PHYTOPHTHORA DIVERSITY IN THE CAPE FLORISTIC REGION

by

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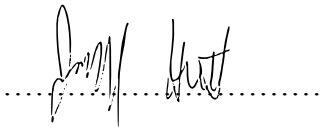
DECLARATION

I, the undersigned, hereby declare that the thesis herewith submitted for the degree *Philosophiae Doctor* to the University of Pretoria contains my own independent work.

This work has not been submitted for any degree at any other University.



Joseph Michael Hulbert

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I dedicate this thesis, the product of my time in South Africa, to those I have sacrificed the most time away from while abroad: my wife, parents and sisters. While I resent the time away and the day-to-day events I have missed in their lives, I have grown in heart and the experience has cultivated me into a better husband, son and brother. Now, I look forward to turning the page from this chapter of growth and exploration in South Africa to a new chapter of homecoming as a better person and family member.

TABLE OF CONTENTS

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PREFACE

The anthropogenic movement of *Phytophthora* species threatens the bioeconomy and biodiversity of nations throughout the world. Investing in biosecurity is consequently critical, especially in countries that contain hotspots biodiversity. However, countries such as South Africa face greater challenges for monitoring and managing *Phytophthora* invasions because of their developing economies. Therefore, the purpose of the work undertaken in this thesis was to reveal the diversity and distribution of *Phytophthora* species present within the Cape Floristic Region. Furthermore, to investigate the potential merit of engaging the public as a reduced-cost approach to enhance the biosecurity of countries like South Africa.

The content of this thesis is divided into a literature review and three research chapters. The primary content of each chapter pertains to *Phytophthora*, but the discussion varies between ecology and biosecurity. Overall, the aim of this collection of work was to reveal the potential threats of *Phytophthora* species from a conservation perspective and experiment with methods of public engagement to increase the breadth of our findings and simultaneously explore opportunities for education.

Discussions of the merit of citizen science for enhancing biosecurity are provided throughout the body of this work. But the citizen science component of the project was not part of each chapter. An introduction section has also been included because the literature review does not fully introduce or connect the entire body of work. Furthermore, the content in the Appendix is either relevant to the engagement activities offered through Cape Citizen Science or it demonstrates the leadership of the Author within the citizen science community in South Africa. At the time of submission of this thesis for examination, the Introduction Chapter had been submitted for consideration as a short review, Chapters 1 and 2 were published, Chapter 3 had been submitted for consideration and Chapter 4 was being refined for submission.

Chapter 1 is a summary of *Phytophthora* species descriptions and the literature relevant to *Phytophthora* disease epidemics emerging from the urban environment. The purpose of this manuscript was to highlight the importance of monitoring the urban

environment in the context of *Phytophthora* invasions and was presented and published in a special issue of *Biological Invasions*.

Chapter 2 follows from the literature review in Chapter 1 and summarizes the findings of our research conducted in four botanical gardens in South Africa. *Phytophthora* diversity was estimated by baiting rhizosphere samples collected with botanical garden staff. The purpose of this study was to demonstrate the value of surveying botanical gardens for early detection and baseline diversity data generation. This work has been published in the journal *Urban Forestry and Urban Greening*.

Chapter 3 presents an analysis of the association between three *Phytophthora* species and the health of five tree species in the southern Afrotemperate forests of South Africa. The relationships between the presence of *Phytophthora* species, the plant communities and the health of the tree species were evaluated using multivariate community analysis and generalized linear mixed models, respectively. The purpose of this study was to distinguish between the associations of the plant communities with an introduced species and a putatively native species of *Phytophthora*.

Chapter 4 presents the results of the public engagement in Cape Citizen Science. The *Phytophthora* diversity data are summarized based on the type of engagement and other qualitative information to provide recommendations for methods of engagement in similar programs. The purpose of this chapter was to demonstrate the merit of citizens science programs to enhance biosecurity in economically developing countries.

SUMMARY

The biodiversity in the Cape Floristic Region of South Africa faces many threats from anthropogenic sources such as the trade associated with our globalized economy. Aggressive plant pathogens in the genus *Phytophthora* are of particular concern because of their capacity to invade and change plant communities and their frequent dissemination via global trade. The fact that South Africa has a developing economy with many socioeconomic challenges, the capacity to monitor the abundance of plants imported for pests and pathogens is largely inadequate. Consequently, low cost methods to enhance post-border surveillance for the emergence of *Phytophthora* species are critically needed in South Africa. Therefore, the major aims of the research conducted in this thesis were to: 1) reveal the diversity and potential threats of *Phytophthora* species already present within the Cape Floristic Region, and 2) provide information and an example of an approach to enhance the biosecurity with public engagement in South Africa. Through independent sampling and a newly developed citizen science program, this body of work revealed the presence of seventeen described *Phytophthora* species, one informally described *Phytophthora* species, and three putative hybrids. Seven of these *Phytophthora* species were not previously known to occur in South Africa. The work also revealed a relationship between the invasion of *P. cinnamomi* and the health of trees and evidence is provided for dissimilarity between invaded and non-invaded plant communities. In addition, through a synthesis of modern *Phytophthora* species descriptions and a diversity study in botanical gardens, it was possible to provide evidence for the importance of surveying urban environments. Then as a means to demonstrate the potential of public engagement to enhance biosecurity, the findings from activities in Cape Citizen Science have been summarized. In this case it was possible to show that nine of the *Phytophthora* species were recovered only because of citizen participation. Cumulatively, this thesis has advanced the base of knowledge regarding the presence and consequences of *Phytophthora* species in the Cape Floristic Region. In this sense, they also provide valuable information and a model system to enhance biosecurity in South Africa as well as in countries with similar economies.

INTRODUCTION

Alleviating the threats of *Phytophthora* in South Africa with citizen science

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Abstract

The biodiversity in South Africa faces many threats from anthropogenic sources such as our globalized economy. New approaches are needed to increase the biosecurity capacity of the country to prevent and manage invasive species introductions. *Phytophthora* species are of particular concern because of their ability to invade and change ecosystems, but not much is known about the diversity or threats of these species in natural environments of South Africa. Given the lack of knowledge and capacity to monitor and manage these species in South Africa, a citizen science program to monitor *Phytophthora* species would be particularly valuable. Citizen science programs can simultaneously increase levels of biosurvellience and provide important baseline information. Therefore, encouraging citizen science programs is a promising approach to enhance the biosecurity in South Africa.

Biodiversity conservation in South Africa

South Africa is home to two of the 25 most important biodiversity hotspots required to conserve on the planet (Myers et al. 2000), the Cape Floristic Region (CFR) and the Succulent Karoo. The CFR is the richest based on its size (Goldblatt 1997, Goldblatt and Manning 2002, Born et al. 2006, Schnitzler et al. 2011) and therefore arguably the "hottest" hotspot in the world. For example, the region accounts for only 4% of the total land area of South Africa, but contains 42% of the vascular plant species in the country (Goldblatt 1997). Furthermore, 65% of the species in the CFR are endemic (Rebelo and Siegfried 1990, Goldblatt 1997) with most of the diversity (70%) belonging to a single biome referred to as the Fynbos (Rebelo and Siegfried 1990). These unique characteristics are critical to protect and they provide unmatched opportunities to study organismal biodiversity.

There have been many calls to conserve the biodiversity of the CFR (Rebelo and Siegfried 1990, McDonald and Cowling 1995, Milton et al. 1999, Cowling et al. 2003, Schurr et al. 2012), but the system still faces many threats (Richardson et al. 1996, Rouget et al. 2003, Latimer et al. 2004). Some of the most important present-day threats to the fynbos include urbanization and cultivation for intensive agriculture (Rebelo and Siegfried 1990, Cowling et al. 2003, Rouget et al. 2003), climate change (Richardson et

al. 1996, Midgley et al. 2002, 2003, Rouget et al. 2003, Williams et al. 2005), invasive plants (Richardson et al. 1989, Holmes and Cowling 1997, Richardson 1998, Yelenik et al. 2004), and invasive plant pathogens and pests (von Broembsen and Brits 1986, Linde et al. 1997, Jacobsen et al. 2012). In summary, there are many emerging threats to the biodiversity of the CFR and research to advance our understanding of the drivers and consequences are needed now more than ever before.

Threats to the biodiversity of South Africa from invasive species are especially acute because the country has a developing and emerging economy (WEO 2019). Nuñez and Pauchard (2009) noted that countries with lower economic development face greater challenges to control invasive species because they have fewer resources, smaller pools of educated volunteers and even smaller scientific communities. Therefore, methods to add capacity to monitor and detect invasive species are especially needed in South Africa.

Biosecurity in South Africa

The prevention and management of invasive species follow the same general biosecurity principles and frameworks, but new approaches are needed. In general, countries can invest in actions to reduce the effects of biological invasions through pre- and post-border strategies (Leung et al. 2002, Pyšek and Richardson 2010, Epanchin-Niell 2017, Carnegie and Nahrung 2019). Pre-border strategies can include risk assessments, including sentinel plantings, and regulations on pathways such as Phytosanitary Measure 15 (Haack et al. 2014, Barham 2016, Eschen et al. 2019). However, pre-border biosecurity measures are especially limited in preventing invasions of plant pathogens because of the overwhelming number of unknown species, the complexity in taxonomy and genetic diversity, and the frequent exchange of genetic material between species (McTaggart et al. 2016). Furthermore, once an organism has made it past the borders, options to reduce the effects are limited by the ability to detect it and react rapidly (Westbrooks et al. 2014, Carnegie and Nahrung 2019, Hansen et al. 2019). Therefore, initiatives that increase monitoring capacity and promote the early detection of novel epidemics or outbreaks are important components of biosecurity frameworks.

The strength of plant biosecurity measures in South Africa is unclear. Measures related to plant health are implemented and overseen by the Department of Agriculture, Forestry and Fisheries (DAFF), which is mandated by the Agricultural Pests Act (Act 36) from 1983. However, the policies and guidelines followed for plant biosecurity measures are unclear (ASSAf 2015 p. 49). In addition, although a Plant Health (Phytosanitary) Bill was drafted to enhance phytosanitary measures in 2012 (Joemat-Pettersson 2012) and subsequently introduced to the National Assembly multiple times, version B7-2017 was withdrawn on May 16, 2018 (PMG 2019) and has not been enacted. Furthermore, a recent report produced by the Academy of Science South Africa noted deficiencies for implementing the current legislation by highlighting the "inadequate infrastructure and capacity, in particular the capacity to inspect facilities and identify plants at border control points" (ASSAf 2015 p. 40). Therefore, approaches to enhance biosecurity in South Africa are critically needed.

The trade of living plants for planting is well known as major pathway of invasive pests and pathogens (Brasier 2008, Aukema et al. 2010, Liebhold et al. 2012). While the capacity of South Africa to implement legislation at its borders is questionable (ASSAf 2015 p. 40), the country generally requires phytosanitary certificates, import permits, and conducts import inspections for living plants imported for planting (Eschen et al. 2015). However, according to the South African Revenue Service, the country imported 'live trees and other plants' (Customs and Excise Tariff Chapter 6 commodities) into 20 district offices in 2018 (SARS 2019) and DAFF only reported inspection expenditures of R377,556,000 in their fiscal 2017/2018 report (DAFF 2018). Therefore, assuming each port received equal funding for inspections, each office had an annual budget of close to R19,000,000 (~$1,275,000 USD) to conduct inspections, pay staff, and run facilities in 2018. This demonstrates the economic challenges that South Africa faces for preventing the introductions of invasive pests and pathogens.

Phytophthora in the Cape Floristic Region

Of particular concern to the bioeconomy and biodiversity of South Africa are *Phytophthora* species. The consequences of their introductions have displaced populations of people by causing disease in staple crops such as potatoes in Ireland

(Turner 2005), driven irreversible change in plant communities (Weste et al. 2002, Shearer et al. 2007), killed millions of trees (Rizzo et al. 2002) and added substantial annual financial costs to food production (Guenthner et al. 2001). In addition, many *Phytophthora* species have spread throughout much of the world (Grünwald et al. 2012, Burgess et al. 2016). Together the effects and their potential to spread demonstrate the need to study *Phytophthora* species and limit their destructive capacity.

The negative effects of *Phytophthora* species in agricultural, horticultural and plantation forestry productions have been comprehensively reviewed in South Africa (Nagel et al. 2013), but not much is known about their effects in the country's natural ecosystems such as the Cape Floristic Region. One exception is the role of *Phytophthora cinnamomi* as the cause of protea root rot in the fynbos biome (van Wyk 1973, van der Merwe and van Wyk 1973, von Broembsen 1984, von Broembsen and Kruger 1985). *Protea* species are also important horticultural species so there was also an abundance of research conducted to control the pathogen during that period of study (von Broembsen 1985, von Broembsen and Brits 1986, 1990). But at that time it was presumed to be indigenous because of the presence of both mating types, its occurrence in pristine environments in disassociation with roads or foot paths, and the evidence of resistance in some plants in the region (von Broembsen 1984, von Broembsen and Kruger 1985, Zentmyer 1985). However, it was later demonstrated that *P. cinnamomi* had been introduced into South Africa (Linde et al. 1999), but its effects on the native flora have not since been documented or monitored. Knowledge of other *Phytophthora* species present in the Cape Floristic Region is seldom, but Bezuidenhout et al. (2010) identified *P. multivora*, *P. parvispora*, and the informally described species *P. sp.* emzansi from isolates collected from a cultivated indigenous species in region. However, it is unclear if they were introduced via pathways within the agricultural practices or whether they were previously present and only emerged as problems because the land conversion cultivated favourable conditions. Further research regarding the diversity and roles of *Phytophthora* in the fynbos biome are needed.

A similar history and pattern of research has occurred in the forest biome of the Cape Floristic Region. Again, *P. cinnamomi* was identified as the driver of the decline of *Ocotea bullata* in the southern Afrotemperate forests in the mid 1980s (Lübbe and

Geldenhuys 1990, Lübbe and Mostert 1991), but research about other *Phytophthora* species in this biome is limited. Oh et al. (2013) isolated *P. multivora* from a dying *Rapanea melanophloeos* tree in the Harold Porter National Botanical Garden and Bezuidenhout et al. (2010) characterized *P. capensis* from isolates collected from another tree species indigenous to southern Afrotemperate forests. Together these studies indicate there are possibly three species affecting trees in the forest biome of the Cape Floristic Region, but again, more research is needed to understand their roles, threats, or effects and infer if the region could be a possible centre of origin.

Our understanding of the roles of *Phytophthora* species in their natural environment is poorly understood (Hansen 1999). Although *Phytophthora* species emerge as destructive agents of change when introduced to naïve environments, they are generally benign in their native range because they have co-evolved with the associated plant community (Hansen 2008, Brasier 2012). Some species have been suggested to complete their entire lifecycle as saprophytes, contributing to ecosystems as natural recyclers (Marano et al. 2016), while other species may be opportunists in a race to overcome plant defences or equalize competitive advantages (Hansen 1999, Reeser et al. 2011). Nevertheless, studying *Phytophthora* species in their native range provides important opportunities to infer species distributions and origins, understand genetic variation and evolution, identify potential biological controls, assessing host species resistance, and to predict the effects of changing environments (Zentmyer 1985, 1988, Stukenbrock and McDonald 2008, Goss et al. 2014).

Conditions where *Phytophthora* species become problematic arise all too frequently due to the substantial and growing global trade in living plants (Brasier 2008, Jung et al. 2016, Simamora et al. 2017). In addition, even plants produced for restoration activities have aided the movement of *Phytophthora* species into natural systems (Rooney-Latham et al. 2018). It is therefore critical to monitor the international and domestic movement of plants for the emergence of novel disease epidemics.

Managing Phytophthora invasions

Many plant disease epidemics caused by *Phytophthora* have originated from a common pathway, the global trade of plants for planting. Evidence for the threat of this

pathway has been documented in many regions of the world (Brasier 2012, Bienapfl and Balci 2013, Jung et al. 2016, Simamora et al. 2017), and even asymptomatic plants can carry disease initiating propagules (Shishkoff 2007, Migliorini et al. 2015), which is exacerbated by the use of suppressive fungicides. Therefore, countries that import plants for planting consequently risk the introduction of *Phytophthora* species and should incorporate precautions around the pathway.

An effective means to protect biodiversity from *Phytophthora* species would be to adopt the Montesclaros Declaration (IUFRO Division 7.00.00 2011, http://www.iufro.org/science/divisions/division-7/70000/publications/montesclaros-declaration/) which calls for a halt to all international trade of living plants (Hansen 2008, Brasier 2008, Liebhold et al. 2012). However, this is commonly regarded as impractical and biosecurity agencies continue to enable trade between geographically separated areas (Hulme 2009, Santini et al. 2013). Furthermore, the industries involved in the plant trade continue to grow despite the consequential threats that already outweigh our capacity to monitor and control global borders (Liebhold et al. 2012). Therefore, methods to enhance monitoring efforts post-border are consequently greatly needed.

Monitoring urban environments

Monitoring for post-border introductions is a difficult and expensive task compared to monitoring pathways. However, there are specific post-border areas that can be prioritized to increase efficiency, such as ports-of-entry and amenity plantings (Carnegie and Nahrung 2019). In general, many of the green spaces and ornamental industries in urban environments may provide opportunities to detect an invasion prior to its escape into the natural environment (Paap et al. 2017). In this regard, programs that target these areas for monitoring may provide the best chance for the control or elimination of post-border detections.

There is specific merit in monitoring the urban environments for the emergence of plant pathogens such as *Phytophthora* species. For example, many *Phytophthora* species have been described based on type specimens recovered from urban environments (Hulbert et al. 2017) and surveillance in areas such as botanical gardens and urban forests have revealed high levels of diversity (Barber et al. 2013, Riddell et al. 2019, Hulbert et

al. 2019). It is also ideal to monitor these areas because of the presence of ornamental plant trading industries and consumers. Therefore, it is also critical to incorporate caution within the international plant trade and closely monitor areas of the urban environment (e.g. major ports or areas new ornamental plant nurseries) to detect the post-border emergence of *Phytophthora* species.

Monitoring with citizen science

Monitoring for the emergence of novel disease outbreaks in post-border scenarios could be enhanced through the participation from non-scientists as citizen scientists (Thomas et al. 2017, Baker et al. 2018). Citizen science is a term used to denote research that involves the public and artisans in the collection of data. This approach has substantial merit for monitoring invasive species because of the inclusion of large numbers of people and consequently increased monitoring coverage at relatively low cost (Meentemeyer et al. 2015, Bates et al. 2015, Thomas et al. 2017).

There are many initiatives that engage citizens in invasive species monitoring globally. For example, more than 150 projects were listed online in 2017 (Hulbert 2017). However, most of these projects focus on invasive aquatic species, plants, or mammals. There are fewer citizen science programs focused on monitoring plant pests or pathogens, but many projects are emerging to monitor threats to agricultural commodities and natural ecosystems because of growing recognition of the added capacity at relatively low-costs (Ryan et al. 2018). Specifically engaging stakeholders in plant related industries has also been suggested as a means to raise awareness and enhance biosurvellience (Marzano et al. 2016).

Although these programs generally share in the objectives to recruit ‘many eyes’ (Thomas et al. 2017), the methods and approaches differ. For example, some programs offer in-person training or workshops, which can increase data quality and educational outcomes in invasive species monitoring projects (Gardiner et al. 2012), others offer training resources online (Newman et al. 2010), and some invite contributions and participation without training. Nonetheless, the level of training provided or required in a citizen science program represents just one example of how the programs differ. Other examples of major differences could be whether a program accepts physical samples or

verifies observations shared online, recruits artisans or the general public, provides targeted areas for collection or allows collection from backyards. Therefore, although many citizen science projects focused on invasive species exist, the best methods or frameworks to enhance biosecurity with citizen science continues to be explored.

Citizen science and Phytophthora

Globally there are at least five active citizen science programs designed to facilitate research concerning *Phytophthora* species. Cape Citizen Science (http://citsci.co.za) and *Phytophthora*-Citizen Science (http://phytophthora.se/) are programs to survey the diversity of *Phytophthora* species, while the Sudden Oak Death (SOD) Blitz (https://nature.berkeley.edu/garbelottowp/) and Oak Mapper (http://www.oakmapper.org/) programs monitor for known invasive species or specific disease hosts. Similarly, the *Phytophthora* Stream Monitoring Program (https://ppo.puyallup.wsu.edu/sod/monitoring/streams/) was designed to reveal the diversity of *Phytophthora* and monitor for *P. ramorum,* but it focuses on sampling waterways. The Kauri Rescue program (http://kaurirescue.org.nz/) is distinct from other such programs because it provides kits for citizens to test different methods of treating *P. agathidicida*-infected trees on their properties. Cumulatively, these programs illustrate the diversity of approaches and rationale to engage citizens in *Phytophthora* research, but the number of active programs demonstrates there must be merit to engaging the public.

Cape Citizen Science

The pilot project of Cape Citizen Science (https://citsci.co.za/) was initiated to facilitate research regarding the diversity and distribution of *Phytophthora* species in two biomes of the CFR: the fynbos and the southern Afrotemperate forest (See Appendix). Since its establishment, the program has grown to host information and resources for other projects relevant to plant health to remain relevant over a longer period, such as the outbreak of the polyphagous shot hole borer (Paap et al. 2018). Overall, the program has demonstrated there are communities and research needs in South Africa that can be integrated through the citizen science approach.

Conclusions

South Africa hosts exceptional centres of biodiversity that are critical to protect. However, the country faces many unique challenges for preventing and managing invasive species because of its limited economic development and generally underfunded biosecurity capacity. Therefore, methods to enhance the biosecurity are critically needed. Furthermore, because of the clear threat of *Phytophthora* species to the biodiversity and bioeconomy of South Africa, as well as the general deficiency in knowledge about the diversity or effects of *Phytophthora* species within the Cape Floristic Region, more research is needed to provide baseline information. Therefore, because citizen science can enhance biosecurity at relatively low costs by increasing sampling coverage while simultaneously raising awareness and empowering citizens to participate in biosurveillance, the continued support for programs like Cape Citizen would be particularly valuable in South Africa.

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CHAPTER ONE

Urban environments provide opportunities for early detections of *Phytophthora* invasions

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Abstract

Globalization has increased the frequency of inadvertent introductions of plant pathogens. Many catastrophic invasions of both natural and agricultural systems have been initiated through anthropogenic dissemination pathways. *Phytophthora* species are a group of invasive plant pathogens causing many of the most important plant disease epidemics. A review of *Phytophthor*a species descriptions published following the publication of the first DNA-based *Phytophthora* phylogeny was conducted to highlight patterns of recent introductions and to provide insights for early pathogen detection initiatives. Seventy-two publications from 2001-2016 describing 98 *Phytophthora* species were evaluated. Of the 91 species with geographic location isolation data, 22% of species were described from type specimens isolated from urban environments, 33% from agricultural environments and 45% from natural environments. Within the urban environment, ornamental plant trading nurseries were the most important sources. Specifically, for *Phytophthora ramorum*, a species causing multiple epidemics globally, the largest proportion of first report publications were from urban environments, including nurseries. Nearly a quarter of the species descriptions were based on isolates from the urban environment, including ornamental nurseries, and the majority of the first reports for *P. ramorum* were also from this environment. We therefore suggest that detection programs for invasive plant pathogens within the urban environment would be valuable. In this regard, specialized monitoring and citizen science projects that target urban areas where live plant-trading industries are concentrated would be particularly effective to both promote early detection and to facilitate a rapid response to new species invasions.

Introduction

Natural environments are becoming increasingly homogenized due to globalization and the anthropogenic movement of species. The spread of invasive plant-pathogenic organisms such as *Phytophthora* species (Oomycetes) is a global concern for nature conservation because of epidemics such as sudden oak death (USA), ramorum blight (UK), *Phytophthora* dieback (AUS) and protea root rot (RSA). *Phytophthora cinnamomi*, for example, has been described as one of the most destructive plant

pathogens in the world (Brasier 1996, Burgess et al. 2016), metaphorically referred to as a 'biological bulldozer' in Australia (Hardy et al. 2007, Scott et al. 2013). It is thus included in the International Union for the Conservation of Nature (ICUN) list "100 of the World's Worst Invasive Alien Species" (Lowe et al. 2000).

Improving methods to control invasive species is important for several reasons. Invasive species threaten the biodiversity of natural systems (Vitousek et al. 1996, Crowl et al. 2008, Gaertner et al. 2009), and consequently the functions of and services provided by the ecosystems, ultimately affecting human health (Pejchar and Mooney 2009, Pyšek and Richardson 2010, van Wilgen et al. 2012, Donovan et al. 2013). Biodiversity is suggested to be the world's greatest resource (Wilson 1989) and is essential to maintain ecosystem functions and services (Mace et al. 2012). Controlling invasive species is important because they have been shown to drive biodiversity loss (Wilson 1989, Vitousek et al. 1996) and degrade ecosystems (Pyšek and Richardson 2010, Hooper et al. 2012).

Microscopic invasive organisms such as plant pathogens provide serious challenges when attempting to manage invasions. This is partly because there are large numbers of undiscovered and undescribed species that cannot be controlled with current regulatory approaches and biosecurity practices based on taxonomy (Brasier 2008, McTaggart et al. 2016, Crous et al. 2016). Many biosecurity programs use biogeographic information to focus their monitoring efforts, but even for known invasive species, the distribution data are often incomplete (Scott et al. 2013). For example, the origins of many *Phytophthora* species remain unknown. Furthermore, there are many different pathways of movement (e.g. nursery plant trade, wood packaging material, residual soil on equipment), each with their own management complications.

Countries actively managing invasive species have limited options for investing time and energy, and the available options are highly dependent on the invasion stage of the organism. These investments can be made in several management strategies, including: prevention, monitoring and detection, eradication, containment (treatment or management to slow the spread of species), mitigation, or restoration. Of these strategies, prevention has been widely described as the investment that is most economical and most likely to be successful (Leung et al. 2002, Chornesky et al. 2005, Hulme 2006, 2009,

Hansen 2015, Wingfield et al. 2015, Faulkner et al. 2015). However, for already introduced organisms, the first steps in addressing the problem are detection followed by monitoring (Pyšek and Richardson 2010).

Early detection and monitoring of invasive species requires many trained observers. Citizen science initiatives provide new quantitative approaches to investigate the distribution and abundance of organisms across space and time with minimal costs (Bonney et al. 2009, Dickinson et al. 2010, Gallo and Waitt 2011). Such initiatives have been used for the early detection and monitoring of invasive species (Crall et al. 2015) and are suitable for surveying *Phytophthora* species (Meentemeyer et al. 2015), possibly benefiting resource-limited agencies and economies.

To determine priorities for invasive plant pathogen early detection and monitoring efforts, we reviewed recent published studies that describe *Phytophthora* species in order to identify the environment and the economic status of the country in which each species was isolated. We further reviewed first reports of *P. ramorum* to identify the types of environments in which this pathogen has been found throughout its distribution. Finally, we summarized four citizen science projects that facilitate *Phytophthora* research as examples of programs that could be implemented to increase our understanding of the effects of *Phytophthora* species and our ability to manage their invasions. Comprehensively, this review presents a partial summary of recent *Phytophthora* species surveys and provides justification to survey urban environments while proposing citizen science as an ideal tool.

Review of Phytophthora species described after 2001

This review is comprised of all studies between 2001 and 2016 that include descriptions of *Phytophthora* species. We chose to limit the review to the period following the publication of the first ITS-based phylogeny for *Phytophthora* by Cooke et al. (2000) in order to limit inaccuracies in descriptions based on morphology and other less quantitative characters. Numerous species have been described during this period because of increased numbers of *Phytophthora* surveys and several species have been reclassified due to the improved tools available for identification of species (Scott et al. 2013).

Data were based on type specimens that were used to describe the *Phytophthora* species primarily because this ensured accuracy of species identity. Furthermore, type specimens are typically the first isolates collected for a given species. Where "type" information was not provided in a publication, locality and environmental data were compiled from descriptions of disease occurrence and habitat. In a few cases, the authors acknowledged that the type specimen was not the first isolate collected (Hansen et al. 2009) or that an isolate had been collected previously at a different location and had been included to complete the description (Bertier et al. 2013). In the case where holotype specimens had not yet been designated (e.g. *Phytophthora* taxon parsley, *Phytophthora* taxon castitis), geographic information was based on the first isolate collected (Bertier et al. 2013). In some cases, additional information was compiled by using documented NCBI GenBank (https://www.ncbi.nlm.nih.gov/) or CBS accession numbers (http://www.westerdijkinstitute.nl/Collections/).

