Observations and Pathogenicity Experiments on *Ganoderma zonatum* in Florida

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Ganoderma butt rot of palms, caused by *Ganoderma zonatum*, is a widespread and serious disease in Florida, USA. The means by which the fungus invades healthy palm trunks is not known. However, infection appears to begin at the center of the base of palm trunks and spreads outward and upwards from there, eventually producing a hard, shelf-like structure that is the basidiocarp (conk). Basidiospores produced within the conk may be the primary method for spreading the fungus in the urban landscape. Wilt symptoms are caused by the fungus rotting the xylem, as well as other trunk tissue, in the lowest 1.5 m of trunk. It is believed that most, if not all, woody palms are susceptible to this disease. Experimental attempts to inoculate healthy trees with this fungus have generally been unsuccessful.

Palms are found throughout Florida (USA) in both natural and landscaped settings. They are not grown for agronomic purposes, but are important as ornamental plants. When a list of the top ten diseases of Florida ornamentals was compiled in 1997, Ganoderma butt rot of palms was listed as number two (Schubert et al. 1997). The fungal genus *Ganoderma* is a group of wood-decaying basidiomycete fungi that are found throughout the world on all types of wood – gymnosperms, hard- and softwood dicots, and palms (Gilbertson & Ryvarden 1986). The genus is greatly debated at the species level; Miller et al. (1999) described it as "currently chaotic." This has been due to species names being added and then dropped, an inconsistency in criteria used for identification

1 (above). *Sabal palmetto* (sabal palm) with wilted and desiccated leaves due to *Ganoderma zonatum* infection.

2 (right). *Syagrus romanzoffiana* (queen palm) dying from *Ganoderma zonatum*. Only the spear and one other leaf remain green.

3. Basidiocarp (conk) of *Ganoderma zonatum*. Note glazed reddish-brown top surface and white undersurface. The "straight" side of the conk is directly attached to the trunk. There is no "stem" or "stalk" that attaches the conk to the trunk.

purposes at the species level, and the fact that morphological characteristics change based on environment (Moncalvo et al. 1995).

In Florida, Ganoderma butt rot is a lethal disease that appears to be caused by the fungus *Ganoderma zonatum* Murrill. Another fungal name that was associated with this disease in the first half of the 20th century was *Ganoderma sulcatum* Murrill (Murrill 1902, 1908; Childs & West 1953). Murrill indicated in his papers that both species were associated with dead wood in Florida, and that they were very similar in appearance. Recently, these two species have been grouped together as one, *G. zonatum* (Gilbertson & Ryvarden 1986). Although *G. zonatum* is often referred to as a member of the *G. lucidum* complex, it is a distinct species from *G. lucidum* (Adaskaveg & Gilbertson 1989). Information is available regarding the morphological and cultural characteristics of *G. zonatum* (e.g., Adaskaveg and Gilbertson 1989; Adaskaveg and Gilbertson 1988; Gilbertson & Ryvarden 1986), but little is known about the disease it causes. A similar basal stem rot of oil palms is widespread, but is caused by a different *Ganoderma* species, presumed to be *G. boninense* (Miller et al. 1999). This pathogen does not occur in North America. Considerable information is available on this disease in Southeast Asia, so we have relied heavily on this information as a basis for initiating research in Florida. This article is a compilation of our personal observations and pathogenicity research results obtained since 1994.

I. OBSERVATIONS

Symptoms and Signs

Ganoderma zonatum is a white rot fungus that produces numerous enzymes that allow it to degrade (rot) woody tissue, primarily lignin and cellulose (Adaskaveg et al. 1991). As the fungus destroys the palm wood internally, the xylem (water-conducting tissue) will eventually be affected. Therefore, the primary symptom that may be observed is a wilting, mild to severe, of all leaves but the spear leaf (Figs. 1 and 2). Other symptoms can best be described as a general decline – slower growth and off-color foliage. However, these symptoms alone should not be used for diagnosis of Ganoderma butt rot, since other disorders or diseases may also cause these symptoms. Only when these symptoms are accompanied by the development of the basidiocarp (conk) (Fig. 3) can the palm be confirmed as affected by *G. zonatum*. Also, it is commonly observed that basidiocarps will form prior to any obvious wilting or decline symptoms.

