Chapter III

Characterizing forest root- and butt-rot fungi in Yap, Palau, Pohnpei, Kosrae, Guam and Saipan.

By: Phil Cannon, Ned B. Klopfenstein, Mee-Sook Kim, Yuko Ota, Norio Sahashi, Robert L. Schlub, Roger Brown, Sara M. Ashiglar, Amy L. Ross-Davis, John W. Hanna.



Figure 1. Ned Klopfenstein and Rodasio Samuel admiring the mucilaginous mycelial crust of the aggressive *Phellinus noxius* fungus attacking a breadfruit tree (*Artocarpus altilis*) on Pohnpei.

Abstract: *Ganoderma* and *Phellinus* are two common fungal genera causing butt-rot on trees growing on USA-affiliated islands of the western Pacific. Although these fungi can be quite prevalent, especially in some older mangrove stands, it appears that the majority of infections caused by these fungi leads to severe rotting of the heartwood but do not kill the living tissues of the sapwood, cambium and phloem. Thus, the usual consequence of infection by butt-rot fungi in this part of the world is a loss of structural strength in the bole, but not mortality to the tree. Three notable exceptions were found to this general observation. One is where typhoon winds or storm surges hit mangroves, in which case, butt-rot-afflicted trees are exceptionally prone to breakage and toppling. A second exception is when typhoon-strength winds break or otherwise wound previously healthy trees, subsequently enabling butt-rot fungi

to colonize heartwood and spread via root contact to neighboring trees. The third exception is when *Phellinus noxius,* the cause of brown root rot, is the pathogen affecting a stand. This aggressive pathogen can kill trees outright. Although a number of different butt-rot fungi occur in these islands, not much was known about their classification prior to this trip. In this foray, efforts were made to collect the full range of sporophores (conks, fructifications) that were found in association with butt-rotted trees, and isolations were made of these collected fungi onto the appropriate culture media. When the butt rotter appeared to be *P. noxius*, efforts were focused to collect samples for isolation. Molecular genetics techniques were then used at the Rocky Mountain Research Station (USDA Forest Service) for preliminary identifications of fungi that had been collected and isolated.

Introduction

From September 1-23, 2013, the authors collectively surveyed butt-rot fungi in natural forests, agroforestry settings and urban forests in the western Pacific islands of Palau, Yap, Guam, Saipan, Pohnpei and Kosrae. The participation of Drs. Yuko Ota and Norio Sahashi (Appendix I) on Guam, Saipan and Pohnpei was especially useful during this survey, because they have been working on a similar, but much more intensive, survey focusing on *Phellinus noxius* on of all of the Ryukyu islands of Japan over the past 14 years (Sahashi *et al.* 2012). In this present survey, local forestry staff on all islands (Chapter I) were key in helping find the butt-rot fungi. For years, they have noted butt-rot problem areas and they were also able to obtain local permission to visit, survey, and sample most of these areas. They also assisted with the collection of the samples during this trip, including the identification of the host species and the characterization of the infection foci.

On the sites where fungal samples were collected, the following stand parameters were evaluated: date of survey, collector, elevation, GPS points, host species, and other information relating to site characteristics, impact of the disease and extent of the problem (Table 1). Fruiting bodies (conks) were collected where possible (note: *P. noxious* rarely forms these sporocarps, but many other butt-rot fungi found on this trip produced them in abundance). Samples of infected wood were also collected. In the evenings, impromptu phytopathology laboratories were set up and used to make fungal isolations onto slants and/or plates with Potato Dextrose Agar (PDA) or a more selective media (which included benomyl) in accordance with methods detailed by Sahashi *et al.* (2012). Isolations were made from both fruiting bodies and infected wood, as appropriate. In addition, some sections of fruiting bodies and/or infected wood were also dehydrated in an air drier at 60° C for 8 hours.

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Figure 2. Rudy Estoy helps the sporophore (conk, fructification) collection exercise in Guam.

Collected samples in both culture form (plates and slants) and herbarium form (dried conks and wood) were then sent in accompaniment with APHIS permits to the USDA Forest Service - Rocky Mountain Research Station (RMRS), Forest Pathology Laboratory in Moscow, Idaho.

