Botanical gardens provide valuable baseline *Phytophthora* diversity data

J.M. Hulbert[ab], T. Paap[ac], T.I. Burgess[d], F. Roets[b] and M.J. Wingfield[a]

[a]Forestry and Agricultural Biotechnology Institute (FABI), Department of Plant and Soil Science, University of Pretoria, Pretoria, 0002, South Africa
[b]Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, 7600, South Africa
[c]South African National Biodiversity Institute, South Africa
[d]Centre for Phytophthora Science and Management, Environmental and Conservation Sciences, Murdoch University, Perth, 6150, Australia

***** Corresponding author at: Private Bag X20, FABI, University of Pretoria, Pretoria, 0002, South Africa.
joey.hulbert@fabi.up.ac.za

Highlights

• Eight *Phytophthora* species and one informal species were recovered by baiting.
• Three putative hybrids were also recovered.
• Three species had not been reported in South Africa previously.
• Urban gardens provide opportunities for early detection and baseline data collection.

Abstract

Phytophthora species are important plant pathogens especially due to their ability to invade and change ecosystems. However, information regarding their diversity and distribution is not available in many parts of the world. In these areas, surveys of botanical gardens can provide opportunities to detect novel plant-microbe interactions on both indigenous and exotic plants. Three botanical gardens and one historical urban garden in the Western Cape Province of South Africa were surveyed to establish baseline information of *Phytophthora* species diversity in the Cape Floristic Region. Eight described species (*P. amnicola, P. asparagi, P. capensis, P. cinnamomi, P. chlamydospora, P. lacustris, P. multivora* and *P. tropicalis*), the known but as yet unnamed *P.* sp. emzansi and 3 putative hybrids were recovered. Forty eight of 103 samples collected were positive for *Phytophthora* species and *P multivora* was the most

frequently isolated species. Three species (*P. amnicola, P. asparagi* and *P. tropicalis)* had not previously been reported in South Africa, although hybrid progeny of *P. amincola* had been found in two previous studies. These results highlight the value of botanical gardens as areas for baseline data collection and early warning systems.

Keywords

International Plant Sentinel Network, IPSN, urban environments, early detection, *Phytophthora cinnamomi, Phytophthora multivora*

1. Introduction

Biological invasions of plant pests and disease-causing microbes have substantial negative effects both economically and ecologically (Pimentel et al. 2001; Pautasso et al. 2014; Lovett et al. 2016). Yet, novel plant-pest interactions continue to emerge mainly due to an increase in globalization and in the trade of plants for planting (Liebhold et al. 2012; Santini et al. 2013). Consequently, advanced techniques for early detection and monitoring are critically needed to account for the unprecedented and growing levels of trade (Liebhold et al. 2012; Hurley et al. 2016), especially in countries without statutory plant health monitoring programs.

Initiatives such as the International Plant Sentinel Network (IPSN) can reduce the impacts of invasive species and the resources the network provides can be applied at local and global scales. The IPSN was established with the purpose of coordinating information exchange and sentinel research between gardens around the world (Eschen et al. 2019) and it provides resources such as pest identification guides for gardens to use locally. It coordinates the detection of novel threats to plant species through establishing

and monitoring plantings outside of their native range as ‘sentinels’ in gardens (Barham et al. 2015) and can be extended to countries without statutory monitoring such as South Africa (e.g. Paap et al. 2018). Actively participating in and applying the tools and resources provided by the IPSN can assist these countries to increase the capacity for pest surveillance, protect their indigenous flora, contribute to research and provide valuable information that can limit the consequences of globalization (Barham et al. 2016; Packer et al. 2017).

Botanical gardens provide opportunities for the early detection of novel plant-pest interactions and they generate baseline data for species diversity. These gardens are often present in urban spaces, and are therefore, under pressure from local trading hubs of material such as ornamental plants (Hulbert et al. 2017; Paap et al. 2017). They are also custodians of outstanding plant collections, often featuring species collected widely within countries and from abroad, thus presenting novel opportunities for new plant-pest interactions (Britton et al. 2010; Hulme 2011). Furthermore, diverse collections of exotic plants located near natural environments can serve as ‘sentinel’ hosts (Webber 2010; Barham et al. 2016; Eschen et al. 2019). Many of these collections are also major attractions for tourism and are visited frequently by people who could be inadvertent carriers of pathogen propagules (e.g. rust spores on clothes or the soles of their shoes). Therefore, botanical gardens are important sites to survey for the detection of novel plant-pest interactions and to identify species likely to be present in the region.

While surveying botanical gardens is important globally, there is an exceptional need for such work in South Africa for three reasons. Firstly, the country does not have a statutory monitoring program for quarantined organisms in the domestic plant trade. If an

alien species is introduced to the country unnoticed, its presence will likely not be recognized until the effects become obvious. Secondly, South Africa is home to the world's richest biodiversity hotspot for its size, the Greater Cape Floristic Region (Goldblatt 1997; Cowling et al. 2003; Born et al. 2006). It is therefore important to protect the endemic and threatened species in this region. Thirdly, there is little information about the diseases of many of the country's indigenous plant species relative to other parts of the world (Crous et al. 2006; Marincowitz et al. 2008). Fortunately, South Africa is home to nine National Botanical Gardens (NBG) operated and maintained by the South African National Biodiversity Institute (SANBI) and many additional public and private gardens. Collectively, these gardens provide many opportunities to detect and monitor potential threats to indigenous and exotic plant species, with specific representation of Mediterranean ecosystems in the Western Cape Province (WCP). Recent surveys in these gardens have already revealed the presence of the invasive polyphagous shot hole borer (Paap et al. 2018), demonstrating the merit of surveying these gardens, but there is little other information available regarding the plant diseases present in these gardens and only recent investments have been made to a support a pest monitoring program (Paap et al. 2018).

