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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.

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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/).

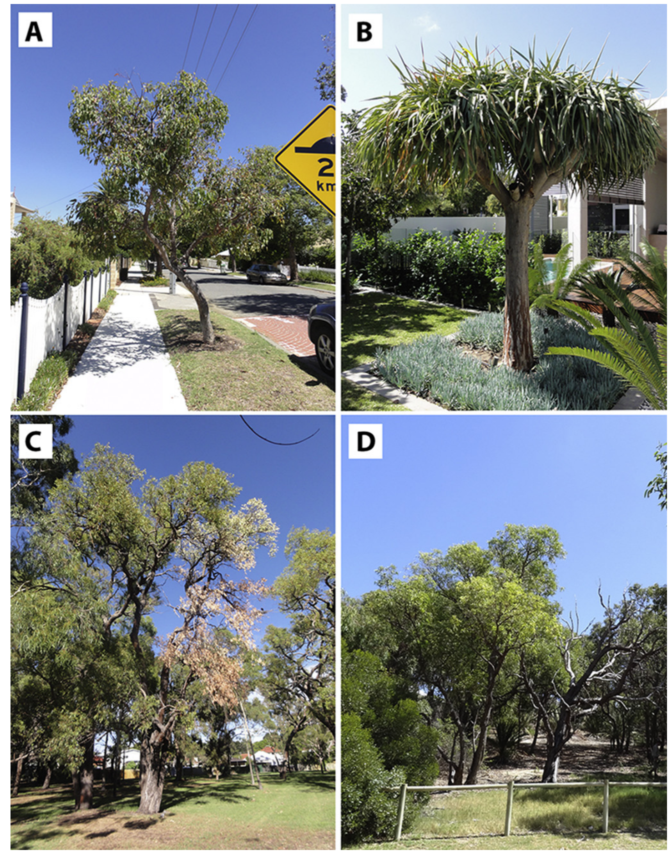

Fig. 1. Land-use categories where *Phytophthora* species were isolated from declining specimens (A) *C. ficifolia* growing on a street verge, (B) *D. draco* within a newly landscaped garden, (C) *E. marginata* growing within an irrigated parkland, (D) *C. calophylla* growing within a remnant bushland.

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).

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