Once a clean culture isolate was obtained, the next step towards getting its identification was to extract the DNA from the culture, followed by the use of the polymerase chain reaction (PCR) technique to make thousands to millions of copies of selected regions of that isolate's DNA, such as ITS (internal transcribed spacer) and other DNA regions. These amplified DNA regions were sequenced for each isolate and the resulting sequence information was "blasted" against the GenBank database (<u>http://blast.ncbi.nlm.nih.gov/Blast.cgi</u>) to compare the closest database match, based on DNA sequence similarity, which helps to determine the isolate's most probable identity. Using this approach, the genus- or species- level identities of most of these fungal samples can be determined. However, a match with GenBank sequences is not sufficiently close for some of the fungal isolates; this may provide an opportunity to describe a new fungal species or add new DNA sequences for an identified species to the GenBank database.



Figure 3. Part of the *Phellinus noxius* survey team (dubbed the "dream team" by the senior author). Front row left to right: Mee-Sook Kim, USA Ambassador Dorothea-Maria "Doria" Rosen, Yuko Ota; Back row: Phil Cannon, Gibson Santos, Rodasio Samuel, Ned Klopfenstein, and Norio Sahashi (photo by Arlene Rosenkrans). Not shown are Robert "Bob" Schlub, Roger Brown and Roland Quitugua (Guam); Vic Guerrero, Manny Tenorio and Arnold Route (Saipan); Francis Ruegorong (Yap); Ann Kitalong (Palau); Blair Charley, Erick Waguk, Maxson Nithan and Leonel Sigrah (Kosrae); and Konrad Engleberger (Pohnpei).



Figure 4. Yuko Ota (left) and Norio Sahashi (right) using their well-honed techniques to isolate *Phellinus noxius* and other wood-rot fungi onto culture media for subsequent mailing to the USA and Japan, where DNA-based molecular techniques are used to help identify the fungal species.

Initial Results

From the surveys conducted on each of the islands it appears that many butt-rot infections on the USA-affiliated islands of the western Pacific are either being caused by *Phellinus* and/or *Ganoderma* spp. Table 1 contains the list of many of the fungal specimens and isolations that were made during this survey of these six islands (a total of 246 samples were collected).

Table 1. List of fungal collections made during the in September 2013 foray of forest butt-rotting pathogens in Yap, Palau, Pohnpei, Kosrae, Guam, and Saipan. This list includes the sample (or isolate) reference number, the preliminary fungus identification, the island where collected, the name of the collector, the name of the host tree or substrate and a very brief summary of the collector's notes.

RMRS	Fungal Species	Place of	Collector	Host Tree	Collector's
Isolate No.	Identified	Origin			Notes
Palau 1F		Palau	Phil Cannon	mangrove	near Capitol
Palau 2F		Palau	Phil Cannon	Casuarina	Burger Hut
Palau 3F	Coriolopsis sanguinaria	Palau	Phil Cannon	Rhizophora	girdled Airai
Palau 4F	Ganoderma orbiforme	Palau	Phil Cannon	Rhizophora	girdled Airai
Palau 5F	Fusarium sp.	Palau	Phil Cannon		girdled Airai
Palau 6F	Ganoderma gibbosum	Palau	Phil Cannon	Stump	Nat. Hist. Mus.
Palau 7F.1	Trichoderma sp.	Palau	Phil Cannon	mangrove	girdled Airai
Palau 7F.2		Palau	Phil Cannon		
Palau 8F.1	<i>P. noxius</i> -like culture	Palau	Phil Cannon	stump	Nat. Hist. Mus.
Palau 8F.2		Palau	Phil Cannon		
Palau 8W		Palau	Phil Cannon	stump	Nat. Hist. Mus.
Palau 9P	Trichoderma sp.	Palau	Phil Cannon	mangrove	girdled airai
Palau 10P	Pestalotiopsis sp.	Palau	Phil Cannon	mangrove	girdled airai
Palau 11P	Fulvifomes sp.	Palau	Phil Cannon	mangrove	Airai Village
Palau 12P	looks like Fulvifomes	Palau	Phil Cannon	Rhizophora	Airai Village
Palau 13P		Palau	Phil Cannon		Nat. Hist. Mus.

