Pathogenicity of *Ganoderma* Species on Landscape Trees in the Southeastern United States

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Abstract

The genus *Ganoderma* contains species that are associated with dead and declining host trees. Many species have been described as pathogens in literature, because anecdotally, the presence of fruiting bodies on living trees has been widely associated with a general decline in tree health. Few studies have investigated the pathogenicity of *Ganoderma* species on landscape trees in the southeastern U.S. Pathogenicity tests were used to determine the pathogenicity of *G. curtisii*, *G. meredithiae*, *G. sessile*, and *G. zonatum* on young, healthy landscape trees (*Pinus elliottii* var. *elliottii*, *P. taeda*, *Quercus shumardii*, *Q. virginiana*, and *Butia odorata*) common to the southeastern U.S. Inoculations were made by drilling into the sapwood of the lower bole and inserting wooden dowels that were infested with each *Ganoderma* species. In two field experiments, 11 to 12 months post inoculation, trees had no visual, external symptoms of disease. There were differences in the extent of internal xylem discoloration near the site of inoculation in comparison with the mock-inoculated control in experiment 1, but there were no differences relative to the control in experiment 2. In both experiments, *G. sessile* was the only species that was successfully reisolated from the pine and oak hosts. Although disease symptoms were not obvious, the reisolation of *G. sessile* outside the inoculation point was a significant finding, and suggests that this species was capable of infecting healthy sapwood. *G. sessile* constitutively produces chlamydospores within its vegetative mycelium, which may contribute to its persistence in the discolored sapwood. These data suggest that the *Ganoderma* species tested, following trunk wounding, are not pathogens of young, actively growing landscape trees that only possess sapwood. The establishment of these fungi using alternative infection courts (e.g., roots) and their interactions in older living trees (e.g., trees with heartwood) needs investigation to better understand their effects on tree health.

Ganoderma is a large, diverse, globally distributed genus of the Basidiomycota that contains species that cause a white rot on a variety of tree species. The taxonomy of the laccate (varnished or polished) *Ganoderma* species is confusing, and for the past century, a majority of authors that have reported findings on *Ganoderma* in the United States have used the name *G. lucidum sensu lato* for any laccate *Ganoderma* species growing on hardwood trees (Adaskaveg and Gilbertson 1987; Gilbertson and Ryvarden 1986; Pirone 1957; Zhou et al. 2015). It is now known through phylogenetic studies that *G. lucidum sensu stricto* (Curtis) Karst is native to Europe and parts of China (Hennicke et al. 2016; Zhou et al. 2015), but not North America. In North America, the *G. lucidum sensu lato* species complex consists of many species that are currently being elucidated using molecular phylogenetic methods (Hong and Jung 2004; Moncalvo et al. 1995; Wang et al. 2012; Zhou et al. 2015). Due to these taxonomic revisions, studies that have focused on the ecology and general biology of *Ganoderma* species in North America need to be reevaluated. It is currently unclear which *Ganoderma* species were examined in many earlier studies, and whether conclusions drawn from studies on *G. lucidum sensu lato* can be applied across all *Ganoderma* species formerly included under this name.

White rot fungi, including species of *Ganoderma*, possess enzymes that break down cellulose, hemiceullulose, and lignin, the major structural components of wood (Schwarze et al. 2000). Species of *Ganoderma* are capable of both simultaneous decay, the process where cellulose and lignin are gradually decayed over time, and selective delignification, where lignin is preferentially decayed over cellulose (Blanchette 1984). Previous studies have suggested that trees with simultaneous decay have more wood strength loss and are more concerning for tree safety compared with trees where fungi have caused selective delignification (Schwarze and Ferner 2003). While *Ganoderma* species can be found on living trees, infected trees often die due to windthrow or breaks caused by a loss of strength and rigidity due to decay of the cellulose and lignin of the lower bole or root flare (Sinclair and Lyon 2005). Because of this, when *Ganoderma* fruiting bodies are found on living trees, these trees are often deemed hazardous and removed, preemptively, without doing other advanced assessments of decay such as sounding, resistance drilling, and sonic tomography etc.

