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Elucidating wood decomposition by four species of *Ganoderma* from the United States

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ABSTRACT

The laccate (shiny or varnished) *Ganoderma* contain fungi that are important wood decay fungi of living trees and decomposers of woody debris. They are also an important group of fungi for their degradative enzymes and bioprocessing potential. Laboratory decay microcosms (LDMs) were used to study the relative decay ability of *Ganoderma curtisii, Ganoderma meredithiae, Ganoderma sessile,* and *Ganoderma zonatum,* which are four commonly encountered *Ganoderma* species in the U.S., across four wood types (*Pinus taeda, Quercus nigra, Quercus virginiana,* and *Sabal palmetto*). Generally, all *Ganoderma* species were able to decay all types of wood tested despite not being associated with only certain wood types in nature. *G. sessile,* on average caused the most decay across all wood types. Among the wood types tested, water oak (*Q. nigra*) had the most mass loss by all species of *Ganoderma.* Scanning electron microscopy was used to assess micromorphological decay patterns across all treatments. All *Ganoderma* species simultaneously decayed wood cells of all wood types demonstrating their ability to attack all cell wall components. However, *G. zonatum* caused selective delignification in some sclerenchyma fibers of the vascular bundles in palm (*S. palmetto*) as well as in fibers of water oak. In addition, *G. zonatum* hyphae penetrated fibers of palm and oak wood causing an unusual decay not often observed in basidiomycetes resulting in cavity formation in secondary walls. Cavities within the secondary walls of fibers gradually expanded and coalesced resulting in degradation of the S2 layer. Differences in colony growth rates were observed when *Ganoderma* species were grown on medium amended with water soluble sapwood extracts from each wood type. *G. meredithiae* had enhanced growth on all media amended with sapwood extracts, while *G. curtisii, G. sessile* and *G. zonatum* had slower growth on loblolly pine extract amended medium.

1. Introduction

Ganoderma Karst. is a large and diverse genus of wood decay fungi that contains species that cause white rot of the roots and lower trunk of trees belonging to many plant families (Murrill, 1902, 1908; Schwarze and Ferner, 2003; Zhou et al., 2015). White rot fungi possess enzymatic and non-enzymatic processes that breakdown cellulose, lignin and other structural components of wood and may degrade these components simultaneously or may selectively attack some cell wall components (such as lignin and hemicellulose) over cellulose (Blanchette, 1984a, b, 1991; Eriksson et al., 2012). Interspecific variation in wood decay rates exists within the genus *Ganoderma* and *in vitro* decay rates are proportional to the *in vitro* growth rates of isolates of a given species in axenic culture (Blanchette, 1984b; Adaskaveg and Gilbertson, 1986a; Adaskaveg et al., 1991). For example, isolates that were identified as *Ganoderma lucidum (sensu lato)* caused approximately 20 and 45 % more mass loss in grape and silver leaf oak wood blocks, respectively, relative to isolates of *Ganoderma tsugae* Murrill over a 20 week period of incubation, and the isolates *G. lucidum* grew 2–3 times as fast as isolates of *G. tsugae* in culture (Adaskaveg and Gilbertson, 1986a). Some wood decay fungi selectively degrade lignin, while others simultaneously decay all structural wood sugars (Blanchette, 1991). *In vitro* decay studies have shown that

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some *Ganoderma* species, such as *Ganoderma oregonense* and *G. tsugae*, selectively delignified and simultaneously decayed wood cells, while others, such as *Ganoderma meredithiae* simultaneously decayed cells with only localized areas of moderate delignification (Blanchette, 1984a, 1991; Adaskaveg et al., 1990). In addition, *Ganoderma zonatum* mostly simultaneously decayed wood cells, but also delignified localized areas of some cell walls (Adaskaveg et al., 1990).

