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A diverse range of *Phytophthora* species are associated with dying urban trees

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ABSTRACT

Surveys of dying vegetation within remnant bushland, parks and gardens, and streetscapes throughout the urban forest of Perth and the South-west of Western Australia revealed symptoms typical of those produced by *Phytophthora* species. A total of nine *Phytophthora* species, including *P. alticola*, *P. multivora*, *P. litoralis*, *P. inundata*, *P. nicotianae* and *P. palmivora* were isolated. In addition, three previously undescribed species, *Phytophthora* aff. *arenaria*, *Phytophthora* aff. *humicola* and *Phytophthora* sp. *ohioensis* were isolated. Isolates were recovered from a wide range of native and non-native host genera, including *Agonis*, *Allocasuarina*, *Brachychiton*, *Calothamnus*, *Casuarina*, *Corymbia*, *Dracaena*, *Eucalyptus*, *Ficus*, *Pyrus and Xanthorrhoea*. *Phytophthora multivora* was the most commonly isolated species. Out of 230 samples collected 69 were found to be infected with *Phytophthora.* Of those 69, 54% were located within parks and gardens, 36% within remnant bushland, and 10% within streetscapes. These pathogens may play a key role in the premature decline in health of the urban forest throughout Perth, and should be managed according to the precautionary principle and given high priority when considering future sustainable management strategies.

Introduction

The rapid global expansion of the human population is primarily focused in urban and peri-urban areas, and this process poses a major threat to the existence of remaining trees and forests. Urbanisation causes the urban heat island (UHI) effect, whereby cities can be several degrees warmer than surrounding rural areas as a result of increased convection and re-radiation of heat by the replacement of vegetation with impervious surfaces such as concrete, asphalt and bricks (Armson et al., 2012; Hardin and Jensen, 2007; Onishi et al., 2010). The removal of trees can also have adverse effects on biodiversity (Davis et al., 2012; Heterick et al., 2012; Stagoll et al., 2012), soil health (Knight et al., 2013; Rao et al., 2013), air quality (Martins, 2012), carbon sequestration (Liu and Li, 2012) and human health (Donovan et al., 2013). It is therefore very important that we conserve and sustainably manage the vegetation that remains within the urban forest.

When considering factors that may cause premature decline of trees and forests, Manion (1991) proposed a disease decline spiral, grouping these factors into predisposing, inciting and contributing. Notably, the urban environment was listed as a factor that predisposes trees to premature decline. Trees already predisposed to premature decline are susceptible to other factors that may incite or trigger a decline in health. These inciting factors can be diverse and include abiotic factors such as water stress, heat stress, or airborne pollution (Wang et al., 2011), or biotic factors such as pests (Aukema et al., 2011; Raupp et al., 2006) and diseases (Grasso et al., 2012; Jacobi et al., 2013; Minorsky, 2003; Yulia, 2011).

One of the most important genera of pathogens causing disease and mortality of trees and forests worldwide is *Phytophthora* de Bary. Species within this genus have been attributed to the worldwide decline and dieback of Mediterranean trees and forests (Balci and Halmschlager, 2003; Greslebin et al., 2007; Maloney et al., 2005; Scott et al., 2009). The majority of studies into the association of *Phytophthora* species with trees have focused on woodlands and forests within natural ecosystems, with very few studies within the urban environment. The few studies that have contributed to this knowledge have predominantly targeted nurseries, or plants other than trees (Bulajic et al., 2010; Mrazkova et al., 2011).

Australian cities have experienced a sustained period of rapid urban growth (McDougall and Maharaj, 2011), and this growth will continue to pose major challenges for the sustainable management of the urban forests due to environmental constraints (McGuirk and Argent, 2011) such as availability of water, and adequate space for developing root systems. The remnant vegetation that remains is

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under increasing pressure from factors related to this urbanisation, and over recent years, many trees throughout Australian cities have suffered a continuing decline in their health. The aim of this study was to determine whether *Phytophthora* species were associated with trees exhibiting crown decline in urban and peri-urban sites of Western Australia, including the capital city Perth.