Numerous studies have reported *Ganoderma* as a causal agent of disease, associated with death and a general decline, in various tree species (Adaskaveg et al. 1991, 1993; Bhadra 2015; Coetzee et al. 2015; Elshafie et al. 2013; Fernando 2008; Glen et al. 2009; Mahmood 1970; Toole 1966), while fewer studies have successfully completed Koch's postulates to prove the causality of *Ganoderma* in tree decline (Adaskaveg and Gilbertson 1987; Deflorio et al. 2008; Elliott and Broschat 2001; Hidayati et al. 2014; Nicolotti et al. 2004; Pirone 1957; Toole 1966). For example, Nicolotti et al. (2004) performed pathogenicity tests with *G. adspersum* on young linden (*Tilia* sp.) and horse-chestnut (*Aesculus* sp.) in Italy, where *G. adspersum* was inoculated into drilled holes in the lower bole. Two years post-inoculation, decay columns were observed originating from the inoculation sites on all trees inoculated with this method, and the pathogen was reisolated or confirmed with a molecular method. Similarly, in New York, Pirone (1957) conducted pathogenicity tests where infested agar plugs or pieces of fruiting body tissue of *G. lucidum sensu lato* were placed into holes drilled into lower trunk tissue of young *Acer platanoides* (Norway maple), and in both cases, infection occurred and symptoms were observed. On the contrary, although *G. zonatum* is considered a destructive palm pathogen (Elliott and Broschat 2001), no infections or disease were observed when palms were inoculated with *G. zonatum* in Florida, despite the authors using several different inoculation methods.

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The current paradigm of the disease life cycle of Ganoderma butt and root rot of living trees is that basidiospores (primary inoculum) initiate infections in wounds of roots of the lower boles of trees (infection courts) and cause decay, which leads to a general decline in tree health (Sinclair and Lyon 2005). Recent surveys of basidiomycete endophytes in trees have revealed that wood decay fungi, including *Ganoderma* species, can be found in sound wood of living trees presumably living as endophytes, which adds another putative step to the general life cycle of this potential root and butt rot pathogen (Martin et al. 2015; Parfitt et al. 2010; Song et al. 2017). Infection and establishment of a given *Ganoderma* species on a particular host appears to be somewhat host-group specific, wherein specific *Ganoderma* species are found predominately on certain groups of hosts (i.e., conifers, hardwoods, or palms) (Gilbertson and Ryvarden 1986). For example, *G. zonatum* is associated with palms, *G. meredithiae* is associated with pines, and *G. curtisii* is associated with hardwoods (Adaskaveg and Gilbertson 1988; Elliott and Broschat 2000; Gilbertson and Ryvarden 1986).

Tree species have evolved mechanisms of chemical and physical resistance to pathogenic decay organisms in living sapwood (Adaskaveg et al. 1991; Baietto and Wilson 2010; Scheffer and Cowling 1966). Living trees can actively compartmentalize infections and wounds. Active compartmentalization can vary among tree species and within individual tree species across different environmental conditions (Boddy and Rayner 1983; Shigo and Hillis 1973). Furthermore, trees respond to wounds or infections in sapwood by producing antimicrobial chemicals such as resins, tannins, and phenols, which impede the growth of many pathogenic decay fungi (Scheffer and Cowling 1966). For example, pines produce resins and antimicrobial chemicals such as pinosylvins and monomethyl ethers, following wounding, fungal infection, insect attack, or desiccation of wood (Jorgensen 1961). Similarly, oak trees produce phenolic compounds in sapwood following colonization by fungi or insects or wounding of cambium, and there are differences in decay resistance across different oak species (Deflorio et al. 2008; Scheffer and Morrell 1998; Shigo 1985). In addition to pure chemistry defense tactics, defense to bark beetles and infectious fungi in trees is dynamic and progressive, where the defense strategy involves repellency, defending and killing the foreign agents, and finally compartmentalization if the invading agent is tolerant of initial defenses (Franceschi et al. 2005).