The taxonomy of *Ganoderma* species in North America is problematic and currently under study. In North America, many laccate (varnished) individuals that occur on hardwoods have been labeled historically as *G. lucidum sensu lato* (Atkinson, 1908; Adaskaveg and Gilbertson, 1986a, b, 1989; Gilbertson and Ryvarden, 1986; Moncalvo et al., 1995). Molecular phylogenetic investigations now show that *G. lucidum sensu stricto* (Curtis) Karst. is found native to Europe and possibly parts of Asia (Nobles, 1965; Zhou et al., 2015; Hennicke et al., 2016). In addition, species such as *Ganoderma curtisii* (Berk.) Muriill and *Ganoderma sessile* Murrill, which were once lumped into *G. lucidum sensu lato,* have been shown to be quite distinct from each other and neither are conspecific with members of the *G. lucidum s.s.* clade, which includes the temperate North American species *G. oregonense* Murrill and *G. tsugae* Murrill (Adaskaveg and Gilbertson, 1988; Zhou et al., 2015).

In addition to differences in the decay ability of various white rot fungi, tree species can differ in their chemical characteristics and physical resistance to decay (Scheffer and Cowling, 1966; Adaskaveg and Gilbertson, 1986a; Adaskaveg et al., 1991; Baietto and Wilson, 2010). Living trees can actively compartmentalize infections and wounds, but the efficiency of this defense strategy can be different between tree species (Shigo and Hillis, 1973; Boddy and Rayner, 1983). In addition, defense chemicals such as resins, phenols, and tannins can be produced in wood, especially after damage to the living sapwood, which can impede the growth of many decay fungi (Scheffer and Cowling, 1966). Pines produce resins and antimicrobial chemicals such as pinosylvins and monomethyl ethers, following wounds, insect attacks or desiccation of wood (Jorgensen, 1961). Oak trees produce phenolic compounds in sapwood following colonization by fungi or insects, wounding of cambium, and possibly desiccation, and it there are differences in decay resistance across different oak species (Shigo, 1985; Scheffer and Morrell, 1998; Deflorio et al., 2008). Since heartwood has little active response growth, relative to sapwood, antimicrobial chemicals are deposited in wood cells naturally, when sapwood dies and forms heartwood (Schwarze et al., 2013). In many types of trees the heartwood is chemically more resistant than sapwood due to deposited extractives (e.g. phenolic compounds) that are composed of decay resistant chemicals (Schwarze et al., 2013). 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, relative to beech and sycamores (Deflorio et al., 2008). Sapwood of conifers is on average more resistant to decay relative to sapwood of hardwood trees (Baietto and Wilson, 2010). Lastly, trees with high wood density such as mesquite, have inherently higher wood decay resistance, likely due to a larger concentration of antimicrobial extractives due to greater surface area of the more dense woods (Scheffer, 1973; Adaskaveg and Gilbertson, 1986a). In a previous study focusing on *in vitro* relative decay of *Ganoderma* species, isolates of *G. lucidum* were incapable of decaying mesquite wood (density = 0.71 g/cm^3), while mass loss of approximately 60 % was observed on less dense wood such as grape (0.37 g/cm^3) (Adaskaveg and Gilbertson, 1986a) (http://db.worldagroforestry.org).

It is likely that certain *Ganoderma* species have evolved to have an affinity for certain tree groups. For example, *G. zonatum* Murrill has only been found in association with the decay of palm wood and *G. tsugae* is typically associated only with hemlock trees (Blanchette, 1984a; Gilbertson and Ryvarden, 1986; Elliott and Broschat, 2001). In addition, *G. meredithiae* Adask. & Gilb. was originally described as a unique species distinguishing it from *G. curtisii* and *G. lucidum* by having an affinity for pines and growing at a slower rate in culture (Adaskaveg and Gilbertson, 1988). For most taxonomic works, knowledge of host species can be an important diagnostic criterion for identification of some species of *Ganoderma* (Gilbertson and Ryvarden, 1986).

Due to taxonomic confusion, reevaluations of functional differences between common *Ganoderma* species are needed to better understand the biology of this cosmopolitan group of wood decay fungi. The major objectives of this research are to i) determine quantitative and qualitative differences in decay between commonly observed *Ganoderma* species in the United States across multiple wood types, and ii) investigate the role that water-soluble sapwood extracts have on the linear growth rates of *Ganoderma* species.