Phytophthora species are important plant pathogens of particular concern because they are responsible for many plant disease epidemics globally (Hansen et al. 2012; Hansen 2015; Jung et al. 2018; Sena et al. 2018). Species in this group were first reported as the cause of potato late blight and responsible for the Irish potato famine (Goss et al. 2014), but more recently recognized for driving epidemics such as sudden oak death (Rizzo and Garbelotto 2003), kauri dieback (Waipara et al. 2013), and *Phytophthora*

dieback in jarrah forests (Weste and Taylor 1971; Weste and Marks 1987; Shearer et al. 2004). In the WCP of South Africa, little is known about the diversity of *Phytophthora* species in indigenous plant environments and our knowledge is limited to research from the 1970-1990s focused on *Phytophthora cinnamomi* (van Wyk 1973; van der Merwe and van Wyk 1973; von Broembsen 1984; Knox-Davies et al. 1987). However, one additional study reported another species, *Phytophthora multivora*, from a botanical garden included in the present study (Oh et al. 2013), but little is known regarding the ecology of this species in South Africa.

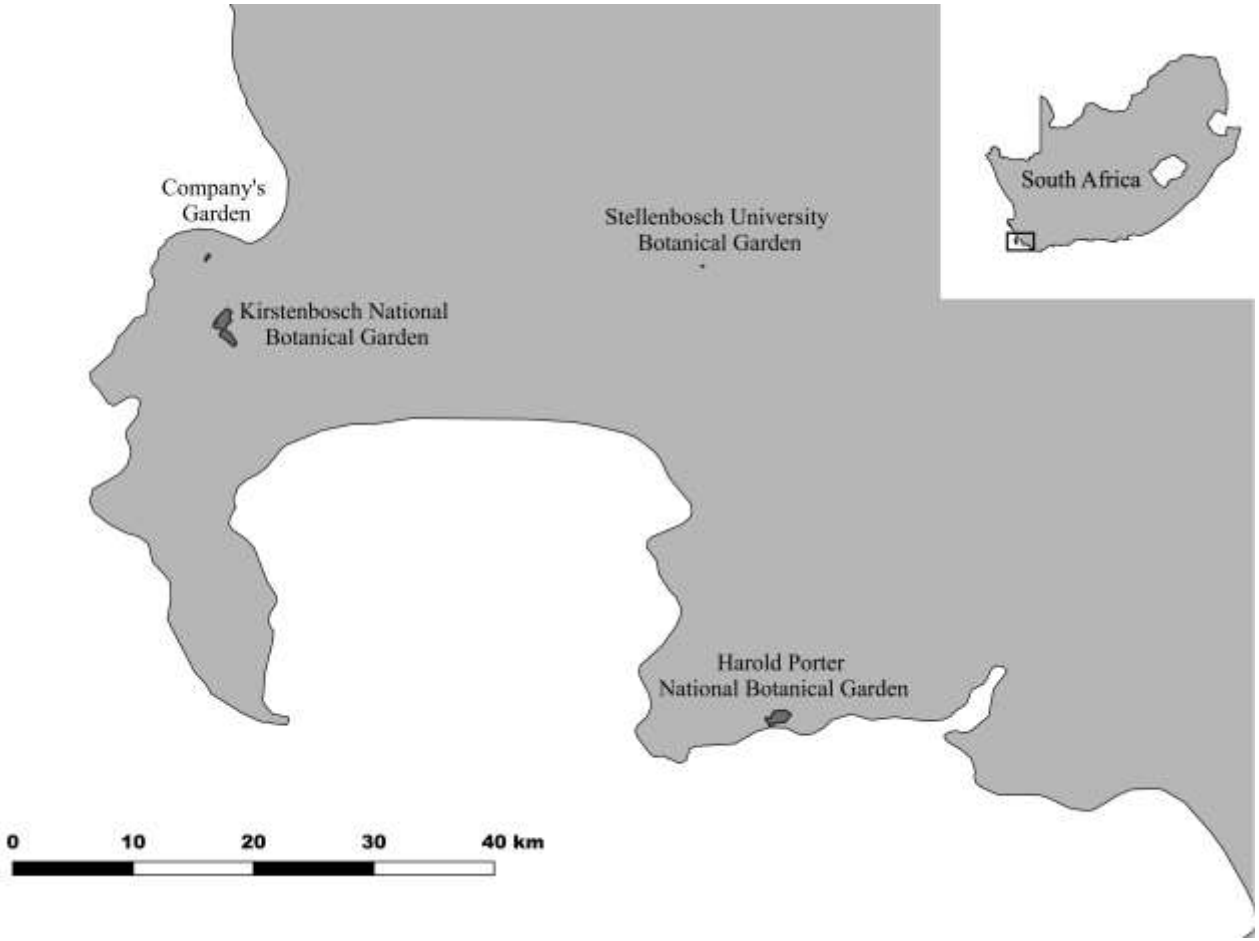
In this study, we report the findings of *Phytophthora* surveys in three botanical gardens of the Western Cape Provence (WCP). We also surveyed the Company's Garden, a historical urban garden in Cape Town. The objective of this study was to identify the *Phytophthora* species present in these gardens because there is a lack of baseline information regarding pathogen species diversity in the WCP. The study was conducted in close collaboration with garden staff, incorporating their knowledge and familiarity with the plant collections, and with the aim of raising awareness of the damage caused by invasive plant pathogens and best practices for their management. We hypothesized we would find species which had not been reported in South Africa previously because of the unique combination of hosts from mixed origins, their locality, and the high levels of tourism in the intensely managed garden settings.

