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Brown Root Rot Disease Caused by *Phellinus noxius* on U.S.-Affiliated Pacific Islands

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Cover: Typical expression of *Phellinus noxius* on a ka tree. USDA Forest Service photo by Philip G. Cannon.

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Abstract

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Phellinus noxius is a fungus that causes brown root rot disease in tree and shrub species of subtropical and tropical regions. It has infected and rapidly killed hundreds of important tree and shrub species in these regions. It is widespread in the Pacific Region including all the island states of American Samoa, the Federated States of Micronesia (Chuuk, Kosrae, Pohnpei, and Yap), the Republic of Palau, the Commonwealth of the Northern Mariana Islands (Islands of Rota and Saipan), and Guam. This report explains how to detect brown root rot disease and its causal pathogen (*P. noxius*) by recognizing the signs and symptoms of the disease as well as using molecular techniques. It discusses several characteristics of *P. noxius* that make it such a destructive pathogen in the areas where it occurs and explains how the hot, humid, and sometimes stormy conditions that occur on these islands contribute significantly to disease spread and development. This publication also describes multiple approaches that have been attempted to control this fungus along with science-based information for consideration when trying to manage the pathogen.

Keywords: *Phellinus noxius*, basidiomycete, tree pathogen, Pacific Islands, extreme tropical weather conditions, typhoons, control methods.

Preface

This report is about the effects of *Phellinus noxius*, a fungus that causes brown root rot disease, also known as black sock disease. This disease infects and kills hundreds of tree species in the U.S.-Affiliated Pacific Islands and many countries in the tropics and subtropics. The main objectives of this report are to (1) provide resource managers and people living and working in these islands with sufficient information to recognize brown root rot disease and identify its causal pathogen (*P. noxius*), and (2) provide options for managing this disease.

This report consists of four chapters. Chapter 1 describes *P. noxius* and the type of damage it causes on most of these island states. Chapter 2 describes the signs and symptoms of brown root rot disease and explains how the disease spreads and kills so many forest trees in this region. Chapter 3 focuses on the molecular genetic studies of *P. noxius* and explains how these studies are helping us understand *P. noxius* behavior in different environments. Chapter 4 discusses options for managing this pathogen on the U.S.-Affiliated Pacific Islands.

Most of the information in this report is derived from experience with *P. noxius* in the U.S.-Affiliated Pacific Islands; however, other regions, such as Australia, Hong Kong, and Taiwan, were also visited to learn about methods those regions were testing or using to manage *P. noxius.* Reports concerning this fungal pathogen in the world literature were also consulted.

Chapters 1, 2, and 4 of this report were written mainly for land managers on the Pacific Islands to familiarize them with *P. noxius* and present options for managing its harmful impact. Chapter 3 is considerably more scientific than the other three chapters and is key to understanding the biology and spread of this fungal pathogen. It is also helpful for determining which disease control measures will be potentially effective.

Contents

Chapter 1: An Introduction to *Phellinus noxius* in the U.S.-Affiliated Pacific Islands

Philip G. Cannon[1]

Introduction

Phellinus noxius is a fungus that infects and causes rot in the roots and lower trunks of hundreds of tree species, generally resulting in tree mortality within months after infection. *Phellinus noxius* is severely affecting the health of tens of thousands of trees in natural forests, agroforests, urban forests, and forest plantations in the tropics and subtropics of the Western Pacific Region (fig. 1.1).

Most of the fieldwork described in this publication occurred in the U.S.-Affiliated Pacific Islands (fig. 1.1). These islands include American Samoa; the Federated States of Micronesia (FSM), including the island states of Chuuk, Kosrae, Pohnpei, and Yap; the Republic of Palau; the Commonwealth of the Northern Mariana Islands (CNMI), including the islands of Rota and Saipan; and Guam (fig. 1.1).

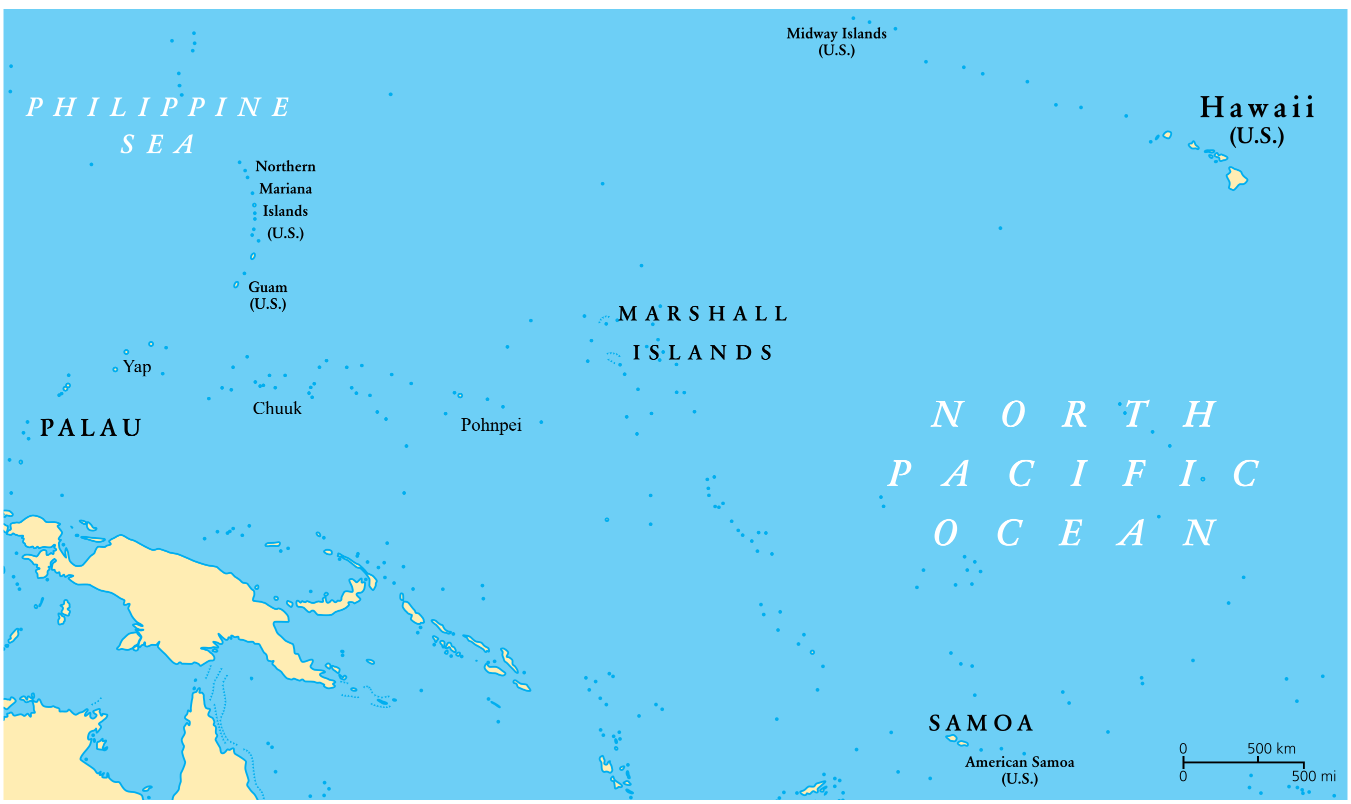

Figure 1.1—U.S.-Affiliated Pacific Islands.

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Phellinus noxius is known by several names (see app. 1); however, its current scientific name, *Phellinus noxius*, will be used throughout most of this report because of its familiarity to most readers. "Brown root rot" is the most often used common name for the disease caused by *P. noxius* and will also be used in this report (see app. 1).

The main objective of this report is to provide resource managers and people living and working in these islands with information useful for identifying *P. noxius* and options for managing the brown root rot disease caused by the fungus.

To manage brown root rot disease caused by *P. noxius*, we must first recognize the symptoms and signs of this disease and understand how the causal pathogen grows and reproduces. We must also understand how *P. noxius* spreads, infects, and kills trees, and that it can survive for up to a decade after it has killed a host tree. We will also consider previous attempts to manage *P. noxius* to better understand optimal approaches for managing this fungal pathogen.

Pacific Island foresters began to recognize *P. noxius* as a major threat to forest and horticultural trees in the U.S.-Affiliated Pacific Islands after it started killing valuable trees on their lands (fig. 1.2). Most Pacific Island foresters' understanding of how *P. noxius* functions was based largely on limited local experience, and initial approaches to control the disease were ad hoc and generally only marginally effective. Because brown root rot disease continued to expand and kill more trees even after local management methods were applied, it was determined that more effective control measures were needed.

Several regions in eastern Asia, especially Hong Kong, Taiwan, and Japan, have also been facing formidable challenges posed by brown root rot disease, and the shared findings of leading researchers in these regions on this disease will be of great value in the U.S.-Affiliated Pacific Islands, where effective and affordable management options are needed.

Phellinus noxius was first observed in the U.S.-Affiliated Pacific Islands early in the 1980s when Joaquin Tenorio, an entomologist in Saipan, CNMI, noticed that many flame trees (*Delonix regia*) were dying along avenues leading to the Saipan International Airport. He discussed this issue with Michael Robinson, a U.S. Department of Agriculture (USDA) Forest Service representative to CNMI, and they brought Charles Hodges, a Forest Service plant pathologist who worked on forest pathology issues in Hawaii, to Saipan to diagnose the problem (fig. 1.3). When Hodges visited the region, he soon recognized that the disease killing the flame trees was caused by the fungus *P. noxius.* He and Tenorio then published the first article on this pathogen in the U.S.-Affiliated Pacific Islands (Hodges and Tenorio 1984). In the

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Figure 1.2—Ned Klopfenstein and Rodasio Samuel pose by a breadfruit tree on Pohnpei, Federated States of Micronesia, that has been attacked by *Phellinus noxius*. Notice the dark brown mycelium (the "black sock") with a cream-colored edge advancing up the bole on the outside of the tree, one of the obvious signs of *P. noxius* infection.

Photo from North Carolina State University with permission from Charles Hodges

Figure 1.3—Charles Hodges studied many forest pathogens while working for the U.S. Department of Agriculture, Forest Service and diagnosed *Phellinus noxius* as the fungus responsible for the death of flame trees on Saipan in 1984.

1990s, Fred Brooks, a plant pathologist, compiled many keen observations about *P. noxius* while working on American Samoa (Brooks 2002a, 2002b).

In 2008, 24 years after Hodges and Tenorio published their paper, I had recently assumed the position of forest pathologist with the USDA Forest Service, Pacific Southwest Region (California, Hawaii, and U.S.-Affiliated Pacific Islands). In preparation for my first trip to the U.S.-Affiliated Pacific Islands, I wrote to the state foresters on each of the island groups asking if any forest disease problems were evident on their respective islands. If there was a response, it was almost always a polite "No." However, FSM state foresters Erick Waguk on Kosrae and Francis Ruegorong on Yap (fig. 1.4) both said that they had observed the death of a few trees after a "black sock" first appeared at the base of the tree, and then proceeded to grow upwards quickly along the outside of the tree.

During the ensuing 10 years, I visited most of the main U.S.-Affiliated Pacific Islands. While in these regions, with the assistance of local foresters and natural resource experts, I surveyed trees for evidence of disease, collected *P. noxius* samples, and established *P. noxius* cultures from most islands visited. While on Guam and Saipan, during one of these first visits, I collaborated with Yuko Ota and Norio Sahashi (*P. noxius* experts from Japan) and Ned Klopfenstein and Mee-Sook Kim (research plant pathologists with the USDA Forest Service) to establish procedures for collecting, culturing, and shipping the samples. Also, in 2010, I traveled with Brooks to American Samoa where, with help from that area's plant pathologist, Ndeme Atibalentja, and his pathology lab technicians Shalley Tailevai and Emily

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Figure 1.4—Francis Ruegorong (left) and Erick Waguk are the state foresters for Yap and Kosrae, Federated States of Micronesia, respectively. In 2008, they alerted Philip Cannon about a "black sock" growing up the outside of tree boles, which subsequently killed those trees. Here, they are participating in the 2017 Pacific Islands forestry conference, where *Phellinus noxius* and its management were among the topics covered in depth.

Ilaoa, we visited 19 *P. noxius* hot spots on the main island of Tutuila and made dozens of collections and fungal isolations.

During this 10-year period, I collaborated with forest pathologists skilled in molecular genetics of fungal pathogens and visited other countries with experience in attempts to control and manage brown root rot disease (see app. 2 for a list of collaborating foresters and forest pathologists, many of which are mentioned in this publication).

Several aspects of *P. noxius'* biology are important to understand. One is that *P. noxius* can grow extremely fast, both on the outside and inside of infected trees and from the root system of an infected tree to the root system of a nearby healthy tree. This fungal pathogen can also spread across larger distances via spores moving in air currents and infect healthy trees, especially trees that have been wounded. More details about these aspects of the biology of *P. noxius* will be explored in chapter 2. Another important aspect of this fungus is that its host range is unusually broad: *P. noxius* can infect hundreds of different tree species, including most of the important species growing on the Pacific Islands. In addition, *P. noxius* can survive up to

10 years within the decayed root systems of dead trees and as dormant inoculum in organic residue (Chang 1996). When a root system from a healthy tree grows into an old, infected root system or infested root residue, the dormant fungus can become active and quickly infect the root and subsequently kill the tree.

The death of an individual tree or even a forest stand from brown root rot disease can go unnoticed. In fact, the untrained observer may think that sites exhibiting a few scattered trees and weedy vines covering the landscape on some Pacific Islands is a normal condition. In addition, other factors can also contribute to barren landscapes on some islands, including frequent typhoons, repeated wildfires, rampant invasive plant populations, shallow soils, aluminum toxicity of the soil, and pressures from human populations. Nevertheless, by observing the subtle but impactful effect of *P. noxius* as it kills trees within infested areas, one can begin to understand the substantial harmful impact of *P. noxius* on the health of forests and ecosystems of these islands.

Studies in different tropical locales have determined that between 70 and 200 plant species are susceptible to attack by *P. noxius*. Because of incomplete records, it seems likely that, worldwide, more than 400 tree species are susceptible to *P. noxius*. Appendix 3 presents a list of host tree species attacked that was developed for Taiwan (Ann et al. 2002) and is useful for islanders of the Western Pacific Region where many of these same species are found. Appendix 4 presents a list of *P. noxius*-susceptible commercial trees compiled by CABI (2021), and other tree species belonging to these same genera and families may also be susceptible. Brooks (2002b) produced a list of susceptible species based on 20 strip transects through *P. noxius*-infected forests on Tutuila Island, American Samoa (app. 5).

Local Knowledge of *Phellinus noxius* in the U.S.-Affiliated Pacific Islands

American Samoa

Tutuila Island—

The survey contingent looking for *P. noxius* in American Samoa consisted of three forest pathologists, Brooks, Atibalentja, and myself, with forest pathology technicians Ilaoa and Tailevai. We found *P. noxius* in 19 different locations. In some cases, small infection centers of *P. noxius*-caused brown root rot had created 0.1-ha (0.25-ac) openings where no trees were able to survive and where newly infected trees on the periphery of these openings were starting to die. The signs of *P. noxius* on some trees on Tutuila Island, American Samoa were quite impressive (see fig. 3.3 in chapter 3).

Federated States of Micronesia

Kosrae—

Erick Waguk, Floyd Waguk, Maxim Nithan, Leon Sigrah, and Blair Charlie, collaborating foresters and agroforesters from the Kosrae Department of Forestry (see app. 2), contributed substantially to the local body of knowledge on *P. noxius* in the island State of Kosrae, Federated States of Micronesia. Erick Waguk first took me to see a diseased tree, which was the first tree he had observed with the "black sock" symptoms 2 months before my arrival. It was near the junction of Utwe and Island Roads 1.6 km (1.0 mi) north of Utwe town. However, when we arrived at the site, we found only a tall stump; the rest of the tree had somehow disappeared. At the time, neither of us understood that the tree was "missing" because *P. noxius*-caused brown root rot (or "the black sock") had killed it quickly and then rotted the entire bole and root system to the point that the tree trunk could no longer remain standing. The tree had fallen and rotted so quickly that only its remnants could be found lying in the weeds. Later, however, we found a few nearby trees that had been more recently killed by *P. noxius*-caused root rot. And, days later, when Nithan, Kathleen Friday (a collaborating forester), and I arrived at Floyd Waguk's house, we found two raging *P. noxius* epidemics underway in two separate, 20-year-old, 2-ha (5-ac), breadfruit (*Artocarpus altilis*) plantations. In the lower plantation, about 80 percent of the 200 breadfruit trees initially planted had already been killed, and the remaining trees showed symptoms of defoliation from *P. noxius* infection. At the same time, the upper breadfruit plantation looked relatively healthy, though a few trees showed initial symptoms of leaf loss, a condition locally referred to as "musrasr" ("balding" in Kosraen).

When we returned to these same breadfruit tree stands 2 years later, trees in the lower stand had almost completely disappeared; no tree boles remained standing. Only the remnants of a few mushy, brown root rot-decayed stumps were found. By that time, brown root rot disease had also progressed rapidly in the upper breadfruit plantation. There, almost all the trees that had been mostly green and healthy during the previous visit were now dead but still standing.

During our first visit to these diseased breadfruit plantations, Floyd Waguk also provided some important insight into how *P. noxius* could be transferred from one geographic area to another. Waguk said that about 10 years earlier, people from his village had collected root sprouts of breadfruit to send to Lelu Island, the adjacent capitol of Kosrae State, where they were given as a gift for planting. Later, when we visited Lelu Island, we found several dead breadfruit trees, and all the dead trees we examined showed signs and symptoms of brown root rot disease. This finding suggested that some of the gifted root sprouts derived from Waguk's breadfruit plantations may have originated from breadfruit trees that were already infected with *P. noxius*. Comparisons of isolates using molecular genetics (chapter 3) could confirm this possibility.

While on Kosrae, we also determined that spores were another potential mode for initiating *P. noxius* infections. This mode of spread was observed where breadfruit farmers making a trail through the ka (*Terminalia carolinensis* Kaneh) forest in the Yela Forest Reserve had cut large, trail-marking blazes in the buttress roots of about 10 gigantic ka trees. Several of these trees had subsequently become infected with *P. noxius* precisely at the wound sites created by these blaze marks, and several of these trees had already died. In some cases, brown root rot disease had spread from one of the originally infected trees to a neighboring tree via the root systems. This phenomenon of the infected blaze marks was first deduced by Charlie and Nithan while making a global positioning system (GPS) map showing the locations of all *P. noxius*-infected ka in the Yela Forest Reserve. Of special note is that except for trail blazing, all trees in the Yela Forest Reserve are given maximal protection (except for a few ka trees that had been felled to make dugout canoes decades earlier), and no other land use activities occur in this forest. However, this is not the only case where apparent spore infection of ka trees was documented in Yela. In 2013, a rare (for Kosrae) but powerful typhoon came through the island and caused considerable damage to some trees. Where a couple of crowns of ka had been broken off by these winds, *P. noxius* spores had apparently been able to land on and subsequently colonize the resulting wounds. At the time of our visit, the disease was rapidly advancing down the bole from these initial points of infection.

Pohnpei—

Phellinus noxius infections have been found at multiple locations on Pohnpei's main island of Pohnpei. Gibson Santos[2] resided in a beautiful agroforestry hamlet on the southwestern side of this island that unfortunately contained several breadfruit trees that were infected with *P. noxius*. Santos described two novel routes of spread that he had observed for *P. noxius* on some of his neighbor's land.

Use of *Phellinus noxius* for land clearing—

One case involved farmers who wanted to clear forested land for crops without having to fell the trees already growing on the land. In this example, the farmers located a tree infected with *P. noxius*, collected several wallet-size chunks of infected bark from that tree, and then placed one of these chunks on the base of each tree that they wanted to kill. In some instances, this approach was effective, with many of the inoculated trees dying within 3 months. The infected trees usually fell over within a few more months.

[2] Santos, G. 2011. Personal communication. U.S. Department of Agriculture, Forest Service, Natural Resources Conservation Service, Pacific Islands Area, Pohnpei.

These farmers were using *P. noxius* as a biological herbicide, and it was even more effective than a chemical herbicide because it not only killed the tree quickly, but also rotted the tree and its root system so fast that it seldom needed to be removed from the land. Santos also relayed that the disease may also have spread by people intentionally inoculating the trees of other landowners so that their trees would die. However, undesirable consequences were also associated with using *P. noxius* as a land-clearing agent. These consequences will be discussed later.

Additional human-caused spread—
One of the best examples of fungal pathogen movement via infected root sprouts was found in Netti Province, Pohnpei. On the road leading up to the Seven Waterfalls Natural Wonder, dozens of breadfruit trees were dying from brown root rot. We were told that many of these trees originated as root sprouts collected from a *P. noxius*-infected breadfruit stand at the base of the hill; it appears that some of these root sprouts may have been infected with *P. noxius*.

Other known locations of *P. noxius* on Pohnpei included a coffee plantation near Paliker, a village near Pwisehn Malek, aka Chicken **** Mountain (fig. 1.2), in some coffee plantations with vulnerable shade trees, and in a few farms near the Paradise restaurant. All these locations are on the western side of Pohnpei. The presence of *P. noxius* on the east side of this island has not yet been reported. Whether this distribution was due to host-species variability, differences in land use, or weather patterns is unknown. The influence of weather could be important, as during El Niño years, the westerly winds are often associated with extraordinarily heavy rainfall (Landers and Khosrowpanah 2004). The importance of high levels of rainfall is discussed in chapter 2.

Although, the above-mentioned examples may account for the some of the spread of *P. noxius* on Pohnpei, at least one other situation should be considered. In previous years, a United Nations Food and Agriculture Organization food-security project was initiated to establish breadfruit trees on outlying islands in the states of Chuuk and Yap, Federated States of Micronesia. Numerous breadfruit root sprouts were cut from trees on the island of Pohnpei and shipped to these outer islands. Unfortunately, when this project occurred, the risks of using infected *P. noxius* root sprouts were not yet understood, and no precautions were taken to ensure the root sprouts used were from noninfected trees. Currently, efforts are underway to determine if any of the transferred sprouts are showing signs or symptoms of brown root rot disease.[3] Any diseased breadfruit found will be excavated immediately and destroyed, preferably by fire.

[3] Englberger, K. 2020. Personal communication. Environmental specialist, Pohnpei Environmental Protection Agency. Pohnpei State Government. Pohnpei, Federated States of Micronesia.

Chuuk—

My visit to Chuuk State was brief, and only portions of Weno and Tenowas Islands were visited. On this trip, no *P. noxius* was observed. However, after I showed a presentation of *P. noxius* photos, Joakim Wasan, the Secretary of Agriculture for Chuuk, confirmed that he had observed *P. noxius* on the island of Fefan.

Yap—

Yap State's mainland, Yap Proper (FSM), is another island with an interesting history of *P. noxius*. During my first visit to Yap, Francis Ruegorong and Pius Liyeleg, both state foresters, said the main spot on the island for *P. noxius* was right behind the forestry building. This building had previously served as the Yap Agricultural Experiment Station and housed fodder for a fledgling beef industry for a period following World War II. Most of this fodder came from the Philippines and was brought in on pallets. [4] Is it possible that *P. noxius,* originating in the Philippines, was brought into Yap on some of these wood pallets? In chapter 3, we will discuss how molecular genetics could be used to confirm or disprove this hypothesis.

Although Yap's largest concentration of *P. noxius* infections was behind a forestry building (and this infection foci was about 0.5 ha [1 ac]), the fungus was also detected on breadfruit trees near Ruegorong's farm, in the poisonwood (*Rhus*) forest, and in a half-dozen other locations (generally one to four trees per location) near the path leading from the main road to the village of Amin in the province of Gagil-Tamil (home to Yapese forester Andrew Yinfel). It was surprising that more *P. noxius* was not found on Yap. However, Ruegorong mentioned that Yap had experienced exceptionally dry weather for 2 consecutive years, and these dry conditions may have reduced new infections by this fungal pathogen.

Republic of Palau

Because my visit to Palau was brief and had multiple objectives, I observed only a few signs of *P. noxius*. Nevertheless, some trees in the arboretum of the Balau Natural History Museum appeared to have been killed by brown root rot disease, and, by digging into the stump a bit, I observed its classic conks (see chapter 2) from which I made cultures. Isolates of this fungus from Palau were subsequently subjected to DNA-sequence analyses that confirmed that the fungus was *P. noxius*. Also, there were accounts of severely rotted trees falling on cars; these trees potentially failed because their root systems had been killed and rotted by *P. noxius*.

[4] It is still possible to find old pallets laying around this former agricultural import station, although none have been tested for *P. noxius*.

Commonwealth of the Northern Mariana Islands

Rota—

Rota is yet another island where the potential arrival of *P. noxius* from abroad and the impact of storms on disease development can both be considered (fig. 1.5)

During my visit, the two trees immediately outside the front entrance of the Rota airport were infected with *P. noxius* and in the process of dying. In a survey of the entire circumference of the Rota International Airport, 17 additional infection foci were found. Although we found *P. noxius* in many other places surveyed in Rota, the area surrounding the airport had, by far, the highest number of infection sites (fig. 1.6), strongly suggesting the airport was a potential port of entry for *P. noxius* into Rota.

