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Analysis and Assessment of *Phytophthora* Community in Perth

Saad Y. SAAD

Department of Biology, College of Education, Al-Iraqia University, Baghdad, IRAQ

Abstract
Phytophthora species were everywhere and they appeared to be no environmental filtering. Many studies reported that *Phytophthora* can be spread long-distances through moving infested soil by animals or humans along preferred pathways. In this study, analysis and assessment based on field works are presented for the *Phytophthora* community in Perth. Operation parameters of the nanopowder extraction method employing ultrasonic waves; mainly ultrasonic frequency, freezing temperature and application time, are very important to control the minimum particle size of the extracted nanopowder. This extraction method enables to produce nanopowder with high structural purity as the particle size approximately be the same as in the thin film sample.

Keywords: soil; *Phytophthora* ,community structure , Perth

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1. Introduction

Tree decline symptoms associated with *Phytophthora* are frequently associated with more than one species [1]. However, only a few studies have examined the diversity of *Phytophthora* species in a plant community[2] . Indeed, compared with determining community structure in natural ecosystems, evaluating *Phytophthora* community structure in an urban forest and the environmental factors which influence this community is challenging. This could be because, in urban forests, human activities are diverse and constant, which in turn introduce and change the biotic and abiotic factors that predispose urban forests to the introduction, establishment, and spread of new pathogens[3]. In this study, *Phytophthora* species were widespread and there appeared to be no environmental filtering. Many studies have reported that *Phytophthora* can be spread long-distances through moving infested soil by animals or humans along preferred pathways.

2. Materials and Methods

Study area and sample collection

The area investigated was the City of Joondalup located approximately 26 kilometers north of central Perth, Western Australia and surrounded by Wanneroo Road and Lake Joondalup to the east, Beach Road to the south, Amala Park to the north and the Indian Ocean to the west. Joondalup covers an area of 98.9 square kilometers (38.2 sq mi). For the purposes of this research, the study area was delineated by geo-rectified multi-spectral imagery. Between 2012 and 2015, digital multi-spectral imagery (DMSI) was captured annually with a fixed-wing aircraft across all urban bushland in Joondalup, as four spectral bands of data (red, green, blue, and near infrared) at a spatial resolution of 5 m pixels. The image gap was calculated by subtracting 2015-pixel values from that of 2012. An increase in pixel values means an increase in tree health over this period, while a decrease in pixel values means a decline in health. Using GIS, 236 sites from 91 parks where evidence of tree decline was present (Fig 2.1) were established for collecting soil and root samples to determine the presence of *Phytophthora* species. Bulked soil and root sites were collected during summer and autumn in 2014, 2015 and 2016. Each sample was approximately 150g made up of 8-12 scoops of rhizosphere soil collected within a 5m radius from under declining trees.

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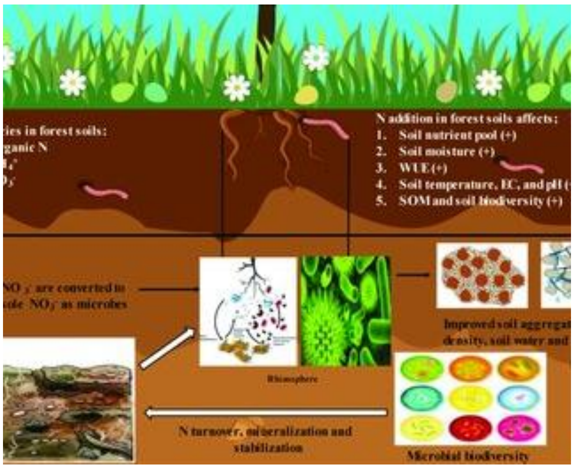
eDNA extraction from fine roots and metabarcoding

Fine roots from each sample were air-dried, and 60-80 g was ground to a fine powder using the TissueLyser LT (Qiagen). After each sample, the grinding tubes were cleaned by detergent (Pyroneg) then rinsed in an acid bath for 5 minutes (HCl 0.4 mM), then rinsed with water and finally sprayed with ethanol 70% and allowed to air dry. All ground samples were stored frozen. Free water was used to grind the controls. DNA extractions were performed using the Mo-Bio PowerPlant DNA isolation kit (Carlsbad, CA) following the manufacturer's protocol. Amplicon pyrosequencing and clustering were conducted as described previously[4]**.**

