DEVELOPMENT OF TOOLS FOR DETECTING, MONITORING, AND MANAGING BROWN ROOT ROT (CAUSED BY *PHELLINUS NOXIUS*) IN THE PACIFIC ISLANDS

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Introduction

The brown root rot pathogen (*Phellinus noxius*) is causing a destructive, mortality-causing disease on diverse tropical trees that are integral to forest ecosystems in the Pacific Islands (e.g., Ann et al. 2002, Brooks 2002, Sahashi et al. 2015, Figure 1). Our previous work indicates that (1) this invasive fungal pathogen is more prevalent and aggressive than previously reported across the Pacific Islands (e.g., Northern Mariana Islands, Federated States of Micronesia, Palau, American Samoa); and (2) multiple genetic groups of this fungal pathogen are present, which indicates that the pathogen can differ across eastern Asia, Australia, and Pacific Islands. To date, *P. noxius* has not been reported in some U.S. states/territories (e.g., Hawaii, Florida, Puerto Rico) where it represents a major invasive threat based on our bioclimatic models (e.g., Figure 2). Currently, human-associated spread of the pathogen into uninfested areas is a major concern exemplified by possible movement via infected plants, shipping containers, and/or soil associated with equipment/vehicles in the Pacific Islands and other U.S. states/territories. Currently, little or no information is available on (1) identification/detection of *P. noxius*, (2) brown root rot disease incidence/severity, pathways of spread, and (3) disease management in regions impacted or threatened by this invasive forest pathogen. Here, we present preliminary approaches to develop molecular probes for detecting distinct genetic groups within *P. noxius*. In addition, we are working to develop DNA-based tools for assessing the effectiveness of management methods for brown root rot disease, which are essential but critically lacking. The DNA-based tools developed from this project will be used to detect and monitor the brown root rot pathogen, while also helping to determine effective treatments to minimize damage from the disease in vulnerable areas within the Pacific Islands. Here, we present preliminary approaches toward developing tools to detect and monitor the soil/rhizosphere inoculum density of invasive *P. noxius* over time and determine the effects of treatments (e.g., urea, lime, urea + lime, and control) for disease management. In addition, other soil microbiota will be also monitored in association with management treatments.

Materials and Methods

Planned experimental site and design:

The project site will be selected among *P. noxius* infection centers within the Pacific Islands (e.g., Guam, Rota, Saipan, or Pohnpei). Approximately 30 infected trees will be selected to serve as center for each treatment plot of ca. 12 m (40 ft) diameter (0.0113 ha). After removing infected trees, including excavating roots and root residues (> 0.6 cm diameter), and collecting pre-treatment samples, one of five treatments will be applied to each plot. The five treatments follow:

1. Urea at a rate of 336 kg/ha (300 lbs/acre)
2. Urea at a rate of 112 kg/ha (100 lbs/acre)

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3. Urea at a rate of 336 kg/ha (300 lbs/acre) plus lime to pH 7.0
4. Urea at a rate of 112 kg/ha (100 lbs/acre) plus lime to pH 7.0
5: Control (untreated)

Our experimental design will be a complete randomized block design with six replications (30 plots = treatment/plot x 5 treatments x 6 replications). Soil probes to record temperature and moisture will be installed at each plot, and soil cores will be taken to determine soil properties.

Figure 1: Phellinus noxius *infection - dried mycelial sheath/crust (Photo: by Fred Brooks).*

Soil extractions, DNA extractions, metagenomic analyses, and tree plantings:

To determine changes in *P. noxium* density over time, soils will be collected before (baseline density of *P. noxius*) and after treatment applications in each plot to quantify *P. noxius* inoculum (e.g., every 3-months for 24 months) as well as to identify soil microbes (fungi and bacteria) associated with potential biological control of brown root rot disease. Metagenomic (metabarcoding) tools will be used for comparing forest soil microbial communities to (1) determine differences among soil treatments, (2) assess how differences relate to the soil properties (chemical and physical), and (3) examine how communities compare across space and time. At 6-month post-treatment, three breadfruit propagules will be planted in each plot to allow monitoring of future *in vivo* assessments of treatment effects on tree infection over the long-term.

qPCR/PCR:

In addition to rDNA-based, DNA barcoding, we plan to develop a DNA probe that distinguishes *P. noxius* from other soil microbes. This particular probe will be used for real-time PCR, which provides detection and quantifies relative pathogen amounts based on the DNA amplification rates. DNA extracted from control soils will also provide a basis for quantifying relative pathogen levels. Real-time PCR will be conducted on each soil sample to determine the relative changes in *P. noxius* levels associated with each soil treatment.

Soil analyses:

Soil temperature and moisture will be recorded over time. Soil samples will also be tested for pH and stored for measuring other soil properties, such as organic matter, bulk density, etc. Soil information will allow comparisons with soil levels of *P. noxius* and other microbes.

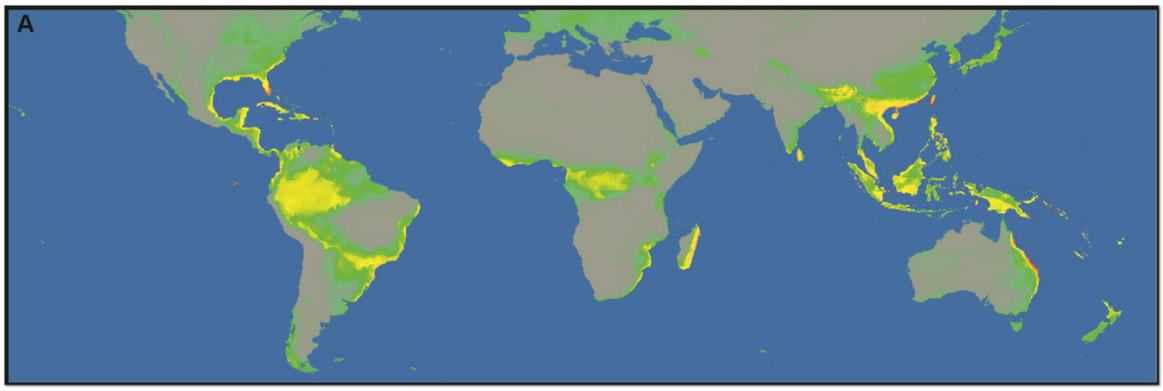

Figure 2: *Maxent bioclimatic predictions of potential distribution (suitable climate space) for* Phellinus noxius *based on all DNA confirmed records of occurrence. Different colors represent probability of risk based on suitable climate space for* P. noxius *(Modified from Stewart et al., in revision). Light green represents predicted suitable climate space, with green, yellow, orange, and red indicating increased suitability, respectively.*

Benefits

The tools developed from this project will be used to determine appropriate soil treatments and associated management methods for minimizing damage from brown root rot disease in vulnerable areas within the Pacific Islands. The scientific information provided by this project is critical to continuing development of sustainable management methods (e.g., fostering natural biological control and/or application of other treatments) for brown root rot disease.

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