Methods

Surveys of trees throughout part of the swan coastal plain (Latitude −31°55′S, Longitude 115°46′E to Latitude −33°39′S, Longitude 115°23′E) of South-western Western Australia were conducted between 2010 and 2012. Trees were selected within areas suggested by individual land managers, and individual specimens were chosen for sampling based upon expression of premature crown decline symptoms. A total of 230 samples were collected from trees and shrubs growing from the Busselton region approximately 200 km south of Perth to the Perth inner suburbs, within three different urban land use categories (1) streetscapes – defined as planted specimens growing along the verge of streets (2) parks and gardens – planted specimens growing within local government parks or private gardens and remnant bushland – natural bushland that existed prior to surrounding urban development. Soil along with symptomatic root and basal stem tissues were collected from trees exhibiting symptoms of crown decline located within streetscapes, parks, gardens, and urban bushlands. Soil and root samples were flooded and baited according to the method of *Aghighi* et al. (2012) and once lesions developed the baits were plated onto NARPH, a selective media for *Phytophthora* species (Huberli et al., 2000). Pure cultures of *Phytophthora* were then isolated by repeated subculturing. Symptomatic root and basal stem materials were washed with de-ionised water, blotted dry and plated onto NARPH. Morphological characters were used to identify isolates to genus level and species where possible. In most cases, identification to species level required the isolation, amplification and sequencing of DNA and subsequent phylogenetic analysis.

DNA isolation, amplification, sequencing and identification

Harvested mycelium of pure *Phytophthora* isolates was frozen in liquid nitrogen, ground to a fine powder and genomic DNA was extracted according to the method described by (Andjic et al., 2007). The region spanning the internal transcribed spacer (ITS1-5.8S-ITS2) region of the ribosomal DNA was amplified using the primers ITS-6 (5′ GAA GGT GAA GTC GTA ACA AGG 3′) (Cooke et al., 2000) and ITS-4 (5′TCC TCC GCT TAT TGA TAT GC 3′) (White et al., 1990). The clean-up of products and sequencing were as described previously (Sakalidis et al., 2011).

The sequence data were edited using Geneious software version 6.0.6 created by Biomatters (www.geneious.com). The sequences were then subjected to a BLAST search on GenBank to find the closest sequence matches and then aligned in Geneious to confirm identity. In this way, six known species and three new taxa were identified. All sequences derived in this study were deposited in GenBank.

For phylogenetic analysis, sequences of representative isolates from each taxa were aligned with those of closely related species obtained from GenBank (http://www.ncbi.nlm.nih.gov/). ITS sequence data were assembled and manually edited as described previously (Jung and Burgess, 2009). Parsimony analysis was performed in PAUP (Phylogenetic analysis using parsimony) v. 4.0b10 (Swofford, 2003) and Bayesian analysis with MrBayes v. 3.1 (Ronquist and Heuelsenbeck, 2003) as described previously (Jung and Burgess, 2009). Alignment files and trees can be viewed on TreeBASE (http://www.treebase.org/).

