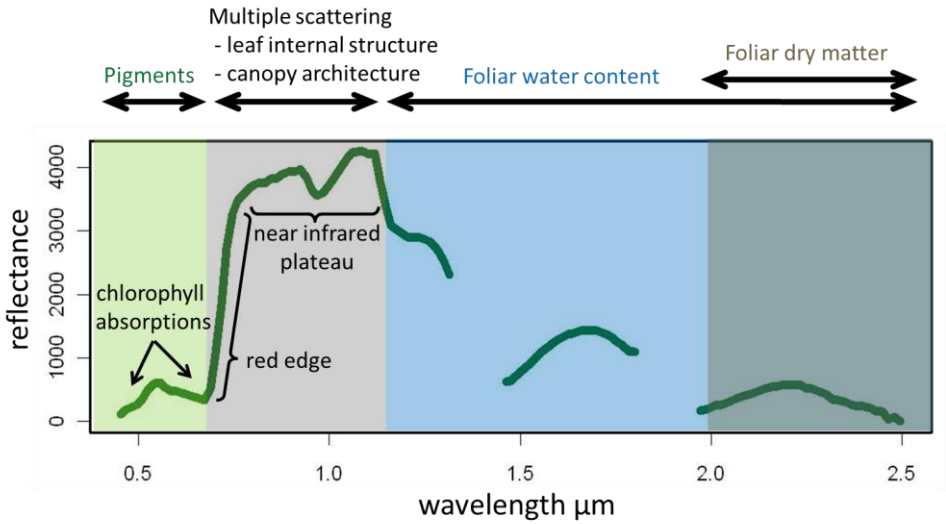

Figure 1.5. Reflectance of light by vegetation in the different areas of the electromagnetic spectrum. Image Margaret Andrew

Though this list is not exhaustive, Table 1.1 summarises some of the uses of reflectance spectroscopy with vegetation. It can be used to assess vegetation health (Chavana-Bryant et al., 2017, Babar et al., 2006, Bolton and Friedl, 2013, Gitelson et al., 2005, Coops et al., 2004). It can also be used to assess stress in vegetation due to

dust storms (Boloorani et al., 2020), drought (Zhang et al., 2017b, Rallo et al., 2014, Dzikiti et al., 2010) or damage caused by drought (Andrew et al., 2016). Reflectance spectroscopy can also be used to detect insect stress with trees (Stone et al., 2001, Delalieux et al., 2007). In plant pathology, reflectance spectroscopy can used to detect a variety of diseases; examples include laurel wilt disease (*Raffaelea lauricola)* with avocado (Abdulridha et al., 2018), *Verticillium* wilt (*Verticillium dahlia)* in olive trees (Calderón et al., 2013), powdery mildew (*Blumeria graminis* f.sp. *tritici*) in two winter grain species (Cao et al., 2015), myrtle rust (*Austropuccinia psidii*) with lemon myrtle trees (Heim et al., 2019), late blight (*Phytophthora infestans*) with potato (Gold et al., 2019), *P. cinnamomi* distribution in heathland (Hill et al., 2009) and *Cercospora* leaf spot (*Cercospora beticola*), powdery mildew (*Erysiphe betae*) and leaf rust (*Uromyces betae*) with sugar beet (Mahlein et al., 2012).

Reflectance spectroscopy measurements can be taken by satellites (i.e., Landsat, MODIS), by using cameras mounted onto light-wing aircraft or unmanned vehicles, or a variety of handheld devices, such as cameras mounted on a platform a particular distance away from the object, or by using a probe, which clips onto the leaves. In plant pathology, most reflectance measurements are taken with portable, handheld device (Table 1.1).

Table 1.1. Applications with reflectance spectroscopy *

Category	Application	Type of device, i.e., satellite, airborne or portable	Reference
Assessing Vegetation health	Assessing wheat yield under irrigation	Portable spectroradiometer (FieldSpec UV/VNIR)	(Babar et al., 2006)
	Forecasting crop yield	Satellite data collected by NASA with MODIS (Moderate Resolution Imaging Spectroradiometer)	(Bolton and Friedl, 2013)
	Leaf aging in Amazonian canopies	Portable spectrometer (FieldSpec Pro), with a contact probe	(Chavana-Bryant et al., 2017)
	Assessment of Eucalyptus crown condition	Field-based spectroradiometer (Personal Spectrometer II) and Compact Airborne Spectrographic Imager 2 (CASI-2)	(Coops et al., 2004)
	Remote estimation of canopy chlorophyll	Dual system Ocean Optics USB2000 radiometer, mounted on a platform	(Gitelson et al., 2005)
Detecting drought stress	Assessing drought disturbance in forests	Aerial surveys, with a hyperspectral camera mounted on a fixed-wing aircraft	(Andrew et al., 2016)
	Detection of water and dust storm stress with Persian oak	ASD FieldSpec-3 spectroradiometer, mounted on a tripod	(Boloorani et al., 2020)
	Determining water status of *Satsuma* mandarin trees	ASD FieldSpec Pro spectroradiometer, mounted on a platform	(Dzikiti et al., 2010)
	Detecting crop water status in mature olive trees	Portable ASD FieldSpec spectroradiometer, mounted on a platform	(Rallo et al., 2014)
	Drought stress with Mediterranean evergreen oaks	Portable LR1 spectroradiometer, mounted on a platform	(Zhang et al., 2017b)

Category	Application	Type of device, i.e., satellite, airborne or portable	Reference
Detecting insect stress	Insect damage with eucalyptus foliage	Field-based spectroradiometer (Personal Spectrometer II)	(Stone et al., 2001)
Detecting disease with vegetation	Laurel wilt disease (*Raffaelea lauricola*) with avocado	Spectroradiometer (SVC HR-1024iTM) with halogen light resources	(Abdulridha et al., 2018)
	Early detection of *Verticillium* wilt (*Verticillium dahliae)* of olive	High-resolution airborne hyperspectral and thermal imagery	(Calderón et al., 2013)
	Detection of powdery mildew (*Blumeria graminis* f. sp. *tritici*) in two winter wheat species	ASD Field Spec Pro spectrometer, mounted on a platform	(Cao et al., 2015)
	Detecting myrtle rust (*Austropuccinia psidii*) with lemon myrtle	Portable spectroradiometer (Spectral Evolution PSR+ 3500), with a leaf probe	(Heim et al., 2019)
	Identifying potato late blight (*Phytophthora infestans*) with potato	SVC HR-1024i field spectroradiometer, with a leaf probe	(Gold et al., 2019)
	Assess distribution of *P. cinnamomi* disease on heathland, by comparing characteristics between healthy and infected vegetation	Multispectral aerial data obtained by SpecTerra (with DMSI)	(Hill et al., 2009)
	Early detection of Cercospora leaf spot (*Cercospora beticola)*, powdery mildew (*Erysiphe betae*) and leaf rust (*Uromyces betae*) with sugar beet	IMSpector (V10E)	(Mahlein et al., 2012)
	Detecting apple scab (*Venturia inaequalis*) with apple trees, by identifying the most important spectral regions for distinguishing between infected and non-infected leaves	FieldSpec Pro JR spectroradiometer	(Delalieux et al., 2007)

Category	Application	Type of device, i.e., satellite, airborne or portable	Reference
Detecting disease with vegetation	Detection of *Phytophthora cinnamomi* infection and water-stress in four Australian native species	ASD FieldSpec-3 spectroradiometer	(Newby et al., 2019)
	Quantifying the severity of *Phytophthora* root rot disease in avocado trees	FLIR ONE Camera attached to a smart phone	(Salgadoe et al., 2018)
	Discrimination between laurel wilt disease (*Raffaelea lauricola*) and freeze damage with avocado	SVC HR-1024 Spectroradiometer;	(Sankaran et al., 2012)

*This list is not exhaustive.

Aims

This study aimed to investigate whether:

- *Phytophthora* is associated with cankered/dying *C. calophylla* across the *C. calophylla* range;

- *Phytophthora* root infection has a detrimental effect on *C. calophylla*;

  The results of the above-mentioned investigations are outlined in:
  Chapter 2: Field Survey, isolation, identification and pathogenicity of *Phytophthora* species associated with *Corymbia calophylla* (marri).

- *Phytophthora* root infection is predisposing *C. calophylla* to secondary pathogens, such as the endemic canker pathogen, *Quambalaria coyrecup*;

  The results of this investigation are outlined in:
  Chapter 4: The role of *Phytophthora* and drought stress in the canker disease of *Corymbia calophylla*.

- Reflectance spectroscopy can be used to determine *Phytophthora*, waterlogging, canker, and drought stress in *C. calophylla*.

  The results of this investigation are outlined in:
  Chapter 3: Reflectance spectroscopy to characterise the response of *Corymbia calophylla* to *Phytophthora* root rot and waterlogging, as well as:
  Chapter 4: The role of *Phytophthora* and drought stress in the canker disease of *Corymbia calophylla*.

Chapter 2: Field survey, isolation, identification and pathogenicity of *Phytophthora* species associated *Corymbia calophylla* (marri)

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Results from this chapter also contributed to the species description paper Paap, T., Croeser, L., White, D., Aghighi, S., Barber, P., Hardy, G.E.St.J., Burgess, T.I. (2017). *Phytophthora versiformis* sp. nov., a new species from Australia related to *P. quercina*. *Australasian Plant Pathology*, 46, 4, 369 – 378.

Abstract

Corymbia calophylla (marri), a keystone tree species in the global biodiversity hotspot of south-western Australia, is suffering decline and mortality associated with a canker disease caused by the endemic fungus *Quambalaria coyrecup*. *Phytophthora* species are frequently isolated from the rhizosphere of *C. calophylla,* and a hypothesis is that *Phytophthora* root infection is predisposing *C. calophylla* to this endemic canker pathogen. Field surveys were conducted in both anthropogenically disturbed and undisturbed *C. calophylla* stands from where a total of 100 rhizosphere soil samples, from both healthy and cankered trees, were collected. *Phytophthora* species were isolated from 26% of the samples collected, with *Phytophthora* incidence significantly higher on disturbed stands than in natural forests (73 and 27%, respectively). Five *Phytophthora* species were recovered, including *P. cinnamomi*, *P. elongata*, *P. multivora*, *P. pseudocryptogea* and *P. versiformis.* Under-bark inoculations with the recovered *Phytophthora* isolates caused significant lesion length differences in excised *C. calophylla* stems. *Phytophthora* isolates responsible for the longest lesion lengths were used in the follow-up pot infestation glasshouse trials. *Corymbia calophylla* response to inoculation with these selected *Phytophthora* isolates varied between *Phytophthora* species and isolates, with some isolates of *P. cinnamomi* and *P. multivora* causing a significant reduction in seedling root volume and often leading to seedling death. This study demonstrates that root disease caused by *Phytophthora* species, especially *P. cinnamomi* and *P. multivora*, can adversely affect *C. calophylla* health. The results in this study can be used to design a dual inoculation trial with the *Q. coyrecup*, and different *Phytophthora* species to investigate if *Phytophthora* root infection predisposes *C. calophylla* to this canker disease.

Introduction

Corymbia calophylla (marri), a keystone tree species in the global biodiversity hotspot in southwest Western Australia (SWWA), has been suffering from a severe canker disease (Fig. 2.1) since the 1960s (Smith, 1970). This canker disease is present across most of the *C. calophylla* range except in drier (Fig. 2.2) and warmer areas to the north and east (Paap et al., 2017a). The disease enters *C. calophylla* stems or

trunk by means of an opening or injury in the bark (Shearer, 1994, Smith, 1970), where it would develop into a canker, often girdling the tree. Disease development is slow, especially on the bigger branches – the ability of *C. calophylla* to exude kino probably slows down disease progress – but the final stages of this disease always lead to the death of the infected tree (Smith, 1970, Paap et al., 2016). The disease affects trees irrespective of height, diameter at breast height (DBH) or crown condition (Paap et al., 2016). The cause of this disease remained unknown (Shearer, 1992), until Paap et al. (2008) identified an endemic pathogen, *Quambalaria coyrecup* as the causal agent.

Figure 2.1. A and B) *Corymbia calophylla* displaying cracks and putative early canker development. The presence of kino indicates the tree is responding to a stress agent. C and D) Cankered *C. calophylla*: In both trees, the canker pathogen has almost girdled the tree trunk and the trees will die eventually. E and F) Healthy *C. calophylla* trees displayed an intact canopy with no flagging and the bark displayed no signs of cracking, kino exudation or cankers.

It is unusual for an endemic pathogen to have such a devastating effect on a co-evolved tree, implying that the natural ability of *C. calophylla* to combat this canker

pathogen is compromised by predisposing biotic or abiotic factors. Possible underlying causes could be due to a warmer, dryer climate (https://www.der.wa.gov.au/your-environment/climate-change), human activities like farming, forest logging, unnatural fire regimes (Jurskis, 2005, Davison, 1997, Paap et al., 2017a) or other pathogens attacking *C. calophylla*. Barber et al. (2013) reported a diverse number of *Phytophthora* species associated with dying urban trees, including *C. calophylla*.

Phytophthora species are often being implicated in tree declines around the world, such as the oak decline in Austria (Balci and Halmschlager, 2003), *Eucalyptus* crown dieback in New Zealand (Dick et al., 2006), *Quercus ilex* (Jung, 2009, Corcobado et al., 2013, de Sampaio e Paiva Camilo-Alves et al., 2013) and English Walnut decline in Europe (Vettraino et al., 2003), beech decline in Central Europe and the *Austrocedus chilensis* mortality in Argentina (Greslebin et al., 2007). The genus *Phytophthora* also has a long history in SWWA, where it has been cited many times as the cause of *Phytophthora* dieback in many endemic plant species (Shearer et al., 2004, Scott et al., 2006, Scott et al., 2012, Hüberli et al., 2002, Simamora et al., 2016, Bunny, 1996, Anderson et al., 2010). *Eucalyptus marginata* (jarrah) trees were reported as dying as early as 1921 (Wallace and Hatch, 1953), but it was not until 1964 that *Phytophthora cinnamomi* was identified as the cause of the dieback in *E. marginata* (Podger et al., 1965). Though recent work debates as to whether *Phytophthora* causes large-scale die-off of *E. marginata*, its pathogenicity to the mid- and understory plants and other trees in the forest remains unquestioned (Davison, 2015). In rehabilitated bauxite mine pits in the jarrah forest, *P. cinnamomi* was recovered from the roots of dead or dying *E. marginata* and *C. calophylla* trees (Hardy et al., 1996) and *P. elongata* was occasionally recovered from the roots of *C. calophylla* on these rehabilitated pits (Bunny, 1996). In the last two decades more than 20 new *Phytophthora* species have been described from SWWA (Burgess et al., 2021). One of these species, *P. multivora,* has been implicated in the decline of *Eucalyptus gomphocephala* (tuart) (Scott et al., 2012). In an investigation of *Phytophthora* species associated with trees exhibiting crown decline in urban and peri-urban sites of SWWA, *P. multivora* was also regularly isolated from *C. calophylla*

(Barber et al., 2013). Given the impact of *Phytophthora* species on root health, they may increase the susceptibility of *C. calophylla* to canker disease.

This study examined the presence and diversity of *Phytophthora* species in healthy and cankered *C. calophylla* grown in disturbed and relatively undisturbed forest stands. Subsequently, pathogenicity trials with the recovered *Phytophthora* isolates were conducted in the glasshouse.

Materials and Methods

Study sites, sampling and Phytophthora isolation procedure

A small-scale preliminary field survey (pilot study) was undertaken in the Boranup and Dunsborough areas (both anthropogenically disturbed stands) to evaluate the feasibility of the project. The purpose was to establish the association of *Phytophthora* species with cankered *C. calophylla* trees.

Soil was collected from the rhizosphere of cankered *C. calophylla* trees, and best practice Centre of Phytophthora Science and Management (CPSM at Murdoch University) soil sampling and baiting methods were used to recover the *Phytophthora* species resident in the soils. Soil and fine roots were bulked by tree. Shovels, axes, and secateurs used to collect the soil and root samples were sterilised with 70% ethanol (70% ethanol, 30% de-ionised water) solution and left to dry between samples. Samples were placed in clearly labelled zip lock plastic bags and stored in cooler bags for transportation to the laboratory. Back at the laboratory, approximately 200 g of each soil sample was placed in plastic containers, flooded, and young, fully emerged leaves of *Scholtzia involucrata*, *Quercus suber*, *Q. ilex*, *Eucalyptus sieberi* cotyledons, germinated lupin (*Lupinus angustifolius*) seedlings and petals of *Hibbertia* species were placed on top of the water, serving as bait for the *Phytophthora* motile zoospores. These containers were kept in a temperature-controlled room (21-25 °C). The leaves were inspected daily for ten days, and any discoloured or necrotic areas of the leaf were excised and plated on NARH agar, a *Phytophthora* selective medium (Hüberli et al., 2000, Simamora et al., 2017). The petri dishes with NARH agar and excised leaf samples were incubated in the dark at

21°C (+/- 1°C). Clean cultures were plated on half strength Potato-dextrose agar (PDA) (Becton Dickinson, Sparks, MD, 9.75 g PDA, 3.7g Grade A bacteriological agar and 500 mL of distilled water) and stored in the dark at constant temperature (21°C, +/- 1°C). *Phytophthora* isolates were identified using the DNA sequence data for the internal transcribed spacer (ITS) rDNA as previously described by Aghighi et al. (2015). All isolates were deposited in the CPSM culture collection, and sequences were submitted to GenBank for future reference.

After confirming the presence of *Phytophthora* spp. in the rhizosphere of *C. calophylla* at these sites, a formal field survey was conducted at five sites across the *C. calophylla* distribution range (Fig. 2.2). These field surveys were conducted from late winter to early summer (southern hemisphere July to November), the appropriate time for *Phytophthora* recovery in SWWA (Morgan and Shearer, 2013) as conditions of both temperature and moisture would be suitable for sporulation (Podger et al., 1965).

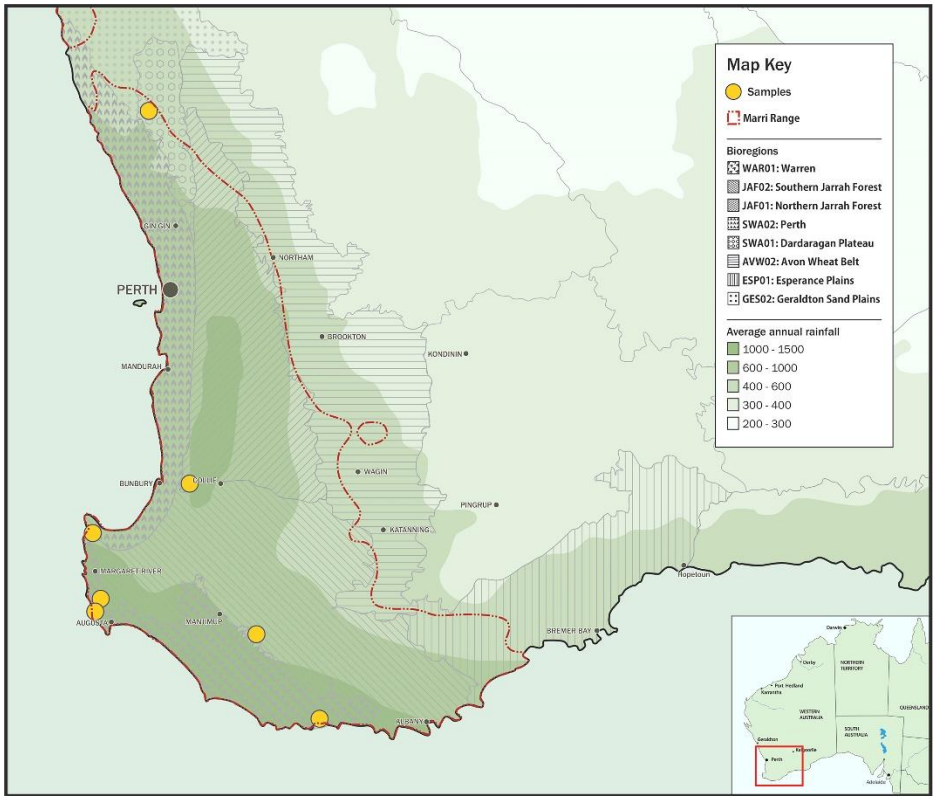

Figure 2.2. Map of sites (yellow dots) located in the southwest of Western Australia where soil samples from the rhizosphere of *Corymbia calophylla* were taken.

Sites ranged from Dandaragan, 170 kilometres north of Perth to Kentdale in the south of Western Australia and covered five bio-subregions (Fig. 2.2). These sites were comparable in terms of age and tree diversity, and all stands displayed presence of cankers. Two stands (approximate 100 x 100 metres) per site in a paired plot design were selected; an intact forest stand and a disturbed (along a road or on a farming property) stand adjacent or very close (within 500 metres) to the intact forest stand (Appendix 2 Table 1). These sites have a typical Mediterranean climate with wet winters and prolonged dry summers.

Ten *C. calophylla* trees were randomly selected in each stand: five healthy and five cankered trees. A healthy *C. calophylla* tree was defined as a tree having a dense crown with the absence of any signs of defoliation (Souter et al., 2009) or flagging of smaller branches, as well as no appearance of cankers on the trunk or branches of the tree. A cankered *C. calophylla* tree had one to many cankers on the trunk or the branches of the tree, displaying the typical symptoms of a canker caused by *Q. coyrecup* (Fig. 2.1) (Paap et al., 2012).

Excised branch trials

The *Phytophthora* isolates recovered from the field survey were screened in two separate under-bark inoculation trials using excised *C. calophylla* branches as described by Hüberli et al. (2002). One-year old green lateral branches, with a mean diameter of 8.0 ± 0.3 mm and 1000 mm in length, were collected from *C. calophylla* trees at Jarrahdale (S 32° 20' 20.4", E 116° 3' 43.2") from six-year-old restored mine sites. The purpose of these branch inoculation trials was to identify the most aggressive isolates to be used in the glasshouse pathogenicity pot infestation trials. The first branch inoculation trial was conducted with 29 isolates (Appendix 2 Table 2) recovered from the field survey. The second branch inoculation trial had 15 isolates of the then undescribed species *P. versiformis* (Paap et al., 2017b), one from the field survey (MJ5) and 14 from the CPSM collection. A *P. cinnamomi* (MHSFH2) and *P. multivora* (TRH5) isolate from the first branch inoculation trial (Appendix 2 Table 2) were also included. The second trial was conducted as *P. versiformis* was only recently being isolated from *C. calophylla* rhizosphere samples from Perth urban parklands and across its SWWA range on a regular basis (Barber et al., 2013, Paap et al., 2017b). Branches inoculated with sterile agar plugs were used as a control treatment in both trials.

Ten replicate branches for each isolate (treatment) were used in both trials. One replicate of each treatment was placed, together with a replicate from each other treatment in a separate plastic bag and kept in a constant-temperature incubator at 24 +/- 2°C for 10 days. When the experiment was terminated, the bark was scraped off from the branches and the length of the exposed necrotic lesion measured. Necrotic material from two randomly selected replicates per isolate, as well the asymptomatic tissue from the control branches, was plated on NARH medium in petri dishes to confirm Koch's postulates.

Glasshouse pathogenicity trials

Two soil-infestation pot trials were performed, the first trial was conducted during the winter and spring (southern hemisphere April to September), and the second

from summer to winter (southern hemisphere January to June). For the first trial 18 isolates were used; the 15 most aggressive *Phytophthora* isolates (as determined from the excised branch assays), and one isolate each of *P. boodjera* (PN12), *P. nicotianae* (PAB10-104) and *P. elongata* (BJi) from the CPSM collection (Appendix 2 Table 2). For the second trial, seven isolates used in the first pathogenicity trial and three *P*. *versiformis* isolates from the CPSM collection were used (Appendix 2 Table 2). A non-inoculated control was included in both trials.

The same protocol and seed from the same seed lot was used for both trials, according to the methods of Aghighi et al. (2015) and Simamora et al. (2016). Six-month old *C. calophylla* seedlings, grown in an evaporative cooled glasshouse (12°C - 25°C) were used for the trials. There were eight replicates per isolate and the pots were placed in a complete randomized block design in. Seedlings were watered daily to container capacity and were fertilised monthly with Thrive® (water soluble, Yates Company, Australia) at one-third the manufacturer's recommended rate.

Vermiculite inocula were prepared according to the method of Simamora et al. (2016). Once the inocula were ready, eight grams of inocula per litre sand were placed near the seedling roots. Inocula viability was confirmed by plating three grams inoculum on NARH medium. Once inoculated, seedlings were watered by hand with de-ionised water to prevent any water splash between pots. To stimulate zoospore production, the containers were flooded immediately after inoculation and then fortnightly for the duration of the experiment according to Aghighi et al. (2015).

Where seedling death resulted, time to death post-inoculation was recorded. After three months, the surviving seedlings were harvested. The sand was washed gently from the roots with de-ionised water, to minimize the loss of the fine roots. The root volume was determined by measuring the amount of water the roots displaced when put into a measuring cylinder filled with water (Pang et al., 2011). The roots and tops of seedlings were dried at 60°C for four weeks and weighed. Necrotic roots and root collars were plated on NARH medium in petri dishes to confirm Koch's postulates.

Data analyses

For the field survey, logistic regression models ('binomial' family with 'logit' link) were used to analyse the data with the aid of the "stats", "vegan", "MKmisc" and "Rmisc" packages in R (R Development Core Team 2015, http://www.R-project.org/). The first model evaluated the effect of stand status on the presence of *Phytophthora and* had stand and stand status (disturbed vs. undisturbed) as the predictor variables and the presence of *Phytophthora* as the response variable. The second model evaluated the effect of the presence of *Phytophthora* on the health of the *Corymbia calophylla* trees and had stand, stand status (disturbed vs. undisturbed) and *Phytophthora* presence (present vs. absent) as the predictor variables and tree status (cankered vs. healthy) as the response variable. The Hosmer-Lemeshow test for goodness of fit was performed on these two logistic regression models, to assess whether the models were a good fit for the data.

For under bark inoculations, the R packages "agricolae", "dplyr" and "Rmisc" (R Development Core Team 2015, http://www.R-project.org/) were used to analyse the data. Data exploration followed the protocol described by Zuur et al. (2010) and indicated the presence of outliers in the lesion length data, thus the lesion lengths were log-transformed. Model assumptions were verified by plotting residual versus fitted values, and the residuals were assessed for any dependency structures between the covariates (Zuur and Ieno, 2016). Differences in lesion lengths between the different *Phytophthora* isolates were assessed using one-way analysis of variance (ANOVA) at $\alpha = 0.05$. Where ANOVAs showed significant differences between treatment means, post-hoc comparisons were performed to explore the differences at $\alpha = 0.05$. The Least Significant Difference (LSD) method, without adjustment for multiplicity was chosen, as the outcome of these tests was used to design follow-up experiments (Milliken and Johnson, 2009).

To determine whether there was a significant difference between *Phytophthora* isolates' ability to cause seedling deaths, the data were analysed using a logistic regression model ('binomial' family with 'logit' link) using the "vegan" and "agricolae" packages in R (R Development Core Team 2015). This model had seedling

status (‘dead’ or ‘alive’) as response variable, and *Phytophthora* isolate as the predictor variable. To illustrate the course of seedling deaths over time for the different *Phytophthora* isolates, survivorship curves were created using Microsoft Excel® for Mac® (2011). Survivorship of the seedlings is represented by a survivorship curve, on which the logarithm of the number of survivors in each age interval is plotted against the age interval (in this case it is the number of days post-inoculation) (Calver et al., 2009).

For the surviving seedlings in the pathogenicity trials, several traits (above-ground dry weight, root volume, root dry weight, height difference at the start and end of the trial, diameter difference (measured at 30 mm above the lignotuber) at the start and end of the trial and the root volume: above-ground dry weight ratio) were assessed. Multivariate analyses techniques were used to analyse the data. Due to the high number of seedling deaths, some treatments were left with few replicates. Because the data were highly heterogeneous, non-parametric multivariate tests were used to explain the effect of the different *Phytophthora* isolates using the freeware statistical package PAST (v. 3.11, Hammer et al. 2001, http://folk.uio.no/ohammer/past). Non-metric multidimensional scaling (nMDS) was used to visualise patterns in the data, aiding in the interpretation of the hypothesis tests. One-way Analysis of Similarity (ANOSIM) was used for hypothesis testing (with 9999 permutations, using 2D dimensions for representing the data). When the global ANOSIM result was significant ($P \leq 0.05$), Similarity Percentage (SIMPER) analysis was used to determine what the contribution of the individual plant traits was to the dissimilarity caused by the different *Phytophthora* isolates (Clarke and Warwick, 2001). These tests were performed using Bray-Curtis similarity (Bray and Curtis, 1957). Prior to analyses, all plant trait variables were range-transformed (0 – 1). This allowed for an equal impact of variables irrespective of measurement scale (Lee et al., 2013). Data for the nMDS plots were grouped together by *Phytophthora* species, but for the ANOSIM test, the data were analysed by *Phytophthora* isolate.

The probability plot approach (Schweder and Spjotvoll, 1982) was applied as a post hoc test, because it corrects the family-wise type 1 error rate for multiple tests but without a substantial penalty in statistical power or inflating the type II error rate.

To assess the relationship between the lesion length in excised stem trials and seedling deaths in the glasshouse, a Pearson product-moment correlation coefficient was computed. This was done by using Microsoft Excel® for Mac® (2011).

Results

Field survey

Corymbia calophylla trees in the disturbed and undisturbed stands varied in health. Disturbed stands at Brunswick, Manjimup and Witchcliffe had more cankered trees than healthy trees. In contrast, the Dandaragan paddock stand (disturbed) had fewer cankered trees than the disturbed stands to the south. Trees grown in the undisturbed stands were mostly healthy (without visible cankers), except at Brunswick (Fig. 2.1).

In total, 33 *Phytophthora* isolates were recovered from 120 soil and root samples (Table 2.1). Five species, namely *Phytophthora cinnamomi*, *P. elongata*, *P. multivora, P. pseudocryptogea* and *P. versiformis* were identified, and these species had sequences identical to those of vouchered isolates (Appendix 2 Table 2). The most prevalent species were *P. multivora* (17 isolates) and *P. cinnamomi* (10 isolates), which were recovered from seven and four stands, respectively. Four isolates of *P. versiformis* were recovered from the Manjimup paddock stand and adjacent road verge, whilst only one *P. pseudocryptogea* isolate was recovered from the Boranup pilot study stand. One *P. elongata* isolate was recovered from the Brunswick paddock stand. When excluding the *Phytophthora* recoveries from the pilot study stands, 73% (19 from 26 isolates) of *Phytophthora* recoveries were from disturbed stands and nearly 42% (11 from 26 isolates) were from cankered trees (Table 2.1).

Phytophthora multivora was more frequently found with disturbed stands and cankered trees (Table 2.1), than with healthy trees or undisturbed stands. *Phytophthora cinnamomi* did not display a preference to a particular type of stand or tree, as it was recovered in almost equal quantities from disturbed (four from 10 isolates) and undisturbed stands, as well as from cankered (four from 10 isolates) and healthy trees.

Table 2.1. *Phytophthora* species recovered from the rhizosphere of *Corymbia calophylla* trees grown in disturbed and undisturbed stands.

Stand name	Stand type	Stand status	Tree Health	Number of isolates				
				P. cinnamomi	***P. elongata***	***P. multivora***	***P. pseudocryptogea***	***P. versiformis***
Dunsborough[1]	Recreational beach area	disturbed	cankered			4		
Boranup[1]	Private paddock	disturbed	cankered			2	1	
Brunswick	State Forest	undisturbed	cankered					
Brunswick	State Forest	undisturbed	healthy					
Brunswick	Private farm paddock	disturbed	cankered			1		
Brunswick	Private farm paddock	disturbed	healthy		1	1		
Dandaragan	Private farm remnant forest patch	undisturbed	cankered					
Dandaragan	Private farm remnant forest patch	undisturbed	healthy					
Dandaragan	Private farm paddock	disturbed	cankered					
Dandaragan	Private farm paddock	disturbed	healthy					
Kentdale	National Park	undisturbed	cankered	1				
Kentdale	National Park	undisturbed	healthy	2				
Kentdale	Road edge of National Park	disturbed	cankered	1		1		
Kentdale	Road edge of National Park[2]	disturbed	healthy	1		3		
Manjimup	State Forest	undisturbed	cankered					
Manjimup	State Forest	undisturbed	healthy					
Manjimup	Private farm paddock	disturbed	cankered					2

Stand name	Stand type	Stand status	Tree Health	Number of isolates				
				P. cinnamomi	***P. elongata***	***P. multivora***	***P. pseudocryptogea***	***P. versiformis***
Manjimup	Private farm paddock[3]	disturbed	healthy			1		2
Witchcliffe	State Forest	undisturbed	cankered	1				
Witchcliffe	State Forest[2]	undisturbed	healthy	2		1		
Witchcliffe	Private farm paddock[2]	disturbed	cankered	1		3		
Witchcliffe	Private farm paddock	disturbed	healthy	1				
Total				10	1	17	1	4

[1] Denotes pilot study stand.

[2] *P. cinnamomi* and *P. multivora* were isolated from the rhizosphere of the same tree host.

[3] *P. multivora* and *P. versiformis* were isolated from the rhizosphere of the same tree host.

The *Phytophthora* recoveries for the pilot study stands (Dunsborough and Boranup) were excluded from the logistic regression model, which validated the effect of stand status on the presence of *Phytophthora.* The model yielded significant results χ^2 (*df* = 5, *N* = 100) = 26.39, P ≤0.01, Hosmer and Lemeshow R^2 = 0.24. (I.e., the stand status predictor significantly improved the model's predictive capability (P ≤0.01) and explained 24% of the variance in *Phytophthora* presence across the different stands.) The Hosmer and Lemeshow test results also confirmed that the model was a good fit for the data, χ^2 (*df* = 8, *N* = 100) = 4.83, P=0.78. The individual *P*-value for the Witchcliffe (P= 0.04) (Table 2.2) stand indicated a significant effect on the distribution of *Phytophthora* in this stand. The presence of *Phytophthora* did not appear to significantly influence the probability of a tree to be cankered, as the logistic regression model validating these effects did not produce significant results, χ^2 (*df* = 7, *N* = 100) = 1.76, P ≥0.05, even after adding an interaction term between *Phytophthora* presence and stand disturbance to the model (i.e., *Phytophthora* abundance was not higher with cankered trees).

Table 2.2. Predictor coefficients for the logistic regression model predicting the presence of *Phytophthora* species with stand status

	b	**SE(b)**	***p*[#]**	**Exp(b) [95% CI]**
Constant	-2.65			
Stand Dandaragan	-16.79	1386.54	0.99	0.00 [NA, 2.80e+25]
Stand Manjimup	0.68	0.84	0.42	1.98 [0.39, 11.60]
Stand Kentdale	1.46	0.81	0.07	4.29 [0.94, 24.40]
Stand Witchcliffe	1.69	0.81	**0.04**	5.41 [1.20, 30.90]
Disturbance	1.46	0.55	**0.01**	4.30 [1.53, 13.40]

R^2 = 0.24 (Hosmer-Lemeshow), Model $\chi^2(1)$ =26.39. [#]*P*-values in bold are significant.

Excised branch trials

For both screening trials, model validation indicated the presence of outliers in the lesion lengths. The two isolates (MHSFH2 and TRH5) repeated between these two trials produced almost identical lesion lengths (Fig. 2.4A, 2.4B). The control branches did not develop necrotic lesions.