2. Methods

2.1. Study areas

Four gardens were surveyed in the WCP of South Africa in the Cape Floristic Region: Kirstenbosch NBG and the Company's Garden in Cape Town, Harold Porter

Figure 1: Map of southwestern South Africa with localities of botanical gardens included in this study.



NBG in Bettys Bay, and Stellenbosch University Botanical Garden (BG) in Stellenbosch (Figure 1). Kirstenbosch NBG and Harold Porter NBG are maintained by SANBI. Kirstenbosch NBG is located on the southeastern slopes of Table Mountain National Park and Harold Porter is a coastal garden on the edge of the Kogelberg Biosphere Reserve. Both of these SANBI-operated gardens are directly adjacent to natural ecosystems and residential areas. In contrast, Stellenbosch University BG and the City of Cape Town Company's Garden are completely surrounded by residential neighborhoods and the central business district, respectively.

2.2. Stakeholder engagement

Samples were collected in close collaboration with staff at each botanical garden. The staff members were involved in the sample collection and garden managers directed sampling efforts to areas of specific interest and provided additional samples (e.g. soil mixes). Each sampling activity was organized in conjunction with Cape Citizen Science (https://citsci.co.za/), and a SANBI initiative to monitor plant health in botanical gardens under the IPSN framework. The surveys commenced with presentations to garden staff to introduce *Phytophthora* and to emphasize the threats of exotic plant pests. Staff also participated in a 'boot wash' and methods to clean tools to improve garden hygiene were demonstrated.

2.3. Sample Collection

Sampling was conducted randomly by generating random points within gardens using research tools in QGIS version 2.10.1. Rhizosphere samples were collected in proximity to unhealthy plants and asymptomatic plants and when possible, irrigation water sources and soil mixes were also sampled. Rhizosphere samples were collected 3-

5cm below the litter layer and after removing the surface layers of mulch or other debris. Soil and fine roots from underneath 2-3 plants near the points were mixed into one sample bag totaling roughly 500 grams. Samples were collected below unhealthy plants in the immediate area if such plants were found.

Additional samples were collected from points of interest to the botanical garden managers such as areas where mother stock plants were grown and garden beds that were recently burned. In Kirstenbosch NBG and Harold Porter NBG, 3-5 samples of potting material or compost were also collected (Table 1). Similarly, one soil sample was collected at the edge of a puddle under a faucet with unfiltered irrigation water at Stellenbosch University BG. One water sample was also collected from a dam used for irrigation in Kirstenbosch NBG and three water samples were collected from various sources of water in Stellenbosch University BG. All samples were set up for *Phytophthora* baiting within garden staff offices immediately after collection. Each sample was added to polyethylene trays and subsequently flooded with de-ionized distilled water. The surface debris were moved aside with paper-towels. Water samples were also placed in trays. Leaves or petals of three plant species (*Rosa* sp. cultivar, *Hedera helix* and *Quercus ilex*) were used as baits and placed on the surface of the water covering the rhizosphere samples. Two-to-three control trays that included only de-ionized water were baited per garden.

Isolations were attempted from all baits regardless of whether symptoms had developed or not. Baits were removed from the trays and blotted dry. Forceps that were used to remove the baits were sterilized between bait species for each sample by dipping them into 95% ethanol and flaming. Tissues were then cut randomly from dry baits

Table 1: Summary of *Phytophthora* recovery data from each botanic garden

Garden[1]	Source	No. Samples	Positive samples	No recoveries+	No. species*
CTCG	Garden bed	11	6	7	2
	Soil mix	0	-	-	-
	Water	0	-	-	-
	Controls	2	0	0	0
HPNBG	Garden bed	25	7	7	3
	Soil mix	3	0	0	0
	Water	0	-	-	-
	Controls	2	0	0	0
KNBG	Garden bed	43	22	28	5
	Soil mix	5	2	2	2
	Water	1	0	0	0
	Controls	3	0	0	0
SUBG	Garden bed	11	7	7	2
	Soil mix	1	1	1	1
	Water	3	3	4	4
	Controls	2	0	0	0

[1]KNBG: Kirstenbosch National Botanical Garden, Cape Town, HPNBG: Harold Porter National Botanical Garden, SUBG: Stellenbosch University Botanical Garden, CTCG: City of Cape Town Company Gardens.
+May be greater than number of positive samples where multiple *Phytophthora* species were recovered from the one sample
*Includes putative hybrids

unless symptoms were visible. In those cases, samples were cut from the advancing margin of the lesions. The tissue pieces were then plated onto NARPH (50mg Nystatin, 200mg Ampicillin, 10mg Rifampicin, 25mg PCNB, and 50mg Hymexezol per 1L DDH_2O and 15g corn meal agar), a medium selective for *Phytophthora* species (Hüberli et al. 2000). Pure cultures of *Phytophthora* were obtained by sub-culturing isolates and maintained on half-strength PDA (20g PDA and 8g agar per 1L DDH_2O) (Scott et al. 2009).

2.4. DNA extraction, amplification, sequencing and analysis

Isolates were grouped based on morphology and DNA was extracted from representative isolates using PrepMan Ultra ® sample preparation reagent or Zymo Research Extraction Kit. The regions spanning the Internal Transcribed Spacer (ITS) 1-5.8S-ITS2 region of the ribosomal DNA were amplified. Amplifications were completed using the ITS4 (White et al. 1990) and ITS6 or DC6 (Cooke et al. 2000) primers using thermal cycles described by Schena et al. (2008). Representative isolates were identified based on DNA sequences using Sanger sequencing.

Sequences were aligned and consensus sequences were compared to an internal dataset of ITS sequences collected from published sources using Geneious version 10.2.3. Blast searches were also made on GenBank to verify the identity of taxa. GenBank accession numbers for selected sequenced isolates are provided in Table 3. Phylogenetic analyses were also conducted in Geneious using maximum likelihood to compare sequences to known and informally described species in the same clades.