2. Materials & methods

2.1. Isolate collections

Isolates of *G. curtisii, G. meredithiae, G. sessile,* and *G. zonatum* were cultured from the sterile context tissue of basidiomata collected in Florida. Cultures were obtained by plating small pieces (<1 cm) of sterile context tissue onto basidiomycete-selective malt extract agar (BSMEA) medium, which was made with a base of malt extract agar (MEA) (Difco Laboratories, Franklin Lakes, NJ) according to the manufacturer's recipe with the addition of streptomycin (100 mg/L), benomyl 95 % (4 mg/L), and lactic acid (1 ml/L). Pure cultures (isolate numbers are listed in Table 1) were maintained on MEA to obtain pure cultures and also stored as infested agar plugs submerged in sterile diH_20 for long term storage. Isolates were initially identified based on the macro- and micromorphological features of basidiomata and these identifications were validated through sequencing the ITS region of the ribosomal DNA (rRNA). DNA was extracted from mycelium of each isolate with the Extract-N-Amp rapid DNA kit (Sigma–Aldrich, St. Louis, MO) per the manufacturer's instructions. Amplification of the ITS region was performed with the primers ITS1f and ITS4b (Gardes and Bruns, 1993) on a MJ Mini thermocycler (BioRad, Hercules, CA) with thermocycling conditions of an initial step of 94 C for 4 min and followed with 37 cycles of 94 °C for 50 s, 55 °C for 50 s, and 72 °C for 1 min that produced an amplicon of ~700–800 nucleotides (White et al., 1990; Gardes and Bruns, 1993). 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 were deposited and accessioned in the GenBank sequence database (Table 1).

Table 1
List of isolates, species name with associated GenBank accession, location of collection, and host information from collection if available.

Isolates	Taxon	State	Host	Genbank
UMN FL60	*G. curtisii*	Florida	no host information	KY767033
UMN FL64	*G. meredithiae*	Florida	*Pinus elliotii* var. *densa*	KY646220
UMN FL10	*G. sessile*	Florida	no host information	KY708884
UMN FL85	*G. zonatum*	Florida	*Serenoa repens*	KY646212

2.2. Wood sources and preparation

Sapwood of *Pinus taeda* (loblolly pine), *Quercus nigra* (water oak), and *Quercus virginiana* (live oak) were sourced from freshly felled, similar sized (~46 cm DBH) and aged trees from the Greater Charlotte, North Carolina area, while *Sabal palmetto* (sabal palm) was sourced from a tree felled from Fort Lauderdale, Florida. The tree species selected for this study were chosen because they all coexist in bottomland forests, are common tree taxa in the Southeastern U.S., and at least one of the *Ganoderma* species used in this study has been collected from these hosts previously (*G. curtisii* and *G. sessile* on oaks, *G. meredithiae* on pines, and *G. zonatum* on palms) (Gilbertson and Ryvarden, 1986; Adaskaveg and Gilbertson, 1988). Wood blocks were cut from the lower 1 m of trunk closest to the soil line and from similar sapwood depth for each tree type to reduce variation within each wood type. All blocks were cut from wood that visually appeared structurally sound with no decay or wounds. Furthermore, negative control blocks were used throughout the study to account for any unforeseen structural problems. Wood was prepared by stripping the bark and cutting 2.54 cm thick planks of wood from sapwood of similar age or growth rings on a gas powered saw mill. Planks of wood were sectioned down into 16.39 cm^3 blocks and 800 mm^3 ($10 \times 40 \times 2$ mm) strips with a chop and band saw. Wood was dried for five days in an incubator set at 50 °C, Wooden blocks were given a unique label and weighed. Prior to use, the wooden blocks and strips were hydrated for 48 h by soaking in diH_2O, and then autoclaved twice for 1 h at 121 C and 103 kPa with 48 h in between autoclave cycles.