Before sapwood dies and is converted to heartwood, which consists of mostly dead cells, antimicrobial chemicals are impregnated into these cells, usually staining the wood a darker color (Schwarze et al. 2000). In many types of trees the heartwood is chemically more resistant than sapwood due to deposited extractives (e.g., phenolic compounds), which are the primary defense against decay in these tissues (Schwarze et al. 2000). In a study focusing on decay in living sapwood of trees, true heartwood-forming species such as oak and Douglas-fir had a higher concentration of phenolic compounds and were more decay resistant after wound inoculation, relative to beech and sycamore (Deflorio et al. 2008). Sapwood of conifers is on average more resistant to decay relative to sapwood of hardwood trees (Baietto and Wilson 2010). Host specialization of *Ganoderma* species could be due to the coevolution of the fungus and host, where *Ganoderma* species have evolved to overcome the plant defenses of its host, and tree species have evolved more efficient defense mechanisms.

Given the previous ambiguity in taxonomy of the North American *Ganoderma* species, and the lack of pathogenicity data for *Ganoderma* species found in the southeastern U.S., research is needed to determine the pathogenicity of these fungi in commonly used landscape trees, where "pathogenicity" is defined as the ability of an organism to cause disease, and "disease" is defined as any negative change to host cell or tissue as a result of continuous perturbation by a pathogen that results in symptom development (Agrios 2005). Knowledge of differences in pathogenicity among *Ganoderma* species on actively growing trees with only sapwood will aid in interpretations of diagnosed infections. The major objective of this study is to investigate the pathogenicity of four *Ganoderma* taxa native to the southeastern U.S. on healthy, actively growing oak (*Quercus shumardii* and *Q. virginiana*), pine (*Pinus elliottii* and *P. taeda*), and palm (*Butia odorata*) trees, which are tree species that coexist as common in landscape plantings in the southeastern United States.

Materials and Methods

Ganoderma isolates. The *Ganoderma* species *G. curtisii*, *G. meredithiae*, *G. sessile*, and *G. zonatum* were commonly observed taxa in a survey (2016–2018) of *Ganoderma* from the southeastern United States across a variety of hosts (data not published). Isolations were made from basidiocarps of each species by taking small pieces (<1 mm^3) of context tissue with a sterile scalpel and placing them onto malt extract agar media (MEA, Difco Laboratories, Franklin Lakes, NJ) prepared according to the manufacturer's recipe with the addition of streptomycin (100 mg/liter) (Fisher Scientific, Waltham, MA), benomyl 95% (4 mg/liter) (Fisher Scientific, Waltham, MA), and lactic acid 95% (1 ml/liter) (Fisher Scientific, Waltham, MA). One isolate of each species was used for this study. Cultures were maintained on MEA slants as working stocks prior to use, and infested agar discs were submerged in sterile water for long term storage (Marx and Daniel 1976). Samples were initially identified based on the macro- and micromorphological features of basidiocarps, and these identifications were validated through sequencing the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA). DNA was extracted from mycelium of each isolate with the Extract-N-Amp rapid DNA kit (Sigma-Aldrich, St. Louis, MO) according to the manufacturer's instructions. Amplification of the ITS region was performed with the primers ITS1f and ITS4 on a MJ Mini thermocycler (BioRad, Hercules, CA) with an initial cycle of 94°C for 4 min followed by 37 cycles of 94°C for 50 s, 55°C for 50 s, and 72°C for 1 min, that produced an amplicon of ~700 to 800 nucleotides in length (Gardes and Bruns 1993; White et al. 1990). Amplicons were purified with Exo-SAP-IT (ThermoFisher, Waltham, MA) according to the manufacturer's instructions. Sanger sequencing was performed using both forward and reverse primers at the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida. Sequences were edited and aligned using Sequencher v. 5.3 (Gene Codes, Ann Arbor, MI). ITS sequences generated here were deposited and accessioned in the GenBank sequence database (Table 1).