3. Results and Discussion

In the present study, forty-five *Phytophthora* phylotypes were detected from 236 sites across 91 urban forest parks sampled in association with different tree species exhibiting decline symptoms (Table 1) as selected by high-resolution multispectral imagery. What stands out from this study is the *Phytophthora* species richness across the urban forest sites sampled and included *Phytophthora* species from eight clades. This provides strong evidence for urban forests being a conduit of potentially invasive *Phytophthora* species into natural environments. It also highlights the role human activities play in this conduit, as in urban areas human activities are numerous and varied. These results compare favorably with the 49 *Phytophthora* species known prior to this study to occur within Western Australia. So, finding all these species within a relatively small urban forest was very interesting. Previously, 25 of these *Phytophthora* species were isolated from urban forests, 21 from agricultural crops (7 from annual crops and pastures and 14 from perennial and forest crops) and 25 from natural environments (Table 1).

P. gonapodyides and *P. fluvialis* are heterothallic (Table 1). *Phytophthora amnicola* was recently described from Western Australia, and was the fourth most frequently isolated species in the present study and produced lesions on 13 of the 15 plant species screened. It is possibly an emerging multi-host pathogen, so the further study is required to confirm its host range and pathogenicity.



Urban forest pathway of *Phytophthora* species

Urban forests are expected to grow in value to society as their importance in heightening peoples' sense of well-being and their contributions to ecosystem services and functions become more appreciated. However, urban forests may represent the main pathway of pathogens to ecosystems outside urban environments. The current study provides evidence for the potential dispersion of *Phytophthora* species from urban to natural ecosystems. Some studies indicated that when an area contained a particular invasive community, it could be a source of spread to new areas[5,6] and this phenomenon is called the bridgehead effect[7]. In the bridgehead effect scenario, urban environments are an important source of spread of *Phytophthora* species into natural ecosystems via major pathways: movement of plants and human activities. For example, *Phytophthora ramorum* was first found in the urban environment[8]. and then spread into other environments where nursery grown plants had been out-planted[9].

4. Conclusion

In concluding remarks, operation parameters of the nanopowder extraction method employing ultrasonic waves; mainly ultrasonic frequency, freezing temperature and application time, are very important to control the minimum particle size of the extracted nanopowder. This extraction method enables to produce nanopowder with high structural purity as the particle size approximately be the same as in the thin film sample. This was confirmed for the thin films prepared by physical vapor deposition methods and techniques. High reliability, high reproducibility and low cost of this method make it an optimum option for the extraction of nanopowders from thin film samples.

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Table (1) Some characteristics of Phytophthora taxa from ITS Clade 6 detected in the present study

Phytophthora species	Clade	Sub-clade	Sex[1]	Chlamyd-ospores
P. amnicola	6	2	Ho	Absent
P. bilorbang	6	2	Ho	Absent
P. crassamura	6	2	Ho	Absent
P. fluvialis	6	2	He	Absent
P. gonapodyides	6	2	He	Absent
P. gregata	6	2	Ho	Absent
P. inundata	6	1	He	Absent
P. kwongonina	6	1	Ho	Absent
P. lacustris	6	2	ST	Absent
P. litoralis	6	2	He	Present
P. moyootj	6	2		Absent
P. rosacearum	6	2,1	Ho	Absent
P. sp. *walnut*	6	1	ST	Absent
P. thermophila	6	2	ST	Present

Table (2) Some characteristics of Phytophthora taxa from ITS Clade 6 detected in the present study

Phytophthora species	Clade	Antheridia	Sporangia
P. amnicola	6		non-papillate
P. bilorbang	6		non-papillate
P. crassamura	6	Paragynous	non-papillate
P. fluvialis	6		non-papillate
P. gonapodyides	6	Amphigynous	non-papillate
P. gregata	6		non-papillate
P. inundata	6	Amphigynous	non-papillate
P. kwongonina	6	Paragynous	non-papillate
P. lacustris	6		non-papillate
P. litoralis	6		non-papillate
P. moyootj	6		non-papillate
P. rosacearum	6	Paragynous	non-papillate
P. sp. *walnut*	6		non-papillate
P. thermophila	6	Paragynous	non-papillate

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