2.3. Laboratory decay microcosms

Laboratory decay microcosms (LDMs) were setup based on the American Society for Testing and Materials (ASTM) Standard D1413-07 ("Standard Test Method for Wood Preservatives by Laboratory Soil-Block Cultures") with slight modifications (ASTM-International, 2007). Jars (237 ml) (Ball Corp., Broomfield, CO) were filled with 123 ml of hydrated Fafard 2S potting soil mix, which was equal parts vermiculite, peat and fertilizer-free potting soil (SunGro, Agawam, MA). The medium was tamped and leveled, and two hydrated 800 mm^3 wood strips were placed on top of the media for each wood type. Lids were placed on the jars with the rubber seal facing up and all LDMs were autoclaved twice for 1 h at 121 C and 103 kPa with 48 h in between autoclave cycles.

Isolates of the four *Ganoderma* species were grown on MEA for 7 d, from which 1 cm^3 agar plugs were excised from the leading edge and used to inoculate the wooden strips inside the sterile LDMs for each treatment, including a negative control (non-inoculated MEA). Inoculated feeder strips were incubated at 28 C in their respective LDM until fully colonized, which was approximately 3 weeks. Blocks for each wood type were sterilized as described previously and then placed on top of the fully colonized wooden strips for each corresponding wood type. All LDMs were incubated for 90 d at 28 °C in the dark. After 90 d blocks were harvested, surface mycelia removed, dried at 50 °C for 5 d, and weighed. Percent mass loss was used as a proxy for total amount of decay, which was calculated for each block by subtracting the final (dry) weight from the initial (dry) weight and dividing by the initial (dry) weight, and multiplying by 100. The experiment was setup in a randomized complete block design with a factorial arrangement of *Ganoderma* taxon and wood type with five replicates, and was repeated twice (Study 1 and 2). Analysis of variance (ANOVA) and separation of means with a Student's T-test were calculated in JMP Pro 12 (SAS, Cary, NC) for each wood type and taxon independently.

2.4. Scanning electron microscopy of decay

In preparation for scanning election microscopy (SEM), small sections were cut from sample wood blocks and infiltrated with TBS™ Tissue Freezing medium™ (Triangle Biomedical Sciences, Durham, NC, USA) using low vacuum and mounted on brass stubs at −20 °C in a freezing microtome (International Equipment Company, Needham Heights, MA, USA). Samples were sectioned to produce a smooth transverse view of the wood and dehydrated by placing in 20, 30, 45 75 and 95 % ETOH for 5 min each. Following dehydration samples were mounted on aluminum stubs and coated with gold/palladium on a Cressington 108auto (Cressington Scientific Instruments, Watford, United Kingdom) sputter coater. Samples were examined using a Hitachi S3500N (Hitachi, Tokyo, Japan) scanning electron microscope.

2.5. Wood density, and water-soluble wood extract assays

In order to determine wood density, *P. taeda* (loblolly pine), *Q. nigra* (water oak), *Q. virginiana* (live oak) and *S. palmetto* (sabal palm) wood, sourced as described previously, were cut into small, approximately 1.5 cm^3, rectangular pieces. Five pieces of each wood type were weighed and individually submerged into a known volume of water in a graduated cylinder. The amount of displaced water was measured to estimate volume. The density was calculated by dividing the mass by the volume of water displaced by each wood piece. The average wood densities were as follows: live oak (0.95 g/cm^3), loblolly pine (0.52 g/cm^3), sabal palm (0.55 g/cm^3), and water oak (0.65 g/cm^3).

Each wood type was standardized to 190 g of wood pieces per 700 ml of diH_2O, which was based on the mass of 100, 1.5 cm^3 pieces of loblolly pine, the least dense wood. Bottles containing the water/wood mixture were incubated in a water bath at 80 °C for 5 h to extract water-soluble chemicals from each type of wood. The solid wood pieces were separated from the liquid by filtering through cheese cloth and 500 ml of the water soluble wood extracts was used as the liquid solvent to make 2 % MEA medium with each wood type. Standard 2 % MEA medium (dH_2O + MEA + agar), which is commonly used for *Ganoderma* isolation and characterization, was used as a baseline control and made based on the manufacturer's instructions (Difco Laboratories, Franklin Lakes, NJ) with an additional 2.5 g of supplemental agar.