Host plants. For each repetition of the pathogenicity tests, 25 large containerized (57 or 114 liter) live oak (*Q. virginiana*), loblolly pine (*P. taeda*-experiment 1), or slash pine (*P. elliottii* var. *elliottii*-experiment 2), Pindo palm (*B. odorata*), and Shumard oak (*Q. shumardii*) were sourced from commercial growers in North Florida. These tree species were selected based on the following criteria: i) commonly planted landscape plants in the southeastern U.S., and ii) trees were known hosts for at least one of the four *Ganoderma* taxa. Individual trees within a species were of similar size; where live oaks were

Table 1. List of *Ganoderma* spp. isolates used in this study with associated GenBank accession, location of collection, and host information from collection if available

Isolate	Taxon	Host type	Location	ITS GenBank accession
UMNFL60	*G. curtisii*	Unknown hardwood	Florida	KY767033
UMNFL64	*G. meredithiae*	*Pinus elliottii*	Florida	KY646220
UMNFL10	*G. sessile*	Unknown hardwood	Florida	KY708884
UMNFL85	*G. zonatum*	*Serenoa repens*	Florida	KY646212

approximately 2.5 to 3.8 cm in diameter, Shumard oaks were approximately 5.1 cm in diameter and pines were approximately 5.1 (experiment 1) and 7.6 cm (experiment 2) in diameter. Pindo palms had no evident pseudobark, and were still in a grass like stage.

Experimental setup and inoculations. For each repetition, the pathogenicity tests were set up in a randomized complete design with a full factorial arrangement, with one isolate of each of the four *Ganoderma* species plus a mock inoculated control across four tree species with five replicates per *Ganoderma*-host combination ($n = 100$). The experiment was staged outdoors on container pads in Gainesville, FL, and the experiment was repeated once with inoculations in the summer (experiment 1) and winter (experiment 2). The average temperatures in Gainesville during the two months postinoculation (establishment phase) was 28.3°C (experiment 1) and 16.1°C (experiment 2) (www.wunderground.com). The optimum growth temperatures of the *Ganoderma* spp. tested are between 25 and 30°C, although all four species grow between 15 and 35°C (Adaskaveg and Gilbertson 1986, 1989). Trees were irrigated daily in the summer months with drip irrigation for 45 min (approximately 400 ml/min), and as needed in the dormant season with automated irrigation to maintain the health of the trees and regulate temperature.

To prepare inoculum, dikaryotic isolates were cultured onto MEA for 7 days and then inoculated into sterilized, hydrated rye grain in half-filled 237 ml glass jars and incubated at 27°C until fully colonized (approximately 3 weeks). Commercially available birch dowels (Fungi Perfecti, Olympia, WA) (0.8 × 2.54 cm) were soaked in distilled H_2O for 24 h and then sterilized by autoclaving for 45 min in 237 ml glass jars at 121°C at 103 kPa. Sterile wooden dowels were infested with grain inoculum of each respective isolate, and were incubated at 27°C for 6 weeks.

Trees were inoculated on 28 June 2016 (experiment 1) and 20 December 2016 (experiment 2) by drilling 2.54 cm into the lower bole of trees with a 0.8 cm diameter drill bit, and placing the infested or noninfested (mock inoculated control) dowels into this void. Two inoculation sites adjacent to each other were made in experiment 2. For palms, a 30 cm long, 0.8 cm diameter drill bit was used to drill through the boots (leaf scars) to a point below the primordial leaves of the meristem, where root initials were forming, then two infested dowels were placed in this void. For each experiment, dowels infested with each species were plated on the day of inoculation on MEA to confirm viability. Paraffin wax was used to seal the inoculation sites to avoid contamination and drying out of inoculum.