Palau 14P	Psilocybe sp.	Palau	Phil Cannon		Nat. Hist. Mus.
	Ganoderma				
Palau 15P	boninense	Palau	Phil Cannon		Airai girdle
Palau 16P	Ganoderma sp.	Palau	Phil Cannon		Airai girdle
Palau 17P	Coriolopsis	Palau	Phil Cannon		Airai tree
	sanguinaria				
	Coriolopsis				
Palau 18P	sanguinaria	Palau	Phil Cannon		girdled Airai
Palau 19P	Trichoderma sp.	Palau	Phil Cannon		P. noxius-like
Palau 20P	P. noxius-like culture	Palau	Phil Cannon		Nat. Hist. Mus.
Palau 21P	<i>P. noxius</i> -like culture	Palau	Phil Cannon		Nat. Hist. Mus.
Palau 22P	<i>P. noxius</i> -like culture	Palau	Phil Cannon		Nat. Hist. Mus.
	Fomitiporia				
Palau 23P	bannaensis	Palau	Phil Cannon	Casuarina	baseball Park
	Fomitiporia				
Palau 24P.1	bannaensis	Palau	Phil Cannon	Casuarina	baseball Park
Palau 24P.2	Chaunopycnis sp.	Palau	Phil Cannon		
Palau 25P	Pestalotiopsis sp.	Palau	Phil Cannon	mangrove	Odamelach
Palau 26P.2		Palau	Phil Cannon	mangrove	Odamelach
Palau 27P	P. noxius-like culture	Palau	Phil Cannon	stump	Nat. Hist. Mus.
Palau 28P	Phellinus noxius	Palau	Phil Cannon	stump	Nat. Hist. Mus.
Palau 29P	P. noxius-like culture	Palau	Phil Cannon	stump	Nat. Hist. Mus.
Palau 30P	P. noxius-like culture	Palau	Phil Cannon	stump	Nat. Hist. Mus.
Palau 31P	<i>P. noxius</i> -like culture	Palau	Phil Cannon	stump	Nat. Hist. Mus
Palau 32P	<i>P. noxius</i> -like culture	Palau	Phil Cannon	stump	Nat. Hist. Mus
Palau 33P	Phellinus noxius	Palau	Phil Cannon	stump	Nat. Hist. Mus
S916-03W.1	Phellinus noxius	Saipan	Yuko Ota	Delonix regia	decline
S916-03W.2	Phellinus noxius	Saipan	Yuko Ota	Delonix regia	decline
S916-05W.1	Phellinus noxius	Saipan	Yuko Ota	Delonix regia	almost dead
S916-05W.2	Phellinus noxius	Saipan	Yuko Ota	Delonix regia	almost dead