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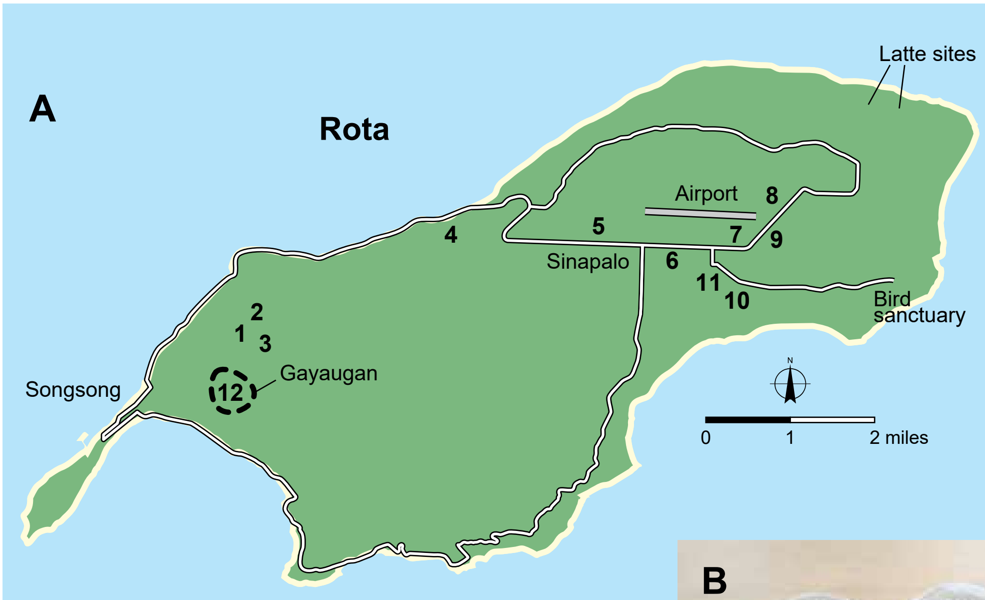
Figure 1.5—James Manglona, state forester for Rota, on his farm with a couple of breadfruit trees that show signs of brown root rot disease caused by *Phellinus noxius*. Infected bark from the root of the tree was taken to a laboratory to isolate and grow *P. noxius* in culture on potato dextrose agar in Petri plates. The DNA from the resulting cultures was extracted and analyzed. Similar samples of infected wood tissues were collected, and fungal cultures were established from samples from more than 100 other infected trees on Rota and other U.S.-Affiliated Pacific Islands during this study.

Saipan—

Saipan is another island where initial infection site for *P. noxius* was near a principal point of entry, the Saipan International Airport. Joaquin Tenorio informed me that one flame tree after another lining the road into this airport became infected by *P. noxius* in the early 1980s. Eventually, the line of affected trees reached about 400 m (0.25 mi) and brown root rot disease had killed almost every flame tree along that distance. This was why Tenorio reached out to Robinson, who in turn brought in Hodges to make the assessment about the disease that was killing the flame trees on Saipan. Their publication (Hodges and Tenorio 1984) documents the impact of *P. noxius* on the flame tree, the official tree of Saipan. The exact source of *P. noxius* in Saipan is still under investigation using molecular genetics (discussed in chapter 3). One theory is that it was brought in on pallets from the Philippines when supplies were brought in by plane following World War II, a similar scenario to that on Yap. Some evidence to support this possibility was a massive buildup of pallet manufacturing during and following World War II in the Philippines. Moreover, *P. noxius* has been found in the Philippines, and massive numbers of pallets are still piled up around the Saipan airport. Today, the manufacture of all pallets worldwide is done with strict requirements for wood treatment; however, this was not the case during World War II and in the decade or so afterward.



Mee-Sook Kim

Figure 1.6—(A) The major roads, airport, and locations where samples of *Phellinus noxius* and other root rot fungi were collected on Rota. These samples were isolated on potato dextrose agar in Petri plates after which fungal DNA was extracted from the cultures for subsequent analyses. (B) Fungal cultures from four different diseased trees on Rota after 1 week of growth. DNA analyses showed that the fungal isolates numbered 4, 7, and 11, were derived from *P. noxius*-infected trees; they grew much faster than the *Ganoderma* isolate. Potential mechanisms responsible for the phenomenal growth of *P. noxius* are provided in chapters 2 and 3. The brown growth in one of the plates labeled "4" is the pseudosclerotial rind that is a characteristic sign of *P. noxius* in culture.

Although the flame trees near the airport and along Flame Tree Road are the most notorious examples of *P. noxius* on Saipan, brown root rot disease is also found on several additional areas, including the grounds of the Agricultural Experimental Research Station, which is part of Northern Marianas College; in the row plantings of trees near the basketball gymnasium of that same college; in some extensive 0.4- to 1.2-ha (1- to 3-ac) infection centers on some flat, idle lands in the Garapan; and along Chalan Pale and Chalan Monsignor Guerrero Roads and Isla Drive. Significant infection centers are also found in Kagman and on Navy Hill. We revisited many of these spots under the guidance of Sid Cabrerra, who was a technician when Hodges and Tenorio performed their initial assessment and who fortunately remembered the locations of some of the original *P. noxius* infection centers.

Typhoon Soudelor (known in the Philippines as Typhoon Hanna) hit Saipan in 2015. It was a devastating hurricane with winds of 290 kph (180 mph) (a category 5 typhoon), and few trees were left standing in some of the areas where *P. noxius* infections were rampant (fig. 1.7). Furthermore, about 6 months after that typhoon, we found a new infection center at Navy Hill where it appeared that most trees had been attacked by *P. noxius* simultaneously because the mycelial crusts on the outside of affected trees (the black sock) had all risen to almost the same height (fig. 1.8).

A survey for *P. noxius* has not been conducted on Saipan since 2018 when the island was struck by Typhoon Yutu (known in the Philippines as Rosita) (fig. 1.9). This was considered the strongest hurricane on earth that year. Photos of the devastation from this storm show that a very high proportion of the trees on Saipan

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Figure 1.7—Five years before this photo was taken of a property on Saipan's Plata-Naguus Drive, hundreds of healthy trees were observed, with just three trees showing evidence of brown root rot disease caused by *Phellinus noxius*. It was previously speculated that *P. noxius* would slowly spread to most of the neighboring trees; however, *P. noxius* spread far more speedily than anticipated owing to two separate typhoons that hit the property, rendering the trees more vulnerable to infection. In addition, once infected, trees were far more vulnerable to windthrow. Large stumps from the previous forest are still visible, but vines have obscured most of the logs and stumps. The standing trees near the rear of the picture were the last to become infected, and had not yet died or fallen over. The palm trees seemed to be less vulnerable to *P. noxius*, but they were not completely resistant.

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Figure 1.8—Manny Tenorio, by a tree at the Ada Estate at Navy Hill, Saipan. The mycelial sheath of *Phellinus noxius* is growing up along the tree base. Removal of all brush, shrubs, and grass from a 0.4-ha (1-ac) site revealed the extent of the disease. Diameters at breast height were recorded of all trees on this site, and their bases were examined for brown root rot. Results of this survey are shown in figure 2.27.

were broken and some toppled completely. This typhoon likely rendered vast areas of Saipan susceptible to colonization of wounded trees by *P. noxius.* Interestingly, *P. noxius* has never been reported in the part of Saipan that lies to the north of Capitol Hill, although methodical surveys for the fungus have been conducted in that area.

Figure 1.9—In this satellite image, the eye of Hurricane Yutu is about to pass over Guam and the Commonwealth of the Northern Mariana Islands.

Guam

Exactly when *P. noxius* reached the U.S. island territory of Guam is unknown; however, plant pathologist George Wall reported finding it soon after his arrival in 1986, and plant pathologist Robert Schlub diagnosed an infected tree in the 1990s. Prior to 2014, Schlub stated that brown root rot had only been rarely detected in urban landscapes and never seemed to spread beyond one or two infected trees. In more recent years, forest pathologist Marjorie Falanruw reported seeing *P. noxius* on trees growing in karst topography near Ritidian National Park. Although karst topography is quite common on Guam, our initial surveys focused on the taller forests at the base of the karst escarpment in that park rather than the shorter, scruffier trees growing on the top of the escarpment.

Fortunately, in 2014, about 20 trained observers helped search for *P. noxius*. This training came about because a Forest Inventory and Analysis (FIA) crew of the USDA Forest Service and an endangered species crew from the University of Guam were both initiating activities in Guam at the same time our *P. noxius* survey group was beginning its survey. Yuko Ota, who was a part of our *P. noxius* survey group, had spent 20 years in Japan studying this fungus and she conducted a training course for these FIA and endangered species teams on how to recognize brown root rot disease and its causal agent, *P. noxius*. For 6 months, both FIA and endangered species teams searched for and recorded the locations of *P. noxius* infection foci on Guam. Both teams recorded the locations where they found *P. noxius* using GPS coordinates, and later provided maps showing the locations of 70 infection foci (fig. 2.29). It is worth noting that strong typhoon winds had pounded Guam 6 to 9 months prior to these surveys.

From the preceding information, *P. noxius* was determined to be present on most of the U.S.-Affiliated Pacific Islands. A stylized representation of the known distribution of *P. noxius* is depicted in figure 1.10.

American Samoa is not shown in this diagram, although *P. noxius* was also found at 19 different locations on Tutuila Island. Additional details about all these sites can be found in trip reports by the author (Cannon 2013, 2014, 2015, 2017, 2019).

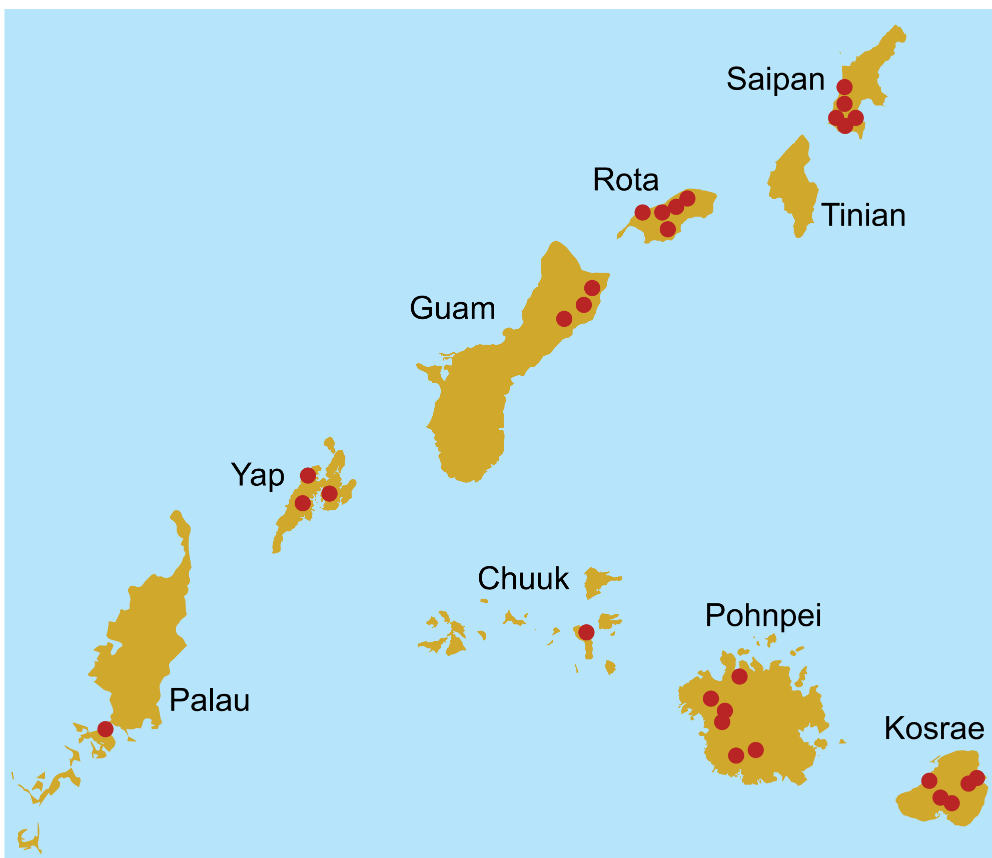

Figure 1.10—Red dots show the locations where *Phellinus noxius* was found in the U.S.-Affiliated Pacific Islands. Note: The shapes of the islands are accurate, but distances and directions between islands are not to scale to facilitate their representation in this diagram.

Chapter 2: Signs, Symptoms, and Etiology of Brown Root Rot Disease and Its Causal Pathogen, *Phellinus noxius*

Philip G. Cannon[1]

Signs and symptoms are features of a disease that can be observed, often with the naked eye. They can indicate what organism, if any, is causing a tree to be diseased. Specifically, signs are features of the disease-causing organism (pathogen) itself that can be observed, whereas symptoms of plant disease are defined as visible effects of the disease on the plant. Symptoms may include detectable changes in color, shape, or function of the plant as it responds to the pathogen-caused disease. Etiology refers to the biological manner by which a disease develops and affects the host, and how the pathogen reproduces and spreads from one host to another.

Signs of *Phellinus noxius*

The most common sign of *P. noxius* and the one most commonly observed is the formation of the black/dark-colored "sock" or mycelial sheath that forms at the base of an infected tree (fig. 2.1). This sock/sheath is a mucilaginous mycelial web of black to orange-colored fungal hyphae that produces exudates, which can

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Figure 2.1—Typical expression of a freshly forming *Phellinus noxius* mycelium on a buttress root of ka tree in Kosrae, Federated States of Micronesia. This is a very clear sign that *P. noxius* is present on this tree. A global positioning system was used to record the location of the infected tree.

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decompose lignin, cellulose, and hemicellulose—the principal chemical components of a tree's wood and bark. The sock/sheath is often most evident during prolonged rainy periods. It can be thick (up to about 2.5 cm or 1 inch), and, when freshly formed, is a deep black or dark colored. It is often accompanied by a 2.5- to 5.1-cm (1- to 2-inch) whitish margin of young, swollen mycelium along its outer edge. Furthermore, this leading edge often exudes beads of liquid, which are likely attributable to profuse respiration by the fungus while its wood-decomposing enzymes degrade the woody components on the outside of the bole of the tree and the bole itself (fig. 2.2). Because this sign is most strongly expressed during extended rainy periods when the fungus is growing the fastest, the tail end of a rainy period is an ideal time to quickly survey numerous trees for *P. noxius*. During the dry season, this sign may be more difficult to observe as *P. noxius* is far less active during dry periods and the black sock fades to grey or tannish brown, a color similar to that of the bark of some tree species in the U.S.-Affiliated Pacific Islands.

Figure 2.2—The leading margin of a mycelial sheath of *Phellinus noxius*. This fungus is growing rapidly, as evidenced by the beads of liquid formed from the actively respiring fungus as it degrades the tree bark.

Another clear sign of *P. noxius* is the fructification or basidiocarp where its spores are produced (figs. 2.3 and 2.4). A limitation to relying on *P. noxius* basidiocarps for identification is that they are not commonly present, especially during dry periods. However, when present, *P. noxius* basidiocarps or other signs can sometimes be found by doing one of the following:

Norio Sahashi

Figure 2.3—A classic conk or shelf-like sporocarp or basidiocarp of *Phellinus noxius*. This sporocarp is reported infrequently on some islands and never on others. Note the lavender-colored on the underside surface of the conk.

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Figure 2.4—Joab Santos shows the top (left) and bottom (right) of a large *Phellinus noxius* basidiocarp (conk) found on a breadfruit tree from his farm on Pohnpei Island, Federated States of Micronesia.

- Roll a log over and observe its underside, where resupinate basidiocarps are typically found, if present (figs. 2.5 and 2.6).
- Dig into the rotten wood at the base of the decayed stump, about 30 cm (1 ft) below the groundline; sometimes perfectly formed shelf-like basidiocarps or conks can be found on the subterranean portion of the stump.
- Chop into the rotten wood of a tree that has died and see if the characteristic orange to brown pseudosclerotial rinds or plates of *P. noxius* are present (fig. 2.7)
- Uproot the tree and observe the undersides of the excavated roots, where resupinate basidiocarps or mycelial crusts of *P. noxius* can sometimes be found.

Another sign occurs when *P. noxius* is growing in contact with the soil. Under these circumstances, the fungus will usually aggregate large amounts of soil into its mycelium, which make the root surface seem very gritty.

Norio Sahashi

Figure 2.5—A resupinate (appressed) basidiocarp of *Phellinus noxius*. Resupinate fructifications such as this one can vary in size from about 12 cm^2 (about 0.73 in^3) to more than 10 m^2 slightly more than 100 ft^2. Note: two layers of fructifications are shown here. The older layer is now black and has stopped producing spores. The younger one is lighter colored and is currently producing spores.

Figure 2.6—After rotating this log slightly from its original position, this striking resupinate *Phellinus noxius* fructification (basidiocarp) was revealed. *P. noxius* fructifications are difficult to find, even by forest health professionals. However, in areas where they are produced, these fructifications can be found at a much higher frequency, when decayed stumps are broken up or when the cool, dark, damp underside of root systems and logs are examined. These fructifications can produce billions of spores.

Besides basidiocarps, several other signs can be reliable indicators of *P. noxius*. The spores of *P. noxius* are also distinctive. They are shaped like a typical basidiospore (ovoid, with average measurements of 4.2 by 3.2 µm); however, they are usually lavender colored, a feature that provides some protection against the ultraviolet light of the sun. When the mycelium of *P. noxius* grows on the outside of the base of a tree, it is generally dark black/brown and grows quickly. The fungus can remain in this active phase throughout the rainy season. The growth of the dark mycelial sheath can sometimes reach heights of 4.85 m (16 ft) on a living tree,[2] but it is found more commonly on the lower 0.3 to 0.9 m (3 to 10 ft) of the tree trunk. Sometimes the mycelial sheath does not even extend above the soil surface. When it does, its position seems to correlate with free water that accumulates in bark depressions at the base of the tree, a result of water flowing down the stem during rainstorms. As the season becomes drier, the color of the mycelial sheath fades to brown, orange-brown, or sometimes a mottled orange and white, yet it remains quite distinctive.

[2] Martinez, M. 2017. Personal communication. Graduate student, University of Guam, Mangilao, Guam 96913.

If detected, these mycelial sheaths or basidiocarps are clear signs of *P. noxius*-infected trees; however, because they usually grow only a short distance up the tree bole, the ground vegetation (fast-growing grasses, vines, and small shrubs) on most Pacific Islands often obscure these signs. Therefore, it is often necessary to remove or displace vegetation around the base of the trees being surveyed to determine if these trees have been infected by *P. noxius*. Without checking all sides of the tree base, some infected trees may be overlooked. Also it is often necessary to check the underside of the roots of dead trees to find evidence of a mycelial crust or resupinate basidiocarp.

The bark is not the only part of an infected tree that is attacked by *P. noxius*. Just inside the bark, at the juncture of the bark and the xylem wood (where the cambial layer once was), a *P. noxius*-infected tree will often have a thin, white layer of mycelium. This is where *P. noxius* has displaced the cambial tissue with a 4-mm (1/8-inch) layer of mycelium. Where *P. noxius* has destroyed and replaced the cambial tissue, the bark can remain in only minimal contact with the tree and can easily be knocked off, exposing the white mycelial layer beneath.

Other signs also are apparent in the sapwood. A white rot of the wood (explained later in the etiology section) is caused by *P. noxius*, and mats and pockets of stringy white mycelium are commonly found throughout the wood colonized by this fungus. Near the final stages of wood decomposition, *P. noxius* begins to form characteristic pseudosclerotial rinds that encase the fungus. These units of rind-encased decayed wood form pods that are typically about 4 to 8 cm^3 (0.24 to 0.49 $inches^3$) in volume (fig. 2.7). These pseudosclerotial rinds usually begin as nonagglutinated hyphae that become agglutinated and then turn into faint orange lines. These lines then turn brown and eventually black and harden as they age further. If the decayed wood could be removed, the pseudosclerotial pods would collectively resemble a three-dimensional matrix, like 3D chicken wire, or a tree-trunk-size collection of orange-to-black pods. As these pseudosclerotial rinds age, they turn progressively darker, become stronger and, when mature, are water resistant and can resist penetration by many fungi and insects, including termites.

It is worth noting that several other species of wood-rotting fungi can also form these pseudosclerotial plates. The exact purpose of these pseudosclerotial rinds or plates or pods is not certain. Similar lines form in trees attacked by the honey mushroom, *Armillaria* spp.; and these rinds (plates, zone lines) are speculated to play a role in protecting *Armillaria* from unfavorable conditions or other fungi that attempt to invade its territory (Worrall 2004).

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Norio Sahashi

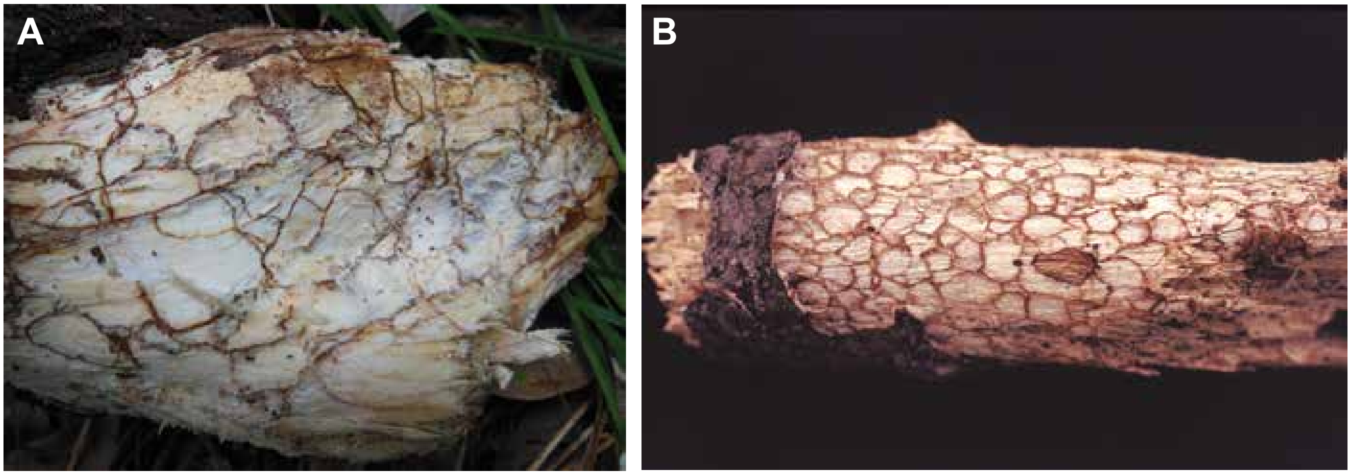

Figure 2.7—Wood infected with *Phellinus noxius* showing the development of pseudosclerotial rinds or plates. In (A), the rinds are just beginning to develop and are still weak; in (B), they are hardened, watertight, and impenetrable by most insects and fungi. The fungus can remain viable in these pods for 2 to 10 years after the tree has died.

During our surveys for *P. noxius*, we collected samples from hundreds of trees showing signs described in the previous paragraphs. Fungal isolations were performed from the sign or the associated wood, and *P. noxius* was established in culture (fig. 2.8). Potato dextrose agar was the most common medium used, although Chang (1995) recommended a more selective medium. DNA from these cultures was then extracted and used for polymerase chain reaction (PCR) and subsequent DNA sequencing to identify the fungus. This procedure is described in more detail in chapter 3. In all cases where a pure fungal culture was obtained, DNA analysis showed a match with *P. noxius* from samples collected from trees that had the signs of *P. noxius* described above. These results provide confirmation that the previously mentioned signs are reliable for identifying *P. noxius* in the field.

Faith Bartz

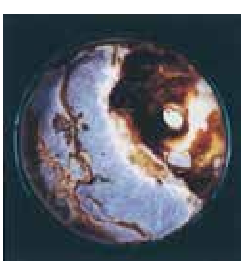

Figure 2.8—An agar Petri plate showing the formation of pseudosclerotial rinds of *Phellinus noxius* in culture. This rind or plate formation involves a proliferation, aggregation, melanization, and suberization of broad hyphae at the interface where zone-line formation takes place. Factors such as oxygen level increases, carbon dioxide decreases, moisture level changes, and wounding reactions have each been studied to determine which of these factors might influence plate formation; however, no definitive conclusions have been reached (Lopez-Real and Swift 1977). (Photo taken by Faith Bartz and provided courtesy of North Carolina State University).

Symptoms of Brown Root Rot Disease Caused by *Phellinus noxius*

The first thing commonly observed on a dicotyledonous tree attacked by *P. noxius* is that its leaves become smaller, turn yellow, dry up, and drop off, resulting in a defoliated tree (in conifers, the first symptom is red needles). Thus, the Kosraean word for this disease, “musrasr” (balding), is especially appropriate. Subsequently, the strength of the wood in the tree weakens quickly as *P. noxius* grows rapidly through the wood. As the brown root rot disease progresses, within a few months, the bark sloughs off from affected trees because the cambium has been destroyed, and then limbs begin to fall beginning with the smallest. These failures occur because *P. noxius* rapidly decomposes all the components of wood, which is typically composed of about 50 percent lignin, 20 percent cellulose, and 30 percent hemicellulose. Decay in the base of trees, and especially in their root systems, causes infected trees to fall over relatively fast.

Besides the symptoms of disease on a single tree, other symptoms include whole areas of forest, from 0.004 to ≥4 ha (0.01 to ≥10 ac) in extent, either with trees being killed progressively or dying all at once (fig. 2.9). Reasons for these stand-level symptoms and some explanation of the different progressions of mortality are provided in the next section.