Destructive harvest, disease assessment, and reisolations. Trees were monitored monthly for disease symptoms and destructively harvested and rated after approximately 12 (experiment 1) and 11 months (experiment 2) of incubation and destructively harvested on 5 July 2017 (experiment 1) and 8 November 2017 (experiment 2). Tree health ratings (1 to 3) were recorded, where 1 was healthy, 2 was symptomatic, and 3 was dead. Trees were cut below the inoculation site and sectioned into approximately 15 cm long sections. Longitudinal cuts were made through the inoculation point with a bandsaw. Each inoculation site on each tree was rated on a 1 to 4 wound response rating scale, where 1 was complete wound closure; 2 was response wood present, but wound not closed; 3 was no wound closure; and 4 was external decay cavity formed in the xylem outside the point of inoculation. In addition, photographs were taken for all longitudinal cross sections of the inoculation sites in order to measure surface area of internal xylem discoloration from the inoculation. Surface area measurements were made using the computer program Assess 2.0 (APS, St. Paul, MN). Finally, isolations were made from the inoculum (dowel) and discolored wood (Fig. 1) from each tree by taking five small sections (<1 mm^3) from the respective locations, surface sterilizing them in 50% sodium hypochlorite solution for 30 s, rinsing in sterile water, blotting dry, and plating them onto MEA amended with benomyl, streptomycin, and lactic acid as described previously. Subcultures of suspect *Ganoderma* isolations were made onto MEA, and identified using morphology. Pindo palms showed no exterior symptoms, and due to the difficulty of sectioning the young palms, they were not destructively harvested.

Statistical analyses. Since the tree health and average wound response rating data were not normally distributed, these data were analyzed using a one-way nonparametric Wilcoxon test, with means separation using Wilcoxon pairwise comparisons in JMP PRO v. 12. Data were analyzed independently for each experiment and tree species, with *Ganoderma* species or mock inoculated control as the independent variable and ratings as the dependent variable. To test for differences in surface area of discoloration with the different *Ganoderma* inoculations relative to the mock inoculated control, analysis of variance (ANOVA) and Dunnett's Means comparison test were calculated independently for each experiment and tree species in JMP PRO v. 12, with *Ganoderma* species or mock inoculated control as the independent variable and area of internal xylem discoloration as the dependent variable.

Results

Disease assessment, wound responses, and reisolations. No symptoms of disease, external lesions, or decay were observed at 11 or 12 months postinoculation on any tree species inoculated with any of the four *Ganoderma* species. Additionally, no symptoms were found on any of the mock inoculated controls. All trees were therefore given a tree health rating of one. However, in experiment 2, all *Q. virginiana* trees, both inoculated and mock-inoculated, died 3 months post inoculation. Presumably, death was due to the inoculation method, which girdled the cambium tissue. Because of this, live oak trees in experiment 2 were removed from the statistical analyses.

In experiment 1, all *Ganoderma* inoculations and mock inoculated controls on live oak, loblolly pine, and Shumard oak had an average wound response rating of one or two, meaning that the inoculation site had completely closed with response wood, or had some response wood present and were in the process of wound closing. Similarly, in experiment 2, loblolly pines and Shumard oaks inoculated with the four *Ganoderma* species and mock inoculated controls had an average wound response rating of one or two. In both experiments, there was no statistical difference ($P > 0.05$) in tree health rating or average wound response rating between trees inoculated with the *Ganoderma* species and mock inoculated controls (Table 2).

There were differences in extent of internal discoloration in some of the *Ganoderma* inoculations relative to the mock inoculated
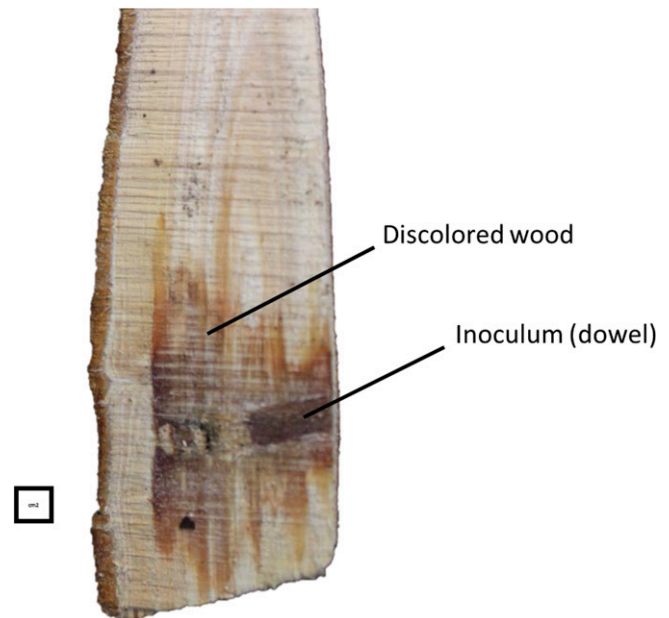