It should be emphasized that this fungus is not restricted to vascular tissue. The palm wilts because the vascular tissue as well as the surrounding tissue has been degraded by the fungus. This becomes evident when a palm that has died or is dying from Ganoderma butt rot is cut down and cross-sections made of the trunk. Figs 4 and 5 are examples of the wood rotting and disease progression pattern observed. The fungus colonizes and degrades the palm trunk tissue closest to the soil line first, expands in diameter at the base and moves up the center or near-center of the trunk. Therefore, the disease progression pattern within the trunk is best described as cone-shaped, widest at the soil line and narrowing to a pinpoint. In general, we do not see the fungus extend more than 1.5 m (~5 ft) up into the palm trunk. Although roots may eventually rot, especially after the palm dies, the lethal damage from the fungus appears to be associated with the trunk degradation. Ganoderma butt rot is a disease of mature palms (i.e., palms with trunks) and has not been observed to affect seedling or juvenile palms in natural or landscape settings.

It was previously thought that the location of the initial basidiocarp formation was the entry point for the fungus, perhaps from a wound to the trunk. We do not believe that is true. The location of the basidiocarp is where the fungus is <u>emerging</u> from the trunk. This means the degradation is occurring internally as the fungus moves from the lower center of the palm to the outside. This is especially evident when a palm suspected of being infected with *G. zonatum*, based on wilt symptoms and proximity to other diseased palms, but without basidiocarps is examined. This means that wounds are <u>not</u> a likely factor in disease initiation. Other external environmental factors associated with the trunk are probably not associated with disease development either, such as too much mulch around the trunk, irrigation heads striking the trunk, flowers or shrubs too close to the trunk, or painting the trunk.

Life Cycle of the Fungus

The basidiocarp or conk is the most easily identifiable structure associated with the fungus. The conk is a specialized mass of fungal growth that originates from mycelia in the palm trunk. Fig. 6 illustrates different stages in the development of the basidiocarp. When the fungus first starts to grow on the side of a palm trunk or palm stump, it is a solid white mass that is relatively soft when touched. It will have an irregular to circular shape and is relatively flat on the trunk or stump.

As the conk matures, a small shelf or bracket will start to form as the basidiocarp begins to protrude from the trunk. It will still be white, both on the

Table 1. Palms documented to have been infected with *Ganoderma zonatum*.

Palm	Source[z]
Acoelorraphe wrightii	FTG
Acrocomia aculeata	FTG
Adonidia merrillii	FTG
Aiphanes sp.	DPI
Arenga engleri	FTG
Arenga tremula	FTG
Arenga undulatifolia	FTG
Attalea sp.	FTG
Bactris major	FTG
Brahea berlandieri	FTG
Brahea brandegeei	FTG
Brahea dulcis	FTG
Brahea edulis × brandegeei	FTG
Butia eriospatha	FTG
Butia capitata	DPI
Carpentaria acuminata	FLREC
Caryota mitis	DPI
Chamaerops humilis	FTG
Coccothrinax sp.	FTG
Cocos nucifera	DPI
Copernicia curtisii	FTG
Dictyosperma album	DPI
Dypsis cabadae	FTG
Dypsis lutescens	DPI, FTG
Elaeis guineensis	TREC
Euterpe edulis	DPI
Gastrococos crispa	FTG
Hyophorbe indica	TREC
Livistona benthamii	FTG
Livistona chinensis	DPI, FTG
Livistona merrillii	FTG
Livistona muelleri	FTG
Livistona saribus	Forestry
Nannorrhops ritchiana	FTG
Phoenix canariensis	DPI, FTG
Phoenix dactylifera	FLREC
Phoenix reclinata	DPI, FTG
Phoenix roebelenii	FTG
Phoenix sylvestris	FLREC
Ptychosperma elegans	TREC
Ptychosperma macarthurii	DPI, FTG
Ptychosperma salomonense	FTG
Roystonea altissima	FTG
Roystonea oleracea	FTG
Roystonea regia	DPI, FTG
Sabal causiarum	FTG, TREC
Sabal mauritiiformis	FTG
Sabal palmetto	DPI, FLREC, FTG
Sabal uresana	FTG
Satakentia liukiuensis	FLREC
Serenoa repens	FTG
Syagrus oleracea	FTG
Syagrus picrophylla	FTG
Syagrus romanzoffiana	DPI, FLREC, FTG
Syagrus sancona	FTG
Syagrus schizophylla	FTG
Syagrus × costae	FTG
Washingtonia robusta	DPI, FTG