Isolates were identified as putative hybrids if the presence of sequence polymorphisms corresponded exactly to expected polymorphisms when comparing in the

sequences of the possible parents. The isolates were identified as putative hybrids when the nucleotides in the possible parents were different whenever there was an ambiguity in the sequence. For example, if the ambiguity was represented by a 'M', one possible parent would contain a 'C' and the other an 'A' at that position of the sequence. In such cases, we identified the isolates as putative hybrids because the polymorphism occurred at the same position as the difference between the two possible parents.

3. Results

3.1. Samples and isolations

A total of 103 samples were baited from the four gardens. Soils from forty-three garden beds and five media samples were baited from Kirstenbosch NBG, 25 garden beds and 3 media samples from Harold Porter BG, 11 garden beds and one soil mix sample from Stellenbosch University BG, and 11 garden beds from the City of Cape Town Company Gardens. In addition one water sample was baited from dam water that is used for irrigation at Kirstenbosch NBG and three water samples were baited at Stellenbosch University BG from filtered and unfiltered water sources, as well as a pond that accumulates water from a nearby river via an urban irrigation system (Table 1).

Phytophthora was recovered from 42 of the 90 sampled garden beds, three of the nine media and soil mix samples, and three of the four water samples. Multiple species were recovered from eight samples resulting in 56 total unique *Phytophthora* isolates. No *Phytophthora* were recovered from the controls.

3.2. Phytophthora taxa recovery

Phytophthora species were recovered from all four botanical gardens (Tables 1 & 2, Figure 2). These included eight formally described species, one known but not yet formally described species and three putative hybrids. Six species (*P. asparagi*, *P. capensis*, *P. cinnamomi*, *P. chlamydospora*, *P. multivora* and *P.* sp. emzansi) were recovered from Kirstenbosch NBG. Four species (*P. amnicola*, *P. cinnamomi*, *P. lacustris* and *P. multivora*) were recovered from Stellenbosch University BG. *Phytophthora cinnamomi* and *P. multivora* were recovered from Harold Porter NBG and *P. tropicalis* and *P. multivora* were recovered from the City of Cape Town Company Gardens.

Five isolates of three putative hybrids were recovered from the gardens. One putative hybrid (*P. pseudocryptogea*/*P. cryptogea*) was recovered from a rhizosphere sample near a seasonal stream in Harold Porter NBG. Neither of the possible parents were recovered from the garden. Two other putative hybrids (*P. amnicola/P. chlamydospora* and *P. hydropathical/P.* sp. maryland) were recovered from the pond water sample in Stellenbosch University BG. One possible parent species, *Phytophthora amnicola*, was also isolated from filtered river water used for irrigation in the same garden. *Phytophthora lacustris* was also recovered from unfiltered water in Stellenbosch University BG.

Phytophthora multivora was the most frequently recovered species (33 samples) followed by *P. cinnamomi* (12 samples; Figure 2). All other species were recovered infrequently, only from single samples, and only from one garden. *Phytophthora multivora* also had the widest distribution and was consistently recovered from the most

Table 2: *Phytophthora* recovery and sample distributions per botanical garden.

Taxon	Garden[1]	Garden bed samples	Soil mix samples	Water source samples
Phytophthora amnicola	SUBG	0	1	1
Phytophthora asparagi	KNBG	1	0	0
Phytophthora capensis	KNBG	1	0	0
Phytophthora chlamydospora	KNBG	0	1	0
Phytophthora cinnamomi	HPNBG	1	0	-
	KNBG	6	0	0
	SUBG	5	0	0
Phytophthora lacustris	SUBG	0	0	1
Phytophthora multivora	HPNBG	5	0	0
	KNBG	19	1	0
	SUBG	2	0	0
	CTCG	6	-	-
P. tropicalis	CTCG	1	0	0
P. sp. emzansi	KNBG	1	0	0
P. amnicola x *P. chlamydospora* hybrid	SUBG	0	0	1
P. hydropathica x P. sp. maryland hybrid	SUBG	0	0	1
P. pseudocryptagea x *P. cryptogea* hybrid	HPNBG	1	0	-