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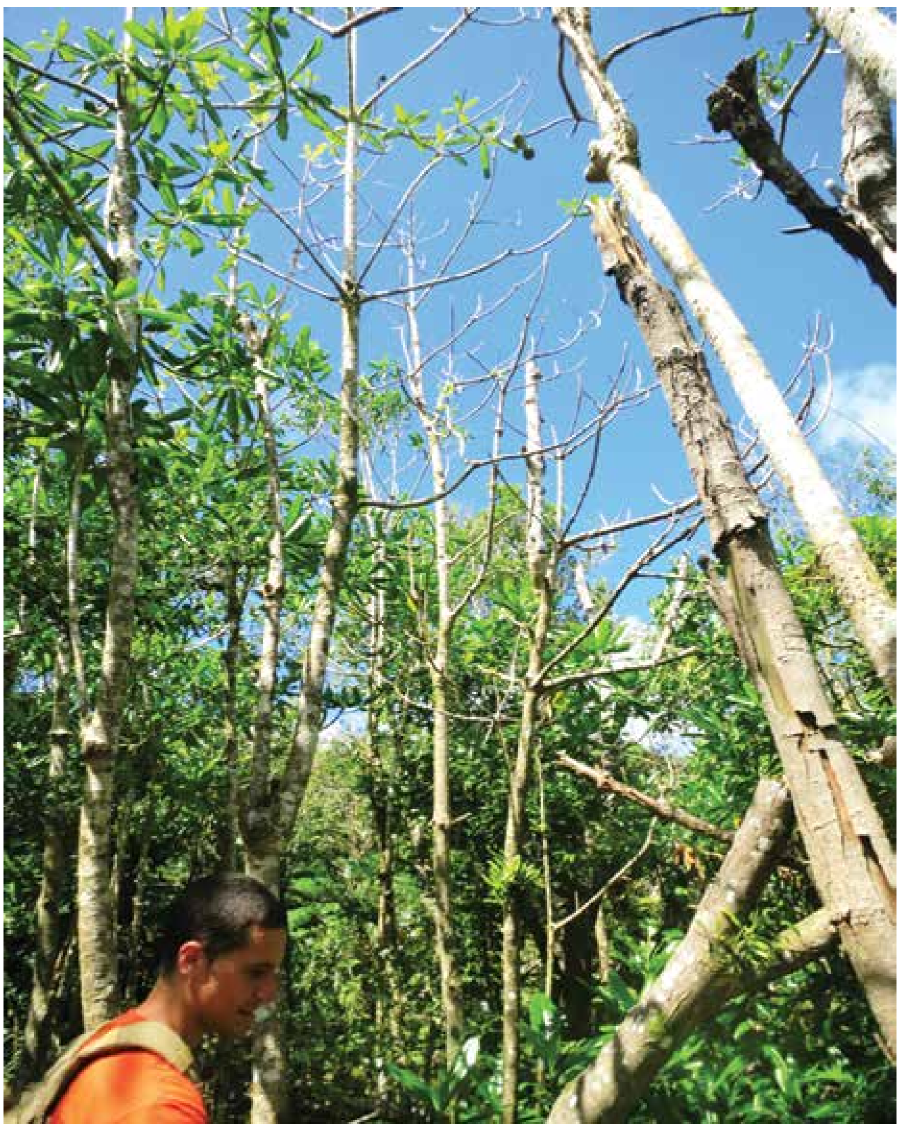

Figure 2.9—Mario Martinez checks out a *Phellinus noxius* infection center on Guam. Trees on the right were the first to die as the fungus spread through their root systems to neighboring trees.

The Etiology of Brown Root Rot Disease Caused by *Phellinus noxius*

Phellinus noxius can spread from one tree to another in three basic ways (fig. 2.10). (1) It can grow from one tree to a neighboring tree through root systems if these root systems come within several centimeters (a few inches) of each other or when the root system of a healthy tree grows into contact with infected plant remnants (figs. 2.11 and 2.12). (2) It can spread via the transport of infected plant material from one location to another (figs. 2.13, 2.14, and 2.15). (3) The fungus can also travel relatively long distances by airborne basidiospores.

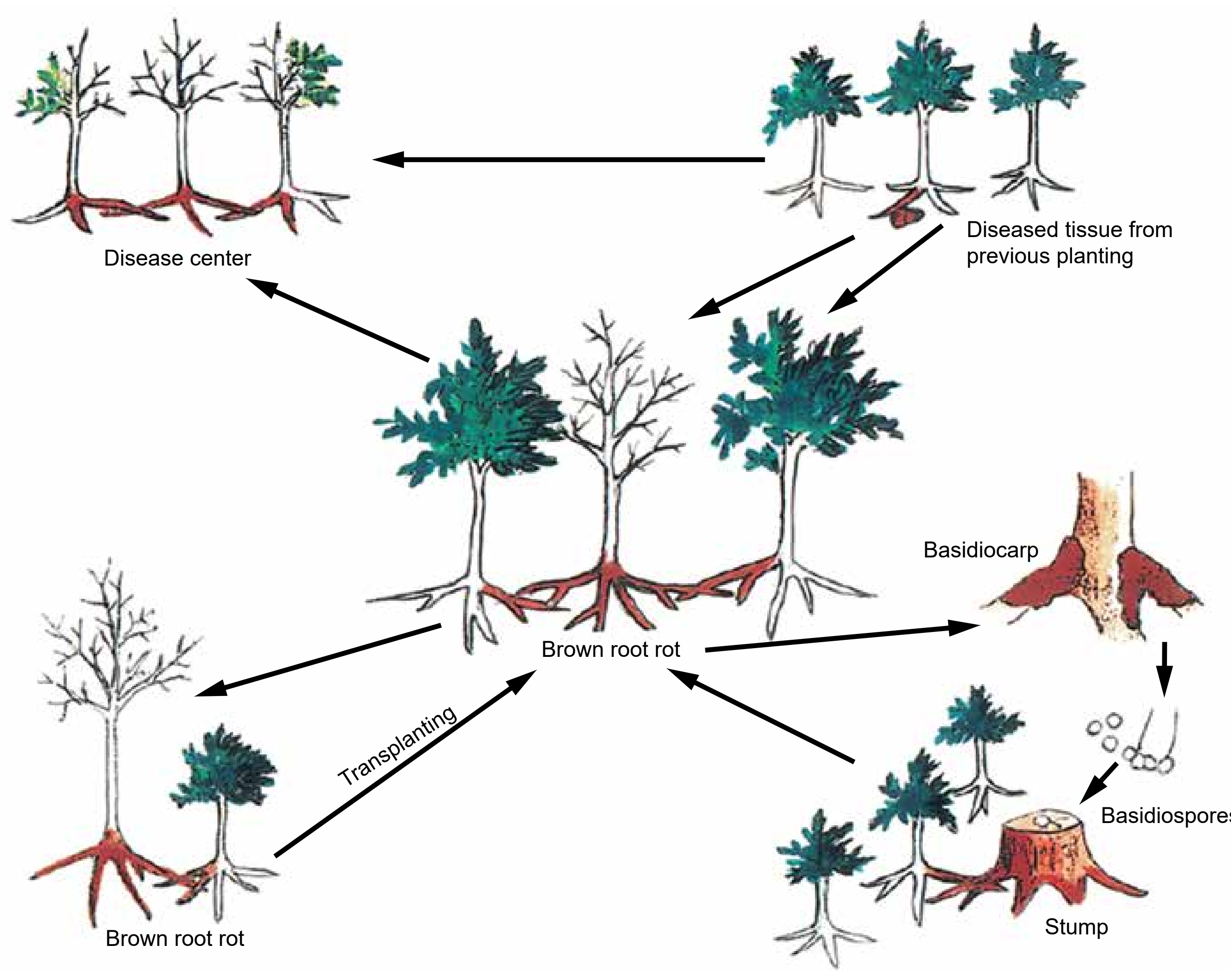

Figure 2.10—Ways that *Phellinus noxius* can spread from one tree to another. Top right, *P. noxius* is spread from infected roots of a previously infected forest stand that was present in the soil to the root system of a healthy tree that grew toward the inoculum. In the top left, *P. noxius* is spreading from an infected tree to neighboring trees via the root systems that are in close contact. Bottom right shows the fungus spreading by spores from a previously infected tree to wounds or stumps in another location. Bottom left shows how transplanting an infected tree can bring the fungus into contact with another tree. (From Ann et al. 2002: original drawing provided courtesy of P.-J. Ann).

Rodasio Samuel

Figure 2.11—This photo demonstrates the *Phellinus noxius'* ability to spread from one tree to another through their root systems. Toward the back is a recently infected breadfruit tree. In the foreground is a large, infected root that extends from the tree shown at bottom right, which appears to be the first tree infected at this location.

Figure 2.12—Fred Brooks observes *Phellinus noxius* on kapok tree on American Samoa. The fungus appears to have grown 66 m (230 ft) over 11 years or 6 m (20 ft) per year through the root systems of these trees that lined Aloau Road.

Konrad Englberger

Figure 2.13—A root sucker of a healthy breadfruit tree that has just been prepared for transfer and planting at a new location.

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Figure 2.14—The small mycelial collar at the base of this root sucker is a sign of brown root rot disease, caused by *Phellinus noxius*. If this root sucker were used to start a breadfruit tree at a new location, it could transport brown root rot disease, likely killing the sucker and infesting the site with *P. noxius* at the new location as well.

The second means of long-distance movement of *P. noxius* is via human-assisted transport of infected vegetative material from one location to another. For example, it is not uncommon for infected but healthy-looking root sprouts collected from a tree in one location to be transported and planted in another location. This occurs frequently among the Pacific Islands as the fruit of the breadfruit tree is the most common staple in the diet of most islanders, and breadfruit root sprouts are a customary gift often shared among family members and friends in another village or country. Of course, if the breadfruit tree at the source location is infected with *P. noxius*, the use of infected root suckers (fig. 2.14) when used as breadfruit tree propagules could allow the spread of brown root rot to new locations.

Figure 2.15—An example of how *Phellinus noxius* can be spread via human-assisted transport of infected root suckers is when, as a gesture of good will, root suckers from breadfruit trees in Utwe Ma (near Utwe). were collected and transported to Lelu Island, where they were planted. Subsequently, all breadfruit trees on the Utwe farm and many of the trees on Lelu Island, a nearby island of Kosrae, died from *P. noxius*-caused brown root rot disease, strongly suggesting that the brown root rot disease was moved with the cut root suckers.

The rate of root-to-root spread of *P. noxius* can vary widely. On American Samoa, under conditions considered to be near optimal for this fungus (warm temperatures and plenty of rainfall) and a highly susceptible tree species, *P. noxius* grew 66 m (233 ft) along a row of kapok trees in 11 years. This suggests that the fungus grew 6 m (20 ft) per year by mycelial growth via root-to-root spread (fig. 2.12). This growth rate would probably be slower under drier conditions or among more resistant host trees.

Phellinus noxius can also spread via basidiospores, which are formed on two different types of fructifications (basidiocarps or fruiting bodies). The classic fungal structure is a shelf-like sporocarp, basidiocarp, or conk (figs. 2.3 and 2.4). Another structure is the "resupinate" basidiocarp, which seems far more common in some regions. The resupinate basidiocarp is most often found on the undersides of infected root systems or decaying logs. It is flat and appressed against the wood, and the basidiospore-forming layer (hymenium) is generally just one layer of pores thick (figs. 2.5 and 2.6). The chance of a single spore infecting a healthy tree is remote. However, other pore-forming members of the Basidiomycota are known to produce billions of spores per day per square centimeter (square quarter inch) of active fruiting structure (Kadowaki et al. 2010). These spores are very small, light, ovoid structures that are suspended in air by Brownian motion and can drift in air currents for days under suitable conditions. Most basidiospores are eventually killed by the ultraviolet rays in sunlight or fall toward Earth because of gravity. Most basidiospores will land on something they cannot colonize, like rock, soil, ocean water, or nonhost plants. Some basidiospores will land on host trees, and a small but significant few will land on tree wounds. If these spores of *P. noxius* germinate in a relatively fresh wound, they can cause a new infection. Factors such as the availability of water can influence spore germination and colonization by mycelia from these spores, too.

Another way brown root rot pathogens can be transported is when infected wood material is taken to a new area and placed in contact with living trees. For example, when thousands of pallets were discarded on the Pacific Islands following World War II, some pallet wood could have harbored wood-rotting pathogens that had previously colonized the wood within the forests where it originated. To this day, pallets are commonly discarded after their cargo has reached its destination (fig. 2.16). If pieces of those pallets harboring *P. noxius* are subsequently discarded near forests or individual trees, this could result in trees becoming infected by the transported *P. noxius*. This mode of pathogen transport would have been especially relevant in the decades before, during, and after World War II before the International Standards for Phytosanitary Measures were established. Today, all new pallets are subjected to required drying and chemical treatments to ensure that wooden components are not potential carriers of pathogens. By international decree, all treated pallets must be marked with the treatment that they received and the name of the treatment plant (fig. 2.17).

Infections originating from *P. noxius* basidiospores are remarkable because many tree species have evolved features that provide protection from fungal infections (figs. 2.18 and 2.19). Tree bark

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Figure 2.16—A modern-day stack of pallets near the airport in Saipan, Commonwealth of the Northern Mariana Islands.

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Figure 2.17—This label guarantees that all wood components of this pallet have been treated in accordance with the International Standards for Phytosanitary Measures.

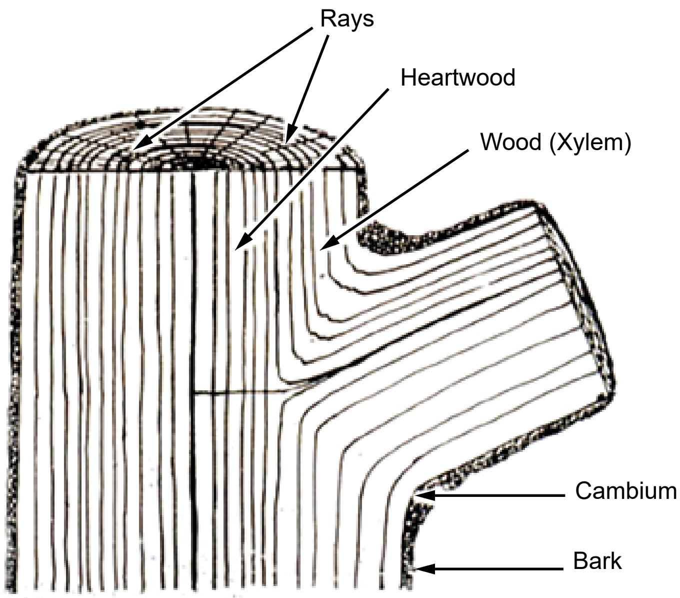

Figure 2.18—Diagram showing the anatomical structures of a tree bole that provide some defense against *Phellinus noxius* infection. The bark generally provides an impenetrable barrier to prevent spore colonization. If the bark is damaged, however, such as by a machete or a broken limb, the resulting wounds render the inner wood vulnerable to fungal infection. None of the defenses inside the tree are as robust as the bark. Diagram courtesy of Martin MacKenzie, USDA Forest Service.

is an especially effective barrier against spores. However, tree bark can be damaged in various ways. And, once the bark is damaged, spores can gain access to the wood inside.

After gaining entrance through the bark layer, spores of *P. noxius* can germinate and result in mycelium that can rapidly spread within the phloem and young xylem (fig. 2.18) because these tissues provide the nutrients needed for mycelial growth, while offering few defenses. When a wood-rotting fungus, such as *P. noxius*, reaches the inner layers of wood, the heartwood, it tends to spread more slowly because most trees deposit waste chemicals (called “extractives”) in dying heartwood cells. Because many extractives are fungistatic, the heartwood of some tree species offers some resistance to wood-rot fungi. However, the composition and concentration of extractives found in heartwood varies significantly among tree species. Many tropical species have low levels of extractives, so their heartwood is more easily colonized.

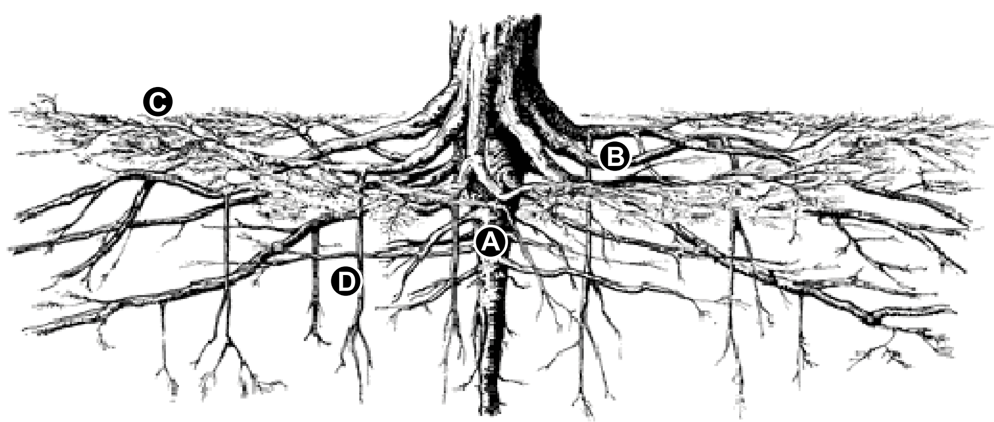

Figure 2.19—Diagram of a tree root system and its defenses. The taproot (A), lateral roots (B), and sinker roots (D) are all protected by bark similar to that formed on the bole of the tree. However, if this root bark becomes damaged or if a sound root comes in contact with a large amount of inoculum of a pathogen like *Phellinus noxius*, these larger roots can easily become infected. The root tips (C) usually harbor symbiotic mycorrhizal fungi, rhizobium, or actinomycetes, which assist in the absorption of water and some nutrients and provide the root tips with some protection against attack by pathogenic fungi in the soil. Drawing courtesy of Elliot Menashe of Greenbelt Consulting.

The rays (fig. 2.18) are rows of cells that radiate from the center to the periphery of the tree. They provide a weak measure of defense by slowing the circumferential spread of *P. noxius*. Ray cells are not found in all tropical tree species, so this defense mechanism does not play a major role in slowing colonization of the bole by the fungus in all trees. Even fewer mechanical barriers exist to restrict vertical fungal growth in a tree, so once inside the bark, *P. noxius* can usually spread rapidly up and down the bole.

When the source of infection is mycelia within an infected root sucker, a piece of infected wood, or an infected root, then the inoculum potential of *P. noxius* will be far greater than that of a spore, which may allow the fungal mycelia to overcome the host tree defenses, such as the bark, of even a healthy tree. These larger inoculum levels also support tree-to-tree spread of *P. noxius* via the root systems.

Although the basidiospores of *P. noxius* are microscopic and cannot typically overcome an intact tree's defenses (figs. 2.18 and 2.19), the basidiospores of *P. noxius* have two ways to successfully overwhelm the normal defense of a tree. First, *P. noxius* produces billions of spores (fig. 2.20), increasing the chance that at least some spores will land on a tree wound and cause subsequent infection. Second, the timing of basidiocarp and spore formation typically coincides with the storm season, which increases the chances of successful infection by spores. Powerful, wet, damaging storms are ideal for spore formation, wound formation on the trees (e.g., limb breakages), spore dispersal, spore survival, and spore germination, which all contribute to the initiation of infections.

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Figure 2.20—A dense cloud containing billions of *Phellinus noxius* spores billowed out from a resupinate fructification (basidiocarp) when this *P. noxius*-infected breadfruit log was gently lifted. Billions more lavender-colored basidiospores remained on the ground. These basidiospores are very light, so they can remain in the air for long periods and travel passively in air currents.

Other situations can also occur to reduce a tree's natural defenses and allow infection by *P. noxius*. For example, in the humid tropics of the Northwest Pacific Basin, limb breakage or damage to the bark can impair a tree's natural defense systems (fig. 2.21).

Although no direct studies have been conducted to determine the extent to which tree wounds or limb breakage predisposes trees to infections caused by *P. noxius*, inferences about the role of wounding in penetration of wood-rot fungi is readily obtained from other fungal species. For example, a survey of 80,000 prune (*Prunus*) trees over 22 years in age growing in one region of California found that 92 percent of these trees had broken limbs, and of these trees with broken limbs, more than 53 percent had *Phellinus tuberculosis* conks (Johnson et al. 2021). That study's data also showed that 73 percent of the colonized trees had at least one broken limb. Although this study does not assess *P. noxius*, it suggests the significance of wounds to infection caused by another wood-rotting species of *Phellinus*.

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Figure 2.21—Maxim Nithan examines a *Phellinus noxius* infection that started on a buttress root of this giant ka tree after spores apparently landed in a wound created by a fresh blaze mark made with a machete. The cut was made as breadfruit farmers marked their way through the Yela conservation area of Kosrae, Federated States of Micronesia. Rotted wood with barely perceptible zone lines in the vicinity of this blaze provided evidence that *P. noxius* basidiospores colonized the exposed wound tissue first. The tree attempted to recover from the wound, as evidenced by the adventitious roots produced near the wound. However, this tree is unlikely to recover from this well-established infection.

Under hot and wet conditions, the outer mycelial sheath of *P. noxius* can grow vigorously up the outside bark of a tree. While this is occurring, *P. noxius* is often also advancing rapidly in the cambial layer, just inside the bark. In some cases, we have observed that this inner sheath of *P. noxius* mycelium eventually replaces the entire cambial layer (fig. 2.22). The inner mycelial sheath is usually white to cream colored, but often pale orange zone lines are apparent. These zone lines are typically up to about 2.5 cm (1 inch) apart and tend to form a loose weblike design.

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Figure 2.22—A forester on Kosrae, Federated States of Micronesia, peels the infected bark from the log of a ka tree killed by *Phellinus noxius*. All the cambial tissue has been degraded and replaced by a white layer (inner sheath) of *P. noxius* mycelium, allowing the tree bark to be easily removed. The signature orange “chicken-wire” mesh of the zone lines of *P. noxius* were barely visible when this photograph was taken.

As *P. noxius* continues to grow within a tree, the next tissue colonized and destroyed is the xylem (fig. 2.18). The function of this tissue is to conduct water and nutrients from the soil to the crown of the tree. Without functional xylem, the crown is no longer supplied with enough water and nutrients, resulting in smaller new leaves, followed by the yellowing and falling off of the older leaves. The diseased tree then progresses through a balding phase, as the crown loses its leaves. Within months, branches start breaking and falling from the tree. This continual structural failure of the tree begins with the smaller branches and then progresses to the larger branches, and then the entire bole.

Phellinus noxius produces a large array of degradative enzymes that collectively decompose all the structural components in wood, contributing to the rapid deterioration of the tree (see chapter 3). Trees killed by *P. noxius* are estimated to fall apart about 10 times faster than trees killed by drought, fire, or mechanical girdling, which can make *P. noxius*-killed trees especially hazardous.

Sources of Variation in Sign and Symptom Development

The time from initial infection to the appearance of visible signs and symptoms of disease can vary. Noting this, researchers in rubber plantations in Ivory Coast hypothesized that *P. noxius* isolates can vary in virulence. To test this hypothesis, eight isolates of *P. noxius* were each used to colonize 20 short sections of sticks that would serve as inoculum. The researchers then attached one inoculum stick of each isolate to one root of 20 different rubber tree (*Hevea brasiliensis*) seedlings in a well-replicated experiment. The results clearly demonstrated that some isolates of *P. noxius* were significantly more aggressive than others under the test conditions (table 2.1).

Table 2.1—Incidence and severity of attack by eight isolates of *Phellinus noxius* on rubber tree seedlings 5 months after inoculation[a]

	Percentage of 20 rubber tree saplings showing the following characteristics				
Isolate number	**Rhizomorphs on root system**	**Root penetration**	**Foliar symptoms**	**Taproot necrosis**	**Mortality**
2	65	65	5	16	20
4	85	80	5	22	10
7	60	60	5	7	0
31	10	10	0	0	0
32	95	95	5	24	35
35	85	80	15	30	15
39	35	30	0	5	5
45	90	80	20	53	45

[a] A total of 20 inoculum sticks were developed for each of eight isolates of *P. noxius*, and one of these inoculum sticks was tied to the root system of each of 20 rubber tree saplings.
Source: Nandris et al. 1987.

Symptoms of *P. noxius*-caused brown root rot disease often advance at different rates depending on the tree species being attacked. A study in Taiwan found that no seedlings of Ceylon myrtle (*Phyllactis myrtifolius*) were killed at 6 months post-inoculation with *P. noxius*, whereas all Malabar (also known as neem; *Azadiracta* *indica*) seedlings were killed within 2 weeks of inoculation with the same inoculum and dosage (Leung et al. 2020). Generally, brown root rot disease progresses faster on trees with wood of lower specific gravity (lighter dry weight) than on trees with denser (heavier) wood. This is perhaps why some trees, such as kapok, edible fig (*Ficus carica*), and banyan (*F. benghalensis*), seem to be less resistant to colonization by *P. noxius*. However, exceptions to this general rule also occur. Flame tree (*Delonix regia*) is extremely susceptible to *P. noxius*, yet its wood has a relatively high specific gravity (0.8 g/cm^3). In another study, Sahashi et al. (2010) found variation both in the ability of different *P. noxius* genotypes to attack a given tree species, and in the degree of resistance of different tree species to withstand an attack by any known *P. noxius* genotype (table 2.2). In that study, isolates PN100 and PN92 were clearly more pathogenic than isolates PN78 and PN85 overall; however, some species, such as bishop wood (*Bischofa javanica*), were far less susceptible to most isolates of *P. noxius* compared to other species, such as India hawthorne (*Rhaphiolepis indica*).