[z]DPI = *Diseases and Disorders of Plants in Florida* (Alfieri et al., 1994); FTG = Fairchild Tropical Garden, Miami, FL; TREC = University of Florida's Tropical Research and Education Center, Homestead, FL; FLREC = University of Florida's Fort Lauderdale Research and Education Center, Fort Lauderdale, FL; Forestry = Florida's Department of Forestry, Miami, FL.

top and bottom surfaces. Eventually, it will form a very distinct shelf-like structure that is quite hard with a glazed reddish-brown top surface and a white undersurface (Fig. 3). A mature conk will have distinct zones, hence the name *G. zonatum*. The basidiocarp will have a half-moon shape with the "straight" side directly attached to the trunk. This is referred to as a sessile basidiocarp as opposed to one that has a stalk or stipe (Figs 3 and 6). Conks can be up to 20 cm (8 in) at their widest point and 5 cm (2 in) thick. Microscopic basidiospores will be produced in the "pores" present on the underside of the conk. When basidiospores are dropped en mass on a white surface, they will appear brownish-red in color.

The conk is considered an annual conk. Once it stops growing, it does not begin growing and expanding in size at a later date. In southern, subtropical Florida, conks can be produced throughout the year. In northern, temperate Florida, conks are normally only produced during the warmer months of May through October. We have observed that mature, viable basidiospores are produced very early in the formation of the basidiocarp. Spores were produced shortly after the conk began to protrude (form a shelf) from the trunk, while it was still white, and long before the top turned a reddish-brown color. We believe that the spores are the primary means of spread of the fungus, but that has yet to be proven. Recent research with *Ganoderma* in Malaysia determined that *G. boninense* may spread in oil palm plantings through basidiospore dispersal or through contact with long-term residual inoculum (mycelia, etc.) in soil debris (Miller et al. 1999). The genetic

Fig. 4 (top). Cross-sections of lower trunk of *Syagrus romanzoffiana* infested with *Ganoderma zonatum*. Top-left section is bottom section (section 1) and remaining sections progress up the trunk. Note darkening of wood due to fungal degradation (rot). Fig. 5 (bottom). Sections 5 and 6 of Fig. 4 (bottom row, middle) after incubation in a moist chamber for 4 days. White growth is mycelia of *Ganoderma zonatum* and corresponds with the discolored area of the cross-sections.

Fig. 6 (top). Three phases of basidiocarp (conk) development of *Ganoderma zonatum*. The white "button" near the top of picture is the beginning stage of the conk. The lower-right structure is a mature conk. The lower-left structure is also a mature conk, but it is an old one; the underside of the conk is no longer white. Fig. 7 (bottom). Cut palm stump with numerous basidiocarps of *Ganoderma zonatum* forming on it.

information obtained by this research group suggests that the fungus does not spread by direct root-to-root contact between palms.

The diversity and isolation of locations in Florida where Ganoderma butt rot has occurred would support the concept of spores as a primary method of spread. Palms are not a plantation crop in Florida, but are found in two distinct settings. One would include natural groves of non-transplanted sabal palms (*Sabal palmetto*). The other is the urban landscape, where the majority of palms are transplanted from either natural settings in Florida, date groves from south-western USA, or plant nurseries. In urbanized southern Florida, the "soil" upon which developments are built, and in which palms are transplanted, is dredged from man-made lakes. It is devoid of any possible plant debris harboring *G. zonatum*. However, only genetic studies will definitively demonstrate how *G. zonatum* spreads.

After spores are released, it is unclear exactly what happens next. We do not know if the spores become dormant in nature. In a laboratory setting, the spores do not show any dormancy and germinate readily on media after being dropped from the basidiocarp. However, in the real world, they may indeed become dormant or need specific substrates, temperature, relative humidity and so forth for germination to occur. We do know that at some point in time multiple spores do germinate, mate and produce mycelia that will go through three distinct stages of development – primary, secondary and tertiary. The basidiocarp or conk is composed of the tertiary mycelia.