We treated *Phytophthora* species having type specimens recovered from planted forests of exotic tree species as isolations from agricultural environments because they are intensively managed. Type specimens recovered from ornamental plant nurseries and garden centers were considered to be from urban environments. Conversely, we classified isolations from plant production nurseries as isolations from agricultural environments because they were producing stock for restoration of native species or reforestation of exotic species. Although some ornamental nurseries may occur in rural areas, we chose to classify these as urban environments for several reasons. We assume that the majority of plant trading nurseries and garden centers are within or close to urban environments, and that ornamental plants are most likely to be purchased for planting in urban and residential areas, rather than natural or agricultural environments. Furthermore, the majority of species description manuscripts do not provide enough detail to distinguish the locality of the nurseries from which the isolates were recovered. Further review of the species descriptions from urban environments was conducted to identify whether species had subsequently been isolated from natural environments. When species descriptions did not state whether a species had also been detected in natural environments, a literature search was conducted to further verify whether additional detections occurred later.

A comprehensive review of the literature yielded 73 publications describing 98 *Phytophthora* species. Fifteen species in these publications were taxonomic revisions or reclassifications of previously informally described species, while five species had only "informal" descriptions (e.g. *Phytophthora* taxon parsley). Geographic location data associated with the isolation of the type specimen were provided in the descriptions for 91 species between 2001-2016. Of the seven descriptions that did not provide clear geographic location data for the isolation of the type specimen, two included isolates from urban environments as additional specimens for the species description.

***Phytophthora* species in the environment**

In our review of the literature, type specimens for 20 species were recovered from urban environments. These recoveries represented 22% of the species with geographic location data associated with the isolation of the type specimen described between 2001 and 2016 (Table 1, Figure 1). Of these species, one was recovered from a botanical garden (Henricot et al. 2014), one was recovered from ornamental trees along an urban street (Brasier et al. 1993, Hansen et al. 2015), and four additional species were recovered from public spaces (Belbahri et al. 2006, Grünwald et al. 2012b, Scanu et al. 2013).

In a few cases, the location of the isolation of the type specimen was ambiguous. For example, location data for the type specimen of *P. parvispora* was described as an ornamental planting, which we considered as a public space because of the park-like setting in the host images (Scanu et al. 2013). Other locations included in our designation as a public space were described as an alleé (Grünwald et al. 2012b) and an alder stand within a town (Belbahri et al. 2006).

The remaining 14 species with type specimens from urban environments were recovered from ornamental plant nurseries or garden centers (Figure 2), representing the most important source of type specimens from the urban environment. The majority of species from urban environments were described with type specimens from ornamental nurseries, including their irrigation reservoirs. In addition, 10 of these species were also recovered in ornamental nurseries elsewhere (Table 1). This finding is not surprising considering that the trade of living plants is widely recognized as the primary pathway for

many plant pathogen invasions (Brasier 2008, Liebhold et al. 2012), especially for *Phytophthora* species (Jung et al. 2016b).

Thirty species of *Phytophthora* were described with type specimens from agricultural environments in 15 countries (Figure 1), representing 33% of the species included in this review. Five *Phytophthora* species (*P. alticola*, *P. captiosa, P. fallax*, *P. frigida,* and *P. pinifolia)* were isolated from plantations of non-native trees (Dick et al. 2006, Maseko et al. 2007, Durán et al. 2008). Some of these species may have been introduced into the agricultural systems in which they were recovered, but others may represent native species that were first detected on exotic hosts (Wingfield et al. 2015). As a result of both pathways, *Phytophthora* species continue to emerge as important pathogens in agricultural systems and preventing their movement and establishment is necessary in the mitigations of their deleterious effects.

The remaining 42 *Phytophthora* species, representing 45% of the species in this review that had type specimen geographic location data, were described based on isolates from natural environments in at least 13 countries (Figure 1). While this finding reflects the substantial diversity of *Phytophthora* in natural environments, many of these species could have been isolated during surveys of other invasive *Phytophthora* species (Burgess et al. 2009), or in exploratory surveys led by international researchers searching for the origin of newly described species (e.g. Jung et al. 2016a; Jung et al. 2017).

In theory, all *Phytophthora* species originate from a natural environment, but only become invasive "open-ended experiments in evolution" once transported and exposed to new environments and hosts (Hansen 2008, 2015, Brasier 2008). Although generally benign because of their coevolution with their hosts in native habitats, surveys of natural environments and the subsequent description of species before they "escape," is critical for establishing baseline datasets for each country or region and for global biosecurity initiatives aimed at prevention (Burgess et al. 2017). Increased exploration of habitats that have not been studied is needed to reveal the full diversity and current distributions of *Phytophthora* species (Hansen et al. 2012).

Eight of the 20 species described based on isolates from urban environments have also been reported in natural environments (Table 1). To find this large proportion is concerning because of the strong link between *Phytophthora* species dissemination and

the trade of living plants, giving these species opportunities to move through urban environments or be dispersed around the world. Jung et al. (2016b) reported that 49 *Phytophthora* species are widespread throughout the nursery trade in Europe. If our results are representative of this situation, it would mean that as many as 20 of these European nursery-associated species will also be found within natural environments, possibly as a result of invasions from urban environments, including ornamental nurseries. Although we did not assess the direction of movement between nurseries and the natural environment for the eight species in our review, this proportion illustrates the potential risk of *Phytophthora* invasions into natural environments for countries engaged in the trade of ornamental nursery stock.

The abundance of *Phytophthora* species within ornamental nursery settings is a major concern as plant trading nurseries are the most important source of *Phytophthora* invasions because they are linked to global shipping networks (Brasier 2008, Jung et al. 2016b). *Phytophthora* species have been recovered from soil of potted plants (Davison et al. 2006 p. 20), irrigation reservoirs (Ghimire et al. 2011, Yang et al. 2016), and hundreds of different hosts with novel plant-microbe interactions (Moralejo et al. 2009, Jung et al. 2016b). Nurseries also provide opportunities for hybridization between species (Brasier 2001, Brasier et al. 2004), where progeny can have novel host specificities and effects (Érsek et al. 1995, Brasier et al. 1999). Novel plant-microbe interactions are often unpredictable and can serve as a new means for escape into the natural environment. For example, *Phytophthora* species on weeds or other new hosts can be cryptic and infections do not always induce symptoms on the host (Denman et al. 2009, Migliorini et al. 2015). The asymptomatic plants may then be transported allowing for inadvertent pathogen movement, potentially on a global scale. Regardless of the setting in the urban environment, targeting these invasions before they escape into natural environments is critical for the protection of biodiversity and natural resources.

Phytophthora species in economies around the world

Utilizing the classification of the International Monetary Fund World Economic Outlook (WEO 2016), it was possible to characterize the economic environments where *Phytophthora* species have been described. Based on type specimens, 44 species were

collected in countries with major advanced economies, 34 species from advanced economies and 13 species were from countries with emerging markets and developing economies (Figure 3). Only one species (*P. polonica*) had a type specimen recovered from an urban environment in a country with an emerging market and developing economy (Belbahri et al. 2006).

The positive correlation between the number species descriptions and IMF economy classifications could suggest that countries with emerging markets and developing economies are under-surveyed. An alternative explanation may be that smaller international markets imply a lower risk of invasion due to lower levels of trade or movement of people. Nonetheless, the *Phytophthora* species found in countries with developing economies are of global concern because of phenomena such as the "bridgehead effect" (Lombaert et al. 2010, Scott et al. 2013, Wingfield et al. 2015) that strongly influences subsequent invasions into new environments. The concept of the "bridgehead effect" implies that once an area becomes a sink of an invasive species, it can be a source to other areas (Lombaert et al. 2010).

In terms of global occurrence, *Phytophthora* species were described in only 23 countries between 2001 and 2016. This information suggests that *Phytophthora* species remain to be described from much of the rest of the world. For example, only three species have been described from Africa (*P. capensis*, *P. frigida*, and *P. alticola*). This is of great concern because according to UN trade statistics (data not shown), Africa's exports of living plants are increasing; for example, Jung et al. (2016b) suggested that Africa supplied 3.6 billion plants to the Netherlands, accounting for 83.7% of the imports from overseas in 2010 alone.

Phytophthora ramorum: an example of a globally invasive plant pathogen

A detailed examination was conducted for *P. ramorum* by reviewing first report publications. This species was chosen as a case study because of its international importance as a pathogen, but also because it represents an invasive *Phytophthora* species that was released into an urban environment and subsequently spread to natural environments. The origin of *P. ramorum* remains unknown, but it was described based on isolates from ornamental plant nurseries in Germany and the Netherlands in 2001 and

simultaneously identified as the cause of the ongoing sudden oak death epidemic in the urban environment of California (Garbelotto et al. 2001, Werres et al. 2001, Rizzo et al. 2002, Rizzo and Garbelotto 2003).

The importance of urban and residential monitoring programs such as university extension programs is validated by the establishment story of *P. ramorum*. The initial report of dying tanoaks arose when homeowners asked University of California (UC) Cooperative Extension to investigate the cause of mortality for many trees bordering a creek in Marin County (Garbelotto et al. 2001). This mortality was subsequently found to be associated with the planting of ornamental rhododendrons in the understory (Rizzo et al. 2002). Then, within two years, the pathogen had spread along the creek and up the slopes to the crest of the hill and the first coast live oaks began to die in gardens of Marin County (Garbelotto et al. 2001). The existence of the UC Cooperative Extension program enabled the first report from a citizen and the following detection of the pathogen.

Since its first discovery and description (Werres et al. 2001), 72 first reports for *P. ramorum* have been published from various parts of the world. These reports were made for close to 60 host species in 14 countries on three continents. Additional reports were published when different lineages of *P. ramorum* were found in additional areas (e.g. Garbelotto et al. 2013), but these reports have not been included in the present study. Forty-eight publications representing 58 different host species included unambiguous geographic data that could be used in this review (Table 2).

First reports have been published for *P. ramorum* discoveries in urban, agricultural, and natural environments. Forty-seven of these first reports were for hosts in urban environments, 18 in natural environments, and 5 from agricultural environments. One additional report was from multiple environments in the UK (Table 2, Denman et al. 2005). While this distribution represents the prioritization for monitoring efforts in urban settings, often associated with statutory monitoring of plant nurseries (O'Hanlon et al. 2016), it does not necessarily suggest that *P. ramorum* could not be detected in natural environments. However, only 3 of the 14 countries that contributed first reports have reported *P. ramorum* in non-urban environments.

The majority of first reports of *P. ramorum* were from the two countries with active disease epidemics related to the pathogen: the United States and the United

Kingdom. *Phytophthora ramorum* was discovered in a greater diversity of environments for these countries than all other countries combined (Table 2, Figure 4). In all other countries, the majority of reported first detections occurred in nurseries and subsequent first reports have not been published for discoveries in natural or agricultural environments.

While our results are limited to publications of 'first reports', many other reports of novel *P. ramorum* host interactions exist but were not included in this review (e.g. Hansen et al. 2005). Therefore, the summary presented here is likely an underrepresentation of the current distribution and host range of *P. ramorum*, which is also limited because it is a quarantined species, listed in regulatory or legislative frameworks of more than 60 countries (Sansford et al. 2009). Nonetheless, we suggest that the frequency of recovering and reporting it in urban environments fairly represents a species that emerged through the trade of nursery stock, which is the most important source of *Phytophthora* species dissemination.

Recommendations

The findings of this review are consistent with previous studies regarding the association of *Phytophthora* species with plant nurseries (Moralejo et al. 2009, Jung et al. 2016b, O'Hanlon et al. 2016) and support the consensus that *Phytophthora* species are frequently disseminated via the plant trade. Therefore, we suggest that monitoring urban environments is especially important for countries that are engaged in the international trade of living plants.

We recommend monitoring ornamental nurseries and the surrounding urban environments because we predict a substantial proportion of the currently undiscovered *Phytophthora* species will be found in these areas, given the findings of this review. As many as 500 *Phytophthora* species are estimated to exist (Brasier 2008). Assuming that the results of this review represent future trends, we estimate that approximately 110 species will be described based on type specimens found in urban environments.

Monitoring nurseries and the surrounding urban environments is important because of the severe risk posed by species such as *P. ramorum*, which provides an excellent example of a previously undiscovered *Phytophthora* species first found in the

urban environment with evidence for emergence through the trade of nursery stock (Rizzo et al. 2002, Grünwald et al. 2012a). Focusing monitoring efforts in these areas will promote the early detection and rapid response required to prevent inadvertent movement of *Phytophthora* species into natural and agricultural environments. Furthermore, this focus would enable countries with limited resources for monitoring to detect and control invasive *Phytophthora* species.

It is important to monitor urban environments because they include most ports-of-entry, which are largely recognized as critical areas for early detection of plant pests and pathogens (McCullough et al. 2006, Aukema et al. 2010). The importance of monitoring the urban environment is also supported by a recent study in British Columbia that found greater diversity of *Phytophthora* species in urban environments than natural environments (Dale et al. 2017). The relationship between the increased diversity in urban environments and the proximity to ports-of-entry may be attributed to the trade of nursery stock (Liebhold et al. 2012) and supports the suggested need for increased monitoring in urban areas.

To the best of our knowledge, this review is the first attempt to establish a comprehensive list of *Phytophthora* species that have been discovered in urban environments. However, because we have limited this study to specimens used in species descriptions, our list of species is most likely an underrepresentation and does not reflect the actual number of *Phytophthora* species in urban environments. For example, Barber et al. (2013) recorded five species of *Phytophthora* killing trees in urban environments in Australia. Four of these species were described after 2001, but none of those were described using isolates from urban environments. Unfortunately, there are few studies similar to those of Barber et al. (2013) on which to base a more thorough review of *Phytophthora* in urban environments.

Although one-third of the species included in this review were isolated from agricultural environments, monitoring is usually intrinsic to agricultural production because local growers often report new invasions. For example, most species described from isolates in this environment were described following the identification of problems. However, this scenario also depends on the level of education, access to scientific communities, and the knowledge required to recognize the problem as having a

biological origin. This may not occur in many countries with developing economies. In these cases, raising awareness about the threats and consequences of invasive plant pathogens would promote the reporting of invasions and subsequent species descriptions.

Specifically surveying ornamental nurseries could prevent local invasions of undetected pathogens moving from nurseries to neighboring natural or urban environments and this could also prevent the pathogen from being shipped elsewhere. While detection of an invasive pathogen in a nursery could represent a disruption of the plant-trade pathway (i.e. the host and pathogenic material are destroyed before they are shipped or before they can establish outside), it may also represent a late detection of a completed introduction to the outside environment. The latter possibility is well-demonstrated by the case in Norway, where *P. ramorum* was first detected in a nursery, and was later found on public land that had plantings from the nursery (Herrero et al. 2006). Therefore, monitoring ornamental nurseries should be the priority in all countries because they pose the most immediate risk for dissemination, but broader urban surveys are also needed.

Although detection and monitoring programs should be prioritized in nurseries, we caution that countries should not overlook the importance of pathogen monitoring outside of nurseries. Particularly in areas where nursery detections of aggressive plant pathogens have occurred, monitoring of the nearby urban environment could provide the opportunity to eradicate an invasive plant pathogen before it escapes into a natural environment. Many *Phytophthora* species have been isolated from irrigation reservoirs running from nurseries (Ghimire et al. 2011), and movement from aquatic environments into terrestrial ecosystems has also been observed (Werres et al. 2007, Hulvey et al. 2010). Monitoring these areas in combination with nurseries would provide the best chance to detect a newly introduced *Phytophthora* species before it escapes into other environments.

Monitoring activities in urban environments such as residential neighborhoods or public gardens or arboreta, and natural areas that receive considerable human activity, such as national parks, could also provide opportunities for the early detection of *Phytophthora* species invasions. Several *Phytophthora* descriptions were based on isolates from public spaces and botanical gardens. The importance of monitoring these

areas is recognized and acted upon by the International Plant Sentinel Network (IPSN), a platform to connect gardens with monitoring efforts and exchange information internationally (Barham et al. 2015). These settings within the urban environment are also well suited for citizen science projects, especially in situations where monetary resources are focused on monitoring nurseries or where university extension programs do not exist. Training professionals to monitor ornamental nurseries and to conduct port inspections is important. But training non-scientists through programs such as the IPSN and citizen science projects would maximize detections in broader urban environments that are missed at the first stages (port inspections or in nurseries) of an invasion. In this regard, Brown et al. (2017) recently concluded that incorporating public contributions in plant pest monitoring efforts can maximize the use of resources for regulatory surveys. Therefore, where possible, monitoring ports and nurseries, embracing the IPSN, and establishing citizen science projects would provide the best possible combination of actions for the early detection of *Phytophthora* species within the urban environment.

Citizen Science projects that facilitate research about *Phytophthora* species

Citizen science initiatives can mitigate the effects of invasive species through supporting monitoring in resource-scare countries or countries that underfund such efforts. The methods used in citizen science initiatives broaden sampling distribution and can offset the prohibitive costs of data collection (Bonney et al. 2009, Meentemeyer et al. 2015, Hulbert 2016). Such projects have exceptional merit for monitoring invasive species because of the incorporation of 'many eyes' and greater access to private lands. In this regard, four citizen science projects have been established to survey, monitor and treat *Phytophthora* species. These include: the Sudden Oak Death (SOD) Blitz Program and the *Phytophthora* Stream Monitoring Program in the USA, Kauri Rescue in New Zealand, and Cape Citizen Science in South Africa.

The SOD Blitz program (https://nature.berkeley.edu/garbelottowp/) facilitates the regional monitoring of the Sudden Oak Death pathogen *Phytophthora ramorum* in California. Public contributions to the SOD Blitz program have improved predictive modeling capacity and informed managers of hot-spots for disease emergence in both urban and natural environments (Meentemeyer et al. 2015). The project has also

demonstrated that members of the public are equally capable of recognizing the disease than professionals. This result highlights the value of engaging the public in *Phytophthora* research and promotes non-scientist training programs as a valuable resource for monitoring invasive species in urban and rural environments.

The *Phytophthora* Stream Monitoring Program (https://ppo.puyallup.wsu.edu/sod/monitoring/streams/) is a project in western Washington State that gathers baseline data on *Phytophthora* species in streams in urban and wildland areas. The project was initiated to facilitate early detection of *Phytophthora ramorum,* but has since broadened its focus to survey multiple genera (Elliott et al. 2017). Through engagement of volunteer organizations, landowners, students, and the general public, the project has increased awareness of waterborne plant pathogens and the importance of sanitation.

Kauri Rescue (http://www.kaurirescue.org.nz) is an initiative to engage a broad community in the control of *Phytophthora agathidicida*, which is killing culturally and environmentally important kauri (*Agathis australis)* trees (Weir et al. 2015). Because kauri dieback is an issue in the urban interface, the project invites citizens to test treatment methods (e.g. phosphite application) and thus to determine the best approach for control. Although this project is not necessarily a monitoring program, it demonstrates the merit of involving the public to test hypotheses that seek to reduce the impacts of *Phytophthora* species while also raising awareness of the problem.

Cape Citizen Science (http://citsci.co.za) facilitates research regarding the diversity and distributions of *Phytophthora* in southwestern South Africa. It has also demonstrated that citizens are invaluable for plant disease research in both urban and natural environments. The program uses a model in which participants can contribute by sharing observations of plant disease or by submitting samples for analysis. The project has received many isolates of *Phytophthora* from citizens because it offers training to recognize plant diseases and methods to isolate causal organisms. Specifically in the urban environments, the project and has received samples from both plant trading industries and home gardens. In contrast to the other initiatives, Cape Citizen Science is pioneering methods to engage the public and survey *Phytophthora* diversity in a country with a developing economy.

Conclusions

Because of the frequency and diversity of *Phytophthora* species found within the urban environment, and the potential for these pathogens to move to new environments, we recommend prioritizing monitoring efforts in the urban environment. This focus is especially recommended in countries with limited resources that engage in the trade of plants for planting. The summary provided by this review, coupled with the consensus regarding the risks posed by the trade in living plants, suggests that monitoring nurseries and the greater urban environment provides opportunities to detect invasive *Phytophthora* species before they escape into other environments. While it remains critical to train professionals and specialists to monitor plant-trading nurseries, we recommend citizen science as an approach to offer training for non-scientists, similarly to the IPSN, to monitor and discover *Phytophthora* species in urban and natural environments.

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Tables

Table 1: *Phytophthora* species with type specimens from urban environments.

Species	Location	Also in natural environment	Holotype from nursery	Ornamental host	Ornamental nurseries elsewhere	Country	IMF WEO Economy[1]	Publication
P. pachypleura	Botanical garden	N	N	Y	N	UK	MA	Henricot et al. 2016
P. chlamydospora	Urban street	Y	N	Y	N	UK	MA	Hansen et al. 2015
P. mississippiae	Nursery	N	Y	NA	N	USA	MA	Yang et al. 2013
P. parvispora	Public space	Y	N	N	N	Italy	MA	Scanu et al. 2013
P. niederhauserii	Nursery	N	Y	Y	Y	USA	MA	Abed et al. 2014
P. obscura	Public space	N	N	N	Y	Germany	MA	Grünwald et al. 2012
P. foliorum	Nursery	N	Y	Y	Y	USA	MA	Donahoo et al. 2006
P. ramorum	Nursery	Y	Y	Y	Y	Germany	MA	Werres et al. 2001
P. macilentosa	Nursery	N	Y	NA	N	USA	MA	Yang et al. 2014
P. hydrogena	Nursery	N	Y	NA	N	USA	MA	Yang et al. 2014
P. virginiana	Nursery	N	Y	NA	N	USA	MA	Yang and Hong 2013
P. irrigata	Nursery	Y	Y	NA	Y	USA	MA	Hong et al. 2008
P. polonica	Public space	Y	N	N	N	Poland	E&D	Belbahri et al. 2006
P. aquimorbida	Nursery	Y	Y	NA	Y	USA	MA	Hong et al. 2012
P. hydropathica	Nursery	N	Y	NA	Y	USA	MA	Hong et al. 2010
P. stricta	Nursery	Y	Y	NA	N	USA	MA	Yang et al. 2014
P. hedraiandra	Nursery[a]	N	Y	Y	Y	Netherlands	A	deCock and Lévesque 2004
P. occultans	Nursery[b]	N	Y	Y	Y	Netherlands	A	Man in 't Veld et al. 2014
P. terminalis	Nursery[b]	N	Y	Y	N	Netherlands	A	Man in 't Veld et al. 2014
P. lacustris	Public space[c]	Y	N	Y	Y	UK	MA	Nechwatal et al. 2013

Y = yes, N = no, NA = not available

[1]IMF World Economy Outlook, MA = Major Advanced, A = Advanced, E&D = Emerging and Developing (WEO 2016).

[a]Assumed to be isolated from a nursery because of host and source of cultures (De Cock and Lévesque 2004).

[b]Assumed to be isolated from a nursery because hosts were ornamental and some additional specimens were collected from horticultural centers (Man in 't Veld et al. 2015).

[c]Interpreted as public space because holotype was isolated from ornamental plant near a stream (Nechwatal et al. 2013).

Table 2: Host and demographic data from first report publications for *Phytophthora ramorum*.

Host	Country	IMF WEO Economy[1]	Environment	Year	Source (DOI if available)
Rhododendron sp.	Germany and the Netherlands	A	Urban	1997	Werres & Marwitz 1997
Viburnum sp.	Germany and the Netherlands	A	Urban	2001	10.1016/S0953-7562(08)61986-3
Pseudotsuga menziesii	USA	MA	Natural	2002	10.1094/PDIS.2002.86.11.1274B
Notholithocarpus densiflorus	USA	MA	Natural	2002	10.1094/PDIS.2002.86.4.441C
Rhododendron macrophyllum	USA	MA	Natural	2002	10.1094/PDIS.2002.86.4.441C
Vaccinium ovatum	USA	MA	Natural	2002	10.1094/PDIS.2002.86.4.441C
Viburnum tinus	United Kingdom	MA	Urban	2002	http://www.ndrs.org.uk/article.php?id=006013
Sequoia sempervirens	USA	MA	Natural	2002	10.1094/PDIS.2002.86.11.1274A
Rhodedendron spp.	Spain	A	Urban	2002	10.1094/PDIS.2002.86.9.1052A
Rhodedendron catawbiense	Poland	E&D	Urban	2002	Orlikowski & Szkuta 2002
Viburnum bodnantense	Belgium	A	Urban	2003	10.1094/PDIS.2003.87.2.203C
Trientalis latifolia	USA	MA	Natural	2003	10.1094/PDIS.2003.87.5.599B
Pieris formosa var. forrestii	United Kingdom	MA	Urban	2003	10.1111/j.1365-3059.2003.00894.x
Quercus chrysolepis	USA	MA	Natural	2003	10.1094/PDIS.2003.87.3.315C
Camellia japonica	Spain	A	Urban	2003	10.1094/PDIS.2003.87.11.1396A
Syringa vulgaris	United Kingdom	MA	Urban	2004	10.1111/j.1365-3059.2004.01033.x
Camellia spp.	United Kingdom	MA	Urban	2004	10.1111/j.1365-3059.2004.01028.x
Quercus falcata	United Kingdom	MA	NA[a]	2004	10.1111/j.1365-3059.2004.01079.x
Hamamelis virginiana	United Kingdom	MA	Urban	2004	10.1111/j.1365-3059.2004.01034.x
Rosa gymnocarpa	USA	MA	Natural	2004	10.1094/PDIS.2004.88.4.430
Taxus baccata	United Kingdom	MA	Urban	2004	10.1111/j.1365-3059.2004.01022.x
Camellia japonica	USA	MA	Urban	2004	10.1094/PDIS.2004.88.1.87A
Camellia sasanqua	USA	MA	Urban	2004	10.1094/PDIS.2004.88.1.87A
P. floribunda × *japonica*	USA	MA	Urban	2004	10.1094/PDIS.2004.88.1.87A
Pieris japonica	USA	MA	Urban	2004	10.1094/PDIS.2004.88.1.87A
Pieris japonica × *formosa*	USA	MA	Urban	2004	10.1094/PDIS.2004.88.1.87A
Rhododendron spp.	USA	MA	Urban	2004	10.1094/PDIS.2004.88.1.87A

Viburnum bodnantense	USA	MA	Urban	2004	10.1094/PDIS.2004.88.1.87A
Viburnum plicatum var. tomentosum	USA	MA	Urban	2004	10.1094/PDIS.2004.88.1.87A
Rhodedendron catawbiense 'Grandiflorum'	Slovenia	A	Urban	2004	10.1111/j.1365-3059.2004.01023.x
Viburnum farreri	Slovenia	A	Urban	2004	10.1111/j.1365-3059.2004.01023.x
Viburnum bodnantense	Slovenia	A	Urban	2004	10.1111/j.1365-3059.2004.01023.x
Castanea sativa	United Kingdom	MA	Urban	2005	10.1111/j.1365-3059.2005.01222.x
Quercus ilex	United Kingdom	MA	Multiple[b]	2005	10.1094/PD-89-1241A
Maianthemum racemosum	USA	MA	Natural	2005	10.1094/PD-89-0204C
Rhododendron catawbiense	Norway	A	Urban	2006	10.1094/PD-90-1458B
Pittosporum undulatum	USA	MA	Urban	2006	10.1071/DN06009
Parrotia persica	United Kingdom	MA	Urban	2006	http://www.ndrs.org.uk/article.php?id=013011
Rhamnus purshiana	USA	MA	Natural	2006	10.1094/PD-90-0246C
Adiantum aleuticum	USA	MA	Natural	2006	10.1094/PD-90-0379B
Adiantum jordanii	USA	MA	Natural	2006	10.1094/PD-90-0379B
Griselinia littoralis	United Kingdom	MA	Urban[c]	2007	10.1111/j.1365-3059.2007.01590.x
Magnolia loebneri	United Kingdom	MA	Urban[c]	2007	10.1111/j.1365-3059.2007.01590.x
Magnolia stellata	United Kingdom	MA	Urban[c]	2007	10.1111/j.1365-3059.2007.01590.x
Camellia sp.	France	A	Urban	2007	10.1094/PDIS-91-10-1359B
Pieris japonica	France	A	Urban	2007	10.1094/PDIS-91-10-1359B
Rhodedendron spp.	France	A	Urban	2007	10.1094/PDIS-91-10-1359B
Viburnum bodnantense	France	A	Urban	2007	10.1094/PDIS-91-10-1359B
Viburnum tinus	France	A	Urban	2007	10.1094/PDIS-91-10-1359B
Rhodedendron spp.	Finland	A	Urban	2007	10.1094/PDIS-91-8-1055C
Acer circinatum	USA	MA	Natural	2008	10.1094/PHP-2008-0118-02-BR
Arctostaphylos columbiana	USA	MA	Natural	2008	10.1094/PHP-2008-0118-02-BR
Arctostaphylos manzanita	USA	MA	Natural	2008	10.1094/PHP-2008-0118-02-BR
Ceanothus thyrsiflorus	USA	MA	Natural	2008	10.1094/PHP-2008-0118-02-BR
Corylus cornuta var. californica	USA	MA	Natural	2008	10.1094/PHP-2008-0118-02-BR
Viburnum tinus	USA	MA	Urban	2008	10.1094/PDIS-92-2-0314B
Osmanthus heterophyllus	USA	MA	Urban	2008	10.1094/PDIS-92-2-0314B
Rhodedendron spp.	Serbia	E&D	Urban	2009	10.1111/j.1365-3059.2009.02033.x

Abies magnifica	USA	MA	Agriculture	2010	10.1094/PDIS-94-9-1170B
Larix kaempferi	United Kingdom	MA	Agriculture	2010	10.5197/j.2044-0588.2010.022.019
Vaccinium myrtillus	Norway	A	Urban	2011	10.1094/PDIS-10-10-0709
Trachelospermum jasminoides	USA	MA	Urban	2011	Osterbauer et al. 2011
Abies grandis	USA	MA	Agriculture	2011	10.1094/PHP-2011-0401-01-BR
Rhodedendron spp.	Greece	A	Urban	2011	10.1094/PDIS-08-10-0607
Loropetalum chinense	USA	MA	Urban	2012	10.1094/PDIS-01-12-0062-PDN
Chamaecyparis lawsoniana	United Kingdom	MA	Agriculture	2012	10.5197/j.2044-0588.2012.025.026
Myristica fragrans	India	E&D	Agriculture	2012	Mathew & Beena 2012
Cinnamomum camphora	USA	MA	Urban	2013	10.1094/PDIS-01-13-0096-PDN
Rhododendron spp.	USA	MA	Urban	2014	10.1094/PDIS-10-13-1043-PDN
Viburnum tinus	Italy	A	Urban	2014	10.1094/PDIS-07-13-0767-PDN
Gaultheria procumbens	USA	MA	Urban	2014	10.1094/PHP-BR-13-0109
Notholithocarpus densiflorus	USA	MA	Natural	2016	10.1094/PDIS-10-15-1169-PDN

[1]IMF World Economy Outlook, MA = Major Advanced, A = Advanced, E&D = Emerging and Developing (WEO 2016).
[a]The isolates for this first report were recovered from a mature tree in south-east England in an unspecified environment.
[b]The isolates were recovered from infected trees at 'various woodland and garden sites in the UK' and 'recorded on saplings in nurseries'.
[c]Assumed to be isolated from nurseries because the hosts are ornamental and the author affiliations include DEFRA PHSI.