All *Phytophthora* isolates used in the first screening trial produced lesion lengths (Fig.2.3) that were significantly (P≤0.05) different from the control treatment's lesion lengths (Fig. 2.4A). Post hoc comparisons using the Least Significance Difference test indicated that all isolates (M = 4.11, SE = 0.43) differed significantly at the α=0.05 level from the control (M = 2.3, SE = 0.00). Four *P. cinnamomi* isolates (MHH2, MHSFC4, MHSFH2 and TSFC3) produced the longest lesion lengths and differed significantly (P ≤0.0.5) from the *P. multivora* isolates (MgR2.12, MgR2.3, Mj10, and TRH1B2).

Figure 2.3. *Corymbia calophylla* branches inoculated with different *Phytophthora* species. A) The lesion formed on the branch inoculated with sterile agar did not extend beyond the initial cut in the branch. B) A clear, brown-coloured lesion of the branch inoculated with *P. elongata.* C-D) The branches inoculated with *P. multivora*, showing that lesions covered almost half the length of the branches. E) The branch was inoculated with *P. cinnamomi*, showing the largest lesion length.

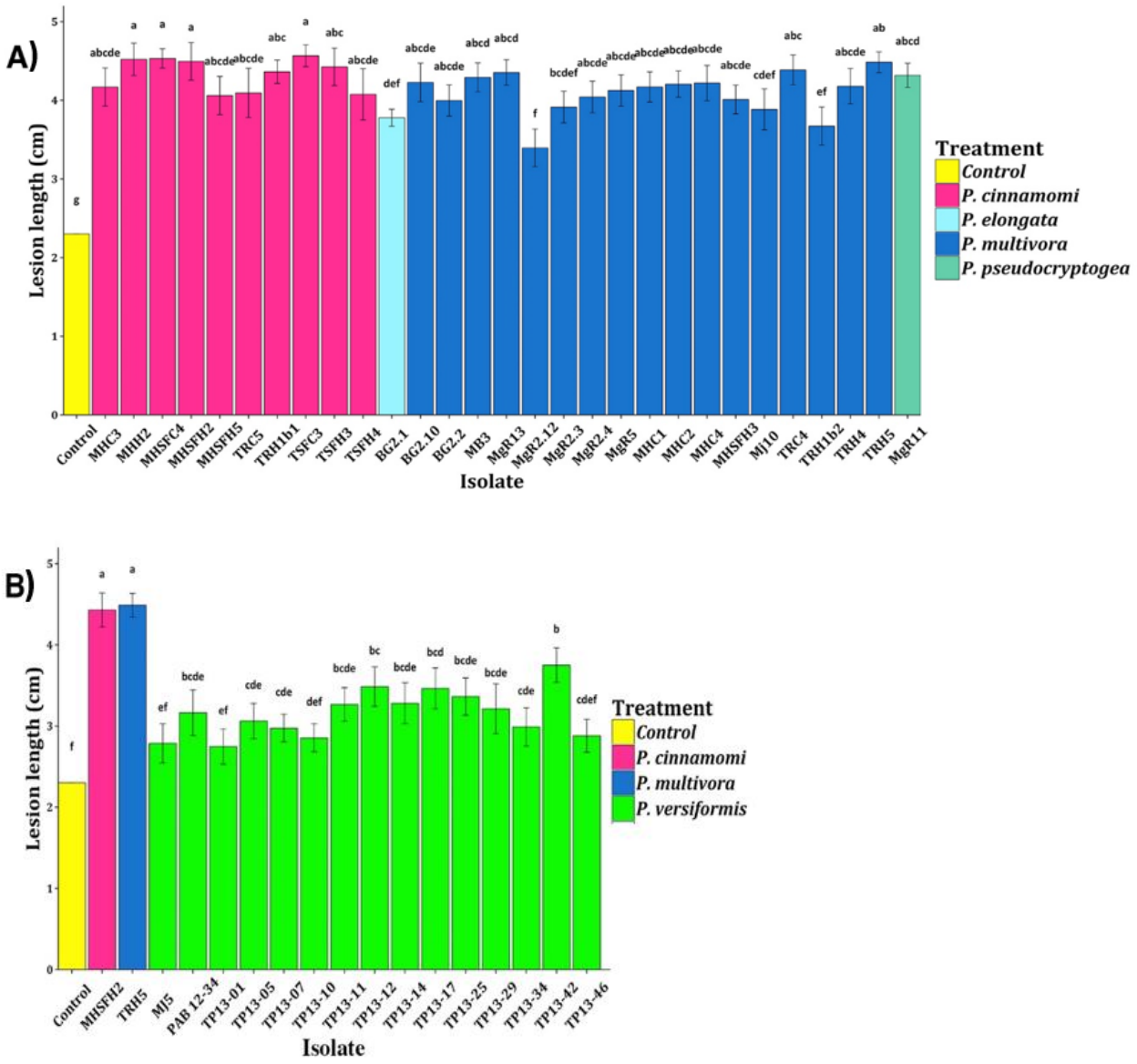

Figure 2.4. Mean (± SE) lesion lengths (cm) developed following under-bark inoculation of *Corymbia calophylla* excised branches with isolates of four *Phytophthora* species. A) Data for the first branch inoculation trial. B) Data for the second branch inoculation trial. Means that were significantly different, based on LSD analysis, are labelled with different letters (n = 10, α ≤0.05).

The second screening trial showed that the *P. versiformis* isolates were not able to cause the same damage to the excised branches as *P. cinnamomi* (MHSFH2) and *P. multivora* (TRH5) (Fig. 2.4B), though ANOVA yielded significant results (P ≤0.05) for lesion lengths produced by these species. Post hoc comparisons using the Least Significance Difference test indicated that all isolates (M = 3.26, SE = 0.49) differed significantly at the α=0.05 level from the control (M = 2.3, SE = 0.00).

Glasshouse pathogenicity trials

Seedlings inoculated with *Phytophthora* (especially *P. cinnamomi* and *P. multivora*) were visibly stressed, stunted in growth, and had lower aboveground mass. The leaves of these seedlings were chlorotic and/or of a lighter green colour than the control seedlings. The root systems of the *Phytophthora* inoculated seedlings were smaller, mostly dark coloured with often-visible necrotic lesions and fleshy white roots were mostly absent. In contrast, the roots of the control seedlings were light brown and with numerous fleshy white roots (Appendix 2 Fig. 1). *Phytophthora* species were recovered from all the inoculated seedling roots, but not from the controls, thus fulfilling Koch's postulates.

Seedling deaths

Except for an isolate of *P. multivora* (MHSFH3) and two isolates of *P. versiformis* (TP13-29 and TP13-42), all isolates caused seedlings to die prior to harvest (18 out of the 21 isolates). In the first pathogenicity trial, 25% of the seedlings had died during the trial, and in the second pathogenicity trial 5% of the seedlings died prior to harvest (Appendix 2 Table 3). A *P. cinnamomi* isolate (MHSFH5) was responsible for the highest number of seedling deaths in the first and second (62.5 % and 25%, respectively) pathogenicity trials. A *P. multivora* isolate (BG2.10) caused 50% seedling deaths, and the *P. cinnamomi* (TSFH4, TRH1B1), *P. elongata* (BG2.1), *P. multivora* (TRC4, TRH4) and *P. nicotianae* (PAB 10-104) isolates killed 37% of seedlings before harvest in the first trial, but they did not kill any seedlings in the second trial. The generalized linear model (GLM), validating the ability of the *Phytophthora* isolates used in the first pathogenicity trial to cause seedling deaths before harvest, did not produce significant results (χ^2 (*df* = 18, *N* = 152) = 24.77, P ≥0.05, Hosmer and Lemeshow R^2 = 0.14). These results indicated that the *Phytophthora* isolates did not differ significantly from each other in their ability to kill the *C. calophylla* seedlings. Only five seedlings died in the second trial: too few for statistical evaluation.

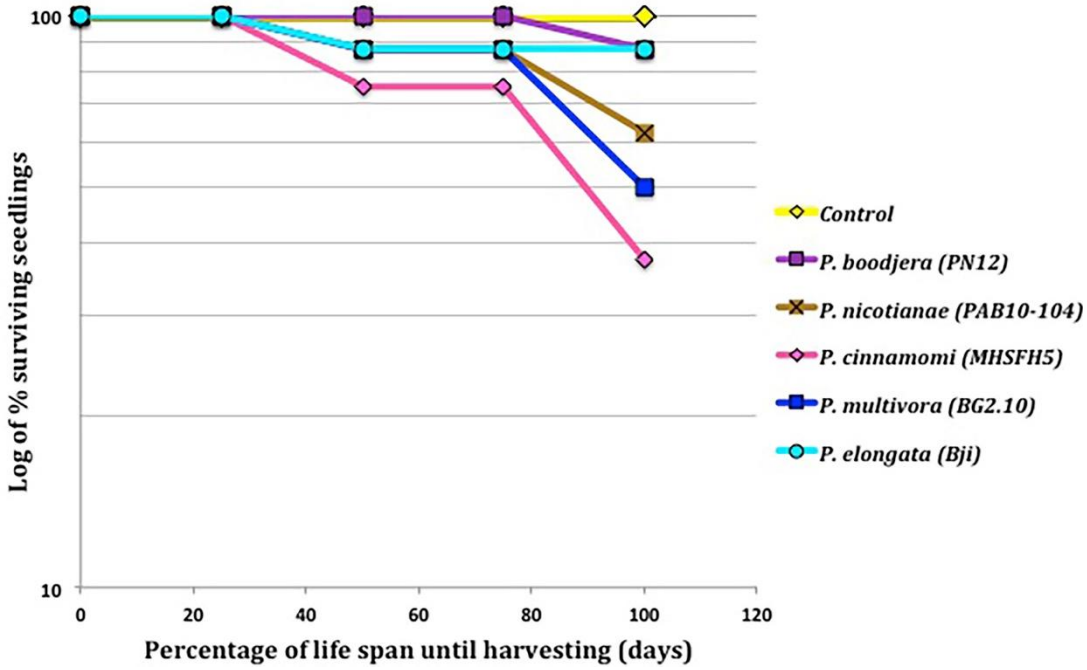

Figure 2.5. Survival curve of *Corymbia calophylla* seedlings with representative *Phytophthora* isolates for the first pathogenicity trial. Though seedlings were affected at different rates, most seedling deaths occurred in the fourth term of the experiment before harvesting. There was no loss of non-inoculated (control) seedlings.

Most of the *C. calophylla* seedling deaths in the first pathogenicity trial occurred in the fourth term of the experiment before harvesting, between 40 and 80 days after inoculation. The survivorship curves followed a Type I pattern, indicating that the P*. cinnamomi* and *P. multivora* isolates caused the highest number of seedling deaths, though the time in which these killed the *C. calophylla* seedlings varied (Fig. 2.5). No post-inoculation deaths occurred in the control seedlings.

Growth response of surviving seedlings

In the first pathogenicity trial, ten out of the 18 isolates caused a reduction in seedling root volume when compared to the control treatment. *Corymbia calophylla* seedlings inoculated with MHSFH5 and TSFH4 (*P. cinnamomi),* TRH4, BG2.10 and TRH1B2 (*P. multivora*), BG2.1 (*P. elongata*) and PAB10-104 (*P. nicotianae*) displayed extensive damage to their root systems (Fig. 2.6). Seedlings also displayed low top dry weights, and regression analysis indicated a positive correlation between the above- and below-ground measurements for the *P. cinnamomi* (R^2 = 0.591) and *P. multivora* treatments (R^2 = 0.685).

Phytophthora isolates of the same species differed in their pathogenicity towards *C. calophylla*, and some isolates seemed to have 'stimulated' seedling growth. Root volumes of seedlings inoculated with MHC4, MHSFH3, MJ10, and TRC4 (*P. multivora*) and MGR11 (*P. pseudocryptogea*) isolates were greater than those of the control seedlings (Fig. 2.6). Treatment with the MHSFH5 (*P. cinnamomi*) and BG2.10 (*P. multivora*) isolates did not result in the greatest reductions of root volumes, even though these two isolates had caused the most seedling deaths in the first pathogenicity trial.

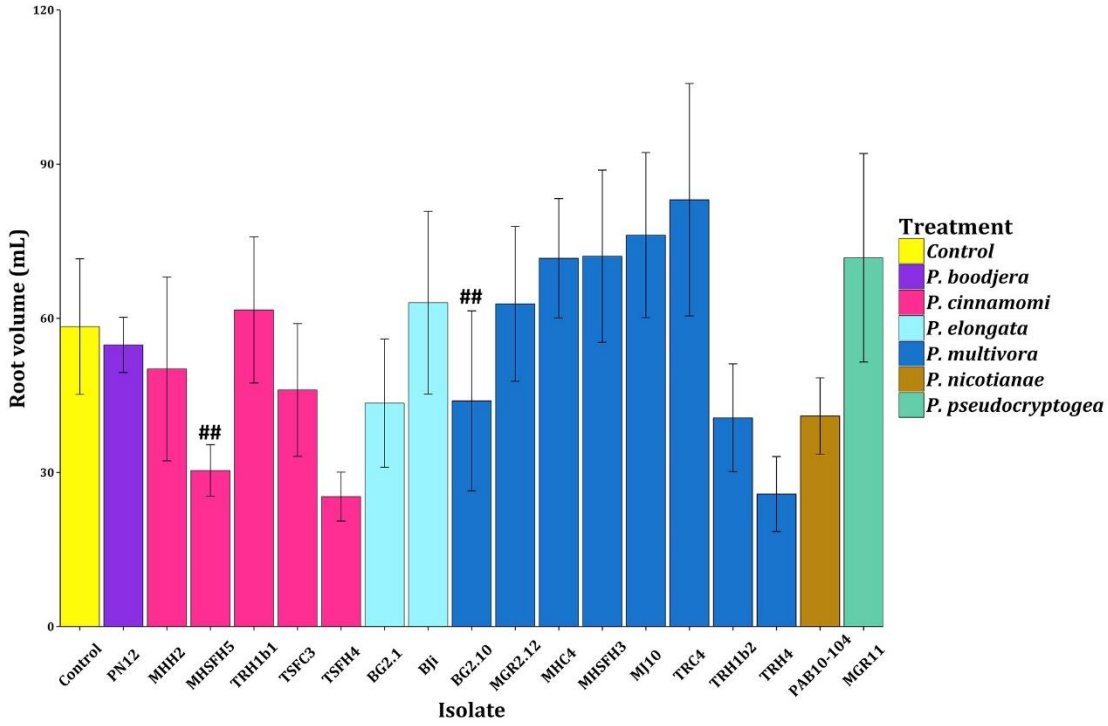

Figure 2.6. Mean (± SE) of root volume (mL) of surviving *Corymbia calophylla* seedlings inoculated with *Phytophthora* isolates in the first glasshouse pathogenicity trial, harvested 12 weeks after inoculation. When compared to the control, treatment with some isolates resulted in a reduction in root volume, whilst other isolates caused an increase in root volume.
These isolates caused the highest number of seedling deaths in the soil infestation trials.

In nMDS, the plant trait variables for the seedlings inoculated with the *P. cinnamomi*, *P. multivora* and to a lesser extent *P. elongata* isolates displayed separation from the rest of the seedlings (stress = 0.131)), suggesting that inoculation with these three *Phytophthora* species resulted in seedlings having different below- and above-ground measurements than the seedlings inoculated with the other species (Fig. 2.7). The seedling traits for the other groups of *Phytophthora* (*P. boodjera*, *P. nicotianae* and *P. pseudocryptogea*) species and the control treatment overlapped (Fig. 2.7).

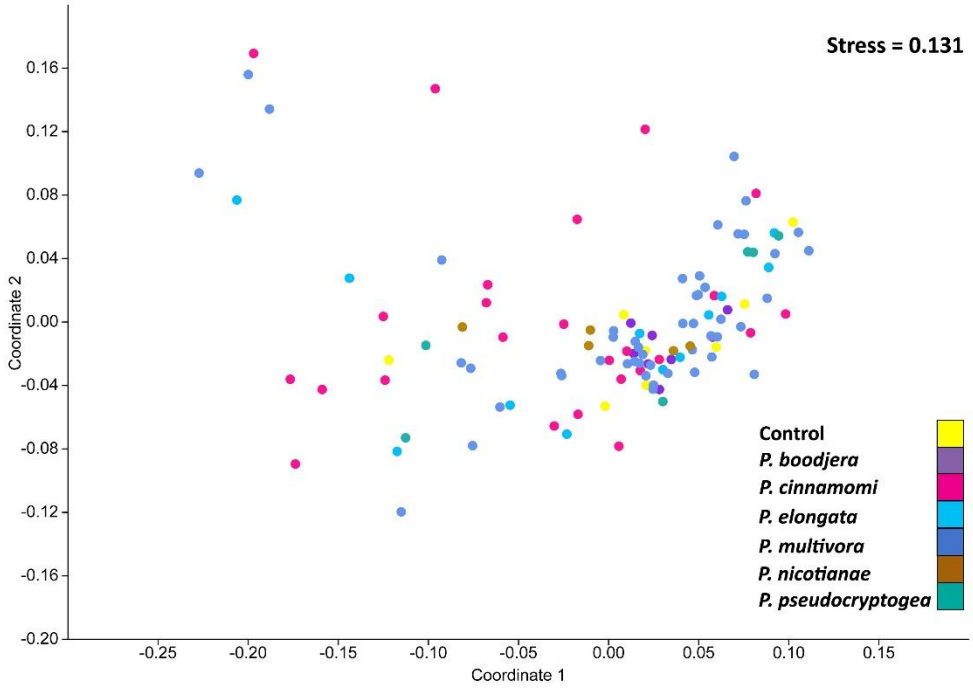

Figure 2.7. An nMDS scatterplot (stress = 0.131), indicating the effect of the *Phytophthora* species on the above- and below-ground plant traits for the *Corymbia calophylla* seedlings used in the first pathogenicity trial. This scatterplot included all biomass variables. The seedling plant traits for the *P. multivora* (blue), *P. cinnamomi* (pink) and *P. elongata* (aqua) treatments were different ('separated') from the other treatments, indicating that inoculation with these three *Phytophthora* species resulted in seedlings having different below- and above-ground measurements than seedlings inoculated with the other species and especially the control.

The ANOSIM results indicated an overall significant but modest (Global *R*: 0.067, P =0.013) effect of the different *Phytophthora* isolates on the seedling traits. The ANOSIM boxplot suggests that these results were handicapped by the large standard errors produced by some of the *Phytophthora* isolates (hence explaining the low R-value). Post hoc testing, as demonstrated by the probability plots indicated that three tests were likely to be significant. These corresponded to comparisons between MJ10 versus TSFH4 (*P. multivora* vs. *P. cinnamomi*), MJ10 versus TSFC3 (*P. multivora* versus *P. cinnamomi*), and TSFH4 versus PN12 (*P. cinnamomi* versus *P. boodjera)*, with *P*-values of 0.001, 0.002 and 0.002, respectively. The results for these tests are presented in Appendix 2 Table 4. Similarity Percentage (SIMPER) indicated that the average dissimilarity between all pairs of intergroup treatments was 36.69%. The SIMPER results also indicated that the aboveground measurements were contributing more to the dissimilarity results than the belowground

measurements (Table 2.3). The height difference contributed 20.78% to the dissimilarity between the *Phytophthora* isolates, whereas the root volume contributed 15.77% and the root volume: top dry weight ratio 12.32%.

Table 2.3. SIMPER results for the *Corymbia calophylla* seedling plant traits for the *Phytophthora* isolates used in the first pathogenicity trial, grouped by plant traits. These results indicate the percentage contribution each plant trait made to the dissimilarity distribution in descending order.

Seedling trait	Average dissimilarity	Contribution %
Height difference	7.624	20.78
Top dry weight	7.287	19.86
Diameter difference	6.915	18.85
Root volume	5.787	15.77
Root dry weight	4.558	12.42
Root volume: top dry weight ratio	4.519	12.32

In the second pathogenicity trial, none of the *Phytophthora* (*P. cinnamomi*, *P. multivora*, *P. pseudocryptogea* and *P. versiformis*) treatments produced significant differences in seedling traits when compared to the controls. nMDS did not indicate a separation of the plant trait variables for seedlings inoculated with the different *Phytophthora* species. In addition to this, the ANOSIM did not indicate an overall significant (ANOSIM, Global *R*: -0.025, P=0.805) effect of the different *Phytophthora* isolates on the seedling traits. For this reason, SIMPER analysis was not conducted.

Comparison of excised branches and plant deaths in soil infestation trials

Overall, there was not a strong relationship between lesion length in excised branches and the number of seedling deaths in the pathogenicity trials (R2=0.06). The relationship for some individual species was stronger, though correlations varied between the different species. The *P. multivora* isolates had a positive correlation (R2=0.17), indicating that the isolates, which had caused the longest lesion lengths in the excised branches, were also responsible for the most seedlings deaths. In contrast, the *P. cinnamomi* (R2 = -0.39) isolates displayed a negative correlation.

Discussion

Field Survey

In this study, the presence of five *Phytophthora* species in the rhizosphere of *Corymbia calophylla* was detected and there was a significantly higher prevalence of *Phytophthora* in anthropogenically disturbed stands. This is in line with previous reports where *Phytophthora* diebacks in Australia have been associated with human activities such as earthworks, logging and wildfires (Marks et al., 1975, Fagg et al., 1986, Davison, 1997). In a recent study where the soils from the rhizosphere of cankered *C. calophylla* on 62 disturbed stands were tested, *Phytophthora* was recovered from 34 of the 62 sites (Paap et al., 2017a). Another study investigating dying urban trees from remnant bushland, parks, gardens and streetscapes in Perth and surrounds, nine different *Phytophthora* species were recovered from the rhizosphere of a wide range of native host species, including *C. calophylla* (Barber et al., 2013). *Phytophthora multivora* was the most frequent species recovered. This recently described pathogen (Scott et al., 2009) is associated with a wide range of dying native host species (including *C. calophylla*) not only in Perth and surrounds, but also throughout the SWWA (Scott, 2011, Scott et al., 2012).

Phytophthora does not always cause disease in native plant communities in SWWA (Ward and McKimm, 1982, Davison, 1997), as it is found in apparently non-diseased stands too (Pratt and Heather, 1973). This was true for the *P. cinnamomi* recoveries in this study too. Though *P. cinnamomi* is regarded as a highly virulent pathogen to many native host plants in SWWA (Shearer and Dillon, 1995), it was also recovered from the undisturbed forests in Kentdale and Witchcliffe (Table 2.1). More studies need to be done to investigate why *C. calophylla* is field resistant to *P. cinnamomi* on these undisturbed forest stands. However, the presence of *P. cinnamomi* in asymptomatic vegetation is well documented and it can persist as a biotroph in several annual and herbaceous hosts (Crone, 2012, Crone et al., 2013). In situations where *Phytophthora* does not act as a primary pathogen, it is often found to be a secondary or contributing factor to forest tree decline (Jurskis, 2005), where other factors such as the loss of mycorrhiza (Ishaq, 2013, Sapsford et al., 2017) or the presence of chemicals due to fertilizer and herbicide practises can have a negative impact on

C. calophylla health (Sapsford et al., 2017), thus making the way for *P. cinnamomi* to become necrotrophic to its host.

Even though *Phytophthora* was recovered more frequently from cankered *C. calophylla* trees, there was no significant relationship between the presence of *Phytophthora* and cankered trees. This could be due to the small number of trees per stand (five healthy and five cankered trees), as well as the large variance in the data. In another study *Phytophthora* was found to be associated with cankered *C. calophylla* on disturbed stands (Paap et al., 2017a), and the presence of *P. cinnamomi* and *P. multivora* (considered pathogenic to *C. calophylla* in this study) increased with increased incidence of cankered *C. calophylla* trees. Paap et al. (2017a) only tested for *Phytophthora* on site level (and not tree level), thus it would be worthwhile to conduct another study with bigger sample sizes to identify the correlation between the presence of *Phytophthora* and canker frequency at the stand level. Also, in this study the *C. calophylla* seedlings were infected with only one *Phytophthora* isolate, but it is possible that in field conditions multiple *Phytophthora* spp. could infect *C. calophylla*. This might lead a synergistic or additive pathogenic effect on seedling vigour. Table 1 has two examples where a couple of *Phytophthora* species were isolated from a single *C. calophylla* tree.

Paap et al. (2017a) found that site-specific climate characteristics were a significant indicator of canker incidence with *C. calophylla.* Canker incidence was higher in the wetter and cooler areas of the *C. calophylla* range and lower in the dryer northern region, suggesting that these areas are conducive to the growth of the canker pathogen, *Quambalaria coyrecup*. These conditions are also beneficial to the development of *Phytophthora* (Morgan and Shearer, 2013), and in the current study there were no *Phytophthora* recoveries from the dryer northern areas (Dandaragan) of the *C. calophylla* range (Fig. 2.2). While it is accepted that temperature and rainfall are important factors in the distribution of pathogens (Desprez-Loustau et al., 2007), *C. calophylla* are also affected by changes in temperature and rainfall (Szota et al., 2011). Changes in temperature and rainfall could also alter the interactions between *C. calophylla* and *Phytophthora*, between *C. calophylla* and *Q. coyrecup* and possibly the interactions between *Phytophthora* and Q*. coyrecup*. Another key factor in the canker incidence could be the distribution of *C. calophylla* – *C. calophylla* populations are becoming increasingly smaller towards the warmer and dryer northern regions of its

distribution, implying that tree density is too low for a canker disease outbreak (Paap et al., 2017a).

Pathogenicity trials

The glasshouse pathogenicity trials performed in this study indicated that some *Phytophthora* species severely impacted *C. calophylla.* Seedling root volumes were reduced, the physical aboveground traits differed substantially between the treatments and seedlings were killed because of the inoculation with *Phytophthora*. These findings are important, as while *C. calophylla* was considered field resistant to *P. cinnamomi* (Cahill and McComb, 1992), except in restored mine sites which suffered temporary ponding and *P. cinnamomi* was shown to kill *C. calophylla* (Hardy et al., 1996), the susceptibility of *C. calophylla* to other *Phytophthora* species was unknown until before this study. While a significant effect of *Phytophthora* on *C. calophylla* could not be demonstrated with the ANOSIM tests, it must be kept in mind that due to the heterogeneity of the pathogenicity data (and the fact that dead plants were removed from the analyses), non-parametric tests were used to explain the effect of *Phytophthora* on the *C. calophylla* seedlings. Non-parametric tests (ANOSIM) are not as powerful as parametric tests. In future research, it would be worthwhile to include more predictors or covariates to explain more of the variance in the data.

Seedlings inoculated with some isolates of *P. cinnamomi* and *P. multivora* died 40 to 80 days post-inoculation, indicating that the *Phytophthora* root infection takes time to kill the seedling roots before the onset of disease symptoms. A *P. cinnamomi* isolate was the most pathogenic as it killed most (62.5%) of the seedlings before harvest. These results are consistent with similar trials conducted using *Phytophthora* species in other eucalypt species. For example, *P. cinnamomi* and *P. multivora* were recovered from the rhizosphere of dying *Eucalyptus gomphocephala* (tuart) and *E. marginata*, and in pathogenicity trials using these two pathogens, a significant loss in seedling root mass was observed (Scott et al., 2009).

Corymbia calophylla response to treatment with *P. boodjera*, *P. elongata*, *P. pseudocryptogea* and *P. versiformis* differed from reports with other hosts. In nurseries, *P. boodjera* was frequently isolated from damped-off eucalypt seedlings and it significantly reduced root biomass of up to two-year-old seedlings (Simamora et al., 2016), though it did

not demonstrate pathogenicity with *C. calophylla. Phytophthora elongata* has been routinely isolated from the roots and collars of dead and dying *E. marginata* in the Northern Jarrah Forest, and in soil infestation trials it showed pathogenicity comparable to that of *P. cinnamomi* (Rea et al., 2010) though again, it was not as pathogenic to *C. calophylla*. *Phytophthora versiformis* did not display pathogenicity towards *C. calophylla*, even though *P. quercina*, a species closely related to *P. versiformis* is considered a predisposing factor in killing fine roots of oaks in Europe (Jung et al., 1999). *Phytophthora pseudocryptogea* was described recently (Safaiefarahani et al., 2015), and though its pathogenicity with potato (*Solanum tuberosum*) tubers was demonstrated, it was not pathogenic to *C. calophylla*. It is important to realise that some hosts have a genetic resistance to certain pathogens, and the mechanism by which this resistance plays off between a pathogen and a host is still poorly understood.

Phytophthora multivora has a high phylogenetic and phenotypic variability, indicating that this pathogen was introduced long ago to Western Australia (Scott et al., 2009). This variability between the different *P. multivora* isolates was reflected in the different pathogenic effects the isolates had on the *C. calophylla* seedlings. Seedlings inoculated with four isolates of *P. multivora* produced greater root biomass than the controls, but at the same time, these isolates had also caused seedlings deaths. This phenomenon could be explained by the hypothesis that *P. multivora* stimulates *C. calophylla* to replace roots faster than it is killing the roots. Infection with some pathogens is known to induce host resistance resulting in better plant defence and occasionally increased root growth (Serrazina et al., 2015, Bailey et al., 2006). A similar observation was made with *E. gomphocephala* in a pathogenicity trial where seedlings inoculated with ECM fungi had lower root mass than when seedlings were inoculated with both ECM fungi and *P. multivora* (Ishaq, 2013). More work needs to be done to investigate the driving factors behind this phenomenon in *C. calophylla*, as it is unusual for *Phytophthora* to seemingly stimulate root growth. It should be considered that these trials were done in a temperature-controlled glasshouse and seedlings received ample water throughout the trial. In the absence of water, seedlings could experience water deficit and die, a situation comparable with the field where *C. calophylla* must compete for water and nutrients during the long dry and warm summer months.

Season and temperature differences also play a role in the pathogenicity of *Phytophthora*. The lower number of seedling deaths for the second pathogenicity trial could be explained by the fact that this trial was done during the colder winter months, when *Phytophthora* growth is not as active as in summer months. Similarly, Morgan and Shearer (2013) investigated the capacity of *P. cinnamomi* to sporulate and release zoospores for soil from the Northern Jarrah Forest collected during the summer, winter and spring. Season did influence the stimulatory capacity of the soils; sporangia count for soils collected in the winter were significantly less than for soils collected in the summer and spring.

There was a poor relationship between the branch inoculation lesion lengths and seedlings deaths in the glasshouse pathogenicity trials. This was in agreement with work done on *E. marginata*, where the length of colonisation including asymptomatic infection of branches was more consistent as a measure of pathogenicity (as observed in soil infestation trials) than lesion length itself (Hüberli et al., 2002). Thus, while a useful rapid methodology for screening many isolates, under bark inoculation of excised stems should not be used to determine pathogenicity, but rather as a way of selecting isolates for more detailed infestation trials with living plants.

Conclusion

In this study, it was demonstrated that *Phytophthora* species have the ability, by infecting *C. calophylla* roots, to affect its health and vigor and even kill it. However, a causal relationship between the *Phytophthora* species and cankered *C. calophylla* trees could not be determined. The canker pathogen, *Quambalaria coyrecup* is considered endemic to SWWA, but the reasons why it becomes invasive to its co-evolved host is not yet fully understood. Perhaps a *Phytophthora* root infection acts as a biotic stressor, thereby compromising the natural defense mechanisms of *C. calophylla*. This opens the need to conduct another experiment where *C. calophylla* seedlings are inoculated with both *Phytophthora* and *Q. coyrecup* to investigate whether a *Phytophthora* root infection does indeed predispose *C. calophylla* to the canker disease.

Chapter 3: Reflectance spectroscopy to characterise the response of *Corymbia calophylla* (marri) response to *Phytophthora* root rot and waterlogging

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Abstract

The health of *Corymbia calophylla* (marri), a keystone tree species in the native forests of southwest Western Australia, has been in decline for the past few decades. *Phytophthora* root disease and waterlogging have often been cited as contributing to this decline. Traditional methods (i.e., field surveys and sampling) of mapping *Phytophthora* root infection in the field are time-consuming and expensive; thus, the potential of reflectance spectroscopy to characterise *C. calophylla* response to *Phytophthora* and waterlogging stress was investigated. Twelve-month old *C. calophylla* plants were infected with either *P. cinnamomi* or *P. multivora* in glasshouse trials and waterlogged for 24 hrs each fortnight. Spectral measurements with a portable high-resolution spectroradiometer were taken weekly. Plant biophysical measurements were taken at harvest time. Normalised difference spectral index (NDSI) was calculated for every combination of reflectance values between 350 nm – 2 500 nm for all time points, correlated with the treatment effects and displayed as heat maps. Narrowband vegetation indices (VIs), utilising different wavelengths of the electromagnetic spectrum, were also calculated from the spectral data. The *Phytophthora* treatments did not cause significant differences with the biophysical measurements in both trials. In the second trial, the waterlogging treatment significantly lowered plant top dry weight ($p = .016$) and diameter ($p = .044$). Reflectance values plotted against wavelength displayed differences between treatments as well as a seasonal trend. The NDSI heat maps indicated that the *P. cinnamomi* and waterlogging treatment effects were correlated with bandwidths in the visible and near -infrared portions of the electromagnetic spectrum (538 – 558 nm, 701 – 709 nm). Six of the VIs (normalised difference nitrogen index 2, anthocyanin reflectance index 1, photochemical reflectance index, Carter index 1, Vogelman index 3 and water band index) were able to track the spectral reflectance changes in the leaves over the ten weeks, confirming the seasonal trend. The interaction effect between *P. cinnamomi*, waterlogging and elapsed time in the first trial was significant for WBI ($p = .010$). This study demonstrates that reflectance spectroscopy holds promise for characterising *C. calophylla* response to waterlogging stress, but more work needs to be done to identify the optimum wavelengths for identifying *Phytophthora* and waterlogging stress with *C. calophylla*.

Introduction

The jarrah (*Eucalyptus marginata* Sm.) forest in the biodiversity hotspot of southwest Western Australia is a dry sclerophyll forest. *Corymbia calophylla* (Lindl.) K.D. Hill & L.A.S. Johnson (marri) is a keystone species in this forest, growing alongside jarrah, contributing to a uniform overstorey. The jarrah trees in the forest were observed to be dying back as early as the 1930's, and in the 1960s *Phytophthora cinnamomi* Rands was identified as the cause of this dieback (Podger et al., 1965, Podger, 1972). Wet field conditions in the jarrah forest favour the spread and sporulation of *P. cinnamomi* spores, as *Phytophthora* dieback in the jarrah forest is often associated with sites where the topography of the sites make the accumulation of water possible. Recent work by Davison (2015) disputes that *P. cinnamomi* is the only cause of the jarrah dieback, and discusses the possibility that the jarrah deaths could have been caused by waterlogging, or the combination of waterlogging damage and *P. cinnamomi* infection (Davison, 1997). Though *C. calophylla* is considered more tolerant to waterlogging than jarrah and field-resistant to *P. cinnamomi* (Shearer and Tippett, 1989a), recent studies have concluded that *C. calophylla* is also susceptible waterlogging damage (Farifr and Aboglila, 2015) and to *P. cinnamomi* infection (Croeser et al., 2018).