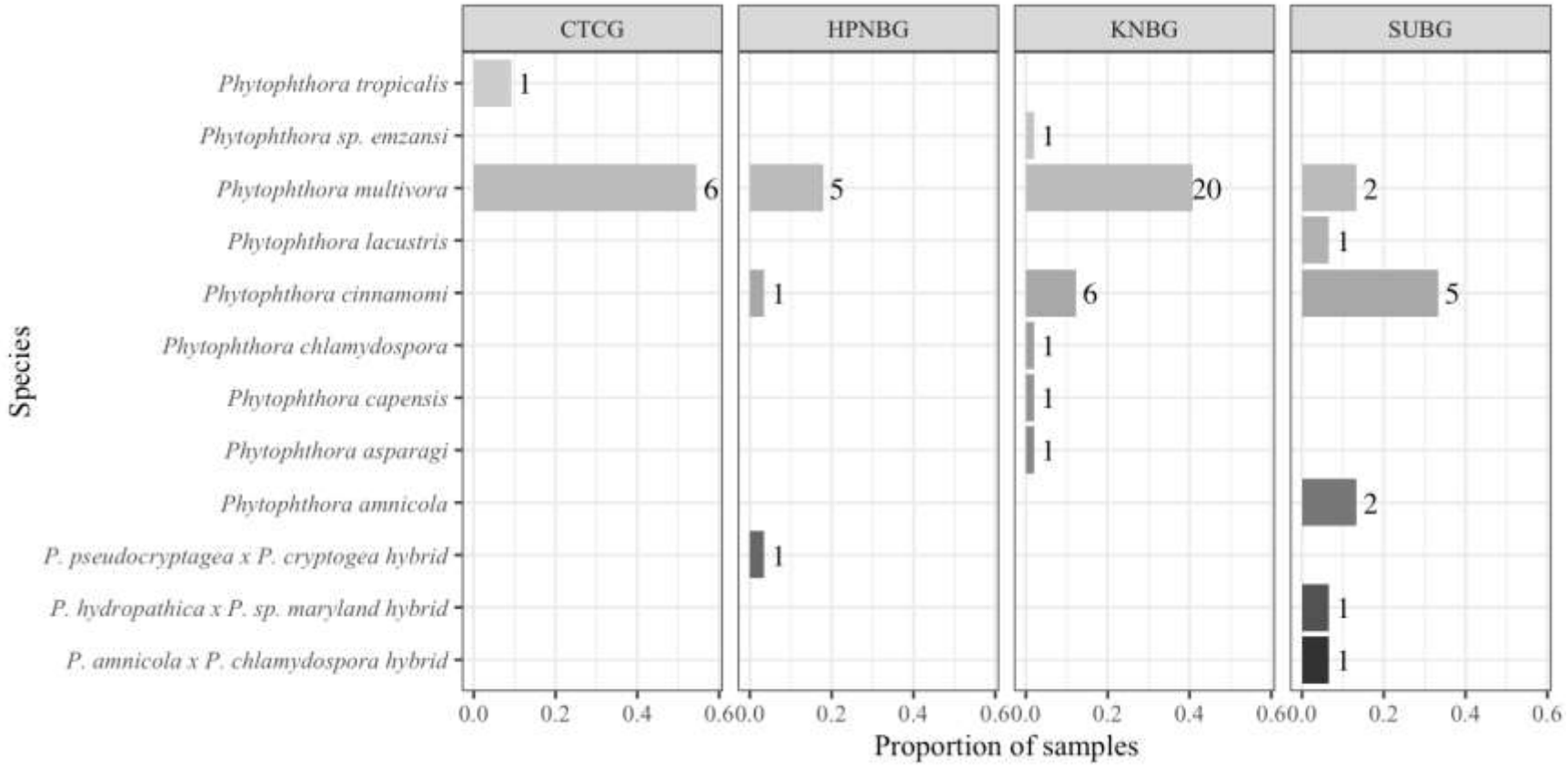
[1]KNBG: Kirstenbosch National Botanical Garden, HPNBG: Harold Porter National Botanical Garden, SUBG: Stellenbosch University Botanical Garden, CTCG: City of Cape Town Company Gardens.

Table 3: Representative Isolates and GenBank Accession Numbers

Taxon	Isolate Number[1]	Garden[2]	Accession Number
Phytophthora capensis	CMW54539	KNBG	MN545900
P. chlamydospora	SS0078*	KNBG	MN545902
P. multivora	CMW54538	KNBG	MN545899
P. cinnamomi	CMW50706	KNBG	MN545367
P. asparagi	CMW50710	KNBG	MN545892
P. sp. emzansi	CMW50975	KNBG	MN545898
P. pseudocryptagea /cryptogea hybrid	CMW50735	HPNBG	MN545897
*P. hydropathica /*sp. *maryland hybrid*	CMW50719	SUBG	MN545894
P. amnicola /chlamydospora hybrid	CMW50718	SUBG	MN545893
P. cinnamomi	CMW50730	HPNBG	MN545370
P. amnicola	CMW50726	SUBG	MN545896
P. lacustris	CMW50720	SUBG	MN545895
P. cinnamomi	CMW50722	SUBG	MN545368
P. multivora	CMW50727	SUBG	MN545369
P. tropicalis	CMW54658	CTGG	MN545901

[1] CMW = culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa
[2]KNBG: Kirstenbosch National Botanical Garden, HPNBG: Harold Porter National Botanical Garden, SUBG: Stellenbosch University Botanical Garden, CTCG: City of Cape Town Company Gardens. *The culture for Isolate SS0078 had died and could not be recovered from long term storage so the isolate is not available in the CMW culture collection.

Figure 2: Proportion of samples with *Phytophthora* recovery in each botanical garden.



Numbers indicate the number of samples where each species was recovered. CTCG: City of Cape Town Company Gardens, HPNBG: Harold Porter National Botanical Garden, KNBG: Kirstenbosch National Botanical Garden, SUBG: Stellenbosch University Botanical Garden.

sampling locations at all four gardens (Table 2). *Phytophthora cinnamomi* was found in three gardens (Kirstenbosch NBG, Stellenbosch University BG, and Harold Porter NBG). *Phytophthora multivora* and *P. cinnamomi* were recovered together in four garden beds in Kirstenbosch NBG, but both were found separately in other samples. *Phytophthora multivora* was recovered from underneath a declining *Sequoia gigantum* tree in Stellenbosch University BG. It was also recovered from the same garden bed samples as *P. asparagi* and *P. capensis* in Kirstenbosch NBG. *Phytophthora chlamydospora* (SS0078) was recovered from 'general mix' potting soil and *P. multivora* was recovered from 'fynbos mix' potting soil at Kirstenbosch NBG. *Phytophthora amnicola* (CMW50726) was recovered from a mixed soil sample at Stellenbosch University BG that included compost, soil from recently diseased plants, and soil under a nearby dripping fawcet of unfiltered river water. *Phytophthora tropicalis* (CMW54658) was recovered from the City of Cape Town Company Gardens in a garden bed that recently had many plants of unhealthy *Rosa* sp. *var* 'Lioness' planted. *Phytophthora multivora* was also recovered from the same sample collected from that garden bed.