Fig. 1. Longitudinal cross section through inoculation site, showing where attempts to recover *Ganoderma* species were made (discolored wood and infested dowel-inoculum). This sample (169) was from *Pinus elliottii* var. *elliottii* inoculated with *G. meredithiae*. The scale box outlined in black measures 1 cm^2.

controls, although few were statistically significant ($P \le 0.05$). When compared with the control, all *Ganoderma* inoculations in experiment 1 had higher average internal discoloration of xylem, but the differences were only significant for inoculations with *G. meredithiae* ($P = 0.05$) and *G. sessile* ($P = 0.02$) on pine, and *G. meredithiae* on Shumard oak ($P = 0.04$) (Table 2). In experiment 2, there was no statistical difference in discolored area of the internal xylem tissues between the inoculations with four *Ganoderma* species and mock inoculated controls. In both experiments, live oak trees had the least amount of discolored area relative to the other trees, while Shumard oaks (experiment 1) and pine (experiment 2) had the most.

With the exception of trees inoculated with *G. sessile*, the inoculated *Ganoderma* species were not successfully reisolated from either the original inoculum (infested dowel) or the surrounding discolored wood (Table 3). In both experiments, *G. sessile* was isolated from the inoculum (infested dowel) and the discolored wood outside the inoculation site from both live oak and Shumard oak, but was only isolated from the infested dowel from the two pine species (Table 3).

Discussion

Throughout the course of both experiments, no external symptoms were observed across four tree species inoculated with any of four *Ganoderma* species, or in the mock inoculated controls. It is generally thought that Ganoderma butt or trunk rot requires wounded roots or trunk tissue as an infection court for successful infection by a *Ganoderma* species (Adaskaveg and Gilbertson 1987; Pirone 1957; Sinclair and Lyon 2005). Although trees were wounded in these experiments and inoculation methods were similar to other published experiments (Deflorio et al. 2008; Nicolotti et al. 2004), the four *Ganoderma* spp. tested were found to not be pathogens of sapwood in these young, actively growing trees with the inoculation method used. Furthermore, all *Ganoderma* and mock inoculated oaks

Table 2. Average total area of discolored wood and external wound response ratings for *Pinus taeda*, *P. elliottii*, *Quercus shumardii*, and *Q. virginiana* inoculated with four *Ganoderma* species

Inoculum	Experiment 1		Experiment 2	
	Discolored area (cm²)[a]	Wound response rating[b]	Discolored area (cm²)	Wound response rating
Quercus virginiana				
Control	4.8 ± 1.1	1	NA[c]	NA
Ganoderma curtisii	6.1 ± 1.1	1	NA	NA
G. meredithiae	6.9 ± 1.0	1	NA	NA
G. sessile	6.9 ± 2.6	1	NA	NA
G. zonatum	7.1 ± 2.0	1	NA	NA
Pinus sp.[d]				
Control	6.1 ± 1.4	1	12.9 ± 1.6	2
G. curtisii	9.4 ± 2.2	1	12.8 ± 3.5	2
G. meredithiae	9.2 ± 1.8*[e]	1	19.1 ± 7.4	2
G. sessile	9.7 ± 2.1*	2	12.1 ± 3.2	2
G. zonatum	8.6 ± 1.5	1	13.5 ± 3.5	2
Quercus shumardii				
Control	4.8 ± 0.9	1	8.0 ± 1.8	1
G. curtisii	10.2 ± 3.1	1	10.2 ± 1.4	1
G. meredithiae	11.1 ± 4.5*	1	11.8 ± 4.5	1
G. sessile	10.1 ± 3.8	1	10.1 ± 1.1	2
G. zonatum	10.1 ± 3.8	1	9.4 ± 2.1	1

[a] Each cell represents averages of replicates where n = 5. Values represent the average discolored area ± the standard deviation.
[b] Values represent the average wound response rating rounded to the nearest rating.
[c] Not analyzed, due to death from inoculation method because of death of hosts due to inoculation method.
[d] *Pinus taeda* in experiment 1, and *P. elliottii* var. *elliottii* in experiment 2.
[e] Values with * are statistically significant ($P \le 0.05$) with Dunnet's test relative to the control.