In recent years more *Phytophthora* species have been described from soils in the jarrah forest (Burgess et al., 2009). Many of these *Phytophthora* species proved to be pathogenic to *C. calophylla* in pot infestation trials (Croeser et al., 2018, Belhaj et al., 2018, Migliorini et al., 2019) . One of these species, *P. multivora* P.M. Scott & T. Jung, which is implicated in the decline of *Eucalyptus gomphocephala* (tuart) (Scott et al., 2009, Scott et al., 2012), was also regularly isolated from *C. calophylla* exhibiting a crown decline in urban and peri-urban sites of SWWA (Barber et al., 2013).

Extensive mapping for *Phytophthora* has been conducted in the jarrah forest since 1978 (Dell et al., 2005). Mapping involves collecting soil and root samples from beneath dying, *Phytophthora*-sensitive native vegetation in the forest, and obtaining *Phytophthora* isolates by baiting methods (Vegetation Health Service, DBCA). This traditional *Phytophthora* mapping in the jarrah forest is time-consuming and expensive; thus, there is interest in exploring options for an alternative method to identify *Phytophthora* infection in these forests.

The use of reflectance spectroscopy to evaluate the condition of vegetation is widely described in the literature (Usha and Singh, 2013, Ustin et al., 2009, Gitelson and Solovchenko, 2018). This technique measures the amount of solar radiation not absorbed by the photosynthetic components (chlorophyll, carotenoids) in the leaves, but which is reflected into the atmosphere. Leaf pigments absorb in the visible area (400 – 700 nm) of the electromagnetic spectrum (EM), multiple scattering of light due to internal leaf structure takes place in the near-infrared (NIR) region (700 – 1 300 nm), foliar dry matter absorbs in the short-wave infrared (SWIR) region (1 300 – 2 500 nm), with the water absorption features at 1 150 – 1 260 nm (Sims and Gamon, 2003), 1 400 and 1 900 nm (Huete, 2004). The sharp increase of reflected light in the ~ 680 – 750 nm range is called the "red edge" region, often cited as an important region to detect vegetation stress (Horler et al., 1983b, Vogelmann et al., 1993).

When a plant is stressed, one of the first responses is the deterioration of its chlorophyll pigments; its photosynthetic apparatus cannot harvest all the visible light it needs for photosynthesis, reflecting a bigger portion back into the atmosphere. This results in reduced photosynthetic capacity and net primary production of carbohydrates (Croft et al., 2014). The reflectance values retrieved from these areas (the visible, near-infrared and short-wave infrared regions of the electromagnetic spectrum) are used to calculate vegetation indices (VIs). These VIs are derived by calculating the ratios of the amount of light reflected at wavelengths sensitive to a particular leaf pigment, internal light scattering or water content of the leaves (Blackburn, 1998), and compare it against a reference wavelength known to not reflect light particular to the specific function of interest.

Reflectance spectroscopy measurements are non-destructive and require less effort than traditional *Phytophthora* mapping. It can serve as an early warning tool to monitor plant response to stressors (Hernández-Clemente et al., 2019), as biochemical composition changes (i.e. pigment condition, water content) in the leaves can be detected before biophysical change (such as canopy thinning) become visible. It can also be applied to detect disease in plants (Usha and Singh, 2013). Recently Heim et al. (2019) reported developing a spectral disease index, LMMR (lemon myrtle-myrtle rust index), designed to detect myrtle rust, *Austropuccinia psidii*, on lemon myrtle trees, with an overall accuracy of 90%. Studies with avocados have demonstrated that VIs using reflectance values in the visible and NIR

regions, could distinguish between leaves from trees infected with laurel wilt (*Raffaelea lauricola*), *Phytophthora* root rot, salinity-damaged leaves and healthy trees (Abdulridha et al., 2016, De Castro et al., 2015). *Phytophthora* root rot severity in avocado could be quantified by using images of avocado tree canopies, taken by a smartphone, combined with canopy reflectance measured by high-resolution Worldview-3 (WV-3) satellite imagery (Salgadoe et al., 2018). Spectral signatures of four potato cultivars, infected with *P. infestans*, displayed similar responses in the SWIR region thus facilitating discrimination between infected and healthy plants (Gold et al., 2019). In work on eucalypt trees, reflectance data have indicated that in some eucalyptus species, anthocyanin pigments are synthesised in response to biotic stresses such as leaf-damaging insects and fungal pathogens (Stone et al., 2001). Spectral measurements could also detect water stress and *P. cinnamomi* infection in two Australian grass and tree species (Newby et al., 2019).

In this study, we used in-vivo foliar reflectance spectroscopy in the VIS, NIR and SWIR regions, to investigate its potential to characterise biophysical and biochemical changes in *C. calophylla* when infected with *Phytophthora* and waterlogged. Should these spectral measurements at leaf scale be successfully characterise these stresses, this methodology could be extended for use at a canopy level, using optical sensors onboard satellites, fixed-wing aircraft or drones.

Materials and Methods

Plant preparation and inoculation

Two trials were conducted, the first from May to September (southern hemisphere late autumn to early spring), and the second from March to July (southern hemisphere early autumn to mid-winter). Solar radiation data were downloaded from Murdoch University's automatic weather station located on campus outside the glasshouse facility. A completely randomised, two-factorial design (*Phytophthora* with waterlogging) was chosen. Plants were inoculated with either sterile inoculum (control) or with *P. cinnamomi* or *P. multivora.* Half of the plants in each treatment group were waterlogged every fortnight for 24 hours. There were 10 replicates per treatment.

Plant preparation and maintenance were conducted, using the same protocol and seeds from the same seed lot described previously (Chapter 2). Two batches of one-year-old plants

planted approximately one year apart and grown in a temperature- and humidity-controlled glasshouse (21-27°C) were used for both experiments. Plants used in the first trial were shorter than in the second trial, they were approximately 55 cm high (39 - 70 cm) with mean stem diameters of 4.6 mm (3.5 – 5.7 mm) at the time of inoculation, whereas plants in the second trial were approximately 64 cm high (44 – 84 cm) with mean stem diameters of 7.6 mm (5.7 – 9.5 mm) at the time of inoculation. A *P. cinnamomi* isolate (MHSFH2, GenBank ID KX120095) recovered from Witchcliffe State Forest, as well as a *P. multivora* isolate (TRH5, GenBank ID KX120119) recovered from Kentdale (Chapter 2) were used to inoculate plant roots, two weeks after potting. The vermiculite inoculum was prepared according to the method of Simamora et al. (2016), and plants were inoculated according to the method described in Chapter 2. An additional *P. multivora* isolate (TRH1B2, GenBank KX120117), also from Kentdale, was included in the second trial. After inoculation, plants were watered daily with deionised water. Deionised water was used for flooding too. All plants were flooded for 24 hrs, two days after inoculation, to stimulate sporangia production and zoospore release. Half of the plants in every treatment group were flooded on a fortnightly basis for 24 hours. The plants were monitored weekly for disease symptoms and the development of chlorotic leaves (an indication of plant stress).

Harvest and biophysical measurements

The plants were harvested ten weeks after inoculation. Various biophysical measurements were taken (Table 3.1) at harvest time. Plant heights and stem diameters (30 mm above the lignotuber) were measured before removing the plants from the pots. The sand was washed off the roots gently with deionised water to minimise the loss of fine roots. The root volume was determined by measuring the amount of water the roots displaced when put into a graduated cylinder and filled with water (Pang et al., 2011). Roots and tops were placed in separate brown paper bags and dried at 60 °C for four weeks in an oven before weighing. Koch's postulates were confirmed by plating necrotic root and lignotuber material on NARH agar, a *Phytophthora* selective medium (Simamora et al., 2017). The *Phytophthora*-inoculated roots returned positive results for *Phytophthora*, whereas the non-inoculated seedlings did not.

Table 3.1. List of biophysical response variables measured at harvest

Category	Response variable	Description
Stem	Diameter	Diameter of stem 30 mm above lignotuber
Stem	Diameter difference	Difference between diameter at the time of inoculation and harvest
Stem	Height	Height of plant
Stem	Height difference	Difference between height at the time of inoculation and at harvest
Roots	Root volume	Root volume of fresh roots
Roots	Root dry weight	Dry weight of roots

Spectral measurements

Reflectance spectra covering the range 350 – 2 500 nm were measured using a portable analytical spectral device (ASD) FieldSpec 4 high-resolution spectroradiometer (Malvern Panalytical Ltd., UK). This instrument was fitted with a handheld contact probe and a leaf-clip attached to the probe. Three internal sensors recorded reflectance with a resolution of 3 nm in the visible (VIS) and near-infrared (NIR) and 8 nm in the short-wave infrared (SWIR) ranges of the electromagnetic spectrum. The probe was fitted with a fibre-optic cable and an integrated halogen light source, with the probe window being 2 cm in diameter. A Spectralon® (~99%, Labsphere, USA) pure white reference disc was used to calibrate the instrument before measurements, and at regular intervals during measurements (after every three plants). The amount of light reflected by the leaves was estimated as a percentage between the maximum amount of light reflected from the surface of the pure white reference disc, and the minimum reference value obtained by blocking all light from the fibre optic. Spectra were interpolated to regular 1 nm intervals using cubic spline interpolation and reduced to 400 – 2400 nm by removing the wavelengths with relative noise at the edges of the spectra. The data were also normalised (continuum removal [CR]) to show the relative intensities (depth) of the water absorption features. The spectral data were converted to ASCII format, using the ViewSpec Pro software (Malvern-Instruments-Limited, 2008).

The spectroradiometer was set up on a table in the glasshouse, next to the benches with the *C. calophylla* plants (Appendix 1 Fig. 1). The plants were randomly selected each time and carried to the table, where the measurements took place. The hyperspectral measurements were taken on fully intact leaves, still attached to their stems. To ensure that the large number of plants received the same amount of light intensity for photosynthesis during

measurements, the measurements were taken between 10:00 and 12:00 and split over two days. For the second trial, only half of the plants in each treatment were measured. The plants in each treatment were divided into two groups randomly, where one group was measured on the first day and the remainder the following day. Baseline measurements were taken immediately prior to inoculation, and then every week for the duration of the experiment. To obtain the reflectance spectra, the youngest fully expanded leaf of a plant was clipped into the leaf clip assembly and three measurements, one each at the top, middle and end parts on the adaxial side of the leaf were taken. Care was taken not to include the centre vein or edges of the leaves during the measurements. These measurements were averaged per plant after each session.

As it was unknown which vegetation indices (VIs) would correlate best with the treatment effects in *C. calophylla*, fifty-seven narrowband VIs were calculated using reflectance values in the different regions of the electromagnetic spectrum (VIS, NIR, SWIR). These VIs included indices relating to canopy greenness (nitrogen content), chlorophyll content (photosynthetic capacity or efficiency), leaf pigments such as chlorophyll, carotenoid, and anthocyanin content (as an indication of senescence) and water content in the leaves. A complete list of all calculated VIs can be found in Appendix 1 Table 1.

Statistical analyses

Statistical analyses were done using R (R-Core-Team, 2019). The R add-on packages "dplyr"(Wickham et al., 2018), "ggbiplot" (Vincent, 2011), "gplots" (Warnes et al., 2020), "ggplot2"(Wickham, 2016), "lattice" (Sarkar, 2008),"nlme" (Pinheiro et al., 2018), "PerformanceAnalytics" (Peterson and Carl, 2018), "RColorBrewer" (Neuwirth, 2014) and "tidyverse" (Wickham, 2017) were used for more specialised analyses.

Reducing the number of response variables

Some of the selected multivariate analysis techniques could not cope with the large (six biophysical and 57 VIs, Table 3.1, and Appendix 1 Table 1) number of response variables (the number of response variables were almost the same as the number of observations). Highly collinear variables were excluded from the analysis by calculating their variance inflation factors (VIF), and only those variables that displayed VIF values < 10 (Quinn and Keough, 2003) were retained. A total of 33 variables remained, of which a final number of 15

variables were selected (Table 3.2), four biophysical variables, and 11 vegetation indices (VIs). The selected biophysical response variables selected were expected to represent the response of *C. calophylla* to the treatment effects best. Inspection of the reflectance against bandwidth plots and the heat maps indicated that the treatment effects mostly separated in the visible and short-wave infrared regions of the electromagnetic spectrum. Vegetation indices making use of these regions were selected as well as those often cited in the literature (i.e. anthocyanin reflectance index-a [ARI1] (Cao et al., 2015), normalised difference index [NDVI] (Abdulridha et al., 2018, Calderón et al., 2013) , photochemical reflectance index [PRI] (Newby et al., 2019, Calderón et al., 2013) and normalised difference water index [NDWI] (Abdulridha et al., 2018)) (Appendix 1 Table 1). Four of the selected VIs (anthocyanin reflectance index-1 (ARI1), red-green ratio (RG-ratio), PRI, Carter index 1 (CTR1)) used the reflectance of light in the visible portion, three (Vogelman index-3 (VOG3), normalised difference water index (NDWI), WBI) in the near-infrared portion, two (normalised difference nitrogen index 2 (NDNI2), cellulose absorption index (CAI)) in the short-wave infrared portion of the electromagnetic spectrum, and two indices (light curvature index-3 (LI3), NDVI) used light in both the visible and near-infrared portions of the electromagnetic spectrum.

Table 3.2. The final 15 response variables selected for use in further analyses

Category*	Response variable***	Description	Area in EM**
Biophysical	Diameter	Diameter 3cm above lignotuber	N/A
Biophysical	Height	Height of plant	N/A
Biophysical	Root volume	Volume of the roots	N/A
Biophysical	Top dry weight	Dry weight of above-ground mass	N/A
Greenness: canopy nitrogen	NDNI2	Vegetation index	SWIR
Leaf pigment	ARI1	Vegetation index	VIS
Leaf pigment	RG-ratio	Vegetation index	VIS
Light-use efficiency (LUE)	LI3	Vegetation index	VIS/NIR
Light-use efficiency (LUE)	PRI	Vegetation index	VIS
Photosynthetic potential	CTR1	Vegetation index	VIS
Photosynthetic potential	NDVI	Vegetation index	VIS/NIR
Red-edge Index	VOG3	Vegetation index	NIR
Dry/Senescent vegetation	CAI	Vegetation index	SWIR
Water content	NDWI	Vegetation index	NIR
Water content	WBI	Vegetation index	NIR

* For more information on the selected vegetation indices, see Appendix 1 Table 1, **Electromagnetic spectrum,
*** NDNI2 (normalised difference nitrogen index 2), ARI1 (anthocyanin reflectance index 1), RG-ratio (red-green ratio), LI3 (light curvature index-3 (LI3), PRI (photochemical reflectance index), CTR1 (Carter index 1), NDVI (normalised difference vegetation index), VOG3 (Vogelman index 3), CAI (cellulose absorption index), NDWI (normalised difference water index), WBI (water band index).

Analyses of biophysical measurements

Multivariate analysis of variance (MANOVA) was used on the data collected at each harvest, to test the treatment effects simultaneously across the four biophysical (root volume, diameter, height and top mass dry weight) response variables (Quinn and Keough, 2003). The model used for data in the first experiment had *P. cinnamom*i, *P. multivora* TRH5 and waterlogging as predictors, with interaction effects for *P. cinnamomi* and *P. multivora* TRH5 with waterlogging, respectively. For the second experiment, the model used for the data had the same layout, except that the *P. multivora* TRH1B2 treatment and its interaction effect with waterlogging were added too. Where the overall multivariate effect for the model was significant, subsequent univariate ANOVAs were conducted to explore the treatment effects on the response variables individually. Bonferroni adjustment to correct for multiplicity was applied to the resulting p-values.

Analyses of spectral data

Last measurements taken at harvesting

The spectral reflectance values, using the last measurements taken just before harvest, were plotted against the wavelengths for both trials, and inspected visually for any differences between the treatments.

Time-based measurements

The data from all dates for each experiment were pooled together for this part of the data analyses. All possible two-band combinations of the normalised difference spectral index (NDSI) (equation 1):

$$\boldsymbol{NDSI} = \frac{(\lambda_i - \lambda_j)}{(\lambda_i + \lambda_j)} \qquad \text{(Equation 1)}$$

were calculated, where λ is the reflectance value at wavelength *i* and *j*, after the method of Gold et al. (2019). Pearson correlation coefficients (*r*) were calculated between treatments and these NDSI combinations, to identify which regions of the electromagnetic spectrum best correlated with the treatment effects on *C. calophylla*. These correlations were plotted as a wavelength-versus-wavelength NDSI heat map, to visualise important spectral regions for distinguishing plant responses towards the treatments. The NDSI correlations were coloured on a gradient from red to blue – the strongest correlated NDSI combinations were coloured in red, and the least correlated coloured in blue. The 10 most correlated NDSIs were tabulated to identify the wavelength regions most associated with the treatment effects.

Linear mixed-effects models, using the VIs, were used to investigate changes in the spectral reflectance over time due to the treatment effects. The best model to characterise the response over time within the subjects (observation), and the variation in time trends between subjects (Pinheiro and Bates, 2000), had the treatment effects (*P. cinnamomi*, *P. multivora* TRH5 and waterlogging) as fixed effects, with time (measured in weeks) as a covariate. The second trial's model had the additional *P. multivora* TRH1B2 isolate as a treatment effect too. Interaction terms between the different *Phytophthora* treatments and waterlogging were added to the models as adding these interaction terms significantly improved model performance by lowering the Akaike information criteria (AIC) (Sakamoto et al., 1986). To determine the appropriate random effects, the protocol outlined by Zuur et al.

(2009b) was followed, which indicated that the optimal structure should include a random intercept (over time) for each plant. Model residuals displayed heterogeneity issues, and these were corrected for by modelling the data against its error distribution using the varPower variance structure (Zuur et al., 2009a). Likelihood ratio tests were used to assess the significance of individual covariates. Bonferroni post-hoc testing to correct for multiplicity was also applied (Perrett et al., 2006).

Results

Biophysical measurements

Plants inoculated with *Phytophthora* displayed symptoms of stress (chlorotic leaves and thinner canopies) by the end of the two trials, though plants inoculated with *P. multivora* TRH5 in the first trial appeared healthier than plants inoculated with *P. cinnamomi*: these plants were taller and displayed larger root volumes and top dry weights than the control plants (Fig. 3.1 A, C & D, Appendix 3 Table 1). In the second trial, however, inoculation with *P. multivora* TRH5 resulted in stunted growth with the non-waterlogged plants as these plants were shorter and displayed smaller diameters and top dry weights (Fig. 3.2 B-D, Appendix 3 Table 1).

The *P. cinnamomi* treatment in the first trial also resulted in stunted growth of the plants, as the waterlogged *P. cinnamomi* plants displayed smaller diameters and top dry weights than the other plants (Fig. 3. 1B-C, Appendix 3 Table 1). Boxplots of the four biophysical variables (Fig. 3.2 B-D) from the second trial indicated that the diameter, top dry weight and height for the waterlogged *P. cinnamomi* infected plants were less than those of the other treatments, suggesting this interaction effect impaired the growth pattern of these plants (Appendix 3 Table 1).

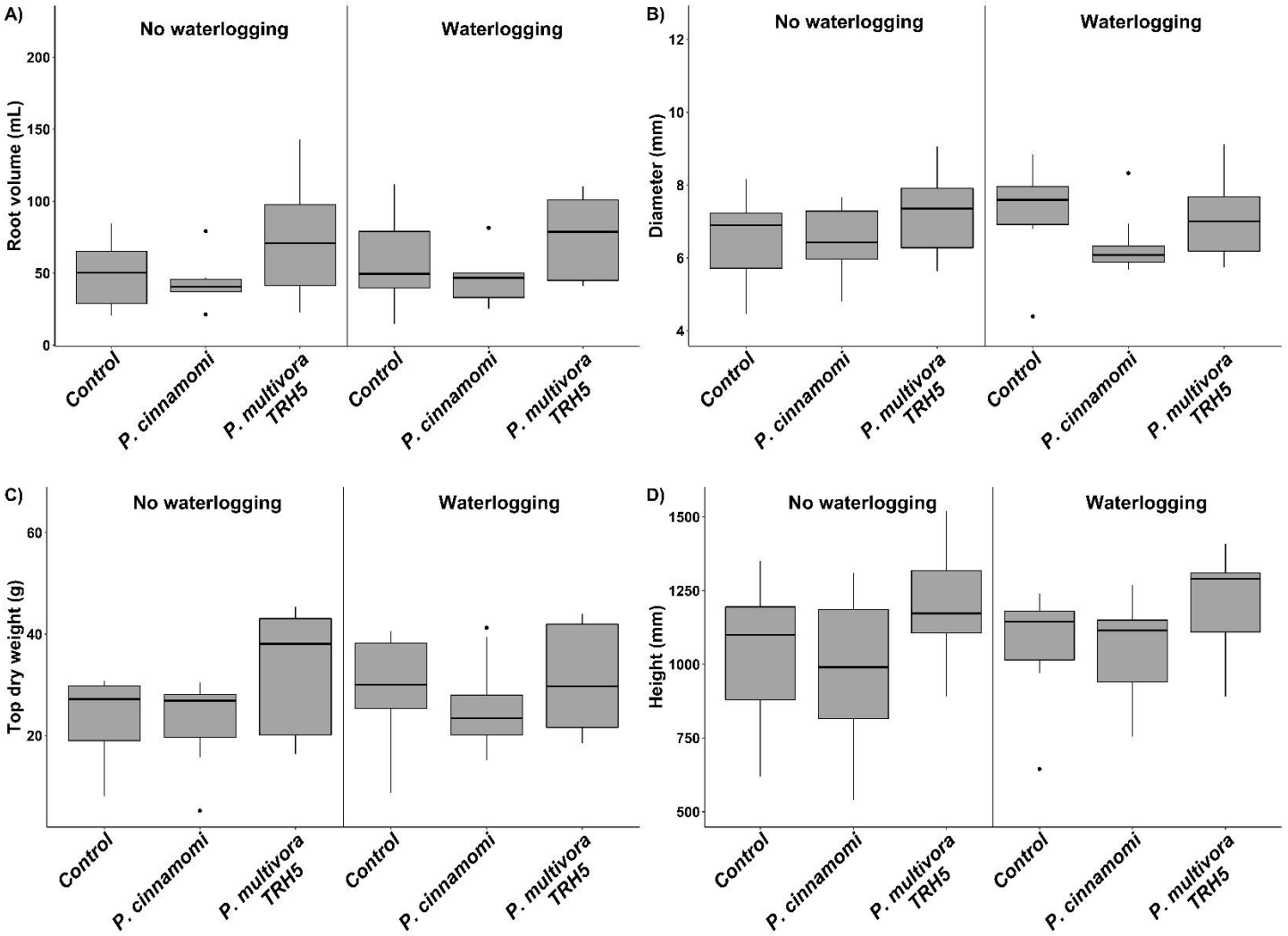

Figure 3.1. Boxplots, displaying the central tendency of data for the biophysical response variables, with first quartile, median (black line), and third quartile as well as outliers at the conclusion of the first trial, for A) root volume, B) diameter, C) top dry weight, and D) height. Plants were inoculated with *P. cinnamomi* and *P. multivora* TRH5 and waterlogged every fortnight for 24 hours.

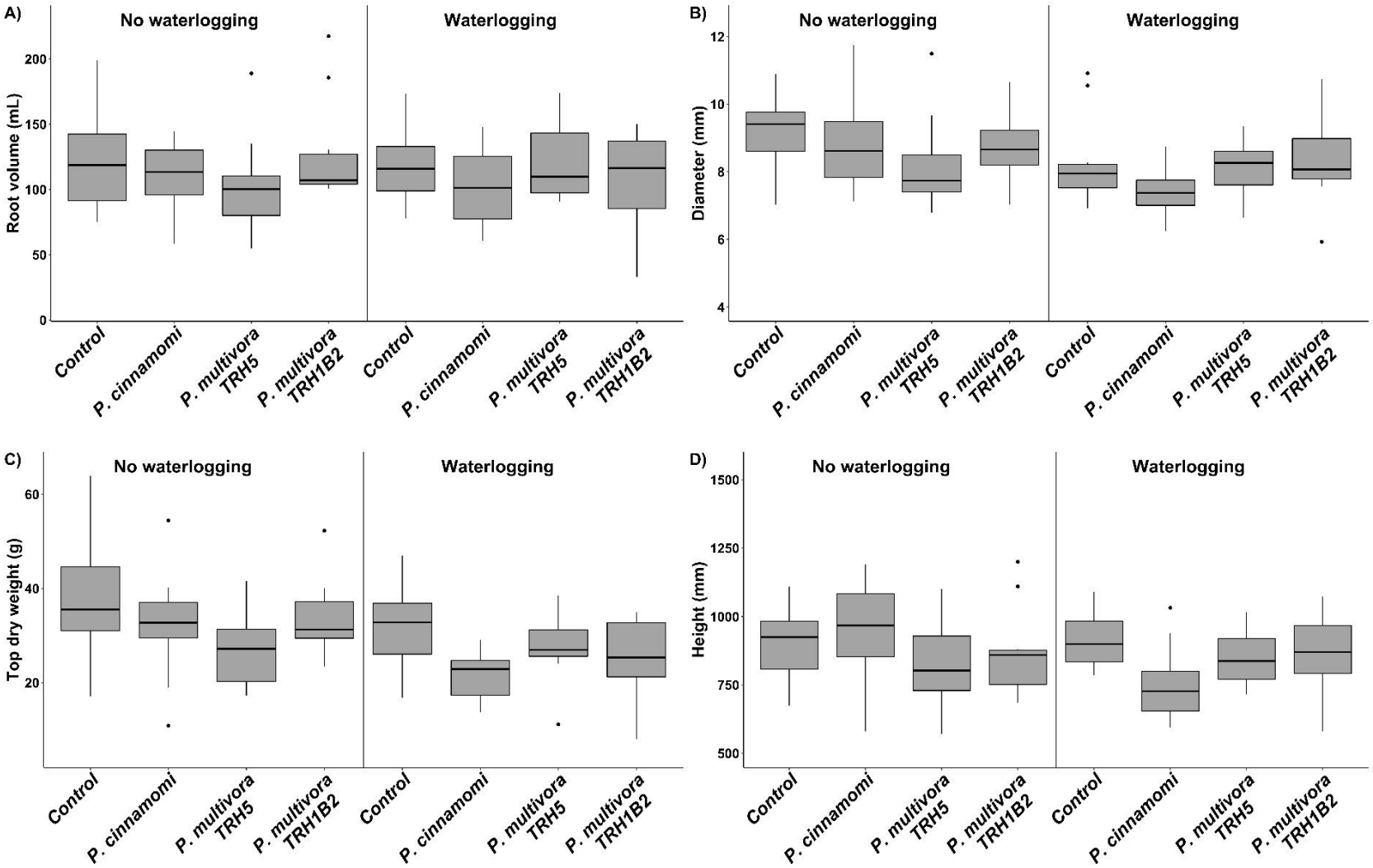

Figure 3.2. Boxplots, displaying the central tendency of data for the biophysical response variables, with first quantile, median (black line), and third quantile as well as outliers at the conclusion of the second trial, A) root volume, B) diameter, C) top dry weight, and D) height. Plants were inoculated with *P. cinnamomi*, *P. multivora* TRH5, and *P. multivora* TRH1B2 and waterlogged every fortnight for 24 hours.

The mean proportions of the biophysical variables differed between the two trials at the conclusion of the trials. Plants in the second trial (mean 866 cm, range 758 – 946 cm) were shorter across all treatments than plants in the first trial (mean 1091, range 977 – 1205cm) at the conclusion of the trials. The root volumes in the second trial (mean 114 mL, range 101 – 127 mL) were almost double the root volumes in the first trial (mean 59 mL, range 44 – 76 mL) (Fig. 3.1A-D, 3.2A-D, Appendix 3 Table 1). This implies that the plants in the second trial experienced less vegetative growth than the plants in the first trial, as these plants had started approximately 10 cm taller than the plants in the first trial.

The biophysical response variables for both trials displayed heterogeneous standard errors, as well as extreme outliers. Root volume for the first trial displayed a non-normal distribution, thus it was log transformed. Using Pillai's trace, the global *p*-value for the first trial was not significant (V = 0.39, F (20, 168) = 0.91, p =.58), and univariate ANOVAs were therefore not performed on this data.

The global *p*-value for the MANOVA (using Pillai's trace) for the second trial's biophysical variables was significant (V = 0.53, F (28, 288) = 1.57, p =.04). The global multivariate p-values (Type II sum of squares) for the treatment effects indicated that the waterlogging treatment was highly (V = 0.22, F (4, 69) = 4.79, p = .002) significant, but not for the *P. multivora* TRH5 (V = 0.13, F (4, 69) = 2.50, p = .06) and *P. cinnamomi* (V = 0.12, F (4, 69) = 2.13, p = .07) treatments (Table 3.3). The *P. multivora* TRH1B2 treatment (V = 0.09, F (4, 69) = 1.68, p = .16) was also not significant.

Table 3.3. Global multivariate effects (Type II sum of squares) for the *Phytophthora cinnamomi*, *P. multivora* TRH5, *P. multivora* TRH1B2, and waterlogging treatments on the biophysical variables (root volume, diameter, top dry weight and height) in the second trial

Variables	Pillai's Trace	F	df	p
P. cinnamomi	0.12	2.31	4, 69	.066
P. multivora TRH5	0.13	2.50	4, 69	.050
P. multivora TRH1B1	0.09	1.68	4, 69	.164
Waterlogging	0.22	4.79	4, 69	.002 **
P. cinnamomi & waterlogging	0.06	1.14	4, 69	.345
P. multivora TRH5 & waterlogging	0.02	0.42	4, 69	.794
P. multivora TRH1B1 & waterlogging	0.05	0.85	4, 69	.508

*α ≤ 0.05, **α ≤ 0.01, ***α ≤ 0.001.

Univariate ANOVAs with Bonferroni-adjusted p-values indicated that the waterlogging treatment lowered plant top dry weight (p = .02) and diameter (p = .04) significantly. The

interaction between *P. cinnamomi* (p = .05) and waterlogging was also significant for height (Fig. 3.2D, Appendix 3 Table 2).

Spectral measurements

Last measurements taken at harvesting

Visual inspection of spectral reflectance values plotted against wavelength in the first trial indicated that differences in reflectance values between the controls and the treatments were more noticeable in the visible (VIS) light range (400-700 nm) and short-wave infrared range (SWIR) of the electromagnetic spectrum (EM) (1 300-2 500 nm) (Fig. 3.3A). These differences relate to changes associated with leaf pigments, water content and foliar dry matter. The reflectance spectra for the non-waterlogged control and *P. cinnamomi* plants separated out from the other treatments in the VIS and SWIR regions of the EM, by having higher reflectance values (Fig. 3.3A). The continuum removed (CR) spectral data indicated that the treatments separated at the water absorption feature at 1 450 nm (Fig. 3.3B).

Differences between the control and treatment groups in the second trial, though less profound, were associated with internal leaf structure and foliar dry matter of the leaves (i.e., the NIR (750 – 1 300 nm) and SWIR (1 600 – 1 800 nm) regions of the EM (Fig. 3.3C)). The reflectance spectra for the waterlogged *P. cinnamomi*-inoculated plants separated out from the other treatments in the NIR and SWIR regions of the EM, by having lower reflectance values. The continuum removed (CR) spectral data indicated that the water absorption features at 1 200 nm, 1 450 nm and 1 950 nm displayed small differences between treatments (Fig. 3.3D), with only the non-waterlogged *P. multivora* TRH1B2 treatment separating out at 1 450 nm and 1 950 nm.

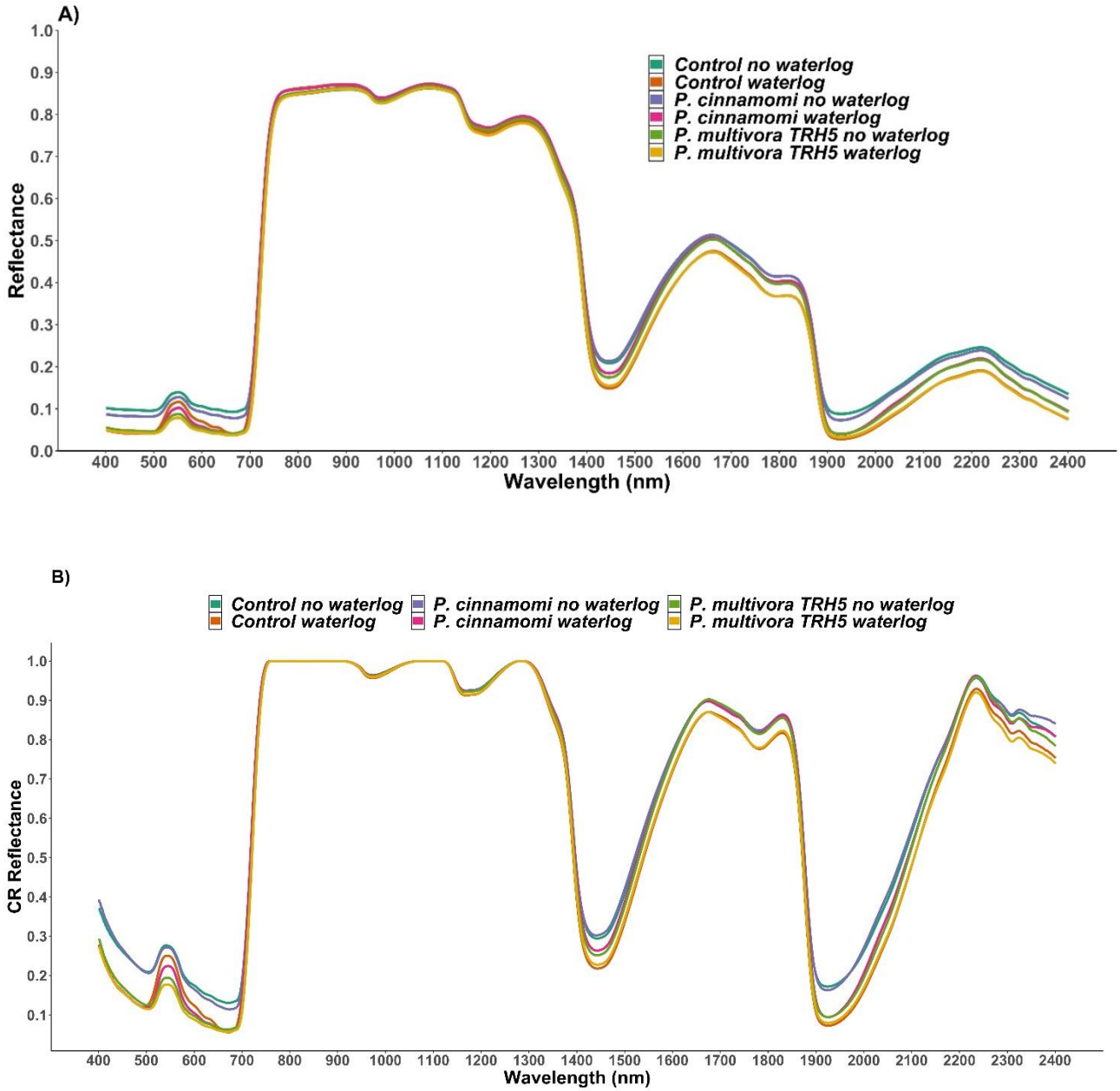

Figure 3.3A-B. Reflectance spectra for plant leaves in the first trial: A) measured at harvesting and B) Continuum removed (CR) reflectance spectra for the same leaves.