4. Discussion

The surveys conducted in this study revealed the presence of three *Phytophthora* species (*P. amnicola*, *P. asparagi* and *P. tropicalis*) that had not been previously reported in South Africa. This result confirms our hypothesis that botanical gardens can be avenues for early detection of potential plant pests. It also emphasizes the value of botanic gardens as sentinel plantings and the opportunity they provide for research with sentinel plants (Wylie et al. 2008; Paap et al. 2017; Eschen et al. 2019). The discovery of *P. tropicalis* was of particular concern due to its wide host range and the many reports of

the pathogen on ornamental hosts (Orlikowski et al. 2006; Hao et al. 2010; Luongo et al. 2013). Similarly, *P. asparagi* was isolated from an Aloe specimen in the Melbourne Royal Botanic Gardens in Australia (Cunnington et al. 2005) and could pose a threat to South Africa's indigenous Aloe spp. This finding was also interesting because Aloe spp. reside in the the same Order as asparagus (Asparagales). In contrast, finding *P. amnicola* was not surprising because three previous studies have identified hybrid progeny indicating that it is common n Sout African rivers (Nagel et al. 2013, 2015; Oh et al. 2013).

Phytophthora multivora was the most commonly isolated species in this study. It was recovered from 33 samples and was present in all four botanical gardens. It was first described in Australia (Scott et al. 2009), where it is suspected to have been introduced (Burgess et al. 2017), possibly from South Africa. It has been recovered from various provinces in South Africa (Oh et al. 2013; Nagel et al. 2015), but little is known regarding its pathogenicity or ecology. In the current study, *Phytophthora multivora* was recovered under a declining *Sequoiadendron giganteum* tree in Stellenbosch University BG. While pathogenicity trials are required to determine if *P. multivora* poses a threat to this species, the finding illustrates the value of surveying botanical gardens and exotic species as sentinel plantings.

Phytophthora cinnamomi was the second most frequently recovered species in this study. Previous studies have identified it as the cause of Protea root rot, silver tree (*Leucadendron argenteum)* decline and stinkwood (*Ocotea bullata*) declines (van Wyk 1973; von Broembsen 1984; Lübbe and Mostert 1991). Based on current knowledge, this species is likely causing the most serious disease problem in the botanical gardens.

Stellenbosch University BG and Kirstenbosch NBG had high levels of diversity of *Phytophthora* species or putative hybrids. This is most likely because of their proximity to residential neighborhoods and the associated pressure from anthropogenic disturbance and sources of inoculum such as ornamental plantings. Stellenbosch University BG also utilizes river water, which enters the garden via the Mill stream, a municipality-controlled diversion of the Eerste River during the dry summer months. Two of Cooke's (2000) ITS Clade 6 species (*P. amnicola* and *P. lacustris*), one putative Clade 6 hybrid and one putative Clade 9 hybrid were recovered from the three sources of water used in the garden filtered and unfiltered river irrigation, and a pond connected to the Mill Stream. All of the water samples in the garden contained *Phytophthora*, indicating the importance of waterways as pathways into managed environments. *Phytophthora amnicola* was also recovered from a sample that included mud directly underneath an unfiltered water tap in this garden. Here, the sources of irrigation and the locality of the garden presented a unique opportunity for the detection of putatively novel hybrids and the first report of a *Phytophthora* species.

Both a putative hybrid and a possible parent species were identified in our surveys of Stellenbosch University BG. Intraspecific *Phytophthora* hybridization is known to occur when previously geographically isolated species come into contact, commonly occurring in water sources and the ornamental trade of plants (Brasier 2001; Yang et al. 2014; Nagel et al. 2015). An additional putative hybrid was recovered from Harold Porter NBG. Interestingly, the isolate CWM50735 was recovered from a rhizosphere sample, yet in close proximity to a small stream. Additional research is needed to reveal whether the putative hybrid is capable of infecting adjacent flora.

The majority of species found in this study are classified in the ITS Clade 6 of Cooke (2000). These included *P. lacustris, P. chlamydospora, P. asparagi, and P. amnicola*, plus one of the putative hybrids (CWM50718) (Cooke et al. 2000; Yang et al. 2017). The presence of *P. amnicola* and its putative hybrid progeny *P. amnicola x P. chlamydospora* (CWM50718) in the same garden suggests that botanical gardens could provide opportunities for hybridization to occur. As Brasier et al. (1999) have previously concluded, this could lead to accelerated pathogen evolution, and provides further support for monitoring phylogenies in botanical gardens. Therefore, botanic gardens should be monitored for the emergence of hybrids with novel traits because they may provide opportunities for hybridization.

This study has generated baseline data that can be used for further research to aid garden management and conservation. The discovery and first reports of the possible exotic *Phytophthora* species supports the merit of including botanical gardens in early warning systems such as the IPSN. The research has also presented an opportunity to engage and empower garden staff to take action to prevent the spread of microbes within and between gardens (e.g. boot washes, tool sterilization and propagation hygiene). Collectively, this study demonstrated that surveying botanical gardens can achieve the major objective of the IPSN to provide valuable information about plant health and raise awareness of garden staff (Barham et al. 2015).

5. Conclusions

The results of this study represent a baseline of data pertaining to the *Phytophthora* species diversity present within the WCP. Many plant species in the Greater Cape Floristic Region are endangered, but the effects of *Phytophthora* species on

these plants are largely unknown and require further research. The merit of the IPSN is demonstrated by the first reports generated in this research and our close collaboration with garden staff. This relationship has led to the detection and diagnosis of many additional plant health issues and highlights the benefits of building capacity in botanical gardens and enhancing biosecurity practice.