S916-04W.1	P. noxius-like culture	Saipan	Yuko Ota	Delonix regia	green tree
S916-04W.2	P. noxius-like culture	Saipan	Yuko Ota	Delonix regia	green tree
S916-06W.1	Phellinus noxius	Saipan	Yuko Ota	Delonix regia	gym, dead
S916-06W.2	Phellinus noxius	Saipan	Yuko Ota	Delonix regia	gym, dead
S916-01W	Fulvifomes sp.	Saipan	Yuko Ota	Casuarina	>40yrs
S916-02W	Ganoderma	Saipan	Yuko Ota	Casuarina	host healthy
	gibbosum				
S916-02		Saipan	Yuko Ota	Casuarina	host healthy
S916-07W.1	Phellinus noxius	Saipan	Yuko Ota	Mangifera	dead Kagman
S916-07W.2	Phellinus noxius	Saipan	Yuko Ota	Mangifera	dead Kagman
S916-07C	P. noxius-like culture	Saipan	Yuko Ota	Mangifera	dead Kagman
S917-06W.1		Saipan	Yuko Ota	Delonix regia	dead
S917-06W.2	Phanerochaete	Saipan	Yuko Ota	Delonix regia	dead
	sordida				
\$917-06W.3	Scedosporium sp.	Saipan	Yuko Ota	Delonix regia	dead
S917-06W.4	Scedosporium sp.	Saipan	Yuko Ota	Delonix regia	dead
S917-07W.1	Phellinus noxius	Saipan	Yuko Ota	Delonix regia	living
S917-07W.2	Phellinus noxius	Saipan	Yuko Ota	Delonix regia	living
S917-02		Saipan	Phil Cannon	Thespesia	beach Road
S917-03C		Saipan	Phil Cannon	Thespesia	beach Road
S917-01C.1	Trichoderma sp.	Saipan	Phil Cannon	Casuarina	beach Road
S917-01C.2	Trichoderma sp.	Saipan	Phil Cannon	Casuarina	beach Road
S917-01C.3	Fulvifomes sp.	Saipan	Phil Cannon	Casuarina	beach Road
S917-04		Saipan	Phil Cannon	Casuarina	beach Road
S917-08C		Saipan	Phil Cannon	Delonix regia	dead
Yap 1	Cerrena sp.	Үар	Phil Cannon	Areca	betel nut
				catechu	
Yap 2	Psilocybe sp.	Үар	Phil Cannon	Cocos	leaf spot
Үар ЗА	Phellinus noxius	Үар	Phil Cannon	chestnut	Andrew conk
Yap 3F	Phellinus noxius	Үар	Phil Cannon	chestnut	Andrew conk

Yap 4.1	Lenzites/Trametes	Үар	Phil Cannon	Betel Pep	Chool Village
	sp.?				
Yap 4.2	Fusarium sp.				
Yap 5A	Phellinus noxius	Үар	Phil Cannon	Mangifera	near For. Hdqr.
Yap 5B	Phellinus noxius	Үар	Phil Cannon	Mangifera	near For. Hdqr.
Yap 5C.1		Үар	Phil Cannon	Mangifera	near For. Hdqr.
Yap 5D		Үар	Phil Cannon	Mangifera	near For. Hdqr.
Yap 5E		Үар	Phil Cannon	Mangifera	near For. Hdqr.
Yap 5F2	Phellinus noxius				near For. Hdqr.
P919-08W.1	Rigidoporus vinctus	Pohnpei	Yuko Ota	Artocarpus	healthy
P919-08W.2		Pohnpei	Yuko Ota	A. altilis	healthy
P919-08W.3	Wrightoporia	Pohnpei	Yuko Ota	A. altilis	Healthy
	tropicalis				
P919-08W.4	Phellinus noxius	Pohnpei	Yuko Ota	A. altilis	healthy
P919-08W.7	<i>P. noxius</i> -like culture	Pohnpei	Yuko Ota	A. altilis	healthy
P919-02W.1	Phellinus noxius	Pohnpei	Yuko Ota	Ficus tinct.	Gibson's farm
P919-02W.2		Pohnpei	Yuko Ota	Ficus tinct.	Gibson's farm
P919-02W.4		Pohnpei	Yuko Ota	Ficus tinct.	Gibson's farm
P919-02W.5		Pohnpei	Yuko Ota	Ficus tinct.	Gibson's farm
P919-02W.7	Phellinus noxius	Pohnpei	Yuko Ota	Ficus tinct.	Gibson's farm
	Abundisporus				
P919-02W.10	roseoalbus	Pohnpei	Yuko Ota	Ficus tinct.	Gibson's farm
P919-02W.11		Pohnpei	Yuko Ota	Ficus tinct.	Gibson's farm
P919-02W.12		Pohnpei	Yuko Ota	Ficus tinct.	Gibson's farm
	Wrightoporia				
P919-07W.1	tropicalis	Pohnpei	Yuko Ota	A. altilis	Yellow Kitti
P919-07W.2	Phellinus noxius	Pohnpei	Yuko Ota	A. altilis	Yellow Kitti
P919-07W.4	<i>Fusarium</i> sp.	Pohnpei	Yuko Ota	A. altilis	Yellow Kitti
P919-07W.5	Phellinus noxius	Pohnpei	Yuko Ota	A. altilis	Yellow Kitti
P919-07W.9	Ceriporia lacerata	Pohnpei	Yuko Ota	A. altilis	Yellow Kitti