Table 3. Frequency of recovering *Ganoderma* species from inoculum (infested dowel) and discolored wood of trees 11 to 12 months post inoculation

	Experiment 1		Experiment 2	
	Inoculum	Discolored wood	Inoculum	Discolored wood
Quercus virginiana inoculum control	-[a]	-	-	-
G. curtisii	-	-	-	-
G. meredithiae	-	-	-	-
G. sessile	++[b]	+	++++	++++
G. zonatum	-	-	-	-
Pinus sp.[c] inoculum control	-	-	-	-
G. curtisii	-	-	-	-
G. meredithiae	-	-	-	-
G. sessile	++	-	+++	-
G. zonatum	-	-	-	-
Quercus shumardii inoculum control	-	-	-	-
G. curtisii	-	-	-	-
G. meredithiae	-	-	-	-
G. sessile	++++	++++	++	+++
G. zonatum	-	-	-	-

[a] - indicates that reisolation was unsuccessful.
[b] + indicates successful reisolation, where each + indicates success in one replicate, and +++++ indicates success for five replicates per *Ganoderma* species/host tree combination.
[c] *Pinus taeda* in experiment 1, and *P. elliottii* var. *elliottii* in experiment 2.

and pines had wounds that were completely or partially closed over, which suggests that active compartmentalization was occurring.

The internal xylem discoloration observed across all tree species and inocula appeared to be the result of wounding, and not *Ganoderma* infection. Trees respond to wounding of the sapwood by producing antimicrobial compounds that often stain the wood and function by deterring decay organisms from establishing (Deflorio et al. 2008; Scheffer and Morrell 1998; Shigo 1985). Antimicrobial chemicals are also naturally produced in sapwood when it converts to heartwood (Schwarze et al. 2000). In addition to plant defense chemicals discoloring wood, bacteria and wood staining fungi are often prevalent in wounded sapwood tissues (Scheffer and Cowling 1966).

Previous studies have shown *Ganoderma* species to be poor competitors in the soil (Bakshi et al. 1976; Rees et al. 2007). In addition, for successful infection, *Ganoderma* propagules have to be in contact with or in close proximity (1 to 2 cm) to the infection court (Adaskaveg and Gilbertson 1987; Bakshi et al. 1976; Rees et al. 2007). Although our inoculations were placed into the infection court (i.e., trunk wound), no visible decay was observed. The internal xylem discoloration, which was presumably produced by antimicrobial plant defense chemicals (e.g., phenolics) or the colonization of wood staining microbes, could help explain the unsuccessful infections with *G. curtisii*, *G. meredithiae*, and *G. zonatum* and the lack of decay with all *Ganoderma* species in the oak and pine trees. Plant defense chemicals could have made the sapwood uninhabitable, while wood staining microbes could have outcompeted the *Ganoderma* species. It is also possible that the inoculation method was not the appropriate method and it failed for other reasons. This is based on the unsuccessful reisolation attempts with *G. curtisii*, *G. meredithiae*, and *G. zonatum* from the infested dowels after 11 to 12 months of incubation in the infection court.