Host and Geographic Range

In the *Diseases and Disorders of Plants in Florida* (Alfieri et al. 1994), five non-palm species are listed under *G. zonatum*. It is quite possible that *G. zonatum* was not a pathogen of these non-palm species, but rather a saprobe that grew on the wood after the tree had died. According to Adaskaveg et al. (1991), the only authenticated reports of *G. zonatum* are from palms. However, there has been one report in Florida of *G. zonatum* associated with a cycad. Still, palms should be considered the primary hosts of this fungus. Table 1 lists all of the palms known to have died from Ganoderma butt rot in Florida. This list was compiled with information obtained from numerous sources in Florida, as indicated in the table. Although not all palm species known to be growing in Florida have succumbed to this disease, the majority of those species not on this list are relatively uncommon in Florida. Therefore, just because a palm is not listed in this table, it is not safe to assume that the palm is resistant to the disease. In fact, it is probably more logical to assume that all palms are susceptible to *G. zonatum*. The only possible exceptions would be palm species that do not form woody trunks – e.g., *Sabal minor*, some *Chamaedorea* spp. Since *G. zonatum* kills by degrading wood, these palm species may not have any suitable tissue to serve as a substrate for the fungus.

The distribution of *G. zonatum* in Florida is shown in Fig. 8. This information was obtained from many of the same sources as listed for the palm host range and by contacting University of Florida Cooperative Extension Service faculty in each county. While the disease is more prevalent in the southern half of the state where palms are in greatest abundance, it is certainly not restricted to that area.

Environments or Practices that Encourage Disease Development

To date, we have observed no common environmental conditions or landscape management practices that favor the development of Ganoderma butt rot. The disease has been observed in natural settings (palms never transplanted) and in highly-maintained, transplanted landscapes. It has been observed on palms that have been maintained very well nutritionally (no nutrient deficiencies) and on palms that were severely stressed by deficiencies. The disease has been observed in well-drained settings and in swamps. The fungus has killed trees that had no apparent mechanical injuries and those that had been severely damaged by weed trimmers. Soil type appears to have no relationship with disease either as diseased palms have been observed on deep sands (both silica and calcareous), muck (peat), and limestone rock. There has been no simple discernible pattern to provide clues as to why palms become infected and die from *G. zonatum*.

Management Considerations

In general, the fungus will be located in the lower 1.5 m of trunk. This has two implications. First, this means that only this lower trunk portion should not be chipped and used for mulch. If possible, the diseased section should be placed in a legal landfill or incinerated. The remaining portion of the palm trunk could be immediately chipped and used for mulch in the landscape.

Secondly, only the lower 1.5 m of trunk will need to be protected from the fungus. However, typical xylem-limited, systemic fungicides will not be effective unless they are capable of spreading beyond the vascular tissue and protecting all the wood in the lower portion of the trunk. No fungicide will be effective once the basidiocarps have formed, since a large percentage of the trunk is already destroyed. Since we have no means of predicting or determining which palms are infected with *G. zonatum*, this effectively eliminates the use of fungicides as a control method, either preventively or curatively, for the present time.

Since basidiospores are probably the primary method of spreading the fungus, we urge people to monitor their palms closely, especially after a palm has died or been removed for any reason. The fungus will readily colonize and degrade palm stumps or dead palm trunks (Fig. 7). Once it becomes established on this dead wood, it will normally produce basidiocarps with millions of basidiospores. We had never observed *G. zonatum* as a pathogen on the Fort Lauderdale Research and Education Center (FLREC) property until March 1993 when it first killed a *Sabal palmetto*. Interestingly, this palm was only ~ 30 cm away from a sabal palm stump, left when a healthy palm had been cut down 5 years earlier for research purposes. However, we had observed *G. zonatum* basidiocarps on this stump as early as August 1991. More recently at the FLREC, we observed *G. zonatum* basidiocarps on dead *Carpentaria acuminata* trunks. These palms had died from other causes. We also noticed that there was a *Carpentaria* palm in this same planting, ~ 1 m away, that appeared wilted. When it was cut down, the center of the basal trunk area was already rotted from *G. zonatum*. We have also observed and been told on numerous occasions of Ganoderma butt rot occurring on *Dypsis lutescens* (areca palm) after some of the mature canes had been removed. In all probability, the fungus moves in on the dead stumps of these palms and spreads from there to living canes.