Table 2.2—Mortality results for seven different host species inoculated with six different *Phellinus noxius* isolates

	Percentage of seedlings killed by each *P. noxius* isolate					
	PN78	**PN81**	**PN83**	**PN85**	**PN92**	**PN100**
Bischofia javanica	10	30	0	0	10	30
Calophyllum inophyllum	10	10	10	0	10	40
Casuarina equisetifolia	20	0	10	20	50	50
Cinnamomum japanocum	30	20	20	0	0	70
Melia azaderach	0	20	10	0	40	50
Podocarpus macrophyllus	10	60	20	0	80	50
Rhaphiolepis indica	10	40	50	40	80	30

Source: Modified from Sahashi et al. 2010.

Fully grown tree species also vary in their susceptibility to *P. noxius*. In a study of four host species for their susceptibility to *P. noxius*, Mohd Farid et al. (2009) found that teak (*Tectona grandis*) was the most susceptible species, followed by Pará rubber tree (*Hevea brasiliensis*), ironwood (*Casuarina equisetifolia*), and acacia species (*Acacia excelsa* and *A. mangium*). Few studies have been conducted on the susceptibility of tree species to *P. noxius*, perhaps because well-replicated trials on the virulence of *P. noxius* genotypes and resistance of tree species requires considerable time, effort, and resources.

A simpler approach that yields results more quickly was performed by Wu et al. (2011). They visited an area of China where many different tree species were growing in areas known to have *P. noxius* infection centers. They established transects through these infection centers and recorded the health of every tree and then determined the inoculum potential of *P. noxius* present in the soil around each tree. The prevalence of brown root rot disease in a tree species with a root system that overlapped with the root systems of other diseased trees corresponded to a greater susceptibility rating. The susceptibility index scores for trees found in this part of China (Wu et al. 2011) were listed as follows:

Tree genus and species	Common name	Susceptibility index score
Aleurites moluccana	Candlenut	3.29
Antirhea chinensis	Bois goudron	3.38
Aralia chinensis	Chinese angelica tree	0
Averrhoa carambola	Star fruit	1.92
Bridelia monoica	Pop gun seed	7.67
Casuarina equisetifolia	Ironwood	0.71
Cinnamomum burmanii	Cinnamon	5.05
Craibiodendron spp.	Temple bell	0
Ficus hispida	Hairy fig	0
Ficus simplicissima	Asian fig	4.56
Ficus variegate	Red-stem fig	2.63
Helicteres angustifolia	Shan zhu ma	0
Ligustrum sinense	Chinese privet	0
Litsea monopetala	Many flowered litsea	3.83
Livistona chinensis	Chinese fan palm	27.15
Mallotus apela	Kamela tree	0
Mallotus paniculitus	Turn-in-the-wind	2.21
Microcos paniculata	Garden mint	3.02
Schefflera octophylla	Dwarf umbrella tree	0
Sterculia lanceolata	Scarlet sterculia	7.27
Syzygium jambos	Rose apple	0
Syzygium levinei	Shan pu tao	0

No studies on the susceptibility of tree species to *P. noxius* have been conducted on any of the U.S.-Affiliated Pacific Islands. On repeated occasions, however, it has been obvious that breadfruit, flame tree, mango (*Mangifera indica*), loquat (*Eriobotrya japonica*), and at least some *Ficus* trees appear to be highly susceptible to *P. noxius*. Other trees, such as *Leucaena* sp., appear to be moderately susceptible, while coconut palms (*Cocos nucifera*) appear to be attacked only occasionally. More work is needed to identify susceptible and resistant tree species as this information is key to maintaining an arboreal component in many areas of the U.S.-Affiliated Pacific Islands.

The Importance of Weather in Brown Root Rot Disease Caused by *Phellinus noxius*

The mycelium of *P. noxius* appears to grow faster during wet weather, especially on the outside of the bark and undersides of root systems. In addition, the fungus appears to form more basidiocarps and basidiospores in wet weather than in dry weather. Cloudy or humid weather may also support longer survival of airborne basidiospores. Furthermore, basidiospores that land on an open tree wound likely have a greater chance of successful germination and establishment under humid conditions.

Because rainfall is an important factor in determining when and where *P. noxius* infection could occur, knowledge of factors that contribute to the amount of rainfall on northwestern Pacific Islands is useful. A map from the National Oceanic and Atmospheric Administration shows the general distribution of rainfall in this part of the Pacific (fig. 2.23). The map shows that large differences in average annual rainfall occur among islands in the northwestern Pacific area. Near Kosrae, Federated States of Micronesia (FSM), the oceans receive 457 cm (180 inches) of rainfall per year, while the ocean near Yap, FSM receives 305 cm (120 inches), and the ocean near Saipan, Commonwealth of the Northern Mariana Islands receives only 203 cm (80 inches).

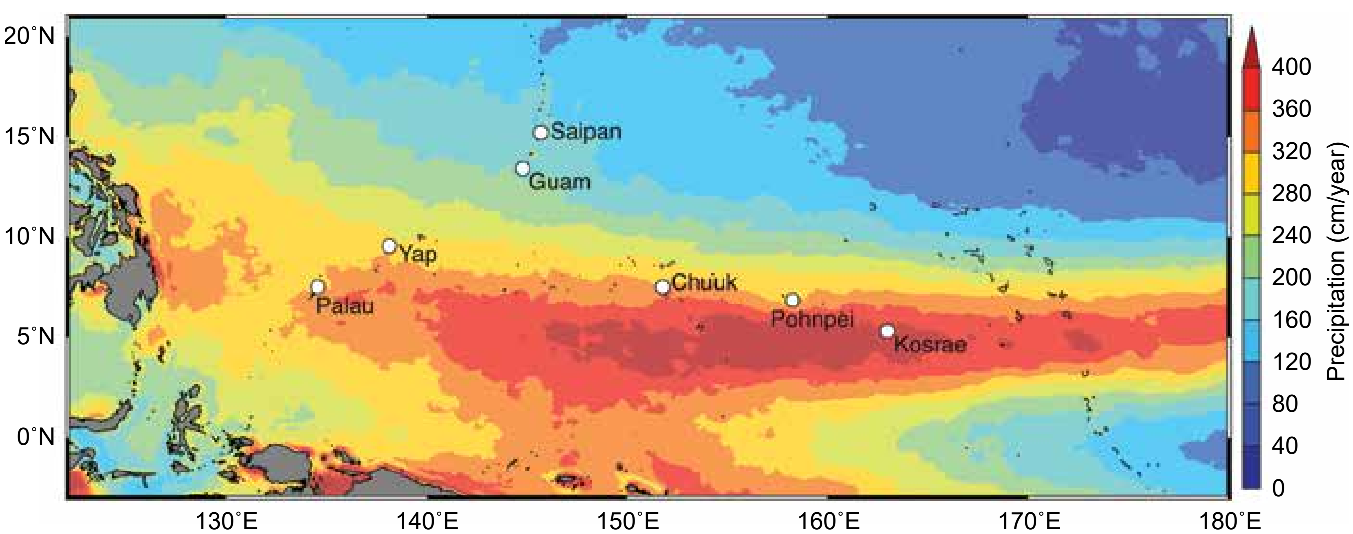

Figure 2.23—Mean annual, over-ocean-water rainfall in Micronesia. Image adapted from WERI annual report (2002: 78).

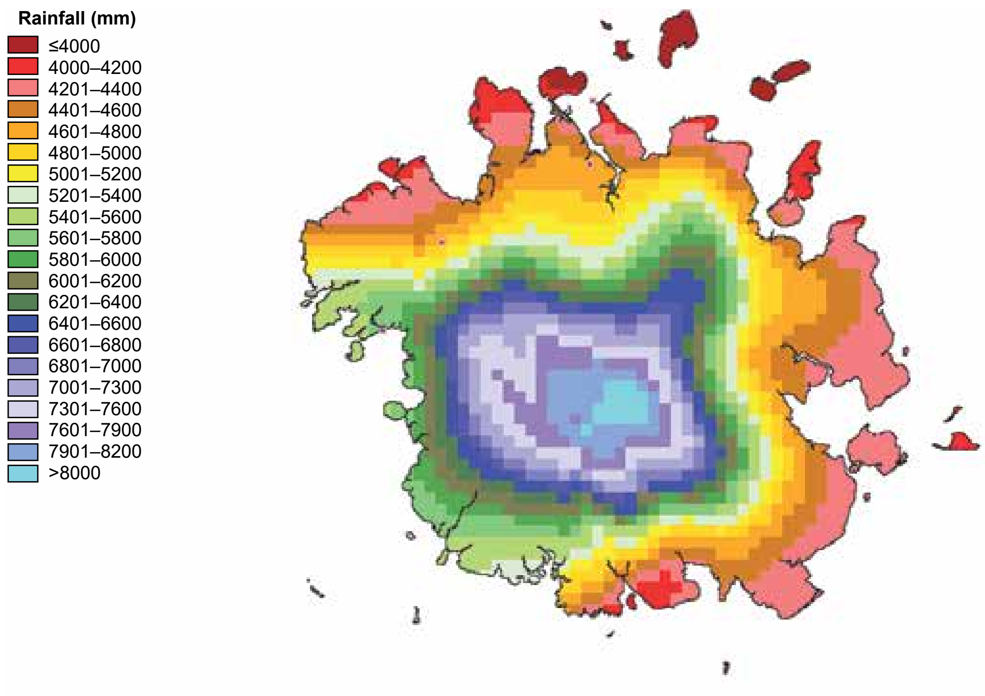

Figure 2.24—Estimated annual rainfall for Pohnpei, Federated States of Micronesia, prepared by the Spatial Climate Analysis Service, Oregon State University, using the Precipitation-elevation Regression Independent Slopes Model (PRISM) analysis (Daly et al. 1994). Note that the rainfall in the central highlands is estimated to be nearly twice that of the coastal area, and the rainfall on the west side of the island is greater than on the east side. Used with permission from Chris Daly, Oregon State University, Spatial Climate Analysis Service.

However, the amount of rainfall over the ocean near an island is not the only factor influencing the amount of rain that reaches the ground at a given location on an island. The topography of the island can play a significant role, especially at locations with higher altitudes, such as those with a high volcanic mountain at their core (fig. 2.24). Rain-bearing winds for the island of Pohnpei, FSM, typically come from the west, so the western side of the island usually averages about 630 cm (248 inches) of precipitation annually. The eastern side of the island, however, receives only 430 cm (169 inches) of rain. The highest part of this island receives 830 cm (327 inches) of rain; few places on earth receive this amount of rainfall (fig. 2.24)

Violent storms and even typhoons may enhance conditions for the growth and spread *P. noxius* because of the ample water they deposit on the land (fig. 1.7). Storms in this part of the Pacific Ocean can deliver rainfall levels of 13 cm (5 inches) per hour, and typhoons may be up to 1609 km (1,000 mi) in diameter. When typhoons come close to land, their movement may slow to 4.8 to 8 kph (3 to 5 mph) during which these storms can deliver heavy rainfall for several days. Therefore, Saipan, CNMI, and Yap, FSM, usually receive lower annual rainfall than other islands in the western Pacific; however, these islands can receive high levels of rainfall about every 3 to 5 years when they are exposed to typhoons. This additional moisture could greatly influence the growth and infectivity of *P. noxius*.

Typhoons are most common in the northwestern Pacific Ocean from August to October, when ocean surface temperatures commonly surpass 32 °C (90 °F), the critical level for typhoon formation (fig. 2.25). The Northwest Pacific Basin has the greatest number of typhoons on earth (USDC NOAA 2021). It seems probable that the spread of *P. noxius* is related to the occurrence of typhoons directly affecting the islands. In contrast, trees in other parts of the tropical world where typhoons (also known as hurricanes in the North Atlantic, central North Pacific, and eastern North Pacific) are not as common, may suffer less damage from *P. noxius* than prevailing temperatures and rainfall levels would suggest.

Figure 2.25—Typhoon frequency in the northwestern Pacific is especially high. This graph represents all typhoons from 1980 to 2005; tracks in red and yellow represent the most powerful typhoons, category 5. The Hawaiian Island chain can be seen at the right side of this image. Most of the U.S.-Affiliated Pacific Islands discussed in this report lie directly below these typhoon tracks. Each dot is the location of the eye of the storm at 6-hour intervals. Created using WikiProject Tropical cyclones/Tracks. The background image is from NASA. Tracking data are from the National Hurricane Center.

As global warming continues, it seems likely that even more trees will become vulnerable to attack by *P. noxius*, especially among islands in the Northwest Pacific Basin. Climate modeling efforts by Knutsen et al. (2015) and Zhang et al. (2017) have studied the potential of typhoons becoming more intense in the Northwest Pacific Basin by the end of the current century. Both found that the intense super typhoons (categories 4 and 5) were likely to become more common and more intense in this region, although their projections on the numbers of mid-level typhoons (categories 1 and 2) differed somewhat. Knutsen et al. (2015) projected a slight decrease in the number of categories 1 and 2 storms, whereas Zhang et al. (2017) projected an increase in storm activity at these levels.

The influence of wind on brown root rot disease deserves special consideration, as wind can greatly influence the incidence of wounding to a tree. As windspeeds of tropical storms surpass 161 kph (100 mph), the chance of damage to branches and root systems progressively increases. Where these breaks occur on the limbs and roots, the bark can no longer protect the internal woody tissues of the tree. Rizzo and Harrington (1988), found that occasional wind gusts of 21.4 m/sec (70 ft/sec) were sufficient to cause damage to conifer roots when these trees were growing on rocky soils. The windspeeds during a typhoon are often more than twice this speed and can remain high for several hours. Figures 2.26 through 2.30 suggest that storm damage to roots of trees can promote the development of new *P. noxius* infections of trees.

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Figure 2.26—A fresh orange, brown, and white mycelial sheath of the butt-rotting fungus, *Phellinus noxius*, advances up the outside bark of a pengua tree (*Macaranga thompsonii*) near Ritidian, Guam. During the fierce winds of Typhoon Dolphin in 2015, the bark of roots growing in the sharp karst limestone were sliced and cracked open as the trees were whipped back and forth. This wounding of the root bark and wet conditions likely allowed *P. noxius* basidiospores to establish and colonize the tree. Several hundred other trees were similarly affected in this area.

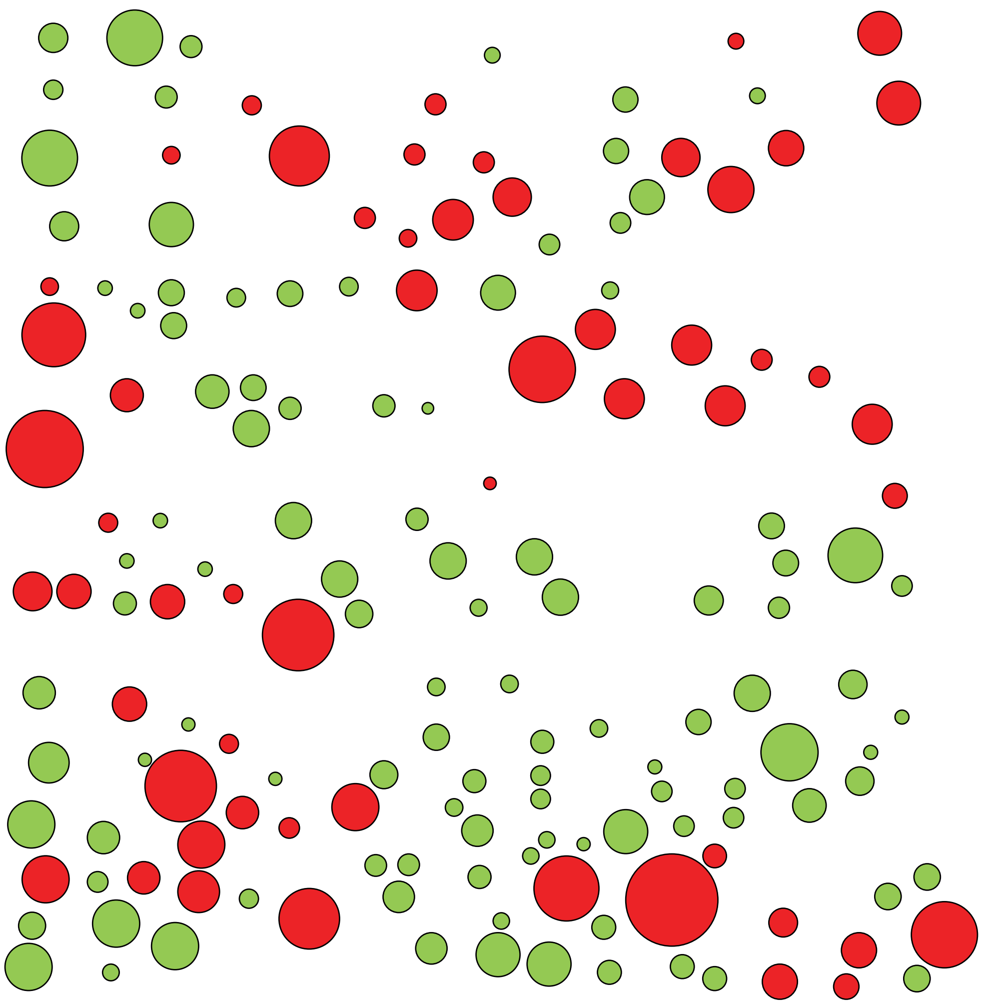

Figure 2.27—The distribution and diameter of healthy and *Phellinus noxius*-infected trees on a Navy Hill property in suburban Saipan, Commonwealth of the Northern Mariana Islands. The largest circles represent trees with diameters of 91 cm (36 inches), and the smallest trees have diameters of 10 cm (4 inches). Green circles indicate trees that appeared healthy; red circles indicate trees that showed signs (black/dark-colored sock or mycelial sheath) of *P. noxius* infection; some, but not all, of these trees were already dead. The plot center for this property is at N15°17.814' E 145°41.923'. Note: this drawing is not to scale. Distances between trees was deliberately reduced so that all trees in this site could be represented. *P. noxius* will likely continue expanding through the tree root systems, and all trees at this site may soon become infected and die.

Mario Martinez

Figure 2.28—Locations of *Phellinus noxius* infection centers near Anderson Air Force Base, Guam. Mario Martinez and John Horneg, graduate students at the University of Guam, did the surveys to find these centers and determine their global positioning system locations for this map. Most of these infection centers involved 8 to 20 *P. noxius*-infected trees.

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Figure 2.29—Mario Martinez stands in one of the *Phellinus noxius* infection centers he found near Anderson Air Force Base, Guam.

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Figure 2.30—The underside of this pengua (*Macaranga mappa*) tree root has a resupinate fruiting body (basidocarp) of *Phellinus noxius* where the roots were in contact with water perched on limestone rock.

After a Tree Dies From *Phellinus noxius*-Caused Brown Root Rot Disease

One feature of *P. noxius* that makes it especially difficult to manage is that even after a tree dies from *P. noxius*-caused brown root rot disease, the fungal pathogen can continue to survive in the remaining bole section and root system of the tree. Estimated survival time ranges from 1 to 10 years (CABI 2021). All factors contributing to this longevity are not known, but the pseudosclerotial rinds that form around units or pods of *P. noxius*-decayed wood may play a key role (fig. 2.7). These rinds can help deter other fungi, bacteria, termites, and wood ants, thereby protecting the *P. noxius* fungus within these pods. Thus, *P. noxius* remains a constant threat for causing future infections, especially when a healthy root from a neighboring tree grows close to the remnants of an infected root system. Additionally, extremely wet weather from heavy rains and storms can encourage *P. noxius* to produce basidiocarps on dead trees, providing another surge of basidiospores adding to the difficulty of managing this pathogen.

Forest-Level Impacts of *Phellinus noxius*

As mentioned previously, *P. noxius* can attack many wounded trees simultaneously and spread quickly via contact between the root systems of neighboring trees during favorable weather. This tree-to-tree spread can result in patches of infected forest trees. Significantly, these *P. noxius*-caused patches of brown root rot disease often appear to stop expanding after the dry weather returns. Disease spread that seems to occur during the same time and at different locations (and spreads rapidly at each location) and then slows, pauses, or stops is referred to as a random episodic event.[3] Tropical storms seem to be a common element linked to the initiation of random episodic events that involve *P. noxius*. When storms with high winds occur, some trees boles break (boles that have V-shaped limb junctures, with bark between leading shoots, as well as species that form limbs in whorls, are especially prone to this kind of breakage). In addition, some trees lose limbs, and some wind-blown trees collide with each other, resulting in bark breakage and exposure of the unprotected xylem beneath. Storms can also cause numerous wounds to root systems, especially in rocky soils, when trees are rocked back and forth. From 1981 to 2020, annual averages of 25.9 storms (sustained winds more than 17 m/sec), 13.9 typhoons (sustained winds more than 33 m/sec), and 4.4 super typhoons (sustained winds more than 50 m/sec) were recorded in the Northwest Pacific Basin (USDC NOAA 2021). Where these typhoons and especially the super typhoons made direct hits on any of these islands, hundreds of thousands of trees sustained massive damage. Figure 1.5 shows some of the more typical results of typhoon damage that can occur in a forest already infested with *P. noxius*, and figures 1.8 and 2.27 illustrate the heavy levels of *P. noxius* infection that can occur during the 6 months or so following a severe typhoon. At Navy Hill in Saipan, CNMI, 56 percent of trees in a 0.4-ha (1.0-ac) plot were infected by *P. noxius* within the 6 months after Typhoon Sudolor pounded the area in 2015.

Similarly, a 2016 storm on Guam caused sporadic damage to thousands of trees near Anderson Air Force Base (fig. 2.28). Individual trees became infected with *P. noxius* during this time and the disease subsequently spread to neighboring trees. However, this epidemic slowed considerably when drier weather returned.

Although powerful typhoons, possibly more than any other event, are more conducive to development of a *P. noxius* epidemic, less powerful storms, of which there are many in the Northwest Pacific Basin, can also contribute significantly to the development of brown root rot disease. Waves of *P. noxius* mycelial growth up

[3] Harrington, T. 2015. Personal communication. Professor of forest pathology, Iowa State University, Department of Plant Pathology and Microbiology, Ames, IA 50011.

the tree correspond to periods when free water accumulates on the surface of the tree bole, which is largely derived from stem flow during storms.

When the signs and symptoms of brown root rot are readily apparent, the spread of the disease is easy to document. After the trees die and fall over, however, the pattern and extent of spread are more difficult to determine. Fallen, decomposing trees and their root systems often become buried in a morass of vines and other overgrowth of plants. Yet, it remains important to know the location of trees killed by brown root rot disease as *P. noxius* may survive in the fallen trees and their root systems (for up to 10 years), and most other tree species will not survive on this site until the *P. noxius* inoculum is completely eradicated.

Another scenario can occur when inoculum potential of *P. noxius* that is built up in association with one storm, increases the likelihood of more infections following subsequent storms. This scenario appears to have happened in a rural area on Plata-Naguus Drive on Saipan, CNMI (fig. 1.7). By 2014, three widely dispersed trees had become infected with *P. noxius* in a 0.4-ha (1-ac) forest that separated two properties. After 1 year, the disease had spread to about 20 trees in the area and was still spreading quickly. By 2017, almost all trees in the area were infected, which compromised their root systems and subsequently led to their being easily blown over by Typhoon Sudelor. The few trees that remained standing at that time also had the black mycelial sheath indicative of *P. noxius* infection, indicating they, too, would likely soon fall.

As these first two chapters have described, *P. noxius* is greatly affecting many tree species among the U.S.-Affiliated Pacific Islands of American Samoa, the Federated States of Micronesia, the Commonwealth of the Northern Mariana Islands, Guam, and the Republic of Palau. Trees can become infected in diverse ways, but the end result of any *P. noxius* infection is almost always tree death. Examination of site conditions suggest that newly initiated infection centers of *P. noxius* are commonly the result of basidiospore-derived colonization of a wound. Transport of *P. noxius*-infected root suckers and the casual dumping of infected wood materials can also be major contributors to the establishment of new infection centers. Root-to-root spread of *P. noxius* is a significant mechanism for the expansion of these infection centers.

The impact of brown root rot disease can vary widely based on the number of infected trees and their spatial relationship. Sometimes, only a single tree is infected, but this infection often spreads to neighboring trees, as was observed on Guam. Finally, infection centers can coalesce and kill most of the trees in a stand, as occurred near Plata-Naguus Drive (fig. 1.7) on Saipan, CNMI. Weather, especially conditions brought by storms and typhoons, appears to have had a significant influence on brown root rot disease. Violent weather can result in tree wounds that can be colonized by basidiospores. Heavy rainfall events can contribute to greater basidiospore production, spore germination, colonization of wounds, and increased mycelial growth on and within infected trees. Because most of the islands in the Northwest Pacific Basin receive so much rain, often associated with powerful storms, the islands of the Federated States of Micronesia, the Commonwealth of Mariana Islands, and Guam are especially vulnerable to major damage from *P. noxius*-caused brown root rot disease.

Chapter 3: Molecular Genetics of *Phellinus noxius*

Philip G. Cannon, Ned B. Klopfenstein, Mee-Sook Kim, Jane E. Stewart, and Chia-Lin Chung[1]

Molecular genetics studies provide critical information to establish the scientific foundation for many conclusions reached about *Phellinus noxius*. The following are some of the more important conclusions being drawn from these studies:

- DNA sequencing provides a reliable method to identify *P. noxius.*
- *P. noxius* possesses considerable genetic variation, and the species is composed of multiple genetic groups that may display different ecological behaviors.
- *P. noxius* can spread via airborne spores and by vegetative growth through root-to-root contact.
- *P. noxius* has numerous genes that encode diverse wood-degrading enzymes (e.g., enzymes that degrade cellulose, hemicellulose, and lignin), and other enzymes that help metabolize sugars and other compounds from the degraded wood for use in hyphal growth.