Representative isolates of *G. curtisii, G. meredithiae, G. sessile* and *G. zonatum* used in the decay experiment were also used for this experiment. Isolates of each species were grown on MEA for 7 d, and an 8 mm plug was excised from the leading edge of the actively growing culture of each isolate with a cork borer and plated on each of the five media types (live oak amended-MEA, loblolly pine amended-MEA, sabal palm amended-MEA, water oak amended-MEA, and 2 % MEA control). Isolates were incubated at 28 °C for 5 d on all media types at which point the colony diameter was measured in two directions perpendicular to each other and averaged for each isolate. This experiment was setup in a randomized complete block design with a factorial arrangement of *Ganoderma* taxon and media type with 3 replications of all treatments. ANOVA and Tukey's HSD means separations were calculated in JMP Pro 12® for each *Ganoderma* taxon independently due to an interaction.

3. Results

3.1. Laboratory decay microcosm studies

Differences in decay across fungal–wood combinations were evident after 90 d of incubation in the LDMs. The control wood blocks had the lowest levels of mass loss (0–9 %) compared to all

Ganoderma taxa. The mass loss of the control wood blocks can be attributed to changes to wood during preparation (drying, hydrating, autoclaving, etc.). Host species ranked from least to most decay resistant were: water oak, sabal palm, loblolly pine, and live oak (Fig 1). Water oak was the least decay resistant wood tested with an average percent mass loss with all taxa of 44.2 % (Study 1) and 23.1 % (Study 2). Live oak wood was the most resistant with an average percent mass loss with all taxa of 13.6 % (Study 1) and 11.3 % (Study 2), and *G. zonatum* had very limited decay in live oak wood (Fig 2).

While percent mass loss was somewhat similar for each wood type decayed by *G. curtisii*, *G. meredithiae*, *G. sessile*, and *G. zonatum*, there were some differences. *G. zonatum* had very limited decay in live oak wood, having only 0.45 % (Study 1) and 3.17 % mass loss (Study 2). Similarly, loblolly pine showed some decay resistance to *G. zonatum*, which caused on average 9.5 % (Study 1) and 11.8 % mass loss (Study 2) (Fig 2). *G. sessile* consistently caused the greatest average percent mass loss across all wood types (27.8 % in Study 1 and 21.6 % in Study 2), while *G. zonatum* caused on average the least percent mass loss across all wood types (19.8 % in Study 1 and 15 % mass loss in Study 2) (Fig 1). Although the smallest percent mass