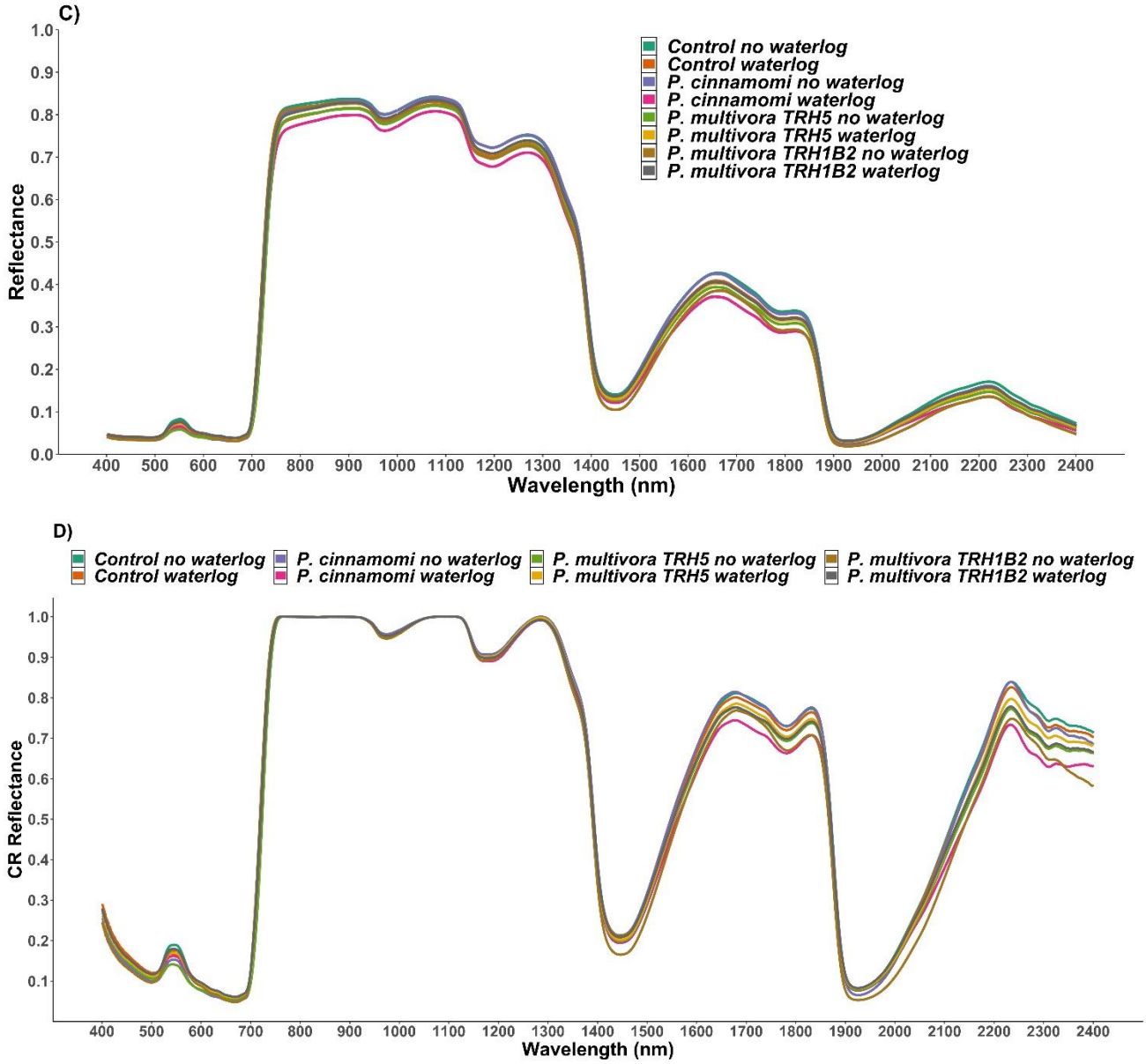

Figure 3.3C-D. Reflectance spectra for plant leaves in the second trial: C) measured at harvesting and D) Continuum removed (CR) reflectance spectra for the same leaves.

Time-based measurements

Data from all time points were used to visualise important spectral regions for distinguishing plant responses towards treatments, by plotting the correlations between the reflectance data and treatments as a wavelength-versus-wavelength NDSI heat map. The NDSI correlations were coloured on a gradient from red to blue; the best correlated NDSI combinations were coloured in red and the least correlated NDSI combinations in blue.

The heat maps produced different results for the two trials. For the first trial, NDSIs making use of the bands in the visible portion of the electromagnetic spectrum (400-700 nm) displayed the strongest correlations (red colour) with the treatments combined (Fig. 3.4A). When separating the data into *Phytophthora*-infected and control plants, very weak correlations between disease status and NDSIs were observed (Fig. 3.4B). However, when the data were separated between the waterlogged and non-waterlogged treatments, stronger correlations between NDSIs and the waterlogging treatment (red colour) were observed across all wavelengths (Fig. 3.4C), especially in the visible and SWIR portion of the electromagnetic spectrum.

The NDSI heat map for the second trial did not display correlations (mostly blue in colour) with the combined treatments in any of the wavelength regions (Fig. 3.5A). The same is true when the data were separated between the *Phytophthora* and control treatments (Fig. 3.5B). The heat map for visualising the correlations between the NDSIs and the waterlogging treatment on the plants, however, displayed weak correlations in the SWIR region of the electromagnetic spectrum (1 300-2 500 nm) (Fig. 3.5C).

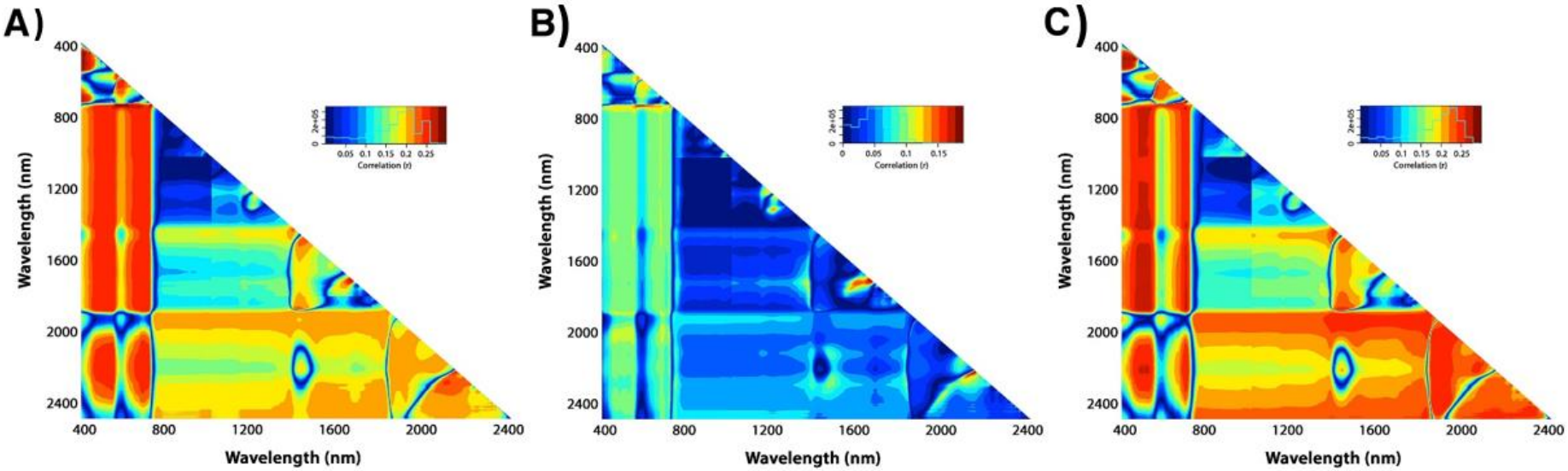

Figure 3.4. Correlation matrices (heat maps) showing the coefficients of determination (R^2) (Pearson's correlations) for all dual wavelength combinations (normalised difference vegetation indices) in the range of 400 – 2 500 nm, across all dates for the first trial: A) all treatments combined, B) *Phytophthora* treatment, and C) waterlogging treatment. The red colour indicates a strong correlation between the reflectance data and the relevant treatments (A, B or C), and blue indicates a weak to no correlation.

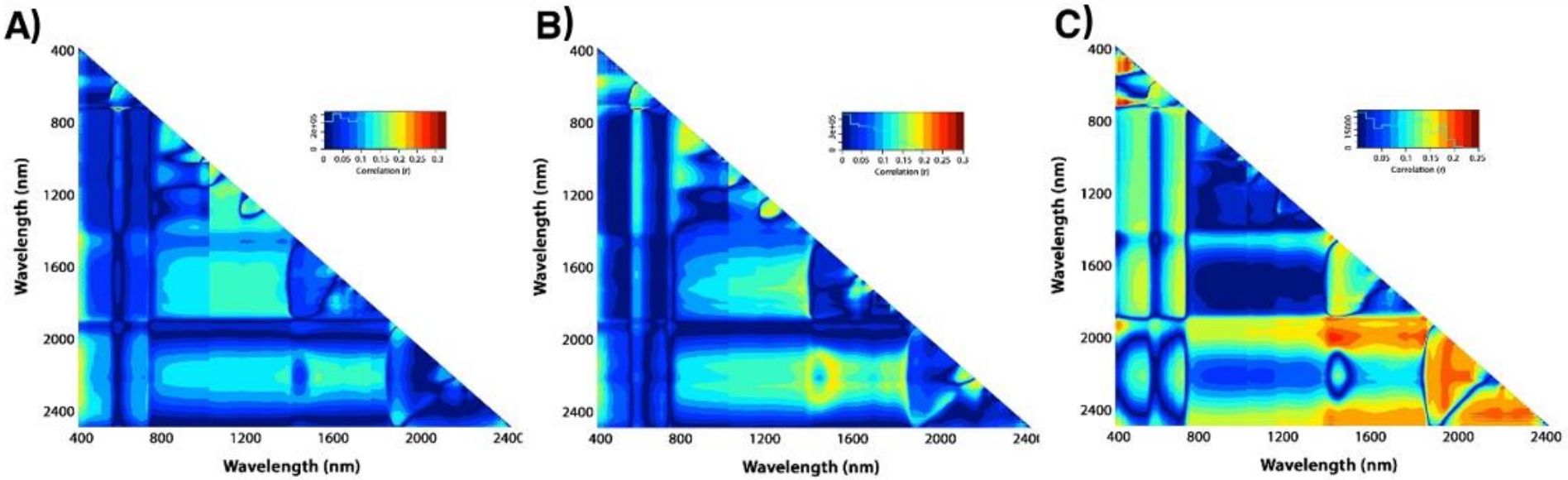

Figure 3.5. Correlation matrices (heat maps) showing the coefficients of determination (R^2) (Pearson's correlations) for all dual wavelength combinations (NDSIs) in the range of 400 – 2 500 nm, across all dates for the second trial, A) all treatments combined, B) *Phytophthora* treatment, and C) waterlogging treatment. The red colour indicates a strong correlation between the reflectance data and the relevant treatments, and blue indicates a weak to no correlation.

The wavelengths of the 10 most correlated NDSIs with treatment effects are listed in Table 3.4; for both trials the first wavelength fell within the visible portion (538 – 544 nm for the first trial, 538 – 558 nm for the second trial), and the second wavelength in the NIR portion (708 – 709 nm for the first trial, 701 – 702 nm for the second trial) of the electromagnetic spectrum. This implies that both wavelengths are within the regions of the electromagnetic spectrum where the chlorophyll pigments absorb less light (the first wavelength is within the 'green light' region (500 – 600 nm), and the second wavelength is within the "red edge" region (> 700 nm)).

Table 3.4. List of spectral bandwidths combinations with the highest correlations to the combination of the *Phytophthora* and waterlogging treatments for both trials. All these bandwidths fall within the visible portion of the electromagnetic spectrum.

First trial		Second trial	
Wavelength 1	**Wavelength 2**	**Wavelength 1**	**Wavelength 2**
539	708	556	702
541	708	557	702
540	708	555	702
539	709	554	702
542	708	553	702
538	709	539	701
541	709	552	702
540	709	558	702
544	708	538	701
543	708	540	701

Boxplots of six of the eleven selected VIs (NDNI2, ARI1, PRI1, CTR1, VOG3 and WBI), when comparing the values between the first and tenth week of the trial, displayed a significant trend over the ten weeks. These trends differed between the two trials. NDNI2 values decreased for only the *P. cinnamomi*-treated plants in the first trial (Fig. 3.6A) but decreased for all plants in the second trial (except for the waterlogged *P. multivora* TRH1B2 treatment) ($p < 0.00$) (Fig. 3.7A, Table 3.6). ARI1 values (Fig. 3.6B) remained relatively constant in the first trial (except for the waterlogged *P. multivora* TRH5 plants) but increased for all treatments in the second trial ($p < 0.00$) (Fig. 3.7B, Table 3.6). PRI values in the first trial increased for all treatments ($p < 0.00$) (Fig. 3.6C, Table 3.5) but decreased in the second trial ($p < 0.00$) (Fig. 3.7C, Table 3.6). CTR1 values remained relatively constant for both trials (Fig. 3.6D, Fig. 3.7D), except for the *P. multivora* TRH1B2 treatment in the second trial, which increased. VOG3 values increased for all treatments in the first trial ($p < 0.00$) (Fig. 3.6E, Table 3.5), but not for all plants in the second trial – values for the non-waterlogged control, waterlogged *P. multivora* TRH5 and non-waterlogged *P. multivora* TRH1B2 treatments decreased significantly ($p < 0.00$) (Fig. 3.7E, Table 3.6). WBI values increased for all treatments in the first trial ($p < 0.00$) (Fig. 3.6F, Table 3.5) but decreased for only the *P. multivora* TRH5 and *P. multivora* TRH1B2 values in the second trial (Fig. 3.7F).

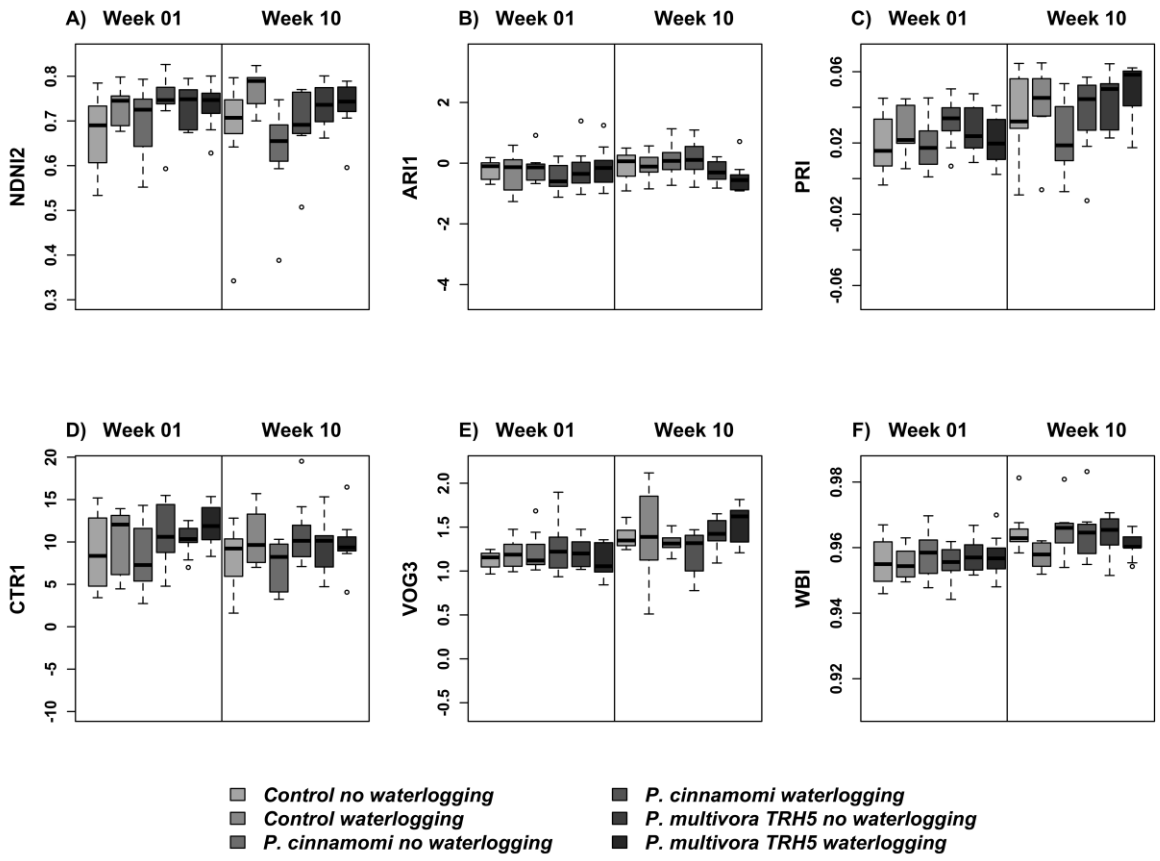

Figure 3.6. Boxplots, displaying the central tendency of data for the vegetation indices, with first quantile, median (black line), and third quantile as well as outliers for the first trial. These were plotted for weeks 1 and 10, from left to right, for A) NDNI2 (normalised difference nitrogen index 2), B) ARI1 (anthocyanin reflectance index 1), C) PRI (photochemical reflectance index), D) CTR1 (Carter index 1), E) VOG3 (Vogelman index 3) and F) WBI (water band index).

The results of the mixed effects models, analysing the temporal component of the spectral data, indicated that the main treatment effects (*P. cinnamomi*, *P. multivora* TRH5, *P. multivora* TRH1B2, and waterlogging) did not cause any significant differences in the VIs between the treated plants and the controls for both trials. The time covariate was significant for PRI, VOG3 and WBI for the first trial, and for the second trial, it was with NDNI2, ARI1, LI3, PRI, NDVI, VOG3 and CAI (Tables 3.5 and 3.6). The water band [index (WBI) displayed a significant (p=.01) response towards the combined effect of *P. cinnamomi* and waterlogging in the first trial (Table 3.5), indicating an exacerbating effect on the plants when the two treatments are combined. As with the biophysical response variables, these data were plagued with heterogeneous standard errors and extreme outliers (Fig. 3.6A-F, 3.7A-F).

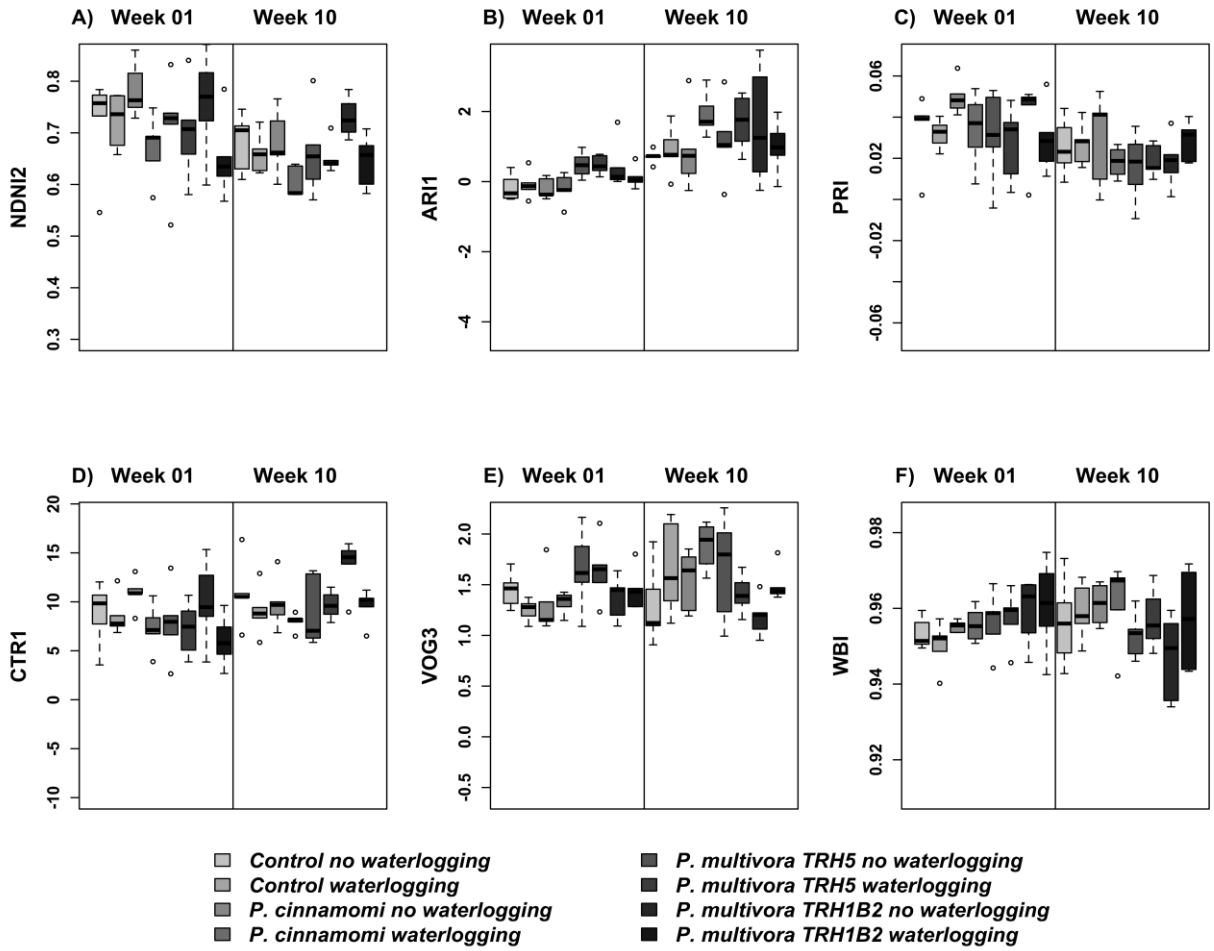

Figure 3.7. Boxplots, displaying the central tendency of data for the vegetation indices, with first quantile, median (black line), and third quantile as well as outliers for the second trial. These were plotted for weeks 1 and 10, from left to right, for A) NDNI2 (normalised difference nitrogen index 2), B) ARI1 (anthocyanin reflectance index 1), C) PRI (photochemical reflectance index), D) CTR1 (Carter index 1), E) VOG3 (Vogelman index 3) and F) WBI (water band index).

Solar radiation during the trials

The daylight length and solar radiation levels were consistent with the season in which the trials were conducted. During the first trial, solar radiation levels remained relatively constant, except towards the last few weeks when there was an increase in light intensity as the season changed from mid-winter (July) to late winter (August) (Appendix 3 Fig. 1A). Solar radiation levels decreased during the second trial at a constant pace (Appendix 3 Fig. 1B), as the experiment ran from early autumn (March) into mid-winter (July). Daylight length also changed because of the changing seasons (Appendix 3 Table 3, becoming shorter as the season went into the winter months in the second trial).

Table 3.5. Responses of the vegetation indices[#] towards the treatment effects in the temporal component of the data in the first trial. Bonferroni correction for multiple tests was applied; significant *p*-values are listed in bold (with the original *p*-values before Bonferroni correction in brackets next to the adjusted *p*-values.)

Vegetation Index#			Treatment							
	Category	Electromagnetic spectrum region^	*P. cinnamomi*	*P. multivora* TRH5	Water-logging	Week	*P. cinnamomi*, waterlogging	*P. multivora* TRH5, waterlogging	*P. cinnamomi*, waterlogging, week	*P. multivora* TRH5, waterlogging, week
NDNI2	Canopy greenness	SWIR	p=1.0 (0.40)	p=.55 (.18)	p=.85 (.28)	p=.58 (.19)	p=1.0 (.65)	p=1.0 (.59)	p=.10 (.03)	p=.68 (.23)
ARI1	Leaf pigment	Visible	p=1.0 (.78)	p=1.0 (.79)	p=1.0 (.55)	p=.86 (.29)	p=1.0 (.99)	p=.90 (.30)	p=.97 (.32)	p=.16 (.05)
RG-ratio	Leaf pigment	Visible	p=1.0 (.94)	p=.12 (.04)	p=.39 (.13)	p=1.0 (.99)	p=1.0 (.78)	p=.77 (.26)	p=.95 (.32)	p=1.0 (.72)
LI3	Light-use efficiency (LUE)	Visible	p=1.0 (.90)	p=.24 (.08)	p=.58 (.19)	p=.46 (.15)	p=1.0 (.88)	p=.99 (.33)	p=1.0 (.66)	p=1.0 (.58)
PRI	Light-use efficiency (LUE)	Visible	p=1.0 (.81)	p=.64 (.22)	p=.42 (.14)	**p<.00*** (.00)**	p=1.0 (.97)	p=.78 (.26)	p=1.0 (.59)	p=.11 (.04)
CTR1	Photosynthetic potential	Visible	p=1.0 (.94)	p=.07 (.02)	p=.25 (.08)	p=1.0 (.87)	p=1.0 (.98)	p=.79 (.24)	p=.72 (.40)	p=1.0 (.35)
NDVI	Photosynthetic potential	Visible/NIR	p=1.0 (.86)	p=.21 (.07)	p=.45 (.15)	p=1.0 (.41)	p=1.0 (.76)	p=.74 (.25)	p=1.0 (.53)	p=1.0 (.99)
VOG3	Red-Edge Index	Visible	p=1.0 (.75)	p=1.0 (.93)	p=1.0 (.66)	**P=.04 (.01)**	p=1.0 (.52)	p=1.0 (.53)	p=.26 (.09)	p=.60 (.20)
CAI	Senescence	SWIR	p=1.0 (.94)	p=.53 (.18)	p=1.0 (.48)	p=1.0 (.91)	p=1.0 (.69)	p=1.0 (.86)	p=.43 (.15)	p=.59 (.20)
NDWI	Water content	NIR	p=1.0 (.77)	p=1.0 (.98)	p=1.0 (.88)	p=1.0 (.46)	p=1.0 (.66)	p=1.0 (.94)	p=1.0 (.48)	p=1.0 (.89)
WBI	Water content	NIR	p=1.0 (.95)	p=1.0 (.96)	p=1.0 (.93)	**p<.00*** (.00)**	p=1.0 (.81)	p=1.0 (.91)	**p<.01** (.00)**	p=.60 (.20)

For more information on the selected vegetation indices, see Appendix 1 Table 1. **α ≤ 0.01, ***α ≤ 0.001.

^ SWIR = shortwave infrared, NIR = near-infrared

Table 3.6. Responses of the vegetation indices[#] towards the treatment effects in the temporal component of the data in the second trial. Bonferroni correction for multiple tests was applied; significant *p*-values are listed in bold (with the original *p*-values before Bonferroni correction in brackets next to the adjusted *p*-values.)

Vegetation Index#			Treatment							
	Category	**Electro-magnetic spectrum region^**	***P. cinnamomi***	***P. multivora* TRH5**	***P. multivora* TRH1B2**	**Water-logging**	**Week**	***P. cinnamomi*, waterlogging**	***P. multivora* TRH5, waterlogging**	***P. multivora* TRH1B2, waterlogging**
NDNI2	Canopy greenness	SWIR	p=1.0 (.60	p=1.0 (.95)	p=1.0 (.75)	p=1.0 (.64)	**p<.00*** (.00)**	p=.11 (.03)	p=1.0 (.80)	p=.84 (.21)
ARI1	Leaf pigment	Visible	p=1.0 (.47)	p=1.0 (.26)	p=.16 (.04)	p=1.0 (.66)	**p<.00*** (.00)**	p=1.0 (.25)	p=1.0 (.25)	p=1.0 (.93)
RG-ratio	Leaf pigment	Visible	p=1.0 (.83)	p=1.0 (.54)	p=1.0 (.33)	p=1.0 (.62)	p=.17 (.04)	p=.79 (.20)	p=1.0 (.77)	p=1.0 (.54)
LI3	Light-use efficiency(LUE)	Visible	p=1.0 (.85)	p=1.0 (.96)	p=1.0 (.72)	p=1.0 (.50)	**p=.04 (.01)**	p=1.0 (.61)	p=1.0 (.49)	p=1.0 (.87)
PRI	Light-use efficiency(LUE)	Visible	p=1.0 (.96)	p=1.0 (.61)	p=1.0 (.24)	p=1.0 (.58)	**p<.00*** (.00)**	p=.52 (.13)	p=1.0 (.43)	p=1.0 (.53)
CTR1	Photosynthetic potential	Visible	p=1.0 (.85)	p=1.0 (.69)	p=1.0 (.61)	p=1.0 (.37)	p=.57 (.15)	p=1.0 (.47)	p=1.0 (.36)	p=1.0 (.44)
NDVI	Photosynthetic potential	Visible/NIR	p=1.0 (.60)	p=1.0 (.81)	p=1.0 (.39)	p=1.0 (.52)	**p<.00*** (.00)**	p=1.0 (.67)	p=1.0 (.81)	p=1.0 (.79)
VOG3	Red-Edge Index	Visible	p=1.0 (.87)	p=1.0 (.49)	p=.81 (.20)	p=1.0 (.61)	**p<.00*** (.00)**	p=.80 (.20)	p=1.0 (.48)	p=1.0 (.26)
CAI	Senescence	SWIR	p=1.0 (.44)	p=1.0 (.72)	p=1.0 (.28)	p=1.0 (.93)	**p<.00*** (.00)**	p=.48 (.12)	p=1.0 (.98)	p=1.0 (.45)
NDWI	Water content	NIR	p=1.0 (.79)	p=1.0 (.83)	p=1.0 (.41)	p=1.0 (.94)	p=1.0 (.41)	p=1.0 (.93)	p=1.0 (.94)	p=1.0 (.95)
WBI	Water content	NIR	p=1.0 (.76)	p=1.0 (.68	p=1.0 (.49)	p=1.0 (.89)	p=1.0 (.28)	p=1.0 (.89)	p=1.0 (.84)	p=1.0 (.94)

For more information on the selected vegetation indices, see Appendix 1 Table 1. ***α ≤ 0.001.

^ SWIR = shortwave infrared, NIR = near-infrared

Discussion

Effect of Phytophthora *and waterlogging on the biophysical and spectral reflectance characteristics of* C. calophylla

In this study, there were no significant differences between plants inoculated with *Phytophthora* and the control plants at a biophysical level (i.e., diameter, height, root volume, and root dry weight), except for the interaction between *P. cinnamomi* and waterlogging on plant height (p = .05) in the second trial. This is in contrast with previous studies, where *P. cinnamomi* and *P. multivora* severely affected *C. calophylla*, even leading to plant death (Chapter 2). A possible explanation could be that the plants in this study were more resistant to *Phytophthora*, as the plants were six months older than the plants in Chapter 2. Day length could also have played a role here. In Chapter 2, the trials were run during the summer months, whereas the trials in this study were run from early autumn through winter to early spring. Very little research on the effect of phototrophy on *P. cinnamomi* and *P. multivora* pathogenicity was conducted, but studies done with *P. infestans* on different potato cultivars suggest that not all host response variation to this pathogen could be explained by host differences (Sujkowski, 1986, Mihovilovich et al., 2010). More work needs to be done to understand the effect of day length on *P. cinnamomi* and *P. multivora* pathogenicity on *C. calophylla*.

With the spectral measurements in the first trial, when the reflectance values were plotted against wavelength, the *Phytophthora* treatments absorbed more light in the VIS and SWIR (1 900 – 2 400 nm) regions than the non-waterlogged control treatment. The waterlogged *P. cinnamomi* treatment in the second trial separated out from the other treatments in the NIR and SWIR regions, also absorbing more light in these regions. This may indicate structural damage to the photosynthetic apparatus in the leaves of these plants, as photosynthetic pigments are unable to harvest light for photosynthesis in the NIR and SWIR regions. In the Newby et al. (2019) study, different reflectance spectra were also visible in the VIS and NIR regions of the EM for the *P. cinnamomi*-inoculated plants for two of the species considered highly susceptible to *P. cinnamomi*. This could indicate that *P. cinnamomi* infection with susceptible hosts does result in damage of the photosynthetic components in a plant but more work needs to be done in this area, especially for

C. calophylla, as the *C. calophylla* plants in this study were not as susceptible to *P. cinnamomi* as the plants in the Newby et al. (2019) study.

The NDSI heat maps displayed weak correlations between *Phytophthora* and reflectance values in all spectral regions for both trials. Recent work with NDSI heat maps and *P. infestans* infection indicated differently. Spectral reflectance measurements were taken on leaves of four different potato cultivars infected with *P. infestans*. These measurements were taken on the asymptomatic parts of the infected leaves. The NDSI heat maps displayed consistent correlations with infection status, particularly in the SWIR region and around 1 000 nm (Gold et al., 2019). Perhaps this could be ascribed to the difference in aetiology between *P. infestans* and *P. cinnamomi* as host defence mechanisms would work differently for leaf and root pathogens. The specific response of *C. calophylla* to infection with *P. cinnamomi* could also have contributed to these results. In studies with *E. marginata* (jarrah) and *C. calophylla*, the levels of cytokinins in the xylem exudate of the susceptible jarrah were significantly reduced within three days of infection with *P. cinnamomi*, but no reduction in cytokinin levels was observed for the field resistant *C. calophylla* (Cahill et al., 1986). More work needs to be done to determine the specific biochemical changes in *C. calophylla* when infected with *Phytophthora*, and then focus on determining the wavelengths involved with these compounds/pigments.

In the second trial, waterlogging significantly reduced *C. calophylla* top dry weight (p = .016) and diameter (p = .044). Though the anatomy of its leaves enable *C. calophylla* to regulate its water content, even while experiencing adverse water potentials (Colquhoun et al., 1984, Szota et al., 2011), these findings are in line with previous studies, where *C. calophylla* plants under flooded conditions in a glasshouse experiment had significantly less total dry weight and smaller diameters than the control plants (Farifr and Aboglila, 2015).

When reflectance values were plotted against wavelength for the waterlogging treatment, the water absorption features at 1 450 nm and 1 950 nm (both in the SWIR region) displayed differences between the treatments for the first trial. This is consistent with previous studies (Newby et al., 2019), as a number of water-based vegetation indices make use of reflectance in this area. The corresponding NDSI heat map displayed stronger correlations in the VIS and SWIR regions too. Reflectance in the visible area of the electromagnetic spectrum indicates leaf pigment condition in the leaves, implying that

photosynthesis was affected by the waterlogging treatment. Similar observations have been made for three *Eucalyptus* spp. (Florentine and Fox, 2002) and *Zea mays* (Ren et al., 2016), where waterlogging significantly reduced photosynthetic capacity. The plants in the second trial were less affected by the waterlogging treatment as there were small differences between treatments at the water absorption features, and their waterlogging heat map displayed no correlations in the VIS and weak correlations in the SWIR regions too.

The vegetation indices used in this study also did not display significant differences between the *Phytophthora* and waterlogging treatments, except in the first trial, where the WBI was significant for the interaction effect between *P. cinnamomi* and waterlogging ($p = 0.01$). A possible explanation could lie in the bandwidths that were used in the vegetation indices. Most of the VIs were narrowband indices, using reflectance values of very specific bandwidths (i.e., for PRI it is reflectance at 531 nm and 570 nm, Appendix 1, Table 1), and it may be that these bandwidths are not optimised for *C. calophylla*. Various other reasons could also have influenced these results, i.e., seasonal changes in the leaves, genetic variability in the plants (as the seed was collected from the native forest), and internal processes in the plant cells. Light-induced chloroplast movement (also called photo relocation) in the cells of the leaves may cause different pathways for reflected and transmitted radiation, which in turn could cause spectral peaks that are slightly outside the optimised level, thus affecting the measurements (Gamon et al., 1990). A study by Main et al. (2011) confirms this, stating that chlorophyll in some types of leaves is easily saturated with light, and suggested that ”off-centre” wavelengths should be used for chlorophyll content changes of those leaves. A different approach for the use of VIs with *C. calophylla* is needed, such as the use of continuum removed reflectance spectra and NDSI heat maps, to refine the selection of bandwidths to devise VIs specific to *C. calophylla*.