Therefore, monitor your palms in Florida, and other areas of the south-eastern USA where the fungus has been documented, for the basidiocarps. Remove the basidiocarp (conk) and place in a trash receptacle that will be incinerated or delivered to a landfill. Do not place in trash that will be recycled in the landscape. The earlier the basidiocarp is removed (i.e., before it becomes a distinct shelf-like structure), the less likely that spores will be released into the environment. If you have never observed Ganoderma butt rot on the property, monitoring the palms once every six months will be adequate. Once you have observed the basidiocarps on palms or have a palm cut or fall down for any reason, monitor the palms at least once a month.

It has been observed repeatedly that a replacement palm planted into the same site where a palm died from Ganoderma butt rot also eventually succumbs to this disease. This would indicate that the fungus does survive in the soil, either as infected plant material or spores. Therefore, it is never recommended to plant another palm in the same location as the previous *Ganoderma*-infested palm. In general, no other plant tree species grown in Florida (pines, oaks, woody shrubs, etc.) are affected by *G. zonatum*. Therefore, these would be acceptable replacements. We have no definitive information on the length of time for *G. zonatum* survival in the soil, but it should be measured in multiple years, not months. It may be as long as 10 to 30 years. It is certainly not less.

If an infested site must be replanted with a palm, follow these guidelines. Remove the stump and all roots from the site, and then fumigate the soil. This can be done by a professional pesticide applicator using a legally registered fumigant for the landscape. An example would be the product dazomet (trade name = Basamid). If the palm was located in a site surrounded by concrete (e.g., a street median), remove all of the old soil, bring in new soil and then fumigate. However, none of these procedures guarantees the new palms will remain free of *Ganoderma zonatum*.

II. RESEARCH

Introduction

Although it may appear obvious that *G. zonatum* is a pathogen of palms in Florida, a fungal pathologist would like to have this confirmed. The term used for the process that confirms pathogenicity is "Koch's Postulates." This requires isolating the fungus from a host with typical symptoms, obtaining a pure culture of the fungus, using this culture to inoculate a healthy host, and then observing the development of typical symptoms and re-isolating the same fungus. While this may be a simple task when dealing with small annual plants, it is not simple when dealing with mature palms. Furthermore, we would like to have a system that would consistently provide us with a method for artificially inducing the disease in healthy palms in order to conduct experiments relative to control tactics, fungal life cycle and disease cycle.

Researchers working with the *Ganoderma* disease on oil palms in Southeast Asia have examined pathogenicity of *G. boninense* using *Elaeis guineensis* (African oil palm) seedlings. Both natural inoculum (Navaratnam & Leong 1965) and artificial inoculum (Hashim et al. 1993; Sariah et al. 1994) were used and were successful in inducing disease. However, as Hashim et al. (1993) point out, reproducing these experiments has not always been possible.