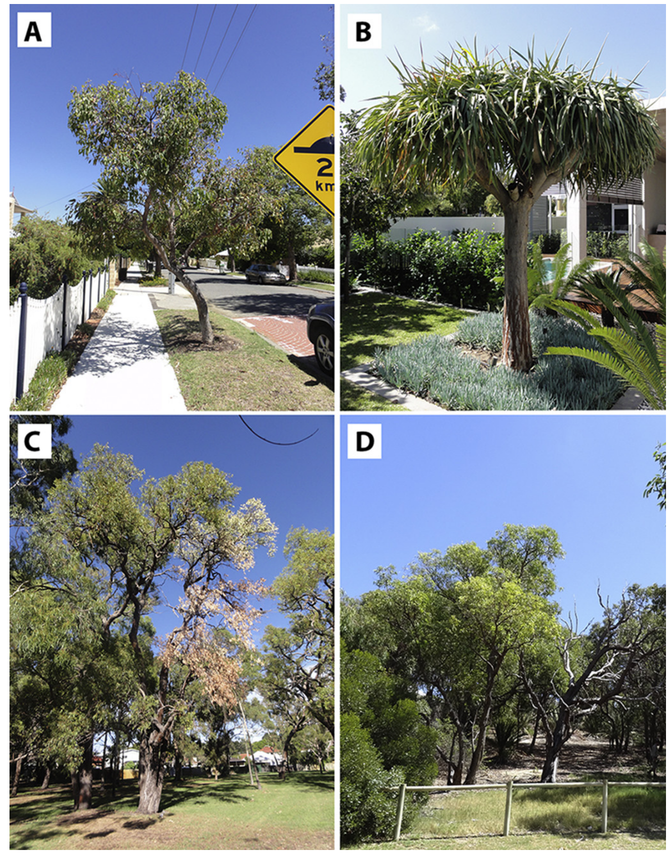

Fig. 1. Land-use categories where *Phytophthora* species were isolated from declining specimens (A) *C. ficifolia* growing on a street verge, (B) *D. draco* within a newly landscaped garden, (C) *E. marginata* growing within an irrigated parkland, (D) *C. calophylla* growing within a remnant bushland.

Results

Hosts and symptoms

Phytophthora species were isolated from exactly 30% ($n = 69$) of samples, with 10% of these occurring within streetscapes (Fig. 1A), 54% of these located within government parks and private gardens (Fig. 1B and C) and 36% within remnant bushland (Fig. 1D; Table 1).

Hosts included the following Australian natives with number of positive specimens for each listed in brackets; *Agonis flexuosa* Lindl. (23), *Allocasuarina* L.A.S. Johnson (1), *Brachychiton populneus x acerifolius* (1), *Calothamnus* Labill. (1), *Casuarina obesa* Miq. (4), *Corymbia calophylla* (Lindl.) K.D. Hill & L.A.S. Johnson (10), *C. citriodora* (Hook.) K.D. Hill & L.A.S. Johnson (1), *C. ficifolia* (F.Muell.) K.D. Hill & L.A.S. Johnson (4), *Eucalyptus camaldulensis* Dehnh. (1), *E. gomphocephala* DC. (7), *E. marginata* D.Don ex Sm. (8), *E. robusta* Sm. (1), *E. rudis* Endl. (1), *Eucalyptus* L'Hérit. (2), *Ficus microcarpa* var. *hillii* (F.M.Bailey) Corner (1), *F. macrophylla* Desf. Ex Pers. (1), *Grevillea* sp. (2), *Grevillea thelemanniana* Hugel ex Endl. (1), and *Xanthorrhoea preissii* Endl. (1), and non-natives, *Dracaena draco* L. (1), *Pyrus ussuriensis* Maxim. (1). A range of crown health symptoms was observed on these specimens, including one or more of the following: branch tip dieback, foliar chlorosis and necrosis, entire crown collapse, crown wilting, crown thinning, and sudden death (Fig. 2A and B). Further investigation of many specimens revealed necrotic lesions on fine feeder roots, lateral roots or basal stem, and complete death of some roots (Fig. 2C and D).

Table 1
Summary of *Phytophthora* isolations by host species.

Host species	*P. alticola*	*P. aff. arenaria*	*P. aff. humicola*	*P. inundata*	*P. litoralis*	*P. multivora*	*P. nicotianae*	*P. ohioensis*	*P. palmivora*
Agonis flexuosa		2 (P)				21 (18 R, 3P)			
Allocasuarina sp.						1 (P)			
Brachychiton populneus x acerifolia						1 (P)			
Calothamnus sp.						1 (R)			
Casuarina obesa			2 (P)	1 (P)	1 (P)				
Corymbia calophylla						7 (4R, 3P)		3 (1R, 2P)	
Corymbia citriodora						1 (P)			
Corymbia ficifolia		3 (P)					1 (S)		
Dracaena draco									1 (P)
Eucalyptus camaldulensis						1 (P)			
Eucalyptus gomphocephala		5 (P)				2 (P)			
Eucalyptus marginata	1 (P)	3 (P)				4 (P)			
Eucalyptus robusta						1 (P)			
Eucalyptus rudis						1 (P)			
Eucalyptus sp.						1 (R)	1 (S)		
Ficus microcarpa var. hilli		1 (P)							
Ficus macrophylla						1 (P)			
Grevillea sp.							2 (S)		
Grevillea thelemanniana							1 (P)		
Xanthorrhoea preissii						1 (P)			

Landscape setting: P, parks & gardens; R, remnant natural bushland; S, streetscape. Where *P* and *R* co-occur for an isolate and species, the ratio is shown.