Figures

Figure 1: *Phytophthora* holotype distribution data for each country between 2001 and 2016.

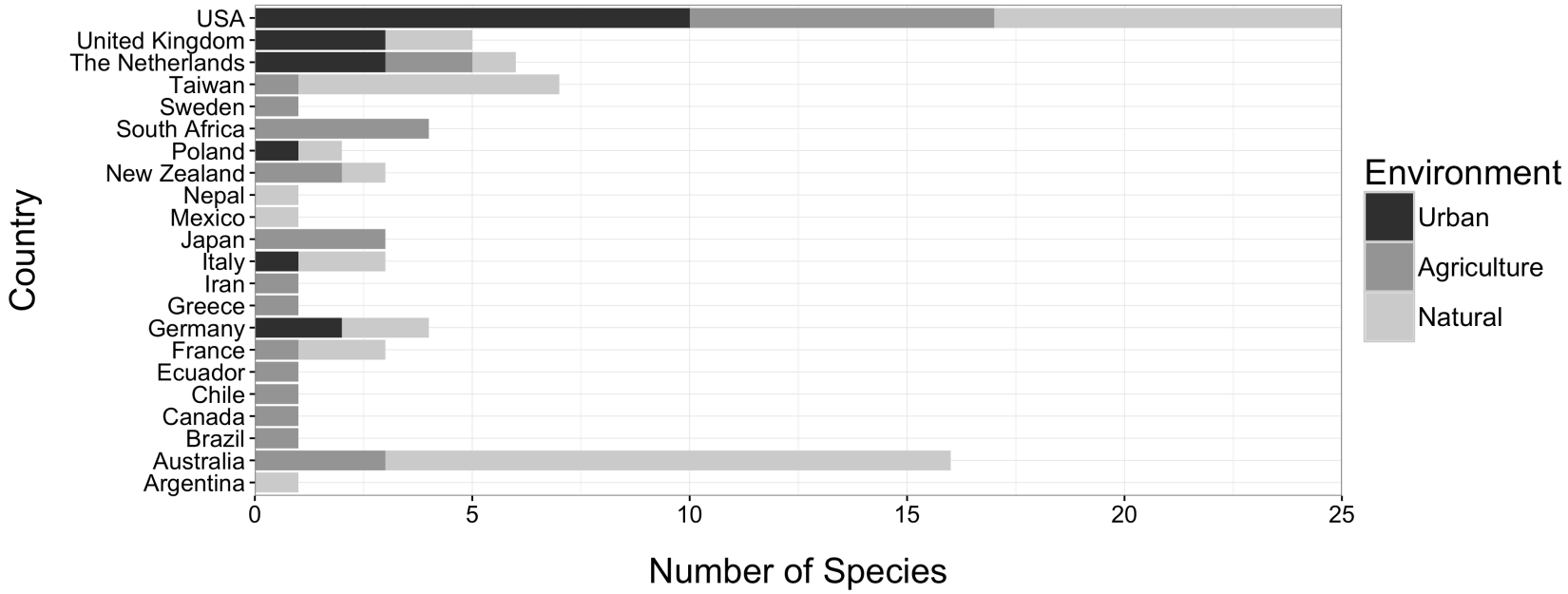


Figure 2: *Phytophthora* holotype distributions in urban environments for each country.

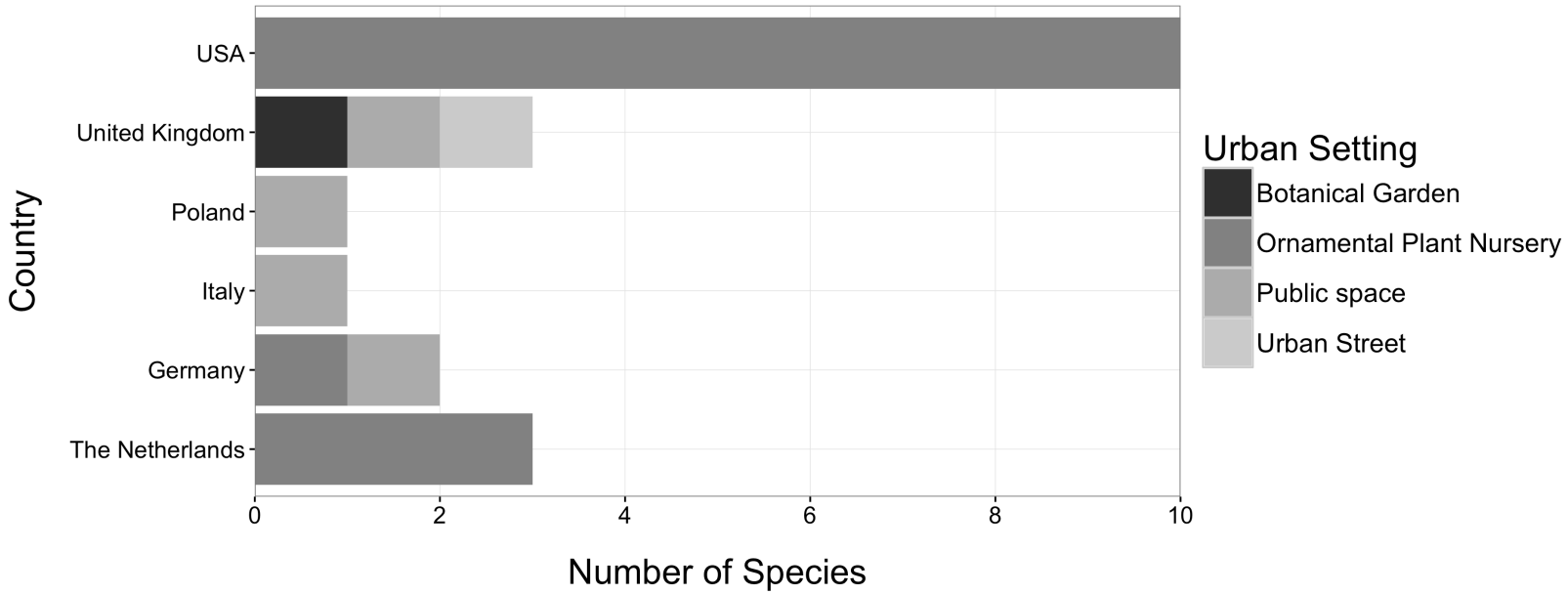


Figure 3: *Phytophthora* holotype distribution data within WEO economy classes between 2001 and 2016.

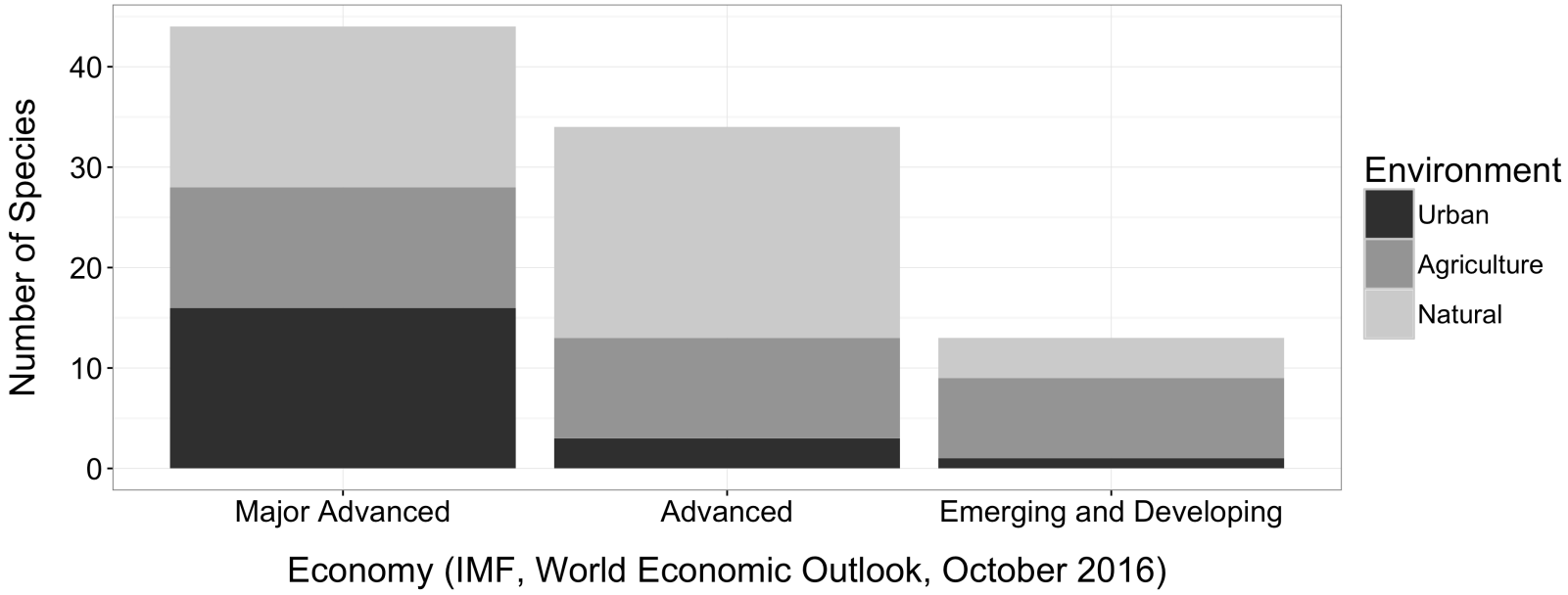
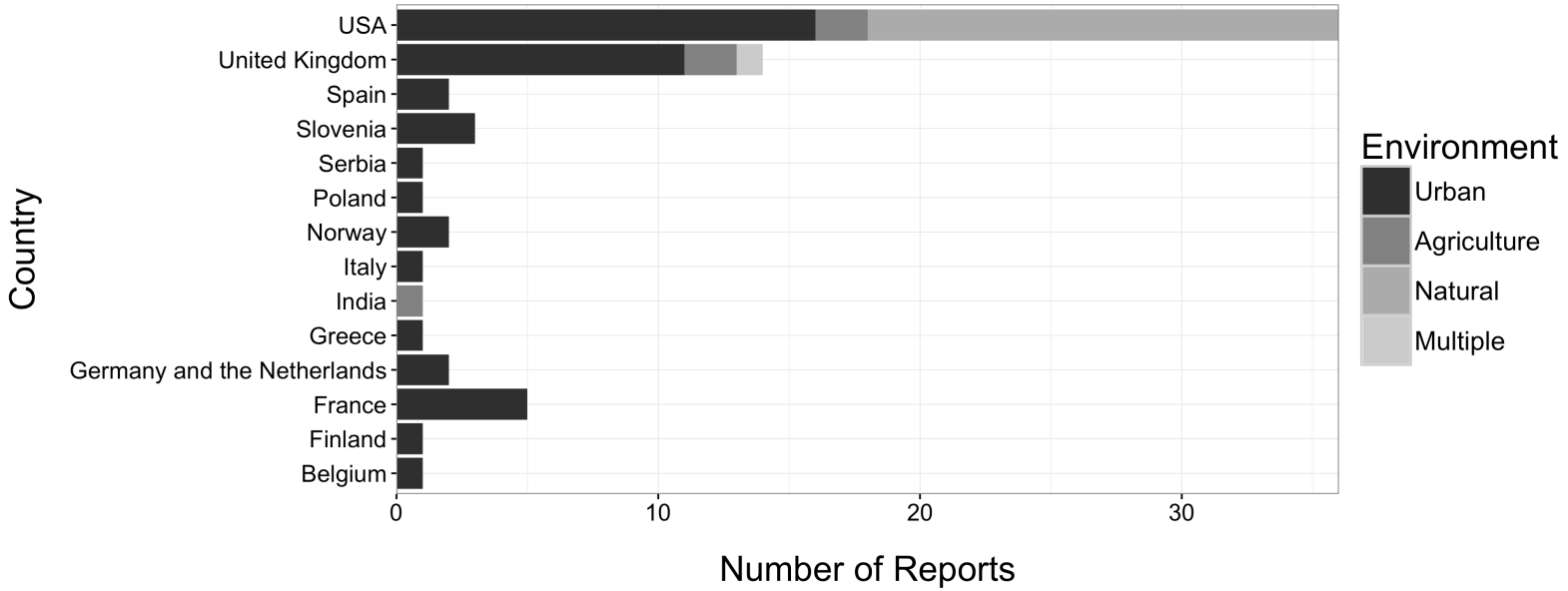


Figure 4: *Phytophthora ramorum* first report distributions by country.



CHAPTER TWO

Botanical gardens provide valuable baseline *Phytophthora* diversity data

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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.

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.

Methods

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 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.

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.

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 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).

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.

Results

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.

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 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.

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).

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.

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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

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.

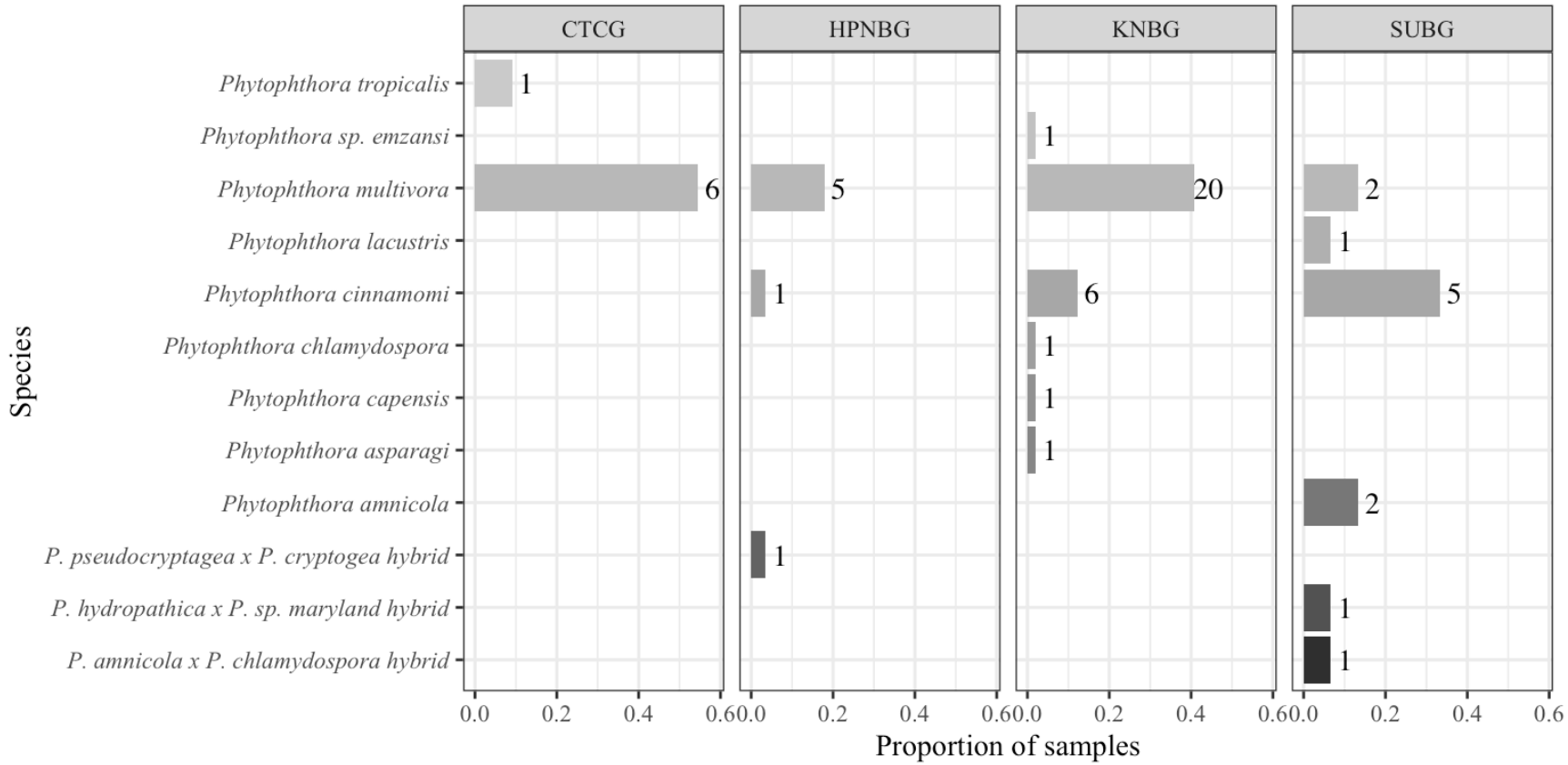
Table 3: Representative Isolates and GenBank Accession Numbers.

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

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.

CHAPTER THREE

Exotic and putatively native *Phytophthora* species are associated with distinct southern Afrotemperate forest communities

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Abstract

Phytophthora species are important forest pathogens, often studied following their anthropogenic movement into naïve ecosystems. However, the function of these species in their native environments is less well understood and opportunities to contrast the impacts of exotic *Phytophthora* species with those of native species are uncommon. In this study, we investigated the diversity of *Phytophthora* species within a native forest ecosystem in southern Africa and compared the plant community relationships of an introduced *Phytophthora* species with two putatively native species. Three described *Phytophthora* species and one informally recognized species (*P. cinnamomi*, *P. multivora*, *P. capensis* and *P.* sp. emzansi) were recovered by baiting rhizosphere samples from 75 plots in five southern Afrotemperate forest fragments. All three known *Phytophthora* species were recovered from at least four of the forest fragments while *P.* sp. emzansi was recovered from only two forests. We found significant dissimilarity between plant communities in plots containing the introduced species *P. cinnamomi* and the putatively native species *P. multivora. Phytophthora cinnamomi*, *P. multivora*, and *P. capensis* were associated with distinct plant communities compared to plots where no *Phytophthora* species were recovered. A difference was also identified when comparing the levels of dispersion between plant communities in plots with and without *Phytophthora* species recovery. These data suggest *Phytophthora* species may contribute cumulatively to the overall beta-diversity in Afrotemperate forest fragments and each species may occupy a distinct niche. While this could be due to each *Phytophthora* species causing disease in specific tree species, there was no evidence for a relationship between the presence of the putative native *P. capensis* or *P. multivora* and the crown health of the native trees. This may indicate a long coevolutionary and community filtering history between these native organisms. In contrast, there was evidence for a relationship between the presence of the exotic *P. cinnamomi* and the crown health of trees, most likely because the plant communities have not co-evolved with this relatively recently introduced pathogen.

Introduction

Phytophthora species are important pathogens of forest trees well known for the diseases they cause outside of their native ranges. Sudden oak death (Rizzo and Garbelotto 2003), kauri die-back (Waipara et al. 2013), *Phytophthora* die-back in *Banksia* woodlands (Burgess et al. 2017b), and Protea root rot (von Broembsen 1984) provide examples of diseases in native plant communities caused by exotic *Phytophthora* species. The species driving these epidemics are well known as agents of change in infected plant communities, however, little is known with regard to their function in the ecosystems where they coevolved with their hosts (Reeser et al. 2011, Hansen 2015, Laliberté et al. 2015).

Native plant pathogens are recognized as strong regulators of patterns and processes in natural forests. Fundamentally, plant pathogens are thought to maintain natural processes such as succession by causing disease (Castello et al. 1995). Other studies have suggested plant pathogens contribute to plant community diversity in their natural environments through ecological processes such as negative density dependence (Bagchi et al. 2011, Laliberté et al. 2015), compensatory response (Bradley et al. 2008), negative feedback (Mills and Bever 1998, Petermann et al. 2008, Mangan et al. 2010), and gap formation (Hansen and Goheen 2000).

The Janzen-Connell hypothesis proposes that host-specific predators such as pathogens maintain tree diversity in tropical forests as functions of distance and density (Janzen 1970, Connell 1971). Here, pathogens directly affect plant growth rates, survival, and reproduction and influence plant community composition at local levels (Bever et al. 2012). Support for this hypothesis has been revealed through the effects of soil pathogens on patterns of seedling mortality (Packer and Clay 2000, Bagchi et al. 2010, Sarmiento et al. 2017) and patterns of spatial distributions of canker development and mortality in tropical trees (Gilbert et al. 1994). These mechanisms likely constitute the complexity of multi-species and highly structured forest ecosystems, but the contributions of most pathogenic microbe taxa (e.g. *Phytophthora*) in natural systems have not been well studied because they are cryptic where they have co-evolved with their native hosts (Brasier 2008, Hansen 2015).

Plant pathogens are known to contribute to plant community diversity and structure (Bagchi et al. 2014, Bever et al. 2015, Sarmiento et al. 2017). However, the

community effects vary between scales from the stand level (alpha diversity) to sites across a geographic area (beta diversity) (Bishop et al. 2010, Fukami and Nakajima 2013). For example, negative feedback mechanisms such as the Janzen-Connell effect increases alpha diversity by allowing species to coexist at the stand level (Bever et al. 2012), but this process is also suggested to reduce beta diversity as the plant communities converge (Fukami and Nakajima 2013). Beta diversity is a critical concept to manage ecosystems for the conservation of biodiversity and in order to understand the functioning of ecosystems (Legendre et al. 2005). Therefore, further work is needed to understand the role and contributions of different pathogens in large-scale patterns of plant diversity (Bever et al. 2015).

Phytophthora species are widespread, abundant and diverse in forest ecosystems where they have evolved into separate ecological assemblages (Hansen et al. 1986, Hansen 2015). Some species, for example *P. ramorum*, *P. pluvialis*, and *P. pinifolia,* are specialized fine-twig and foliar pathogens and are often recovered from forest canopies (Rizzo et al. 2002, Durán et al. 2008, Dick et al. 2014). Other species such as *P. multivora* and *P. cinnamomi* are soilborne and cause disease as fine-root feeders and stem canker pathogens (Newhook and Podger 1972, Cahill et al. 2008, Scott et al. 2009). Although confined to the roots and woody tissues, above ground symptoms of infection by root and stem pathogens can include canopy die-back, stunting, thinning, wilting and chlorosis (Podger 1972, Cahill et al. 2008).

A third ecological assemblage of *Phytophthora* species exists within waterways (Hansen 2015). Many species in these environments, particularly those from phylogenetic Clade 6 (Jung et al. 2011), are recognized as opportunist pathogens (Reeser et al. 2011) or saprophytes (Marano et al. 2016). In general, these groups are referred to as ‘assemblages’ rather than ‘communities’, because there is insufficient information to infer whether they are interacting together or with other organisms in the ecosystem (Reeser et al. 2011).

There is limited information on *Phytophthora* diversity and distribution for Africa. For example, only three of the more than 160 *Phytophthora* species known to science have been described from Africa (Hulbert et al. 2017), and all were collected from disturbed environments in South Africa (Maseko et al. 2007, Bezuidenhout et al.

2010). Specifically in native southern Afrotemperate forests, a few studies have noted the decline of a single native host, *Ocotea bullata*, caused by *P. cinnamomi* (Lübbe and Geldenhuys 1990, Lübbe and Mostert 1991), a pathogen introduced to these areas (Linde et al. 1999, Oh et al. 2013). However, its effects on other forest species and the diversity and effects of other *Phytophthora* species in these forests remains poorly understood.

Introduced *Phytophthora* species are often singular causes of change in forest communities. For example, *Phytophthora ramorum* is functionally eliminating mature *Notholithocarpus densiflora* trees from mixed conifer-hardwood forests in California and Oregon (Cobb et al. 2012), *P. agathidicida* is responsible for the loss of young and old (upwards of 1500 years) *Agathis australis* trees in native forests of New Zealand (Scott and Williams 2014, Weir et al. 2015), and *P. lateralis* has changed riparian communities by killing *Chamaecyparis lawsoniana* along infected streams in California and Oregon (Hansen et al. 2000). The selective plant species mortality caused by each these *Phytophthora* species is effectively filtering the overall diversity of these forests into novel and tolerant communities, and may result in localized extinctions. This selection process is undoubtedly determined by a combination of the host species susceptibility, the host-specificity and virulence of the pathogen, and the suitability of the environment (Shearer et al. 2004, Benítez et al. 2013, Belhaj et al. 2018). Therefore, the impact of newly introduced *Phytophthora* species into an environment with suitable and susceptible hosts can be a devastating, while in an ecosystem where the host and the pathogen have coevolved the impact is likely to be patchy, e.g. with local conditions such as poor soil drainage (Lübbe 1991, Rhoades et al. 2003), or limited to a particular life stage (Simamora et al. 2017) or general stage of succession (Hansen 1999). In this regard, we expect introduced *Phytophthora* species to be associated with different plant communities compared to native *Phytophthora* species.

In this study, we report findings from a survey of the rhizosphere in five southern Afrotemperate forest sites in South Africa. *Phytophthora cinnamomi* is present in these forests (Lübbe and Mostert 1991) and provides a novel opportunity to contrast the effects of an exotic species with putatively native species. We expected to find support for a relationship between the presence of the *Phytophthora* species and the crown health of forest trees, indicating some level of parasitism was occurring with negative effects on

the host. The primary objectives were to reveal the diversity of *Phytophthora* species present at these sites and to test hypotheses regarding their associations with the plant communities and disease symptoms. The following hypotheses were tested: (1) plots with *Phytophthora* species have different plant communities than plots where no *Phytophthora* species were recovered due to mechanisms such as the Janzen-Connell effect, (2) plant communities associated with *P. cinnamomi* are different from plant communities where putatively native *Phytophthora* species occur because *P. cinnamomi* was introduced to these systems and is a relative 'newcomer' (Linde et al. 1999), (3) the presence of *Phytophthora* (especially *P. cinnamomi*) is associated with decreased tree crown health because this is a common symptom of root decay (Podger 1972, Blaschke 1994), (4) the presence of *Phytophthora* is associated with reduced tree regeneration and the recruitment of seedlings because of mechanisms hypothesized Janzen and Connell (Janzen 1970, Connell 1971), and (5) *Phytophthora* species would be associated with increased alpha diversity and decreased beta diversity because of community convergence as suggested by Fukami and Nakajima (2013).

Methods

Study Area

Five sites were selected in native, recently undisturbed and naturally occurring forest fragments between Sir Lowry's Pass and Storms River in the Western and Eastern Cape Provinces of South Africa, respectively (Figure 1). Sites were selected to represent a range of abiotic conditions for native southern Cape mixed evergreen Afrotemperate forests. These forests are complex multi-species and highly structured ecosystems (Seifert et al. 2014) receiving year-round rainfall (Lübbe and Mostert 1991). These naturally fragmented forests are the smallest (only covering 0.1% of the land area), but most widely distributed vegetation formation in South Africa (Geldenhuys and Mucina 2006). The sites were selected in forest fragments ranging in size from 65 to 1035 hectares in size. Additional information concerning the forest sites selected for this study is provided in Table 1.