Effect of seasonal changes on the biophysical and spectral reflectance measurements

Although the plants were of the same age, the results from the two trials displayed seasonal differences on both the biophysical and spectral reflectance levels. At the end of the first trial, plants displayed smaller root volumes, but they were taller than in the second trial, even though the *C. calophylla* plants used in the first trial were shorter at the onset of the trial than the plants in the second trial. This could be due to a phototropic effect on the plants in the second trial; *C. calophylla* displays a short-day response to photoperiodism,

where vegetative growth gives way to flowering as the days get shorter (December to May). Light intensity and daylight length also changed over the ten weeks in both trials. During the first trial, conducted from late autumn to early spring, the light intensity fluctuations remained relatively constant, except towards the end of the trial when it started to increase again. It is then when the *C. calophylla* plants would have started to prepare for the new growth season, and resources would have been directed towards stem elongation and leaf flush. The second trial was conducted from early autumn to mid-winter. During this time both the light intensity and daylight length decreased at a constant pace. This is a time when photosynthetic rate would be less as leaf pigments such as chlorophyll would degenerate, stem growth slowed down, and more resources are directed towards enhancing root systems.

Reflectance values plotted against wavelength also displayed a seasonal trend. In the first trial, there were bigger differences between treatments in the VIS (400 - 700 nm) and SWIR (2 000-2 500 nm) portions of the electromagnetic spectrum. These areas are associated with leaf pigment content and foliar dry matter (protein, lignin, and cellulose content), respectively, also indicative that these plants were preparing for the new growth season by starting to produce new chlorophyll pigments. The spectral reflectance measurements in the second trial displayed differences in the NIR (750 – 1 300 nm) and SWIR (1 500 – 1 900 nm) regions of the electromagnetic spectrum. These areas are associated with the internal leaf scattering of light and the foliar dry matter of the leaves, thus confirming that the plants were adjusting to the shorter day lengths and lower night temperatures, thus vegetative growth would have slowed down, and the chlorophyll condition would have deteriorated too.

Six of the vegetation indices displayed seasonal trends over time in both trials (i.e., NDNI2, ARI1, PRI, CTR1, VOG3 and WBI). The NDNI2, a VI related to nitrogen content in the leaves, displayed a downward trend for most treatments over the ten weeks in the second trial, but not during the first. This index is a revised version of the normalised difference nitrogen index (Serrano et al., 2002), and was created to estimate canopy nitrogen content in wetlands (Wang and Wei, 2016). Nitrogen content in the leaves is strongly related to the condition of chlorophyll pigments (Daughtry et al., 2004); a lower nitrogen content in the leaves signals reduced/deteriorated chlorophyll pigments (Wang and Wei, 2016). The plants

in the second trial were harvested in mid-winter when they were not growing actively, thus photosynthesis was at a lower rate than in spring and summer, hence the downward trend in nitrogen content in the leaves. This is in line with the results from the biophysical measurements, as the plants in the second trial were shorter and had bigger root volumes than in the first trial (i.e., less vegetative growth was taking place).

ARI1 is another VI displaying a seasonal trend. This index was devised to estimate the anthocyanin content in intact maple, cotoneaster, dogwood and pelargonium leaves (Gitelson et al., 2001). It remained relatively constant for the *C. calophylla* plants in the first trial but increased for plants in the second trial. Anthocyanin accumulation occurs in senescing and stressed vegetation (Gitelson et al., 2001), though some plants display higher ARI1 values during the early growing season too (Gitelson et al., 2001, Vergara-Díaz et al., 2018). These results agree with those for the biophysical (root volumes and plant height) and NDNI2 values, where the plants in the second trial may not have been actively growing in the mid-winter; thus, their chlorophyll content could have been reduced/deteriorated. This implies that the leaves anthocyanin content may have been more prominent at the time of measurement in the second trial.

In this study, a clear seasonal trend was observed for the PRI (photochemical reflectance index) in both trials. PRI was originally developed for estimating changes in the xanthophyll cycle, i.e., the ability of vegetation to use absorbed light efficiently for photosynthesis (light use efficiency (LUE) (Gitelson et al., 2015). PRI values increase when vegetation is healthy (thus actively growing) (Zhang et al., 2017a), but decrease when vegetation is stressed. The first trial's PRI values increased over the ten weeks for all treatments but decreased in the second trial. This implies that the plants in the second trial experienced a reduction/deterioration of chlorophyll over the ten weeks, whereas plants in the first trial were starting to prepare for spring by developing new chlorophyll pigments.

Another index displaying a seasonal trend was CTRI1 (Carter, 1994), this is a simple ratio index and was developed to indicate plant stress. Its value increases as the chlorophyll content in the leaves decreases due to stress. CTR1 values were mostly unaffected in the first trial but increased for both the waterlogged and non-waterlogged *P. multivora* (TRH1B2)-inoculated plants in the second trial. This implies that the spectral measurements

were able to detect stress in the plants inoculated with *P. multivora* (TRH1B2), even though this could not yet be quantified by the biophysical measurements.

VOG3 (Vogelmann et al., 1993) also displayed a seasonal trend. This VI makes use of reflectance values in the red-edge portion (680-750 nm) of the electromagnetic spectrum. When plants are stressed, their chlorophyll absorption feature usually shifts to shorter wavelengths (i.e. blue) (Horler et al., 1983a), resulting in increased VOG3 values as more light is reflected. VOG3 values increased over time for plants in both trials, implying that they became more stressed, though this was not confirmed by the statistical analyses in this trial. There was an exception as VOG3 values for the non-waterlogged control and non-waterlogged *P. multivora* (TRH1B2)-plants did not increase in the second trial.

The last VI to display a seasonal trend, WBI, was developed to indicate the water status in plant leaves (Penuelas et al., 1993). A higher WBI content resembles higher water content in the leaves, indicating that plants are less stressed. This index seems to work better at the canopy level when plants wholly cover the soil (Penuelas et al., 1993). In the present study, WBI values in the *C. calophylla* leaves increased over time, except for both the waterlogged and non-waterlogged *P. multivora* TRH5 and *P. multivora* TRH1B2 inoculated plants in the second trial. Plants in both trials were watered daily to container capacity and flooded fortnightly for 24 hours and then drained; thus, a decrease in water content in the leaves was highly unlikely unless due to a specific response of the plants to the *P. multivora* treatments.

The VIs used in this study could be used as a guideline for developing new vegetation indices to discriminate *Phytophthora* and waterlogging stress with *C. calophylla*. The method described in Heim et al. (2019) for developing a vegetation index to detect myrtle rust (on the leaves), could be adapted to develop these new VIs for *C. calophylla*, though this method should take the epidemiology of *Phytophthora* root disease into consideration. However, previous studies with other plant species have confirmed a strong relationship between VIs and pigment content in the leaves and measuring the chlorophyll and anthocyanin content in the *C. calophylla* leaves could also contribute to the selection of optimal bandwidths for algorithms specific to *C. calophylla*.

Performing this study in the warmer spring and summer months would ensure that the effect of *Phytophthora* on *C. calophylla* would be more severe as conditions for the pathogen would have been optimal; the slowing down in plant growth in these trials could have lessened the amount of root exudates that could have attracted *Phytophthora* zoospores to infect the roots. Also, the lower temperatures could have resulted in a reduced amount of zoospores in the soil (Morgan and Shearer, 2013), thus leading to the reduced effect of *Phytophthora* on the plants. If the effect of *Phytophthora* on *C. calophylla* can be more detrimental, it would aid in correlating the biophysical measurements with the spectral measurements too, which could not be done in this study.

The* P. multivora *anomaly

The two *P. multivora* treatments caused variable responses with the *C. calophylla* plants. The *P. multivora* plants in the first trial were taller and with larger root volumes than the control plants, but not in the second trial. The WBI values for the *P. multivora* TRH5 and *P. multivora* TRH1B2 treatments differed from the other treatments (*P. cinnamomi* and control) in the second trial. Also, both the NDNI2 and CTR1 values for the *P. multivora* TRH1B2 treated plants at ten weeks in the second trial were higher than for the other treatments, but at the same time had lower VOG3 values. These results are in accordance with results of Croeser et al. (2018), where these two *P. multivora* isolates also caused variable responses in the *C. calophylla* plants. A possible explanation could be that *P. multivora* was introduced to Western Australia long ago, as it displays high phylogenetic and phenotypic variability (Scott et al., 2009). This would enable them to adapt to local conditions and hosts, resulting in variable pathogenic responses with their hosts.

Conclusion

Even though the effect of *Phytophthora* on *C. calophylla* was not as detrimental as in previous studies, the spectral signatures were able to differentiate between different *Phytophthora* and waterlogging treatments in the visible and shortwave-infrared regions of the electromagnetic spectrum in the first trial, and in the near-infrared and shortwave-infrared regions in the second trial. The effect of the waterlogging treatment in both trials was observed in the water absorption features on the reflectance vs. wavelength plots, as well as in the heat maps. Six VIs, NDNI2, ARI1, PRI, CTR1, VOG3 and WBI were able to track the effect of the seasonal changes in the leaves over the ten weeks of the trials. Reflectance

values in the visible and short-wave infrared regions of the electromagnetic spectrum proved to be important spectral regions for *C. calophylla*. Reflectance spectroscopy with *C. calophylla* holds the potential to be used in future studies, though more work needs to be done to select optimum bandwidths for *C. calophylla*. The reflectance against wavelength plots (continuum removed or not) and NDSI heat maps would prove to be a valuable tool for this purpose. Once the optimum bandwidths specific to *C. calophylla* were selected, the next step would be to extend the reflectance spectroscopy measurements at leaf level to canopy level.

Chapter 4: The role of *Phytophthora* and drought stress in the canker disease of *Corymbia calophylla* (marri)

Abstract

Corymbia calophylla (marri), an iconic keystone species in the northern jarrah forest of south-western Australia (SWWA), is suffering from a canker disease caused by an endemic fungus *Quambalaria coyrecup*. It is unusual for an endemic pathogen to have such a detrimental effect on a co-evolved host, unless host defence mechanisms have been compromised. This study investigated the role of *Phytophthora cinnamomi* root infection and drought stress in predisposing *C. calophylla* to this canker disease, and whether these two stresses work synergistically to intensify the effect of the canker pathogen on *C. calophylla*. The roots of two-year-old *C. calophylla* plants were inoculated with *P. cinnamomi* in pot infestation trials, and eight weeks later in the stems with the canker pathogen *Q. coyrecup*. Half of the plants were exposed to drought stress for the duration of the trial. Biophysical variables related to plant responses to the treatments were measured at harvesting. Reflectance spectroscopy measurements with a portable high-resolution spectroradiometer were also taken weekly. Normalised difference spectral index (NDSI) was calculated for every combination of reflectance value between 350 nm and 2 500 nm for all time points and correlated with treatment effects and displayed as heat maps. Fifty-seven vegetation indices (VIs), utilising wavelengths from different regions in the electromagnetic spectrum, were also calculated from the spectral data. Neither *P. cinnamomi* nor the drought stress treatments exacerbated the effect of the canker pathogen on the plants. Plant diameter and canker volume increased significantly ($p < .001$) due to the canker treatment. The NDSI heat maps indicated that wavelengths in the visible and shortwave infrared portions of the electromagnetic spectrum displayed the strongest correlations with the *P. cinnamomi* and drought stress treatments. For the canker treatment, it was the shortwave infrared portion. Six of the VIs responded significantly ($p < .01$) to the drought stress treatment: CTR1 (Carter index 1), RDVI (renormalised difference vegetation index), NDWI (normalised difference water index), NPQI (normalised phaeophytinization Index), PRI (photochemical reflectance index), RG-ratio (red-green ratio index). RDVI was also sensitive to the canker treatment ($p < .001$), and CTR1 to the *P. cinnamomi* and drought stress treatment ($p < .001$). Reflectance spectroscopy was able to track biochemical changes in *C. calophylla* leaves due to inoculation with *P. cinnamomi*, *Q. coyrecup* and drought stress, but more work needs to be done to identify optimum wavelengths specific to *C. calophylla*.

Introduction

Corymbia calophylla (marri), an ecologically important, eucalypt-type tree species in the northern jarrah forest of southwest WA, is experiencing considerable decline due to a canker disease. Reports of a canker disease among *C. calophylla* date back as early as the late 1930s, but have become more widespread over recent years, raising concern over the causes of increased disease incidence (Shearer, 1992, Shearer, 1994, Paap et al., 2008). These cankers are present on *C. calophylla* across a wide range of rainfall regions, vegetation, and soil types, though fewer cankers are observed in the dryer and warmer areas of the *C. calophylla* range (Chapter 2) (Paap et al., 2017a). Paap et al. (2008) identified the endemic fungal pathogen *Quambalaria coyrecup* as the cause of this canker disease. The canker presents itself on trunks, branches and twigs of trees of all ages, and once canker symptoms become evident, the trees do not appear to recover (Paap et al., 2012). It is a perennial canker, i.e., the pathogen grows actively in the tree for a certain period in a year before the tree responds for the remainder of the year by trying to wall it off. It is more evident in anthropogenically disturbed areas, such as remnant stands bordering cleared land such as road verges or paddocks (Paap et al., 2018). A thirteen-year study of canker incidence in *C. calophylla* indicated that trees on anthropogenically disturbed sites (along road verges and paddocks) presented more cankers (50.7% were cankered) than their counterparts in the forest areas (14.7% were cankered). Canker incidence also increased at a faster rate in trees on anthropogenically disturbed sites (Paap et al., 2016). Disease incidence and mortality were also significantly lower on road verges bordering a forest area as opposed to paddocks and continued to decrease further into native forest stands. In addition to this, canker incidence was significantly related to the proportion of non-native vegetation in a 100-m-radius circle surrounding the survey sites (Paap et al., 2017a).

The exact aetiology of this canker disease in *C. calophylla* is still unknown. It is likely that *Q. coyrecup* enter *C. calophylla* via injury or other openings in the bark, where it possibly lives as an endophyte, until changed conditions in the *C. calophylla* host or environment cause this fungus to become pathogenic (Shearer et al., 1987, Burgess and Wingfield, 2002, Mehl et al., 2017, Old, 2000). It is unusual for an endemic pathogen to have such a devastating impact on a native host, implying that there are other factors that affect *C. calophylla* health and vigour, thus predisposing *C. calophylla* to this canker disease.

Phytophthora root disease is frequently associated with declining *C. calophylla*, and it is hypothesised that *C. calophylla* health could be compromised by *Phytophthora* root infection. In field surveys conducted on sites with cankered *C. calophylla* trees, *Phytophthora* was recovered from 34 of the 62 sites (Paap et al., 2017a). In Chapter 2, five *Phytophthora* spp. (*P. cinnamomi*, *P. elongata*, *P. multivora*, *P. pseudocryptogea* and *P. versiformis*) were recovered from eight of the 10 sites with cankered *C. calophylla*. Barber et al. (2013) also isolated nine *Phytophthora* spp. from underneath unhealthy or dying *C. calophylla* in peri-urban sites and raised the possibility that these pathogens could be involved in the premature decline of an urban forest in SWWA. While *C. calophylla* is considered field-resistant to *P. cinnamomi* (Cahill et al., 1992), various *Phytophthora* spp. (including *P. cinnamomi*) impaired growth and killed the plants in glasshouse trials (Chapter 2) (Belhaj et al., 2018). Hardy et al. (1996) also found that *P. cinnamomi* was associated with dying *C. calophylla* on rehabilitated bauxite mines, when the collars of trees were exposed to temporary inundation.

Negative impacts of climate change in the jarrah forest can have an impact on *C. calophylla* health and vigour too. Though *C. calophylla* canker incidence and related mortality were higher in the cooler and wetter areas of its range, these areas are also associated with increased temperatures and reduced rainfall due to climate change (Paap et al., 2017a). Shearer et al. (1987) also reported that high incidence and severity of cankers were associated with environmental stress, particularly drought. A positive correlation between drought and disease in forest trees is often reported in the literature (Desprez-Loustau et al., 2006), as drought can affect plant physiological status by predisposing them to disease and favouring attack by pathogens (Bostock et al., 2014). *Eucalyptus* plantations that are subject to environmental stress such as drought, waterlogging, temperature extremes or defoliation by insects are more susceptible to infection by the canker pathogens *Endothia gyrosa* (=*Holocryphia eucalypti*) and *Botryosphaeria ribis* (=*Neofusicoccum australe*) (Old et al., 1990, Old, 2000). There is also evidence that fungal species, usually living as saprophytes and/or endophytes within their host, take advantage of compromised tree defence mechanisms caused by drought (Desprez-Loustau et al., 2006). The saprophytic fungi *Cytophoma pruinosa* and a *Fusicoccum* sp. caused cankers in the upper crown branches and main stems of drought stressed trees (Manion and Lachance, 1993). The opportunistic

pathogens *Chalara paradoxa* and *C. radicola* also became aggressive and caused serious damage to date-palm trees in Kuwait, often leading to tree death when their host trees became stressed due to drought conditions and salinity (Suleman et al., 2001).

Field surveys for mapping the extent of *C. calophylla* canker and dieback can be labour-intensive and time-consuming, and therefore alternative surveillance options need to be explored. Research on reflectance spectroscopy and hyperspectral imagery to assess vegetation health is often reported in the literature (Croeser et al., 2021). This technique allows for collecting data about plant health, without destroying the plant, by measuring changes in the light reflectance patterns of the plant's photosynthetic components.

When a plant is stressed, its photosynthetic components are unable to absorb all the visible light it receives, and it, therefore, reflects a bigger portion of light back into the atmosphere. Plant photosynthetic components absorb light in the visible (400 – 700 nm) region of the electromagnetic spectrum, with the chlorophyll pigments at 400 – 500 nm (blue region) and at 650 – 700 nm (red region), carotenoids at 460 – 480 nm and anthocyanins at ~530 nm (Croft and Chen, 2018). In the near-infrared region (700 – 1 300 nm), most light is reflected by the internal structure (mesophyll structure) of the leaf (Blackburn, 2007). The transition from strong absorption by chlorophyll pigments in the visible region to reflectance in the NIR region (~ 690 – 750 nm) is called the "red-edge" region. The red edge is an important region for determining plant stress, as the position of the maximum slope is particularly sensitive to changes in the chlorophyll content of the leaf (Curran, 1989, Horler et al., 1983b, Vogelmann et al., 1993). In the shortwave infrared region (1 300 – 2 500 nm) of the electromagnetic spectrum, light is absorbed by leaf water content and foliar dry matter (proteins, cellulose, lignin, and other leaf components), with water absorption features at 1 450 nm and 1 950 nm. The reflectance values obtained from these measurements are used to compute vegetation indices (VIs) by calculating the ratios of light reflected at wavelengths sensitive to a function of interest (i.e., chlorophyll or water content) and a reference wavelength known for not reflecting light particular to the function of interest (Blackburn, 1998).

Reflectance spectroscopy can serve as an early warning tool to indicate stress with vegetation (Hernández-Clemente et al., 2019), as changes to pigment condition and water content in the leaves can be detected before biophysical change (like chlorotic leaves,

thinning canopy) become visible (Ehleringer and Sandquist, 2006). Reflectance spectroscopy is also often applied to detect disease in plants. In studies on avocados, it could detect laurel wilt (Sankaran et al., 2012, Abdulridha et al., 2016), and *Phytophthora* root-rot disease (Salgadoe et al., 2018). It could also detect *P. cinnamomi* infection and drought stress in two Australian grasses and two tree species (Newby et al., 2019). Heim et al. (2019) used hyperspectral measurements to design a spectral index for detecting myrtle rust (*Austropuccinia psidii*) with lemon myrtle (*Backhousia citriodora*) trees in Australia. In Chapter 3, hyperspectral measurements were used to track changes in the pigment condition of *C. calophylla* leaves due to seasonal changes and waterlogging stress (Croeser et al., 2021).

This study aimed to investigate whether:

i) *Corymbia calophylla* infected with *P. cinnamomi* is more susceptible to canker disease caused by the endemic pathogen *Q. coyrecup*;

ii) drought stress also predisposes *C. calophylla* to the canker disease caused by *Q. coyrecup*;

iii) these two stresses (*P. cinnamomi* infection, drought stress) work synergistically to make *C. calophylla* more susceptible to the canker disease; and

iv) reflectance spectroscopy with a handheld spectroradiometer can be used to identify *Q. coyrecup, P. cinnamomi* and drought stress in *C. calophylla*.

Materials and Methods

Plant preparation and inoculation

The trial was carried out from December to April (southern hemisphere summer to autumn) and ran over sixteen weeks. A completely randomized, three-factorial design (*P. cinnamomi*, *Q. coyrecup* and drought stress) was chosen. There were eight treatment groups in total (Appendix 4 Fig. 1) and 10 replicates per treatment group. Plants were inoculated with either *P. cinnamomi* or *Q. coyrecup* or with both *P. cinnamomi* and *Q. coyrecup*. Control plants were inoculated with sterile inoculum. The plants were watered daily. After inoculation with *P. cinnamomi*, half the plants in each treatment group (*P. cinnamomi*, *Q. coyrecup*, *P. cinnamomi* and *Q. coyrecup*, non-inoculated control) were watered to 50% container capacity (i.e., drought stressed), whilst the other half received full water

treatment. To calculate 50% container capacity, 10 pots were filled with dry sand, weighed and the average weight of these 10 pots calculated. These pots were then watered until the water drained freely from the pots. Once water was not dripping from the pots anymore, the pots were weighed, and the average weight calculated again. The difference in weight between the pots with dry and wet sand was the amount of water needed to fill the pots to container capacity; this amount of water was halved to calculate the amount of water to fill the containers to 50% capacity.

Plant preparation and maintenance were conducted, using the same protocol and seeds from the same seed lot as described in Chapters 2 and 3. Six months after sowing the seeds, the plants were transplanted to 100 mm polyurethane free-draining pots containing pasteurized river sand and allowed to grow for another six months. The plants were transplanted again, this time into 175 mm free-draining polyurethane pots and transferred to a temperature- and humidity-controlled glasshouse (21-27°C). The plants were fertilized every four weeks with half-strength Thrive® and watered daily to container capacity. Osmocote® Plus (Native Gardens) (containing 17.0% total nitrogen, 1.6% total phosphorus, 8.7% potassium, 3.7% sulphur, and micronutrients) was applied (15 g per plant) every three months.

Eight weeks before inoculation with *P. cinnamomi*, the plants were transplanted to 250 mm polyurethane free-draining pots. At the time of inoculation, the plants had a mean height of 1869 cm (1268 – 2290 cm), with a mean stem diameter of 17.5 mm (14.08 – 22.98 mm). A *Phytophthora cinnamomi* isolate (MHSFH2, GenBank ID KX120095) recovered from Witchcliffe State Forest was used to inoculate plant roots. The vermiculite inoculum was prepared according to the method of Simamora et al. (2016), and the plants inoculated according to the method described in Chapter 2. Once inoculated, plants were watered by hand with deionised water to prevent any water splashing between pots. All plants were flooded for 24 hours two days after inoculation to stimulate sporangia production and zoospore release.

Eight weeks after inoculation with *P. cinnamomi*, the plants were under-bark inoculated with *Q. coyrecup* (isolate S45 from the CPSM collection). An incision (5 x 12 mm) was made 40 cm above the lignotuber, and the inoculation was done according to the method of Yulia et al. (2014). Briefly, this involved placing a 5 mm diameter colonised disc, cut from the

margin of actively growing *Q. coyrecup* colonies, and placed mycelial face down onto the inoculation point, covered with sterile moist cotton and wrapped in Parafilm® (Bemis Company, INC, Neenah, WI, United States) and beige Scotch® masking tape (3M, St. Paul, MN). Ten days after inoculation, the Parafilm®, cotton wool and masking tape were removed. The plants were monitored weekly for disease symptoms and development of visible lesions.

Harvest and Biophysical Measurements

The plants were harvested eight weeks after inoculating with *Q. coyrecup*. Various biophysical measurements were taken at harvest (Table 4.1). Plant heights and stem diameters (30 mm above the lignotuber) were measured before removing the plants from the pots. The sand was washed off the roots gently with deionised water to minimise the loss of fine roots. The root volume was determined by measuring the amount of water the roots displaced when put into a graduated cylinder and filled with water (Pang et al., 2011). Roots and tops were placed in separate brown paper bags and dried at 60°C for four weeks in an oven before weighing. Stems (cankered and control) were excised 10 cm above and below the incision areas, placed in sealed plastic bags, and stored in a cold room (4°C) until used in the assessment. The length, width, and diameter of the lesion as well as the volume (by water displacement) were measured (Table 4.1). Koch's postulates were confirmed by plating necrotic root and stem material on NARH agar, a *Phytophthora* selective medium (Simamora et al., 2017).

Table 4.1. List of biophysical response variables measured at harvest.

Category	Response variable	Description
Canker	Lesion volume	Volume of cankered part of the stem
Canker	Lesion length	Total length of canker lesion
Canker	Lesion width	Total width of canker lesion
Canker	Lesion diameter	Stem diameter at biggest part of the canker
Stem	Diameter	Diameter of stem 3cm above lignotuber
Stem	Final height	Final height of plant
Stem	Height difference	Calculated from the initial and final height
Roots	Root volume	Root volume of fresh roots
Roots	Root dry weight	Dry weight of roots

Spectral measurements

A portable Analytical Spectral Device (ASD) FieldSpec 4 high-resolution spectroradiometer ("Malvern Panalytical," n.d.), with a handheld probe was used to measure leaf reflectance in the *C. calophylla* leaves. A Spectralon® (~99%, Labsphere, USA) pure white reference disc was used to calibrate the instrument before measurements and at regular intervals during measurements (after every three plants) (see Chapter 3 for further details).

Spectra were interpolated to regular 1 nm intervals using cubic spline interpolation and reduced to 400 – 2400 nm by removing the wavelengths with relative noise at the edges of the spectra. The data were also normalised (continuum removal [CR]) to show the relative intensities (depth) of the water absorption features. The spectral data were converted to ASCII format, using the ViewSpec Pro software (Malvern-Instruments-Limited, 2008).

The spectrometer was set up on a table, next to the benches with the *C. calophylla* plants, in the glasshouse (Appendix 1 Fig. 1). The plants were randomly selected each time and carried to the table, where the measurements were taken. The hyperspectral measurements were taken on fully intact leaves, still attached to their stems. To ensure that the large number of plants received the same amount of light intensity for photosynthesis during measurements, the measurements were taken between 10:00 and 12:00 and split over two days. The plants in each treatment group were divided randomly into two groups; one group was measured on the first day and the remainder the following day. Baseline measurements were taken immediately prior to inoculation with the canker pathogen, and then weekly for the duration of the experiment (see Chapter 3 for further details).

Fifty-seven narrowband VIs were calculated using reflectance values in the different regions of the electromagnetic spectrum (VIS, NIR, SWIR) (Appendix 1 Table 1). These VIs included indices relating to canopy greenness (nitrogen content), chlorophyll content (photosynthetic capacity or efficiency), leaf pigments such as chlorophyll, carotenoid, and anthocyanin content as an indication of senescence and water content in the leaves.

Data analyses

Statistical analyses was done using R (R-Core-Team, 2019). The R add-on packages “dplyr”(Wickham et al., 2018), “ggbiplot” (Vincent, 2011), “ggplot2”(Wickham, 2016), “cowplot” (Wilke, 2018), “lattice” (Sarkar, 2008),“nlme” (Pinheiro et al., 2018),

"PerformanceAnalytics" (Peterson and Carl, 2018) and "tidyverse" (Wickham, 2017) were used for more specialised analyses.

Reducing the number of response variables

Some of the selected multivariate analyses techniques could not cope with this large number of variables (the number of response variables were almost the same than the number of observations). The number of response variables were reduced according to the method in Chapter 3. Finally, 16 response variables were retained: four biophysical variables and 12 vegetation indices (VIs). Five of the selected VIs (anthocyanin reflectance index-1 (ARI1), red-green ratio (RG-ratio), photochemical reflectance index (PRI), Carter index 1 (CTR1), and normalised phaeophytinization index (NPQI)) made use of the reflectance of light in the visible portion, four (renormalised difference vegetation index (RDVI), Vogelman index 3 (VOG3), normalised difference water index (NDWI), and water band index (WBI)) in the near-infrared portion, one (cellulose absorption index (CAI)) in the shortwave infrared portion and two (light curvature index-2 (LI3), and normalised difference vegetation index (NDVI)) in both the visible and near-infrared portions of the electromagnetic spectrum (Table 4.2).

Analysis of biophysical measurements

Multivariate Analysis of Variance (MANOVA) was performed on the data collected at harvest, to test for treatment effects simultaneously across the four biophysical (canker volume, diameter, height, and root volume) response variables (Quinn and Keough, 2003). The model used had "*P. cinnamomi*", "*Q. coyrecup*" and "drought stress" as predictors. Interaction effects for all three predictors were included too. Time until harvest was added as a covariate to adjust for non-uniformity in harvest day (it was expected canker sizes would be larger for plants that were harvested later). The overall multivariate effect for the canker treatment was significant; thus, two-sample t-tests with Bonferroni adjustment to correct for multiplicity were applied to identify which response variables were responsible for the significant results.

Analyses of spectral data

Last measurements taken at harvesting

The spectral reflectance values, taken at harvest time, were plotted against the wavelength, and inspected visually for any differences between the treatments.

Time-based measurements

Because the VIs such as PRI can reflect short-term changes in the leaves (in the case of PRI, it is the de-epoxidation state of the xanthophyll) as well as long-term changes (for PRI the carotenoid/chlorophyll and β-carotene/chlorophyll ratios) (Filella et al., 2009, Gitelson et al., 2017), the reflectance data from all dates were pooled together for this part of the data analyses, to investigate the long-term trends on leaf biochemical composition due to the treatments.

NDSI (normalised difference spectral index) heat maps were created, by calculating all possible two-band combinations (Gold et al., 2019), according to the method described in Chapter 3. Pearson correlation coefficients (*r*) were calculated between treatment effects and these NDSI combinations to identify which regions of the electromagnetic spectrum best correlated with the treatment effects on *C. calophylla*. These correlations were plotted as wavelength-vs-wavelength NDSI heat maps and coloured on a gradient from red to blue; the strongest correlated NDSI combinations were coloured in red, and the least correlated coloured in blue. The 10 most correlated NDSIs were tabulated to identify the wavelength regions most associated with the treatment effects.

Linear mixed effects models were used to investigate changes in the spectral reflectance due to the treatment effects over the 16 weeks. The best model to characterise the response over time within the subjects, and the variation in time trends between subjects (Pinheiro and Bates, 2000), had the treatment effects ('*P. cinnamomi*', '*Q. coyrecup',* and 'drought stress') as fixed effects, with time (measured in weeks) as a covariate. Interaction terms between the *P. cinnamomi, Q. coyrecup,* and drought stress treatments were added to the models (adding these interaction terms significantly improved model performance by lowering the Akaike Information Criteria (AIC) (Sakamoto et al., 1986)). To determine the appropriate random effects, the protocol outlined by Zuur et al. (2009b) was followed,

which indicated that the optimal structure should include random intercept (over time) for each plant. Model residuals displayed heterogeneity issues, and these were corrected for by modelling the data against its error distribution using the “varPower” variance structure (Zuur et al., 2009a) (the “varPower” variance structure allow variance to increase proportionally to a covariate i.e., an increase in the covariate is associated with an increase in the variance). Likelihood ratio tests were used to assess the significance of individual covariates. Bonferroni post-hoc testing to correct for multiplicity was applied too (Perrett et al., 2006).

Table 4.2. List of the final 16 response variables selected for use in further analyses*

Type of response variable	Response variable	Area in electromagnetic spectrum	Description of the response variable
Biophysical	Canker volume	NA	The volume of lesioned part of the stem, 10 cm above and below the incision areas
Biophysical	Diameter	NA	Diameter 3cm above lignotuber
Biophysical	Height	NA	Height of plant at harvesting
Biophysical	Root volume	NA	Root volume of fresh roots
Vegetation Index	Anthocyanin reflectance index-1 (ARI1)	Visible	Measures the anthocyanin content in the leaves, which is usually more visible in stressed and senescing vegetation.
Vegetation Index	Red-green ratio (RG-ratio)	Visible	Useful to detect dead/dying trees, as it measures the ratio between the chlorophyll and carotenoid/anthocyanin content in the leaves.
Vegetation Index	Light curvature index-3 (LI3)	Visible / Near-infrared	An indicator of light use efficiency (LUE) of the leaves, which is the ability of vegetation to use absorbed light efficiently for photosynthesis.
Vegetation Index	Photochemical reflectance index (PRI)	Visible	An indicator of light use efficiency (LUE) of the leaves, which is the ability of vegetation to use absorbed light efficiently for photosynthesis. Different wavelengths are used to calculate PRI and LI3 values.
Vegetation Index	Carter index-1 (CTR1)	Visible	An indication of the chlorophyll condition of the plants (i.e., photosynthetic potential).
Vegetation Index	Normalised difference vegetation index (NDVI)	Visible / Near-infrared	An indication of the condition of the photosynthetic area; it quantifies the photosynthetic capacity of the leaves (i.e., photosynthetic potential).
Vegetation Index	Renormalised difference vegetation index (RDVI)	Near-infrared	An indication of the chlorophyll condition of the plants (photosynthetic potential).
Vegetation Index	Vogelman red-edge index-3 (VOG3)	Near-infrared	Related to the variation in total chlorophyll content of the leaves, especially in canopies (red-edge index).
Vegetation Index	Cellulose absorption index (CAI)	Shortwave infrared	Used to indicate dry or senescent vegetation, i.e., could indicate stress with vegetation.
Vegetation Index	Normalised phaeophytinization Index (NPQI)	Visible	Used to indicate dry or senescent vegetation, i.e., could indicate stress with vegetation.
Vegetation Index	Normalised difference water index (NDWI)	Near-infrared	An indication of the water content in the leaves.
Vegetation Index	Water band index (WBI)	Near-infrared	An indication of the water content in the leaves.

*For more information on the selected vegetation indices, see Appendix 1 Table 1.

Results

Biophysical measurements

The plants inoculated with *Q. coyrecup* all developed cankers. Some of these cankers girdled 50% of the plant's stem or more, affecting the plant's water and nutrient uptake (Fig. 4.1, Fig. 4.2).