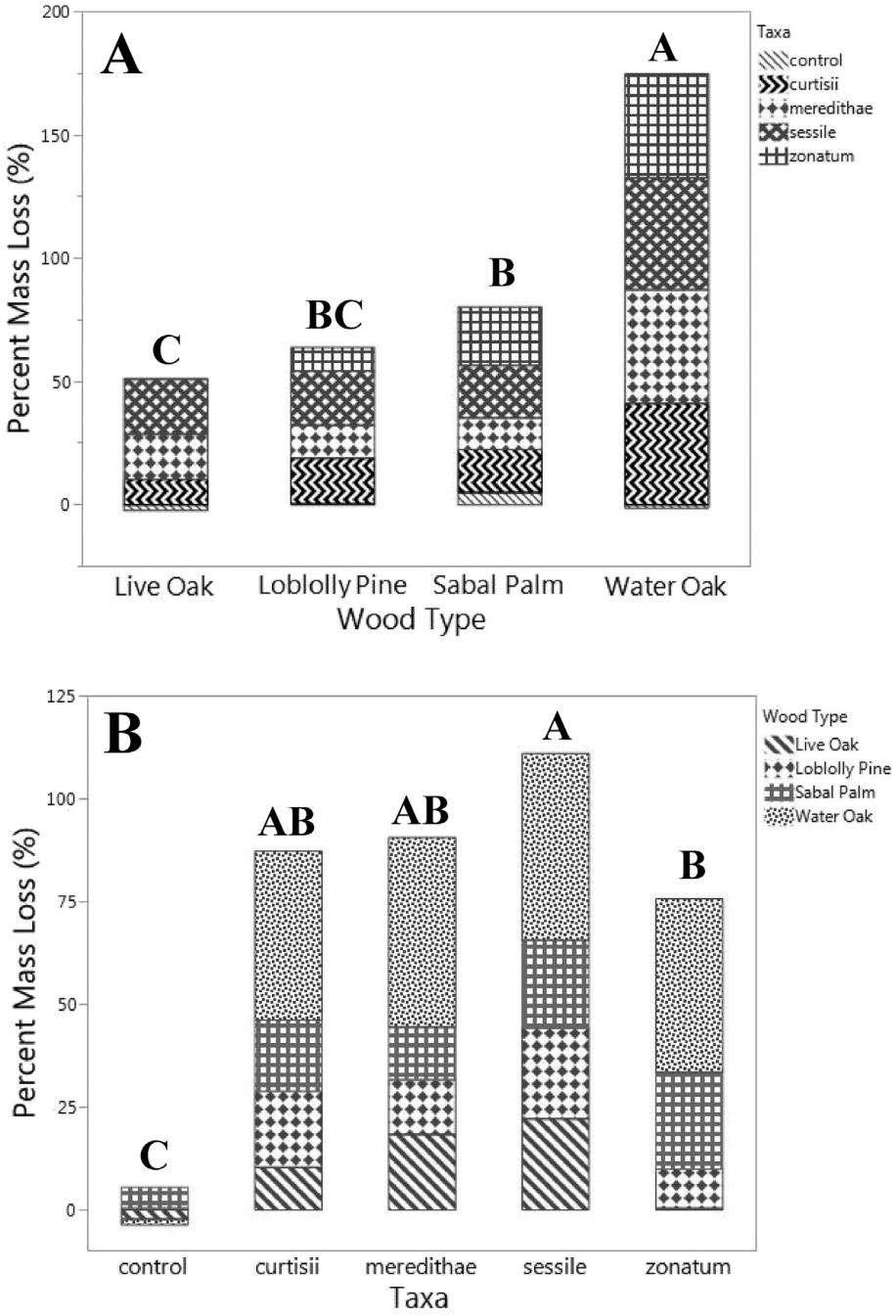

Fig 1. Percent mass loss in different wood types decayed by *Ganoderma* taxa. (A) Percent mass loss of the various wood types showing the relative amounts of decay from study 1, where different letters represent different means separation scores using a Student's T-test across all wood types (B) Percent mass loss of the various wood types showing the relative amounts of decay caused by the various *Ganoderma* species from study 1, where different letters represent means separation scores using a Student's T-test across all *Ganoderma* species and control.