Acknowledgments

This project was made possible by public supporters that 'backed' our crowd funding campaign (DOI:10.18258/2066). This study also benefitted from support provided by the South African Department of Science and Technology Centre of Excellence in Tree Health Biotechnology and the South African National Department of Environment Affairs through the South African National Biodiversity Institute's Biological Invasions Directorate. We thank garden managers Mashudu Nndanduleni, Adam Harrower, Anthony Hitchcock, Jane Forrester, Karen Wall, Rory Phelan and Martin Smit for their support and interest. We also acknowledge Dr Tanay Bose, Ms Michelle Agne, Mr Vule Mukwevho and Dr Donghyeon Lee for their technical assistance.

Competing Interest Statement

The authors of this work have no competing interest to declare.

References

Barham E, Sharrock S, Lane C, Baker R (2016) The International Plant Sentinel Network: a tool for Regional and National Plant Protection Organizations. EPPO Bull 46:156–162. doi: 10.1111/epp.12283

Barham E, Sharrock S, Lane C, Baker R (2015) An International Plant Sentinel Network. Sibbaldia J Bot Gard Hortic 13:83–98

Born J, Linder HP, Desmet P (2006) The Greater Cape Floristic Region. J Biogeogr 34:147–162. doi: 10.1111/j.1365-2699.2006.01595.x

Brasier CM (2001) Rapid evolution of introduced plant pathogens via interspecific hybridization. BioScience 51:123–133. doi: 10.1641/0006-3568(2001)051[0123:REOIPP]2.0.CO;2

Brasier CM, Cooke DEL, Duncan JM (1999) Origin of a new *Phytophthora* pathogen through interspecific hybridization. Proc Natl Acad Sci 96:5878–5883. doi: 10.1073/pnas.96.10.5878

Britton K, White P, Kramer A, Hudler G (2010) A new approach to stopping the spread of invasive insects and pathogens: early detection and rapid response via a global network of sentinel plantings. N Z J For Sci 40:109–114

Burgess TI, White D, McDougall KM, et al (2017) Distribution and diversity of *Phytophthora* across Australia. Pac Conserv Biol. doi: 10.1071/PC16032

Cooke DEL, Drenth A, Duncan JM, et al (2000) A molecular phylogeny of *Phytophthora* and related Oomycetes. Fungal Genet Biol 30:17–32. doi: 10.1006/fgbi.2000.1202

Cowling RM, Pressey RL, Rouget M, Lombard AT (2003) A conservation plan for a global biodiversity hotspot—the Cape Floristic Region, South Africa. Biol Conserv 112:191–216. doi: 10.1016/S0006-3207(02)00425-1

Crous PW, Rong IH, Wood A, et al (2006) How many species of fungi are there at the tip of Africa? Stud Mycol 55:13–33. doi: 10.3114/sim.55.1.13

Cunnington JH, de Alwis S, Pascoe IG, Symes P (2005) The 'asparagus' *Phytophthora* infecting members of the Agavaceae at the Royal Botanic Gardens, Melbourne. Australas Plant Pathol 34:413–414. doi: 10.1071/AP05034

Eschen R, O'Hanlon R, Santini A, et al (2019) Safeguarding global plant health: the rise of sentinels. J Pest Sci 92:29–36. doi: 10.1007/s10340-018-1041-6

Goldblatt P (1997) Floristic diversity in the Cape Flora of South Africa. Biodivers Conserv 6:359–377. doi: 10.1023/A:1018360607299

Goss EM, Tabima JF, Cooke DEL, et al (2014) The Irish potato famine pathogen *Phytophthora infestans* originated in central Mexico rather than the Andes. Proc Natl Acad Sci 111:8791–8796. doi: 10.1073/pnas.1401884111

Hansen EM (2015) *Phytophthora* species emerging as pathogens of forest trees. Curr For Rep 1–9. doi: 10.1007/s40725-015-0007-7

Hansen EM, Reeser PW, Sutton W (2012) *Phytophthora* Beyond Agriculture. Annu Rev Phytopathol 50:359–378. doi: 10.1146/annurev-phyto-081211-172946

Hao W, Richardson PA, Hong CX (2010) Foliar blight of annual vinca (*Catharanthus roseus*) caused by *Phytophthora tropicalis* in Virginia. Plant Dis 94:274–274. doi: 10.1094/PDIS-94-2-0274A

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

Hulbert JM, Agne MC, Burgess TI, et al (2017) Urban environments provide opportunities for early detections of *Phytophthora* invasions. Biol Invasions 1–16. doi: 10.1007/s10530-017-1585-z

Hulme PE (2011) Addressing the threat to biodiversity from botanic gardens. Trends Ecol Evol 26:168–174. doi: 10.1016/j.tree.2011.01.005

Hurley BP, Garnas J, Wingfield MJ, et al (2016) Increasing numbers and intercontinental spread of invasive insects on eucalypts. Biol Invasions 18:921–933. doi: 10.1007/s10530-016-1081-x

Jung T, Pérez-Sierra A, Durán A, et al (2018) Canker and decline diseases caused by soil- and airborne *Phytophthora* species in forests and woodlands. Persoonia - Mol Phylogeny Evol Fungi. doi: 10.3767/persoonia.2018.40.08

Knox-Davies PS, van Wyk PS, Marasas WFO (1987) Diseases of *Protea*, *Leucospermum* and *Leucadendron* recorded in South Africa. Phytophylactica 19:327–338

Liebhold AM, Brockerhoff EG, Garrett LJ, et al (2012) Live plant imports: the major pathway for forest insect and pathogen invasions of the US. Front Ecol Environ 10:135–143. doi: 10.1890/110198

Lovett GM, Weiss M, Liebhold AM, et al (2016) Nonnative forest insects and pathogens in the United States: Impacts and policy options. Ecol Appl 26:1437–1455. doi: 10.1890/15-1176

Lübbe WA, Mostert GP (1991) Rate of *Ocotea bullata* decline in association with *Phytophthora cinnamomi* at three study sites in the Southern Cape indigenous forests. South Afr For J 159:17–24. doi: 10.1080/00382167.1991.9630390