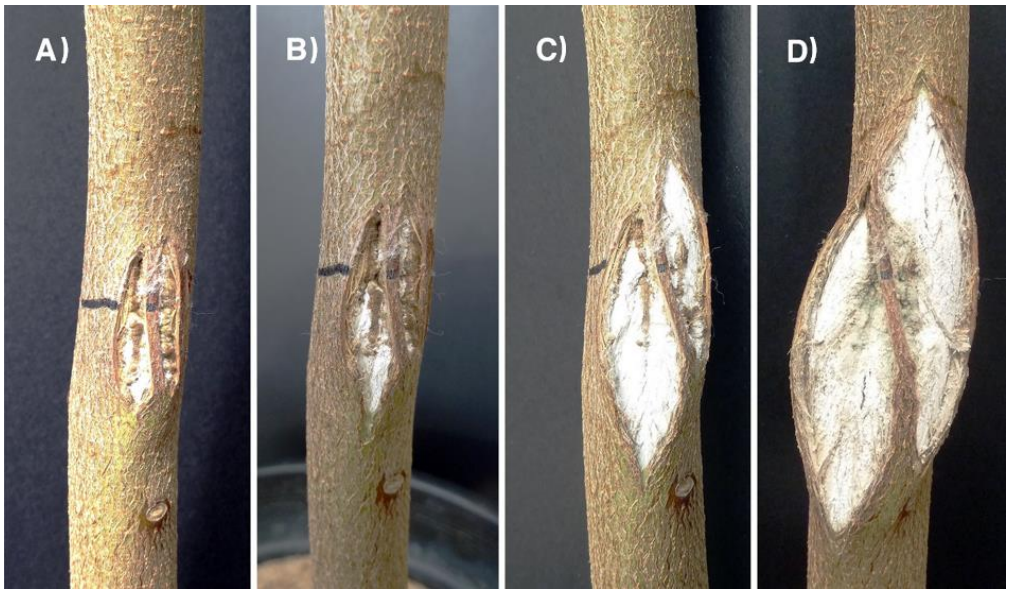

Figure 4.1. Stem of a C*orymbia calophylla* plant at A) two weeks, B) four weeks, C) six weeks, and D) eight weeks after inoculation with the canker pathogen, *Quambalaria coyrecup*. By eight weeks, the canker pathogen had girdled more than 50% of the stem. Spores developed by week two, as indicated by the white area.

Using Pillai's trace, the global *p*-value for the MANOVA for the four biophysical variables was significant (V = 0.75, $F(28, 248) = 2.05$, $p = .002$), but only the canker treatment had a significant global multivariate effect (V = 0.41, $F(4, 58) = 10.21$, $p < .001$) (Table 4.3)

Table 4.3. Global significant multivariate effects (Type II sum of squares) for the *Phytophthora*, canker (*Q. coyrecup*) and drought stress treatments on the biophysical variables (canker volume, diameter, height, root volume).

Variables	Pillai's Trace	*F*	*df*	*p*[#]
Phytophthora	0.11	1.71	4, 58	.16
Canker	0.41	10.21	4, 58	**< .001**[***]
Drought stress	0.04	0.59	4, 58	.67
Day harvested	0.11	1.73	4, 58	.16
Phytophthora, canker	0.09	1.39	4, 58	.25
Phytophthora, drought stress	0.07	1.14	4, 58	.35
Canker, drought stress	0.07	1.08	4, 58	.37
Phytophthora, canker,drought stress	0.04	0.58	4, 58	.68

***α ≤ 0.001. [#]P-values in bold are significant.

The results for the two-sample t-tests indicated that the treatment with the canker pathogen *Q. coyrecup* had a highly significant effect on the canker volume $t(53.31) = 5.98$, $p < .0001$ and diameter $t(67.97) = 3.87$, $p = .0008$ (Fig. 4.2, Table 4.4); the mean values for both these variables increased because of the canker treatment.

Table 4.4. Results of the two-sample t-tests (α ≤ 0.05) run on the effect of treatment with the canker pathogen *Q. coyrecup* on the biophysical variables (root volume, canker volume, diameter, and height). The *p*-values were adjusted with Bonferroni corrections to correct for multiplicity.

Variable	Canker treatment				
	t	*df*	*p*	Adj. *p* (Bonf.)[@]	Means (0,1[#])
Root volume (mL)	2.01	66.91	.048	.19	302.92, 355.57
Height (mm)	0.75	65.81	.46	.99	1887.15, 1844.67
Diameter (mm)	3.87	67.97	.0002	**.0008**	16.61, 18.28
Canker volume (mL)	5.98	53.31	<.0001	**<.0001*** **	19.50, 27.62

***α ≤ 0.001. [@]P-values in bold are significant.
[#]Mean values for the control (0) and canker-treated (1) plants

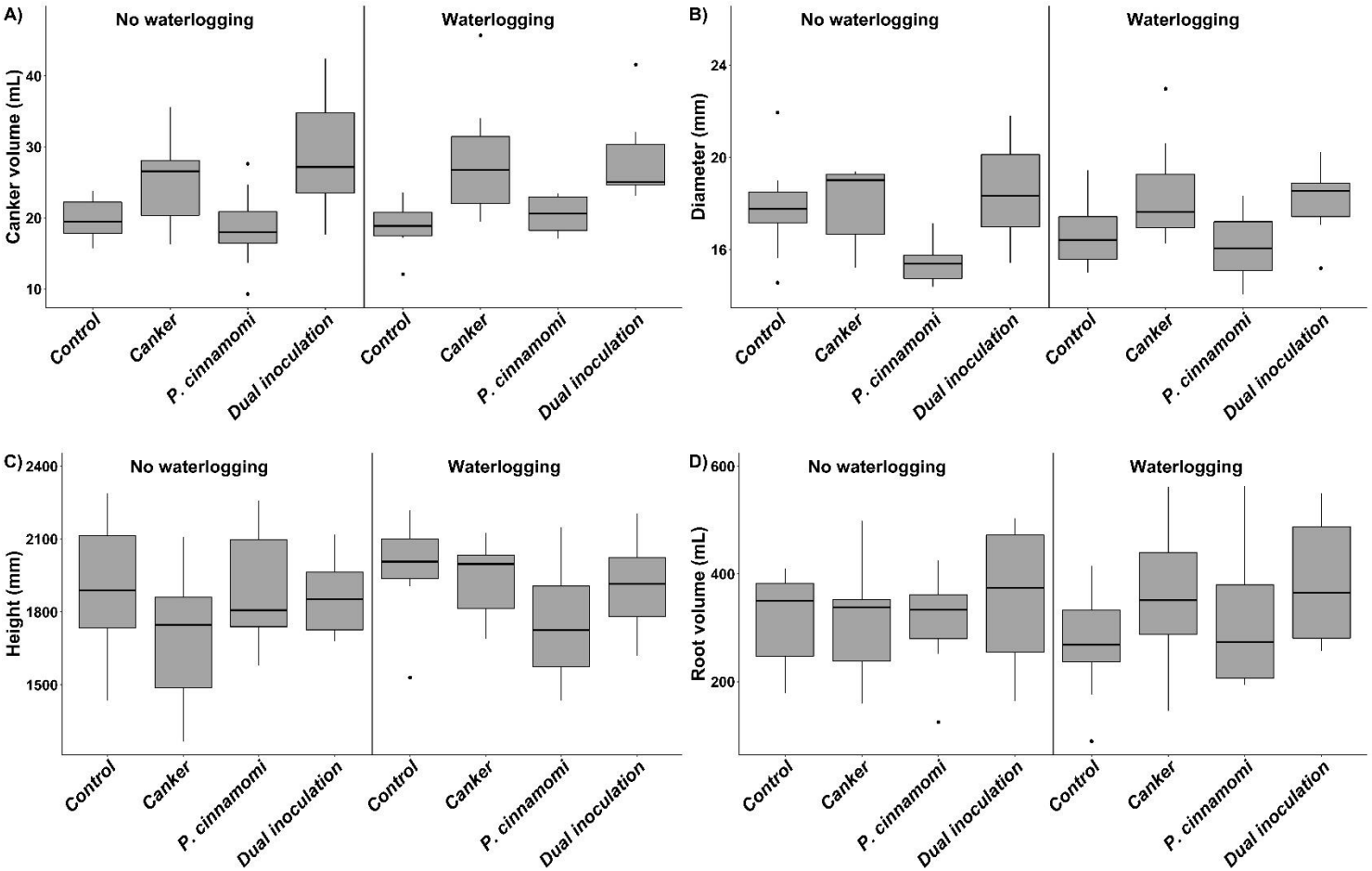

Figure 4.2. Boxplots with minimum, median and maximum values of the four biophysical variables for *Corymbia calophylla*, as measured at harvest: A) canker volume, B) stem diameter, C) height, and D) root volume. Plants were inoculated with either *Phytophthora cinnamomi*, the canker pathogen *Quambalaria coyrecup,* or both pathogens combined. Once the plants were inoculated with *P. cinnamomi*, half of the plants were subjected to a drought stress treatment for the remainder of the trial.

Boxplots of the four biophysical variables (Fig. 4.2) indicated that the *Phytophthora*-only plants displayed a stunted growth pattern, as they were shorter (1 854 cm) than the other plants (1 874 cm) (Appendix 4 Table 1). The canker volume for these plants (24.14 mL) were bigger than the other plants (23.28 mL) too (Appendix 4 Table 1).

Plants receiving the water stress treatment displayed stunted growth too, as they were shorter (1 836 cm) than the plants receiving the full water treatment (1 900 cm) (Fig. 4.2, Appendix 4 Table 1). These plants also displayed symptoms of stress during the first part of the trial, as they had less leaves than the full watered plants. Their leaves displayed symptoms of chlorosis too and often wilted. During the latter part of the trial, these plants had seemingly adjusted to the drought stress treatment, as their leaves looked healthier and greener.

Spectral measurements

Last measurements taken at harvesting

Visual inspection of the spectral reflectance values plotted against wavelength indicated that differences in reflectance values between treatment groups were more profound in the visible (VIS) (400 – 700 nm) and shortwave infrared (SWIR) (1 300 – 2 500nm) regions of the electromagnetic spectrum (EM) (Fig. 4.3A). These differences are associated with leaf pigments, and water content and foliar dry matter respectively. The continuum removed (CR) spectra did not indicate separation between the treatments at any of the water absorption features (1 250 nm, 1 450 nm, or 1 950 nm) (Fig. 4.3B)

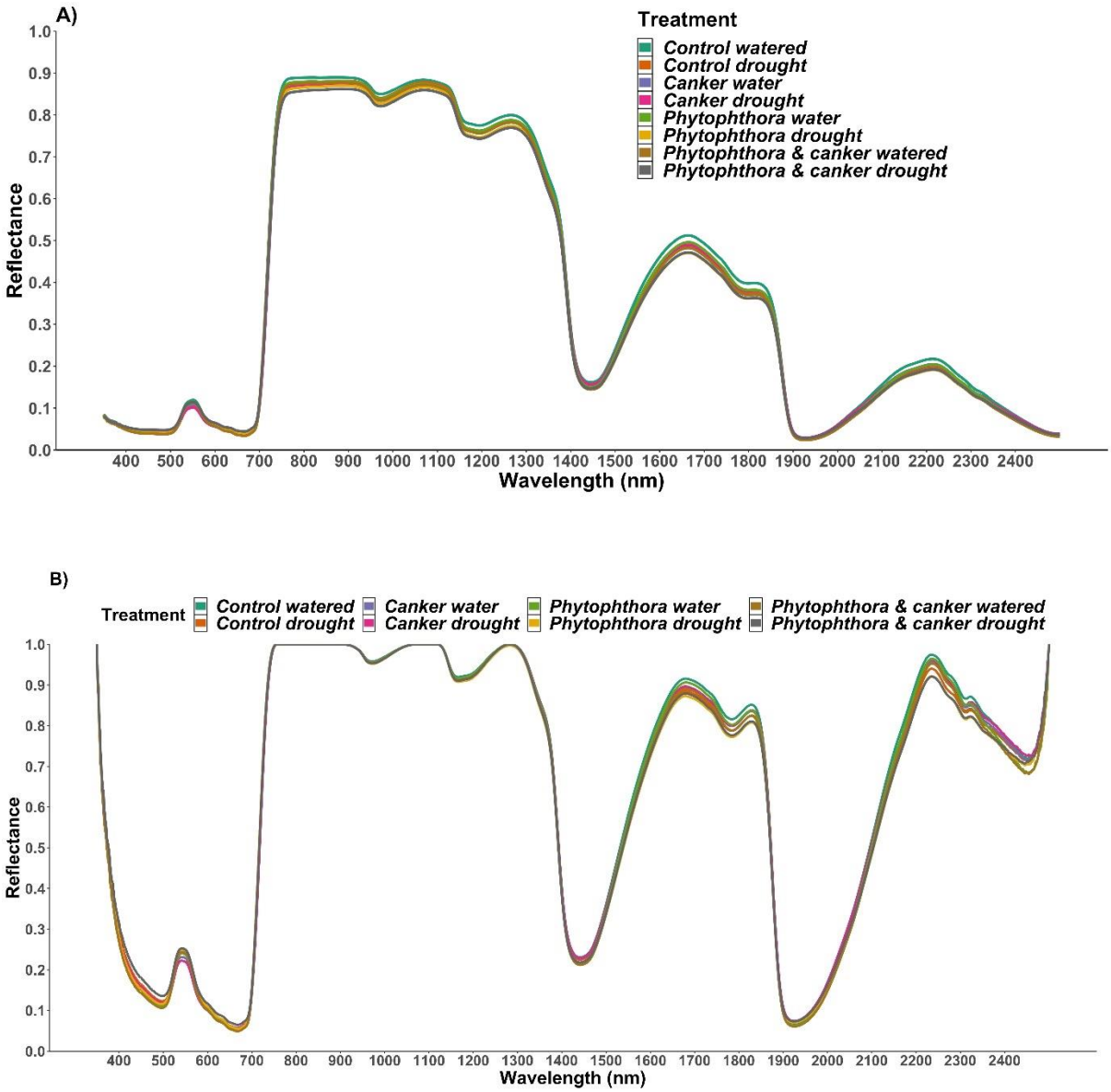

Figure 4.3. Reflectance values plotted against wavelength as measured immediately before harvest, at 350 – 2 500 nm of the electromagnetic spectrum.

Time-based measurements

Data from all time points were used to visualise important spectral regions for distinguishing plant responses towards the treatment effects by plotting the correlations between the reflectance data and treatments as a wavelength-vs-wavelength NDSI heat map. The NDSI correlations were coloured on a gradient from red to blue; the best correlated NDSI combinations were coloured in red, and the least correlated coloured NDSI combinations in blue.

NDSIs using wavelengths in the visible portion (400 – 700 nm) of the electromagnetic spectrum displayed the strongest correlations when all the treatments were

combined (*Phytophthora* + canker + drought stress; Fig. 4.4A) (thus dividing the data between the plants receiving any of the three treatments and the full-watered control plants). When the spectral data were separated between the cankered and non-cankered plants (i.e., the one group included plants receiving the canker treatment irrespective of the other treatments, and the second group had all the plants which were not inoculated with *Q. coyrecup*), the strongest correlations between NDSIs and the canker treatment were displayed in the shortwave infrared region of the electromagnetic spectrum (Fig. 4.4B). When separating the data into *Phytophthora* inoculated and non-*Phytophthora* infected plants (now discriminating between *Phytophthora*-inoculated and non-inoculated plants), the strongest correlations between infection status and spectral reflectance were observed in the visible region of the electromagnetic spectrum (Fig. 4.4C). When the spectral data were separated between the drought stress and full-watered plants (where the first group included drought stressed plants irrespective if they were inoculated or not, and the other group had all the well-watered plants), the correlations were also the strongest in the visible region of the electromagnetic spectrum (Fig. 4.4D).

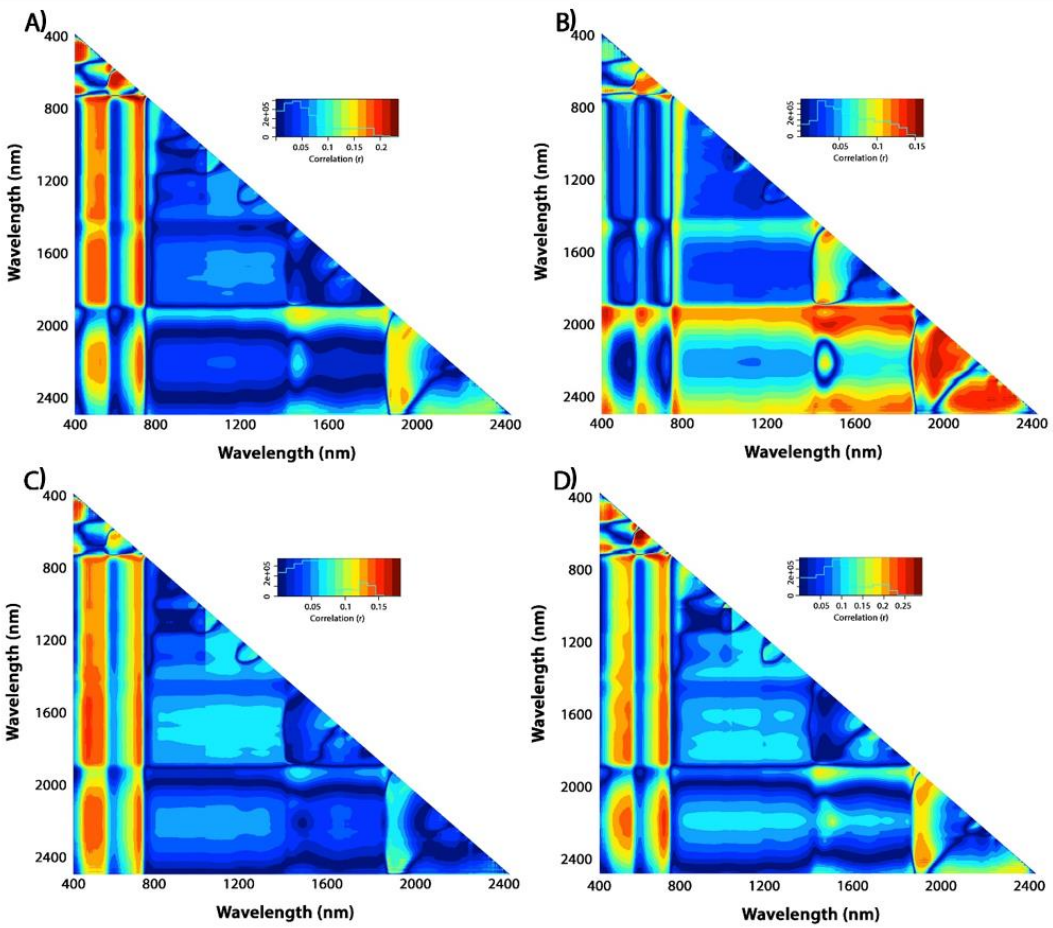

Figure 4.4. Correlation matrices (heat maps) showing the coefficients of determination (R^2) (Pearson's correlations) for all dual wavelength combinations (NDSIs) in the range of 400 – 2 500 nm for A) all treatments combined, B) *Q. coyrecup* canker treatment, C) *Phytophthora* treatment, and D) the drought stress treatment. The red colour indicates a strong correlation between the reflectance data and the treatments, and blue indicates a weak to no correlation.

The wavelengths of the first 10 NDSIs, which correlated most with treatment effects, are listed in Table 4.5. For the *Phytophthora* treatment, both the first and second wavelengths fell within the visible portion (370 – 417 nm) of the electromagnetic spectrum. The first wavelength is before the violet region, where the photosynthetic pigments absorb a small fraction of the light, and the second wavelength is within the blue region of the electromagnetic spectrum where the chlorophyll a, d, and f pigments absorb light (chlorophyll at ~ 430 nm, chlorophyll d and f at ~ 410 nm) (Croft and Chen, 2018).

With the drought stress treatment, both the first and second wavelengths fell within the green area (541 – 581 nm) of the electromagnetic spectrum. The anthocyanin

pigments absorb at ~ 540 nm, but neither the chlorophyll nor carotenoid pigments absorb light at 580 nm.

For the *Q. coyrecup* canker treatment, both wavelengths for the first five NDSIs fell within the SWIR portion (2 303 – 2 306 nm) of the electromagnetic spectrum. This area is associated with higher light reflectance by the foliar dry matter in the leaves. The next five wavelength combinations fell within the visible and SWIR regions of the electromagnetic spectrum (370 nm for the first wavelength and 1 919 – 1 923 nm for the second wavelength). Very little absorption of light takes place at 370 nm, but the second wavelength is related to the water absorption feature, usually at 1 9 00 – 1 950 nm.

Table 4.5. List of spectral bandwidths combinations with the highest correlations to the *Phytophthora*, *Q. coyrecup* canker and drought stress treatments for both trials.

Treatment					
Phytophthora		***Q. coyrecup* canker**		**Drought stress**	
Wavelength 1	Wavelength 2	Wavelength 1	Wavelength 2	Wavelength 1	Wavelength 2
370	397	2303	2305	543	580
370	396	2303	2306	542	580
370	402	2304	2306	543	581
370	403	2304	2305	542	581
370	399	2303	2306	544	580
370	417	370	1921	545	580
370	411	370	1922	546	580
370	410	370	1920	543	579
370	418	370	1919	541	580
370	401	370	1923	544	581

The results of the mixed models, analysing the temporal component of the spectral data, indicated that eight of the twelve selected VIs (ARI1, RG-ratio, CTR1, NDVI, RDVI, CAI, NPQI, and WBI), when comparing VI values between the first and sixteenth week of the trial, displayed a significant ($p < .01$) trend (Table 4.6) over time. RG-ratio, PRI, CTR1, RDVI, NPQI, and NDWI displayed significant ($p < .01$) responses towards the drought stress treatment (Fig. 4.5, Table 4.6). CTR1 was also sensitive to the *Phytophthora* treatment ($p <. 0001$), and RDVI to the *Q. coyrecup*

canker treatment (p = .0001) (Fig 4.5, Table 4.6). VIs sensitive towards the combined effects of treatments were CTR1 and NPQI, displaying a highly significant response towards the *Q. coyrecup* canker + drought stress treatments (p < .0001 for both), and PRI and CTR1 towards the *Phytophthora* + canker + drought stress (p = .030 and p < .0001, respectively) treatments (Table 4.6). These results reflect a compounding effect of the combined treatments on the response variables (Fig. 4.5).

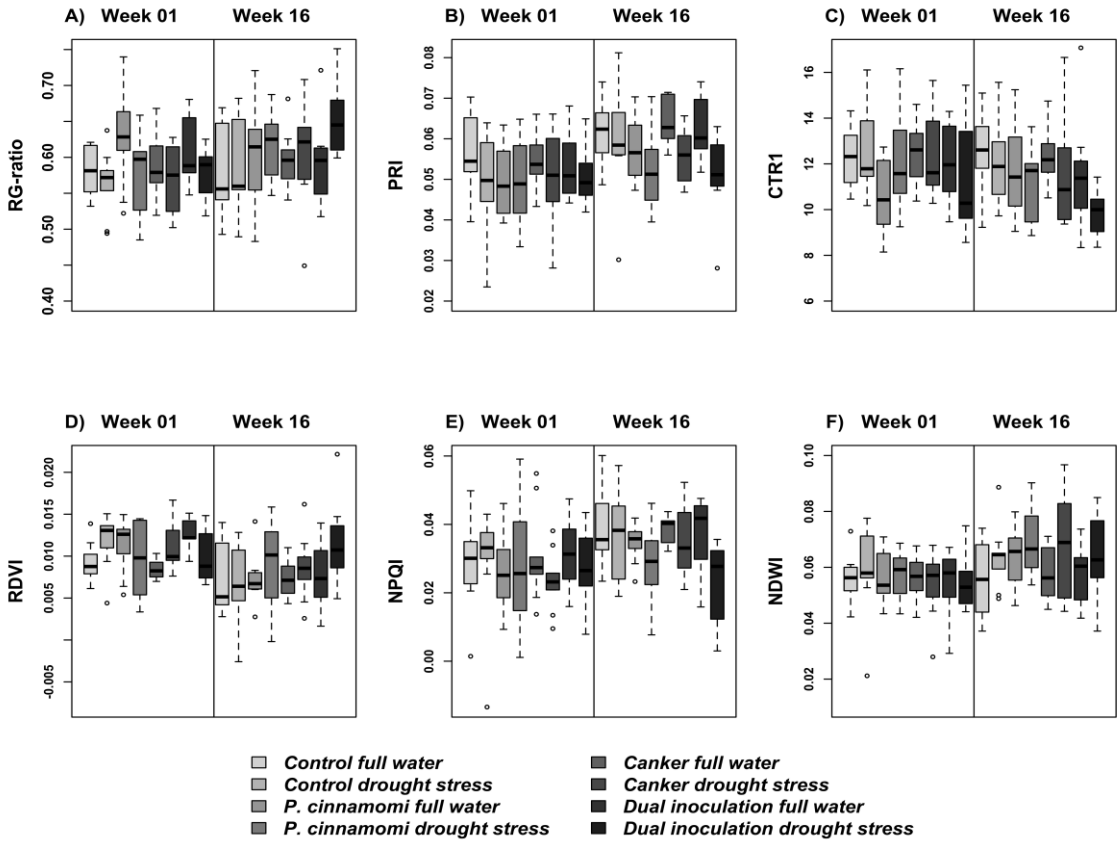

Figure 4.5. Box plots with minimum, median, and maximum values for the vegetation indices plotted for weeks one and sixteen, after treatment with *P. cinnamomi*, the canker pathogen *Q. coyrecup* and drought stress, from left to right, for A) RG-ratio (Red-Green ratio), B) PRI (photochemical reflectance index), C) CTR1 (Carter index 1), D) RDVI (renormalised difference vegetation index), E) NPQI (normalised phaeophytinization index), and F) NDWI (normalised difference water index).

Table 4.6. Responses of the Vegetation Indices[#] towards the treatment effects (*P. cinnamomi*, the canker pathogen *Q. coyrecup* and drought stress) in the temporal component of the data. Bonferroni correction for multiple tests was applied to the resulting *p*-values. Significant *p*-values after Bonferroni correction are in bold, with the original *p*-values in brackets.

Vegetation Index[#]			Treatment							
	Category	Electro-magnetic spectrum region^	*P. cinnamomi*	*Q. coyrecup*	Drought stress	Week	*P. cinnamomi*, *Q. coyrecup*	*P. cinnamomi*, drought stress	*Q. coyrecup*, drought stress	*P. cinnamomi*, *Q. coyrecup*, drought stress
ARI1	Leaf pigment	Visible	*p*>1.0(.602)	*p*>1.0(.447)	*p*>1.0(.604)	***P*<.0001*** **	*p*=.054(.018)	*p*=.495(.165)	*p*>1.0(393)	*p*>1.0(.931)
RG-ratio	Leaf pigment	Visible	*p*=.471(.157)	*p*=.135(.045)	***p=.018***(.006)	***P*<.0001*** **	*p*>1.0(.804)	*p*>1.0(.857)	*p*=.177(.059)	*p*>1.0(.354)
LI3	Light-use efficiency (LUE)	Visible	*p*=.570(.190)	*p*>1.0(.727)	*p*=.075(.025)	***P*=.003(.001)**	*p*>1.0(.728)	*p*>1.0(.933)	*p*=.228(.076)	*p*=.249(.083)
PRI	Light-use efficiency (LUE)	Visible	*p*=.072(.024)	*p*>1.0(.334)	***p*<.0001*** **	*p*>1.0(.146)	*p*=.144(.048)	*p*=.066(.022)	*p*=.789(.263)	***p=.030***(.010)
CTR1	Photosynthetic potential	Visible	***p<.0001******	*p*=.150(.050)	***p*<.0001*** **	***P*=.003(.001)**	*p*=.306(.102)	*p*>1.0(.466)	***p<.0001******	***p<.0001******
NDVI	Photosynthetic potential	Visible/NIR	*p*=.834(.278)	*p*>1.0(.644)	*p*=.096(.032)	***P*<.0001*** **	*p*>1.0(.831)	*p*>1.0(.573)	*p*=.282(.094)	*p*=.393(.131)
RDVI	Photosynthetic potential	NIR	*p*=.594(.198)	***p<.0001******	***p<.0001******	***P*=.009(.003)**	*p*>1.0(.415)	*p*>1.0(.535)	*p*>1.0(.901)	*p*>1.0(.611)
VOG3	Red-Edge Index	Visible	*p*=.681(.227)	*p*=.072(.024)	*p*=.063(.021)	*P*=.318(.106)	*p*>1.0(.389)	*p*>1.0(.496)	*p*=.630(.210)	*p*>1.0(.517)
CAI	Senescence	SWIR	*p*>1.0(.858)	*p*=.312(.104)	*p*=.750(.250)	***P*<.0001*** **	*p*>1.0(.999)	*p*>1.0(.481)	*p*=.597(.199)	*p*>1.0(.960)
NPQI	Senescence	Visible	*p*=.474(.158)	*p*>1.0(.571)	***p<.0001******	***P*<.0001*** **	*p*>1.0(.756)	*p*>1.0(.686)	***p<.0001******	*p*=.156(.052)
NDWI	Water content	NIR	*p*=.390(.130)	*p*=.201(.067)	***p=.012***(.004)	*P*=.795(.265)	*p*>1.0(.799)	*p*=.906(.302)	*p*=.741(.247)	*p*=.195(.065)
WBI	Water content	NIR	*p*>1.0(.596)	*p*>1.0(.579)	*p*=.744(.248)	***P*=.003(.001)**	*p*>1.0(.808)	*p*>1.0(.537)	*p*>1.0(.514)	*p*>1.0(.470)

For more information on the selected vegetation indices, see Appendix 1 Table 1. *** $\alpha \leq 0.001$. ^ SWIR = shortwave infrared, NIR = near-infrared

Discussion

Did P. cinnamomi *and drought stress predispose* C. calophylla *to the canker disease?*

From these results, *P. cinnamomi* root infection did not predispose *C. calophylla* to the canker disease, as the canker volumes for dual-inoculated plants (inoculated with both *Phytophthora* and *Q. coyrecup*) were no bigger than the canker volumes for the plants inoculated only with *Q. coyrecup*. This was unexpected as many *Phytophthora* species are associated with cankered *C. calophylla* (Chapter 2) (Barber et al., 2013, Paap et al., 2017a). The drought stress treatment also did not predispose *C. calophylla* to this *Q. coyrecup* canker disease, as this treatment also did not result in bigger canker volumes. This contrasts with the general view that drought weakens a tree's defence mechanisms, making them more susceptible to pathogens. The ash dieback caused by the fungal pathogens *Cytophoma pruinosa* and *Fusicoccum* sp. was induced by drought (Hibben and Silverborg, 1978). These fungi were already present in the bark of the unstressed trees but caused cankers once these trees were predisposed by drought. The timing of drought seems to be of importance, though. In a separate study with *C. calophylla* and *Q. coyrecup*, even though canker development was greater in the well-watered saplings than in droughted saplings, canker development was the greatest in plants subjected to drought stress before inoculation (Hossain et al., 2018). The *C. calophylla* plants in the present study were drought stressed only after inoculation with *P. cinnamomi*; thus, it is possible that these plants were not sufficiently stressed before inoculation with the canker pathogen. *Eucalyptus globulus* plants were also more susceptible to the canker-causing fungal pathogen *Neofusicoccum eucalyptorum* when the plants were inoculated after being subject to a drought stress treatment (Barradas et al., 2017).

The effect of the treatments on the biophysical responses of C. calophylla

The canker treatment impaired plant growth, as the plants inoculated with *Q. coyrecup* were shorter than the other plants. These plants also had significantly larger stem diameters, possibly due to kino vein formation for combatting the canker

pathogen. These findings are in line with a previous study (Paap et al., 2018), where cankered *C. calophylla* on the road verges had larger diameters at breast height.

The plants inoculated with *P. cinnamomi* also displayed impaired growth, but they were not as adversely affected by *P. cinnamomi* as seen in Chapter 2. In Chapter 3, the effect of *Phytophthora* was also not as detrimental to *C. calophylla* as observed in Chapter 2. However, in Chapter 2, infecting the roots of six-month-old *C. calophylla* seedlings with either *P. cinnamomi* or *P. multivora* led to a significant reduction in seedling root volume and even seedling death. The *C. calophylla* plants used in the pot infestation trials in Chapter 3 and this present study were older than the plants used in Chapter 2; thus, they could have been more resistant to the effect of a *P. cinnamomi* infection. This is in line with the study of Cahill et al. (2008), which found *C. calophylla* in the field was resistant to *P. cinnamomi*. However, the *C. calophylla* plants in the Cahill et al. (2008) study was of mixed age (some much older than one year) and growing in natural forests, thus other micro-organisms in the soil may have inhibited the effect of a *P. cinnamomi* infection in these plants.

The effect of the drought stress treatment on *C. calophylla* biophysical attributes was, even though initially detrimental to *C. calophylla*, also not significant. This could be due to the anatomy of *C. calophylla* leaves. *Corymbia calophylla* can regulate adverse water potentials through a mechanism of osmotic adjustment (Ruthrof et al., 2015); it appears to close stomata early, thus maintaining a high water status during drought conditions (Szota et al., 2011, Colquhoun et al., 1984). In this study, it also appeared that the *C. calophylla* plants adjusted to the droughted conditions, especially towards the second half of the trial.

The effect of the treatments on the spectral reflectance measurements

Measurements taken at harvesting

Visual inspection of spectral values plotted against wavelength indicated that differences between treatments in spectral reflectance were mostly associated with the shortwave infrared regions (1 300 – 2 500 nm) of the electromagnetic spectrum, where the *Phytophthora*-treated plants could be differentiated from the other treatments. Reflectance in the SWIR region is indicative of the water content and the

amount of foliar dry matter in the leaves. In Chapter 3, where the *C. calophylla* plants were infected by *P. cinnamomi* and *P. multivora*, differences between treatments were more noticeable in both the visible and shortwave infrared regions of the electromagnetic spectrum. There were small differences between treatments at the water absorption features at 1 450 nm and 1 950 nm in this study, while in Chapter 3 with the first trial, these differences were more obvious.

Time-based measurements

The NDSI heat maps for the *P. cinnamomi* and the drought stress treatments displayed the biggest correlations in the visible portion of the electromagnetic spectrum. The wavelengths of the ten NDSIs correlating most with these treatments also fell within this region, indicating that the photosynthetic capacity of the *C. calophylla* plants were affected. In Chapter 3, the wavelengths of the ten NDSIs correlating most with the *P. cinnamomi*, *P. multivora*, and waterlogging treatments also fell in the visible region of the electromagnetic spectrum.

The NDSI heat maps for the canker treatment displayed the biggest correlations in the shortwave infrared region, an area associated with leaf water content and foliar dry matter. The wavelength combinations of the ten NDSIs correlating most with this treatment also fell within this region, as well as in the visible region. This may indicate that the ability of the *C. calophylla* plants to take up water and nutrients was impaired because of the canker treatment; towards the conclusion of the trial, the canker pathogen had girdled many of the *C. calophylla* plants. The *C. calophylla* plants would have been unable to maintain a proper photosynthetic rate too.

Six of the vegetation indices used in this study displayed significant responses to the three main treatment effects, i.e., for *P. cinnamomi* (Carter Index 1 (CTR1)), for canker (renormalised difference vegetation index (RDVI)), drought stress (RG ratio (red-green ratio)), photochemical reflectance index (PRI), Carter index 1 (CTR1), renormalised difference vegetation index (RDVI), normalised phaeophytinisation index (NPQI), and normalised difference water index (NDWI)). Four of these VIs (RG-ratio, PRI, CTR1, NPQI) make use of light in the visible portion of the electromagnetic spectrum, and the remaining two (RDVI and NWDI) make use of the near-infrared

portion. RDVI and PRI were sensitive to the drought stress treatment, and the bandwidths used in their algorithms are within the same region as the 10 most correlated NDSI bandwidth combinations for drought stress. The bandwidths used for CTR1, the only VI sensitive to the *P. cinnamomi* treatment, are also within the same region as the bandwidths for the 10 most correlated NDSI combinations for the *P. cinnamomi* treatment.