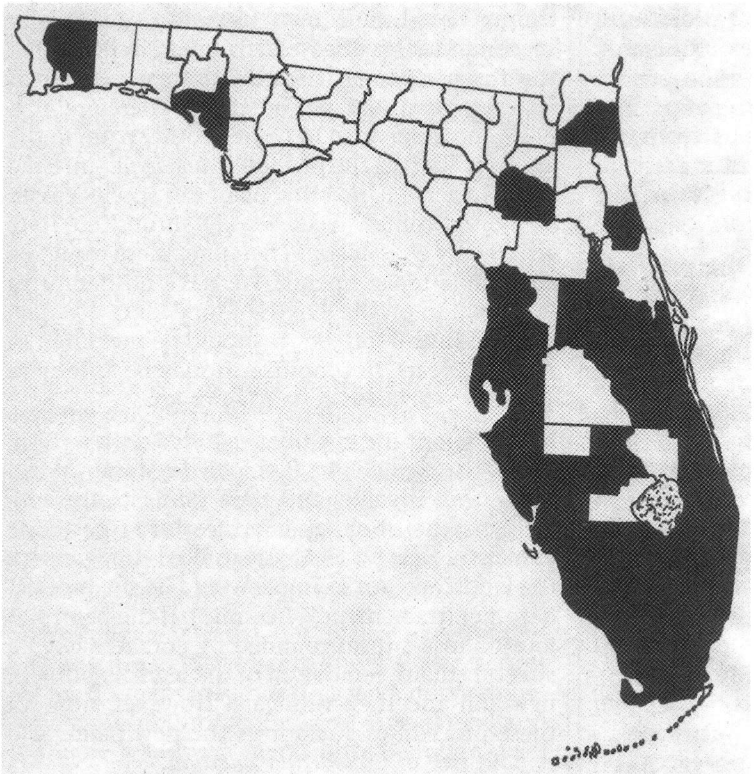

Fig. 8. This map illustrates those Florida counties where Ganoderma butt rot, caused by *Ganoderma zonatum*, has been reported on palms. Information was obtained from the Florida Department of Plant Industry, University of Florida's Plant Disease Clinics and County Extension Faculty.

Materials and Methods

Field experiments with natural inoculum and mature palms

A *Syagrus romanzoffiana* (queen palm) with Ganoderma butt rot was obtained in Palm Beach County, Florida, USA. This palm had basidiocarps present on the lower trunk that were confirmed as *G. zonatum*. The lower portion of the trunk was rotted. This section (~ 0.5 m) was cut into 10–15 cm cross-sections with a chainsaw, and then broken into different size pieces (chunks, splinters, etc.) by hand. These pieces were mixed with the sawdust in a large garbage can and the lid replaced. This inoculum remained at air temperature in a shade-house for 14 days. A pure culture of the pathogen was obtained from the basidiocarp and the wood.

A block of 28 *Syagrus romanzoffiana*, 20–25 years old, were used for the experiment. Half of the palms were transplanted on 28 September 1994, on 6 m centers, to a site adjacent to the original block. Half (7) of each palm group, transplanted vs. non-transplanted, was inoculated with the infested palm trunk material on 10 October 1994. The inoculum was divided into 14 equal portions (~ 2 L volume per tree). For each inoculated transplanted palm, 3 equidistant holes were dug 15 cm from the trunk base and about 8–10 cm deep (until roots were contacted). The inoculum was divided among the holes and recovered with soil. For inoculated non-transplanted palms, the inoculation procedure was similar except that we avoided contacting or damaging any roots. The non-inoculated palms were not disturbed. Transplanted palms were maintained using standard procedures. Once they were established, no extra care (fertilizer or water) was provided these palms or the non-transplanted palms.

Shade-house experiments with natural and artificial inoculum and seedling palms

Two experiments were initiated to determine the best method for inducing disease by *G. zonatum* on seedling palms. The use of artificial and natural inoculum was examined as well as size, amount and location of inoculum. These experiments were initiated in June 1995.

For Experiment I, three palm species in the seedling phase, *S. romanzoffiana* (queen palm), *Phoenix roebelenii* (pygmy date palm), and *Elaeis guineenis* (African oil palm), were inoculated with either artificial or natural inoculum. Artificial inoculum was made following the method of Sariah et al. (1994). Two isolates of *G. zonatum* were used, one from *P. reclinata* (GAN-9) and the other from *S. romanzoffiana* (GAN-10). *Bischofia javanica* wood was obtained and cut into 5 × 7.5

cm pieces, with the bark removed. These pieces were sterilized for 90 min. Individual pieces were then placed separately in heat-sealable pouches and sterilized again for 90 min. Three days later, 25 ml of sterile, cooled, but not yet solidified, malt extract agar (1.5% malt extract plus 2% Bacto agar) was added to each pouch. Pouches were rotated to evenly coat the wood pieces as the agar solidified. The next day half the pouches were inoculated with GAN-9 and the other half with GAN-10. The *G. zonatum* isolates had been grown on potato dextrose agar in petri plates for 15 days. Fungal growth in each plate was cut into 5 × 5 mm pieces, and one-quarter of the pieces were added to each pouch. Pouches were incubated at 28–32 C for 4 weeks. Natural inoculum was obtained from a *G. zonatum*-infested *Sabal palmetto* trunk. The wood was cut into 12.5 × 25 × 50 mm pieces.