Identification

A total of nine species of *Phytophthora* were isolated and identified using a combination of morphological characters and DNA sequence data (Table 1; Fig. 3–phylogenetic tree). A total of 44 isolates of *P. multivora* P.M. Scott & T. Jung were recovered from a wide range of hosts, including *A. flexuosa*, *Allocasuarina* sp., *B. populneus x acerifolius*, *Calothamnus* sp., *C. calophylla*, *C. citriodora*, *E. camaldulensis*, *E. gomphocephala*, *E. marginata*, *E. robusta*, *E. rudis*, *F. macrophylla*, *Pyrus ussuriensis*, and *Xanthorrhoea preissii.* Another described species, *P. nicotianae* Breda de Haan, was confirmed from five specimens collected from *C. ficifolia*, *Eucalyptus* sp., *Grevillea* sp., and *G. thelemanniana. Phytophthora palmivora* (E.J. Butler) E.J. Butler was only collected once from *D. draco*, as were *P. litoralis* T.

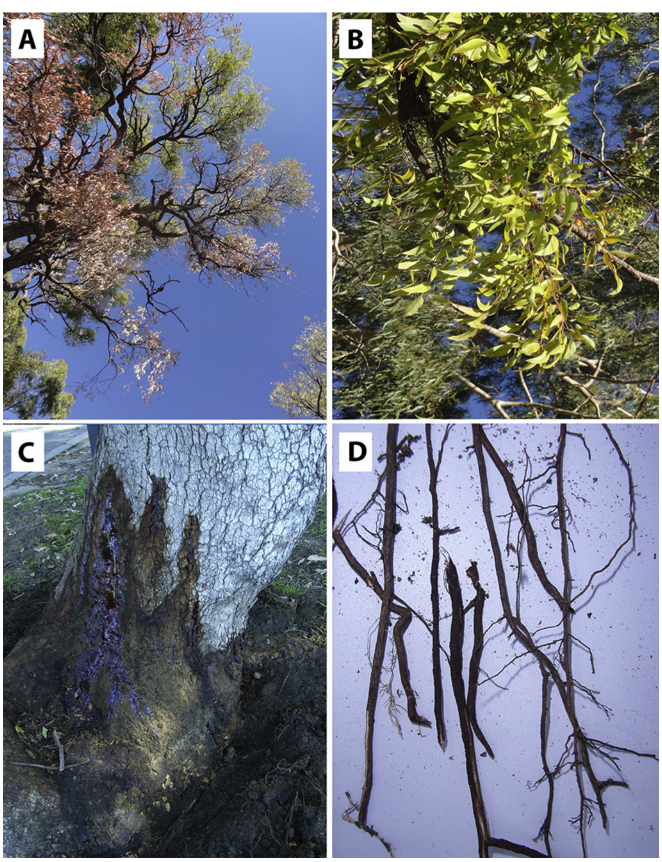

Fig. 2. Examples of symptoms of specimens from which *Phytophthora* was isolated (A) Crown thinning and foliar necrosis of *E. marginata*, (B) Interveinal chlorosis of *E. marginata*, (C) Basal lesion and exudation of kino on *C. calophylla*, (D) Necrotic fine roots of *E. marginata*.