The Carter Index1 (CTR1) vegetation index outperformed the other vegetation indices. It was sensitive to the main effects of the *P. cinnamomi* and drought stress treatments, to the interaction between canker and drought stress as well as to the interaction between *P. cinnamomi*, canker and drought stress. This VI was developed to indicate plant stress (Carter, 1994), and should be considered in future use with *C. calophylla*.

Another vegetation index to hold promise for future use with *C. calophylla* is the photochemical reflectance index (PRI). This VI was sensitive to the drought stress treatment, as well as the combined effect of *P. cinnamomi*, canker and drought stress. PRI was originally developed to measure the ability of vegetation to absorb light efficiently for photosynthesis, but is increasingly being used to indicate drought stress in vegetation too (Gitelson et al., 2017). Inoculation with either *P. cinnamomi* or *Q. coyrecup* could lead to reduced uptake of water. Adding the drought stress treatment would have compounded the effect on the plants, which can be seen in the PRI values in this study; PRI values for the drought stressed *P. cinnamomi*, *Q. coyrecup* and dual-inoculated plants were all lower than their well-watered counterparts.

The renormalised difference vegetation index (RDVI) was the only index sensitive to the canker treatment. It was also sensitive to the drought stress treatment. This index makes use of the near-infrared portion (760 nm – 800 nm) of the electromagnetic spectrum, but borders on the red-edge region (700 – 750 nm). The red-edge region is a crucial region for determining stress in plants. More work needs to be done to determine the specific wavelengths for the red-edge region in *C. calophylla*, as perhaps the red-edge region of *C. calophylla* does actually include 760 nm; sometimes vegetation indices making use of ”off-centre” wavelengths are

better related to chlorophyll content (Croft et al., 2014), as these would vary according to the leaf type and specific vegetation species.

Two of the other vegetation indices (RG-ratio, NPQI), sensitive to the drought stress treatment, measure the relationship between the chlorophyll and anthocyanin content (Gamon and Surfus, 1999), and between the chlorophyll a and carotenoid content (Penuelas et al., 1995b), respectively. This relates to measuring the degradation of the chlorophyll pigments in the leaves due to stress. NPQI was also sensitive to the combined effect of the canker and drought stress treatments. This index was initially developed to assess the different levels of mite attack on apple trees (Penuelas et al., 1995b).

NDWI was also sensitive to the drought stress treatment. This vegetation index is sensitive to changes in the liquid water content of vegetation (Gao, 1996). This may indicate that the water content in the *C. calophylla* leaves were affected by the drought stress treatment, even though *C. calophylla* is considered resilient against water potential changes in its leaves.

The six VIs sensitive to the treatments in this study could be used as a basis for developing indices to discriminate *P. cinnamomi*, *Q. coyrecup,* and drought stress with *C. calophylla*. However, more work is required to refine wavelength selections specific to *C. calophylla*, such as measuring the chlorophyll, carotenoid and anthocyanin content in the leaves and comparing with VI values. Once the optimal wavelengths for *C. calophylla* are determined, more work should be done to develop spectral indices as an indication of the onset of these stresses, to enable management to make informed decisions on the management of these stresses

Corymbia calophylla *canker is a complex disease*

Tree declines are caused by complex interactions between biotic and abiotic factors (Manion and Lachance, 1993). *Corymbia calophylla* canker disease is also an example of a complex disease, where disease incidence cannot be attributed to a single cause. In the case of *C. calophylla* canker, a combination of factors, i.e., anthropogenic disturbances, presence of root-rot pathogens such as *Phytophthora,* and changed climate conditions (warmer and dryer climates) can work

synergistically, thereby exacerbating the effect of *Q. coyrecup* on this tree. Other factors can also affect disease development, i.e., light conditions, soil pH, soil moisture, humidity, and oxygen concentration, but these have not been explored in *C. calophylla* yet. There is increasing evidence that disturbances and stresses on trees do not act independently (Desprez-Loustau et al., 2006). Thus, interdisciplinary studies are required to assess forest health. In the meantime, control measures should focus on managing stress in these trees by planting drought- and pathogen-resistant *C. calophylla* where possible (Old, 2000). Recent trials with *C. calophylla* seedings grown from seed from different populations from a wide range of climate origins within WA, indicated that some populations are more drought- or disease-tolerant (Ahrens et al., 2019).

Conclusion

In this study, *C. calophylla* plants infected by *P. cinnamomi* were not more susceptible to canker disease caused by the endemic pathogen *Q. coyrecup*. The effect of drought also did not predispose *C. calophylla* to the canker disease, nor did the combined effect of *P. cinnamomi* and drought stress. Reflectance spectroscopy was able to discriminate between the three different treatments (*P. cinnamomi*, *Q. coyrecup,* and stress stress). Reflectance values from the visible and shortwave infrared proved to be important spectral regions for cankered and drought stressed *C. calophylla*. Six vegetation indices (RG-ratio, PRI, CTR1, RDVI, NPQI and NDWI) displayed significance towards the treatments. Reflectance spectroscopy holds promise as an assessment tool for determining these stresses in *C. calophylla*, though more work is needed to select bandwidths optimal for *C. calophylla*. Should the spectral measurements at the leaf scale be successful in identifying these stresses in *C. calophylla*, this methodology could be trialled for use at landscape level with unmanned vehicles or fixed-wing aircraft fitted with cameras, thus aiding in managing these stresses in *C. calophylla*.

Chapter 5: General Discussion

Prior to this study, the effect of *Phytophthora* on *C. calophylla* and its role in *C. calophylla* canker disease, caused by *Q. coyrecup*, was largely unknown. This study not only established the association of *Phytophthora* with cankered and healthy *C. calophylla* but demonstrated its detrimental effect on *C. calophylla* too. Inoculation with *Phytophthora* significantly reduced root volumes and top dry weights, and even killed some of the plants. However, it could not be demonstrated that *Phytophthora* is predisposing *C. calophylla* to the canker disease, as dual inoculation with both *Phytophthora* and *Q. coyrecup* did not result in bigger cankers. The drought stress treatment added to the dual inoculation trial also did not predispose *C. calophylla* to the canker disease, as this treatment also did not result in increased canker lesions too.

This is the first time that the use of reflectance spectroscopic measurements, to determine the effect of *Phytophthora* and the canker pathogen on *C. calophylla*, has been explored. This study demonstrated that reflectance spectroscopic measurements were able to track biochemical changes in the *C. calophylla* leaves because of the treatments, as well as biochemical changes in the *C. calophylla* leaves due to the seasonal effects. Bandwidths in the visible and shortwave infrared regions of the electromagnetic spectrum proved to be important regions for characterising *C. calophylla* response to *Phytophthora*, canker, waterlogging and drought stresses.

Phytophthora association with cankered *C. calophylla*

In the field surveys conducted across the *C. calophylla* range, five *Phytophthora* species were recovered from the rhizosphere of both healthy and cankered *C. calophylla*. These were *P. cinnamomi*, *P. elongata*, *P. multivora*, *P. pseudocryptogea* and a novel species *P. versiformis* (Chapter 2) (Paap et al., 2017b). *Phytophthora multivora* and *P. cinnamomi* were the most abundant species recovered, and consequently the reason why they were examined in more detail in this study.

Both *Phytophthora* recoveries and canker incidence were higher on anthropogenically disturbed stands. Seventy three percent (19 from 26 isolates) of *Phytophthora* recoveries were from anthropogenically disturbed stands. Recent

work with *Phytophthora* (Barber et al., 2013, Khdiar et al., 2020) also demonstrated that *Phytophthora* species are associated with declining vegetation in urban and peri-urban (anthropogenically disturbed) stands in the Perth metropolitan area. Though canker incidence with *C. calophylla* was higher on anthropogenically disturbed stands, due to the design of the field trials (five healthy and five cankered trees were sampled from both healthy and an adjacent anthropogenically disturbed stand, and not according to the proportion of the number of cankered and healthy trees on a particular stand), this could not be established statistically. However, a separate study found that canker incidence was significantly greater with *C. calophylla* growing on anthropogenically disturbed stands than on undisturbed forest stands (Paap et al., 2016). Loss of mycorrhiza, as well as chemical run-off due to the use of pesticides and herbicides could be important factors in the establishment of *Q. coyrecup* on anthropogenically disturbed stands (Sapsford et al., 2017).

Site-specific climatic characteristics played a role in both the *Phytophthora* recoveries and the canker incidence with *C. calophylla*. The optimum temperature to germinate and grow for *P. cinnamomi* is 24-28 °C (Morgan and Shearer, 2013), and for *P. multivora* 25 °C (Scott et al., 2009). *Phytophthora* zoospores also need water to distribute and germinate (Morgan and Shearer, 2013). These climatic preferences were reflected in the number of *Phytophthora* recoveries made from the different stands visited in this study. *Phytophthora* recoveries were higher in the cooler and wetter areas (Witchcliffe, temperature 16-27 ˚C, mean annual rainfall 1 000 mm, http://www.bom.gov.au/wa/forecasts/perth.shtml) of the *C. calophylla* range, with no recoveries from the warmer, dryer stands to the northern margin (Moora, temperature 17-35 ˚C, mean annual rainfall 461 mm, http://www.bom.gov.au/wa/forecasts/perth.shtml). Canker incidence was also higher in the cooler and wetter areas of the *C. calophylla* range and lower in the warmer and drier areas, which is in line with a previous study (Paap et al., 2017a). *Quambalaria coyrecup* spores also need water to germinate, and it is likely that they need water to distribute to other hosts too (i.e., with rain splash, which is the case with its related shoot-blight *C. calophylla* pathogen, *Q. pitereka*).

Even though *Phytophthora* was recovered more frequently from cankered *C. calophylla* (nearly 42%, 11 from 26 isolates), the relationship between *Phytophthora* recoveries and cankered trees in this study was not significant. The small sample size (5 healthy and 5 cankered trees per stand), as well as the large variance in the data could have contributed to these results. In a previous study, the relationship between the recovery of *Phytophthora* and cankered *C. calophylla* was established, although this study was done on a site and not tree level (Paap et al., 2017a).

The effect of *Phytophthora* on *C. calophylla*

In the first two glasshouse pathogenicity trials (Chapter 2), *C. calophylla* plants were inoculated with the recovered *Phytophthora* species (*P. cinnamomi*, *P. elongata*, *P. multivora*, *P. pseudocryptogea*, *and P. versiformis*) from the field trials as well as other species (*P. boodjera*, *P. nicotianae*) from the CPSM collection. The results of these trials demonstrated that some *Phytophthora* species had a harmful effect on *C. calophylla* plants, by reducing their top dry weight and root volume. Inoculation with *Phytophthora* resulted in seedling death too; in the first trial 25% of the seedlings had died prior to harvest. The species responsible for seedling death were *P. boodjera* (12.5%), *P. cinnamomi* (62.5%), *P. elongata* (37.5%), *P. multivora* (37.5%)*, P. pseudocryptogea* (25%) and *P. nicotianae* (37.5%). In the two follow-up glasshouse pathogenicity trials (Chapter 3), conducted with only *P. cinnamomi* and *P. multivora*, *Phytophthora* did not have the same detrimental effect on the plants than in the first two trials (Chapter 2). The *C. calophylla* plants used in the latter two trials were six months older than the plants in the first two trials, thus it might be that they were more resistant to *Phytophthora* than their younger counterparts were. The season could also have played a role in the pathogenicity of *Phytophthora*. The follow-up two trials were conducted during late autumn and winter, when the colder temperatures and shorter daylight lengths might have inhibited both *C. calophylla* and *Phytophthora* growth. Future research should include studies to investigate the effect of daylight length on *Phytophthora* pathogenicity and development, as currently this topic is largely unexplored.

There was no relationship between the pathogenicity of the recovered *Phytophthora* isolates and the condition (anthropogenically disturbed or undisturbed) of the site from where it was recovered. The most pathogenic isolate was a *P. cinnamomi* isolate, recovered from the undisturbed Witchcliffe native forest (it had killed 62.5% of its plants before harvest). Two other *P. cinnamomi* isolates, recovered from the undisturbed Kentdale forest were also responsible for *C. calophylla* deaths (37.5% and 25%, respectively). Seemingly, the presence of *P. cinnamomi* within the rhizosphere of *C. calophylla* in these forests did not result in *Phytophthora* dieback in these forests too. This might be possibly due to soil types that are suppressive to *P. cinnamomi* (Shearer and Crane, 2013). In addition, because *P. cinnamomi* is able to live as a biotroph within their hosts, thus not affecting its host adversely (Crone, 2012), it might be that these *C. calophylla* trees were already infected with *P. cinnamomi* but that this infection did not result in dieback (yet). This suggests that *Phytophthora*, like many other tree pathogens, can become pathogenic to its host when conditions for either the host or the inhabiting *Phytophthora* species change (Sturrock et al., 2011, Old, 2000, Desprez-Loustau et al., 2006).

Phytophthora predisposing *C. calophylla* to canker disease

Even though *P. cinnamomi* inhibited the growth of the *C. calophylla* plants (shorter than the control plants, and with smaller diameters too) (Appendix 4 Table 1), it could not be demonstrated that *P. cinnamomi* predisposed *C. calophylla* to the canker disease. Dual inoculation of *C. calophylla* with *P. cinnamomi* in the roots and *Q. coyrecup* in the stems did not result in bigger cankers when compared to cankers from plants that were inoculated with only the canker pathogen.

It could also not be demonstrated that the drought stress treatment predisposed *C. calophylla* to the canker disease, as this treatment also did not result in bigger cankers, nor did the interaction between *Phytophthora* and the drought stress treatment. This is in contrast with another study, where canker development was greater in well-watered seedlings than in seedlings which experiencing drought stress (Hossain et al., 2018). However, the timing of the drought stress treatment may be important, as in the Hossain et al. (2018) study, seedlings that were

subjected to a drought stress treatment before inoculation with *Q. coyrecup* had the greatest canker development. The *C. calophylla* plants used in the dual inoculation trial were subjected to drought stress conditions only after they were inoculated with *P. cinnamomi*. The possibility that drought stress could alter the physiological status of *C. calophylla*, thus affecting its resilience against *Phytophthora* and especially the canker pathogen should be explored in more detail in future.

Phytophthora multivora displays variable responses in trials

Phytophthora multivora did not always perform consistently in the trials with *C. calophylla*. In two pot infestation trials in Chapters 2 and 3, inoculation with *P. multivora* TRH1B2 resulted in reduced root volumes in both trials. In the two branch inoculation trials (Chapter 2), *Phytophthora multivora* TRH5 also performed consistently (lesion lengths of 48 mm and 47 mm, respectively). However, in the first pot infestation trial (Chapter 3), inoculation with another *P. multivora* isolate, *P. multivora* TRH5, appeared to have stimulated root growth as these plants displayed bigger root volumes than the control seedlings, but in the second pot infestation trial the inoculated plants displayed reduced root volumes (Chapter 3).

Inoculation with four of the other *P. multivora* isolates used in Chapter 2 also resulted in increased root volumes (MHC4, MHSFH3, MJ10, TRC4), whilst the remaining *P. multivora* isolates in the trial (BG2.10 and TRH4) resulted in reduced root volumes.

Phytophthora multivora, previously mis-identified as *P. citricola*, is considered to have been introduced to Australia (Burgess et al., 2019). It has been recovered extensively from managed as well as native ecosystems (Burgess et al., 2017), and it being considered an emerging problem to West Australian ecosystems (Migliorini et al., 2019). *Phytophthora multivora* displays a high phylogenetic and phenotypic variability (Scott et al., 2009), which could enable it to adapt to local conditions and hosts, resulting in variable pathogenic responses with their hosts. It might also be that in some instances, the presence of *P. multivora* stimulates its host to produce new roots faster than at the rate that *P. multivora* is killing them. In trials with two cultivars (Genghis and Savannah) of winter wheat (*Triticum aestivum*), disease-

induced root growth was observed for both cultivars when few roots were infected with *Gaeumannomyces graminis* var. *tritici*, but root growth was inhibited when many roots were infected (Bailey et al., 2006). Root production was also stimulated when inoculum density was low but inhibited in high inoculum density conditions.

It must be kept in mind that in the present study, the trials were performed in controlled glasshouse environments, thus the effect of *P. multivora* may be more detrimental to *C. calophylla* under field conditions, where the plants need to compete for water and nutrients too. Seasonality (temperature, daylight length and light intensity) might play a role in the pathogenicity of *P. multivora* too; *P. infestans* displayed a phototropic behaviour with potato (Mihovilovich et al., 2010). More work needs to be done to investigate the factors influencing the variable behaviour of *P. multivora* with disease-induced root growth in plants.

Five pot infestation trials were conducted in this study. Table 5.1 provides a summary of these trials. The first two pot infestation trials were conducted to investigate the effect of *Phytophthora* on *C. calophylla*, using isolates recovered from the field surveys as well as vouchered isolates from the CPSM collection. The second two pot infestation trials were conducted to investigate the potential of reflectance spectroscopy to characterise *C. calophylla* response to *Phytophthora* and waterlogging stress. The fifth pot infestation trial was a dual inoculation trial with both *P. cinnamomi* and the canker pathogen *Q. coyrecup*, to investigate whether a *Phytophthora* root infection can predispose *C. calophylla* to *Q. coyrecup* canker disease.

Table 5.1. Summary of the five trials conducted during this study.

	First trial	Second trial	Third trial	Fourth trial	Fifth trial
Relevant chapter	**Chapter 2**	**Chapter 2**	**Chapter 3**	**Chapter 3**	**Chapter 4**
Purpose	Determine association of *Phytophthora* species with cankered and healthy *C. calophylla* across the *C. calophylla* range. Investigate the effect of *Phytophthora* on *C. calophylla*.	Investigate the effect of *Phytophthora* on *C. calophylla*.	Investigate the potential use of reflectance spectroscopic measurements, to characterise biochemical changes in *C. calophylla* leaves due to *Phytophthora* and waterlogging stress.	Investigate the potential use of reflectance spectroscopic measurements, to characterise biochemical changes in *C. calophylla* leaves due to *Phytophthora* and waterlogging stress.	Investigate if *Phytophthora* is predisposing *C. calophylla* to canker disease, by conducting a dual inoculation trial with both *P. cinnamomi* and *Q. coyrecup.* Investigate the role of drought stress in *C. calophylla* canker in combination with *Phytophthora*.
Treatments	Conduct field surveys across the *C. calophylla* range and collect soils from the rhizosphere of healthy and cankered *C. calophylla*, on both anthropogenically disturbed and undisturbed stands. Inoculation of *C. calophylla* roots with the recovered *Phytophthora* species, as well as *Phytophthora* isolates from the CPSM collection (18 isolates in total). A fortnightly waterlogging regime was added too.	Inoculation of *C. calophylla* roots with 10 *Phytophthora* species used in the first trial. A fortnightly waterlogging regime was added too.	Inoculation of *C. calophylla* roots with either a *P. cinnamomi* or a *P. multivora* (TRH5) isolate. A fortnightly waterlogging regime was added too. Weekly reflectance spectroscopic measurements were taken too, for the duration of the trial.	Inoculation of *C. calophylla* roots with either a *P. cinnamomi* or one of two *P. multivora* (TRH5, TRH1B2) isolates. A fortnightly waterlogging regime was added too. Weekly reflectance spectroscopic measurements were taken too, for the duration of the trial.	Inoculation of *C. calophylla* with *P. cinnamomi* in the roots and *Q. coyrecup* in the stems. A drought stress treatment was added after the plants were inoculated with *P. cinnamomi*. Weekly reflectance spectroscopic measurements were taken too, for the duration of the trial.

	First trial	Second trial	Third trial	Fourth trial	Fifth trial
Results	Five *Phytophthora* species were recovered from healthy and cankered *C. calophylla* during the field surveys. In glasshouse pot infestation trials, various *Phytophthora* isolates displayed the ability to impair *C. calophylla* growth and even kill it.	In glasshouse pot infestation trials, various *Phytophthora* isolates displayed the ability to impair *C. calophylla* growth and even kill it.	In pot infestation trials, *Phytophthora* did not display the same pathogenicity than in the first two trials. Reflectance spectroscopic measurements were able to track biochemical differences in the *C. calophylla* leaves due to the waterlogging treatment, as well as seasonal changes.	In pot infestation trials, *Phytophthora* did not display the same pathogenicity than in the first two trials. Reflectance spectroscopic measurements were able to track biochemical differences in the *C. calophylla* leaves due to seasonal changes.	Neither inoculation with *P. cinnamomi* nor the drought stress treatments demonstrated a predisposing effect of *C. calophylla* to the canker disease. Reflectance spectroscopic measurements were able to track biochemical changes in the leaves due to the treatments (*P. cinnamomi*, *Q. coyrecup*, drought stress).
Journal	Chapter 2 published in Forest Pathology, 2018.	Chapter 2 published in Forest Pathology, 2018.	Chapter 3 published in Forestry: An International Journal of Forest Research, October 2021.	Chapter 3 published in Forestry: An International Journal of Forest Research, October 2021.	Prepared for submission to Forest Pathology.

Reflectance spectroscopy with *C. calophylla*

The reflectance spectroscopic measurements were able to detect changes in the *C. calophylla* leaves due to the treatments, as well as due to seasonal changes. When reflectance values were plotted against wavelengths, the *P. cinnamomi* treatment separated out from the other treatments in the visible region of the electromagnetic spectrum for all three spectral trials. These results correspond with another recent study, where reflectance spectroscopy measurements at leaf scale were also able to detect the effects of *P. cinnamomi* on two native grass and two native tree species; and the reflectance spectra for *Phytophthora*-inoculated plants also separated out in the visible region of the electromagnetic spectrum (Newby et al., 2019). The NDSI heat map for *Phytophthora* did not display any correlations in the first two spectral trials (Chapter 3), but the *Phytophthora* NDSI heat map in the dual inoculation trial (Chapter 4) displayed correlations in the visible portion of the electromagnetic spectrum. Reflectance in the visible portion of the electromagnetic spectrum is indicative of the photosynthetic ability and light use efficiency of the chlorophyll pigments in the leaves (Blackburn, 2007), thus these results suggest that the photosynthetic abilities of *C. calophylla* were altered/impaired due to *Phytophthora* infection.

Differences in the water absorption features at 1 450 and 1 950 nm (both in the SWIR region) were visible for the *C. calophylla* plants subjected to the waterlogging treatments, especially in the first spectral trial. This is because the leaves of the waterlogged plants likely had a higher water content, thus it reflected less light back into the atmosphere. In addition, the NDSI heat maps for the waterlogging treatment also displayed stronger correlations in the SWIR region of the electromagnetic spectrum.

The NDSI heat map for waterlogging for the first spectral trial also displayed correlations to reflectance values in the visible portion of the electromagnetic spectrum too. This is indicative that the waterlogging treatment may have affected the plant's ability to photosynthesize, which was the case with three *Eucalyptus* species during waterlogging conditions (Florentine and Fox, 2002).

The reflectance spectra for the plants subjected to the drought stress treatment did not differentiate out from the full water treatment, though the drought stress NDSI heat map displayed correlations in the visible potion of the electromagnetic spectrum. *Corymbia calophylla* has the ability to regulate the water potential in its leaves during drought stress conditions (Szota et al., 2011); this might have resulted in *C. calophylla* photosynthesis not as negatively affected by the drought stress than by the waterlogging conditions.

Reflectance values for the canker treatment did not differentiate out from the other treatments, though its NDSI heat map displayed stronger correlations in the shortwave infrared region of the electromagnetic spectrum. Changes in this region could indicate a change in dry leaf matter (Daughtry et al., 2004), which could have been the result of the canker pathogen girdling the stems - thereby not only affecting the flow of water in the stems, but also resulting in cells in the leaf tissue of the *C. calophylla* dying off.

Vegetation indices in the first two spectral trials (Chapter 3) did not result in any significant differences between the treatments, but they did display a seasonal trend. These were related to the nitrogen content (normalised difference nitrogen index 2 (NDNI2) (Wang and Wei, 2016)) and anthocyanin pigment content (anthocyanin reflectance index (ARI1) (Gitelson et al., 2001)) in the leaves, to the light use efficiency i.e., how effective is the photosynthesis process (photochemical reflectance index (PRI)(Gamon et al., 1992)) and photosynthetic cell structure i.e., photosynthetic ability (Carter Index 1(CTR1) (Carter, 1994)) in the leaves, a red-edge index (Vogelman red edge index 3(VOG3) (Vogelmann et al., 1993)), which is indicative of biological stress, and the water-content (water band index (WBI) (Penuelas et al., 1993)) in the leaves.

The vegetation indices for the first trial indicted that the *C. calophylla* plants were preparing for the new growth season by the time they were harvested (late winter and early autumn), as their values were indicative of new vegetative growth happening. The plants in the second trial, however, were harvested in mid-winter and thus, the values were indicative of a lower photosynthetic rate, i.e., fewer

resources would have been allocated to vegetative growth and photosynthesis too. All these indices (NDNI2, ARI1, PRI, and CTR1) made use of bandwidths in the visible portion of the electromagnetic spectrum, except for VOG3 and WBI, which uses the near-infrared region.

In contrast to the first two spectral trials, vegetation indices for the dual inoculation trial (Chapter 4) displayed significant responses towards all the treatments. CTR1 was sensitive towards the *P. cinnamomi* treatment, RDVI (renormalised difference vegetation index i.e., photosynthetic cell structure or photosynthetic ability) (Haboudane et al., 2004) towards the canker treatment, RG-ratio (red-green ratio index i.e., relationship between chlorophyll and anthocyanin pigments) (Gamon and Surfus, 1999), PRI, CTR1, RDVI, NPQI (normalised phaephytinization index i.e., dry or senescent vegetation) (Penuelas et al., 1995b) and NDWI (normalised difference water index)(Gao, 1996) towards the water-stress treatment. Four of these VIs (RG-ratio, PRI, CTR1, and NPQI) made use of bandwidths in the visible portion of the electromagnetic spectrum, which demonstrates that the photosynthetic capability and efficiency of the plants were impaired by the treatments. The remaining two vegetation indices (RDVI, NDWI) make use of near-infrared bandwidths. Reflection in the NIR portion of the electromagnetic spectrum is also an indication of the photosynthetic capability of a plant – a plant with more chlorophyll will reflect more light in the NIR region than a plant who is unhealthy.

The Carter Index 1 (CTR1) should be considered for future use in *C. calophylla*, as it featured in all three spectral trials. CTR1 was initially developed to indicate plant stress (Carter, 1994) by gaining information on the photosynthetic ability of the leaves. This vegetation makes use of the visible portion of the electromagnetic spectrum, and the NDSI heat maps indicated that this area of reflectance is important to *C. calophylla*.

PRI (photochemical reflectance index) should also be considered for future use in *C. calophylla*, as it featured in all three the spectral trials too. PRI was initially developed to measure the ability of vegetation to absorb light efficiently for photosynthesis (Gamon et al., 1992), but is increasingly being used to indicate water-stress with vegetation (Gitelson et al., 2017). PRI is a versatile index, as it can reflect

short-term changes in the leaves (the de-epoxidation state of the xanthophyll), as well as long-term changes (the carotenoid/chlorophyll and B-carotene/chlorophyll ratios) (Filella et al., 2009, Gitelson et al., 2017). This might explain why the results with PRI in this study (Chapters 3 & 4) differs from another study, where PRI was found not to be a significant indicator of the ability of *C. calophylla* to withstand drought and pathogen stresses (Ahrens et al., 2020). In the Ahrens et al. (2020) study only one set of measurements were taken (March – late summer), thus reflecting short-term changes in the leaves; weather conditions on the day (i.e., cloud cover and temperature) could have affected the results. Reflectance spectroscopic measurements in this study (Chapters 3 & 4) were done over a period of 12 - 16 weeks, thus reflecting long-term changes in the pigments (the carotenoid/chlorophyll and B-carotene/chlorophyll ratios). This indicates that PRI might be effective for use in *C. calophylla* to monitor long-term effects of drought and pathogen stresses. It should be kept in mind though that in the Ahrens et al. (2020) *C. calophylla* seedlings were screened for genetic resistance against drought and pathogen stress, whereas in this study (Chapters 3 & 4) the effect of *Phytophthora*, waterlogging, drought stress and canker on the biochemical composition of *C. calophylla* leaves was investigated.

Corymbia calophylla canker is a complex disease

In this study it could not be determined that *Phytophthora*, drought stress or the combination thereof are predisposing *C. calophylla* to canker disease. *Quambalaria coyrecup* canker with *C. calophylla* is a complex disease; it is likely the result of several factors, both biotic and abiotic and cannot be attributed to a single cause. The influence and interaction of these factors in this canker disease could be best described by a modified disease spiral (Manion, 1991), especially when combined with the results from other studies on *C. calophylla* (Fig. 5.1). Anthropogenic activities (Chapter 2) (Paap et al., 2016, Paap et al., 2018), genetic predisposition (Ahrens et al., 2019), excess soil nutrients (Sapsford, 2017), drought (Hossain et al., 2018), poor draining soils (waterlogged) (Chapter 3) (Farifr and Aboglila, 2015) and a warming climate (Paap et al., 2017a) could act as predisposing factors on *C. calophylla*. Inciting factors could be a *Phytophthora* root infection (Chapter 2) and

decreased mycorrhiza richness (Sapsford, 2017), with a contributing factor, the presence of the native fungal pathogen *Q. coyrecup* (Paap et al., 2008). A drying and warmer climate could stress the *C. calophylla* trees, but potentially benefit pathogens like *Phytophthora* and *Q. coyrecup*. *Phytophthora*, likely introduced by anthropogenic activities, could cause the loss of fine-feeder roots in *C. calophylla*, thereby resulting in long-term reduced uptake of micro- and macro-nutrients. The loss of mycorrhizal connections, also due to the loss of the fine-feeder roots caused by a *Phytophthora* infection, could result in the reduced uptake of nutrients provided by the ectomycorrhizal fungi (ECM) too. Long-term nutrient deficiencies in the trees could weaken the defence mechanisms of *C. calophylla*, thereby making them even more vulnerable to the canker pathogen.

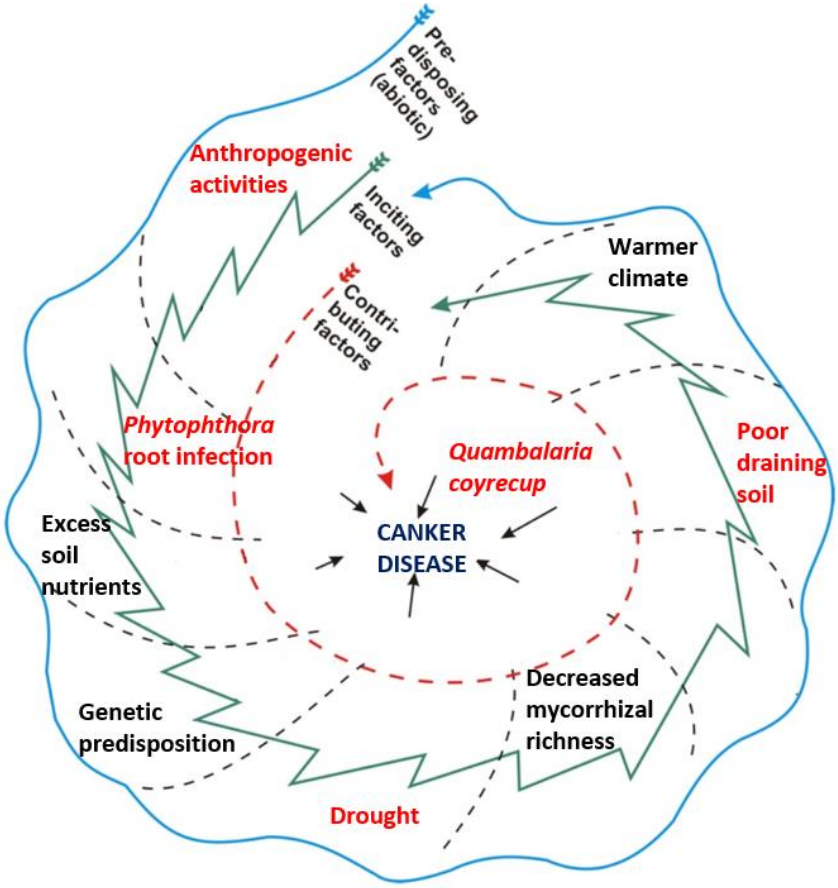

Figure 5.1. *Corymbia calophylla* disease spiral. Factors in red were addressed in this study, and factors in black were addressed in previous studies.

Future work

The glasshouse pathogenicity trials in this study tested the recovered *Phytophthora* isolates individually. Future work should include trials with combinations of these *Phytophthora* species, with and without *Q. coyrecup*, as more than one *Phytophthora* species are often isolated from the rhizosphere of a single tree. In this study, both *P. cinnamomi* and *P. multivora* were isolated from a single tree on the Kentdale and Witchcliffe stands, and both *P. multivora* and *P. versiformis* from a single tree from the Manjimup stand (Chapter 2 Table 2.1). These findings are in line with recent work, where metabarcoding detected that multiple *Phytophthora* species can be present in the rhizosphere of *C. calophylla* and other native vegetation with single plants (Gyeltshen et al., 2021, Khdiar et al., 2020).

Future work with *C. calophylla* should also investigate the role of key nutrient deficiencies in predisposing *C. calophylla* to this canker disease. These investigations should include trials with cankered and healthy *C. calophylla*, where *Phytophthora* is present or not. Mycorrhizal fungal richness should also be included in these trials, as changes in the rhizosphere of *C. calophylla* could also have an influence in predisposing these trees to canker disease.

Seasonal variation plays an important role in *Phytophthora* and the canker disease with *C. calophylla*. Future work should be conducted in conditions more suitable for disease development, i.e., spring, and early summer. The effect of daylight length on *Phytophthora* pathogenicity should also be explored. Currently, very little research exists on the effect of phototropic differences on pathogens.

Future work should also include trials on different options (fungicides and phosphite applications) to control *Phytophthora* and *Q. coyrecup* with *C. calophylla* too. Although the use of phosphite is advised for controlling *Phytophthora* with *Eucalyptus marginata* and *Banksia grandis* (Scott et al., 2015), it was observed that it can aid in the ability of *C. calophylla* to combat *Q. coyrecup* too, by enhancing its defence mechanisms (pers. comm. G. Hardy)..

Even though the CTR1 and PRI vegetation indices, together with the visible region of the electromagnetic spectrum, were identified as important vegetation indices for

future use in *C. calophylla*, more work needs to be done to determine the optimum wavelengths specific to *C. calophylla*. The use of continuum reflectance spectra and NDSI heat maps can provide valuable information in this regard. Once the optimum wavelengths for *C. calophylla* are established, the next step would be to extend the work to hand-held measurements in the field, and then to remote sensing of canopies using unmanned vehicles or fixed-wing aircraft.

The data in this study were plagued with large standard errors and heterogeneous variances, which is often the case with biological systems. Perhaps a data-driven approach such as machine-learning (Ferreira et al., 2016, Gold et al., 2019) or classification (Abdulridha et al., 2018) would be better suited to analyse this type of data, as the Frequentist approach is often too stringent and relies heavily on its assumptions of normality, equal variances and independent observations (Quinn and Keough, 2003).