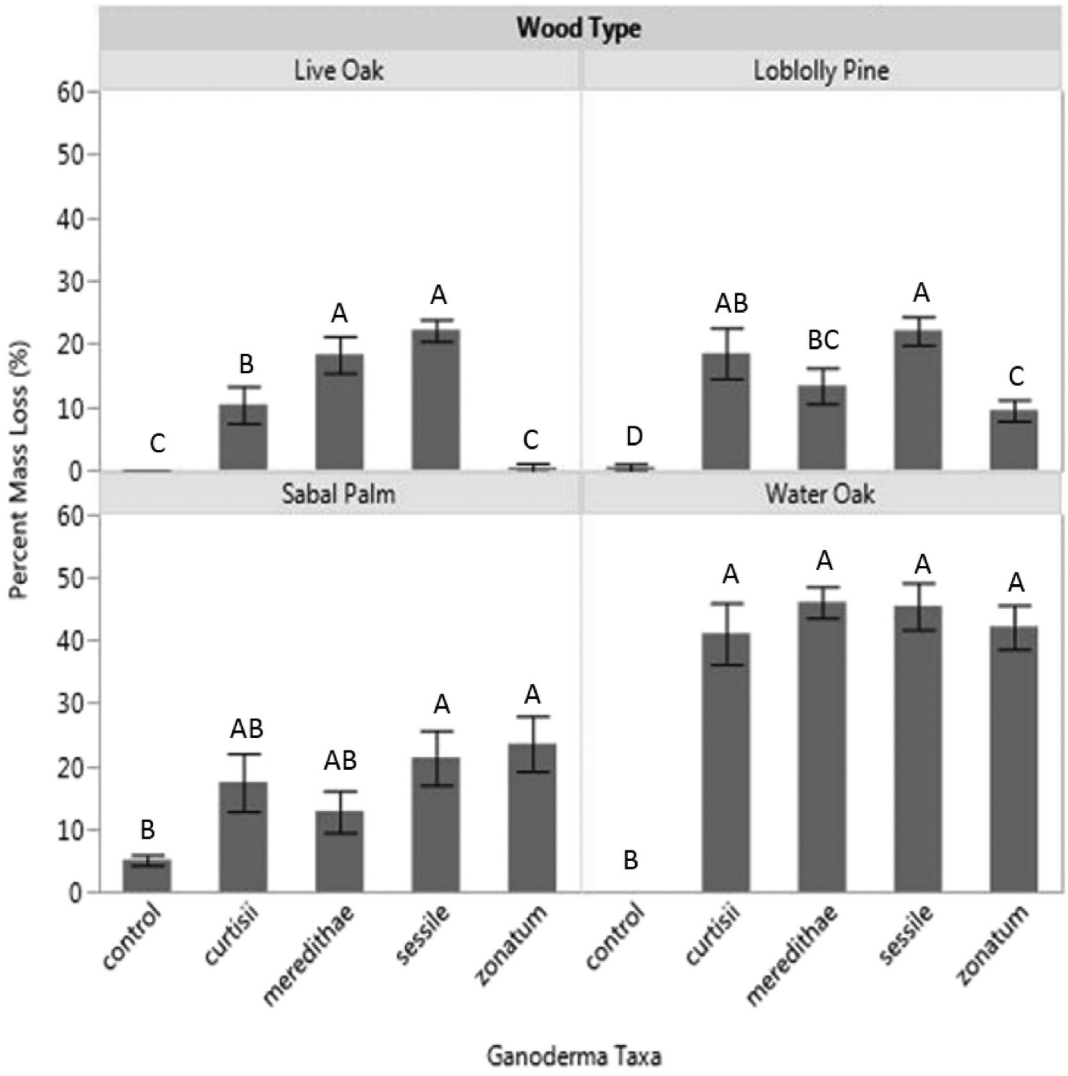

Fig 2. Percent mass loss of four wood types caused by the different *Ganoderma* species tested. Error bars represent standard error of the mean, and different letters represent different means separation scores using a Student's T-test independently for each wood type across each *Ganoderma* species.

loss was observed with *G. zonatum* across all woods, this could be attributed to the limited decay of pine and negligible mass loss of live oak wood. *G. zonatum* did cause the highest percent mass loss on sabal palm wood in Study 1, relative to the other *Ganoderma* taxa; although not statistically significant.

3.2. Scanning electron microscopy (SEM) of decay

Transverse sections of non-decayed wood observed with SEM show the typical arrangement of wood cells for each wood type, *P. taeda* (loblolly pine), *Q. nigra* (water oak), *Q. virginiana* (live oak), and *S. palmetto* (cabbage palm) as expected (Fig 3). Loblolly pine wood consisted of early wood, and late wood tracheids and ray parenchyma cells. The early wood consisted of large cells with thin cell walls, where the late wood consisted of small, densely packed cells with thick cell walls. While live oak wood consisted of a much denser arrangement of cells compared to the water oak wood, the pattern of cell arrangement was similar, where large, thin-walled parenchyma cells were arranged evenly amongst a matrix of thicker walled fiber cells. In addition, large thick-walled xylem vessel cells were evenly arranged in the matrix of fibers and parenchyma cells. In transverse sections of cabbage palm wood vascular bundles were evenly spaced in a matrix of parenchyma cells. The vascular bundles had two to three thick-walled xylem vessels that were adjacent to thin-walled phloem cells directly above a large matrix of thick-walled sclerenchyma fibers.