Luongo L, Galli M, Vitale S, et al (2013) First Report of *Phytophthora tropicalis* on Rhododendron in Italy. Plant Dis 97:1385–1385. doi: 10.1094/PDIS-04-13-0445-PDN

Marincowitz S, Crous PW, Groenewald JZ, Wingfield MJ (2008) Microfungi occuring on'Proteaceae'in the Fynbos. CBS Fungal Biodiversity Centre

Nagel JH, Gryzenhout M, Slippers B, et al (2013) Characterization of *Phytophthora* hybrids from ITS clade 6 associated with riparian ecosystems in South Africa and Australia. Fungal Biol 117:329–347. doi: 10.1016/j.funbio.2013.03.004

Nagel JH, Slippers B, Wingfield MJ, Gryzenhout M (2015) Multiple *Phytophthora* species associated with a single riparian ecosystem in South Africa. Mycologia 13–380. doi: 10.3852/13-380

Oh E, Gryzenhout M, Wingfield BD, et al (2013) Surveys of soil and water reveal a goldmine of *Phytophthora* diversity in South African natural ecosystems. IMA Fungus 4:123–131. doi: 10.5598/imafungus.2013.04.01.12

Orlikowski LB, Trzewik A, Wiejacha K, Szkuta G (2006) *Phytophthora tropicalis*, a new pathogen of ornamental plants in Poland. J Plant Prot Res 46:103–109

Paap T, Beer ZW de, Migliorini D, et al (2018) The polyphagous shot hole borer (PSHB) and its fungal symbiont *Fusarium euwallaceae*: a new invasion in South Africa. Australas Plant Pathol 47:231–237. doi: 10.1007/s13313-018-0545-0

Paap T, Burgess TI, Wingfield MJ (2017) Urban trees: bridge-heads for forest pest invasions and sentinels for early detection. Biol Invasions 19:3515–3526. doi: 10.1007/s10530-017-1595-x

Packer JG, Meyerson LA, Richardson DM, et al (2017) Global networks for invasion science: benefits, challenges and guidelines. Biol Invasions 19:1081–1096. doi: 10.1007/s10530-016-1302-3

Pautasso M, Schlegel M, Holdenrieder O (2014) Forest Health in a Changing World. Microb Ecol 1–17. doi: 10.1007/s00248-014-0545-8

Pimentel D, McNair S, Janecka J, et al (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. Agric Ecosyst Environ 84:1–20. doi: 10.1016/S0167-8809(00)00178-X

Rizzo DM, Garbelotto M (2003) Sudden oak death: endangering California and Oregon forest ecosystems. Front Ecol Environ 1:197–204

Santini A, Ghelardini L, De Pace C, et al (2013) Biogeographical patterns and determinants of invasion by forest pathogens in Europe. New Phytol 197:238–250. doi: 10.1111/j.1469-8137.2012.04364.x

Schena L, Duncan JM, Cooke DEL (2008) Development and application of a PCR-based 'molecular tool box' for the identification of *Phytophthora* species damaging forests and natural ecosystems. Plant Pathol 57:64–75. doi: 10.1111/j.1365-3059.2007.01689.x

Scott PM, Burgess TI, Barber PA, et al (2009) *Phytophthora multivora sp. nov.*, a new species recovered from declining Eucalyptus, Banksia, Agonis and other plant species in Western Australia. Persoonia 22:1–13. doi: 10.3767/003158509X415450

Sena K, Crocker E, Vincelli P, Barton C (2018) *Phytophthora cinnamomi* as a driver of forest change: Implications for conservation and management. For Ecol Manag 409:799–807. doi: 10.1016/j.foreco.2017.12.022

Shearer BL, Crane CE, Cochrane A (2004) Quantification of the susceptibility of the native flora of the South-West Botanical Province, Western Australia, to *Phytophthora cinnamomi*. Aust J Bot 52:435. doi: 10.1071/BT03131

van der Merwe JJH, van Wyk PS (1973) Hosts of *Phytophthora cinnamomi* in the Western Cape Province of South Africa. Plant Dis Report 57:1005–1006

van Wyk PS (1973) Root and crown rot of silver trees. J South Afr Bot 39:255–260

von Broembsen S (1984) Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa, with special reference to the south-western Cape Province. Phytophylactica

Waipara NW, Hill S, Hill LMW, et al (2013) Surveillance methods to determine tree health, distribution of kauri dieback disease and associated pathogens. N Z Plant Prot 66:235–241

Webber J (2010) Pest Risk Analysis and Invasion Pathways for Plant Pathogens. N Z J For Sci 12

Weste G, Marks GC (1987) The Biology of *Phytophthora cinnamomi* in Australasian Forests. Annu Rev Phytopathol 25:207–229. doi: 10.1146/annurev.py.25.090187.001231

Weste G, Taylor P (1971) The invasion of native forest by *Phytophthora cinnamomi*. in Brisbane Ranges, Victoria. Aust J Bot 19:281–294

White TJ, Bruns T, Lee S, et al (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc Guide Methods Appl 18:315–322

Wylie FR, Griffiths M, King J (2008) Development of hazard site surveillance programs for forest invasive species: a case study from Brisbane, Australia. Aust For 71:229–235. doi: 10.1080/00049158.2008.10675040

Yang X, Richardson PA, Hong C (2014) *Phytophthora ×stagnum nothosp. nov.*, a New Hybrid from Irrigation Reservoirs at Ornamental Plant Nurseries in Virginia. PLoS ONE 9:e103450. doi: 10.1371/journal.pone.0103450

Yang X, Tyler BM, Hong C (2017) An expanded phylogeny for the genus *Phytophthora*. IMA Fungus 8:355–398. doi: 10.5598/imafungus.2017.08.02.09