More work needs to be done to help these iconic trees to survive in an ever-changing world of climatic conditions and anthropogenic disturbances. Control options for managing this canker disease should include the selection of canker- and *Phytophthora* resistant lines. Genetic trials are currently in progress with *C. calophylla*, to assess adaptive variation in growth and resistance against both *Q. coyrecup* and *Q. pitereka.* Although, too early to determine responses to the canker pathogen, research has demonstrated that there is large variation between provenances/families to the introduced shoot blight pathogen (Ahrens et al., 2019, Ahrens et al., 2020). However, the selection of canker- and *Phytophthora* resistant *C. calophylla* lines need to consider drought and heat tolerance too, as these favourable traits could be excluding each other.

Another control option is to offer training, raising the awareness on the spread of pathogens, and providing the skills on how to maintain biosecurity-hygienic practises when entering and exiting native forests. The Green Card Training program, run by the Dieback Working Group (https://www.dwg.org.au/green-card-training/) and the Department of Biodiversity, Conservation and Attractions is invaluable in this regard, as it provides the skills and knowledge to participants on how to reduce the spread of *Phytophthora* on-ground. This program is considering to include training on the

risk of spreading myrtle rust, caused by the highly virulent fungus *Austropuccinia psidii* (Carnegie, 2015), in the program too.

Conclusion

From the work in this study, it was demonstrated that various *Phytophthora* species are associated with both healthy and cankered *C. calophylla*. *Corymbia calophylla* canker and *Phytophthora* were more abundant in anthropogenically disturbed stands. *Phytophthora* can impair *C. calophylla* growth, even kill it, but it could not be demonstrated that it predisposes *C. calophylla* to canker disease. It could also not be demonstrated that drought predisposes *C. calophylla* to canker disease, though the timing of the drought treatment might have confounded these results. Reflectance spectroscopic measurements can detect changes in the photosynthetic abilities of *C. calophylla* leaves due to *Phytophthora* and *Q. coyrecup* infection, as well as to the waterlogging and drought stress treatments. The visible region of the electromagnetic spectrum was shown to be an important region for *C. calophylla*, as most of the vegetation indices responding to the treatments made use of reflectance in this area. Two vegetation indices to explore for future use in *C. calophylla* are the Carter Index-1 (CTR1) and the Photochemical Reflectance Index (PRI), but more work needs to be done to refine bandwidths specific to *C. calophylla*. NDSI correlation heat maps can be used to aid in this regard. In conclusion, this study has contributed to more 'stepping-stones' to our understanding of the canker disease which affects Western Australia's beloved, iconic *C. calophylla*. More work is required to understand what additional biotic and abiotic factors might be involved in predisposing *C. calophylla* to this endemic pathogen and the canker disease it causes.

Appendices

Appendix 1

Figure 1. Glasshouse setup during the spectral trials. The ASD FieldSpec spectrometer was setup in front of the glasshouse. The *Corymbia calophylla* plants were carried to the spectrometer, and the measurements taken by clipping the leave probe onto the youngest, fully expanded leave.

Table 1. The 57 spectral vegetation indices calculated from the hyperspectral data collected in this study

Vegetation index and category	Short name	Formula	Reference
Indices indicating Greenness:			
Canopy nitrogen			
Normalized difference nitrogen index-1	$NDNI_1$	Log(1/R1510)–log(1/R1680) / Log(1/R1510)+log(1/R1680))	(Serrano et al., 2002)
Normalised difference nitrogen index-2	$NDNI_2$	Log(R1680/R1510)/Log(1/R1680 +R1510)	(Wang and Wei, 2016)
Photosynthetic area: cell structure			
Carter index -1	CTR1	R695/R420	(Carter, 1994)
Carter index-2	CTR2	R695/R760	(Carter, 1994, Coops et al., 2004)
Carter index-3	CTR3	R605/R760	(Carter, 1994)
Carter index-4	CTR4	R710/R760	(Carter, 1994)
Green chlorophyll index	CI_{green}	R790/R550-1	(Gitelson et al., 2005)
Green normalized difference vegetation index	gNDVI	(R790–R550)/(R790+R550)	(Li et al., 2014, Gitelson and Merzlyak, 1996)
Leaf chlorophyll index	LCI	R850–R710/R850–R680	(Pu, 2009, Datt, 1999)
MERIS terrestrial chlorophyll index	MTCI	R750–R710/R710–R680	(Dash and Curran, 2004, Li et al., 2014)
Modified normalized difference vegetation $index_{705}$	$mNDVI_{705}$	((R750–R705)/(R750+R705–2R445)	(Sims and Gamon, 2002, Datt, 1999)

Vegetation index and category	Short name	Formula	Reference
Modified normalized difference vegetation index$_{755}$	mNDVI$_{755}$	(R755–R730)/(R755+R730)	(Mutanga and Skidmore, 2007, Croft et al., 2014)
Modified simple ratio index$_{705}$	mSRI$_{705}$	(R750–R445)/(R705–R445)	(Datt, 1999, Sims and Gamon, 2002)
Normalized difference vegetation index	NDVI	(R800–R680)/(R800+R680)	(Rouse Jr et al., 1974)
Pigment specific normalized difference$_a$	PSND$_a$	(R810–R676)/(R810+R676)	(Blackburn, 1998)
Pigment specific normalized difference$_b$	PSND$_b$	(R800–R635)/(R800+R635)	(Blackburn, 1998)
Pigment specific simple ratio$_a$	PSSR$_a$	R810/R676	(Blackburn, 1998)
Pigment specific simple ratio$_b$	PSSR$_b$	R800/R635	(Blackburn, 1998)
Renormalized difference vegetation index	RDVI	(R800–R760)/sqrt(R800+R670)	(Haboudane et al., 2004)
Simple ratio index$_{680}$	SRI$_{680}$	R800/R680	(Rouse Jr et al., 1974, Sims and Gamon, 2002)
Simple ratio index$_{705}$	SRI$_{705}$	R750/R705	(Sims and Gamon, 2002)
Structure insensitive pigment index	SIPI	(R800–R445)/(R800–R680)	(Penuelas et al., 1995a)
Sum greenness index	SG	Mean reflectance of 500nm to 599nm range	(Gamon and Surfus, 1999)
Red edge indices			
Gitelson & Merzlyak index-1	GM1	R750/R550	(Gitelson and Merzlyak, 1994, Gitelson and Merzlyak, 1996)
Gitelson & Merzlyak index-2	GM2	R750/R700	(Gitelson and Merzlyak, 1994, Gitelson and Merzlyak, 1996)

Vegetation index and category	Short name	Formula	Reference
Normalized difference red edge	NDRE	R790–R720/R790+R720	(Li et al., 2014)
Red edge chlorophyll index	$Cl_{red\ edge}$	R790/R720-1	(Li et al., 2014, Gitelson et al., 2005)
Red edge lower slope	RE_{ls}	R710–R690/710-690	(Coops et al., 2004)
Red edge position (linear extrapolation method)	REP_{lem}	700+40[(((R670+R780)/2)-R700)/	(Cho and Skidmore, 2006)
		(R740–R700)]	(Guyot et al., 1992, Tian et al., 2011)
Red edge total slope	RE_{ts}	R740–R690/740–690	(Coops et al., 2004)
Red edge normalized difference vegetation index	reNDVI	(R750–R705)/(R750+R705)	(Gitelson and Merzlyak, 1994, Sims and Gamon, 2002)
Vogelman red edge index-1	VOG1	R740/R720	(Vogelmann et al., 1993)
Vogelman red edge index-2	VOG2	(R734– R747)/(R715+R726)	(Vogelmann et al., 1993)
Vogelman red edge index-3	VOG3	(R734–R747)/(R715-R720)	(Vogelmann et al., 1993)
Indices indicating Stress:			
Dry or senescent vegetation			
Cellulose absorption index	CAI	0.5(R2000–R2200)/R2100)	(Daughtry et al., 2004)
Normalised phaeophytinization index	NPQI	R415–R435/R415+R435	(Penuelas et al., 1995a)
Normalized difference lignin index	NDLI	log(1/R1754)–log(1/R1680)/ log(1/R1754) + log(1/R1680)	(Serrano et al., 2002)
Normalized pigment chlorophyll index	NPCI	(R680–R430)/(R680+R430)	(Penuelas et al., 1995a)

Vegetation index and category	Short name	Formula	Reference
Plant senescence reflectance index	PSRI	(R680–R500)/R750	(Sims and Gamon, 2002)
Leaf pigment activity/Light use efficiency			
(Carotenoid: chlorophyll content)			
Light curvature index-2	LI2	R440/R690	(Lichtenthaler et al., 1996)
Light curvature index-3	LI3	R440/R740	(Lichtenthaler et al., 1996)
Photochemical reflectance index	PRI	(R531–R570)/(R531+R570)	(Gamon et al., 1992, Garbulsky et al., 2011)
Scaled PRI	sPRI	(PRI+1)/2	(Rahman et al., 2001)
Leaf pigments			
(Carotenoids or anthocyanin content)			
Anthocyanin reflectance index-1	ARI1	(1/R550)–(1/R700)	(Gitelson et al., 2001)
Anthocyanin reflectance index-2	ARI2	R800((1/R550)–(1/R700))	(Gitelson et al., 2001)
Carotenoid reflectance index	CRI	R800(1/R520–1/R550)	(Gitelson et al., 2002, Manjunath et al., 2016)
Carotenoid reflectance index-1	CRI1	(1/R510)–(1/R550)	(Gitelson et al., 2002)
Carotenoid reflectance index-2	CRI2	(1/R510)–(1/R700)	(Gitelson et al., 2002)
Red-green ratio index	RG-ratio or GSI	Mean reflectance of 600nm to 699nm range/ Mean reflectance of 500nm to 599nm range	(Gamon and Surfus, 1999, Coops et al., 2004)

Vegetation index and category	Short name	Formula	Reference
Indices indicating Water content:			
Disease stress water index	DSWI	R802+R547/R1657+R682	(Pu, 2009)
Moisture stress index	MSI	R1599/R819	(Hunt and Rock, 1989)
Normalized difference infrared index	NDII	(R819–R1649)/(R819+R1649)	(Hardisky et al., 1983)
Normalized difference water index	NDWI	(R857–R1241)/(R857+R1241)	(Gao, 1996)
Normalized water index-1	NW1	(R970–R900)/(R970+R900)	(Babar et al., 2006)
Normalized water index-2	NW2	(R970–R850)/(R970+R850)	(Babar et al., 2006)
Water band index	WBI	R970/R900	(Penuelas et al., 1993)
Water band index	WB	R900/R970	(Penuelas et al., 1995a, Penuelas et al., 1993)

Appendix 2

Figure 1. Root size of *Corymbia calophylla* seedlings 12 weeks after inoculation. A). The root mass of a non-inoculated control *C. calophylla* seedling. Roots are light brown, and no necrosis can be seen. The root ball contains several white fleshy roots. B). Root mass reduction of a *C. calophylla* seedling inoculated with *P. cinnamomi*. Most of the fine roots are lost. Roots are dark coloured, indicating necrosis of the roots. C). Root ball reduction of a *C. calophylla* seedling inoculated with *P. multivora*. Roots are dark coloured, and necrosis can be seen. The root mass contains very few white fleshy roots and most of the fine roots were lost.

Table 1. Details of the stands surveyed in southwest of Western Australia.

Stand Name	Bioregion [1]	Stand type	Condition[2]	GPS coordinates (D/M/S)	
Boranup[3]	Warren	Private paddock	Disturbed	34°05'31S	115°03'23E
Brunswick	Jarrah Forest	State Forest	Moderate	33°14'40S	115°56'20E
Brunswick	Jarrah Forest	Private farm paddock	Disturbed	33°14'43S	115°56'14E
Dandaragan	Swan Coastal Plain	Private farm remnant forest patch	Moderate	30°31'00S	115°42'09E
Dandaragan	Swan Coastal Plain	Private farm paddock	Disturbed	30°30'59S	115°42'08E
Dunsborough[3]	Swan Coastal Plain	Recreational beach area	Disturbed	33°34'14S	115°05'02E
Kentdale	Warren	National Park	Undisturbed	34°52'53S	117°01'32E
Kentdale	Warren	Road edge of National Park	Disturbed	34°53'24S	117°02'12E
Manjimup	Jarrah Forest	State Forest	Moderate	34°16'54S	116°28'01E
Manjimup	Jarrah Forest	Private farm paddock	Disturbed	34°17'22S	116°27'06E
Witchcliffe	Warren	State Forest	Undisturbed	34°01'45S	115°09'20E
Witchcliffe	Warren	Private farm paddock	Disturbed	34°01'48S	115°09'06E

[1]IBRA 7 - Interim Biogeographic Regionalisation for Australia, version 7 and Sub-regions (www.dpaw.gov.au).

[2]'Disturbed' indicates an anthropogenically disturbed stand, "Undisturbed" indicates a natural forest stand with little or no anthropogenic activities evident on the stand and "Moderate" indicates a natural forest stand that has some evidence of anthropogenic disturbance.

[3] Denotes pilot study site.

Table 2. *Phytophthora* isolates used in *Corymbia calophylla* (marri) excised branch under-bark inoculation and soil infestation pathogenicity trials in glasshouse.

			Branch Inoculation		Soil Infestation	
Isolate name	***Phytophthora* species**	**GenBank no.**	**1**	**2**	**1**	**2**
PN12[1]	*P. boodjera*	MF541547			✓	
MHC3	*P. cinnamomi*	KX120093	✓			
MHH2	*P. cinnamomi*	MF541548	✓		✓	
MHSFC4	*P. cinnamomi*	KX120094	✓			
MHSFH2	*P. cinnamomi*	KX120095	✓	✓		
MHSFH5	*P. cinnamomi*	KX120096	✓		✓	✓
TRC5	*P. cinnamomi*	KX120068	✓			
TRH1B1	*P. cinnamomi*	KX120099	✓		✓	
TSFC3	*P. cinnamomi*	KX120100	✓		✓	
TSFH3	*P. cinnamomi*	KX120101	✓			
TSFH4	*P. cinnamomi*	KX120102	✓		✓	✓
BG2.1	*P. elongata*	KX120103	✓		✓	✓
BJi[1]	*P. elongata*	MF541549			✓	
BG2.10	*P. multivora*	KX120105	✓		✓	
BG2.2	*P. multivora*	KX120104	✓			
M-B-3	*P. multivora*	KX120106	✓			
MGR13	*P. multivora*	MF541550	✓			
MGR2.12	*P. multivora*	KX120109	✓		✓	
MGR2.3	*P. multivora*	KX120107	✓			
MGR2.4	*P. multivora*	KX120108	✓			
MGR5	*P. multivora*	KX120110	✓			
MHC1	*P. multivora*	KX120112	✓			
MHC2	*P. multivora*	KX120113	✓			
MHC4	*P. multivora*	KX120114	✓		✓	
MHSFH3	*P. multivora*	KX120097	✓		✓	✓
Mj10	*P. multivora*	KX120115	✓		✓	
TRC4	*P. multivora*	KX120116	✓		✓	
TRH1B2	*P. multivora*	KX120117	✓		✓	✓
TRH4	*P. multivora*	KX120118	✓		✓	✓
TRH5	*P. multivora*	KX120119	✓	✓		
PAB 10-104[1]	*P. nicotianae*	KC748453			✓	

Isolate name	*Phytophthora* species	GenBank no.	Branch Inoculation		Soil Infestation	
			1	2	1	2
MGR11	*P. pseudocryptogea*	KX120120	✓		✓	✓
MJ5	*P. versiformis*	KX011271		✓		
PAB 12-34[1]	*P. versiformis*	KX062205		✓		
TP13-01[1]	*P. versiformis*	KX011281		✓		
TP13-05[1]	*P. versiformis*	KX011273		✓		
TP13-07[1]	*P. versiformis*	KX011274		✓		✓
TP13-10[1]	*P. versiformis*	KX011275		✓		
TP13-11[1]	*P. versiformis*	MF170963		✓		
TP13-12[1]	*P. versiformis*	MF170964		✓		
TP13-14[1]	*P. versiformis*	KX011276		✓		
TP13-17[1]	*P. versiformis*	KX011282		✓		
TP13-25[1]	*P. versiformis*	KX011285		✓		
TP13-29[1]	*P. versiformis*	KX011277		✓		✓
TP13-34[1]	*P. versiformis*	KX011283		✓		
TP13-42[1]	*P. versiformis*	KX011278		✓		✓
TP13-46[1]	*P. versiformis*	KX011279		✓		
Control	Sterile agar		✓	✓		
Control	Sterile vermiculite				✓	✓

[1] Isolates from the CPSM (Centre for Phytophthora Science and Management) collection.

Table 3. Percentage of *Corymbia calophylla* seedlings in the two glasshouse pathogenicity trials that died post-inoculation with different *Phytophthora* isolates.

		Trial 1[1]		Trial 2[1]	
Isolate	**Species**	**Number of deaths**	**% Mortality**	**Number of deaths**	**% Mortality**
PN12	*P. boodjera*	1	12.5	0	0
MHH2	*P. cinnamomi*	1	12.5	0	0
MHSFH5	*P. cinnamomi*	5	62.5	2	25
TRH1B1	*P. cinnamomi*	3	37.5	0	0
TSFC3	*P. cinnamomi*	2	25	0	0
TSFH4	*P. cinnamomi*	3	37.5	0	0
BG2.1	*P. elongata*	3	37.5	0	0
BJi	*P. elongata*	1	12.5	0	0
BG2.10	*P. multivora*	4	50	0	0
MGR2.12	*P. multivora*	1	12.5	0	0
MHC4	*P. multivora*	1	12.5	0	0
MHSFH3[2]	*P. multivora*	0	0	0	0
Mj10	*P. multivora*	1	12.5	0	0
TRC4	*P. multivora*	3	37.5	0	0
TRH1B2	*P. multivora*	2	25	0	0
TRH4	*P. multivora*	3	37.5	0	0
PAB 10-104	*P. nicotianae*	3	37.5	0	0
MGR11	*P. pseudocryptogea*	2	25	0	0
TP13-07	*P. versiformis*	0	0	1	12.5
TP13-29[2]	*P. versiformis*	0	0	0	0
TP13-42[2]	*P. versiformis*	0	0	0	0
Control		0	0	2	25

[1]Includes only seedlings that have died post-inoculation and before harvest.
[2] Inoculation with these three isolates did not result in any seedling deaths.

Table 4. ANOSIM results, displaying differences in *Corymbia calophylla* (marri) seedling plant traits for all *Phytophthora* isolates[1] used in the first pathogenicity trial. The values bottom left represent the sequential Bonferroni corrected P-values, while the values on the top right are the corresponding R-values.

	Control	PN12	MHH2	MHSFH5	TRH1b1	TSFC3	TSFH4	BG2.1	BJi	BG10	MGR2.12	MHC4	MHSFH3	MJ10	TRC4	TRH1b2	TRH4	PAB10-104	MGR11
Control		-0.05	0.02	0.19	-0.15	0.21	0.32	-0.10	0.00	-0.09	-0.13	-0.08	-0.04	-0.09	0.02	0.07	0.18	-0.13	0.03
PN12	0.74		0.16	0.54	0.29	0.41	0.61	0.11	0.21	0.48	0.04	0.07	-0.03	0.04	0.38	0.23	0.35	0.12	0.35
MHH2	0.33	0.04		-0.06	-0.05	-0.08	0.00	-0.13	-0.08	-0.16	0.06	0.06	0.03	0.14	0.05	-0.09	-0.08	-0.13	-0.10
MHSFH5	0.18	0.03	0.54		0.18	-0.14	-0.04	0.06	-0.04	0.06	0.32	0.42	0.15	0.49	0.17	0.07	-0.17	0.09	0.06
TRH1b1	0.97	0.03	0.53	0.23		0.10	0.27	-0.12	-0.10	-0.09	-0.03	-0.07	-0.02	0.01	0.00	0.02	0.11	-0.06	-0.04
TSFC3	0.05	0.00	0.72	0.75	0.21		-0.08	0.02	-0.02	0.00	0.25	0.31	0.15	0.43	0.22	-0.06	-0.08	-0.01	-0.03
TSFH4	0.03	**0.00**[2]	0.43	0.50	0.07	0.73		0.07	0.09	0.06	0.31	0.52	0.32	0.58	0.24	0.04	-0.10	0.05	0.12
BG2.1	0.83	0.16	0.91	0.41	0.82	0.35	0.20		-0.05	-0.14	-0.03	0.01	-0.01	0.07	0.05	-0.07	-0.06	-0.14	-0.07
BJi	0.39	0.01	0.81	0.51	0.85	0.45	0.23	0.59		-0.06	0.07	-0.01	-0.03	0.08	-0.06	-0.01	0.01	-0.06	-0.10
BG10	0.67	0.01	0.92	0.31	0.59	0.46	0.28	0.81	0.56		0.01	0.19	0.10	0.25	0.18	-0.06	-0.10	-0.18	-0.02
MGR2.12	0.99	0.24	0.22	0.10	0.46	0.02	0.01	0.56	0.16	0.38		0.03	-0.03	-0.03	0.10	0.12	0.21	-0.09	0.10
MHC4	0.85	0.17	0.20	0.05	0.70	0.01	0.00	0.41	0.46	0.13	0.32		-0.10	-0.06	-0.01	0.16	0.32	0.07	0.01
MHSFH3	0.75	0.60	0.27	0.22	0.50	0.08	0.03	0.44	0.54	0.24	0.60	0.97		-0.05	0.01	0.08	0.20	0.01	0.03
MJ10	0.92	0.27	0.07	0.03	0.37	**0.00**[2]	**0.00**[2]	0.23	0.15	0.10	0.49	0.69	0.67		0.06	0.27	0.42	0.15	0.15
TRC4	0.36	0.01	0.26	0.14	0.42	0.07	0.08	0.25	0.64	0.16	0.19	0.44	0.33	0.27		0.26	0.21	0.20	-0.04
TRH1b2	0.20	0.04	0.78	0.32	0.31	0.65	0.33	0.68	0.42	0.59	0.13	0.10	0.17	0.03	0.03		0.03	-0.09	-0.02
TRH4	0.09	0.01	0.73	0.90	0.13	0.72	0.75	0.65	0.38	0.78	0.05	0.02	0.08	0.01	0.06	0.35		-0.02	0.03
PAB10-104	0.92	0.15	0.90	0.36	0.54	0.46	0.30	0.91	0.59	0.87	0.77	0.26	0.38	0.11	0.05	0.72	0.49		-0.01
MGR11	0.27	0.00	0.82	0.38	0.50	0.50	0.16	0.63	0.91	0.38	0.16	0.35	0.29	0.07	0.65	0.37	0.29	0.41	

[1]See Table 2 for details on isolates. [2]The probability plots indicated that these tests were likely to be significant.

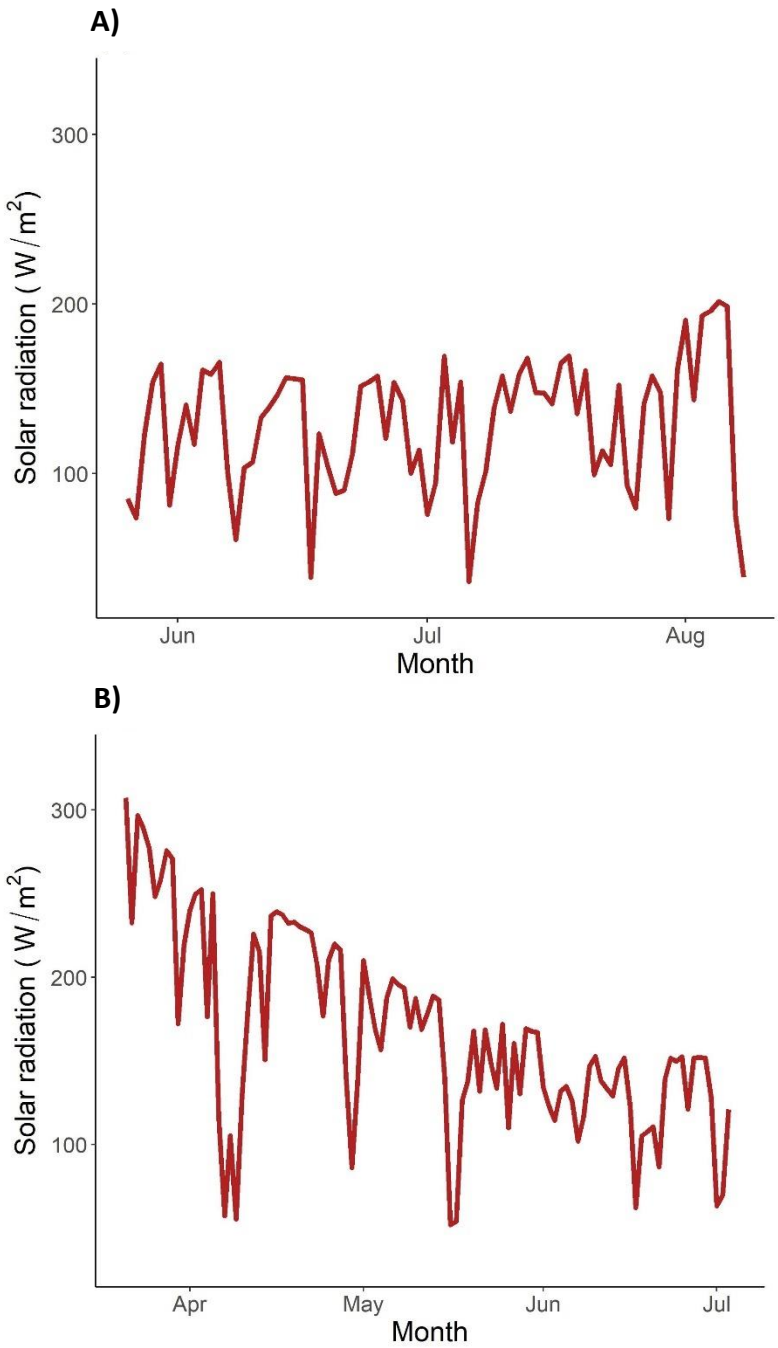

Figure 1. A) Average solar radiation conditions during the first trial and B) during the second trial.

Table 1. Mean and standard errors for biophysical variables in the first and second experiment at harvest for the *C. calophylla* plants, after treatment with *Phytophthora*.

Experiment 1			Experiment 2		
Diameter	**Mean**	**Std. error**	**Diameter**	**Mean**	**Std. error**
Control no waterlog	6.49	0.4	Control no waterlog	9.18	0.33
Control waterlog	7.27	0.42	Control waterlog	8.29	0.43
P. cinnamomi no waterlog	6.48	0.32	*P. cinnamomi* no waterlog	8.86	0.44
P. cinnamomi waterlog	6.36	0.26	*P. cinnamomi* waterlog	7.39	0.23
P. multivora TRH5 no waterlog	7.24	0.38	*P. multivora TRH5* no waterlog	8.22	0.44
P. multivora TRH5 waterlog	7.15	0.34	*P. multivora TRH5* waterlog	8.16	0.25
			P. multivora TRH1B2 no waterlog	8.74	0.31
			P. multivora TRH1B2 waterlog	8.43	0.45
Average diameter	**6.83**		**Average diameter**	**8.41**	
Height	**Mean**	**Std. error**	**Height**	**Mean**	**Std. error**
Control no waterlog	1031.42	79.17	Control no waterlog	901.6	43.4
Control waterlog	1069.38	61.22	Control waterlog	911.6	32.74
P. cinnamomi no waterlog	977.14	88.28	*P. cinnamomi* no waterlog	946.0	63.21
P. cinnamomi waterlog	1059.44	54.78	*P. cinnamomi* waterlog	758.2	43.7
P. multivora TRH5 no waterlog	1205.13	62.36	*P. multivora TRH5* no waterlog	813.8	53.65
P. multivora TRH5 waterlog	1205.56	55.14	*P. multivora TRH5* waterlog	853.3	33.43
			P. multivora TRH1B2 no waterlog	872.8	52.63
			P. multivora TRH1B2 waterlog	858.8	47.96
Average height	**1091.35**		**Average height**	**864.52**	
Root volume	**Mean**	**Std. error**	**Root volume**	**Mean**	**Std. error**
Control no waterlog	49.05	7.94	Control no waterlog	123.30	12.55
Control waterlog	59.36	10.7	Control waterlog	120.85	10.25
P. cinnamomi no waterlog	43.83	5.57	P. cinnamomi no waterlog	108.93	9.26
P. cinnamomi waterlog	49.49	6.37	P. cinnamomi waterlog	101.40	9.32
P. multivora TRH5 no waterlog	73.87	13.7	P. multivora TRH5 no waterlog	103.99	11.77
P. multivora TRH5 waterlog	76.31	8.89	P. multivora TRH5 waterlog	121.20	9.5
			P. multivora TRH1B1 no waterlog	127.51	12.86
			P. multivora TRH1B1 waterlog	106.61	12.18
Average root volume	**58.62**		**Average root volume**	**114.22**	
Top dry weight	**Mean**	**Std. error**	**Top dry weight**	**Mean**	**Std. error**
Control no waterlog	23.38	2.84	Control no waterlog	38.47	4.26
Control waterlog	29.21	3.41	Control waterlog	32.29	2.87
P. cinnamomi no waterlog	22.58	2.87	P. cinnamomi no waterlog	32.39	3.72
P. cinnamomi waterlog	25.57	2.91	P. cinnamomi waterlog	21.58	1.54
P. multivora TRH5 no waterlog	33.05	3.84	P. multivora TRH5 no waterlog	27.08	2.46
P. multivora TRH5 waterlog	30.92	3.22	P. multivora TRH5 waterlog	27.78	2.39
			P. multivora TRH1B1 no waterlog	33.91	2.54
			P. multivora TRH1B1 waterlog	25.47	2.62
Average top dry weight	**27.45**		**Average top dry weight**	**29.87**	

Table 2. Results of univariate ANOVA models on the treatment effects (*P. cinnamomi*, *P. multivora* TRH5) with Bonferroni correction[#] in the second trial with *Corymbia calophylla*.

Variable	*df*	*MS*	*F*	*P*	Adj. *P*[#]
Root volume:					
P. cinnamomi	1, 72	2188.71	1.79	.19	.74
P. multivora TRH5	1, 72	648.26	0.53	.49	1.0
P. multivora TRH1B2	1, 72	251.65	0.21	.65	1.0
Waterlogging	1, 72	233.35	0.19	.66	1.0
P. cinnamomi, waterlogging	1, 72	112.68	0.09	.76	1.0
P. multivora TRH5, waterlogging	1, 72	2781.63	2.28	.14	.54
P. multivora TRH1B2, waterlogging	1, 72	850.36	0.70	.41	1.0
Top dry weight:					
P. cinnamomi	1, 72	222.26	2.63	.11	.44
P. multivora TRH5	1, 72	347.89	4.11	.05	.18
P. multivora TRH1B2	1, 72	323.42	3.82	.55	1.0
Waterlogging	1, 72	765.08	9.04	.00	**.02**
P. cinnamomi, waterlogging	1, 72	142.79	1.69	.20	.80
P. multivora TRH5, waterlogging	1, 72	213.65	2.53	.12	.47
P. multivora TRH1B2, waterlogging	1, 72	12.70	0.15	.70	1.0
Diameter:					
P. cinnamomi	1, 72	2.18	1.60	.21	.84
P. multivora TRH5	1, 72	2.91	2.13	.15	.60
P. multivora TRH1B2	1, 72	0.24	0.17	.68	1.0
Waterlogging	1, 72	9.33	6.83	.01	**.04**
P. cinnamomi, waterlogging	1, 72	4.12	3.02	.09	.35
P. multivora TRH5, waterlogging	1, 72	0.96	0.70	.41	1.0
P. multivora TRH1B2, waterlogging	1, 72	0.82	0.60	.44	1.0
Height:					
P. cinnamomi	1, 72	4133	0.19	.67	1.0
P. multivora TRH5	1, 72	37066	1.65	.20	.81
P. multivora TRH1B2	1, 72	16769	.075	.39	1.0
Waterlogging	1, 72	29108	1.30	.26	1.0
P. cinnamomi, waterlogging	1, 72	149301	6.66	.01	**.048**
P. multivora TRH5, waterlogging	1, 72	5782	0.26	.61	1.0
P. multivora TRH1B2, waterlogging	1, 72	1404	0.06	.80	1.0

($\alpha \leq 0.05/4 = .0125$). *P*-values in bold are significant

Table 3. Average number of daylight length hours in Perth, Western Australia[#].

Month	Daylight length (hrs)
January	13:58
February	13:17
March	12:21
April	11:01
May	10:30
June	10:05
July	10:16
August	10:59
September	11:57
October	12:57
November	13:47
December	14:11

[#] Data from https://geodesyapps.ga.gov.au/sunrise

Appendix 4

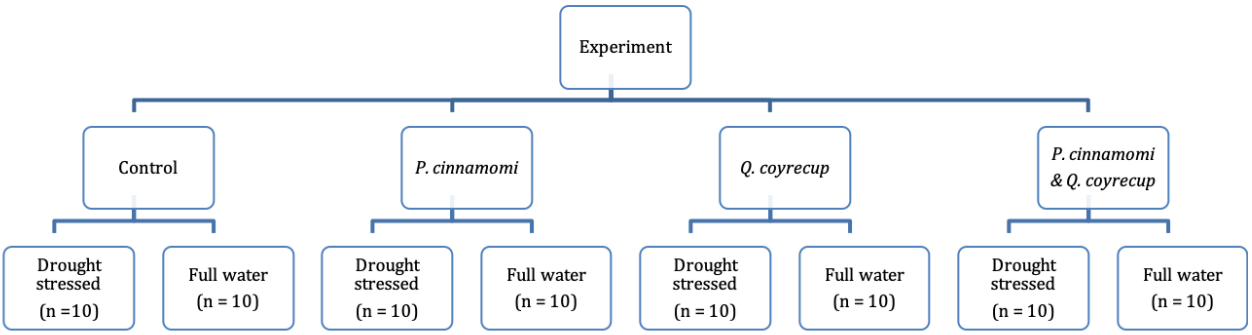

Figure 1. Three-factorial design, (*Phytophthora cinnamomi*, inoculation with the canker pathogen *Q. coyrecup* and drought stress) used in this study.

Table 1. Results of the two Sample t-tests (α ≤ 0.05) run on the effects of the *P. cinnamomi*, the canker pathogen *Quambalaria coyrecup* and drought stress treatments on the biophysical variables (root volume, canker volume, final diameter, and final height). The *p*-values were adjusted with Bonferroni corrections to correct for multiplicity.

Variable	*P. cinnamomi*				*Q. coyrecup*				Drought stress			
	t	*df*	*p*	Means[#] (0,1)	*t*	*df*	*p*	Means[#] (0,1)	*t*	*df*	*p*	Means[#] (0,1)
Root volume	0.72	63.95	0.47	320.98 340.70	2.01	66.91	**0.048**	302.92 355.57	0.13	59.65	0.90	331.90 328.39
Final height	0.36	67.97	0.72	1874.42 1854.47	0.75	65.81	0.46	1887.15 1844.67	1.15	67.93	0.26	1900.00 1836.08
Final diameter	1.52	63.46	0.13	17.79 17.08	3.87	67.97	**0.0002**	16.61 18.28	0.40	66.76	0.69	17.37 17.55
Canker volume	0.51	62.52	0.62	23.28 24.15	5.98	53.31	**<0.0001*** **	19.50 27.62	0.31	65.99	0.76	23.97 23.43

***α ≤ 0.001, P-values in bold are significant

[#]Mean values for the control (0) and treated (1) plants

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