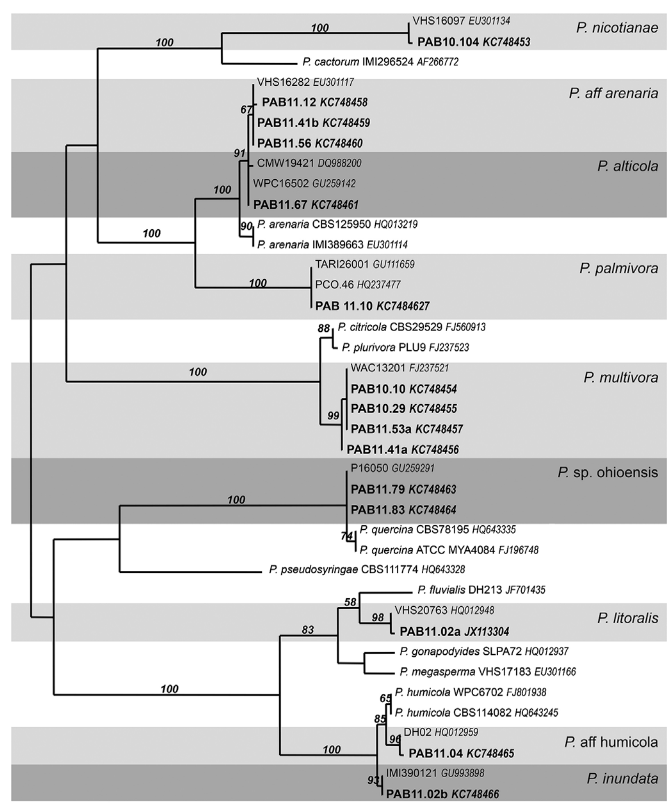

Fig. 3. One of 10 most parsimonious trees of 480 steps (CI = 0.72, RI = 0.94) based on ITS sequence data of *Phytophthora* species isolated in the current study in comparison to reference sequences of known species and taxa. Support for the terminal clades is given as bootstrap values above the lines. GenBank numbers of the selected PAB isolates are given in this tree.

Jung, Stukely & T.I. Burgess and *P. inundata* Brasier, Sánch. Hern. & S.A. Kirk that were both collected from *Cas. obesa*, and *P. alticola* Maseko, Cout. & M.J. Wingf collected from *E. marginata.*

Three undescribed species of *Phytophthora* were collected in the present study. A species closely related to *P. arenaria* A. Rea, Stukely & T. Jung was identified in this study as *P.* aff. *arenaria.* (Fig. 3). A total of 10 isolates of *P.* aff. *arenaria* were identified from *A. flexuosa*, *C. ficifolia*, *E. gomphocephala*, *E. marginata*, and *Ficus microcarpa var. hillii.* A species closely related to *P. humicola* W.H.Ko & Ann was identified in this study as *P.* aff. *humicola* (Fig. 3). Only two isolates were recovered, both from *Cas. obesa* at the same site. The final species identified was *P.* sp. *ohioensis* (Fig. 3), with three isolates recovered from the same host, *C. calophylla*, at three separate locations.

Ecology and distribution

P. multivora was the most widely distributed species, found as far south as Busselton and throughout the Perth metropolitan region. It was isolated from vegetation growing on street verges (4%), in parks and gardens (39%), and the majority were isolated from vegetation growing in remnant bushland areas (54%). The other eight species were found throughout the Perth metropolitan region only. A single isolate of *P.* sp. *ohioensis* was isolated from vegetation in remnant bushland, with the remainder of the isolates of *P.* sp. *ohioensis* and all isolates of the other seven species of *Phytophthora* were recovered from vegetation growing within parks and gardens or streetscapes.

Discussion

The present study has identified nine different *Phytophthora* species associated with dying urban trees in Perth and surrounds. The most common species encountered, *P. multivora*, was recently described by Scott *et al.* (2009) and has been associated with dying plants of a wide range of native host species throughout WA natural ecosystems (Scott et al., 2009). Scott *et al.* (2011) conducted pathogenicity trials to show that *P. multivora* was a fine feeder root pathogen of *E. gomphocephala* and *E. marginata.* The present study has increased the known host range of *P. multivora* to include *Allocasuarina* sp., *B. populneus x acerifolia*, *Calothamnus* sp., *C. calophylla*, *C. citriodora*, *E. camaldulensis*, *E. robusta*, *E. rudis*, *F. macrophylla*, *P. ussuriensis*, and *Xanthorrhoea preissii.* Pathogenicity of *P. multivora* has not been tested on the majority of these species, however, Shearer *et al.* (1988) showed that an isolate of *P. multivora* previously mis-identified as *P. citricola* Sawada, was more pathogenic to *C. calophylla* than *P. cinnamomi* Rands. The present study has shown *P. multivora* to be widespread throughout the Perth urban area in streetscapes, parks and gardens, and remnant bushland, and far more prevalent than any other *Phytophthora* species encountered.