P919-07W.10		Pohnpei	Yuko Ota	A. altilis	Yellow Kitti
P919-07W.12		Pohnpei	Yuko Ota	A. altilis	Yellow Kitti
P919-07W.13		Pohnpei	Yuko Ota	A. altilis	Yellow Kitti
P919-07W.15	Tinctoporellus?	Pohnpei	Yuko Ota	A. altilis	Yellow Kitti
P919-01W		Pohnpei	Yuko Ota	<i>Cananga</i> sp.	stem canker
	Wrightoporia				
P919-06W1A	tropicalis	Pohnpei	Yuko Ota	stump	<i>P. noxius</i> kill
P919-06W1B	Phlebia sp.?	Pohnpei	Yuko Ota	stump	<i>P. noxius</i> kill
P919-03C		Pohnpei	Yuko Ota	Ficus tinct.	not P. noxius
P919-4C	Earliella scabrosa	Pohnpei	Yuko Ota		Gibson's farm
	Abundisporus				
P919-03C.1	roseoalbus	Pohnpei	Yuko Ota	Ficus tinct.	not <i>P. noxius</i>
Entomopath	Conidiocarpus?	Pohnpei	Engleberger	chili pepper	on insect
Kosrae 1.1	<i>Fusarium</i> sp.	Kosrae	Phil Cannon	A. altilis	Max uncle up
Kosrae 1.2	Rigidoporus vinctus	Kosrae	Phil Cannon	A. altilis	Max uncle up
Kosrae 2	Bacillus sp.	Kosrae	Phil Cannon	A. altilis	Max uncle up
Kosrae 3.1		Kosrae	Phil Cannon	Terminalia	Yela River
Kosrae 2.2		Kosrae	Phil Cannon	Terminalia	Yela infected
Kosrae 4	Phellinus noxius	Kosrae	Phil Cannon	Sonneratia	Conk Walk
Kosrae 5	Ganoderma sp.	Kosrae	Phil Cannon	Terminalia	last landing
Kosrae 6	Phellinus noxius	Kosrae	Phil Cannon	A. altilis	Max uncle low
G914-01	G. australe complex	Guam	Klopfenstein	Casuarina	YIGO Exp.
G914-4	G. australe complex	Guam	Klopfenstein	Casuarina	Watson farm
G914-05	Ganoderma sp.	Guam	Klopfenstein	Casuarina	Watson farm
G914-08	G. australe complex	Guam	Klopfenstein	Casuarina	Watson farm
G914-22		Guam	Klopfenstein	Casuarina	Ritidian
G914-12		Guam	Klopfenstein	Casuarina	3/4dead
G914-13		Guam	Klopfenstein	Casuarina	3/4 dead
G914-10		Guam	Klopfenstein	Neisosperma	? disease
G915-01C/W	Phellinus	Guam	Klopfenstein	Thespesia	#1 sample