Forest Plots

Forest plant community data were collected on fifteen 5.7 m-radius plots ($100m^2$) per site spaced at least 15 m apart. Each site included plots established around three individuals of five tree species randomly selected to serve as plot centers. *Cunonia capensis*, *Olea capensis* subsp. *macrocarpa*, *Olinia ventosa*, *Podocarpus latifolius,* and *Rapanea melanophloeos* were selected as focal trees for the study because they were abundant, they represent a diverse phylogenetic assemblage, and they were found in all five forest fragments. Species, crown health, diameter at 1.3 m above the ground, and distance from focal tree were recorded for each tree with a stem diameter greater than 8 cm in the plot. Crown health was estimated as a percentage of the crown with die-back. For example, the loss of leaves from the tips of branches would represent a percentage of crown dieback. In general, the assessments of crown health represented the inverse of the crown conditions presented in Lübbe and Geldenhuys (1990), but were maintained as continuous data rather than as discrete categories. Woody understory species within the plots were identified and recorded, but not quantified. Special reference was made to confirm whether the focal tree species were regenerating within the plots to test for evidence of negative density dependence and the Janzen-Connell hypothesis. For example, detailed determination was made as to whether *R. melanophloeos* seedlings were present in plots established around *R. melanophloeos* trees.

Phytophthora Isolation

Rhizosphere samples were collected from five locations around the base of the focal trees during one week in October 2017 and pooled into a single sample (500-1000 g) for each plot. Samples were collected from 1-10 cm below the litter and duff layer to avoid dominant saprophytes. The samples were kept in unsealed plastic bags at room temperature until they could be processed in the laboratory two weeks later. Spades used for collecting samples were sterilized between plots.

Trays containing 400-600 g of rhizosphere material were moistened 24 h before flooding with 1-1.5 L of double distilled water (DDH_20) with the surface debris displaced against one edge with a paper towel. Four trays containing DDH_20 only were set up as negative controls. Young *Hededra helix* leaves and *Rosa* sp. cultivar petals were added to the water surface to act as bait following methods outlined by Bose et al. (2018).

Symptomatic baits were removed 7-14 days after adding, rinsed in tap water, and blotted dry. The forceps used to remove the baits were sterilized between samples by dipping in 95% ethanol and flaming. Incisions were made from the baits at the advancing margin of lesions and cut pieces were placed on NARPH oomycete selective agar (50 mg Nystatin, 200 mg Ampicillin, 10 mg Rifampicin, 25 mg Pentachloronitrobenzene, and 50 mg Hymexazol per L). The forceps and blades were sterilized between bait types. Baits still asymptomatic after 14 days were considered negative. Baits from control trays were plated even if they were asymptomatic.

Secondary isolations were made the day after the pieces were placed onto the selective agar. If the culture was suspected to overgrow the entire primary plate, a sub-culture was made and the colony and bait tissue were removed from that section of the plate. A final isolation was made from a hyphal tip of all colonies onto half-strength potato dextrose agar (20g per 1L) and left to grow at room temperature in the dark.

DNA Extraction, Amplification, and Sequencing

Sixteen-day-old cultures on half-strength PDA were tentatively identified based on colony morphology. One to three representative cultures of each *Phytophthora* morphotype (depending on availability) were used for species identification using DNA-sequence analyses. Isolates represented by more than three cultures were selected for DNA sequence assessment even if they were not suspected to be *Phytophthora* species. DNA was extracted, amplified and sequenced for all selected isolates.

Mycelium was scraped from 20-day-old cultures into 1.5ml Eppendorf ® tubes for DNA sequence analysis. DNA was extracted from the mycelium by adding 60µl Prepman Ultra ® DNA extraction buffer (Applied Biosystems, Cheshire, United Kingdom), heated to 96°C and crushed using melted pipette tips. The Internal Transcribed Spacer (ITS) gene region was then amplified with methods consistent with Schena et al. (2008) using ITS4 and ITS6 primers (White et al. 1990, Cooke et al. 2000). Forward and reverse sanger sequences provided by the Stellenbosch University Central Analytic Facility were aligned using MUSCLE alignment in Geneious version 10.2.3 (Kearse et al. 2012) and consensus sequences were initially compared with an internal dataset containing ITS sequences curated for published Oomycetes using Blast. They

were subsequently verified using the nucleotide database on GenBank. Accession numbers for selected isolates are provided in Table 2.

Statistical Analyses

The association of *Phytophthora* species with the plant communities was assessed using multivariate analysis. The presence or absence of each plant and *Phytophthora* species in each plot was retained in a binary dataset. Data for living plants in the overstory and understory were combined for the plant community analysis. All aspects of the analysis were conducted in R version 3.3.0. Rare plant species, i.e. plant species occurring in less than 5% of the plots, were removed from the dataset using the R-package: labdsv version 1.8-0 (Roberts 2016) to increase the confidence of the tests for differences by reducing the weight of rare species (Clarke and Green 1988, Poos and Jackson 2012) and increase the performance of our distance measures (Chao et al. 2005).

The relationships between *Phytophthora* species recovery and the plant communities (between-factor beta diversity, as defined by Pryke et al. (2013)) were tested using PERMANOVA (Anderson 2001) with 9999 permutations in the Vegan package version 2.4-4 and explored with ordination using nonmetric multidimensional scaling (NMDS) (Oksanen et al. 2018). Differences (dissimilarity) between plant communities associated with *Phytophthora* species were calculated using the Jaccard similarity index because it is appropriate for binary (presence/absence) data (Boyle et al. 1990, Boyce and Ellison 2001, Chao et al. 2005) and has been commonly used to quantify taxonomic change in communities (Olden and Rooney 2006). This method was used to test for differences in plant communities where *Phytophthora* species were recovered compared to communities where *Phytophthora* species were not recovered. We also evaluated the contrasts between plant communities associated with each species in plots where only a single *Phytophthora* species was recovered. For example, plant communities associated with plots where only *P. cinnamomi* was recovered were compared with those in plots where only *P. multivora* was recovered.

A metric for alpha diversity was compared by estimating species richness with the Shannon-Weaver index using the diversity function in the Vegan package. The index values were compared between plots with and without *Phytophthora* using a Welch two-

sample t-test after checking the appropriateness of a parametric test. Within-factor beta diversity (species turnover among sites, as defined by Pryke et al. (2013)) was evaluated using a permutation test of multivariate homogeneity of group dispersions (PERMDISP) to determine if the mean distances between sites (as defined by their plant communities) and the centroids of the representative groups (those groups with and without *Phytophthora* and again with each *Phytophthora* species separately) differed between groups using the permutest.betadisper function with 9999 permutations in Vegan (Anderson et al. 2006). For example, we tested whether plant communities from plots where *Phytophthora* was recovered had more or less dispersed plant communities than plots where *Phytophthora* was not recovered. Finally, the labdsv package was used to identify host species that were indicators of the presence and absence of *Phytophthora* with the procedure developed by Dufrêne and Legendre (1997).

The relationship between the percent canopy die-back of each tree and the presence of *Phytophthora* was investigated using a mixed effects model. Here, lme4 (Bates et al. 2012) was used to perform a generalized linear mixed effects analysis of the relationship between the proportion of unhealthy trees and the recovery of *Phytophthora* species. A tree was characterized as unhealthy if it had greater than 10% canopy die-back. This level of die-back was selected because it typically represented observations of branch tip die-back or bare tips of terminal shoots. The mixed effect model included a fixed effect parameter representing the recovery of *Phytophthora* in three groups: (1) no *Phytophthora* recovered, (2) *P. cinnamomi* recovered, and (3) other *Phytophthora* species recovered. This parameter was selected to represent the data after comparing AIC values for models with different parameters for *Phytophthora* (see supplementary text). A parameter representing a random intercept for the effect of the plot number nested in the forest site was also included, as well as a parameter with a random intercept for the effect of tree species. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity. P-values were obtained by likelihood ratio tests of the full model with the *Phytophthora* parameter against the model without the *Phytophthora* parameter in question. The model was also refit after evaluation of Wald estimates indicated there was a relationship between *P. cinnamomi* and the proportion of unhealthy trees. Again, a likelihood ratio test was used to confirm the relationship using a parameter with two

groups instead of three (*P. cinnamomi* absent vs *P. cinnamomi* present) with the same random effects.

The relationship between the presence of regeneration (seedling and juvenile tree recruitment) of the focal tree species and the presence of *Phytophthora* species was tested using another generalized linear mixed effect model. The model included the presence of regeneration as the response variable, the three-group parameter for *Phytophthora* as a fixed effect, and a random intercept for focal tree species. Again, P-values were obtained by likelihood ratio tests of the full model with the *Phytophthora* parameter against the model without the *Phytophthora* parameter in question.

Results

Plant species

A total of 54 plant species were identified in the experimental plots. Thirty-six plant species were identified in the overstory (Appendix Table 1) and 53 species were identified in the understory with all but one of the overstory plants (*Diospyros dichrophylla*) also found in the understory. Twelve species occurred in less than 5% of the plots and were removed from the vegetation data set prior to the analyses and creation of the ordination plots. Ten of the species removed were from the overstory (Appendix Table 1). A total of 687 trees were measured in the plots, but 46 dead snags could not be reliably identified to species and were therefore excluded from analyses.

Canopy dieback values varied between species. Twenty-four species from the overstory had unhealthy trees at proportions greater than 0.1 (Figure 3). Twelve of those species had mean canopy die-back values greater than 10% (Figure 4). *Cassine peragua* subsp. *peragua*, *Cunonia capensis*, *Halleria lucida*, and *Pterocelastrus rostratus* had mean canopy dieback values greater than 20% and the proportion of trees from those species characterized as unhealthy ranged from 0.38 to 0.64. *Pterocelastrus tricuspidatus* was one of the most abundant species (65 trees) but also had the second highest proportion (0.63) of trees characterized as unhealthy (Figure 3). Twenty-eight *Ocotea bullata* trees were measured and a proportion of 0.46 of the trees were characterized as unhealthy with a mean dieback value of 10.6%.

Phytophthora *species*

Baits in the control trays were asymptomatic and no colonies representative of *Phytophthora* were isolated. Seven plots had asymptomatic baits at the end of the baiting cycle and were recorded as negative for *Phytophthora*. *Phytophthora* was not recovered from 26 plots (4 plots in Grootvadersbosch, 6 plots in Kleinbos, 1 plot in Oubos, 9 plots in Witelsbos, and 6 plots in Woodville). At least one *Phytophthora* species was recovered from 49 of the plots. A single *Phytophthora* species was recovered from 31 plots (4 plots had *P. capensis* only, 9 plots had *P. cinnamomi* only, 1 plot had *P.* sp. emzansi, and 17 plots had *P. multivora* only), two species were recovered from 16 plots (11 plots contained *P. capensis* and *P. multivora*, 2 plots contained *P. capensis* and *P. cinnamomi*, 2 plots contained *P. cinnamomi* and *P. multivora*, and 1 plot contained *P.* sp. emzansi and *P. multivora*), and three species (*P. cinnamomi*, *P. multivora*, and *P. capensis)* were recovered from two plots. Four species were recovered from two forest sites (Figure 2). *Phytophthora* sp. emzansi was recovered from only two plots in total (2.7%), one plot from each Oubos and Witelsbos forest fragments. The low recovery of this species did not permit its inclusion in statistical analyses or ordination. *Phytophthora cinnamomi* was recovered from 15 plots (20%) in total, but it was not recovered in the Woodville forest site. *Phytophthora multivora* was recovered from 35 plots (46.7%) and all five forests. *Phytophthora capensis* was also recovered in all five forest sites from 19 plots (25.3%).

Relationships between plant communities and Phytophthora *species*

The presence of at least one *Phytophthora* species did not affect the species richness (alpha diversity) of the plant communities within the experimental plots (Welch t-test, t=0.3518, p=0.7264). However, evidence was found for dissimilarity between plant communities associated with and without any *Phytophthora* species (between-factor beta diversity) (PERMANOVA, $F_{1,54}$= 2.6058, p=0.0038) (Figure 5). There was also evidence for dissimilarity between plant communities in plots containing each *Phytophthora* species separately and those where *Phytophthora* species were not recovered (PERMANOVA, $F_{3,52}$=2.1228,p=0.0007). Analysis of model contrasts revealed differences in plant communities between plots without any *Phytophthora* species and plots containing *P. cinnamomi* (p=0.0093), *P. capensis* (p=0.0177), and *P. multivora*

(p=0.0004) (Figure 6). Evidence was also found for differences in plant community composition when comparing plots containing *P. cinnamomi* to plots containing *P. multivora* (p=0.0088) and when comparing plots containing *P. multivora* to plots containing *P. capensis* (p=0.0011). However, differences in plant community composition between plots containing *P. capensis* compared to those containing *P. cinnamomi* were less evident (p=0.0521).

Plant communities from plots where any *Phytophthora* species was recovered had greater dispersion (within-factor beta diversity) than the plant communities from plots where *Phytophthora* species were not recovered (PERMDISP $F_{1,54}$= 5.8463, p=0.0196). Similarly, there was evidence for different levels of dispersion when including plots with each *Phytophthora* species separately and plots without *Phytophthora* species recovery (PERMDISP $F_{3,52}$= 4.262, p=0.0103). However, analysis of the pairwise comparisons only revealed evidence for differences in dispersion between plots where *P. capensis* was recovered compared to plots where no *Phytophthora* species were recovered (p=0.0469), plots where *P. cinnamomi* was recovered (p=0.0005), and plots where *P. multivora* was recovered (p=0.0002). Here, plots with *P. capensis* had smaller mean distances between sites and the group centroid than any of the other groups. The dispersion did not differ between plots with *P. multivora* and plots with *P. cinnamomi* (p=0.4828), nor between either of those species and the plots without *Phytophthora* species (p=0.2095, 0.1634, respectively).

Indicator species analysis revealed two plant species *(Gonioma kamassi* and *Pterocelastrus tricuspidatus)* were present when all *Phytophthora* species were absent (Table 3). In contrast, *Diospyros whyteana* and *Apodytes dimidiata* were indicators of the presence of least one *Phytophthora* species. No species could be identified as indicators for the absence of *P. cinnamomi*, however its presence was linked to *Cunonia capensis*. Three different species (*Diospyros whyteana*, *Cassine schinoides*, and *Platylophus trifoliatus*) were identified as indicators of the presence of *P. multivora* and four other species (*Olea capensis subsp. macrocarpa*, *Elaeodendron croceum*, *Sparrmannia africana*, and *Rothmannia capensis)* were indicators for the presence of *P. capensis* (Table 3).

Canopy decline and Phytophthora

The canopy health of trees was not linked to the three levels of *Phytophthora* recovery (no *Phytophthora* species, *P. cinnamomi*, and other *Phytophthora* species) in the initial model (Chisq chi=4.4579, p=0.1076). However, further analysis of the Wald estimates of the parameter revealed *Phytophthora cinnamomi* to be an important level (z=-2.042, p=0.0412). Therefore, when the model was refit with a binary parameter (present/absent) representing the recovery of *P. cinnamomi* alone, it was then possible to confirm a relationship between *P. cinnamomi* and the probability of a tree being characterized as unhealthy (Chisq chi=4.2272, p=0.03978). Furthermore, the relationship was negative, and trees were less likely to be characterized as unhealthy if *P. cinnamomi* was present.

Effect of Phytophthora *on regeneration*

Three of the focal tree species (*Olea capensis* subsp. *macrocarpa*, *Podocarpus latifolius* and *Rapanea melanophloeos)* were found to be regenerating in at least half of the plots sampled (Figure 7). However, regeneration for both *Cunonia capensis* and *Olinia ventosa* was found only in two plots (13.3%). No evidence emerged for a relationship between *Phytophthora* and the presence of focal tree regeneration for any of the focal tree species (Chisq chi=0.7108, p=0.7009). There were also no differences between groups after evaluation of the Wald estimates of the individual levels of the *Phytophthora* group parameter. Therefore, the relationship between *Phytophthora* and the seedling recruitment of the five focal tree species in this study remains unclear.

Discussion

This study represents the first attempt to explore possible microbial drivers of plant diversity in southern Afrotemperate forests. Four *Phytophthora* species were recovered from experimental plots and three were associated with distinct plant communities. The recovery of *Phytophthora cinnamomi* from four forests provided an opportunity to contrast the associated communities with those of two other putatively native species: *P. capensis* and *P. multivora*. These results further support the contribution of plant pathogens to the complexity of plant communities in natural systems

(Reynolds et al. 2003, Bagchi et al. 2014, Bever et al. 2015). They also demonstrate the different effects of exotic and native *Phytophthora* species on plant communities.

Relationships between plant communities and Phytophthora *species*

Plant communities associated with *Phytophthora* were different from those without *Phytophthora* (between-factor beta diversity). The *Phytophthora* species are either contributing to plant community dynamics through mechanisms such as the Janzen-Connell effect or the distinct plant communities provide selective environments for *Phytophthora* species colonization and survival. If the former explanation is correct, these *Phytophthora* species are major contributors to ecosystem dynamics. The results of this study were also congruent with our second hypothesis that the plant communities associated with *P. cinnamomi* were distinct from those associated with other *Phytophthora* species. There was evidence for a difference between communities associated with *P. cinnamomi* and *P. multivora*, but there was insufficient information for *P. capensis*. The difference between communities associated with *P. cinnamomi* and those associated with *P. multivora* are likely because *P. cinnamomi* was introduced to these ecosystems (Linde et al. 1999) and *P. multivora* is either native or has been present for much longer. In this scenario, the introduction of an exotic species results in a fundamental change to the plant community and structure of a forest. An alternative explanation for the differences in the communities between *Phytophthora* species is possible differences in their host specificity and aggression. Both *P. cinnamomi* and *P. multivora* are known as generalists capable of infecting many hosts (Shearer et al. 2007, Scott et al. 2009), but the overlap in host susceptibility has not been investigated and it is likely a complex mixture of host specificity and virulence with varying strengths of the interactions between hosts, *Phytophthora* species and environments (Shearer et al. 2007, Fukami and Nakajima 2013, Sarmiento et al. 2017, Belhaj et al. 2018). This could also explain why the plant communities associated with the two putatively native species, *P. multivora* and *P. capensis,* are different.

The Janzen-Connell framework is the predominant theory explaining the coexistence of many species at local levels in tropical systems (Mangan et al. 2010, Bever et al. 2012, 2015, Sarmiento et al. 2017) and the conspecific predation mechanism

is said to increase alpha diversity and reduce within-factor beta diversity (Fukami and Nakajima 2013). However, in this study there was no difference in mean species richness between plots with and without *Phytophthora* species. In contrast, the communities in plots associated with *Phytophthora* had significantly larger dispersion distances, indicating there was greater beta-diversity among the plant communities from plots with *Phytophthora* compared to the plant communities in plots without. Thus, our findings do not support our hypothesis of negative plant-soil feedback, which would have the opposite effect. Although *Phytophthora* species are associated with distinct plant communities in these forest fragments, the mean plant species richness is maintained, and the overall beta diversity is increased. We did not find evidence for differences in dispersion (within-factor beta diversity) between communities associated with *P. cinnamomi* or *P. multivora* and the communities in the plots without *Phytophthora*. However, when all of the communities from plots containing *Phytophthora* were pooled, the dispersion levels were greater in communities containing *Phytophthora*. Each *Phytophthora* species may contribute little to the overall beta diversity individually, but the effects of *Phytophthora* species cumulatively may sum into a heterogeneous forest ecosystem by creating a mosaic of plant communities through filtering out different susceptible species.

Relationships between plant species and Phytophthora species

Each *Phytophthora* species had different indicator species of their presence suggesting host preference or differing susceptibilities. Additional research such as pathogenicity trials would be especially valuable for validating these findings and strengthening our understanding of the roles of these *Phytophtohra* species. The indicator species analysis complements the community analysis where each *Phytophthora* species is associated with distinct plant communities. Although, being considered an indicator for the presence of a *Phytophthora* species implies the persistence of plants despite its presence, some of these indicator species were noticeably unhealthy. For example, *Cunonia capensis* was identified as an indicator for the presence of *P. cinnamomi*, but it also had one of the highest proportions of unhealthy trees and a relatively high mean canopy dieback value. This result represents a negative relationship between *P.*

cinnamomi and *Cunonia capensis*, but more work such as pathogenicity trials is needed to confirm this possible interaction. Similarly, but less alarming, *Olea capensis* subsp. *macrocarpa* was an indicator of the presence of *P. capensis*, but a proportion of 0.29 of trees were characterized as unhealthy and it had a mean dieback of 8.4%. *Phytophthora capensis* has been recovered from at least two species, including the roots of *Curtisia dentata*, an native tree present in 13 of our plots (Bezuidenhout et al. 2010), but it is not yet known if it can infect *Olea capensis* subsp. *macrocarpa*.

Two plant species (*Gonioma kamassi* and *Pterocelastrus tricuspidatus*) were identified as indicators of the absence of all three *Phytophthora* species. This either suggests they are unsuitable hosts for these *Phytophthora* species, or alternatively, they are especially susceptible to all three *Phytophthora* species and no longer present in areas where the *Phytophthora* species occur. Interestingly, *Pterocelastrus tricuspidatus* had the second highest proportion of unhealthy trees and a relatively high mean percent canopy die-back. This result most likely suggests other factors such as the presence of a different pathogen, an insect pest, or an acute susceptibility to changes in the environment (e.g. drought) could be contributing to its decline.

Canopy decline and Phytophthora

Unexpectedly, trees were more likely to be healthy in plots containing *P. cinnamomi* in this study. Possibly *P. cinnamomi* has already impacted the plant communities, thereby indirectly promoting the health of the remaining trees that are either tolerant species or tolerant individuals from a susceptible species. *Phytophthora cinnamomi* has been present in these forests for many decades at least. But it is likely to have been introduced hundreds of years ago when the Dutch began to use Cape Town as a port for trade routes with Indonesia in the mid 1600s (Bruijn 1980), which is near the suspected origin of *P. cinnamomi* in New Guinea (Old et al. 1984, Zentmyer 1988, Arentz 2017). The subsequent introduction of *P. cinnamomi* into these forest sites could therefore have caused shifts in the dominant species, indirectly stimulating the growth of other species in the community. This mechanism was discussed in the review by Bever et al. (2012), who noted the effect of a pathogen on the growth rate of a species could indirectly stimulate the growth rate of other species in the community. In this scenario,

indirect effects such as gaps or reduced canopy closer can favor the species remaining on the infected sites (Hansen 1999, Bishop et al. 2010). Many studies in Australia have noted shifts in the dominant species following the invasion of *P. cinnamomi* (Shearer and Dillon 1996, Laidlaw and Wilson 2003, Weste 2003, Bishop et al. 2010), which could explain the distinct plant communities associated with *P. cinnamomi*.

Alternatively, trees are actually experiencing decline from another factor such as drought, which would reduce the chances of recovering *P. cinnamomi* in the rhizosphere. For example, Weste and Marks (1987) attributed soil dryness as an explanation for the difficulty in isolating *P. cinnamomi* and Dunstan et al. (2016) noted the highest recovery of isolates were from samples with the highest soil moisture in their survey in Australia. In addition, the proportion of positive records of *P. cinnamomi* has also been linked to climatic indices used in distribution projection models (Burgess et al. 2016). However, if this scenario were accurate, we would also have expected to identify a pattern with the other *Phytophthora* species we recovered.

Phytophthora capensis and *P. multivora* were not associated with the probability of characterizing trees as healthy or unhealthy. This finding is as expected if the pathogens had co-evolved in these systems or if they had been previously introduced long before commencement of the current study and already selectively removed the susceptible species. Conversely, *Phytophthora cinnamomi* has been introduced to South Africa in 'recent times' (Linde et al. 1999), likely within the last few hundred years. The mechanism explaining their association with distinct plant communities is apparently more cryptic and complex. It is possible the effect of these species is limited to a specific life stage. For example, Simamora et al. (2017) found host susceptibility to endemic pathogens decreased as plants grew older. This concept has been well studied in the forests of Oregon, where a native root pathogen (*Phellinus weirii*) increases landscape-scale (beta) diversity by causing pockets of mortality of susceptible species and promoting colonization of a tolerant species (Hansen 1999, Hansen and Goheen 2000). In this regard and in consideration of the indicator species associated with *P. multivora*, *Pterocelstrus tricuspidatus* may be susceptible and therefore selectively removed, allowing *Diospyros whyteana* establishment. These mechanisms could explain why *P. capensis* and *P. multivora* were associated with distinct plant communities.

Phytophthora *effects on regeneration*

A final mechanism of reproductive success was tested to explain the distinction between communities with and without *Phytophthora* species. In this study, some species had low regeneration (*Olinia ventosa* and *Cunonia capensis)* while others had relatively high levels of regeneration (*Olea capensis* subsp. *macrocarpa*, *Podocarpus latifolius* and *Rapanea melanophloeos)*, but neither could be linked to the presence or absence of *Phytophthora* species at the time of sampling. Therefore, the results did not support the hypothesis for a relationship between the presence of *Phytophthora* and levels of regeneration of the focal tree species. This could be due to differences in plant responses to plant pathogens as suggested by Fukami and Mifuyu (2013) or the possible associations with other beneficial microbes such as arbuscular mycorrhiza (AM). All three of the species with relatively high levels of regeneration are noted to have AM associations (Hawley and Dames 2004) and AM associations have been shown to protect tree roots from the pathogens (Wehner et al. 2011) and counteract the Janzen-Connell effect (Liang et al. 2015). These associations could therefore be the reason we could not detect a relationship between *Phytophthora* and the presence of regeneration in our plots.

Phytophthora *species origins*

Unlike *P. cinnamomi*, it is not known whether *P. capensis*, *P. multivora*, or *P.* sp. emzansi have been introduced into South Africa, but our findings may support the hypothesis that they are native in this system. For example, *P. capensis* and *P. multivora* had greater distributions and were recovered more frequently than *P. cinnamomi*. *Phytophthora capensis* and *P. multivora* were also recovered together from 11 plots, where *P. cinnamomi* was only recovered from 4 plots with either of the other species. In addition, *P. multivora* was associated with a plant community distinct from the plant community associated with *P. cinnamomi*. Also, no evidence was found for a connection between the presence of these *Phytophthora* species and decreased crown health. Finally, *P. capensis*, *P. multivora*, and *P. sp.* emzansi are all closely related (Yang et al. 2017). It is consequently plausible these species have radiated from a common ancestor from this area as has been suggested with subclades in other countries (Burgess et al. 2017a).

Collectively, these results may be the best indicators presently available for the origins of these *Phytophthora* species.

Although *P. multivora* and *P. capensis* are relatively 'well-behaved' in the forests sampled in this study, they could become the next destructive species in forests elsewhere in the world (Hansen 2015). For example, *P. multivora* has a global distribution and it has recently emerged as a pathogen of native species in Australia (Scott et al. 2009, Burgess et al. 2017b), where disease severity suggests the pathogen has been introduced. It is also suspected to be involved in oak decline in the Czech Republic (Mrázková et al. 2013) and Hungary (Szabó et al. 2000), and has also been recovered from laurel forests of the canary islands (Rodríguez-Padrón et al. 2018). *Phytophthora capensis* was described from South Africa (Bezuidenhout et al. 2010), but it has also been found in natural ecosystems in Taiwan (Jung et al. 2017) and Australia (Burgess et al. 2017b). *Phytophthora* sp. emzansi is known only from South Africa, but it has not been formally described because only two isolates were recovered in original study (Bezuidenhout et al. 2010), with a few more isolates from other locations recovered later (Oh et al. 2013). The known distributions of these species and the difference between their associations in the forest fragments provide insights into their origins, but insufficient evidence to make confident assertions.

Conclusions

Phytophthora cinnamomi was associated with a distinct plant community. However, we did not recover this pathogen from all of the forest sites sampled even though it has been present in this system for many decades (Lübbe and Mostert 1991). We therefore suggest there is merit in preventing its further spread and containing this pathogen in areas where it is known to occur. Regrettably, this study represents another example of the changes caused by this highly invasive and globally distributed oomycete (Burgess et al. 2016, Sena et al. 2018). It also emphasizes the importance of supporting biosecurity initiatives to prevent the introduction of *Phytophthora* species into natural systems.

Although more research is needed to identify the mechanisms that explain the differences and higher dispersion in the plant communities associated with each of the

Phytophthora species recovered in this study, it remains possible they are responsible for the differences in plant communities observed. If this were true, it would mean these species are contributing to the regional heterogeneity or beta-diversity of the system. In general, the niche of each putatively native *Phytophthora* species identified in this study remain unknown, likely because they have co-evolved in a cryptic manner, but also potentially because this study was too limited to detect the mechanisms these *Phytophthora* species contribute to the system.