Five previously described species, *P. nicotianae*, *P. palmivora*, *P. alticola*, *P. inundata* and *P. litoralis*, were all recovered from hosts planted in streetscapes or parks and gardens only, not remnant vegetation. *P. nicotianae* has a wide host range and distribution throughout the world, and in WA has been found associated with a wildflower farm (Boersma et al., 2000), natural vegetation (Burgess et al., 2009), nurseries (Hardy and Sivasithamparam, 1988), and potting mix of nursery plants imported into WA from eastern Australia (Davison et al., 2006). The present study is the first published account of *P. nicotianae* associated with dying plants in the Perth urban area, and from the host genus *Corymbia.*

P. palmivora is commonly found throughout the sub-tropics causing serious diseases on food trees like cocoa and durian (Appiah et al., 2004). Although *P. palmivora* has been isolated from potting mix of nursery plants imported to WA from QLD, NT and NSW (Davison et al., 2006), to our knowledge there have been no published accounts of *P. palmivora* occurring in natural or planted ecosystems within WA.

Phytophthora alticola has only previously been recorded from South Africa and Swaziland associated with diseased and dying *E. badjensis* Beuzev & Welch, *E. dunnii* Maiden, *E. macarthurii* H. Deane & Maiden and *Acacia decurrens* Willd. growing within plantation forests (Maseko et al., 2007). It was shown to be pathogenic to young *E. dunnii* growing in the field, and was believed to be a causal agent of dieback and early death of cold-tolerant *Eucalyptus* species in South Africa. It is very closely related to *P.* aff. *arenaria.* This new record for Australia raises questions about the origin of the species and its pathogenicity to native Australian vegetation.

Phytophthora inundata, as the name suggests, has a tendency to be found in areas of regular flooding. It has been recorded from host genera *Salix*, *Olea*, *Prunus* and *Vitis* in Europe and South America (Brasier et al., 2003), and more recently from soil around carrot and parsley crops in Victoria (Cunnington et al., 2006), soil, dying and dead native vegetation in WA (Stukely et al., 2007), and it was the most commonly isolated *Phytophthora* species from streams and waterways baited throughout Perth and the South-west of WA (Huberli et al., 2013). Like *P. inundata*, *P. litoralis* is also most commonly found in water-logged areas, and has been recovered from dying *Banksia* species in native ecosystems and from streams (Jung et al., 2011). In the present study, both *P. inundata* and *P. litoralis* are newly recorded from the host genus *Casuarina* and from dying plants in the Perth urban area. It is of interest to note that the site where they were recovered is within 30 m of the Swan River and is susceptible to regular flooding events.

Three undescribed species of *Phytophthora* were identified in the present study: *Phytophthora* aff. *arenaria*, *Phytophthora* aff. *humicola* and *Phytophthora* sp. *ohioensis. P.* aff. *arenaria* was commonly isolated from parks and gardens and streetscapes associated with dying specimens of *Eucalyptus*, *Corymbia*, *Agonis* and a *F. microcarpa var. hillii.* The sample collected beneath *Ficus* was part of a bulked sample, therefore the specimen from which the isolate was recovered may have belonged to *C. ficifolia* or *E. gomphocephala.* This species is closely related to the recently described *P. arenaria* (Rea et al., 2011), which was described from *Banksia* Linn., *Hibbertia hypericoides* (DC.) Benth. and *E. drummondii* Benth. in South-west WA, and to *P. alticola* isolated from cold tolerant *Eucalyptus* species at one location in South Africa. Prior to the present study *Phytophthora* aff. *arenaria* had only been recently found at a single *Eucalyptus* seedling nursery in WA (M. Stukely DEC, pers. comm.). Our survey is therefore the first account of *Phytophthora* aff. *arenaria* outside nurseries, and on the genera *Corymbia* K.D. Hill & L.A.S. Johnson and *Agonis* (DC.) Sweet.

Phytophthora aff. *humicola* was isolated from *Cas. obesa* in the same location as *P. inundata* and *P. litoralis.* This species is closely related to *P. inundata* and therefore has developed life strategies to cope with the periodic flooding events (Jung et al., 2011). The present study is the first published record of *Phytophthora* aff. *humicola* associated with dying vegetation, all previous recoveries were from stream baiting (Huberli et al., 2013). *Phytophthora* sp. *ohioensis* is an undescribed species only recorded previously in North America and is most closely related to *P. quercina* T.Jung, a pathogen of oaks. In the present study it was recorded from a single host, *C. calophylla*, at three locations in remnant bushland and parks and gardens.