Approaches in molecular genetics, metagenomics, and metabarcoding offer promise of further insights into disease caused by *P. noxius.* These genetic approaches may assist in developing disease management methods by examining pathogen inoculum levels and changes in microbial communities in response to management treatments.

The growth and spread of *P. noxius* via root-to-root contact among adjacent trees, basidiospores, or relocation through human-related activities (e.g., the movement of infected breadfruit [*Artocarpus altilis*] root suckers or infected wood products) can be readily investigated. The impact of *P. noxius* on tree health can also be observed as wood decay progresses and tree structure is weakened. Molecular genetics can also provide additional insights on pathogen detection, movement, and disease development. Currently, several approaches are available that can be applied to the study of *P. noxius*, or brown root rot disease:

- DNA-based diagnostic tools to detect and identify *P. noxius.*

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- Phylogenetic studies to determine genetic groups and evolutionary relationships within *P. noxius.*
- Population genetic studies to determine gene flow and geographic movement of *P. noxius.*
- Genomic and transcriptomic studies into pathogenicity genes and other genes associated with growth and survival of *P. noxius.*
- Soil metagenomics and metatranscriptomic studies to examine soil and rhizosphere levels of *P. noxius*, biocontrol agents, and other associated soil microbes and their biological functions, and their interactions with environmental factors.

Recent applications of these molecular genetic approaches to study *P. noxius*, along with information generated from these applications, will be briefly covered in this chapter.

DNA-Based Diagnostic Methods to Detect and Identify *Phellinus noxius*

Fungal identifications for our survey of *P. noxius* on all the U.S.-Affiliated Pacific Islands included in this report (except Chuuk) were based on isolation and culture of butt-rot fungi from various forest trees, the isolation of DNA from these cultures, and the subsequent use of the appropriate DNA sequencing procedures to identify the species of fungus involved, especially *P. noxius*. Details on this process are provided below.

These fungi were isolated from recently infected woody tissue or basidiocarps (sporocarps, fruiting bodies, fructifications, conks, sporocarps, or basidiomata). Resupinate basidiocarps were often plentiful, relatively easy to find, and distinctive enough for observational identification (figs. 2.5 and 2.6). Conks, or shelf-like basidiocarps, were less common, but distinctive and more commonly found after periods of heavy rain (figs. 2.3 and 2.4). The chance of finding these conks can be improved by burrowing into the base of stumps killed by *P. noxius*-induced brown root rot disease. The heartwood at the base of the tree appears most susceptible to decay, so a hollow chamber is commonly formed in the center of the stump and below the ground line. The dark, humid conditions in these chambers are apparently conducive to conk production. Basidiocarps are optimal material for isolating *P. noxius*, according to plant pathologists Yuko Ota and Norio Sahashi, who have dedicated much of their professional careers to studying this fungal pathogen.

To isolate and establish *P. noxius* in culture for DNA extraction, Ota and Sahashi begin by surface sterilizing a small portion of the basidiocarp. Then they excise a small piece (ca. 5 × 2 × 1 mm) of this basidiocarp and use this to inoculate the culture medium. A preferred medium for isolating this fungus was developed by Tsai et al. (2017); it consists of potato dextrose agar (PDA) with 100 mg/L streptomycin and 10 mg/L benomyl. After a *P. noxius* isolate is established in the culture, it is subcultured onto the surface of a cellophane/nylon membrane that is then placed on top of the medium in a Petri plate. After about 1 week in culture at 28 °C, about 0.5 g of mycelium is collected by gently scraping the membrane surface and placing it in either liquid nitrogen or a DNA extraction buffer. After grinding the mycelium, DNA is then extracted using various methods, such as a CTAB (hexadecyltrimethylammonium bromide) method (Ibarra Caballero et al. 2019) or various commercially available DNA-extraction kits, such as the ZR Fungal/Bacterial DNA MiniPrep Kit™ (Zymo Research, Irvine, California) (Stewart et al. 2020, Tzean et al. 2016).

DNA-based detection and identification of *P. noxius* frequently relies on the use of polymerase chain reaction (PCR), which amplifies (multiplies) the desired target region of DNA. Standard target regions include the 28S region of rDNA (nuclear large subunit; LSU) and internal transcribed spacers 1 and 2 with 5.8S regions of rDNA (ITS) regions. These regions can be amplified using primer pairs LR0R (Rehner and Samuels 1994)/LR5 (Vilgalys and Hester 1990) and ITS1/ITS4 (White et al. 1990), respectively. For specific detection of *P. noxius*, the primer pair GIF (5'-GCCCTTTCCTCCGCTTATTG-3') and GIR (5'CTTGATGCTGGTGGGTCTCT-3') have been used to amplify a specific 653-bp DNA fragment within the ITS region (Wu et al. 2009). PCR products (amplicons) are confirmed using agarose gel electrophoresis or an automated DNA fragment analyzer. Subsequently, PCR products are treated, by enzyme-mediated inactivation of excess dNTPs and primers (e.g., ExoSAP-IT® PCR Product Cleanup) in preparation for sequencing of the target region. DNA sequencing is typically performed by various commercial companies that offer this service (Stewart et al. 2020). The DNA sequences of the target regions (e.g., LSU, ITS) from each fungal isolate are compared to sequences from GenBank with the basic local-alignment search tool (BLASTN), to confirm the identification of *P. noxius* to species level. This DNA-based diagnostic approach, when used on the fungal isolates we collected as described in chapter 2, helped reveal that *P. noxius* was more prevalent on the U.S.-Affiliated Pacific Islands (i.e., American Samoa, Commonwealth of the Northern Mariana Islands [CNMI], Federated States of Micronesia [FSM], Guam, and Republic of Palau) than previously reported.

Phylogenetic Studies to Define Genetic Groups Within *Phellinus noxius*

Recently, phylogenetic studies were conducted on a large set of *P. noxius* isolates from geographically diverse locations, including the U.S.-Affiliated Pacific Islands, Australia, and eastern Asia (Stewart et al. 2020). This study was notable because it included 95 isolates from various U.S.-affiliated island states: American Samoa, CNMI (Saipan), Guam, FSM (Kosrae, Pohnpei, and Yap), and Palau. The other isolates in the study were from Japan, China (Taiwan and Hong Kong), Malaysia, and Australia. For each isolate, genetic sequencing was attempted at four loci: LSU; ITS; partial translation elongation factor -1 alpha (*tef1*); and partial RNA polymerase II, second largest subunit (*rpb2*). Details of this investigative process are provided in Stewart et al. (2020). Some important results of these analyses are shown in figures 3.1 and 3.2.

DNA sequences from each locus, such as ITS and 28S, are useful for phylogenetic analyses and can determine evolutionary and genetic relationships among the *P. noxius* isolates (Stewart et al. 2020). Additional DNA sequences for comparison are usually available in public databases, such as GenBank (https://www.ncbi.nlm.nih.gov/genbank/), or in association with published studies (Chung et al. 2017, Tsai et al. 2017). Various approaches and software are available for DNA sequence-based phylogenetic analyses. Leung et al. (2020) explain how to diagnose brown root rot disease and highlight phylogenetic variation among *P. noxius* isolates.

As discussed in the previous chapter, symptoms, and especially signs, are primary indicators of *P. noxius* as the cause of a disease, or mortality. However, morphological characteristics provide little or no evidence of genetic variation within *P. noxius* or the virulence of a given isolate. In their genetic analyses of *P. noxius* isolates, Stewart et al. (2020) used a *tef1*-based phylogeny to distinguish genetic groups of *P. noxius* (fig. 3.2). Some interesting patterns emerged from that analysis. For example, one haplotype (H3), present in Palau, Pohnpei, Kosrae, Saipan and Guam, was also present on the Ogasawara Islands of Japan. In addition, some *P. noxius* isolates from Guam and Saipan also appeared to be closely related, as would be expected because of their close geographic proximity. When haplotypes are identical or very similar on different islands, it suggests that *P. noxius* from one island may have been moved, or moved itself via spores, to another island. However, obtaining many *P. noxius* samples from both islands would help to determine on which island the fungus originated.

Figure 3.2 also shows that *P. noxius* isolates from western Pacific regions were contained within three widely defined clades: eastern Asia (Japan; Taiwan and Hong Kong, China; and Malaysia); western Oceania/Japan/Taiwan (including Australia

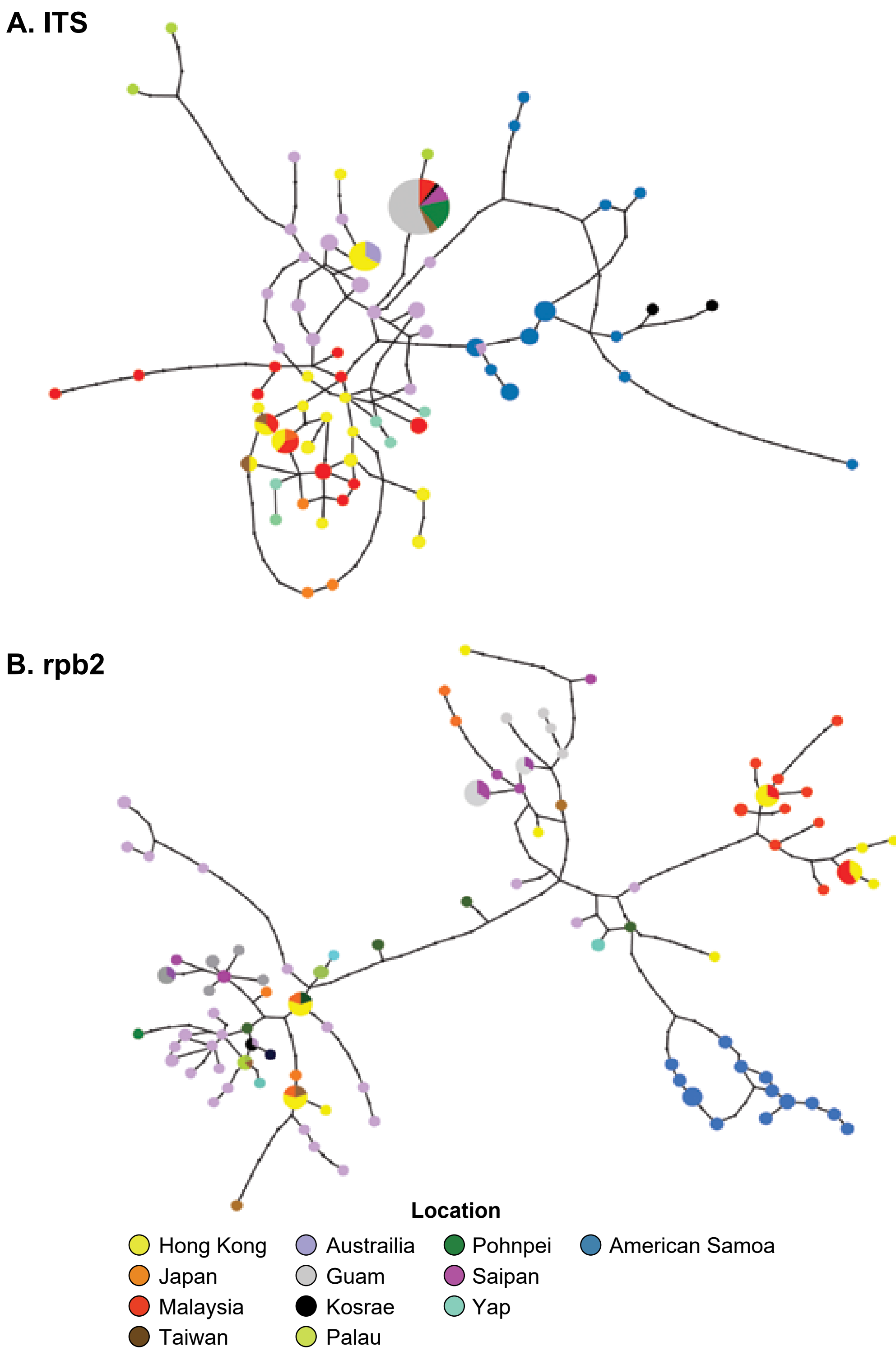

Figure 3.1—Haplotype networks of *Phellinus noxius* based on partial RNA polymerase II and second largest subunit (*rpb2*) concatenated sequences. Circle color represents origin of the isolates. Circle size is proportional to the number of isolates in each haplotype. Small, solid dots represent inferred intermediate haplotypes in the network. Pie charts indicate proportional representation of haplotypes. The number of mutations separating the haplotypes are reflected as segment lengths connecting the circles. (Figure adapted from Stewart et al. 2020)

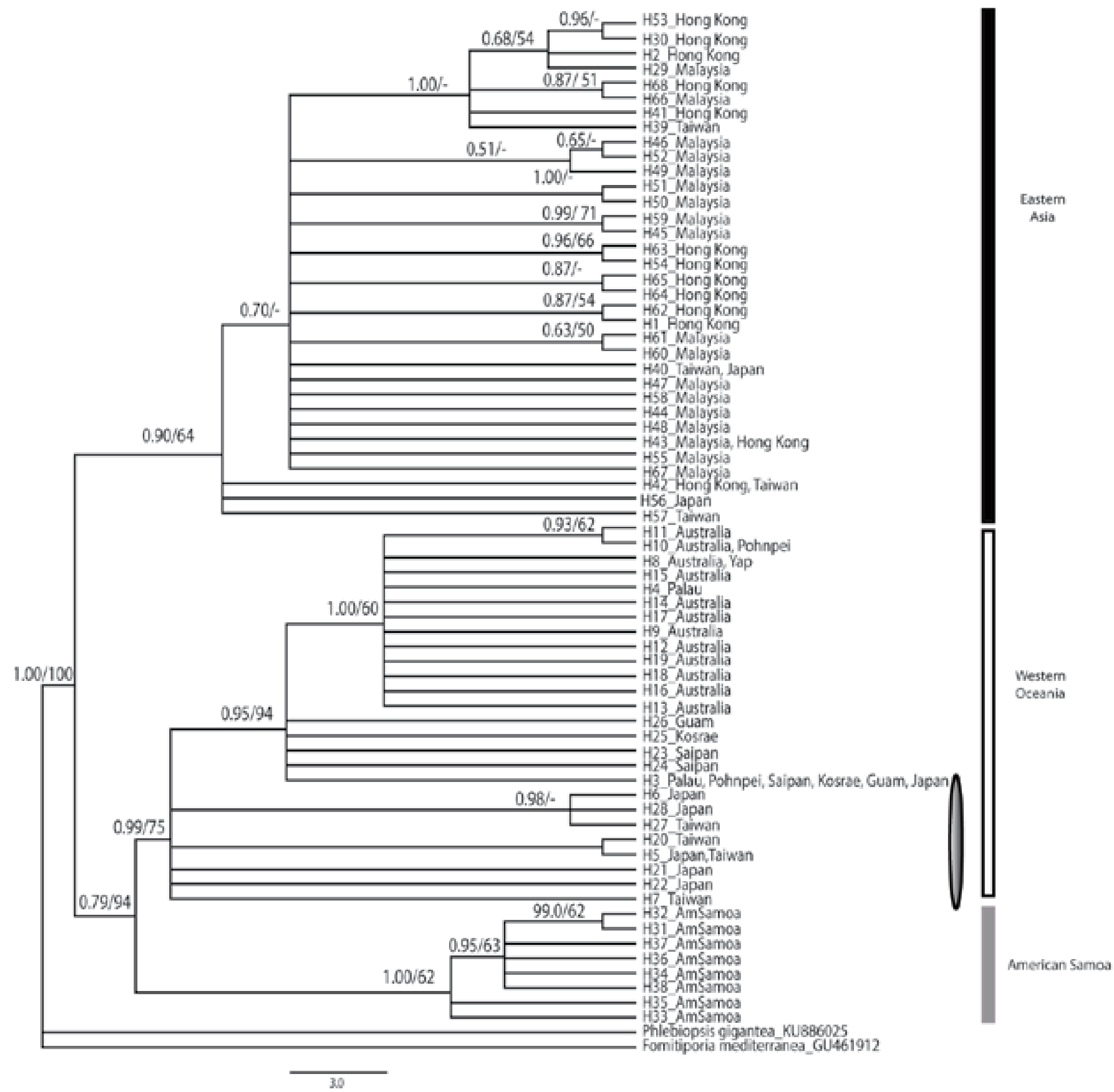

Figure 3.2—Phylogeny of *Phellinus noxius* isolates, based on Bayesian analysis of partial translation factor -1 alpha (*tef1*) sequences, with *Phlebiopsis gigantea* and *Formitoporia mediterranea* serving as outgroups. Node support is indicated as posterior probability/bootstrap. Distinct clades are presented as bars representing eastern Asia, Oceania/Japan/Taiwan, and American Samoa. Some isolates from Japan and Taiwan are grouped within the clade representing western Oceania isolates, which is illustrated by the vertical oval. (Figure from Stewart et al. 2020)

and the U.S.-Affiliated Pacific Islands of Palau, Guam, Saipan, Yap, Pohnpei, and Kosrae); and a distinct southern Oceania group from American Samoa. Isolates from one clade may behave more similarly ecologically, compared to isolates in either of the other two clades. For example, isolates from the genetically distinct American Samoa clade might be expected to appear and behave similarly, compared to isolates from the eastern Asia or western Oceania/Japan/Taiwan clades, but this has not yet

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Figure 3.3—The fascinating growth pattern of a thick, black, mucilaginous, mycelial crust of *Phellinus noxius* (known locally as "limu mea") growing up the outside of a ylang-ylang (*Cananga odorata*) tree (known locally as "moso'oi") near American Samoa Community College. The growth continually expands radially and eventually envelops the entire base of the tree. Each ridge of growth seems to correspond to a rainfall event and high humidity, which are common in American Samoa and probably conducive to the growth of this fungus.

been confirmed. The general appearance (e.g., mycelial growth pattern) of *P. noxius* on American Samoa is exemplified in figure 3.3, which appears quite different from the *P. noxius* in Pohnpei (fig. 1.1). It would be worthwhile to determine if responses to control measures are influenced by phylogenetic differences among *P. noxius* isolates. To date, no studies other than bioclimatic modeling have been conducted to determine if ecological behaviors are associated with distinct clades of *P. noxius*.

Population Genetic Studies Can Determine Gene Flow and Movement of *Phellinus noxius*

Population genetics is a subfield of genetics that considers genetic comparisons within and among populations. Population genetic studies of *P. noxius* are of interest because they can provide insight into the movement and spread history of this pathogen. For example, the genetic diversity of a fungus is expected to be high in the area where it originated. Conversely, the fungus typically has less genetic diversity in areas where it was recently introduced. This phenomenon is referred to as a genetic bottleneck, or "founder effect" (Dean and Ballard 2004) and occurs when all the fungal genotypes in a population are not introduced to a new region. Based on this principle, the area that has the larger number of fungal genotypes is considered as a potential source of the fungus. Furthermore, the pattern of fungal genotypes in each region can provide insight into direction of spread for the fungus. These principles are based on the assumptions that all, or most, of the genetic variation in the invaded locations are a subset of all the genetic variation from the source location.

Population genetics can also be used to determine other aspects associated with spread of the fungus. For example, vegetative or clonal (nonsexual) spread would result in a fungus at one location having the same genotype as a fungus at another location. In the case of root-rot fungi, the same fungal genotypes on adjacent trees is an indication of growth of the mycelium from an infected tree growing onto the root of previously healthy trees. Distant trees with the same fungal genotype suggest spread by the physical movement of the vegetative fungus, including human-mediated transport, such as moving *P. noxius*-infected root suckers from one location to another or placing infected wood near a previously healthy tree.

Sexual reproduction and the spread of basidiospores, however, is suggested when the fungal genotype identified on one tree is distinct from the genotype found on another tree that is growing relatively close to the other infected tree.

Recent population genetic studies of *P. noxius* in Taiwan and Japan have produced interesting insights. From sequence-based analyses of DNA from *P. noxius* samples in Taiwan, fungal dispersal was attributed to both vegetative and basidiospore spread, and the highly diverse genotypes within the *P. noxius* populations attributed to a bipolar heterothallic reproductive system (Chung et al. 2015, 2017). Vegetative transmission has generally occurred through tree-to-tree spread via contact between separate root systems. Transmission by basidiospores was evident in new infection foci with different genotypes than those associated with other neighboring infection foci. Studies with invasive *Heterobasidion irregulare* basidiospores in Europe showed that although most airborne spores cause new infections generally close to the source (ca. 1.3 km year^{-1} or 0.8 mi year^{-1} (Gonthier et al. 2007), some spores, however, can apparently travel much farther (Gonthier et al. 2014), suggesting that *P. noxius* basidiospores might survive long-distance dispersal. In general, *P. noxius*-infected trees growing close to each other are most likely a result of vegetative spread through their roots. Conversely, separate areas of infection are most likely caused by sexually produced basidiospores (Akiba et al. 2015, Hattori et al. 1996). Understanding the mechanisms by which disease spreads is needed to develop and apply the most effective methods for monitoring and controlling *P. noxius* (see management section, chapter 4).

Focused population genetic studies of *P. noxius* specifically, have not yet been conducted for individual U.S.-Affiliated Pacific Islands, in part because the number of samples collected to date have been too small to support a robust study. When more samples are available, population genetic studies will help clarify the spread and ecology of *P. noxius*, both within an island and elsewhere. As tree-to-tree spread is often evident (fig. 2.11) and as basidiocarps are found on most/all the U.S.-Affiliated Pacific Islands, these transmission mechanisms (vegetative and airborne basidiospores) are both likely occurring in these regions. Until more definitive information is available, control measures for any island should consider that *P. noxius* is spreading both vegetatively by root-to-root contact and by airborne basidiospores.

In a previous study, 43 *P. noxius* samples were collected from some U.S.-Affiliated Pacific Islands (Stewart et al. 2020), and phylogenetic relatedness among these samples was discussed in the previous section of this chapter. However, more samples of *P. noxius* from each location and higher resolution genetic markers (e.g., RAD-seq: Restriction site Associated DNA Sequencing) are needed to determine the history of how *P. noxius* has moved throughout the Pacific regions. Important questions are yet to be addressed. For example, *P. noxius* exists in the Philippines (CABI: 2021), and wood pallets came to Guam, Yap, and Saipan from the Philippines after World War II. Isolates of *P. noxius* from the Philippines, however, have not been collected and genetically analyzed so it remains undetermined if the Philippines could have been the source of *P. noxius* on Guam, Yap, and Saipan. Similarly, isolates of *P. noxius* from Saipan and the Ogasawara Islands of Japan appear to be genetically related (Stewart et al. 2020). A more focused study is needed to determine if the fungus was transferred from Saipan to the Ogasawara Islands of Japan or vice-versa. To answer critical questions about the evolution and movement of *P. noxius* among the Pacific Islands, larger numbers of *P. noxius* samples and high-resolution genetic markers are needed to identify the genetic bottlenecks, gene flow, and evolutionary history of this pathogen.

Population genetics can also be used in conjunction with bioclimatic modeling to predict geographic locations with suitable climatic conditions for a population to survive. To conduct bioclimatic modeling, the precise latitude and longitude for each fungal isolate is needed. A bioclimatic model of 179 locations of *P. noxius* isolates from eastern Asia, Australia, and the U.S.-Affiliated Pacific Islands used 19 climatic variables to predict the global regions that are at risk from different genetic groups of *P. noxius*. These regions include a large portion of the South American continent, Southeastern United States (e.g., Florida) and Hawaii, some parts of western Africa, eastern Asia, the eastern Australia coast, and many of the Pacific Islands (Stewart et al. 2020). Figure 3.4 shows that the differences in predicted distribution for the two genetic groups (eastern Asia for fig. 3.4c and western Oceania/Japan/Taiwan for fig. 3.4d) may reflect differences in ecological behavior between these two genetic groups. These data strongly suggest that different genetic groups of *P. noxius* pose distinct invasive threats. Geographic areas should consider different genetic groups of *P. noxius* and their suitable climate space when developing regulations to prevent the importation of invasive pathogens. For example, Florida and Hawaii are predicted to have a suitable climate for *P. noxius*, though the pathogen has not been reported in either region. This example shows how genetic characterization of forest pathogens coupled with bioclimatic modeling can help manage forest diseases. These types of information can be used to establish quarantine and other regulatory policies for management of *P. noxius*-caused brown root rot disease.