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Tables

Table 1: Site locality and description data for the forest sites sampled in this study.

Forest Site	Latitude	Longitude	Fragment Size (ha)	Site Description
Oubos	-34.080344	19.829460	65.1	Relatively undisturbed, privately owned natural forest patch adjacent to citrus orchard.
Grootvadersbosch	-33.982826	20.822967	548.3	Forest recreation site maintained by Cape Nature.
Kleinbos	-33.935733	22.135145	206.3	Forested land owned and actively harvested by Mossel Bay Municipality.
Woodville	-33.936389	22.623478	1035.3	Forested land owned and actively harvested by South African National Parks Board.
Witelsbos	-33.989547	24.097755	765.8	Forested land owned by South African National Parks Board, historically harvested.

Table 2: Isolate data for representative isolates collected from the rhizosphere of the focal trees at the specified locations.

Isolate	Species	Forest	Focal tree	Site	Lat	Long	ITS Asscession
ATF266	*Phytophthora multivora*	Woodville	*Olinia ventosa*	WV04	-33.932669	22.622927	
ATF509	*P. multivora*	Kleinbos	*Podocarpus latifolius*	KB5	-33.935179	22.133191	
ATF048	*P. cinnamomi*	Witelsbos	*C. capensis*	WEB10	-33.985484	24.100357	
ATF407	*P. cinnamomi*	Grootvadersbosch	*C. capensis*	GVB05	-33.981590	20.824287	
ATF610	*P. capensis*	Kleinbos	*Afrocarpus falcatus*	8-2	-33.934757	22.132654	
ATF229	*P. capensis*	Kleinbos	*Olea capensis* subsp. *macrocarpa*	KB07	-33.934396	22.132841	
ATF571	*P. sp. emzansi*	Woodville	*A. falcatus*	4-1	-33.931492	22.622779	
ATF634	*P. sp. emzansi*	Kleinbos	*A. falcatus*	8-1	-33.934521	22.132844	

Table 3: Significant plant indicator species for the presence or absence of each and all *Phytophthora* species.

Phytophthora Species	Status	Plant Species	Indicator value[1]	Frequency[2]	Pvalue[3]
All species lumped	Absent	*Gonioma kamassi*	0.485725615	38	0.008
All species lumped	Absent	*Pterocelstrus tricuspidatus*	0.445342474	30	0.003
All species lumped	Present	*Diospyros whyteana*	0.433839069	19	0.005
All species lumped	Present	*Apodytes dimidiata*	0.2446470	15	0.045
P. cinnamomi	Present	*Cunonia capensis*	0.416260163	17	0.005
P. multivora	Absent	*Gonioma kamassi*	0.43964452	38	0.021
P. multivora	Absent	*Pterocelstrus tricuspidatus*	0.438848462	30	0.004
P. multivora	Present	*Diospyros whyteana*	0.389225589	19	0.004
P. multivora	Present	*Cassine schinoides*	0.26187804	12	0.003
P. multivora	Present	*Platylophus trifoliatus*	0.151515152	5	0.012
P. capensis	Present	*Olea capensis subsp. macrocarpa*	0.520653713	45	0.030
P. capensis	Present	*Elaeodendron croceum*	0.379799339	28	0.037
P. capensis	Present	*Sparrmannia africana.*	0.324667089	14	0.013
P. capensis	Present	*Rothmannia capensis*	0.269987947	8	0.012

[1]Indicator values for significant species discovered with INDVAL function in R-package labdsv.
[2]Number of plots that the species was observed
[3]Probability of obtaining observed indicator value or higher over 1000 iterations.

Figures

Figure 1: Distribution of the five forest sites sampled across the southern Cape of South Africa.

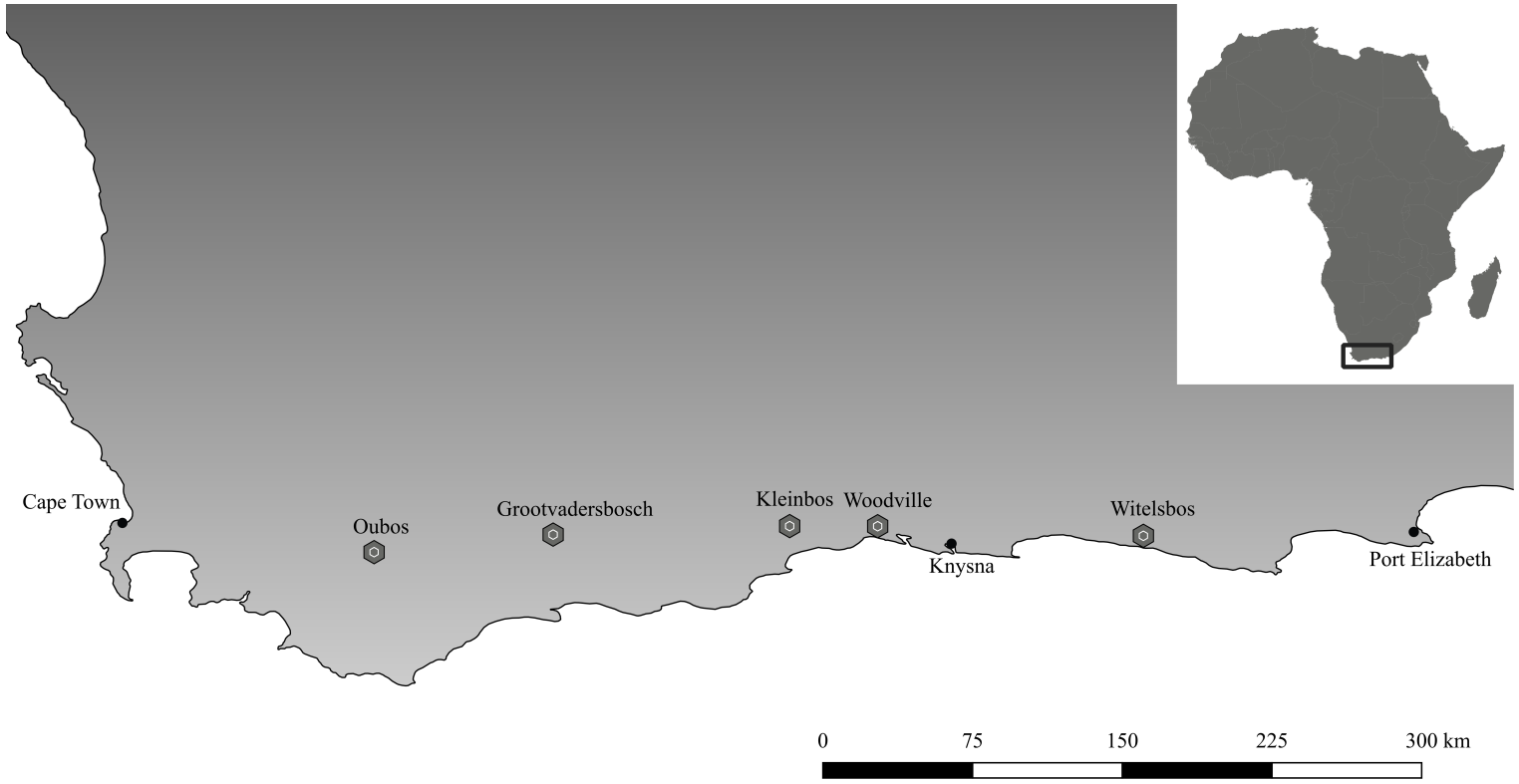
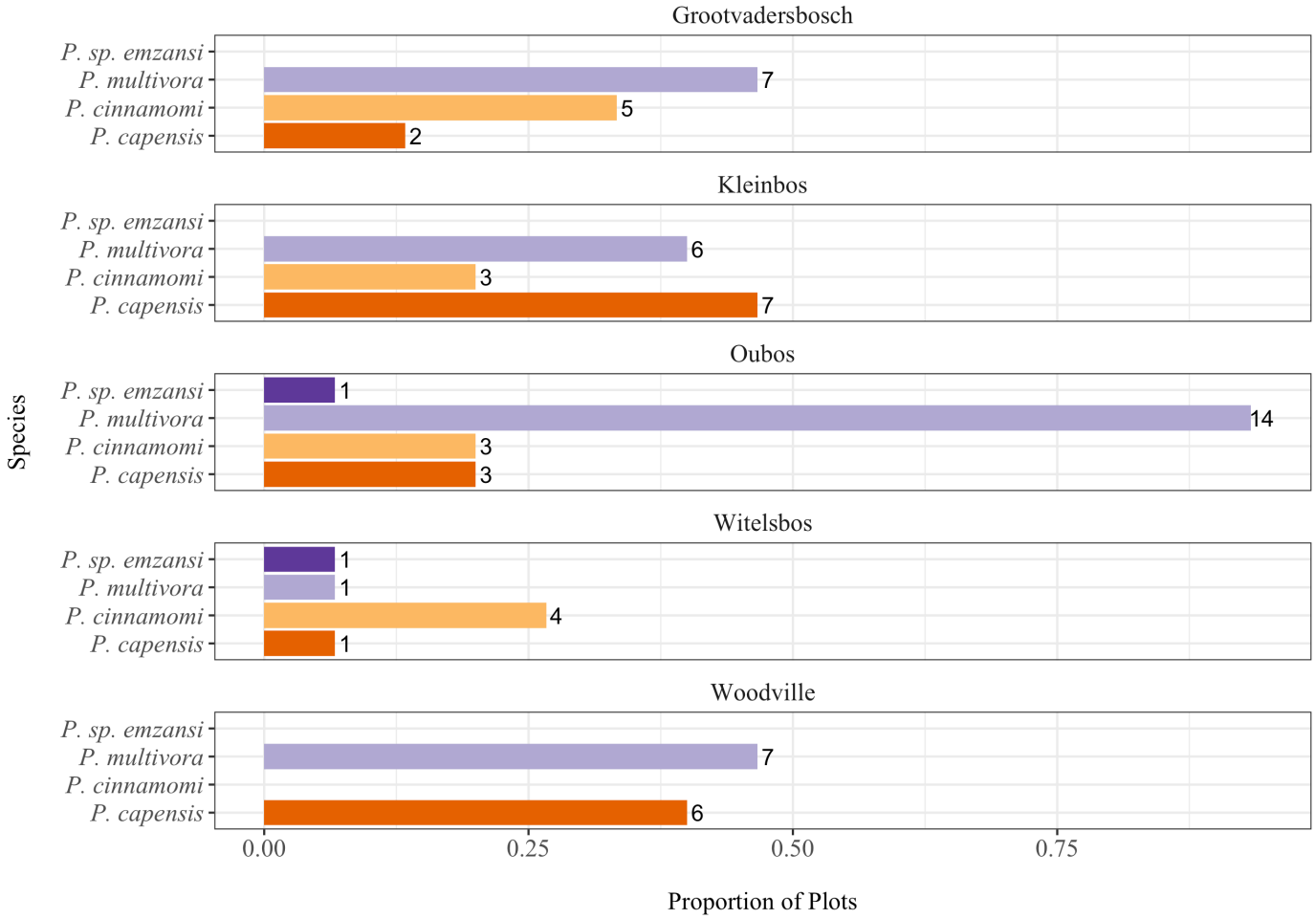
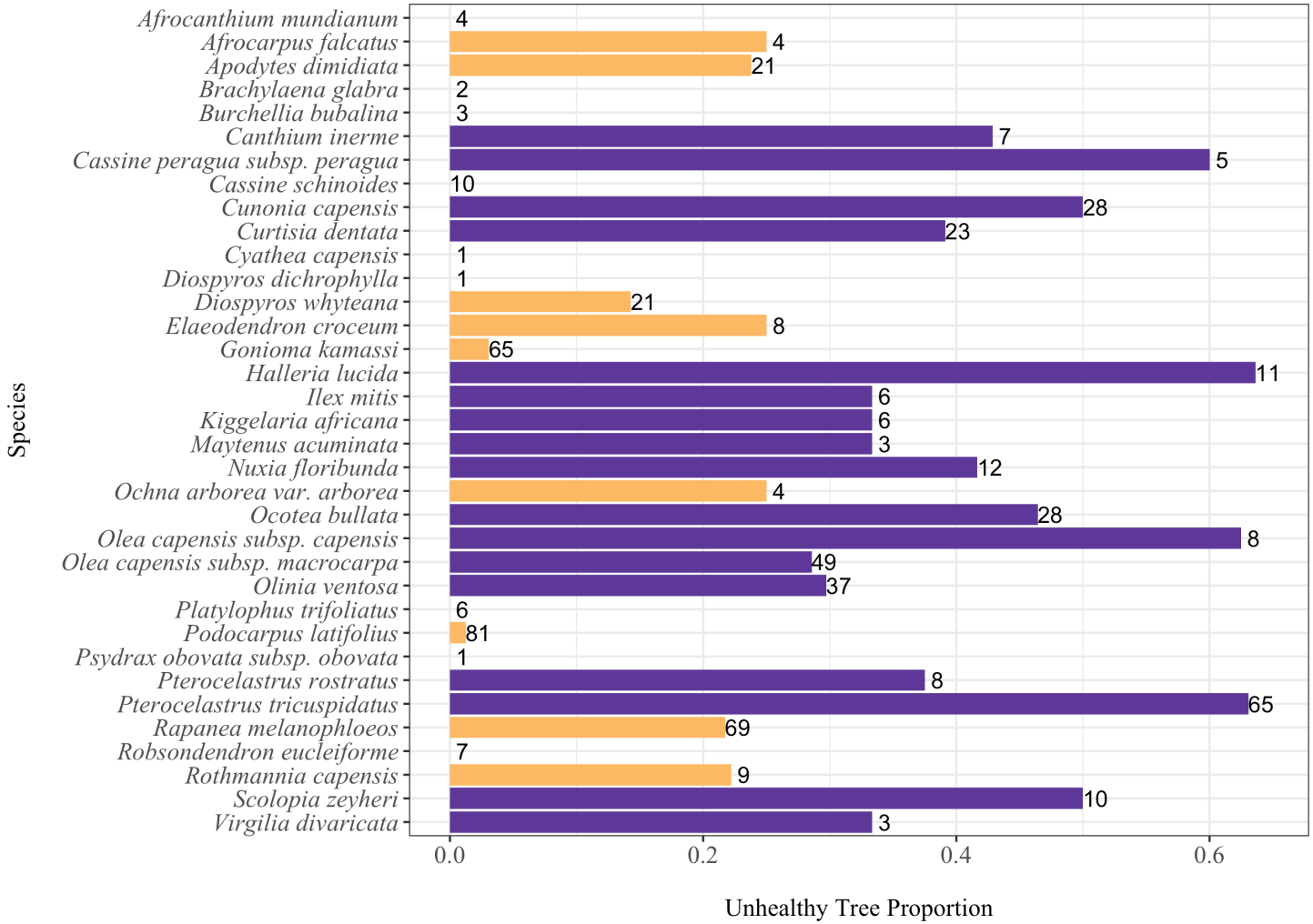


Figure 2: Proportion of plots containing each *Phytophthora* species from each forest.



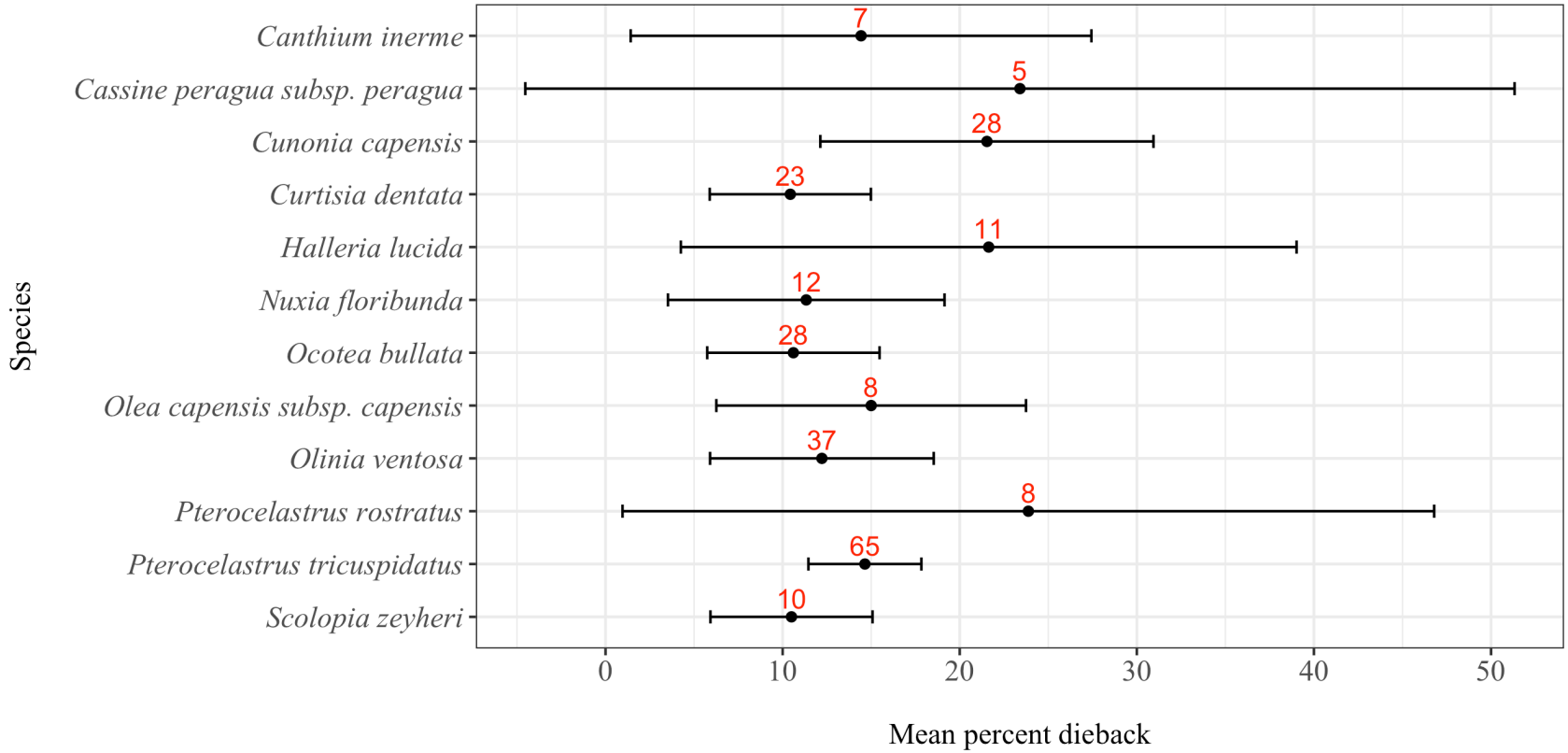
Numbers represent the number of plots where *Phytophthora* was recovered per forest.

Figure 3: Proportions of trees that were unhealthy (canopy dieback >10%) for species in overstory.



Dark colored bars indicate species with proportions greater than 0.25. Numbers represent the number of trees measured per species.

Figure 4: Mean percent canopy dieback for tree species with a mean percent canopy dieback greater than 10.



Numbers indicate the number of trees measured. Error bars are 95% confidence intervals for the mean.

Figure 5: Ordination plot of NMDS values for plant species at sites where at least one *Phytophthora* species was isolated (red) versus sites without recovery of *Phytophthora* species (black).

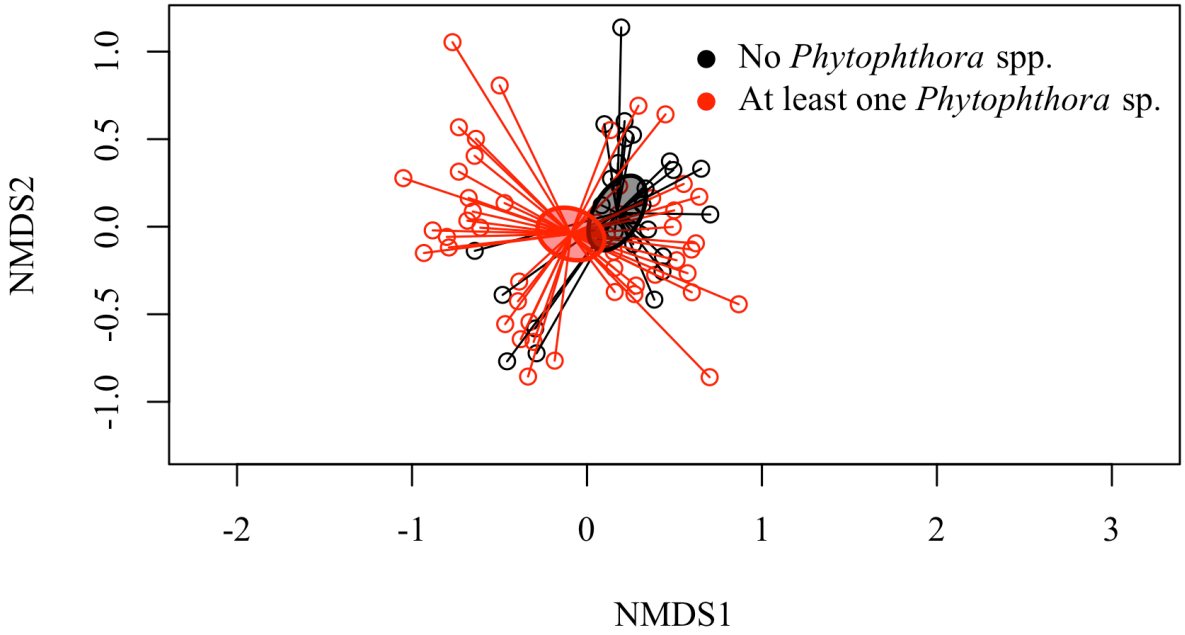
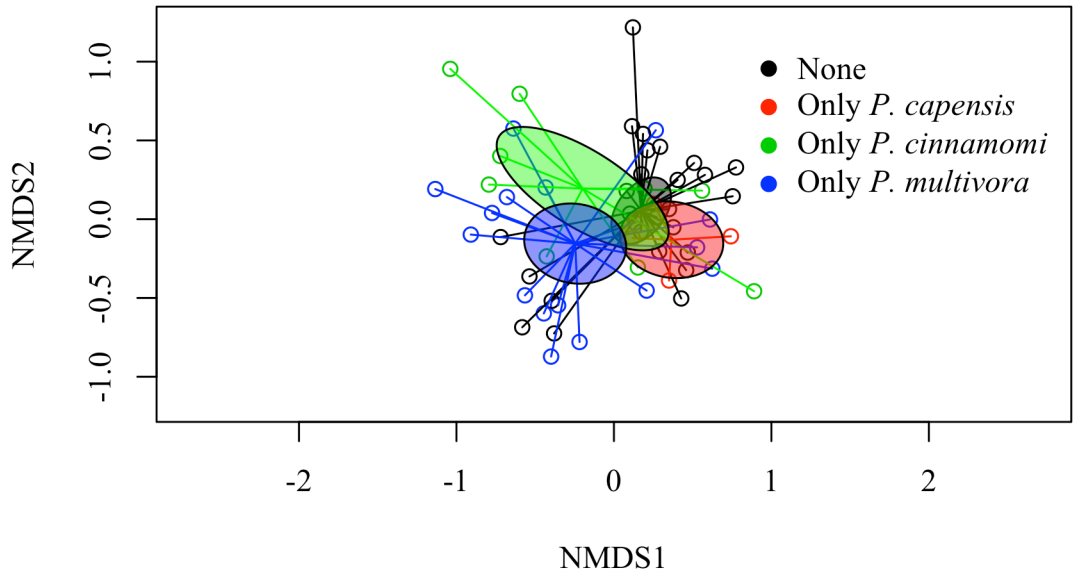
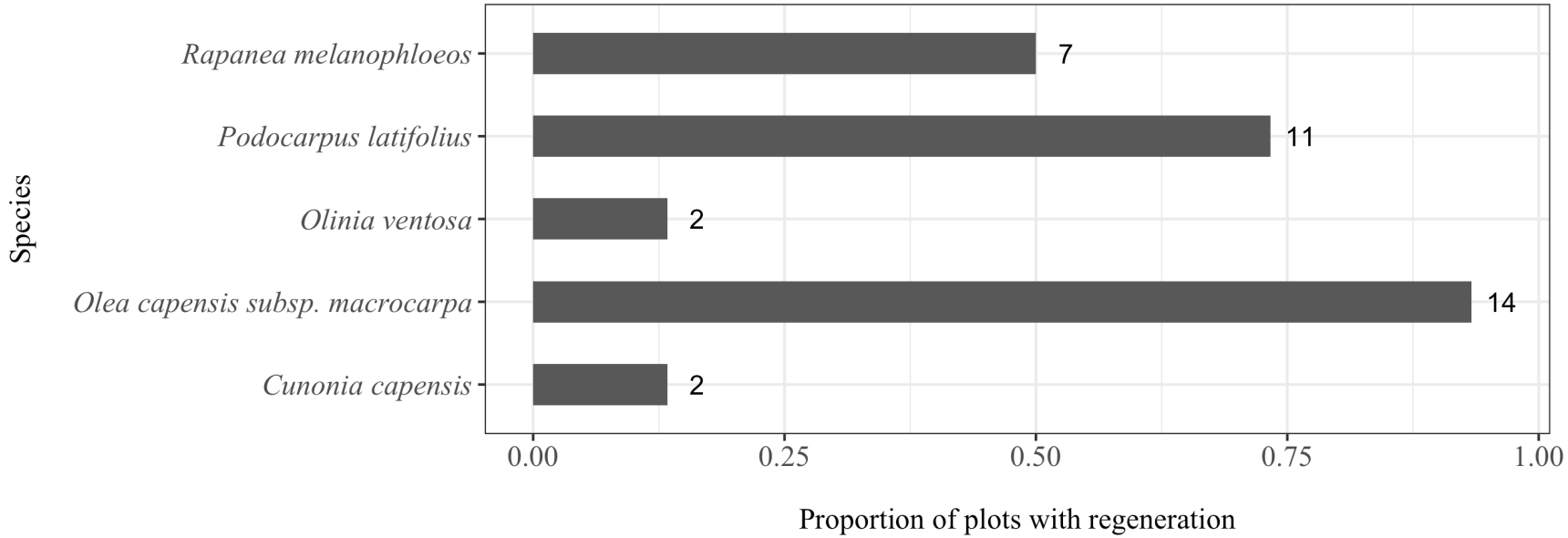


Figure 6: Ordination plot of NMDS values for plant communities in sites where each *Phytophthora* species was recovered individually.



Black: plots where *Phytophthora* spp. were not recovered, red: plots where only *P. capensis* was recovered, green: plots where only *P. cinnamomi* was recovered, and blue: plots where only *P. multivora* was recovered.

Figure 7: Proportion of plots with regeneration for each focal tree species.



Number represents the number of plots where regeneration was present.

CHAPTER FOUR

Citizen science can enhance biosecurity in countries with developing economies

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Abstract

Plant pathogens such as *Phytophthora* species continue to emerge as causal agents of novel disease epidemics because of the global movement of plants and plant products. The introduction of these organisms is often undetected because of lack of biosecurity capacity, especially in regions with developing economies. Citizen science initiatives can decrease the frequency and risk of pathogen establishment by enhancing early detection and raising risk awareness. In this study, we initiated a citizen science program to detect and measure *Phytophthora* diversity in the Western Cape Province of South Africa. Citizens contributed to research through four primary methods: sample submissions, unhealthy plant reports, invited extension visits, and through educational hikes and workshops. Six-hundred and sixty-six samples were collected with or submitted by citizen scientists. Sixteen *Phytophthora* species and one informally described species were recovered. Four species had not been found in South Africa previously. Citizen science added capacity to sample collection. For example, 13.3% of the samples could not have been collected without citizen participation, 26.2% could have been collected, but the costs were offset by citizen participation, and an additional 4.8% were only collected following citizen invitations. Nine *Phytophthora* species were recovered only via citizen participation and the highest *Phytophthora* recovery proportions were from rhizosphere samples submitted for diagnostics by professionals in horticulture or conservation. The results show there is merit of involving non-scientists in plant disease research. They also demonstrate citizen science can increase the capacity for the early detection of microscopic organisms.

Introduction

Methods to enhance biosecurity are needed to alleviate the increasing threats of our global connectivity. The trend of international trade and consequently the number of forest pest and pathogen invasions is increasing (Hulme 2009, Santini et al. 2013, Hurley et al. 2016, Scott et al. 2019), but even now there is not sufficient capacity to monitor major pathways of biological invasions in developed economies such as the US (Liebhold et al. 2012). Furthermore, regulatory and systematic monitoring for introductions post-

border is expensive (Parnell et al. 2015, Freer-Smith and Webber 2015, Cunniffe et al. 2016) and can be cost-prohibitive in developing and emerging economies (Nuñez and Pauchard 2009, Early et al. 2016). In addition, although many pathways for tree pest and pathogen invasions (e.g. live plants, wood pallets) have been documented (Brasier 2008, Liebhold et al. 2012, Haack et al. 2014), many stakeholders and consumers who drive demand of these pathways are unaware of the risk (Marzano et al. 2015, 2016). Therefore, programs that increase monitoring capacity and raise awareness at relatively low costs would enhance biosecurity and alleviate the threats of globalization.