The recovery in our study of most *Phytophthora* species from parks and gardens, and the previous record of some of these species only from nurseries (i.e. *Phytophthora* aff. *arenaria*, *P. palmivora*), or from overseas (i.e. *P. alticola* and *P.* sp. *ohioensis*) raises concerns about the introduction of these pathogens from other countries, and into amenity areas via nursery plants. The higher proportion of positive samples from parks and gardens when compared to remnant bushland and streetscapes may be simply due to a greater sampling effort in these environments, or related to their environmental conditions or history. For example, most parks and gardens are irrigated throughout summer as opposed to remnant bushland. Summer irrigation is conducive to the survival, spread and infection of soil-borne root pathogens such as *Phytophthora*. Historically, introduction of nursery specimens and soil media for earthworks and landscape development are more likely to have occurred in parks and gardens than in remnant bushland, and as such, had a greater potential for introduction of soil-borne pathogens. These are general assumptions that require further investigation and research.

Decline in health and rapid death of urban vegetation in parks and gardens is widespread throughout the Perth metropolitan area. The cause of the great majority of this decline and death of vegetation is not diagnosed and management is limited in most cases to ongoing pruning of deadwood, entire tree removal, and replacement with advanced trees. This management strategy is unsustainable in the long-term, is cost-prohibitive, and detrimental to the environment due to the ongoing impact of the heavy machinery used for pruning, removal, mulching and its transport, and the loss of mature canopy cover. A carbon footprint analysis of the management of urban trees over a 50 year period in Germany showed an increase in mortality of trees from 0.5% to 4% reduces the net carbon sequestration by more than 70% (Strohbach et al., 2012).

At present, very little is known about the pathogenicity of most of the *Phytophthora* species we isolated. Regardless, the precautionary principle should be applied to their management, as is carried out for *P. cinnamomi*, considered one of the major threats to biodiversity in Australia (Dunstan et al., 2010). Particular emphasis should be placed upon the management of *Phytophthora* species in nurseries, and the prevention of movement throughout the environment, as it is known that *Phytophthora* species can hybridise and form new species with the capacity to cause devastation to new hosts. Examples include *P. alni* Brasier & S.A. Kirk, a hybrid of *P. cambivora* (Petri) Buisman and a *P. fragariae*-like sp. that killed approximately 10% of the alders within Britain and has spread throughout France and Bavaria, and other hybrids between *P. cactorum* (Lebert & Cohn) J. Schröt., *P. nicotianae*, and *P. hedraiandra* De Cock & Man (Érsek and Nagy, 2008).

Once a pathogen such as *Phytophthora* has entered a new environment like a park or garden, it is almost impossible to eradicate. The prevalence of such pathogens, particularly those like *Phytophthora* that can survive long periods in the soil and can be moved readily, poses an ongoing threat to surrounding vegetation and to newly planted replacement specimens. Prevention of the introduction of pathogens is much more cost-effective than the ongoing management. If vegetation is impacted by *Phytophthora*, treatment with chemicals such as phosphorous acid (phosphite) to the stems or foliage may be effective as an ongoing control (Scott et al., 2013; Shearer and Fairman, 2007). The success of such treatments requires accurate diagnosis and is usually more effective as a preventative rather than a curative (Sandler et al., 1986), and the effectiveness of such chemicals may vary depending on the *Phytophthora* species and host (Shearer and Fairman, 2007).