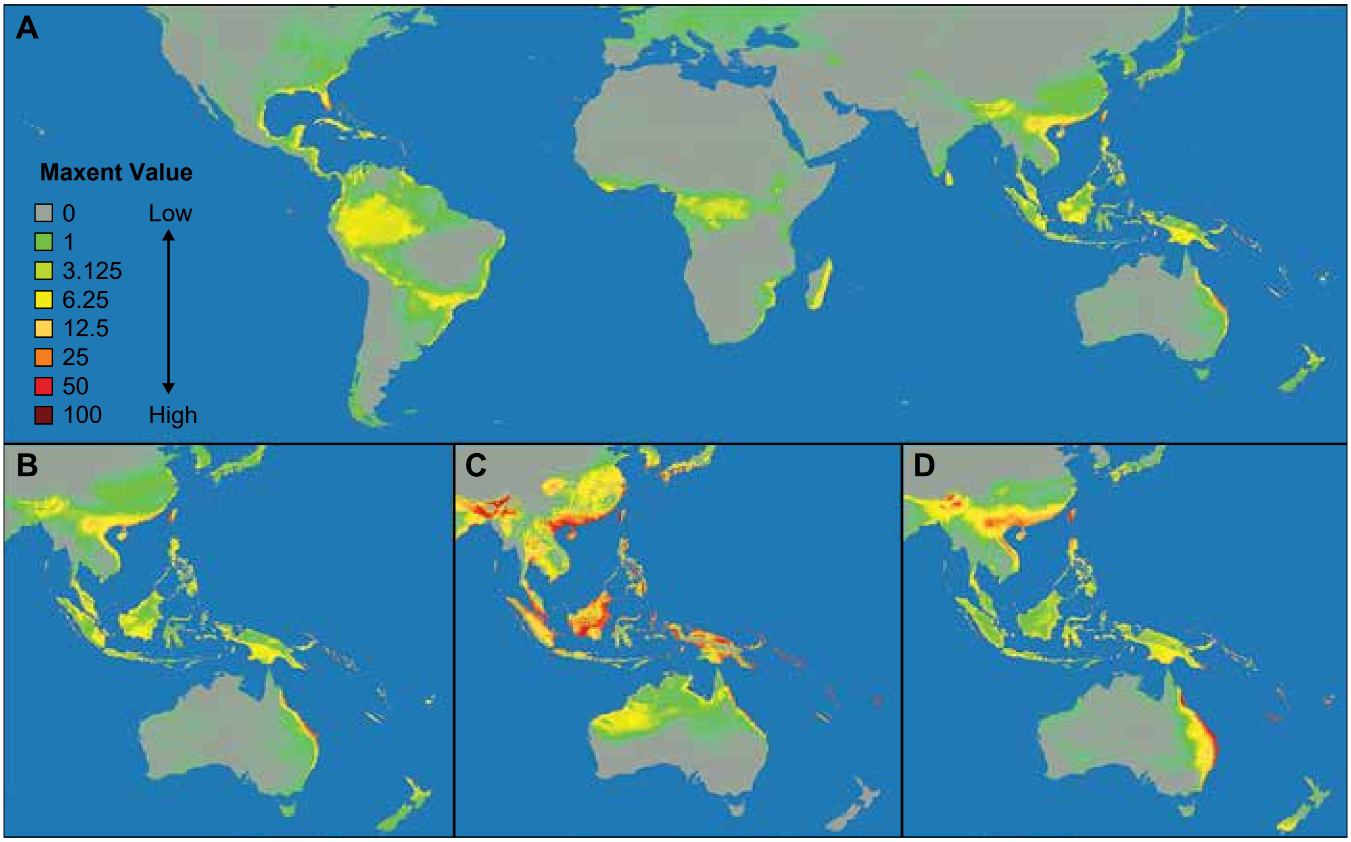

Figure 3.4—Predicted potential distribution (suitable climate space) of *Phellinus noxius* based on Maxent bioclimatic models. Maps 3.4a (global) and 3.4b (regional), 3.4c = eastern Asia (Group 1), and 3.4d = western Oceania/Japan/Taiwan (Group 2), are all based on confirmed occurrences of the *P. noxius*. Increasing Maxent values (probability) of suitable climate space (climate niches) for *P. noxius* are reflected as color shades, with increasing probability as colors range from green to red (figure from Stewart et al. 2020).

Genomic and Transcriptomic Studies Can Provide Insights Into Genes of Interest

Several institutions have conducted genomic sequencing of *P. noxius* (Ibarra Caballero et al. 2020). These efforts have improved our understanding of why *P. noxius* can attack such a wide range of tree species and cause rapid tree death and decomposition. Collaborative work among institutions and comparisons of *P. noxius* genomes have provided insights into its ecology, pathogenicity, and ability to cause bark and wood decomposition on living trees (Ibarra Caballero et al. 2020). Researchers discovered that some isolates were more aggressive than others in Taiwan, and then identified enzyme-encoding genes associated with increased tree mortality and wood decomposition (Chung et al. 2017, Ibarra Caballero et al. 2020). Chung et al. (2017) also determined that 74 percent of the predicted genes of *P. noxius* are the same as at least one other basidiomycete.

Genomic sequencing revealed that *P. noxius* has at least 488 genes that encode the production of carbohydrate- and lignin-metabolizing enzymes. The number of carbohydrate-active enzymes (CAZymes) produced by *P. noxius* is significantly higher than the currently known number possessed by other related fungi. These genomic features of *P. noxius* are assumed to be responsible for its wide host range, including tree species with highly varied wood properties, and its capacity to quickly kill and decompose these hosts (Ibarra Caballero et al. 2020).

Enzymes associated with lignin degradation are among the enzymes encoded within the *P. noxius* genome. These enzymes contribute to the ability of *P. noxius* to cause a white rot (a rot caused by the degradation of all major structural components of wood: cellulose, hemicellulose, and lignin) and allow the fungus to attack and decompose a wide array of hardwood and coniferous trees.

The use of both genomics and transcriptomics can identify the genes in a fungus and determine which genes are expressed under specific developmental and environmental conditions, such as wood colonization and degradation. Before discussing these studies, it is important to understand why wood decomposition is a critical biological function.

Wood is an attractive substrate to micro-organisms such as fungi because it is so abundant (385 gigatons worldwide) (Bar-On et al. 2018). As wood forms (one of the results of photosynthesis), a tremendous amount of the sun's energy is captured and stored as carbon in the form of cellulose, hemicellulose, and lignin. This energy is available to any organism that can overcome a tree's defenses and successfully degrade these compounds.

Cellulose and hemicellulose are basic molecules. They are a linkage of many glucose-derived molecules into one large molecule. Although lignin is also composed of glucose-based molecules, it includes other components, and its complex structure is in turn bonded with cellulose and hemicellulose. Fungi that can decompose wood and derive energy for their own metabolism must therefore possess the capacity to decompose the bonds within cellulose, hemicellulose, or lignin. Fungi that are only able to decompose cellulose and hemicellulose create a brown rot because of the remaining lignin which is brown. In contrast, some fungi have additional wood-degrading enzymes and can degrade all three components of wood, leaving a whitish residue. *Phellinus noxius* is one of these fungi, which are known as white rot fungi.

Ibarra Caballero et al. (2020) have done extensive studies on genes encoding carbohydrate-decomposing enzymes in three *P. noxius* isolates from three geographic areas: Pohnpei, Hong Kong, and Japan. They found many enzymes contributing to the decomposition of cellulose, hemicellulose, and lignin (table 3.1). Collectively, these enzymes allow *P. noxius* to decompose the woody structures of diverse tree species.

We can only speculate why *P. noxius* has genes encoding so many different wood-degrading enzymes. One hypothesis is that tropical and subtropical forests are typically composed of dozens to hundreds of tree species with diverse wood properties and defense systems. While *P. noxius* likely requires one set of enzymes to attack/decompose one tree species under certain environmental conditions effectively, it likely requires a slightly different set of enzymes to attack/decompose another tree species under certain environmental conditions. The capacity to attack different tree species would be especially useful for *P. noxius*, which spreads by root-to-root contact within forests where the neighboring trees are frequently of a different species. Another speculation is that the extensive arsenal of *P. noxius* enzymes may allow it to overwhelm defense systems of some susceptible tree species (e.g., flame tree [*Delonia regia*]), which would also allow it to develop sufficient inoculum potential to easily infect other tree species.

Another interesting feature of *P. noxius* that could favor its rapid growth rate and spread is that it carries seven times more copies of the beta-glucan synthase gene than other basidiomycetes (Chung et al. 2017). This is consistent with the observation by Ann et al. (2002) that this fungus can grow about seven times faster than other basidiomycetes. Note: 1,3-Beta-glucan synthase catalyzes the formation of a beta-1,3-glucan polymer that is a major component of the fungal cell wall. The reaction catalyzed is as follows:

UDP-glucose + {(1,3)-beta-D-glucosyl}(N) = UDP + {(1,3)-beta-D-glucosyl}(N+1).

Therefore, not only is *P. noxius* well equipped with genes encoding enzymes that break down all components of wood, it is also able to use these products of wood degradation to rapidly synthesize its own hyphae and other tissues. Genomic and transcriptomic studies provide additional molecular evidence of potential invasive risks of *P. noxius* associated with the infection and degradation of diverse woody species.

Soil Metagenomic and Metatranscriptomic Studies

Soil metagenomic and metatranscriptomic methods can increase our understanding of soil microbial communities and their ecological function by determining the microbes present, their associated genes, and gene expression in relation to the environment (Stewart et al. 2018, 2021). These methods have not yet been used to understand the *P. noxius* pathosystem but could provide valuable information for management of brown root rot disease. These techniques were recently applied to *Armillaria solidipes*, the cause of Armillaria root disease in the United States. They

Table 3.1—Predicted secreted proteins, proteases, and plant cell wall (PCW) carbohydrate- and lignin-degrading enzymes annotated in *Phellinus noxius* genome assemblies

Enzyme	*P. noxius* isolates[a]		
	P919-02W.7	OVT-YTM/97	FFPR1411160
Total secreted proteins	562	497	518
Small secreted proteins	319	274	273
Proteases	61	54	64
PCW carbohydrate-degrading enzymes	112	102	106
Cellulose degrading	52	44	45
Hemicellulose degrading	31	35	32
Pectin degrading	38	34	38
Lignin- and lignin-derivative-degrading enzymes	30	26	28
AA1 Laccases	8	7	7
AA2 Peroxidases	13	9	12
AA3	5	5	4
AA4	0	0	0
AA5	3	2	2
Cytochrome P450	1	3	3

[a] *P. noxius* isolates from Pohnpei, P919-02W.7; Hong Kong, YTM/97; and Japan, FFPR141160.
Source: Ibarra Caballero et al. 2020.

provided information for the potential biological control of Armillaria root disease (Lalande 2019, Stewart et al. 2021). In general, metagenomics involves sequencing and analyses of DNA from all life forms, especially microbes, found in a soil or wood sample. This process can determine which microbial species are present, the relative level of each microbial species, and which genes are expressed under environmental conditions at a given place and time. This information is then used to assess microbial activities associated with healthy and diseased forest trees, which is explained further by Lalande et al. (2019) and Stewart et al. (2021).

Metabarcoding uses diagnostic DNA sequences to identify and establish the relative levels of each microbial species in a sample. It can also be used to determine the influence of various management techniques on the inoculum density of pathogens, such as *P. noxius*. Metabarcoding can examine the environmental or management influences on native microbes associated with natural biological control methods. This approach may be useful in developing tactics to manage brown root rot disease. An advantage of this approach is that not only can it determine if *P. noxius* is present in a soil or rhizosphere sample, it also can provide a quantitative estimate of how much *P. noxius* DNA is present. A limitation of this technique for *P. noxius* is that this fungus has never been isolated from soil (Wu et al. 2020). Therefore, metabar coding for *P. noxius* may require samples from roots and the decayed remnants of root systems. Other applications of metagenomics are discussed in the management section in chapter 4 below, including its potential use for estimating the amount of *P. noxius* inoculum remaining in the soil after a treatment (e.g., fungicides, urea applications, pH changes).

Metatranscriptomics is based on the extraction and sequencing of mRNA produced by all life forms in a sample. It allows an examination of gene expression within microbial communities at specific time points. This approach can provide critical information on the ecological functions of microbes in relation to environmental conditions and the conditions that support healthy ecological functions within forests, which can help develop management approaches for brown root rot disease and maintaining healthy forests.

Chapter 4: Management Options for *Phellinus noxius*

Philip G. Cannon[1]

The need to manage brown root rot disease, caused by *Phellinus noxius*, is quite compelling. *Phellinus noxius* can infect and destroy hundreds of tree species, it has already spread widely through most islands in the western Pacific, and it can survive and remain infective on a site for 2 to 10 years after it has killed a tree. Three important questions must be answered to successfully manage brown root rot disease: (1) Where does the disease exist? (2) How is the pathogen spreading? (3) Which treatments will be most effective for disease management at a given location?

Where Does *Phellinus noxius* Exist?

We have a general idea of where *P. noxius* exists on some of the U.S.-Affiliated Pacific Islands (fig. 1.10). As discussed in chapters 2 and 3, we can improve our understanding of *P. noxius* distribution by making additional field surveys for visible signs and symptoms and by using DNA-based approaches in forests not yet surveyed for this pathogen.

Several factors can hinder surveys at many locations within the U.S.-Affiliated Pacific Islands. Property issues on some islands can make it difficult to obtain permission to access a property. A scarcity of roads, difficult terrain, and extreme heat and humidity also present a challenge for survey crews. However, it still possible to survey many forested areas on these islands. When the purpose of the survey is explained, local landowners are often glad to accept a survey crew and may even help with the survey. The forestry units on each of the U.S.-Affiliated Pacific Islands typically know the forested areas of their islands well. They know how to approach local landowners about conducting this type of survey, and usually know which landowners would prefer not to have a survey performed on their land.

When considering potential survey methods for an area, a zig-zag technique or some form of random sampling theoretically provides the best coverage. In practice, however, we found that using local trails for travel allowed us to survey far more trees without the need to cut through dense vegetation to reach predetermined sample points. Sampling along trails is also practical because *P. noxius* has a greater chance of becoming established in wounded trees, which are more readily found along trails. Blaze marks, breakage of the bark on roots resulting from foot traffic, and clearing brush with machetes are common sources of tree wounds. Even while using trails, however, considerable ground must be traversed to inspect

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many trees. For this reason, a two-stage, stratified sampling approach can be used to improve efficiency. In the first stage, while walking along a trail, the surveyor quickly surveys numerous trees to determine the presence of dead and dying trees, or clusters of dead trees that have general symptoms resembling *P. noxius* attack. Then, in the second stage, the surveyor closely examines these subsets of dead trees and trees in the surrounding areas for additional symptoms and signs of *P. noxius*.

Before conducting these surveys, the survey crew must be well instructed on how to recognize the signs and symptoms of brown root rot disease and become familiar with the survey methods. These surveys often depend on assistance from local residents. A small number of these residents may be asked to help lead the search, and possibly decide which trails to follow. All participants should be instructed briefly on the survey method and how to recognize brown root rot disease. For example, if available, it is useful to select an infected tree as a demonstration for discussion before the start of the survey. Once the survey begins, a sequence of tree-associated characteristics can be observed. First, the surveyor should look for one or several trees together with dead or dying tops. Next, the surveyor should carefully examine the bases of each of these trees for the black/dark-colored to orange-colored sock/sheath or other signs of *P. noxius* (see signs and symptoms in chapter 2).

One way to potentially save time and improve success at finding *P. noxius* infection centers is to coordinate surveys with the weather. Weather-based coordination may not be as important for some islands, such as Kosrae and Pohnpei (Federated States of Micronesia [FSM]), that receive high levels of rainfall every year (fig. 2.24); however, this approach may help on islands, such as Saipan and Rota, (Commonwealth of the Northern Mariana Islands [CNMI]), Yap (FSM), and the Republic of Palau, where dry spells can last for several years. The best time for finding the black/dark-colored to orange-colored socks/sheaths of *P. noxius* seems to be about 3 to 6 months after a period of heavy rainfall (especially after a typhoon makes a direct hit on the area), because the additional rainfall will facilitate growth and the formation of the signs of *P. noxius*.

Another factor important to consider when planning a survey is that it is difficult to determine if dead trees were killed by *P. noxius*-caused brown root rot disease when the disease occurred more than a few years earlier. The rapid rotting caused by *P. noxius* can lead to the disintegration of causal evidence of tree infections, which makes it difficult to confirm *P. noxius*-caused mortality. Although the signs and symptoms of *P. noxius* activity may not be apparent, the inoculum may still be viable in the remains of these trees that reside on or beneath the soil surface and are protected by the pseudosclerotial rinds (see chapter 2).

Two approaches are available to determine if brown root rot disease was recently present in an area. One approach is to examine the site for large numbers of stumps and fallen logs (fig. 1.5). A second approach is to ask local landowners if trees were previously present on the land. In either case, it will also be necessary to chop through vegetation and debris, find the tree bases, and then determine if brown root rot is associated with the disappearance of the trees. If brown root rot disease occurs on the site, some stumps may still be relatively easy to find and should be checked for signs that *P. noxius* inoculum is present. (Note: If it is determined that an area contains *P. noxius*, it will be important to treat the area for infected stumps and remnant root systems before trying to grow trees there.)

An important step in managing *P. noxius* is to map the infested areas. A good example of a useful map is one developed by Blair Charlie and Maxim Nithan, two leading foresters in the Kosrae Island Resource Management Authority in Kosrae, FSM. They mapped all the *P. noxius*-infected ka (*Terminalia ivorensis*) trees observed in the Ka Forest Reserve of the Yela conservation area using global positioning system (GPS) coordinates (fig. 4.1). Other examples include a map of all known infected and noninfected trees in an area, as was produced for the Ada Estate at Navy Hill, Saipan (fig. 2.28), a map of infection centers at Anderson Air Force Base, Guam (fig. 2.29), and a map of several infection centers on Rota (fig. 1.9a). A map delineates the extent of the brown root rot disease, helps determine how it is spreading, and helps to assess the effort needed to treat for *P. noxius* in that area.

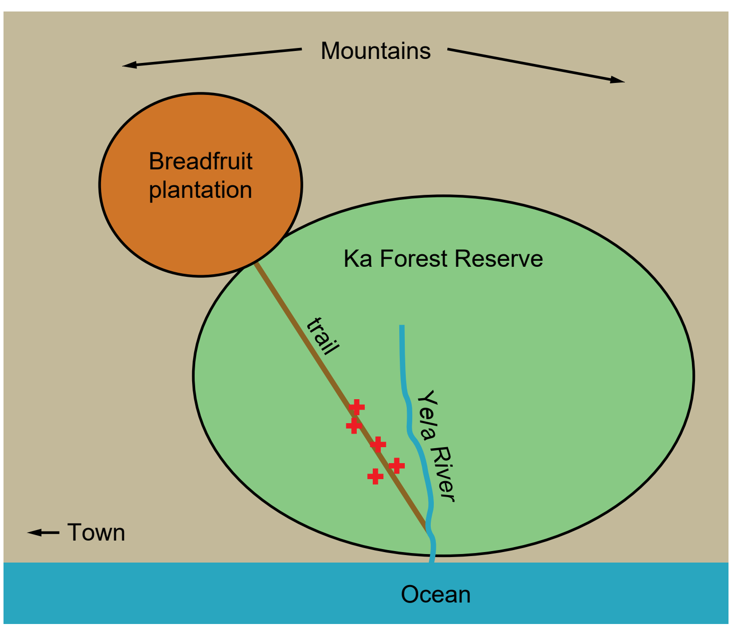

Figure 4.1—Blair Charlie and Maxim Nithan mapped all of the ka trees in the Ka Forest Reserve of the Yela conservation area on Kosrae Island, Federated States of Micronesia. From this map, they marked the locations of *Phellinus noxius*-infected trees, found that the infected trees were generally in a line, and determined that several large ka trees and their root systems would require treatment for the brown root rot disease. The positions of the infected ka trees are indicated by the red crosses.

How Does *Phellinus noxius* Spread Through an Infection Site?

It is important to determine how *P. noxius* is spreading within the site and the extent of the infection centers because these factors help determine the range of treatment options that will be effective and the scale of the management effort needed. The etiology section of chapter 2 describes ways by which *P. noxius* can spread from a single tree to infect large stands of trees; and the molecular genetics methods used in chapter 3 provided evidence that spread occurs by vegetative tree-to-tree (root-to-root) growth and through infection via airborne spores. Successful management techniques must consider both kinds of spread.

What Treatment Options Will Be Most Efficient?

Over the years, people have attempted to control *P. noxius* by various means. Trials to manage the disease have been conducted by landowners, local communities, governmental research institutions, and universities. Table 4.1 summarizes various measures used to control *P. noxius* over the past 20 years and their estimated relative effectiveness in order of least to most effective. More information about each of these control methods and the basis for evaluating the effectiveness of each is provided in the ensuing text.

Table 4.1—Measures used to control *Phellinus noxius* and their effectiveness

Methods to control brown root rot disease	Effectiveness of control methods
Do not control *Phellinus noxius* and accept the consequences.	In an infested area where all trees have been killed by *P. noxius* and no new trees develop from natural regeneration, the inoculum potential should attenuate completely in about 10 years (Chang 1996) unless new trees seed into the area or are planted and later become infected.
Treat trees recently attacked by *P. noxius* with fire (fig. 4.2), smoke, mangrove mud (fig. 4.3), or ginger shampoo (fig. 4.4).	Each of these techniques has some degree of effectiveness, but not enough to completely control the pathogen. Evaluations of these approaches and their effectiveness are described below and in Cannon (2015, 2017).
Use of other agents that help destroy *P. noxius*, such as termites or carpenter ants.	Some effect, but not enough to completely control the disease (Cannon 2015, 2017).
Use of genetically resistant tree species when planting new trees.	Modest to considerable effectiveness (Sahashi et al. 2010).
Make soil unfavorable for *P. noxius* by using lemon grass, fungicides, fertilizers, urea/lime, etc.	Modestly to fully effective (Cannon 2014, 2019; Chang and Chang 1999; Liao et al. in press)
Prevent colonization of cut stump surfaces by applying borax or other compounds.	Possibly effective, but this technique has not yet been evaluated for *P. noxius*, although it can work very well for some root rot pathogens (Kliejunas 1989).
Use biocontrol agents, including other fungi associated with *P. noxius*, such as *Trichoderma* strains.	Modest to considerable effectiveness, but does not provide complete control (Chou et al. 2019, Ribera et al. 2016, Schwarz et al. 2012).
Use endophytic fungi.	No effective endophytes have been found to date (Pang 2020).

Table 4.1—Measures used to control *Phellinus noxius* and their effectiveness (continued)

Methods to control brown root rot disease	Effectiveness of control methods
Prevent infection foci from expanding by digging trenches; or using physical means such as 18-inch-wide (45-cm-wide) plastic or metal sheets; or placing chemicals, such as urea, lime, pig manure, etc. in these trenches.	Considerable effectiveness, but limited in the area it can protect (Cannon 2019, Chang and Chang1999).
Prevent tree wounding.	Considerable effectiveness, but very difficult to implement (Johnson et al. 2021).
Do not disseminate infected wood.	Considerable effectiveness, but it does not offer complete control (Liebhold et al. 2012).
Flood the soil for at least 1 month.	Effective (Chang 1995), but rarely usable because the site must be concave enough to hold water .
Treat the root area with fungicides, such as dazomet, triadimefon, fuchchromin, or procloroz.	Effective (Fu et al. 2012); however, these fungicides must be registered for use in a country before they can be applied.
Destroy zone lines in infected residual stumps, roots, and logs by chipping pathogen-infected wood into small pieces so it can be readily decomposed by other micro-organisms.	Effective (Cannon 2019).
Remove infected stumps and root systems.	Fairly effective (Thies et al.1994).
Rid the soil of all visible traces of inoculum.	Effective (Wu et al. 2020).
Do not use infected planting stock.	Effective to a substantial degree (Sahashi 2010).
Integrated pest management: use combinations of the effective methods above.	Completely effective integrated treatment.

Methods Used to Control *Phellinus noxius*

Below are brief descriptions of control measures for *P. noxius*, from the simplest to the more complex. Costs of each control measure vary depending on labor and operational costs on the different islands. Generally, human labor is the most important cost factor, so to establish the basic cost, we use "person days" of work needed to apply a treatment for many of these control methods. We also describe techniques that seem most appropriate for specific situations among the U.S.-Affiliated Pacific Islands. Also noted are special situations, such as when infected trees in public places become hazardous because they could fall on people or property.

Pacific Island farmers and other landowners surveyed in this report observed *P. noxius* growing at the base of trees on their property, resulting in the trees dying. Because they were losing many high-value trees to brown root rot disease, they realized the need to control *P. noxius*. Treatments landowners typically relied on for this purpose included applying fire or smoke to the base of the tree and scraping off the mycelial sheath before treating the infected tree base with mangrove mud or ginger shampoo. Brief descriptions of these treatments follow.

Fire and Smoke

One method used to kill *P. noxius* is to cover the base of an infected tree with about 0.144 m^3 (5 ft^3) of moderately dry brush and then igniting it with the intent of killing the fungus from the flame and smoke from the fire. The smoke component is usually relied on more than the fire because the heat and flames can damage the base of trees.[2] This smoke treatment can result in a temporary disappearance of the visible signs of *P. noxius*, but infected trees treated in this manner died the following year where this treatment was applied on Pohnpei and Kosrae.

Fire Alone

About 4 kg (10 lb) of dry firewood is placed at the base of an infected tree and ignited (fig. 4.2). This fire treatment cannot save the infected tree from dying, but it can kill some of the inoculum in the butt of the tree.[3]

J.B. Friday

Figure 4.2—Valentino Orhaitil (left) and Francis Ruegorong inspect work by their Yap forestry colleague, Andrew Yinfel, to control *Phellinus noxius* along a path in Gagil-Tamil, Yap, Federated States of Micronesia. During the dry season, Yinfel piled about 4 kg (10 lb) of firewood at the base of this tree and ignited the pile. Although fire appeared to kill the *P. noxius*, the tree still died.