Of particular concern are *Phytophthora* species. Many diseases in natural systems (e.g. sudden oak death, *Phytophthora*-dieback in *Banksia* woodlands, and port-orford cedar decline) are consequences of the movement and introduction of *Phytophthora* species to new environments (Podger 1972, Linde et al. 1999, Hansen et al. 2000, Grünwald et al. 2012). The rate at which this is now occurring is unprecedented and linked to increased levels of global trade and the inadvertent movement of disease initiating propagules (Brasier 2008, Hansen 2015, Scott et al. 2019). Shipping of infected horticultural stock is a major pathway of *Phytophthora* dissemination between continents (Brasier 2008, Santini et al. 2013, Jung et al. 2016, 2018). However, despite an awareness of this risk, countries continue to engage in the international trade of horticultural stock because of the economic value and consequently risk the emergence of serious plant disease problems. Therefore, new methods to alleviate the propagule pressure from trade are critically needed to limit the negative effects of *Phytophthora* species invasions.

Citizen science is an approach that can enhance biosecurity (Brown et al. 2017, Thomas et al. 2017, Baker et al. 2018). Many citizen science programs exist because the approach can increase sampling coverage over space and time, provide access to private areas and offset prohibitive data collection costs (Bonney et al. 2009, Meentemeyer et al. 2015). Citizen science initiatives also simultaneously achieve educational outcomes (Bonney et al. 2009, 2016) and in some cases, have increased the awareness of participants about the impacts of invasive species and anthropogenic disturbance (Jordan et al. 2011, Branchini et al. 2015). The success of engaging the public in *Phytophthora* research has also been demonstrated—see Meentemeyer et al. (2015) and Lione et al. (2017)—and there are ongoing initiatives globally. Therefore, because these initiatives

can offset costs, raise awareness, and increase coverage (especially in urban areas), the citizen science approach has considerable merit for alleviating the threats of forest pathogens such as *Phytophthora* species.

Citizen science may have exceptional merit in developing economies without taxpayer funded monitoring programs. For example, Nuñez et al. (2009) suggests developing economies face greater challenges for managing invasive species because of limited access to education, greater difficulty in organizing volunteers, smaller scientific communities and lack of baseline data. On the other hand, developed countries with programs such as university extension and statutory monitoring have greater capacity to detect invasions once an organism has crossed the border (Burgess 2003). For example, the first report of sudden oak death in the USA was examined by university extension services in California (Garbelotto et al. 2001, Rizzo et al. 2002), but the detection may not have occurred as rapidly without that service and many developing countries do not have this biosecurity infrastructure (Ryan et al. 2018). South Africa, for example, does not have taxpayer-funded programs or statutory capacity to monitor the domestic trade of live plants for plant diseases. This is of concern because of the exceptional floral diversity (Goldblatt 1997, Myers et al. 2000) and the economical benefit of the live plant export market in South Africa (SARS 2019). Therefore, programs that enhance the capacity of post-border biosecurity are critically needed in South Africa to protect its biodiversity and bioeconomy.

South Africa is an example of a country that needs additional initiatives to increase its biosecurity because it is listed as a country with an 'emerging market and developing economy' by the World Economic Outlook Database (WEO 2019) and it continues to engage in the international trade of horticultural stock. According to the South African Revenue Service, live trees and other plants (materials listed as Customs and Excise Tariff Chapter 6 commodities) were imported into South Africa via roads, rail, maritime vessels, aircrafts and mail, and reported by 39 district offices (points of entry) since 2010 (SARS 2019). This is concerning considering the Department of Agriculture, Forestry and Fisheries (South Africa's regulatory agency), only reported inspection expenditures of R377,556,000 (~$25,380,000 USD) in their fiscal 2017/2018 report for their Agricultural Production, Health and Food Safety Programme (DAFF

2018). Assuming each district office received an equal budget for inspection, each office was limited to an annual budget of R9,681,000 (~$650,600 USD) to inspect live plants imported into South Africa during the last fiscal year. South Africa is therefore an example of a situation where the economy does not provide sufficient capacity to effectively prevent invasive plant pest and pathogen introductions.

In this study, we explored methods of engaging the public in research about *Phytophthora* species to enhance the biosecurity of South Africa. Citizens in the Cape Floristic Region biodiversity hotspot were invited to contribute to the research through four methods: online unhealthy plant reports, sample submission for diagnosis, educational hikes and workshops, and extension activities. We hypothesized citizen contributions would enhance the breadth of the research because other programs have demonstrated increased sampling capacity from citizen participation (Meentemeyer et al. 2015, Lione et al. 2017). We aimed to provide recommendations for other programs based on the findings of the different methods. Overall, the purpose of study was to determine if and how citizen science programs could add capacity to biosecurity efforts, especially in a country without current taxpayer investments in post-border monitoring such as university extension or regulatory monitoring.

Methods

Cape Citizen Science

Cape Citizen Science (https://citsci.co.za) was initiated in the Western Cape Province of South Africa in 2016 through support from the Forestry and Agricultural Biotechnology Institute at the University of Pretoria and based in the Department of Conservation Ecology and Entomology at Stellenbosch University. The pilot study was designed to reveal the diversity and distribution of *Phytophthora* species in the Greater Cape Floristic Region. Citizens were invited to participate in the research through four primary methods. Additional outreach activities were organized to raise project awareness and a few citizens and youth also volunteered in the laboratory (Hulbert and Roets 2018). Two marketing phases (‘Go Outside for Science’ and ‘The Cape Town Hypothesis Test’) were also designed to inspire public interest, but engagement was too low to produce meaningful results because of inherent challenges discussed in Hulbert et

al (2019b). In all cases, submissions were invited for the purpose of contributing to research. For example, even though we provided diagnostics to identify the *Phytophthora* species in submitted samples, most submissions (especially from nature reserves) were contributed with the motivation to contribute to research and reveal the diversity, confirm or deny the possibility of a new invasion, or understand their role in the decline of planted species (e.g. samples submitted from home gardens).

Public Engagement

Unhealthy plant reports

Citizens were invited to report unhealthy plants in urban and natural areas using two already existing online tools. Reports were initially collected on Ispot (https://ispotnaturalist.org) (Hulbert 2016), but our emphasis shifted to iNaturalist (https://inaturalist.org) in early 2018 following a general shift in the greater conservation community to accommodate a smartphone application (Pers. comm. T. Rebelo 2018). In both tools, projects were created to curate observations made by citizens. Individuals could add an observation to the project on Ispot by adding the tag 'dyingfynbos' to the observation. Similarly, citizens contributed to the iNaturalist project by joining the project and then tagging the project in the observation. We were also able to add a few pre-existing unhealthy plant reports by searching for the words 'dying', 'dead', 'unhealthy', and 'sick' on Ispot. In one case, a citizen contacted the authors via email and was subsequently asked to add observations to the iNaturalist project.

Further exploratory analysis of the observations was conducted by comparing the plant species to the host lists of *Phytophthora cinnamomi* provided in von Broembsen (1984). Similarly, the plant species were also compared with the redlist data provided and curated by the South African National Biodiversity Institute (2017). Observations that could not be identified to species on iNaturalist were not compared to these databases.

Workshops and youth hikes

Ten workshops were organized at 9 locations with stakeholders that involved a half-day training and sampling activity. Activities were organized with agencies such as South Africa National Parks (SANParks), South African National Biodiversity Institute

(SANBI), the Western Cape Provencal conservation agency Cape Nature, as well as Stellenbosch and Cape Town municipalities and Stellenbosch University. Four activities were organized at three managed gardens, four activities were organized at four national parks and two activities were organized at two nature reserves. Workshops were generally held at stakeholder offices within the national park, nature reserve, managed gardens or municipalities. Each workshop involved a presentation, sampling conducted by and with the staff, and an activity to isolate pathogens from diseased plant tissues. Presentations were designed to raise awareness of common plant disease symptoms and the threats and pathways of exotic plant pathogens with the hope that stakeholders would report observations or submit samples afterward. Samples were collected with and by the staff to obtain plant pathogen isolates and introduce methods of sampling and diagnostics during workshops. We also sampled the rhizosphere with garden staff in three botanical gardens and the results are included in Hulbert et al. (2019a). In all cases, professional staff were asked to write their name on the petri-plates to quantify the number of citizens and samples contributed.

Youth hikes were organized at 11 nature reserves or national parks. One activity was also organized at Kirstenbosch National Botanical Garden. The purpose of these activities was to raise awareness of the field of plant pathology while simultaneously collecting samples to reveal the diversity of *Phytophthora* in the areas where the hikes were organized. The activities were organized similarly to workshops, but presentations also introduced the process of research and the importance of biodiversity and involved hikes into nature reserves or national parks to collect samples. Youth were invited to conduct primary isolations from diseased plant tissues and also asked to write their name on the petri-plates. However, some of primary isolation plates were sent with teachers or group leaders so the youth could observe the microbial growth. Four groups of youth from the South African Education and Environment Project also participated in a study to evaluate the educational merit of participating.

Extension visits

The authors were invited to collect samples from specific properties such as home gardens or subsequent areas of concern in managed gardens or municipalities on a few

occasions. In general, this form of engagement refers to samples collected by the researcher in areas identified by landowners and managers. Here we refer to this form of engagement as extension visits because it provided a similar role to extension programs in developed countries (Ryan et al. 2018), however our investigations were motivated as a form of research rather than service and were limited to testing for the presence of *Phytophthora* species.

Sample diagnostics

The final method of engagement invited citizens to collect and submit samples to our laboratory for *Phytophthora* diagnostics. Citizens and professionals were instructed to collect rhizosphere samples from the base of plants and keep them in unsealed zipblock bags or empty ice-cream containers at room temperature until they could be delivered to us. We received samples from plant nurseries, home gardens, nature reserves and national parks. We also received potted-plants from five plant nurseries.

Research capacity and achievability

Samples were categorized in four categories of research achievability to quantify citizen contributions to the capacity of the research. The four categories were:

- Unachievable without citizen participation,
- Achievable, but required invitation from citizen to collect sample,
- Achievable without citizen participation,
- Achievable, but citizen participation offset the costs of sampling.

Samples submitted from private properties by citizens (e.g. private land and plant nurseries) for diagnostics were considered ‘unachievable without citizen participation’. Samples collected on private properties following an invitation (extension services) from the landowner or property manager were classified as ‘achievable, but required invitation’. In contrast, all samples collected by researchers following invitations (extension services) from nature reserves or managed gardens were categorized as ‘achievable without citizen participation’ because they generally support and encourage

research. All samples collected during educational activities (workshops and youth hikes) were also categorized as 'achievable without citizen participation' because the researchers were present during the activities, demonstrating and working with the participants. Samples submitted by citizens for diagnostics from areas that researchers had permits for (nature reserves, national parks, managed gardens) were considered 'Achievable, but the costs of sampling were offset by citizen participation'.

Sample Collection and* Phytophthora *Isolation

Phytophthora species were isolated from two types of samples. Citizens either performed primary isolations from unhealthy plant material during activities with the authors (workshops and youth hikes) or collected and submitted rhizosphere samples (e.g. bags of soil and fine roots, generally from under unhealthy plants). Primary isolation of *Phytophthora* species were also attempted from unhealthy tissues (e.g. cankers, necrotic areas, decaying root tips, etc.) on plants submitted from plant nurseries or plants examined during extension visits. Most samples collected by youth during hikes were directly isolated from unhealthy plant tissues. In this sample type, excised pieces of the plant tissues were placed onto agar in petri-plates that contained a combination of antibiotics and chemicals to promote *Phytophthora* growth and inhibit other organisms (Erwin and Ribeiro 1996) (15g corn meal agar, 10mg Rifampicin, 25mg PCNB, 25mg Hymexezol, 50mg Nystatin, and 200mg Ampicillin per litre).

Phytophthora species were isolated from the rhizosphere samples by baiting, a method that used healthy plant tissues (*Hedera helix* and *Quercus* sp. leaves or *Rosa* sp. petals) as baits for motile zoospore infection after flooding the soil and fine roots with de-ionized water (Marks and Kassaby 1972). Baiting methods were consistent with those reported in Hulbert et al. (2019a). Symptomatic baits were excised and small pieces from the advanced margin of necrosis were placed onto petri-plates containing the same selective agar mixture. At least two additional trays were set up during each baiting session as negative controls that contained only de-ionized water and the baits. Rhizosphere samples were also tested from the potted plants submitted by plant nurseries.

Phytophthora *identification*

Ten to 16 day old cultures on half strength Potato Dextrose Agar (20g PDA and 8g agar in 1 litre DI water) that resembled *Phytophthora* colonies were grouped based on morphology and colony characteristics and 1-3 representative isolates were identified by amplifying and sequencing the ITS region of extracted ribosomal DNA. DNA was extracted by scraping mycelia from 20 day old cultures into 1.5ml Eppendorf® tubes and adding 60µl Prepman® Ultra Extraction Reagent (Applied Biosystems, Cheshire, United Kingdom) then following methods suggested by the manufacturer. The ITS region was amplified using methods outlined by previous studies with primers ITS4 and ITS6 (White et al. 1990, Cooke et al. 2000, Kroon et al. 2004). Amplicons were sequenced by the Central Analytical Facility at Stellenbosch University, South Africa. Forward and reverse sequences were then pairwise aligned with MUSCLE using Geneious version 10.2.3 (Kearse et al. 2012). The Contigs were then compared to an internal database of curated sequences for published *Phytophthora* species using BLAST and subsequently confirmed by BLAST using the GenBank nucleotide database. Phylogenetic analysis was also completed using the Neighbor-Joining method for species that did not have 100% pairwise identity in our internal dataset also using Geneious version 10.2.3. Overall, the methods to identify the *Phytophthora* species were identical to the methods presented in Hulbert et al. (2019a).

Results

In total, 666 samples were collected with or by citizen scientists from 427 sites. Citizens collected 490 samples and the authors collected an additional 176 samples during engagement activities with citizens. Two hundred and fifty-nine citizens participated in one or more of the engagement methods.

Phytophthora species were recovered from 144 samples submitted or collected through the engagement methods. Sixteen *Phytophthora* species and one informally described species were recovered. No *Phytophthora* species were recovered from the negative controls.

Research capacity and achievability

Three hundred and seventy-three samples (55.7%) collected or submitted were categorized as achievable without citizen participation. An additional 175 samples (26.2%) were categorized as achievable, but the costs were offset by citizen participation. Thirty-two samples (4.8%) were collected following invitations to visit 10 properties and an additional 89 samples (13.3%) were categorized as 'unachievable without citizen participation'.

Eight *Phytophthora* species (including *P.* sp. emzansi) were recovered through methods considered achievable by the researchers without citizen participation (Table 1, Figure 1). Ten *Phytophthora* species were recovered from the samples considered achievable, but had the costs of collecting offset by citizen scientists. Four *Phytophthora* species were recovered from samples collected following invitations from citizens and eight species were recovered from samples categorized as unachievable without citizen participation.

In total, nine *Phytophthora* species were recovered exclusively because of citizen participation in the research (Figure 1). Four species, *Phytophthora chlamydospora*, *P. humicola*, *P. kwongonina*, and *P. megasperma* were recovered only because of citizen contributions that offset the costs of sampling. Two additional species (*P. neiderhauserii* and *P. nicotianae*) were recovered only from samples collected following invitations from citizens. *Phytophthora bilorbang, P. citrophthora,* and *P. cryptogea* were recovered only from citizen contributions considered to be unachievable without their participation.

Engagement method

Online unhealthy plant reports

Seventeen citizen scientists cumulatively shared 113 observations of unhealthy plants from 70 species online. Nineteen of the observations shared online could not be reliably identified to species by the iNaturalist community. However, 72 observations were considered 'research grade' indicating multiple people in the community agreed on the plant species identification by the time the data was accessed. Eighteen of the observations were of four endangered species and an additional 19 observations were of species that are listed as near threatened, vulnerable, or rare.

Thirty-one observations of 13 plant species that are susceptible to *P. cinnamomi* were shared. The remaining 81 observations were of 57 species with unknown susceptibility to the pathogen. Sixteen observations were shared of the three endangered species that are susceptible to *P. cinnamomi* (*Leucadendron argenteum*, *Leucospermum conocarpodendron*, and *Mimetes argenteus*). Individual observations of unhealthy plants from species susceptible to *P. cinnamomi* were shared for two species classified as near threatened (*Curtisia dentata* and *Protea susannae*) and two species classified as vulnerable (*Leucadendron galpinii* and *Leucospermum patersonii*).

Samples were collected from one of the species reported online, *Harpephylum caffrum*. The initial report was for a street tree in Cape Town with substantial gummosis and bleeds extending upward from the base of the tree. The susceptibility of *Harpephylum caffrum* to *P. cinnamomi* is unknown, but *P. neiderhauserii* was directly isolated from the reported tree (Table 2).

Workshops and youth hikes

Seven species were recovered during workshop activities with 49 citizens (Table 1). Five species (*P. amnicola*, *P. asparagi*, *P. capensis*, *P. cinnamomi*, and *P. multivora*) were recovered during workshops with managed garden staff. Four species (*P. capensis*, *P. cinnamomi*, *P. inundata*, and *P. multivora*) and the informal *P.* sp. emzansi were recovered during workshops with national park staff. Only *P. cinnamomi* was recovered during a workshop with nature reserve staff. *Phytophthora* species were directly isolated from 4 (9%) samples and baited from 39 (41%) of the rhizosphere samples collected during workshops (Figure 2).

One hundred and forty-eight youth participated and contributed isolates from 153 samples. *Phytophthora* species were only directly isolated from 3 (2%) of the samples, but 4 (21%) of the rhizosphere samples collected during youth hikes contained *Phytophthora* (Figure 2). *Phytophthora cinnamomi* was recovered from two sites in Jonkershoek Nature Reserve, one site in Helderberg Nature Reserve and three sites in Kirstenbosch National Botanical Garden. *Phytophthora multivora* was also recovered from one site in Kirstenbosch and one site in Helderberg Nature Reserve during youth hikes.

Extension visits

Eighty-six samples were collected from 47 sites during extension visits on 18 properties. Fifty-one of the samples were attempts to directly isolate *Phytophthora* species from unhealthy plant tissues and 38 were collected from the rhizosphere beneath unhealthy plants (Figure 2). Samples were collected from 6 home-gardens, 4 managed gardens, 3 nature reserves, 1 national park, 3 plant nurseries, and 1 urban planting site. Twenty-four samples were collected in home gardens, 25 samples were collected from nature reserves, and 28 samples were collected from managed gardens during extension visits (not including samples collected during workshops).

Phytophthora was only recovered by direct isolation for 2 (4%) samples collected during extension services, but it was recovered from 19 (50%) of the rhizosphere samples collected during extension services (Figure 2). *Phytophthora tropicalis* and *P. multivora* were recovered from managed gardens during extension activities (Table 1). *Phytophthora cinnamomi*, *P. multivora*, and *P. nicotianae* were recovered from urban home gardens. *Phytophthora cinnamomi* and *P. multivora* were recovered from samples collected on the property of a plant nursery. *Phytophthora neiderhauserii* was isolated from an urban street tree (*Harpephylum caffrum*) following an online report mentioned previously (Table 2).

Sample diagnostics

Two-hundred and sixty-eight samples were submitted for diagnosis from 212 sites on 45 properties by 62 citizens. One hundred and twenty-six of those samples were submitted from 24 nature reserves, 43 were submitted from 4 national parks. Forty-six samples were submitted from 4 plant nurseries. The remaining 50 samples were submitted from two managed gardens (11), 9 urban home gardens (26), and 2 private land properties (16).

Phytophthora species were directly isolated from 10 (20%) of the plant samples and 64 (26%) of the rhizosphere samples submitted for diagnostics (Figure 1). Seven *Phytophthora* species (*P. asparagi*, *P. cinnamomi*, *P. humicola*, *P. inundata*, *P. kwongonina*, *P. megasperma*, and *P. multivora*) were recovered from samples submitted

from nature reserves (Table 1). Seven *Phytophthora* species (*P. bilorbang*, *P. capensis*, *P. cinnamomi*, *P. citrophthora*, *P. cryptogea*, *P. multivora*, and *P. tropicalis*) were recovered from samples submitted from plant nurseries. *Phytophthora capensis, P. cinnamomi, P. multivora* and *P.* sp. emzansi were recovered from the samples submitted from national parks and two species were recovered from samples submitted from managed gardens (*P. chlamydospora* and *P. multivora*). *Phytophthora cinnamomi* and *P. multivora* were recovered from samples submitted from urban home gardens, and *P. neiderhauserii* was recovered from a sample submitted from a disturbed area of private land.

Sample-, citizen-, environment- and property-type comparisons

Sample type

Phytophthora species were only isolated from 10% of the 197 direct isolation attempts overall. In contrast, *Phytophthora* species were baited from 39% of the 323 rhizosphere samples. Six *Phytophthora* species were recovered by direct isolation (*P. cinnamomi*, *P. citrophthora*, *P. inundata*, *P. multivora*, *P. neiderhauserii*, and *P. tropicalis*); *Phytophthora citrophthora* was the only species recovered solely from the rhizosphere samples. The other sixteen species including *P.* sp. emzansi were recovered from rhizosphere samples.

Citizen type

Twenty-six individuals who participated were general members of the public and 93 were recognized as professionals. One hundred and forty-six youth also participated. Members of the general public, youth, and professionals directly isolated from 2 (9%), 3 (3%), and 11 (18%) of the samples, respectively. In contrast, *Phytophthora* species were isolated from 5 (10%) and 90 (34%) of the rhizosphere samples collected by members of the general public and professionals, respectively. None of the rhizosphere samples collected by youth were positive for *Phytophthora*.

Overall, 15 *Phytophthora* species, including *P. sp.* emzansi, were recovered from samples submitted by professionals. *Phytophthora cinnamomi* and *P. multivora* were recovered from samples collected and submitted by members of the general public and *P.*

cinnamomi was directly isolated by youth. Nine *Phytophthora* species were recovered from samples collected and processed by the authors.

Environment and property type

Two hundred and ninety samples were collected or submitted from urban areas (e.g. managed and home gardens, plant nurseries, etc.) and 376 samples were from natural areas (e.g. nature reserves and national parks). *Phytophthora* species were recovered from 94 (32%) and 50 (13%) of the samples from urban and natural areas, respectively (Table 1). Twelve species were recovered from urban areas and eight species and *P. sp.* emzansi were recovered from natural areas.

Phytophthora species were directly isolated from 8 (18%) of the plants submitted from plant nurseries, 3 (10%) samples from managed gardens, 2 (10%) samples from urban home gardens, 1 (3%) sample from a National Park, and 3 (2%) samples from Nature Reserves (Table 2). *Phytophtora* species were also recovered from 18 (51%) rhizosphere samples submitted or collected in plant nurseries, 15 (47%) rhizosphere samples from urban home gardens, 45 (39%) rhizosphere samples from managed gardens, 29 (54%) samples from National parks, and 18 (12%) rhizosphere samples from Nature Reserves.

In the urban environment, seven *Phytophthora* species (*P. amnicola*, *P. asparagi*, *P. capensis*, *P. chlamydospora*, *P. cinnamomi*, *P. multivora* and *P. tropicalis*) were recovered from rhizosphere samples and two species (*P. cinnamomi* and *P. multivora*) were directly isolated from samples in managed gardens, including the direct isolation of *P. multivora* from the roots of a *Leucadendron salignum* sample (Table 2). Three species (*P. cinnamomi*, *P. multivora* and *P. nicotianae*) were recovered from rhizosphere samples and *P. cinnamomi* was directly isolated from 2 samples collected in urban home gardens. Six species (*P. bilorbang*, *P.capensis*, *P. cinnamomi*, *P. cryptogea*, *P. multivora*, and *P. tropicalis*) were recovered from the rhizosphere of samples submitted from plant nurseries. *Phytophthora cinnamomi*, *P. tropicalis*, and *P. citrophthora* were directly isolated from the samples submitted from plant nurseries (Table 1).

In natural environments, three species (*P. capensis*, *P. cinnamomi*, and *P. multivora*) and the informal *P.* sp. emzansi were recovered from rhizosphere samples and

two species (*P. cinnamomi* and *P. inundata*) were directly isolated from samples collected in or submitted from national parks. Seven species (*P. asparagi*, *P. cinnamomi*, *P. humicola*, *P. inundata*, *P. kwongonina*, *P. megasperma*, and *P. multivora*) were recovered from rhizosphere samples collected or submitted from nature reserves. *Phytophthora cinnamomi* was also directly isolated from three plant samples collected in nature reserves.

Discussion

Increased capacity from citizen engagement

Inviting non-scientists to contribute to the research increased the capacity to monitor for potential novel *Phytophthora* invasions. Many species were recovered for the first time in South Africa and samples were contributed and collected from properties otherwise inaccessible. These findings confirm our hypothesis that engaging the public would enhance the breadth of our research.

Many *Phytophthora* species were recovered from samples considered unachievable without citizen participation. These contributions led to the first report of *P. bilorbang* in South Africa, a species that is known to occur in Western Australia, Sardinia and the United States presently (Aghighi et al. 2012, Parke et al. 2014, Scanu et al. 2014). Citizen contributions also provided findings of novel plant-microbe interactions. For example, this study presents first reports for isolating *P. cryptogea* from *Olea* spp. and *P. tropicalis* from *Rosa* spp. An online report also led to the recovery of *P. neiderhauserii* from a widely cultivated tree species for the first time. Together these results substantiate the value of citizen science programs to enhance biosecurity, especially in the absence of taxpayer funded monitoring programs such as extension or statutory monitoring.

Best methods to promote Phytophthora *detections by citizens*

Citizen science programs can enhance biosecurity (Bates et al. 2015, Thomas et al. 2017), but the best methods to invite public participation are unclear. This study investigated the merit of four methods to engage the public. Requesting sample submissions and providing diagnostics was the best method for detecting *Phytophthora* species. Fourteen species were recovered from samples submitted to our laboratory.

However, extension services revealed the presence of *Phytophthora* species not recovered through other methods. For example, *P. nicotianae* was recovered from two urban gardens in the region but it was not recovered in the other engagement methods. The online plant reports indicate more research is needed to reveal the drivers of plant mortality in the Cape Floristic Region. Observations of unhealthy plants were shared for 57 species that have not been reported to be susceptible to *P. cinnamomi*. Additional research to investigate the susceptibility of these species to *P. cinnamomi* and other possible factors is needed. In all cases, these methods provided value, but solely providing diagnostics services open to the public may be the best method for quickly and cost effectively collecting baseline *Phytophthora* data.

Environments to maximize early detection

Twelve *Phytophthora* species were recovered from properties in the urban environment and 8 of those species were not recovered from natural environments. These findings support the recognition of urban environments as opportunities for early detection of *Phytophthora* invasions (Hulbert et al. 2017) and these observations may therefore represent early detections of potential threats to the natural environment. Additional research is needed to assess the risk these species pose to natural flora and reduce the likelihood of spread outside of the urban area.

Many species were identified from samples submitted by plant nurseries and *Phytophthora* was recovered from relatively high proportion of these samples. This is consistent with studies in other countries (Migliorini et al. 2015, Jung et al. 2016) and again exemplifies the role of plant nurseries in disseminating *Phytophthora* species. However, the willingness of plant nurseries in the Western Cape Province to submit samples to our research may represent best behavior for proactively managing plant pests. Further, the cooperation between plant nurseries and Cape Citizen Science demonstrate citizen science programs can provide important services for biosecurity in countries without statutory monitoring programs that exist in more developed economies (e.g. O'Hanlon et al. 2016).

In contrast to the urban environment findings, 9 species were recovered from natural areas (nature reserves and national parks) and 5 of those were not recovered from

urban environments. This result could suggest these species are possibly indigenous and have been present in the natural areas for a long time, as has been suggested with *P. capensis* and *P. multivora* (Chapter 3). However, these findings could therefore represent the early emergence of novel disease epidemics caused by species that have not yet been discovered in the urban environment (Hulbert et al. 2017). For example, *P. humicola*, *P. inundata*, *P. kwongonina*, *P. megasperma,* and *P.* sp. emzansi were each only recovered from 2-3 sites in contrast to the high recovery of *P. cinnamomi* and *P. multivora* in the natural environment. It is also possible the natural areas are relatively disturbed. For example, many nature reserves were within the City of Cape Town's boundaries. Alternatively, these species may have been introduced from restoration activities as has been demonstrated recently in the USA (Rooney-Latham et al. 2015, 2018, Sims et al. 2019).