The two hosts from which *Phytophthora* was most commonly isolated during the present study were *A. flexuosa* and *C. calophylla*. *A. flexuosa* (West Australian Peppermint) is the dominant urban tree throughout the older and more prestigious suburbs of Perth, impacting upon the aesthetic values of these areas. The ongoing pro-active and sustainable management of the health of *A. flexuosa* is of critical importance as they also provide important habitat in urban areas to a wide range of fauna and flora endemic to WA, including the mycorrhizal fungus *Inocybe violaceocaulis* Matheny & Bougher (Matheny and Bougher, 2005) and the nationally listed vulnerable species of western ringtail possum, *Pseudocheirus occidentalis* Thomas (Jones et al., 1994, 2004). Diagnosis and management of *Phytophthora* species is important as it can incite premature decline, increasing susceptibility to other contributing factors, or may combine to cause more severe disease (Marçais et al., 2010). The opportunistic pathogen and endophyte, *Neofusicoccum australe* (Slippers, Crous & M.J. Wingf.) Crous, Slippers & A.J.L. Phillips, was recently diagnosed as the responsible agent for crown dieback of *A. flexuosa* in the tuart forest south of Perth, however, it was concluded that the inciting factors leading to the decline were still unknown (Dakin et al., 2010). It is not unreasonable to expect that one or more *Phytophthora* species such as those identified in the present study may have been the inciting factor. The recent arrival in Australia of the exotic rust fungus *Puccinia psidii* G. Winter, commonly known as guava or eucalypt rust, and its rapid spread and impact on native myrtaceous vegetation, including *A. flexuosa*, is of great concern (Morin et al., 2012). The movement of this pathogen into WA from the eastern states of Australia has the potential for widespread devastating impacts on the endemic population of *A. flexuosa*. Maintaining the natural resistance and vigour of the *A. flexuosa* population is therefore of critical importance, and the relationship between *Phytophthora* spp. and the breakdown of this resistance is at present not understood.

Corymbia calophylla (marri) is also a very important endemic tree species throughout Perth and the South-west of WA, where it is one of the most common and widely distributed eucalypts. This iconic tree species provides food and shelter to the nationally listed vulnerable species of *Calyptorhynchus banksia naso* Gould (red-tailed black cockatoo) and *C. baudinii* Lear (Baudin's black cockatoo) (Cooper et al., 2003), and a highly diverse range of non-endemic and endemic invertebrates (Majer et al., 2002). Over the past decade a serious fungal canker disease caused by *Quambalaria coyrecup* Paap, in combination with a foliar disease caused by the closely related *Q. pitereka* (J. Walker & Bertus) J.A. Simpson, have caused extensive decline and deaths of *C. calophylla* throughout South-west WA (Paap et al., 2008). Paap et al. (2008) queried the recent significant increase in the incidence of this disease given its apparent endemism and long-term historical presence since the early 1900s. It was suggested that recent environmental changes and stressors, such as declining rainfall, localised rising groundwater levels, or logging disturbance may be the underlying causes or inciting factors. The collection of *P. multivora* from a number of specimens of *C. calophylla*, a pathogen known to be more pathogenic than *P. cinnamomi* to this host, raises the possibility that, given their prevalence and impact on root health, *Phytophthora* species may incite premature decline and increase the susceptibility of these trees to canker disease.

The successful development of a disease requires the presence of a pathogen, a susceptible host, and suitable environmental conditions. The urban environment in most cases is highly disturbed, and in the majority of parks, gardens and streetscapes in Perth, irrigated and often with compacted soil. Such conditions have been shown to promote the development and impact of *Phytophthora* (Rhoades et al., 2003), and decrease the prevalence of beneficial mycorrhizal fungi. Many of the soils found throughout such parks, gardens and streetscapes are often devoid of the diversity of beneficial microbes required for the development of healthy root systems, as they have been manipulated over many years and managed for the growth of turf rather than trees. There is a clear relationship between the health of native trees and presence of these beneficial bacteria and mycorrhizal fungi in the depauperate sandy soils of WA (Cai et al., 2010; Ishaq et al., 2013; Scott et al., 2012). There is also evidence to suggest that the presence of mycorrhizal fungi can increase the resistance of hosts to *Phytophthora* species (Watanarojanaporn et al., 2011). It is therefore important to ensure soils are well-drained, and use composted soil conditioners, mulches and amendments to improve the soil microbial activity around the rhizosphere of native trees, particularly where *Phytophthora* is known to occur.

In conclusion, this study has highlighted the diversity and distribution of *Phytophthora* species on a wide range of hosts exhibiting crown decline throughout Perth and surrounding urban areas in WA. A great deal more research is required on the taxonomy of the undescribed species, the pathogenicity of the listed *Phytophthora* species to the most commonly planted species of urban trees, the presence of *Phytophthora* species in nurseries, and on sustainable management strategies for the maintenance of a healthy canopy throughout the urban forest. The development of sustainable management strategies has important implications for the environmental and social benefits these urban forests provide.

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