Mangrove Mud

This method entails first scraping the black/dark-colored fungal sheath from the base of the tree with a machete, then covering the scraped area with mangrove mud, which is presumed to contain compounds or micro-organisms detrimental to the growth of the fungal sheath. As shown in figure 4.3, this approach offers some protection, but it appears to be insufficient for stopping the subsequent advance of the fungus for long (fig. 4.3).

[2] Waguk, E. 2015. Personal communication. Chief forester, Kosrae, Federated States of Micromesia, Kosrae Island Resource Management Authority, PO Box 303, Palikir, Administrative Area, Postal Code 96941.

[3] Ruegorong, F. 2018. Personal communication. Yap State Division of Agriculture and Forestry, P.O. Box 463, Yap, Federated States of Micronesia 96943.

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Figure 4.3—Blair Charlie shows the buttress root of a giant ka tree with the “black sock” or dark-colored sheath of a *Phellinus noxius* infection. The external mycelium had been scraped off the root 6 months earlier and one bucket of mangrove mud was smeared over the scraped surface. The procedure appeared to kill *P. noxius* in the treated area, but the fungus continued to grow up the root, past the scraped surface. Mycelial growth during 6 months after treatment spanned the distance between Charlie’s hands.

Ginger Shampoo

The freshly cut flowers of native wild ginger (*Zingiber zerumbet*) are pounded in a container to create a saponin-rich lather known as “ginger shampoo.” This shampoo is spread over the area where the *P. noxius* mycelial sheath has been scraped off at the base of the tree.

It should be noted that in fig 4.4, the *P. noxius* mycelial sheath or black sock was not actually scraped off and the ginger shampoo was not applied to the tree shown in figure 4.4. Within 6 months, the tree died; within 1 year it had fallen over; and within 2 years, the entire bole of the tree was colonized by *P. noxius*. However, a few other breadfruit trees in this same area were given the ginger shampoo treatment. Those trees appeared to recover during the first year, according to Joab Santos; however, those trees also died within subsequent years after treatment.

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Figure 4.4—Gibson Santos (right) shows a mycelial sheath of *Phellinus noxius* growing up the side of a large breadfruit tree on his farm. Joab Santos (left) holds wild ginger flowers that could be pounded to make a saponin-rich lather known as “ginger shampoo. For this treatment, all of the mycelial sheath of *P. noxius* needs to be scraped from the base of the tree before the ginger shampoo would be applied to the entire scraped area to prevent further fungal growth.

Treatment Specific to Banyan Trees

The technique of scraping the mycelial sheath of *P. noxius* away from the tree trunk has proven largely ineffective in controlling this fungal pathogen in western Pacific regions. Not only does the fungus survive, but it also continues to spread, eventually resulting in the death of the tree. However, Jyh-Nong Tsai, a plant pathologist at the Taiwan Agricultural Research Station in Taichung, Taiwan, developed a modified version of this technique, which appears to be effective for banyan (or fig [*Ficus benghalensis*]) trees that show the initial symptoms of infection.

Tsai's technique involved the following steps:

- Scrape off the mycelial sheath of *P. noxius* from the surface of the infected banyan tree using an "open mountain knife" (or machete). Scrape off the outside bark, as well, down to the green layer of the cambial tissue (fig. 4.5).
- Paint the scraped area with a mixture of triadimefon and prochloraz fungicides (discussed later).
- Remove all *P. noxius*-infected roots.
- Treat the soil around the base of the tree with a mixture of triadimefon and prochloraz by injecting 45 to 91 L (12 to 24 gal) of this fungicide solution about 50 cm (20 inches) deep in the soil using a pressurized injection system. Alternatively, add a mixture of urea and lime to the soil (see specific amounts below).
- Form new support trunks by directing aerial roots of the banyan tree so that some are pointed down into soil free of the fungus (fig. 4.6). This can be

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Figure 4.5—Jyh-Nong Tsai using an "open-mountain" knife to scrape the *Phellinus noxius* mycelial sheath down to the layer of green cambial tissue of a banyan tree. This knife is heavy (0.9 kg [2 lb]) and can be used like a draw blade to cut around limbs; it can also probe well into bark crevices. Photo taken in a park near T'aichung, Taiwan.

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Figure 4.6—Jyh-Nong Tsai grasps the aerial roots of of a banyan tree that can be redirected via PVC tubes to locations in the soil where no *Phellinus noxius* occurs, or where all previously infected roots have been carefully removed. Photo taken in a park near T'aichung, Taiwan.

accomplished practically by taking an aerial root dangling from a limb (banyans produce hundreds of such roots), wrapping it around a 2.5-cm (1-inch) diameter PVC tube, and then burying the lower end of the tube in *P. noxius*-free soil where it would be suitable to have a new banyan tree trunk.

Be sure no other *P. noxius*-infected roots are connected to the tree that is to be saved. If a *P. noxius*-infected root is found, severe the root at least 50 cm (20 inches) away (on the tree side) from where the infection is observed (fig. 4.7). The soil in the root trace should also be treated (see below). Tsai noted that this technique may be of use on several Pacific Islands where venerable banyan trees are in early stages of *P. noxius* infection. However, this method is ineffective for managing *P. noxius* in other tree species, according to Tsai.

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Figure 4.7—James Manglona holds a section of a root of this giant banyan tree cut 4.6 m (15 ft) in front of the base of the tree. This root became infected by *Phellinus noxius* when it grew into contact with the root system of a breadfruit tree that had already died from brown root rot disease. This root was cut 18 inches (46 cm) ahead of where the infection was visibly moving toward the base of the banyan tree. Note: close inspection shows that when the root was cut, *P. noxius* was aggregating soil in its mycelium. Urea was subsequently mixed in with the soil in the root trace (hole) from which this root had been extracted. With moisture from the soil, urea is converted into ammonia, which is toxic to *P. noxius*. This method is intended to kill the fungus and prevent it from advancing closer toward the main stems of this banyan tree. Photo taken near Gagani, Rota, Commonwealth of the Northern Mariana Islands..

Limiting the Spread of *Phellinus noxius* in Soil

A trench can be dug around an area of soil contaminated with *P. noxius* to prevent it from moving into the root systems of neighboring trees. In Japan, a root- and fungus-proof metal barrier is placed vertically into the trench. In Taiwan, a thick sheet of plastic is used for the same purpose.[4] A study in the Republic of Indonesia showed that a moderate dose (0.35 g/L) of paclobutrazol (2RS,

[4] Tsai, J.-N. 2019. Personal communication. Plant pathologist, Agricultural Research Institute, Department of Plant Pathology, 250 Kuo Kuang Road, Taichung, Taiwan, Republic of China, 402.

3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol]) retarded the growth of *P. noxius* by 90 percent (Ahmad Nazarudin and Mohd Farid 2019). This result suggests that adding a moderate dose of this fungicide as a soil drench could reduce the spread of *P. noxius*.

When deciding where to place the trench, the general rule is to draw a tentative line in the soil at least one healthy looking tree beyond the current edge of the infection center. Dig the trench 46 cm (1.5 ft) deep, or until a hardpan or the parent rock is encountered.

Several amendments (or structural features) can be added to the trench to make it less penetrable by *P. noxius*. These amendments include urea, which hydrolyses to ammonia that is toxic to the fungus. Urea can be added to the trench at about 0.9 kg per 1.0 m^2 (2 lb per 10 ft^2), mixed with fill dirt, and covered with an additional 10 cm (4 inches) of soil. Pig manure has been used where urea is scarce or too expensive. Both these methods were tried on Pohnpei, and appeared to limit the lateral movement of *P. noxius*. These techniques, however, will have no effect on the *P. noxius* inside the trenched area, and they require considerable effort to install (about 0.5 person days to dig a containment trench for a 200 m^2 [2,000 ft^2] plot).

Use a Fungistatic Ground Cover

Lemon grass is sometimes planted, or encouraged, around the base of trees for protection against *P. noxius*. Lemon grass is reported to have allelopathic properties, but in American Samoa where this approach has been attempted, *P. noxius* still managed to kill at least some trees.

Eliminate or Reduce *Phellinus noxius* Inoculum From a Site

Several approaches are available to reduce or eliminate *P. noxius* from a site:

- Let the land go fallow until *P. noxius* naturally decomposes (this could take at least 10 years).
- Use biocontrol agents to reduce existing pockets of *P. noxius* inoculum.
- Mechanically remove all infected biomass.
- Apply chemicals to kill all *P. noxius* in infected root tissue.
- Asphyxiate all *P. noxius* by flooding the land with water.
- Plant or retain resistant species.
- Use an integrated approach that incorporates two or more of the above options.

Let Land Go Fallow

Letting land go fallow may be the easiest, most economical approach, but it may require a few decades to be sufficiently effective because if this option is implemented, most tree species will not survive on the site until the inoculum of the fungus is sufficiently diminished. This can mean waiting 2 to 10 years before new trees can be planted or allowed to become established. And, if new, naturally regenerated trees become established on the site before the fallow is complete, and subsequently become infected by *P. noxius*, this could mean prolonging the fallowing approach indefinitely.

Biocontrol Methods

Biocontrol methods offer some promise for managing *P. noxius*. During our surveys of several U.S.-Affiliated Pacific Islands, we found several fungi that may have potential as biocontrol agents because their hyphae had displaced *P. noxius* in trees that had been killed by brown root rot disease.

Species of fungi isolated from the wood of flame trees (*Delonix regia*) killed by *P. noxius*-caused brown root rot disease in Saipan included several Basidiomycetes (e.g., *Earliella scabrosa* [fig.4.8], *Annulohypoxylon stygium*, and *Daldinia eschscholtzii*), some ascomycetes (e.g., *Pestalotiopsis thea, Schwanniomyces* spp.,

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Figure 4.8—A fructification of *Earliella scabrosa* found on a flame tree killed by *Phellinus noxius*.

and *Coniothyriacea* spp.), and a yeast (e.g., *Ogataea trehaloabstinens*). The potential of these fungi as biocontrol agents against *P. noxius* is unknown and unstudied.

Trichoderma is another fungal genus known for its ability to destroy Basidiomycetes. Several studies have been conducted to determine if one of the *Trichoderma* species is capable of controlling *P. noxius* (Chou et al. 2019, Ribera et al. 2016, Schwarze et al. 2012). Schwarze et al. (2012) found that some *Trichoderma* species are far more capable of controlling *P. noxius* than are other fungi (table 4.2).

Table 4.2—Potential of biocontrol against four strains of *Phellinus noxius* using volatile organic compounds produced by three species of *Trichoderma*[a]

Biocontrol method	Average *P. noxius* radial growth in millimeters, by strain			
Trichoderma strain	133	144	169	178
Control	9.3	6.5	6.9	1.7
T. atroviride 15,603.1	2.2	1.9	1.0	1.4
T. ghanense 4,428	11.0	6.3	6.1	8.4
T. harzianum 3,967	5.3	5.3	4.4	3.6

[a] Measurements were made of the radial growth of the fungal hyphae of *P. noxius* at 6 days after placing a 5-mm-wide disc of *P. noxius* culture on a Petri dish that had been growing the indicated strain of *Trichoderma*. There were 20 replicates of each combination of the 16 combinations of *P. noxius* x *Trichoderma* treatment. These 20 measurements were then averaged.

Source: Modified from Schwarz et al. 2012.

Using antagonistic *Trichoderma* species for biological control was not effective in controlling *Rigidoporus lignosus*, another white-rot fungus, in field trials with rubber trees (*Hevea brasilensis*) in the Democratic Socialist Republic of Sri Lanka (Jayasuriya and Thennakoon 2007). However, at an experimental level, biological control of *Phellinus* with *Trichoderma* has been successful in Taiwan (Chou et al. 2019). Additional studies could be useful for determining if *Trichoderma* could be used as a biocontrol agent against *P. noxius* in any of the U.S.-Affiliated Pacific Islands.

An experiment was conducted using *Serendipita indica* to determine if this endophytic fungus could help young seedlings of Bishop wood (*Bischofia javanica*) withstand attack by *P. noxius*. In previous experiments, this fungal endophyte had improved the resistance of some plants to other fungal pathogens, but it did not protect Bishop wood from *P. noxius*. Both *S. indica*-inoculated and noninoculated Bishop wood plants were equally susceptible to *P. noxius* infection (Pang 2020). A multitude of other common endophytic fungi have yet to be tested for their potential to protect trees from *P. noxius*.

Use Wood-Boring Insects

Pinhole borers (ambrosia beetles, subfamily Scolytidae, family Curculionidae), carpenter ants, and termites (fig. 4.9) can also displace *P. noxius* in colonized wood. These insects are occasionally found in *P. noxius*-infected trees, and some have been observed penetrating the pseudosclerotial rind that protects the survival structures, or pods, of *P. noxius*. Introducing such insects for the purpose of controlling *P. noxius* is unorthodox and carries some obvious risks that warrant consideration. However, forester Frances Ruegorong reported that he successfully used termites to clean up some *P. noxius*-infected trees on his farm in Yap.

Although the techniques presented above appeared somewhat effective at reducing *P. noxius*, no single technique was able to completely control the fungus. The overall goal is to find a treatment that completely removes *P. noxius* from infected wood and the rhizosphere.

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Figure 4.9—A tree killed by *Phellinus noxius* near Anderson Air Force Base on Guam was subsequently infested by termites that largely destroyed all the wood once infected by the fungus.

Mechanically Removing *Phellinus noxius* Inoculum

Mechanically removing *P. noxious* inoculum entails removing the infected tissue and burning or safely disposing of the infected tissue. This approach has been used numerous times in the U.S.-Affiliated Pacific Islands. The inoculum was removed from a large infection center of *P. noxius* behind the forestry building in Pahniepw, Yap; at the Gibson Santos Estate on Pohnpei (fig. 4.10); near Tofol on Kosrae; and just south of Anderson Air Force Base on Guam. The time and effort required to clean up a site varies. For example, a six-person crew might spend a half day to remove a cluster of ten 15- to 20-cm (6- to 8-inch) diameter trees from a small plot of trees killed by brown root rot disease. This treatment includes removing the tree root systems and piling all the woody material for burning. In contrast, the same-size crew may need a half a day to treat one large breadfruit tree 76 cm (30 inches) in diameter (fig. 4.11). In other cases, such as with giant ka trees on Kosrae, that measure 150 cm (5 ft) in diameter, it might require 2 weeks for a six-person crew to dispose of one infected tree and its root system.

In the above instances, the use of picks, pulaskis, chainsaws, shovels, and axes were useful for extracting the infected trees and their root systems. Infected tree species in other parts of the world, however, might be removed differently. In some situations, where many big trees covering vast acreages have *P. noxius*-infected root systems, excavators are used to remove their large root systems from the soil (fig. 4.12). This approach seems feasible in certain places on some Pacific islands,

John Wichup

Figure 4.10—Cleanup of one 61-cm (24-in) and two 20-cm (8-in) *Phellinus noxius*-infected trees, including the extraction of their root systems, took this crew a half day at the Gibson Santos residence in Pohnpei, Federated States of Micronesia. Picks, shovels, axes, and a wheelbarrow were the only tools used. Most of the root systems were extracted easily. Roots in wet areas tend to grow close to the surface, and a pick was ideal for prying them from the ground. From left to right: Marciano Imar, Rodasio Samuel, Phil Cannon, Romney Panuelo, and Edward Roland.

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Figure 4.11—This 91-cm (3-ft) diameter, *Phellinus noxius*-infected breadfruit tree near Tofol, Kosrae, Federated States of Micronesia, had already fallen, but it still required a half day for a five-person crew with a chainsaw to cut, extract, and pile up its rotten bole and root system. Eric Waguk, State Forester for Kosrae, is manning the chainsaw in this photo.

Thies et al. 1994

Figure 4.12—An excavator removing stumps and parts of the root systems of coniferous trees infected with *Phellinus weirii* in Oregon. After excavation, the stump will be left on the surface to decompose. In a large experiment, removing all infected stumps and the large lateral roots of their root systems (as shown here) reduced the mortality rates of subsequently planted Douglas-fir (*Pseudotsuga menziesii*) trees from 9.1 to 2.4 percent.

especially near towns and roadways. However, some islands do not have excavators, and, if they do, the cost of operating them is high. The scattered distribution of *P. noxius* infection centers would also require moving the excavators from one site or island to another. Finally, many soils on these tropical islands are quite fragile and will deteriorate if heavy equipment is run over them.

Regardless of the technique used to extract the root systems of *P. noxius*-killed trees, small pieces of *P. noxius*-infected wood will break off and remain in the soil. Although this woody inoculum will likely be degraded by soil microorganisms, it can remain infectious for several years.

In Taiwan, the removal of all pieces of the infected root system greater than 1.3 cm (0.5 inch) in diameter from the site is considered full treatment. If handled properly, any remaining inoculum will either break down quickly or can be treated effectively with chemicals. Most infected wood removed from a site can be burned or chipped down to the size shown in figure 4.13 using saws, wedges, and small wood chippers. The main reason for breaking up the infected wood into small chunks (with dimensions smaller than 2.5 cm [1 inch]) in diameter is to assure that the peudosclerotial plates, which protect the *P. noxius*-infected wood from becoming wet or being invaded by other microorganisms and insects, are shattered. That wet, decayed wood decomposes faster than dry, decayed wood supports the concept of the "pod" as the principal survival structure of this fungus. For this reason, the pod structures of *P. noxius*-infected wood encased within the pseudosclerotial plates must be broken down to allow water and microorganisms to invade and displace *P. noxius*.

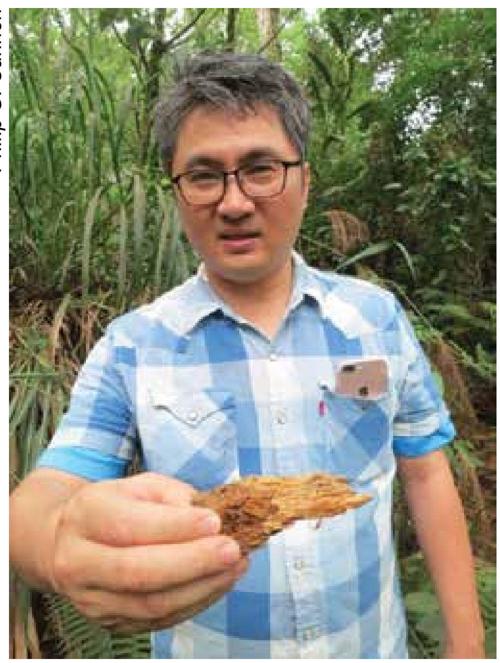

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Figure 4.13—Wen-Wei Hsiao, Forest Health Extension Agent from National Taiwan University, holds a piece of *Phellinus noxius*-infected wood to demonstrate the function of zone lines and the size of wood pieces that allow quick degradation of *P. noxius* by diverse soil micro-organisms. The left half of this piece contains tissue that is so wet, free water can be squeezed from it; the right half is dry. Separating these two halves of the wood piece in an almost imperceptible line, is the *P. noxius* pseudosclerotial plate, or rind.

Sometimes, at sites with hundreds of tonnes of *P. noxius*-infected logs and root systems, a large amount of infected wood is still left on the ground. In these situations, larger equipment may be needed to remove or chip the infected wood. These machines are expensive (about $330,000 USD) according to heavy equipment operator Michael Krauss, of Tree Works, Inc.[5] but could be very cost-effective when

[5] Kraus, M. Tree Works. Agent. Tree Works, Inc., PO Box 277, Hilo, HI 96701.

also used to clean up sites with large amounts of woody debris from storm damage or death from brown root rot disease according to K. Eckhart of Arbol Global.[6]

Chemical Control Methods

Chemical control methods can be used to directly inhibit or kill *P. noxius*, or to stimulate existing biocontrol agents that then hinder or kill the pathogen.

Many fungicidal and other chemicals have been tested for efficacy against *P. noxius*. Initial tests are usually performed on cultures of *P. noxius* in Petri dishes to determine which chemicals impede fungal growth. Tsai has performed much of this work in Taiwan. When testing 60 of the most prominent fungicides used in agriculture, he found that triadimefon and prochloraz were the most effective against *P. noxius*. To inhibit growth of *P. noxius*, Jenn-Wen Huang (vice president of Chung Hsing University in Taichung, Taiwan) developed fuchcromin. This fungicide is commercially available in Taiwan. Fuchromin causes cytoplasmic leakage and death of fungal cells. Dazomet (a substitute for methyl bromide that will not harm the ozone layer) can be used as a fumigant when drenched or injected into the soil to a depth of 51 cm (20 inches). In Taiwan, all these fungicides are regularly used to treat remnants of *P. noxius* that remain after trees and roots are removed from the soil (fig. 4.14). and appear to be very effective at managing *P. noxius*. However, human health and environmental considerations may also be of concern when applying these fungicides. For example, Dazomet is a skin irritant activated by

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Figure 4.14—Taiwan forest research workers stand by an area they just cleared of *Phellinus noxius*-infected trees. First, they felled about 10 eucalyptus trees infected with *P. noxius* by root-to-root spread. Next, they removed the root systems using excavators. Afterward, they drenched the soil with dazomet and covered it with a plastic tarp (shown here) for 3 weeks to keep the volatile components of the treatment in the soil.

[6]Eckhart, K. President and Managing Director, Arbor Global USA/Hong Kong, 326 Lliwahi Loop, Kailua, HI 96734.

moisture, so the product label states it cannot be applied within 0.8 km (0.5 mi) of a school when that school is in session. Also, workers should not handle dazomet when they are perspiring (Fu et al. 2012, O'Malley 2010).

To avoid these hazards, alternatives such as urea can be used (fig. 4.15). When urea is applied to soil with a pH around 7.4, soil microorganisms convert most of the urea to ammonia, which is toxic to most root-rot fungi. Urea should be applied dry to the soil at a level of 3,000 ppm (Chang and Chang 1999), or about 2.7 kg (6 lb) of urea per 1 m^2 (10.8 ft^2) of soil area. When water contacts urea, soil microorganisms will convert it to ammonia, which is toxic to the *P. noxius* that might otherwise survive in the small bits of the infected root system that remain in the soil. Because many tropical soils are acidic (low pH), it may be necessary to add enough lime to the soil to raise it to about pH 7.4 (Chang and Chang 1999). Cost is another consideration when using any of the above-mentioned fungicides/treatments; both the chemicals and the application equipment have associated costs. Cost benefit analyses are recommended when comparing possible control measures.

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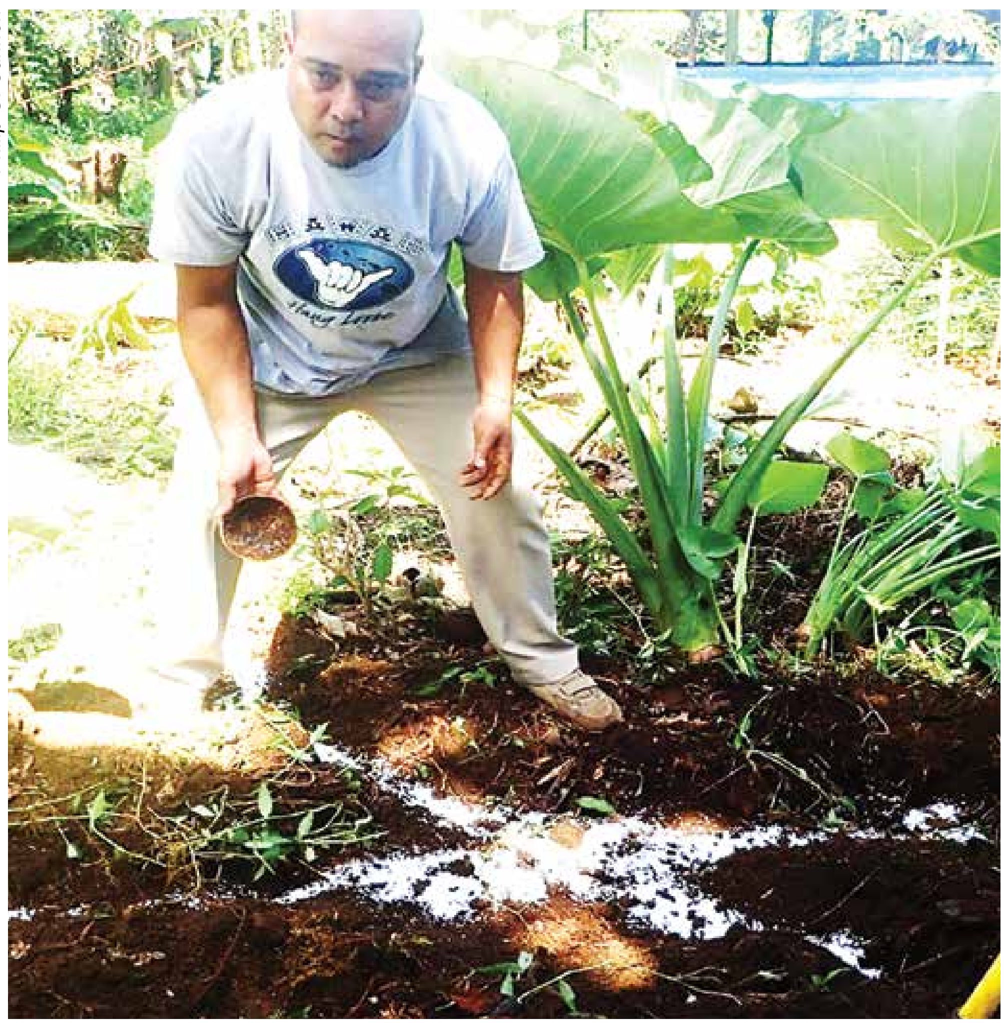

Figure 4.15—In Pohnpei, Federated States of Micronesia, John Wichup applies urea to the root traces left behind when the root system of a large, *Phellinus noxius*-infected breadfruit tree was pulled from the soil. The urea is applied about 10 cm (4 inches) deep and then covered with at least 7.6 cm (3 inches) of soil.