G915-04	Perenniporia	Guam	Klopfenstein	Casuarina	fire killed
	tephropora				
Roger	Xylariaceae?	Guam	Roger	Stump	recent cut
			Brown		
UOG1	Trichoderma sp.	Guam	A. Lehman	Guamia sp.	Sample 1
UOG2		Guam	A. Lehman	Guamia sp.	Sample 1
UOG3	Phellinus noxius	Guam	A. Lehman	Macaranga	Sample 2
UOG4		Guam	A. Lehman	Macaranga	Sample 2
UOG5		Guam	A. Lehman	Macaranga	Sample 2
UOG6	Phellinus noxius	Guam	A. Lehman	Guamia sp.	Sample 4
UOG7	Phellinus noxius	Guam	A. Lehman	Guamia sp.	Sample 4
UOG8	Phellinus noxius	Guam	A. Lehman	Guamia sp.	Sample 4
UOG9		Guam	A. Lehman	Macaranga	Sample 5
UOG10		Guam	A. Lehman	Macaranga	Sample 5
UOG11	Phellinus noxius	Guam	A. Lehman	Macaranga	sp 4 FHA
UOG12	Phellinus noxius	Guam	A. Lehman	Macaranga	sp 4 FHA
UOG13	Trichoderma sp.	Guam	A. Lehman	Intsia bijuga	sp 4 FHA
UOG14		Guam	A. Lehman	Guamia sp.	sp 3 FHA
UOG15	Phellinus noxius	Guam	A. Lehman	Guamia sp.	sp 3 FHA
UOG16		Guam	A. Lehman	Guamia sp.	sp3 FHA
UOG17	Phellinus noxius	Guam	A. Lehman	Ochrosia sp.	dead stand
UOG18	Phellinus noxius	Guam	A. Lehman	Ochrosia sp.	dead stand
UOG19	Phellinus noxius	Guam	A. Lehman	Ochrosia sp.	dead stand
UOG20	Phellinus noxius	Guam	A. Lehman	Ochrosia sp.	dead stand
UOG21	Trichoderma sp.	Guam	A. Lehman	Ochrosia sp.	sp 3 FHA
UOG22	Lasiodiplodia	Guam	A. Lehman	Guamia sp.	dead stand
	theobromae				

Discussion and Next Steps

The total of 246 samples collected during this entire trip may seem like a fairly large collection; however, a collection several thousand times larger would easily have been possible if there had been

no focus on which conks were collected and if there had been ample time. The variation/diversity in forest trees, forest environments and hence all of the associated microflora, including these butt-rot fungi, is extremely rich in this part of the world. Towards keeping this collection at a reasonable level, and to make this collection survey as useful as possible for forest health purposes, two criteria were used to decide which samples to collect. First, to be considered for collection, the fungus had to have associations with butt rot and, second, surveys were focused on the potentially invasive *P. noxius*. In addition, a portion of this survey on Guam was devoted to examinations of ironwood (*Casuarina equisetifolia*), which was recently summarized by Schlub (2013). During this survey, we made a collection of almost every putative *P. noxius* infection that we found and made a collection of ca. one of every 500 fructifications of all other conk-forming basidiomycetes that we came across. As a consequence of these selection criteria, this particular fungal sample collection was heavily biased towards *P. noxius*.

On each island, efforts were devoted toward isolating these fungi onto culture media. For conducting fungal isolations, suitable plant pathology laboratories were available at the universities on Guam and Saipan, and the NRCS building in Pohnpei also had ample working space. On Yap, Palau and Kosrae, make-shift isolation facilities were set up in the senior author's hotel room. For the majority of the samples collected, between 2 and 18 isolations were attempted. Many of these isolations produced clean cultures, but some cultures contained contaminate fungi (e.g., *Trichoderma* spp.) that required that re-isolation of the target fungus. As a consequence, identification work for some of these samples continues to be conducted at the Forest Pathology laboratories at the USDA Forest Service – RMRS and at the Forestry and Forest Products Research Institute, Tsukuba Japan.

The surveys of *P. noxius* are an important follow up to previous reports on the Northern Marianna Islands by Hodges and Tenorio (1984). The reason for focusing on *P. noxius* collections for this survey is two-fold. One, because of the high pathogenicity of this fungus, we wanted to be absolutely certain we were identifying it correctly. The second reason is our interest in the genetic diversity among *P. noxius* isolates from diverse regions. To better understand these genetic relationships we plan to design molecular markers (e.g., microsatellites or Restriction-site Associated DNA Sequences, RADSeq) and evaluate genetic relationships among *P. noxius* isolates. Currently, translation elongation factor (*tef*) 1alpha gene sequences are also being evaluated for potential use in phylogenetic studies. Specific genetic data can be useful both for determining gene flow, where an isolate may have come from and genetic

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diversity, which, in some cases, may help to identify especially aggressive strains of this fungus. *Phellinus noxius* is discussed in more detail within the next chapter (Chapter IV).

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