The *E. guineenis* and *S. romanzoffiana* seedlings were transplanted from 1 L containers into 10 L plastic containers, while the *P. roebelenii* palms were transplanted into 6 L plastic containers. The potting substrate was 50% pine bark, 40% sedge peat and 10% sand. Roots were separated and as much potting mix as possible was removed prior to transplanting into containers with inoculum. All three palm species had the following treatments: 1) artificial inoculum infested with GAN-9, placed below the root ball; 2) artificial inoculum infested with GAN-10, placed below the root ball; 3) natural inoculum placed below the root ball; 4) natural inoculum placed on top of the root ball; 5) uninfested *Bischofia* wood placed on top of root ball; 6) uninfested palm pieces placed on top of the root ball; and 7) no wood pieces. Only one piece of artificial inoculum was placed in the container. Twenty pieces of natural inoculum were placed in each container to obtain a total inoculum volume of 15.5 cm^3. There were 10 replicate containers for each inoculum × palm species treatment.

Palms were examined at 6-month intervals for visible symptoms or signs of the disease. After 19 months, roots and crowns of *P. roebelenii* and *S. romanzoffiana* from each treatment were examined, two replicates per treatment. After visual examination, roots were cut from the crown, washed, surface sterilized, blotted dry and placed on a semi-selective medium for *G. zonatum*.

For Experiment II, five different size pieces of natural inoculum were produced from a *G. zonatum*-infested *Sabal palmetto* trunk: a) 50 × 50 × 50 mm; b) 25 × 25 × 50 mm; c) 12.5 × 25 × 50 mm; d) 12.5 × 25 × 25-mm; and e) 6.25 × 25 × 25 mm. Only *P. roebelenii* palms were used with the same potting substrate as described previously. There were 13 treatments. Each size of inoculum was used as either a single piece per container or as multiple pieces per container to obtain an inoculum volume of 125 cm^3. The remaining 3 treatments were controls with either no inoculum, 1 piece of uninfested palm wood (50 × 50 × 50 mm), or 8 pieces of uninfested palm wood (12.5 × 25 × 25 mm for a 125 cm^3 volume). There were 10 replicate containers for each inoculum volume x size treatment.

Palms were examined at 6-month intervals for visible symptoms or signs of disease. After 24 months, inoculum pieces that were still present were removed, scrubbed clean, surface sterilized, blotted dry and placed on a semi-selective medium for *G. zonatum*.

Shade-house experiment with natural and artificial inoculum and mature or juvenile palms

For Experiment III, nine palm species were transplanted into 38 L plastic containers using the same potting substrate as described previously. Holes, 0.5 cm diam. × 5 cm deep, were drilled into the palm trunk or stem at the soil line (root-trunk interface) or 15 cm above the soil surface. The five *G. zonatum* inoculum treatments were: 1) no inoculum; 2) 1 cm^3 of infested palm wood from *Sabal causiarum*; 3) 1 cm^3 of oat kernel inoculum, made by growing *G. zonatum* on sterilized oat kernels for 4 weeks; 4) 1 cm^3 of basidiospores; and 5) 1 cm^3 of basidiocarp tissue. The palms species were *Phoenix roebelenii, Roystonea regia, Dypsis lutescens, Archontophoenix alexandrae, Veitchia arecina, Ptychosperma elegans, Thrinax radiata, Syagrus amara*, and *Ravenea rivularis*. The *P. roebelenii* palms were mature (9-yr old) and had developed at least 90 cm of clear trunk. The *A. alexandrae, V. arecina*, and *P. elegans* had begun to form a woody trunk, but all other species were without trunk tissue at the time of inoculation. There were six replicates of each palm species × hole location × inoculum type treatment. Palms were examined at 6 month intervals for visible symptoms or signs of disease.

Results

Field experiments with natural inoculum

After 24 months, we observed that two of the seven inoculated, transplanted *Syagrus romanzoffiana* had basidiocarps present on their trunks. Until this point, all of the palms had a similar appearance. Basidiocarps were removed and confirmed as *G. zonatum*. Pure cultures of the pathogen were obtained. These palms were removed with as many roots as possible and replaced with *S. romanzoffiana* juveniles. To date, no other palms in this experiment have died or produced basidiocarps of *G. zonatum*.

Shade-house experiments

No palm was killed by *G. zonatum* or ever exhibited symptoms of the disease in any of the three experiments. The fungus was not detected in the roots examined in Experiment I, nor was it detected in the inoculum examined in Experiment II. We observed that the center of the *Bischofia* wood pieces used in Experiment I were completely degraded leaving a hollow core.