Applying borax to a stump may potentially work for controlling brown root rot disease because this method has been found to be very effective at preventing stump infection and infection foci formation by *Heterobasidion* species under certain circumstances (Kliejunas 1989). However, this approach has not been attempted yet on the U.S.-Affiliated Pacific Islands, largely because most new infection centers of *P. noxius* do not begin with a stump infection, and very few nice round stump surfaces are formed in any of these islands from logging activities.

Regardless of the method used to manage the inoculum on a site, it is important to determine if the treatment is effective. One approach to determine effectiveness is to place small wooden dowels colonized with *P. noxius* into the soil prior to or immediately after treatment. Then, about 3 weeks after a treatment, such as when the tarp is removed after a dazomet treatment, the dowels are extracted and taken to a lab to determine if viable *P. noxius* remains in the dowels. Another method to determine if a *P. noxius* infection center has been successfully treated is to plant a few trees as a bioassay (fig. 4.16). Several small seedlings of a highly susceptible tree species, such as breadfruit, loquat (*Eriobotrya japonica*), or flame trees (*Delonix regia*), are planted at the treated site and monitored for *P. noxius* infection for a few years. If none of the planted trees become infected, the site can be considered safe (or at least low risk) for planting, because the inoculum level of *P. noxius* has been reduced to a level where the threat is minimal.

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Figure 4.16—One of the sites on the National Taiwan University Campus treated for *Phellinus noxius*. An infected tree and its root system were removed and the ground treated with *Trichoderma asperellum* (TA) at 2-week intervals. At 10 months posttreatment, five loquat (*Eriobotrya japonica*) trees were planted on the site as a bioassay. If two of these trees are not infected when removed for close inspection, the site is considered as recovered. Standing behind the loquats, from left to right, are Zong-Chi Wu, Ting-Ting Li, Chia-Lin Chung, and Shean-Shong Tzean, who helped develop and test this technique.

Metagenomics or metabarcoding have potential application for determining the level of *P. noxius* inoculum in the soil. These approaches, respectively, can detect the DNA of all organisms, especially microorganisms, found in a sample, or only DNA unique to *P. noxius*. These methods have management utility for identifying or monitoring microbes associated with biological control of *P. noxius*. With either method, the presence of *P. noxius* in the sample can be detected and the level of inoculum quantified. An important consideration, however, is that soil without any woody tissue is not capable of sustaining *P. noxius* (Wu et al. 2020). Therefore, if a sample of the soil rhizosphere is collected to detect or measure *P. noxius* DNA, the sample should contain some woody root tissue.

Once areas have been successfully treated for *P. noxius*, additional measures can be implemented to reduce the probability of *P. noxius* damage in the future forests.

Flooding the Soil

Flooding the soil with water is another method that has been tried to control *P. noxius*. However, it is rarely usable because the site must be concave enough to hold water (Chang 1995).

Planting or Retaining Resistant Species

Planting or retaining resistant species is another measure that be used to control spread of *P. noxius*. The numerous tree species susceptible to *P. noxius* were discussed earlier. Not all species are attacked in the same manner or to the same extent, however, and a few appear to have especially high resistance (table 2.2). Some characteristics associated with resistance are extremely dense wood, a high extractive content, and an enhanced ability to produce callus following wounding. Some species of palms appear to be less affected by *P. noxius*.

Hazard Trees and *Phellinus noxius*

Every year, thousands of trees with brown root rot disease die or fall over because their decayed root systems lack the structural integrity to hold them upright (fig. 4.17). The consequences of a trees falling over usually are not particularly important, unless they fall on a person or anything of value, such as a car or house. Where *P. noxius*-infected trees could fall and cause harm to lives and property, landowners or land managers in the U.S.-Affiliated Pacific Islands or other regions could benefit greatly from recognizing brown root rot disease and safely removing infected trees before they fall. The wood at the base of *P. noxius*-infected trees can become very weak very fast, and these trees can rot and fall over easily, even before trees are showing other symptoms of brown root rot disease. Special International Society of Arborist Hazard Tree Trainings, sponsored


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Figure 4.17—A dead breadfruit tree with symptoms of advanced brown root rot leaning over a piggery in Kosrae, Federated States of Micronesia, posed a potential hazard to humans and animals. The *Phellinus noxius*-infected tree lost its leaves, and its base was quickly weakened by rapid wood decay, causing it to fall over.

by the USDA Forest Service, are available to all people of the U.S.-Affiliated Pacific Islands. When these courses are conducted in the Pacific Islands, participants learn to recognize and safely remove hazard trees and how to manage *P. noxius*-affected trees.

Report Summary

The primary purpose of this report is to provide people living and working in the U.S.-Affiliated Pacific Islands with enough information to recognize *P. noxius,* evaluate the impact of the resulting brown root rot disease in their location, and consider options for managing it. Chapter 1 introduces brown root rot disease, caused by *P. noxius*, and chapter 2 describes the observable signs and symptoms of brown root rot disease. This information can be used by local forestry and natural resource staff to survey and document the extent of brown root rot disease within forested areas, agroforests, and urban settings. Chapter 2 also describes how *P. noxius* functions in these ecosystems and the conditions under which it can quickly cause a devastating, fast-spreading disease. Chapter 3 discusses the value of using molecular genetics to study *P. noxius*. It also describes the evidence for the short-distance dissemination of *P. noxius* by clonal (e.g., root-to-root) spread, and for long-distance dissemination by airborne spores that result in sexual recombination. Management activities must consider both methods of dispersal.

This chapter (chapter 4) introduces multiple options for managing *P. noxius*, and briefly discusses the benefits and limitations of each management option. Some of the management techniques are quite simple, but, in some cases, are not effective. Others seem to provide an effective disease-management approach but require expensive machinery or chemical applications that may pose hazards to the environment or the people applying them.

The following is an integrated set of practices that might provide practical and low-cost management of *P. noxius* on many U.S.-Affiliated Pacific Islands:

The first step is to remove the *P. noxius*-infected tree or trees from the site, including all the associated root pieces larger than 1.3 cm (0.5 in) in diameter. The entire mass of *P. noxius*-infected wood should be burned. If burning is not possible because of wetness, break the infected wood into pieces that are smaller than the survival structures, or pods, that form in wood decomposed by *P. noxius.*[7]

Apply urea to the root traces of the removed tree at a rate of about 2.7 kg (6 lb) of urea per 1 m^2 (10.8 ft^2) of soil. This assumes that the soil is at or near pH 7.4. Because many soils on the Pacific Islands are more acidic (lower pH) than 7.4, it may be necessary to add lime to the soil in the root trace until a pH near 7.4 is reached (Chang and Chang 1999).

If chemical applications, such as urea and lime, are not added to the soil, extra vigilance is needed to ensure that all the root pieces larger than 1.25 cm (0.5 inch) in diameter are removed from the soil, and adequate time allowed (e.g., a few months to years) before attempting to grow any woody plants in the area that has been treated for *P. noxius*; this should provide enough time to allow the microorganisms in the soil to sufficiently degrade the inoculum.

The decision to reclaim land infested with *P. noxius* should be considered carefully. Management options that include the removal of all infected wood are optimal from a control point of view, but these options may be considered excessive for practical applications. On many islands, however, the amount of land available for farming and agroforestry is limited, so many landowners and communities are often willing to make the necessary effort.

In some situations, especially near suburbs, large tracts of land are infested with *P. noxius* and contain many infected tree boles and stumps killed by the

[7] Hsiao, W.W. 2019. Personal communication. National University of Taiwan, Forest Health Extension, Roosevelt Road, Taipei 10617, Taiwan, Republic of China.

fungus. In this situation, it may be advisable to use heavy machinery to clear *P. noxius*-infected woody debris. The use of an excavator (fig. 4.12) can be effective in this situation (Thies et al. 1994). The use of a masticator, such as the one shown in figure 4.18, could also be especially effective at treating *P. noxius*-infected wood already on the ground.[8]

Mike Krauss

Mike Krauss

Figure 4.18—(A) Side view and (B) rear view of a masticator, which could be especially effective at treating *Phellinus noxius*-infected trees when these trees have already fallen to the ground.

From a global perspective, the key objective for minimizing the costs of disease management is to prevent the entry of an invasive pathogen, such as *P. noxius,* on an island or to actively attempt to eradicate or prevent spread of the pathogen soon after it arrives (Liebhold et al. 2012). Because *P. noxius* is present on nearly all of the U.S.-Affiliated Pacific Islands that were visited for this report, exclusion of the fungus might seem an unlikely possibility. However, because the pathogen has not reached all the western Pacific Islands and is found in only a few spots on many islands, a certain type of quarantine may still have value. Preventing the spread of *P. noxius* within any island will likely take less effort now than in the future, when the pathogen may become more widely established.

Phellinus noxius management is complicated by its aggressive growth and prolific ability to establish new infection centers during periods of heavy rainfall. This seems especially true during typhoon conditions when extremely powerful winds cause major breakage of tree limbs and roots, which creates many infection courts for *P. noxius*.

[8] Eckert, K. 2019. Personal communication. President and managing director, Arbol Global L.L.C., PO Box 1343, Kailua, HI 96734.

Although we cannot readily reduce the number or severity of typhoons that strike an island, we can minimize the spread of *P. noxius* and reduce its infection of trees and the resulting brown root rot disease after a typhoon. For example, surveys for *P. noxius* could be conducted about 3 to 6 months after a heavy typhoon. By this time, the typhoon would have caused its maximum level of wounding; the rainfall could have stimulated maximal germination of *P. noxius* spores and a subsequent rise in infection levels and mycelial growth. These conditions could make it easier to detect *P. noxius*-infected trees and produce maps showing the location and extent of infection centers, and infection centers would still be in an incipient stage which should make them relatively easy to manage.

Public engagement and education are two essential steps in managing *P. noxius*-caused brown root rot disease. These steps may proceed in stages. Initial educational sessions can focus on informing natural resource managers and extension professionals about this pathogen and the disease it causes. Subsequently, these professionals can focus additional extension sessions on brown root rot disease in communities that have been most affected by the disease. Outreach messages, such as those conveyed in the laminated *P. noxius* posters that were circulated on some of the Pacific Islands in 2014, or others that emphasize the importance of minimizing bark damage of trees and avoiding the transfer of *P. noxius*-infected root shoots when moving breadfruit (or other trees) germplasm to new areas, can also help inform the public about how to recognize the pathogen and the importance of avoiding wounding trees. Powerpoint™ presentations and webinars on *P. noxius* and its management are also available.

Our understanding of the biology of *P. noxius* and the best ways to manage its impact is still evolving. Additional research is needed to discover the pathways that have allowed *P. noxius* to establish and spread among the U.S.-Affiliated Pacific Islands. This will require additional sampling and molecular genetics studies, such as were described in chapter 3 (above), Stewart et al. (2020), Ibarra Caballero et al. (2020), and Kozhar et al. (2022). The use of molecular genetic tools for monitoring soil microbial communities can also contribute to the development of management methods that suppress *P. noxius* or encourage naturally occurring soil microbes that provide biological control against *P. noxius*

We must also determine the most cost-effective methods for managing *P. noxius* under different environmental conditions and the societal and political influences associated with tree disease management on each island. To reduce the risk of reinfection by *P. noxius*, the critical amount of time needed between treatment applications and the planting of a new woody species must be determined. Additional knowledge about which tree species can safely be planted in soil treated for *P. noxius*, but still retaining low levels of inoculum, is needed.

Different clones of breadfruit trees may also possess significant variation in the level of resistance against *P. noxius*. Although identifying, developing, and deploying brown root rot disease resistance in breadfruit could take decades, such steps could be especially important toward maintaining a stable supply of breadfruit, which would contribute to food security on many western Pacific Islands. A fringe benefit of using clonal breadfruit material produced via tissue culture is that clonal cuttings from the forest will not be required to propagate breadfruit. This means of propagation would lower the chances of spreading brown root rot disease via rooted cuttings, and it will also reduce the number of wounds created on the roots of the scion trees that could later become infected with *P. noxius*.

All the above-mentioned projects will require political and financial support, plus collaborative efforts among neighboring countries or regions trying to resolve many of these same issues related to brown root rot disease. For these reasons, it is critical to gain the interest and confidence of authorities and organizations that can provide financial and logistical support for conducting *P. noxius* surveys and developing control measures.

Despite the many remaining questions about *P. noxius* and its management, we hope that this report provides sufficient information to help forestry and other natural resource professionals of the U.S.-Affiliated Pacific Islands and other regions recognize *P. noxius*-caused brown root rot disease, evaluate its impact in their forests, and choose the best options available for its management.

Finally, this report provides information about an extraordinary tree pathogen, *P. noxius*, and describes how it can be managed. However, brown root rot disease is not the only challenge faced by forest resource managers in the U.S.-Affiliated Pacific Islands. Hopefully, the information about *P. noxius*-caused brown root rot disease in this report will help gauge the proper effort and funding needed to manage this disease in many situations where *P. noxius* is found in the U.S.-Affiliated Pacific Islands.

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Appendices

Appendix 1: Additional Names for *Phellinus noxius* and the Disease It Causes

Name of disease or pathogen	Origin of name
Black sock disease	A common name for brown root rot disease caused by *Phellinus noxius* in Kosrae, Yap, and Pohnpei, Federated States of Micronesia, because a black mycelial sheath forms at the base of infected trees during the rainy season and then grows upward on the exterior of the tree.
Brown root rot	The name given to the tree disease caused by *P. noxius* in Australia, Hong Kong, Taiwan, and Japan because although the mycelial sheath is initially black, it turns brown and remains brown during the dry season.
Fomes noxius	The original scientific name given to this fungal pathogen by Corner (1932), which is currently known as *P. noxius*.
Limu mea	The common name of *P. noxius* in American Samoa.
Musrasr	Means “balding” and is the common name given to the disease caused by this fungus in Kosrae because it causes affected trees to lose their leaves.
Phellinidium noxium	A scientific name proposed by Bondartseva and Herrera (1992) for the fungal pathogen that is currently known as *Phellinus noxius*.
Chot’ nut atbot	A common name for brown root rot disease on Saipan, Commonwealth of the Northern Mariana Islands.

The full scientific name of *Phellinus noxius* as shown in CABI (2020):

Domain: Eukaryota

Kingdom: Fungi

Phylum: Basidiomycota

Subphylum: Agaricomycotina

Class: Agaricomycetes

Subclass: Agaricomycetidae

Order: Hymenochaetales

Family: Hymenochaetaceae

Genus: *Phellinus*

Species: *noxius*

Appendix 2: Collaborating Foresters and Forest Pathologists and Their Locations

Location	Collaborators
American Samoa	Ndeme Atibalentja, Ian Gurr, Neil Gurr, Shalley Tailevai, Emily Ilaoa, Marc Schmaedick, Simon Stowers, Mary Taufetee
Australia	Geoff Pegg, Paul Barber
Chuuk, FSM	Jaokim Wasan
Guam	Robert Schlub, Ruddy Estoy, Roger Brown, James McConnell, Mario Martinez, John Horeg, Michael Jordan, Sheeka Afiason, Joe Afiason, George Wall
Hawaii, U.S.	James B. Friday, Kathleen Friday, Kevin Eckert, Richard MacKenzie
Japan	Yuko Ota, Norio Sahashi
Kosrae, FSM	Eric Waguk, Leonard Sigrah, Maxim Nithan, Blair Charlie, William William
Palau,ROP	Ann Kitalong, Pua Michaels, Larry Mamus
Pohnpei, FSM	Gibson Santos, Joab Santos, Rodasio Samuel, Konrad Englberger, Arleen Rosekrans, John Wichep, Simon Lipai, Marciano Imar, Romney Panuelo, Edward Roland
Rota, CNMI	James Manglona
Saipan, CNMI	Sid Cabrerra, Vic Guerrero, Joaquin Tenorio, Manny Tenorio, Frank Ada, Dilip Nandwani, Arnold Route
Taiwan, ROC	Chia-Lin Chung, Zong-Chi Wu, Ting-Zhi Lao, Yuen-Yi Pang, Jyn Wen Tsai, Jhin-Hen Yan, Jenn-Wen Huang, Ka-Tung Leung, Shean-Shong Tzean, Tse-Yen Liu, Tun-Tschu Chang, Ting-Zhi Liao, Jyh-Nong Tsai, Wen Wei Hsiao, Chieh-Yin Chen, P.-J. Ann
U.S. Mainland	Charles Hodges, Ned B. Klopfenstein, Mee-Sook Kim, Jane E. Stewart, Michael Robinson, Fred Brooks, Ashley Lehman, Micha Salomon
Yap, FSM	Francis Ruergorong, Marjory Falanruw, Pius Liyagel, Valentino Orhaitil, Tamdad Sulog, Andrew Yinfel

FSM = Federated States of Micronesia; RCP = Republic of Palau; ROC = Republic of China; ROP = Republic of Palau; CNMI = Commonwealth of Northern Mariana Islands; U.S. = United States.

Appendix 3: A *Phellinus noxius* Host List Developed for Taiwan[a]

Host genus and species	Host common name
Host tree:	
Annona montana	Mountain soursop
Annona squamosa	Custard apple
Annona squamosa × *A. cherimola*	Atimoya
Artocarpus heterophyllus	Jack fruit
Averrhoa carambola	Carambola
Dimocarpus longan	Longan
Diospyros kaki	Persimmon
Elaeocarpus serratus	Ceylon olive
Eriobotrya japonica	Loquat
Ficus pumila var. *awkeotsang*	Jellyfig
Litchi chinensis	Litchi
Muntingia calabura	Indian cherry
Pachira macrocarpa	Malabar chestnut
Persea americana	Avocado
Prunus mume	Japanese apricot, plum
Prunus persica	Peach
Pyrus pyrifolia	Pear
Sterculia nobilis	Ping po
Syzygium samarangense	Wax apple
Vitis vinifera	Grape
Ornamental trees:	
Acacia confusa	Taiwan acacia
Actinodaphne pedicellata	Litsea
Aleurites fordii	tungoil tree
Alstonia scholaris	Blackboard tree
Araucaria cunninghamii	Hoop pine
Araucaria heterophylla	Norfolk Island pine
Bauhinia × *hybrid*	Butterfly tree
Bauhinia purpurea	Purple bauhinia
Bauhinia varigata	Orchid tree
Bischofia javanica	Autumn maple tree
Bombax ceiba	Silk cotton
Broussonetia kazinoki	Small paper mulberry
Broussonetia papyrifera	Paper mulberry
Calocedrus formosana	Taiwan incense cedar
Calophyllum inophyllum	Indian poon beauty leaf
Camellia japonica	Camellia
Cassia fistula	Yellow golden shower tree
Casuarina equisetifolia	Ironwood tree

Host genus and species	Host common name
Ceiba pentandra	Kapok
Cerbera manghas	Odollam cerberus tree
Chamaecyparis formosensis	Taiwan red cypress
Chorisia speciosa	Floss silk tree
Chrysalidocarpus lutescens	Yellow areca palm
Cinnamomum kanehirai	Stout camphor
Codiaeum variegatum	Croton
Cryptocarya concinnai	Konishi crytocarya
Cycas taiwaniana	Taiwan cycas
Dalbergia sissoo	Sissoo tree
Delonix regia	Flame tree
Diospyros ferrea var. *buxifolia*	Philippine ebony persimmon
Diospyros oldhamii	Oldham persimmon
Duranta repens	Creeping sky flower
Eucalyptus camaldulensis	Murray red gum eucalyptus
Eucalyptus citriodora	Lemon gum eucalyptus
Eucalyptus grandis	Maiden eucalyptus
Ficus elastica	Rubber plant
Ficus macrocarpa	Small-leafed banyan
Ficus religiosa	Bo tree fig
Firmiana simplex	Chinese parasol
Fraxinus formosana	Island ash
Gardenia jasminoides	Cape jasmine
Grevillea robusta	Silver oak
Hibiscus rosa-sinensis	Hibiscus
Hibiscus schizopetalus	Fringed hibiscus
Hibiscus tiliaceus	Linden hibiscus
Hydrangea chinensis	Chinese hydrangea
Keteleeria davidiana var. *formosana*	Taiwan keteleeria
Kigelia pinnata	Sausage tree
Koelreuteria elegans var. *formosana*	Flame gold rain tree
Lagerstroemia turbinate	Crepe myrtle
Lagerstroemia speciosa	Queen's crepe myrtle
Lantana camara	Lantana
Leucaena leucocephala	White popinac
Liquidambar formosana	Formosa sweet gum
Litsea glutinosa	Indian laurel
Litsea hypophaea	Mountain pepper
Macaranga tanarius	Macaranga
Machilus zuihoensis	Incense machilus
Maesa tenera	Taiwan maesa
Mallotus paniculatus	Turn in the wind
Melaleuca leucadendron	Cajuput tree
Melia azedarach	Chinaberry

Host genus and species	Host common name
Melodinus angustifolius	Narrow leafed melodinus
Michelia compressa	Formosan michelia
Michelia figo	Banana magnolia
Muntingia calabura	Jamaica cherry
Murraya paniculate	Orange jasmine
Neolitsea parvigemma	Small bud neolitsea
Nerium oleander	Oleander
Osmanthus fragrans	Sweet Osmanthus
Palaquium formosanum	Formosan nato tree
Pinus thunbergia	Black pine
Pistacia chinensis	Chinese pistache
Podocarpus macrophyllus	Yew
Pongamia pinnata	Pongamia
Pterocarpus indicus	Rose wood
Rhododendron obtusum	Rhododendron
Roystonea regia	Royal palm
Salix babylonica	Willow
Schefflera octophylla	Scheffera
Sterculia foetida	Hazel sterculia
Swietenia mahogany	Mahogany
Tabebuia chrysantha	Yellow golden bell tree
Taiwania cryptomerioides	Taiwania
Terminalia catappa	Indian almond
Terminalia boivinii	Bovinii
Ulmus parvifolia	Chinese elm
Zelkova serrata var. *serrat*	Zelkova
Special crops:	
Camellia sinensis	Tea
Cinnamomum camphora	Camphor
Cinnamomum zeylanicum	Ceylon cinnamon
Coffea arabica	Coffee
Cordia dichotoma	Cordia
Herbaceous plants:	
Artemisia capillaris	Wormwood
Artemisia princeps	Mugwort
Ipomoea pes-caprae	Morning glory
Lauca indica	Wild lettuce
Melicope merrilli	Melicope
Sauranja oldhami	
Urena lobata	Cadillo

[a] Useful for islanders of the Western Pacific Region.
Source: Ann et al. 2002.

Appendix 4: *Phellinus noxius*-Susceptible Commercial Trees

Tree genus and species	Tree family
Acacia confuse	Fabaeceae
Araucaria cunninghamii	Araucaciaceae
Artocarpus altilis	Moraceae
Bauhinia variegata	Fabaceae
Calophyllum inophyllum	Clusiaceae
Camellia sinensis	Theaceae
Casuarina equisetifolia	Casuarinaceae
Coffea spp.	Rubiaceae
Elaeis guineensis	Arecaceae
Ficus macrocarpa	Moraceae
Garcinia mangostana	Clusiaceae
Hevea brasiliensis	Euphorbiaceae
Koelreuteria elegans var. *formosana*	Sapindaceae
Koelreuteria paniculate	Sapindaceae
Podocarpus macrophyllus	Podocarpaceae
Salix babylonica	Salicaceae
Swietenia mahogany	Meliaceae
Tectona grandis	Lamiaceae
Theobroma cacao	Malvaceae

Source: CABI 2021.

Appendix 5: *Phellinus Noxius*-Susceptible Tree Species Based on 20 Strip Transects Through Infected Forests on Tutuila Island, American Samoa

Genus and species	Family
Rhus taitensis	Anacardiaceae
Spondias dulcis	Anacardiaceae
Cananga odorata	Annonaceae
Cerbera manghas	Apocynaceae
Barringtonia asiatica	Barringtoniaceae
Barringtonia samoensis	Barringtoniaceae
Cordia aspera	Boranginaceae
Canarium harveyi	Burseraceae
Calophyllum neo-ebudicum	Clusiaceae
Terminalia richii	Combretaceae
Diospyros samoensis	Ebenaceae
Flueggea flexuosa	Euphorbiaceae
Glochidion ramiflorum	Euphorbiaceae
Macaranga harveyana	Euphorbiaceae
Macaranga stipulosa	Euphorbiaceae
Adenanthera pavonina	Fabaceae
Inocarpus fagifer	Fabaceae
Intsia bijuga	Fabaceae
Samanea saman	Fabaceae
Hernandia nymphaeifolia	Hernandiaceae
Hibiscus tiliaceus	Malvaceae
Dysoxylum samoense	Meliaceae
Ficus obliqua	Moraceae
Ficus tinctoria	Moraceae
Ficus sp.	Moraceae
Myristica fatua	Myristicaceae
Syzygium inophylloides	Myrtaceae
Syzygium sp.	Myrtaceae
Crossostylis biflora	Rhizophoraceae
Morinda citrifolia	Rubiaceae
Neonauclea forsteri	Rubiaceae
Planchonella grayana	Sapotaceae
Planchonella samoensis	Sapotaceae
Elattostachys falcata	Sapindaceae
Pometia pinnata	Sapindaceae
Pipturus argenteus	Urticaceae

Source: Brooks 2002b.

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