The important findings from both the urban and natural environment illustrate that surveying both environments is vital. Many species were revealed in both environments, but several were only recovered from one or the other. Additional research is needed to investigate the potential threats and pathways of these species to reduce their impacts and prevent their spread between environments. Of particular concern is these results indicate that there are *Phytophthora* species in the urban environment that may not have yet escaped into the natural environment and the future effects of these species is unpredictable.

Value from each sample type

Phytophthora species were more frequently recovered from rhizosphere samples than direct isolation attempts, but the direct isolation attempts provided more information. In general, direct isolation of the pathogen from diseased plant tissues provides additional information about the potential susceptibility of the host plant to the *Phytophthora* species. In contrast, *Phytophthora* species recovered from the rhizosphere does not necessarily confirm that the *Phytophthora* is associated with the actual plant species. In both cases, additional research that includes pathogenicity tests is necessary to confirm relationship between the microbe and the potential host.

Although *Phytophthora* was more frequently recovered from rhizosphere samples, these results are likely still an underestimate of the actual diversity and distribution of *Phytophthora* species in the Cape Floristic Region. For example, many studies have demonstrated lower recovery rates and species diversity from baited samples than from direct sequencing with recently developed molecular techniques (Cooke et al. 2007, Bose et al. 2018, Khaliq et al. 2018, Riddell et al. 2019). Therefore, programs that engage the public to collect rhizosphere samples and then integrate advanced molecular techniques may be the best approach for detecting *Phytophthora* species.

Ideal citizens to engage

The most individuals engaged were youth during educational activities, followed by plant-related professionals and then members of the general public. These professionals recovered *Phytophthora* from samples at higher rates than youth or participants from the general public. Recovery of *Phytophthora* species was especially low during activities organized with youth. However, given the overall higher success of recovering *Phytophthora* from the other rhizosphere samples, it is plausible that youth would have contributed more successful samples if they collected rhizosphere samples rather than direct isolation attempts.

There is merit to engaging youth and other citizens for educational outcomes because it can raise awareness and the more people aware and involved, the better. The main objectives of the workshops and youth hikes were educational although the activities were also designed to recover *Phytophthora* in those areas. Special emphasis was made to provide opportunities to underserved youth in South Africa, which is critically needed (Hulbert and Roets 2018). Increasing awareness of the threats and pathways of *Phytophthora* species may reduce border pressure and the accidental movement of pathogen propagules (Marzano et al. 2015, 2016). Therefore, citizen science initiatives and activities for youth to participate may enhance biosecurity by providing opportunities to achieve research and educational outcomes simultaneously.

Value of citizen science in countries with developing economies

Many of the challenges to countries with developing economies noted by Nuñez and Pauchard (2009) can be overcome by citizen science. For example, here we have demonstrated that citizen science initiatives can generate important baseline data, provide opportunities for informal education, and engage hundreds of volunteers to enhance biosecurity. Many of these results presented above demonstrate a number of threats that would go unnoticed for longer periods without the initiation of Cape Citizen Science. Therefore, citizen science may have exceptional merit for countries where economies and biosecurity capacity are limited. In this regard, we have presented an example of a program with methods that can be revised and adapted to other organisms, systems, and geographies.

Implications for biosecurity

Initiating a citizen science project led to novel discoveries and detections of species that were likely unnoticed because of the insufficient biosecurity capacity. For example, the recovery of *P. tropicalis* from a plant nursery may represent a substantial threat to ornamental plants such as *Rhododendron* and *Cyclamen* varieties in South Africa (Gerlach and Schubert 2001, Hong et al. 2006, Luongo et al. 2013, Leyva-Mir et al. 2016) and provides important information about the domestic plant trade as a pathway. While the results are important for South Africa, the findings are globally relevant because of the ease and frequency of disseminating *Phytophthora* through the global trade (Jung et al. 2016, Hulbert et al. 2017).

Findings in the program have also generated substantial baseline data about the diversity and distribution of *Phytophthora* species in the Western Cape Province. The availability of these data is also enough to compare species distributions. For example, the relatively infrequent recovery of species such as *P. humicola* compared to species such as *P. multivora* or *P. cinnamomi* may suggest that it is a recent introduction into the natural environment. Therefore, the findings from public engagement have enhanced biosecurity in South Africa by providing important information about potential pathways of spread and baseline data on the diversity and distributions of *Phytophthora* species.

Recommendations

Collectively, the public engagement and samples included in Cape Citizen Science have revealed many *Phytophthora* species present in the Western Cape Province of South Africa. These findings illustrate the importance of monitoring urban and natural environments and demonstrate that citizen contributions can increase sampling distributions. In particular, inviting rhizosphere sample submissions for diagnostics from plant-related professionals produced the greatest benefit in terms of efficiency of *Phytophthora* species surveillance. However, involving youth and members of the public provided opportunities to raise awareness of a greater number of citizens. We therefore recommend other projects choose the target audiences, type of samples to be collected and methods of engagement based on the objectives of the research project.

Conclusions

The public engagement and results of this study have demonstrated there are many *Phytophthora* species present within urban and natural environments of the Cape Floristic Region. Many of the species were previously unknown to be associated with the hosts or to be present in South Africa. Now additional research is needed to identify the threats and roles of these species.

Importantly, many of the findings were only revealed because of citizen participation. Therefore, it is clear that inviting contributions enhanced the breadth of the research and advanced knowledge pertinent to biosecurity. These results demonstrate the merit of initiating citizen science programs and engaging the public in efforts to protect plant-based resources, ecosystems and bioeconomies.

Each method of engagement discussed herein had merit and provided an approach to enhance biosecurity. Collectively the methods of Cape Citizen Science have advanced knowledge and raised awareness of citizens in South Africa and this approach can be duplicated in other areas, but expanding on the recommendations discussed above can make improvements. Cape Citizen Science provided an opportunity to compare methods of engagement within a single program, but comparisons across programs is needed to maximize engagement and increase the monitoring capacity of citizen science projects aimed at enhancing biosecurity globally.

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Tables

Table 1: Number of positive samples for each species from each capacity category, engagement method and property type.

Species	Achievable	Achievable, costs offset	Achievable, invitation	Unachievable	Diagnostics	Extension services	Workshops	Youth hikes	Managed gardens[U]	Urban home gardens[U]	National parks[N]	Nature Reserve[N]	Private land[U]	Plant nursery[U]	Urban planting[U]
Phytophthora amnicola	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0
P. asparagi	1	4	0	0	4	0	1	0	1	0	0	4	0	0	0
P. bilorbang	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0
P. capensis	4	12	0	1	13	0	4	0	1	0	15	0	0	1	0
P. chlamydospora	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0
P. cinnamomi	18	9	8	9	18	8	12	6	13	10	8	6	0	6	0
P. citrophthora	0	0	0	2	2	0	0	0	0	0	0	0	0	2	0
P. cryptogea	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0
P. humicola	0	2	0	0	2	0	0	0	0	0	0	2	0	0	0
P. inundata	1	1	0	0	1	0	1	0	0	0	1	1	0	0	0
P. kwongonina	0	4	0	0	4	0	0	0	0	0	0	4	0	0	0
P. megasperma	0	3	0	0	3	0	0	0	0	0	0	3	0	0	0
P. multivora	38	19	6	13	32	12	30	2	36	8	13	8	0	11	0
P. neiderhauserii	0	0	2	1	1	2	0	0	0	0	0	0	1	0	2
P. nicotianae	0	0	3	0	0	3	0	0	0	3	0	0	0	0	0
P. sp. emzansi	1	2	0	0	2	0	1	0	0	0	3	0	0	0	0
P. tropicalis	1	0	0	8	8	1	0	0	1	0	0	0	0	8	0
Total species	8	10	4	8	15	5	7	2	7	3	5	7	1	7	1

[N]=Natural Environment, [U]=Urban Environment

Table 2: Species directly isolated from plant tissues.

Isolate	Final Result	Host	Substrate	Engagement Method
CMW54543	*Phytophthora cinnamomi*	*Leucadendron coniferum*	root collar	Workshop
JMH0112*	*P. cinnamomi*	*Mimetes splenditis*	root collar	Diagnostics
CMW54598	*P. niederhauserii*	*Harpephyllum caffrum*	stem bleed, stem lesion	Extension Services
CMW54601	*P. cinnamomi*	*Mimetes hottentoticus*	stem	Diagnostics
CMW54602	*P. cinnamomi*	*Leucadendron salignum*	roots, root collar	Workshop
CMW54603	*P. multivora*	*L. salignum*	roots, root collar	Workshop
CMW54605	*P. cinnamomi*	*Mimetes fimbriifolius*	root collar	Workshop
CMW54610	*P. citrophthora*	*Rosa* sp.	stem lesion	Diagnostics
NU0004*	*P. tropicalis*	*Rosa* sp.	stem lesion	Diagnostics
NU0064*	*P. cinnamomi*	*Leucadendron* sp. *'blush'*	stem lesion	Diagnostics
CMW54614	*P. cinnamomi*	*Vaccinium* sp. *'blueberry'*	root collar	Diagnostics
NU0071*	*P. cinnamomi*	*Leucospermum sp. var. "High Gold"*	roots	Diagnostics
CMW54615	*P. tropicalis*	*Rosa* sp.	Leaf tip dieback	Diagnostics
CMW54616	*P. inundata*	*Leucadendron salignum*	root collar	Workshop
CMW54617	*P. cinnamomi*	*Leucadendron salignum*	root collar	Workshop
CMW54618	*P. cinnamomi*	*Protea repens*	root collar	Youth Hike
VA0027*	*P. cinnamomi*	*Erica* sp.	root collar	Youth Hike

*Culture not deposited into Mike Wingfield Culture Collection (CMW).

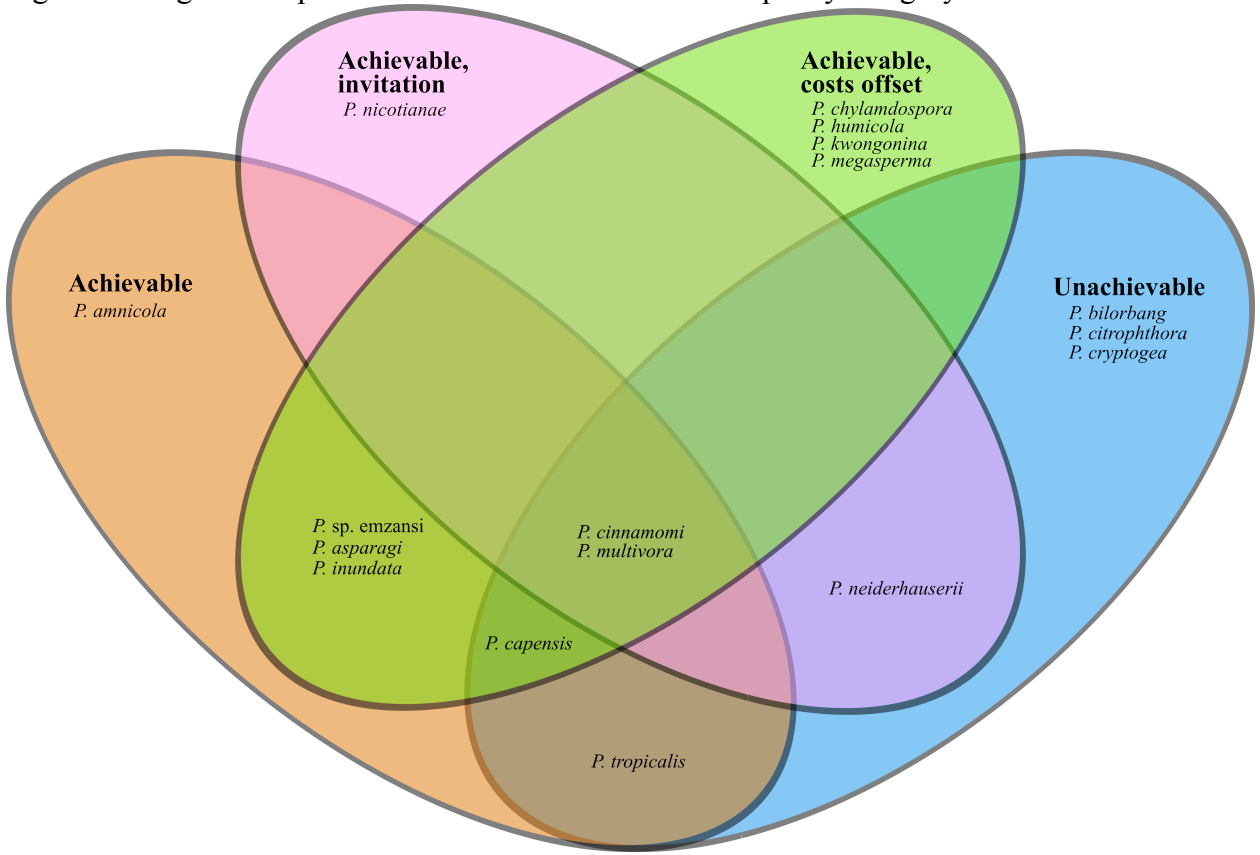
Table 3: Summary data for rhizosphere and direct isolation samples collected or submitted for each property type.

Property type	Direct isolation positive samples	Direct isolation positive sample proportion	Species directly isolated	Rhizosphere positive samples	Rhizosphere positive sample proportion	Species recovered from rhizosphere
Managed garden	3	0.1	2	45	0.39	7
Urban home garden	2	0.1	1	15	0.47	3
National park	1	0.03	2	29	0.54	4
Nature reserve	3	0.02	1	18	0.12	7
Plant nursery	8	0.18	3	18	0.51	6
Private land	0	0	0	1	0.06	1
Urban planting	2	0.33	1	0	0	0
Total	19		6*	126		16*

*Number of distinct species recovered from each sample type.

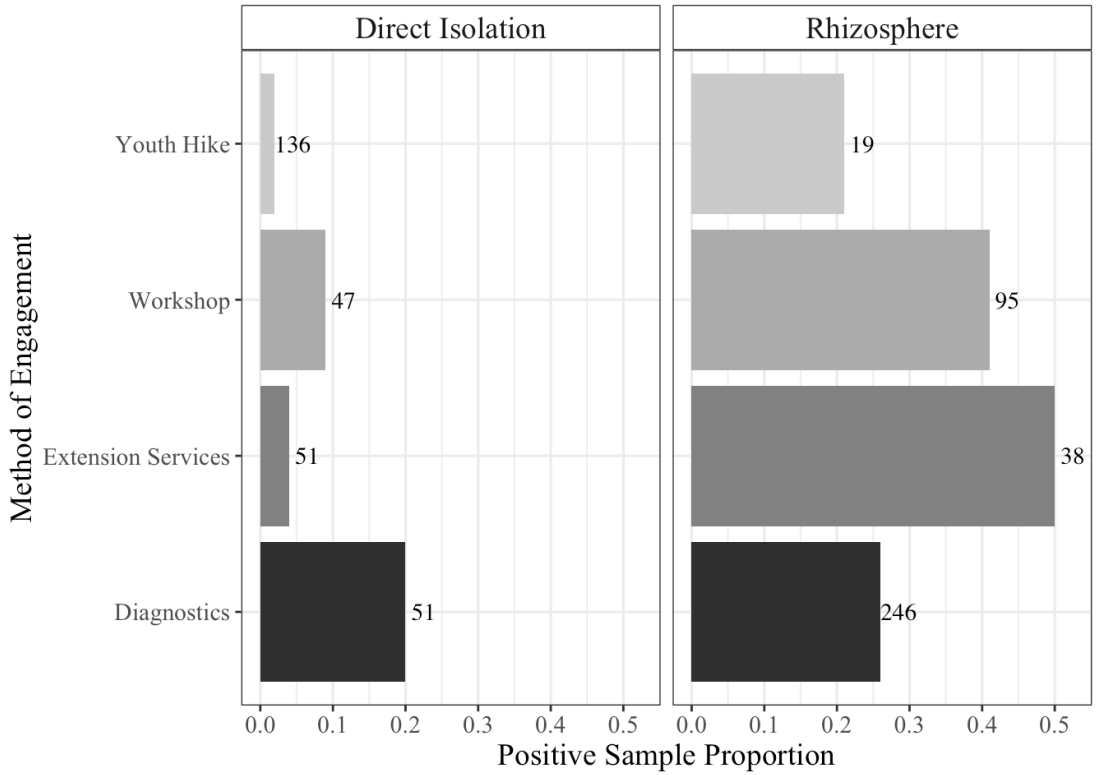
Figures

Figure 1: Diagram of species recovered in each research capacity category.



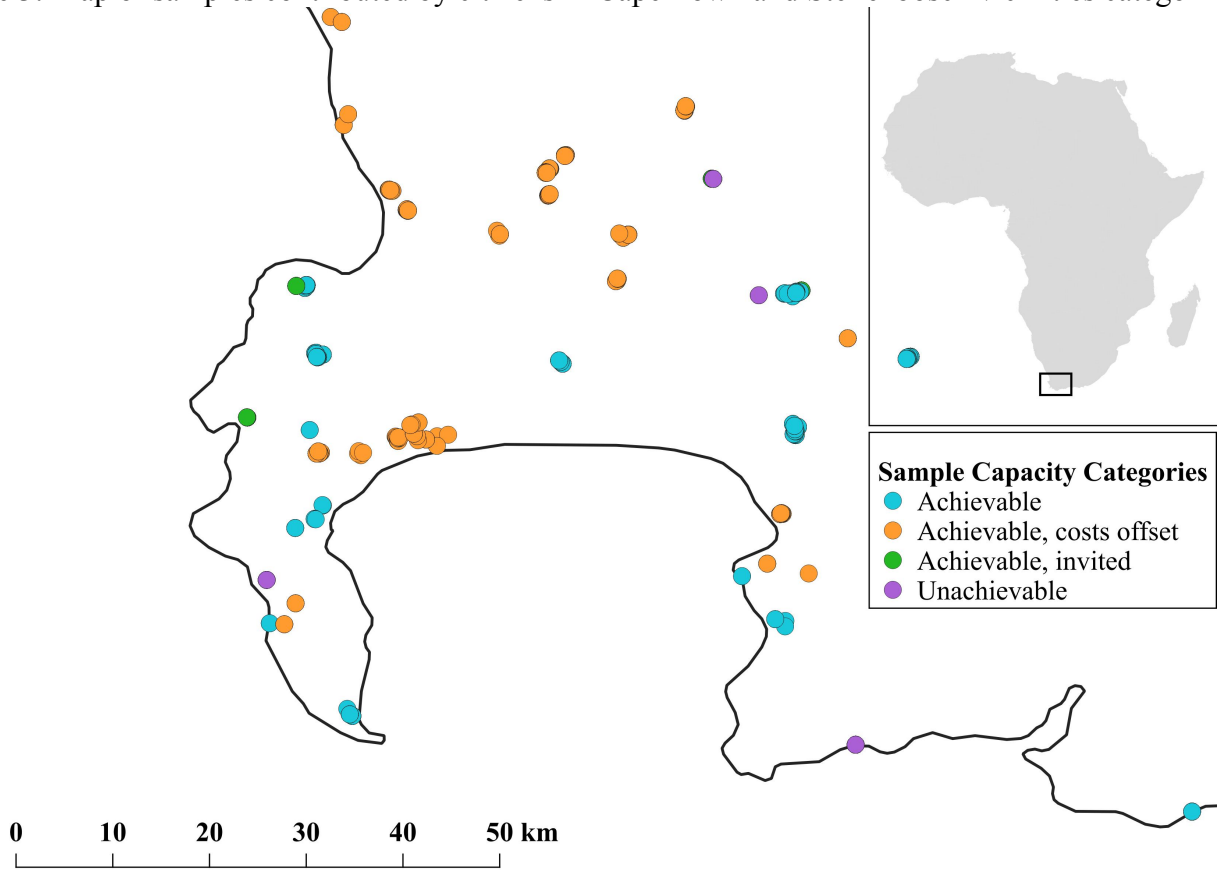
In general, species located toward to the top-right of the diagram would not have been recovered without citizen participation.

Figure 2: Proportion of rhizosphere and direct isolation samples positive for *Phytophthora* from each engagement method.



Numbers next to bars represent total number of samples submitted or collected in each engagement method.

Figure 3: Map of samples contributed by citizens in Cape Town and Stellenbosch vicinities categorized by research capacity.



APPENDIX

Preface

The appendix is composed of materials that were published as commentaries or similar article types and were not peer-reviewed with the exception of the final section. Here the sections are presented in order of publication date, starting with the earliest first. The last section was not prepared for submission outside of this thesis and is only included to provide context for the Cape Citizen Science program.

The first section presents a manuscript that was included as a 'News and Views' piece in the *South African Journal of Science* in 2016. The purpose of the commentary was to highlight three online-tools that were available for creating and curating projects to collect observations of biodiversity at that time.

The second section is a short story of our public engagement efforts that was included as an 'Outside the Tower' piece in *Science Magazine* in 2018. The purpose of the piece was to encourage more science engagement by highlighting an incredible story of three learners who often joined us in the laboratory in Stellenbosch.

The third section is a piece I contributed to share my perspective in a special issue of the *Narratives in Bioethics* journal produced by John Hopkins University Press in 2019. The purpose of the piece was to share my perspective of leading a citizen science project in South Africa as a foreigner.

The fourth section includes a collaborative manuscript that was included as another 'News and Views' piece in the *South African Journal of Science* in 2019. The purpose of this commentary was to share a collaborative perspective about the challenges citizen science project leaders face in South Africa.

The final section included in the appendix is a short summary of the history of Cape Citizen Science. The purpose of this piece was to highlight some of the activities over the past four years and provide an outlook for the program in the future.

Citizen science tools available for ecological research in South Africa

Published as: Hulbert, J. M. 2016. Citizen science tools available for ecological research in South Africa. South African Journal of Science 112.

Citizen Science

Citizen Science is a term for scientific research conducted by non-scientists. Average citizens can participate in research from their home computer, in their own gardens, or in the great outdoors—without any expertise in the field. Many citizen science projects and opportunities exist in South Africa, ranging from monitoring bird migrations to identifying and mapping distributions of fungi. In this article, three citizen science tools available to researchers in South Africa are discussed and three interesting plant focused research projects currently using these tools are introduced.

Incorporating citizen science tools into ecological research provides many benefits. The use of citizen science tools increases sampling distributions, temporally and spatially (Bonney et al. 2009), and engagement of local citizens in research provides 'many scientific eyes' (Dickinson et al. 2012)—especially useful for geographical studies, such as monitoring invasive species or mapping populations of rare species over large spatial scales. Not only does the research benefit from multiple volunteer observers, but also from greater access to private lands (Meentemeyer et al. 2015). Thus, incorporating citizen science tools ultimately increases the feasibility of many research projects through the culmination of these benefits.

Each of the tools discussed below may already have large numbers of citizens actively contributing to projects, available for future research endeavors. However, citizen science is recognised as an inherent avenue for outreach (Meentemeyer et al. 2015), and should only be pursued by projects with a commitment and interest in improving scientific literacy by engaging a greater population. In addition to the recognition of citizen science as a tool for research, it is also recognised as a tool for scientific education. Citizen science projects provide ample outside-of-the-classroom learning opportunities for participants, contributing educational benefits such as skills for accurate data collection, critical thinking, and scientifically informed decision making (Dickinson et al. 2012); ultimately increasing scientific capacity, better informing decisions, and improving social capitol in South Africa.

Citizen Science Tools

Below are three not-for-profit tools available for ecological research projects in South Africa. Each tool is briefly summarised and the method of application in research is introduced. Reviewing the qualities and limitations of each tool is not in the scope of this article. All three tools are web-platforms where citizens can register, upload images and locations, and share observations.

Ispot (www.ispotnature.org)

Ispot is an international platform with a South African based initiative sponsored by the South African National Biodiversity Institute (SANBI). The project launched in South Africa in June 2012, contributing to the nearly 400,000 international observations of 30,000 different species reported by mid 2014 (Silvertown et al. 2015). The project strives to connect citizens to experts in the field through a social network, working together to identify organisms and learn about ecology. Researchers can create 'projects' on *Ispot* where they can collect observations using a tag system. For example, the tag 'dyingfynbos' is a tag used to filter and organise observations for a research project about plant disease in fynbos vegetation. Complexity can also be added to projects on *Ispot* by adding species interactions to observations (i.e. experts and citizens can work together to identify both the bee species and the flower species being pollinated from a single picture) (Pocock et al. 2016).

WhatSpecies (www.whatspecies.com)

WhatSpecies is citizen science tool based specifically in South Africa. A parent who wanted to help her children identify insects and plants launched the platform for people to 'live out their passion and learn about identifying nature' (Pers Comm. Marié Cruywagen, 6 November 2015). The data on the platform are open access and the layout of the website caters well to a young audience. A second version of *WhatSpecies* is expected to launch in late March 2016. The platform strives to engage youth through other forms of social media such as Facebook and blogging and is committed to protecting the ownership of uploaded images. Similarly to *Ispot*, researchers can organise and filter observations for their project using a tag system.

Virtual Museum (http://vmus.adu.org.za)

The *Virtual Museum* is a South African based platform hosted and organised by the Animal Demography Unit at the University of Cape Town. The platform is project oriented and is currently hosting 17 different geographical projects, mapping distributions of organisms ranging from fungi to birds and dung beetles to orchids, each containing many genera. With this tool, researchers create a project and citizens share observations by uploading images and selecting locations specifically for that project. The platform is exceptional with more than a million records of bird distributions since the beginning of the Bird Atlas (SABAP2) project in 2007 (Animal Demography Unit 2015). The data are stored under a creative commons license and registered users can request static maps and species lists.

Selecting a Citizen Science Tool

Each tool discussed above has its own strengths and weakness, highly dependent on the scope of the research project itself. Therefore, it is important to try and consider all three when initiating a citizen science component of a research project in South Africa. Alternatively, researchers could choose to create projects in all three tools discussed, merging observations for their own analysis. All three tools are limited to geographical studies, but each could be used to recruit participants for more interactive projects that involve physical sampling. Data quality, target audience, social engagement and user-abundance (e.g. number of active citizen scientists) are important components to consider when selecting a tool. Both *Ispot* and *WhatSpecies* use crowd-sourcing to identify observations, but observation identifications on the *Virtual Museum* are at the discretion of the project leaders and a selected 'expert panel' by checking photographs. All three tools are suitable for mapping distributions supported by images, but each is limited to projects where organisms can be identified from images (e.g. plants, fungi, and birds, etc.). However, each tool can be used to direct sampling efforts to confirm observations or conduct a more thorough investigation. For example, 'Cape Citizen Science' (discussed below) is a citizen science initiative for a research project to study plant disease throughout the fynbos biome. Because microscopic organisms cause plant disease, images showcasing symptoms of disease cannot be used to conclusively identify

the microorganism. However, the project is currently using two of the tools discussed above to find sampling locations and potentially identify new hotspots of plant disease emergence.

South African Citizen Science Projects

There are many citizen science projects ongoing within South Africa. As mentioned previously, there are 17 different projects that citizens can participate in hosted on the *Virtual Museum* alone. One project from the *Virtual Museum* and two projects on *Ispot* that focus on plant communities are discussed below.

OrchidMap (http://orchidmap.adu.org.za)

OrchidMap is a project hosted by the *Virtual Museum* where citizens can upload images and locations of orchid observations. The purpose of the project is to improve the understanding of the distributions of African orchids. Nearly 3000 geo-referenced records for orchids have been uploaded to the Virtual Museum for this project since it was initiated in September, 2014 (Animal Demography Unit 2015). In contrast to *Ispot* and *WhatSpecies*, the *Virtual Museum* uses a grid system to share location data for individual observations and does not provide explicit coordinates to avoid potential abuse of the platform. This is important when providing access to distributions of rare and endangered species with economical incentives, such as many orchid species. *Ispot* and *WhatSpecies* do allow users to hide location data when sharing observations, but users may be unaware of the risk.

Cape Citizen Science (http://citsci.co.za)

The Forestry and Agricultural Biotechnology Institute initiated *Cape Citizen Science* for a project to survey plant disease in the fynbos biome. The research is designed to focus on a group of microorganisms called *Phytophthora*, which translates from Greek as 'Plant Destroyers'. As part of the initiative, the researchers have created a project on *Ispot* where citizens can contribute observations of dying plants using the tag 'dyingfynbos'. The tag can also be used with observations on *WhatSpecies*. The reported locations will be used to choose sampling locations and is expected to help researchers

and land managers respond quicker to new invasions and diseases. Since the project was initiated, the researchers have also started collaborating with a group out of the Cape Peninsula University of Technology who are studying invasive aboveground fungi in the same system. These research projects will directly benefit from shared observations from citizen scientists and will ultimately contribute positively to the conservation of the biodiversity in the fynbos.