We chose to wound-inoculate on lower trunks, because i) earlier studies had successfully used this method, and ii) it has long been accepted that lower trunk injuries, such as lawn mower injuries, are a prime entry point for *Ganoderma* and other wood decay fungi. However, we did not observe disease development with this method, and in fact were unable to recover the pathogen in most cases. There is precedent in the literature suggesting that fresh, actively growing sapwood is not conducive for pathogen establishment (Blanchette 1991; Boddy and Rayner 1983). Sapwood has a higher moisture content and lower oxygen content than heartwood, which is not optimal for mycelial growth of many wood decay fungi (Boddy 1983; Boddy and Rayner 1983). In a laboratory decay study, *Lentinus edodes* and *Hypoxylon truncatum* decayed oak and beech wood better under moderate moisture conditions in contrast to low and high moisture conditions (Abe 1989). Similarly, there is a positive correlation of lignin degradation and high oxygen contents (Blanchette 1991). Given that sapwood is high in moisture and low in oxygen, and some decay fungi seem to be negatively affected by low oxygen and high moisture conditions, it is not surprising that infections in functional sapwood (living) by decay fungi is unlikely (Boddy and Rayner 1983). Furthermore, sapwood consists of living cells that have an active response to wounding, fungal infections, and beetle infestations, and it is possible that active compartmentalization and plant defense chemicals deterred the fungi from establishing (Blanchette and Biggs 1992).

In a previous inoculation experiment, the reisolation of *G. lucidum sensu lato* was successful from heartwood and pith tissues of inoculated field-grown grapevines, but reisolation was not successful from sapwood tissues (Adaskaveg and Gilbertson 1987). Although healthy sapwood may be a non-preferential tissue as an infection court, as shown previously (Adaskaveg and Gilbertson 1987; Boddy and Rayner 1983), dead sapwood can be rapidly decayed by these four *Ganoderma* species (Loyd et al. 2018). Freshly wounded sapwood may also be a non-preferential infection court, but *G. sessile* was able to infect the sapwood of both oak species. In fact, *G. sessile* was the only species consistently isolated from the sapwood, which we suspect was because it constitutively produces chlamydospores, which aid in survival. These propagules, we suspect, allow *G. sessile*

to persist in healthy trees, and lay dormant as an opportunistic saprophyte. We speculate that this latent opportunistic saprophytic phase persists as an endophyte. This endophytic life stage, where the *Ganoderma* species is living in wood, but not actively decaying wood, seems to be an understudied, yet important part of the *Ganoderma* life cycle (Martin et al. 2015).

Trees in the landscape often experience mechanical wounding from machinery such as lawn mowers and weed eaters, and presumably these wounded tissues can become infection courts for decay fungi, such as species of *Ganoderma*. However, these data suggest that there is a low likelihood of infection immediately following wounding of trees that only possess sapwood or wounds of larger trees that only are in sapwood and don't reach heartwood. The failure of our isolates to establish in fresh trunks raises questions about the entry of wood rot pathogens in trunk injuries. It is likely that such wounds are not immediately conducive to pathogen entry and establishment, but become more so as the wounds age and sapwood within them dies. These data suggest that some factors not present in our study, such as stress, age of tree, or wood type (sapwood versus heartwood) could play a role in enabling pathogenicity. More research is needed to elucidate how and when *Ganoderma* infections establish in wounded trunks and result in decay.

Although others have been successful with trunk inoculations, it is likely that we would have seen more success with root inoculations, given that our trees were young and lacked heartwood. For example, Adaskaveg and Gilbertson (1987) inoculated grape roots with *G. lucidum sensu lato*, and observed that the fungus infected the roots, and decay progressed from roots to the pith and inner discolored wood of the grape vine, only entering the sapwood in later stages of decay. Similarly, Toole (1966) inoculated roots of young green ash (*Fraxinus pennsylvanica*), sweetgum (*Liquidambar styraciflua*), and mimosa (*Albizia julibrissin*) trees with wood block inoculum of *G. lucidum sensu lato* (syn.=*P. lucidus sensu lato*), and had successful infections of all trees and mortality of sweetgum and mimosa trees.

Overall, the four *Ganoderma* species were not sapwood pathogens of the hosts tested, immediately following wounding of the lower trunk tissue. However, the type of infection court, age of tree, and environmental conditions could have played a role in the lack of infections. Inoculations using sealed inoculation sites on wounded roots in young trees and mature trees with heartwood should be completed to fully evaluate the pathogenicity of these *Ganoderma* species on these hosts. The role of tree stress during inoculation by *Ganoderma* species also deserves additional study.

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