Discussion

The only time Ganoderma butt rot was induced was when natural inoculum was used on mature palms. Even then, only 2 of 14 inoculated palms exhibited symptoms and signs (basidiocarps) of the fungus. However, inoculation of field-grown palms is not a good system for completing Koch's postulates. First, working with palms with tall trunks is difficult and expensive. Second, there is no way of definitely determining if these palms were already infected with *G. zonatum*. Our attempts to use seedling palms (i.e., palms without woody trunk tissue) for pathogenicity assays were not successful, whether we used artificial or natural inoculum. We also saw no evidence of root rot or root infection as has been observed with oil palm seedlings inoculated with *G. boninense* (Navaratnam & Leong 1965; Hashim et al. 1993; Sariah et al. 1994). Even the experiments that inoculated mature *Phoenix roebelenii* and various species of juvenile palms with different forms of inoculum placed directly into the trunks were unsuccessful in inducing the disease. However, the observed infection of clumping palms (e.g., *Dypsis lutescens*) that have mature canes removed, the infection of two of the seven transplanted *Syagrus romanzoffiana*, and the pattern of disease development within the trunk suggests that *G. zonatum* probably enters palm trunks via dead palm tissue (dead roots or palm stumps) as a saprophyte and spreads from there into adjacent living woody trunk tissue. Additional experiments are planned to test this hypothesis.

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LITERATURE CITED

ADASKAVEG, J.E. AND R.L. GILBERTSON. 1988. Basidiospores, pilocystidia, and other basidiocarp characters in several species of the *Ganoderma lucidum* complex. Mycologia 80: 493–507.

ADASKAVEG, J.E. AND R.L. GILBERTSON. 1989. Cultural studies of four North American species in the *Ganoderma lucidum* complex with comparisons to *G. lucidum* and *G. tsugae*. Mycological Research 92: 182–191.

ADASKAVEG, J.E., R.A. BLANCHETTE AND R.L. GILBERTSON. 1991. Decay of date palm wood by white-rot and brown-rot fungi. Canadian Journal of Botany 69: 615–629.

ALFIERI JR., S.A., K.R. LANGDON, J.W. KIMBROUGH, N.E. EL-GHOLL AND C. WEHLBURG. 1994. Diseases and disorders of plants in Florida. Bulletin No. 14, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida, USA.

CHILDS, J.F.L. AND E. WEST. 1953. Butt rot of queen palms in Florida associated with *Ganoderma sulcatum*. Plant Disease Reporter 37: 632–633.

GILBERTSON, R.L., AND L. RYVARDEN. 1986. North American Polypores. Volume 1. *Abortiporus – Lindtneria*. Fungiflora, Oslo, Norway.

HASHIM, K., L.T. KWEE AND A.R.A. RAZAK. 1993. Pathogenicity of *Ganoderma boninense* Pat. on oil palm seedlings. In Proceedings 1991 PORIM International Palm Oil Conference – Agriculture (Module I), 418–423. Palm Oil Research Institute of Malaysia, Selangor, Malaysia.

MILLER, R.N.G., M. HOLDERNESS, P.D. BRIDGE, G.F. CHUNG AND M.H. ZAKARIA. 1999. Genetic diversity of *Ganoderma* in oil palm plantings. Plant Pathology 48: 595–603.

MONCALVO, J.-M., H.-H. WANG AND R.-S. HSEU. 1995. Phylogenic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. Mycologia 87: 223–238.

MURRILL, W.A. 1902. The polyporaceae of North America. I. The genus *Ganoderma*. Torrey Botanical Club Bulletin 29: 599–608.

MURRILL, W.A. 1908. *Ganoderma*. North American Flora 9: 118–123.

NAVARATNAM, S.J. AND C.K. LEONG. 1965. Root inoculation of oil palm seedlings with *Ganoderma* sp. Plant Disease Reporter 49: 1011–1013.

SARIAH, M., M.Z HUSSIN, R.N.G. MILLER AND M. HOLDERNESS. 1994. Pathogenicity of *Ganoderma boninense* tested by inoculation of oil palm seedlings. Plant Pathology 43: 507–510.

SCHUBERT, T.S., W.N DIXON, AND R. LEAHY. 1997. Florida's most wanted top 10 diseases. Ornamental Outlook 6(8): 10.