Aliens of the Cape Peninsula (http://www.ispotnature.org/projects/aliens-of-the-cape-peninsula)

Aliens of the Cape Peninsula is a project explicitly on *Ispot*. The aim of the project is to catch new introductions of alien plants and map the current distributions of known alien species. The project page on *Ispot* indicates that there are currently over 800 species of alien plants—a third of the total species present—on the Cape Peninsula and provides a long list of species to look out for. The project was initiated in early January and has already has more than 1000 observations.

Conclusion

The tools discussed above are freely and readily available for implementation into Southern Africa research projects. They are incredible resources useful for improving public well being through educational activities while benefiting future societies with basic research. These tools enable the coupling of science education and hypothesis driven research, benefiting society and the planet by engaging the public in the scientific process.

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Science engagement in South Africa

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Learners from grades 9 to 12 surrounded our Science Week table in the township Khayelitsha, an impoverished community near Cape Town in South Africa. We were conducting outreach for our project Cape Citizen Science (http://citsci.co.za/), an initiative to engage nonscientists in plant disease research in a global biodiversity hotspot. “Can plants get sick, too?” we asked, as the students examined unhealthy plants under dissecting microscopes and held petri-plates containing fungal-like organisms up toward the lights. Late in the day, a grade 10 boy named Dylan approached the table and asked about our work. His hunger to learn and the depth of his questions inspired us to invite him to our lab. He immediately responded, “Can I bring my friends?”

Many researchers in our community met Dylan, Ayebonga, and Ivan during the next year, as they often joined us in our lab to satisfy their curiosity and contribute their time to the scientific process. Their dedication to learning was exemplified by the challenges they overcame to travel to our university; they often spent hours navigating the public transit system of the Western Cape Province, and occasionally faced financial barriers (although we made sure to reimburse them for their efforts).

On his first visit to the lab, Dylan excitedly informed us that it was his first time looking through a microscope. After a few visits, he told us he wants to study microbiology at university. We are grateful to know that we helped empower him to make such a critical decision. However, there are thousands of underprivileged learners in South Africa without these opportunities, whose hunger for knowledge remains unfulfilled and overlooked. We encourage researchers to participate in public engagement programs, especially in countries with emerging economies. Such programs can increase the value and capacity of research beyond science. We also encourage traditional science project leaders to open their programs to citizens, especially those without reliable access to quality science education. Together, we can help science enthusiasts (and potential future scientists) develop critical decision-making and problem-solving skills.

Citizen science improves the ethics of foreign led research

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I often start presentations for youth with a map to point out where I am from. Then, as a clear foreigner, even after three-years of diluting my accent, I ask them: "why did I come to South Africa?" I use my foreign status to emphasize the exceptionalism of the country with particular regard to the biodiversity of the Cape Floristic Region, a global biodiversity hotspot where our project exists. While that connection seems effective, especially for youth who admire western pop culture, the question remains whether I should actually be the one leading these activities and conducting research in South Africa.

Before I could register at the University of Pretoria, the department had to confirm that I would not take the place of a South African. As a PhD student, I receive financial support to cover my costs of living in the form of a bursary. While the confirmation of my place in the university system is almost certainly a question of finances (which are becoming more limited in South Africa), should the university also be concerned whether I am also taking a potential research topic of a South African student?

There are many benefits of international collaboration, but there are also some risks. For example, much of the fun of science is making new discoveries. I usually emphasize to the youth that they may be the first to find a 'new' species in our sample collections together as a form of motivation. However, will my field of science be as fun when every species has been found and described? I have colleagues who travel the world like its a race to describe and name as many fungi or fungus-like organisms as they can. This behavior gets even more ethically shady when searching for internationally regulated pathogens in countries without the capacity to regulate them. But even in my own situation as a PhD student receiving training, am I taking the fun away from a South African down the line? Advancing knowledge is globally important, but some approaches do not benefit local communities.

Ethics are not generally recognized as a motivation to initiate a citizen science project, but training and engaging local communities in scientific discovery can justify research projects led by foreigners. For example, I could have come to South Africa to complete the research I am doing, maybe giving 4-5 presentations to peers each year and having little impact outside of academia, or I could have established a citizen science

project to engage local communities in the research. In this sense, citizen science provided an opportunity to conduct the research I was interested in with a more ethical approach as a foreigner.

Cape Citizen Science (http://citsci.co.za/) is a program that we initiated to aid research for my PhD. We are making progress to incorporate multiple projects under its umbrella, but the research has primarily contributed to our pilot project about plant-killing microbes thus far. The program is almost entirely collections based, asking citizens to contribute physical samples or participate in sampling activities, which have aided our research findings and led to many important discoveries. This research outcome was the primary motivation for us to initiate the citizen science project, but our dedication to education has increased over time.

During a recent workshop I attended, the organizers mapped various citizen science projects on a plane between education and research. They suggested one might develop a project for educational outcomes or research outcomes, but there was no discussion about ethics. What about developing a project for ethical outcomes, are they intrinsic to educational outcomes or are they different? For example, are there differences between citizen science projects that engage impoverished communities or groups that are historically underrepresented in science compared to projects that engage privileged communities, even if they have the same educational objectives?

The first groups of youth we were able to engage in our project were certainly privileged and only later did we establish the partnerships needed to reach kids from impoverished communities. The first activities were in connection with an afterschool group where parents could pay to drop their kids off at a nature reserve to do various naturalist activities. While these youth were 100% engaged and an enormous amount of fun to hunt plant pathogens with, it is difficult to dedicate our time to engage them when we have discovered so many other groups of learners who have never spent a day in a nature reserve.

Our interest in engaging learners in our research has also led to the establishment of several novel partnerships between the university and non-profit organizations. In some cases, these partnerships have transcended our project as we have recommended other students to lead activities or provided group suggestions to other researchers. In this

way, our citizen science project provided an avenue to establish novel partnerships and connect more groups.

Although our motivations to establish a citizen science project were not initially rooted in ethical objectives, the ethics behind engaging local communities have become incentives for me to continue to lead citizen science projects. Ethical objectives can be incorporated in any project and would add value regardless of the place or persons. Establishing Cape Citizen Science provided an opportunity to conduct research more ethically as a foreigner in South Africa, but citizen science projects in any part of the planet could improve their impact by incorporating ethical objectives such as targeting underprivileged groups.

Challenges and solutions to establishing and sustaining citizen science projects in South Africa.

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Citizen science is a term for research that engages non-scientists in the collection and generation of data. Many citizen science projects exist within South Africa and we expect more will be initiated because of their success. In this commentary, we discuss three projects based in, but not confined to, the Western Cape Province (WCP) to provide context for the methods, specific objectives and overall desired impacts. We then identify a few challenges that we have faced from the researcher perspective and we provide recommendations for those interested in initiating a citizen science project in South Africa.

Many projects involve citizen scientists because their participation enhances the breadth of the research through the collection of data over greater spatio-temporal scales. (Delaney et al. 2008, Bonney et al. 2009, Dickinson et al. 2010) However, not all projects in South Africa have purely research objectives, some are education-oriented with specific objectives to empower participants. In some cases, the educational objectives may even be ethically motivated to engage local communities or by the understanding of ubuntu wisdom. Citizen science is therefore a powerful approach to address multiple objectives, but there are many challenges to achieving the desired outcomes that we outline below within the context of an education-oriented project, a research-oriented project, and a project that combines the two.

Iimbovane Outreach Project

The Iimbovane Outreach Project (http://www0.sun.ac.za/Iimbovane) is a science education project based in the DST-NRF Centre of Excellence for Invasion Biology at Stellenbosch University. The word ‘Iimbovane’ translates as ‘ants’ from isiXhosa and represents the project’s engagement of learners in the collection and identification of ants.(Braschler 2009) The project is primarily education-oriented now, but it has led to advances in knowledge about ant diversity throughout the region since it started.(Braschler 2009) Iimbovane supports Grade 10 Life Science teachers and students by enhancing educational outcomes through stimulating workshops and activities that provide resources and hands-on learning about the theoretical biodiversity concepts covered in the Life Science curriculum. Learners are exposed to the various stages of the scientific method and gain practical experience conducting biodiversity studies, all while

advancing knowledge about the diversity of ants in South Africa. Founded in 2005, Iimbovane is the longest running citizen science project that specifically engages youth in South Africa. In this time, Iimbovane has empowered thousands of learners to understand and value the biodiversity of the WCP.

rePhotoSA

rePhotoSA (http://rephotosa.adu.org.za) is the repeat photography project of southern African landscapes. It is a joint project between the Plant Conservation Unit and the Animal Demography Unit at the University of Cape Town. In contrast to Iimbovane, this project is mainly research-oriented. rePhotoSA is founded on one of the largest historical landscape photograph collections in Africa, which currently consists of over 20 000 images.(Scott et al. 2018) Approximately 6 000 photographs have been uploaded to an interactive map available online. Citizen scientists can search for and download historical images to find the exact location from where the original photograph was taken. The primary objective is to enhance the understanding of landscape change over time.(Scott et al. 2018) Ground-based repeat photography has a long history in documenting landscape change and has been used in many research projects to better understand: 1) the drivers of vegetation change in the Karoo,(Masubelele et al. 2015a, 2015b) 2) population changes in plant species, such as the endangered Clanwilliam Cedar(White et al. 2016) and the quiver tree,(Jack et al. 2016) and 3) human impacts on the environment. Contributions to rePhotoSA therefore provide a valuable resource for research and provide a platform to monitor long-term vegetation change into the future.

Cape Citizen Science

Cape Citizen Science (http://citsci.co.za) is a programme co-hosted by the University of Pretoria and Stellenbosch University. The programme receives support from the DST-NRF Center of Excellence in Tree Health Biotechnology and the Forestry and Agricultural Biotechnology Institute. Cape Citizen Science invites citizens (including school groups) to participate through many methods spanning the gradient of education to pure research. Some citizens have learned through workshops and educational hikes, and others have contributed to advance knowledge by submitting physical samples or

reporting unhealthy plants through online tools.(Hulbert 2016) Cape Citizen Science has demonstrated that research projects can provide opportunities for informal education(Hulbert and Roets 2018) and that citizens can contribute to advance scientific discovery in South Africa.

Project Summary

Together these initiatives represent some of the diversity of citizen science projects based in southern Africa. Each project has their own objectives and target groups for participating, but they overlap in overall motivations to engage the public. Each project serves their own niche in the interface between the public and scientists because of the diversity of communities and the availability of resources in South Africa. For these reasons, we suggest that there is great opportunity to establish and sustain citizen science projects in South Africa, but we caution prospective project practitioners to be aware of on-going projects to avoid duplication. The mixture of available resources and communities provides great opportunities for projects to collaborate, share tools and resources. We therefore encourage support for a network of projects to nurture a dialog between project leaders. We anticipate that fostering stronger connections between projects will enhance the quality and breadth of opportunities to empower more people to make observations and critical decisions.

Challenges and solutions to initiating and maintaining a citizen science project

The purpose of this commentary is to identify some of the challenges and potential solutions to citizen science that we have experienced in South Africa. We outline some of the challenges we have faced below by first reviewing available literature and then providing context within the projects discussed above. Understanding these challenges will prepare future projects to overcome barriers and better contribute to advancing knowledge and positively influencing society.

Experimental design

Incorporating effective experimental design can be a major challenge for many citizen science projects. Careful design is critical for collecting adequate sample sizes without fragmentation or sampling bias.(Conrad and Hilchey 2011) One approach to alleviating this challenge is to incorporate a design that targets specific areas for sampling.(Meentemeyer et al. 2015) This design was incorporated in the *Go Outside for Science* phase of Cape Citizen Science. The project asked participants to physically collect samples in randomly selected sampling locations to avoid sampling bias and ensure strong coverage throughout the region. However, this phase could only be promoted to certain groups with permissions to collect samples in protected areas because South Africa has many protected species. Therefore, the strict sampling permission requirements presented a challenge to the implementation of the experimental design. Cape Citizen Science was able to overcome this challenge by engaging professional staff (e.g. nature reserve managers) in the research.

Data quality

Data quality is central to the immediate and long-term success of any citizen science venture. While volunteer participation can create possibilities for otherwise cost-prohibitive projects, careful attention is needed to ensure the data collected by volunteers are high quality.(Crall et al. 2011) Projects can overcome this challenge by providing trainings or workshops for participants(Gardiner et al. 2012) and implementing methods of quality assurance or using tools that are monitored by the greater community such as inaturalist.org.(He and Wiggins 2015) Future projects will also need to work toward overcoming data quality skepticism in the greater science community.(Bonney et al. 2014) Projects that train participants and implement targeted sampling designs together can produce extensive and useful datasets that will help generate support for future projects.(Meentemeyer et al. 2015) We expect more support for citizen science projects will become available if projects can continue to demonstrate that the data collected by citizens are valuable and high quality.

Data quality is particularly important for rePhotoSA. For example, photographs need to be in the exact same location to overlay the images and quantify landscape changes such as the health of individual trees in White et al.(White et al. 2016) To

overcome this challenge, rePhotoSA produced a thorough set of instructions for taking repeat photographs. The project is also currently developing complementary, online video-based tutorials and intends to expand their public engagement to demonstrate how to capture research-grade repeat photographs in the field. Together these resources are examples of methods to improve data quality that can be incorporated into any project.

Project management and sustainability

Another major challenge in South Africa, which may be similar elsewhere, is that many of the citizen science projects are championed by postgraduate students, postdocs, or outreach coordinators that have limited appointments or availability. This challenge emphasises the importance of managing expectations and deciding on the appropriate lifetime for a project. For example, Cape Citizen Science was established to facilitate research for one student's project, but has now grown into an umbrella programme for other projects because of shifts in the scientific capacity, the depletion of project funds, or simply because the research was completed. While it may only take one PhD student to start a citizen science programme, it may take many to keep it going. We therefore recommend that future projects identify the ideal lifetime early on in the project planning process. The apparent deadline may even motivate participation. An alternative solution, employed by Iimbovane, is to hire a project coordinator who is solely dedicated to the project. Such a coordinator can relieve pressure on the researcher and dedicate efforts to sustaining the project over the long-term. While this capacity may not be financially feasible by most projects initially, it is an approach that would help overcome the challenge of sustaining projects or programmes.

Financial support

Limited funding challenges most research in South Africa. However, this challenge has different implications for citizen science programmes. Establishing a citizen science programme may not require a large investment, but the merit of establishing a programme depends on the availability of sustained funding. For example, one-time small grants from the Table Mountain Fund and the Faculty of Agrisciences at Stellenbosch University have both contributed to citizen science projects in the WCP, but

a different funding structure is needed if the projects are to evolve into sustained programmes that host multiple projects. Most financial support for research is project-based, but establishing a citizen science programme to facilitate a single research project may not be worthwhile unless it is sustained across multiple projects. An ideal funding structure in South Africa would involve longer-term support (3–5 years) for a single laboratory to initiate and sustain a programme that could incorporate multiple short-term projects.

Specific costs of each project vary, but the biggest expenses of the projects mentioned above include salaries for project coordinators or bursaries for students and covering the costs of travel (e.g. transporting youth to nature reserves). A few other costs for reference include, but are not limited to, hosting and maintaining datasets on servers, sequencing microbial cultures, general laboratory supplies, and outreach materials. For example, the Iimbovane Outreach Project budgets a large amount toward materials such as workbooks, ant identification keys, and promotional resources. Similarly, Cape Citizen Science tries to provide awards (e.g. plant identification field guides, biodiversity posters, dissecting-kits, etc.) to youth that successfully answer questions after presentations in outreach activities. In general, the costs of these materials and activities are easy to underestimate.

While securing financial support for research may be difficult in general, citizen science programmes may be able to adopt creative approaches to overcome the challenge. For example, Cape Citizen Science has received public support through two crowdfunding campaigns (DOIs: 10.18258/2066 & 10.18258/8690) and other projects have generated support as beneficiaries in 'MySchool MyVillage MyPlanet' (http://www.myschool.co.za/) or through corporate sponsorship. However, even if public or private support is a feasible funding mechanism, connecting with philanthropists in these situations can be challenging. One solution may be to collectively identify the citizen science projects that are present in South Africa, to serve as a repository of projects open to support from philanthropists. Such a repository could be maintained by a government agency or third party (similar to MySchool MyVillage MyPlanet), but the eligibility would need to include small and short-term projects without marketing campaigns or the required designation as a non-profit organisation (NPO).

Project guidance

Alternatively, even if funding is available, some researchers may be uncertain how to use it to achieve educational outcomes. Since citizen science is relatively new in South Africa, universities do not have capacity to guide researchers into the interface between their research programmes and the greater public. A financial incentive (e.g. internal grants) may be the top-down approach to enhancing societal impact, but additional training and guidance is critical for economic efficiency. Enhancing the network between projects, showcasing projects at broader scales, or offering workshops to other faculties interested in initiating projects would increase the efficiency. In the meantime, we encourage those in this position to seek guidance from other projects.

Attracting and maintaining citizen scientists

The number of registrations or people who express interest for citizen science projects may far exceed the number of actively participating members. This phenomenon has been described within the social sciences through the theory of planned behaviour where a gap often exists between intention and behaviour.(Ajzen 1985) Citizens may intend to participate but there are barriers to the behavioural expression of this intention. Some of the attitudinal barriers we have identified, particularly with rePhotoSA, are impatience or confusion with the technicality of taking and uploading a repeat photograph, ambivalence or indifference due to a lack of knowledge about the application of repeat photography, or loss of interest due to insufficient historical images in the participant's area of interest. Citizen scientists who initially struggle to participate in a project are unlikely to try again in the future.(Delaney et al. 2008) This underscores how critical it is to tailor an experience that firstly captures the interest of a potential citizen scientist and then creates a participatory environment that is both intuitive and rewarding.

One approach to recruiting potential participants who may have barriers to getting involved, is to use social media platforms to raise awareness. This approach has been implemented by many citizen science projects where emerging technologies have characterised a new avenue of public engagement.(Newman et al. 2010) rePhotoSA has observed the advantages of social media in cultivating support from the public, equipping

citizens with knowledge of repeat photography protocols, validating citizen repeat photographs, and disseminating the results of scientific studies using repeat photography. Therefore, implementing similar strategies with emerging technologies (such as social media campaigns) should not be overlooked by future projects because it can help overcome challenges to building communities and sharing resources.

Project redundancy

Many citizen science projects can co-exist in South Africa without overlapping because of the diversity and abundance of communities. For example, Cape Citizen Science and Iimbovane can co-exist in the same university and target the same age groups because they work with different communities. Cape Citizen Science has established partnerships with many NPOs, where the Iimbovane works directly with schools and schoolteachers. While both projects strive to provide meaningful engagement opportunities for similar age groups, there are many different communities and groups of learners to engage.(Hulbert and Roets 2018)

Conversely, the diversity of communities can also present opportunities for redundancy. For example, there are at least three online tools where citizens can report observations of biodiversity.(Hulbert 2016) Each of these tools have their own communities of participants who may be unaware of the other communities or tools. This might be a growing pain from emerging technologies and we may see a merger of communities as one tool becomes more popular. However, it is important for projects to take note of and consider using existing tools and platforms rather than creating new ones in the future. In some cases, it may even be feasible to extend an existing tool or project into new communities. Similarly, it is also critical that projects which exist in similar space communicate and work together to avoid redundancy and provide diverse opportunities to communities.

Socioeconomic context

The socioeconomic diversity in South Africa provides great opportunity for citizen science projects to co-exist and serve separate niches in society, but it can also be a challenge for projects to accommodate multiple groups. For example, projects such as

rePhotoSA require access to equipment that can be a barrier to participation for some groups. As an online repeat photography project, citizen scientists need access to a stable internet connection and at least a smartphone, if not a digital camera and tripod. While an online database may increase accessibility to the project for many (Delaney et al. 2008), it may also, together with the type of equipment required, actively exclude others. The unique requirement for high resolution images to be uploaded (≥3Mb) in this project provides a significant constraint for many participants, even those with internet connections, as these can be slow and unstable, especially in rural southern Africa. Despite the limitations, the online format for disseminating historical photographs and receiving repeats is the most efficient at present, but alternatives have been provided on a case-by-case basis. The smartphone may form a potential avenue for data collection in the future and the development of a 'gamified' repeat photography application is being considered. This may provide more opportunities for participation by opening the project up to citizens without access to expensive camera equipment or computers and increase participation with entertainment.

Conclusion

The objective of this commentary is to highlight some challenges we have faced as practitioners of citizen science projects in South Africa. We recognise that additional challenges likely exist for citizen engagement outside of the researcher perspective (e.g. the educator perspective), but we suggest that addressing the challenges herein will promote the sustainability of future citizen science projects. Prospective project leaders are welcome to contact us to join a network of projects in Africa. Although we have identified many challenges, we believe there is ample opportunity to initiate citizen science programmes in South Africa. Cumulatively, our projects demonstrate that the citizen science approach can be applied to achieve many objectives, even simultaneously, across many communities. Although each project serves a distinct niche in society and the research institutes, there are still many communities that do not have opportunities for engagement from local universities or research agencies. We therefore encourage increased support for the establishment and sustainability of citizen science projects.

Such support would provide more South Africans with opportunities for informal education and enhanced citizenship.

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The history and future of Cape Citizen Science

This section of the Appendix is not under preparation to be submitted independently. The content was written by Mr. Joseph Hulbert.

Cape Citizen Science (https://citsci.co.za) is a program that provides opportunities for non-scientists to contribute to ecological research relevant to plant health in the Western Cape Province of South Africa. Since its establishment, the program has diversified to include multiple projects, but the original Pilot Project was designed to enhance research aimed at revealing the diversity and distribution of *Phytophthora* species throughout the Cape Floristic Region. In general, energy was initially directed toward raising awareness for the program, shifted to engagement and data collection, and then followed with dissemination of the findings. In this sense, the completion of this thesis represents the conclusion of the Pilot Project and thereby provides room to grow in new directions.

The idea of engaging the public in research about *Phytophthora* diversity was first proposed during a meeting between the authors at the International Union of Forest Research Organizations (IUFRO) World Congress in Salt Lake City in 2014. At this time, Prof. Mike Wingfield indicated he would support such a project if Mr. Joseph Hulbert joined him as a PhD student at the University of Pretoria. The initial proposals were to focus the research in the forests of the Garden Route, but it was later decided to launch the project in the fynbos because of the higher abundance of populated areas and the proximity to resources such as Prof. Francois Roet's laboratory in Stellenbosch.

In 2015, before Mr. Hulbert had moved to South Africa, he launched the first crowdfunding campaign to raise support for the project. *Discovering plant destroyers with citizen science in South Africa (DOI: 10.18258/2066)* was successfully funded on May 1st, 2015 and provided Cape Citizen Science with financial flexibility from the start. Mr. Hulbert moved to Pretoria in August 2015 and began to raise awareness for the project by building a website and sharing proposals with relevant community leaders and stakeholders. He also visited the Western Cape Province for the first time, contributed popular writing pieces to regional magazines, and applied for sampling permits from Cape Nature, SANParks and a few botanical gardens in the area. The first engagement activity organized through Cape Citizen Science occurred in November 2015 when Mr. Hulbert collected samples at Kirstenbosch National Botanical Garden with the horticulturalist Mashudu Nndanduleni and a homeschool group called Se7en+1.

Efforts to raise awareness for the project continued in 2016 as Mr. Hulbert contributed four pieces of popular writing, provided four presentations to stakeholders such as Cape Nature and SANBI, tabled events such as the Funky Fynbos Festival and the Flower Festival hosted by the Hermanus Botanical Society, and provided resources for four news articles about Cape Citizen Science. He also presented at the regional science conference for the South African Association of Botanists and the presented to science communities such as the Stellenbosch University Department of Plant Pathology and the Invasives in the Cape Discussion Group. Mr. Hulbert moved to Stellenbosch from Pretoria in June 2016 and spent much of the remainder of the year leading youth engagement activities and workshops with stakeholders. During 2016, three youth engagement activities were organized with the EcoRangers in Helderberg Nature Reserve and four Scouts Troops at Hoys Kopie Nature Reserve in Hermanus. Ten workshops were also organized in gardens, nature reserves and national parks with stakeholders such as SANBI botanical garden staff and SANParks rangers. Several home gardens were also visited for sample collections following responses to news articles. Two marketing phases of Cape Citizen Science were also launched during 2016 to inspire public engagement. One phase was called *Go hiking for Science*, which invited citizen scientists to hike to specific randomized areas to collect samples. The other phase was called *Report a dying plant in the fynbos,* which invited citizens to share observations of unhealthy plants throughout the fynbos. In general, both phases yielded little engagement.

More than 200 youth were engaged in activities organized by Cape Citizen Science in 2017. While substantial effort to continue to raise awareness for the project was made (through one public talk, four additional popular writing pieces, four more presentations to stakeholders, three news articles, two radio interviews, presentations to scientific societies such as the South African Association of Botanists and the Southern African Society for Plant Pathology, and tabled events such as Science Forum South Africa and the AIMS Science Week event in Khayelitsha), enormous effort was also made to support and foster engagement of youth from impoverished communities surrounding Cape Town. The success of a second crowdfunding campaign, *Engage Kayamandi Youth in Cape Citizen Science* (DOI: 10.18258/8690), and the procurement of two outreach grants (The Mathre Education Endowment Fund from the American

Phytopathological Society and the Plant Pathology Promotion Fund from the British Society for Plant Pathology) provided specific funding to support youth engagement. As a result, fifteen youth engagement activities were organized during 2017 and celebrated with the production of the video: *Cape Citizen Science: A Year of Empowering Young Minds* (https://youtu.be/PKSI1sxHiEc). Two workshops were also organized with stakeholders and numerous home gardens were visited following citizen invitations to sample. Mr. Hulbert also shared about Cape Citizen Science to an international community by coordinating a session at the IUFRO 125th Anniversary Congress titled: *Early detection and monitoring of invasive forest pests and pathogens with citizen science*.

The effort to raise awareness for the program continued into 2018, but a general shift to diversify projects and share results from the Pilot Project was made. Cape Citizen Science was featured in one news article and one radio interview following the launch of a third marketing phase called *The Cape Town Hypothesis Test*. The purpose of this phase was to directly engage the public in a hypothesis test about the diversity of *Phytophthora* species present in high risk areas of the urban environment (e.g. near ornamental nurseries and ports of entry) compared to the natural areas on the slopes of Table Mountain. Again, this marketing phase yielded little engagement and few citizens were interested, but Mr. Hulbert was invited to give six presentations to scientific communities and help coordinate an International Citizen Science Day fair at Kirstenbosch National Botanical Garden. Three public talks and four youth engagement activities were also organized by Cape Citizen Science in 2018 and additional financial support was secured from the Social Impact Grant provided by the Faculty of AgriSciences at Stellenbosch University. An additional project was also added to the Cape Citizen Science website to provide diversity for participation and add program sustainability upon the completion of the Pilot Project. The *Bearded Beatle Biodiversity* project was presented to teachers during a workshop organized by the Transatlantic Science Education Cooperative and was particularly aimed at school groups. The project was designed to invite youth to sample bark and ambrosia beetles using methods developed by Dr. Jiri Hulcr's program at the University of Florida. The methods were tested out during two of the activities organized with youth during 2018.

In 2019, most of the energy behind Cape Citizen Science was dedicated to analyzing and disseminating results. However, two additional projects were also added to the Cape Citizen Science website to provide information for citizens about the polyphagous shothole borer and raise awareness of an alarming decline of tree ferns (*Cyathea capensis*) in the Garden Route. Both projects were linked to projects created on iNaturlist.org where citizens could report observations of signs or symptoms consistent with the insect pest or decline of the tree ferns. A version of the manuscript summarizing the findings of the Pilot Project was also included in thesis and was under preparation for submission to be considered for publication during the time of this submission. The findings were also shared internationally at the IUFRO World Congress in Curitiba Brazil during another session led by Mr. Hulbert titled: *Forest Health Defenders: empowering citizens to protect forests through research contributions.*

Although the submission of this thesis largely represents the completion of the Pilot Project of Cape Citizen Science, the program is stable and set up to continue sustainably. The authors are committed to maintain relationships with many of the communities previously engaged and will continue to engage with greater citizen science community in the country. Future collaboration and further engagement within the program is welcome. The history of Cape Citizen Science has demonstrated that a post-graduate student is all that is required to champion the development and engagement of the program. While the program continues to host numerous projects relevant to plant health, there is much room to expand and enormous opportunity for parallel projects, especially those interested in engaging citizens throughout the city of Cape Town. Those interested in collaborating are encouraged to contact the authors or join the South African Citizen Science community at https://groups.io/g/CitSciSA.