Scanning electron microscopy (SEM) analysis of all treatments showed that all of the *Ganoderma* species were able to rapidly decay wood cells of each wood type. The pattern of decay development in the live oak (*Q. nigra*) (Fig 4) and water oak (*Q. virginiana*) (Fig 6) wood was similar across all *Ganoderma* taxa (Figs 4 and 6). Large, thin-walled parenchyma cells were degraded first and radiating from these areas of decay a simultaneous attack on the thicker walled fibers followed. No decay was observed in cell walls of xylem vessels. All *Ganoderma* species caused a simultaneous type of decay in the two oak wood types. However, initial decay caused by *G. zonatum* appeared to cause cavities in secondary cell walls, somewhat similar to type 1 soft rot decay, in the water oak, but eventually the cavities coalesced and some secondary walls were removed completely (Fig 7A). All *Ganoderma* species decayed the thin-walled cells of the early wood of pine blocks more than the late wood. The *Ganoderma* species tested appeared to simultaneously decay the wood cells of the pine wood and no preferential lignin degradation was observed (*data not shown*). In

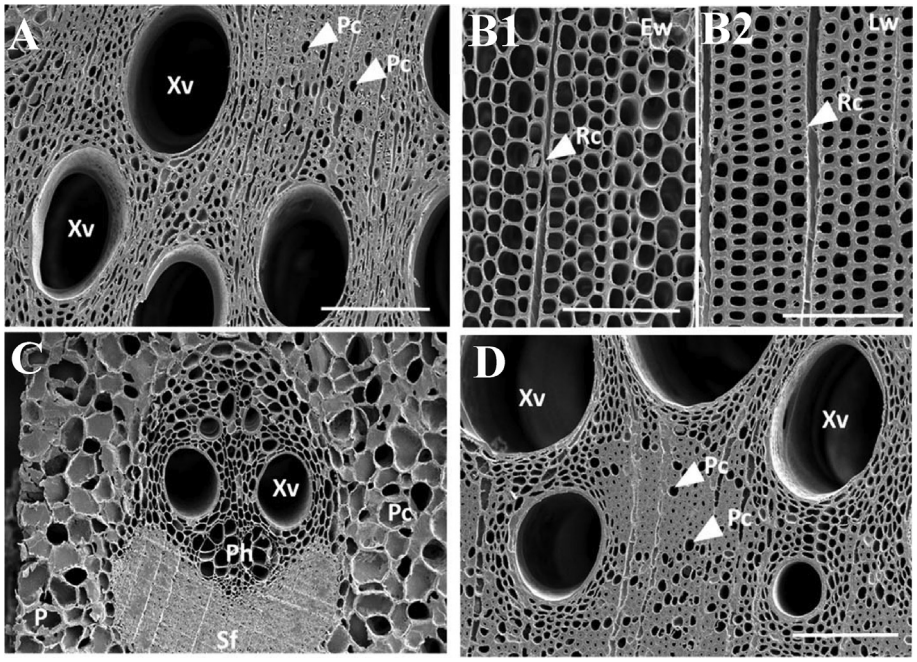

Fig 3. Scanning electron micrographs of transverse sections non-decayed wood control blocks. (A) Live oak (*Quercus virginiana*) wood, showing thin-walled parenchyma cells ("Pc") and large xylem vessels ("Xv"); (B1 and B2) two micrographs of sections of loblolly pine (*Pinus taeda*). (B1) shows wood with large, thin-walled cells of the earlywood ("Ew") and (B2) showing smaller, thick-walled cells of the latewood ("Lw"). Ray cells are indicated with arrows marked "Rc"; (C) cabbage palm (*Sabal palmetto*) wood, showing a vascular bundle, consisting of xylem vessels ("Xv"), phloem cells ("Ph"), and sclerenchyma fibers ("Sf") embedded in a matrix of parenchyma cells ("Pc"); (D) Water oak (*Quercus nigra*) wood showing multiple xylem vessels ("Xv") with parenchyma cells ("Pc") evenly spaced in thicker-walled fibers. Bars = 200 μm.

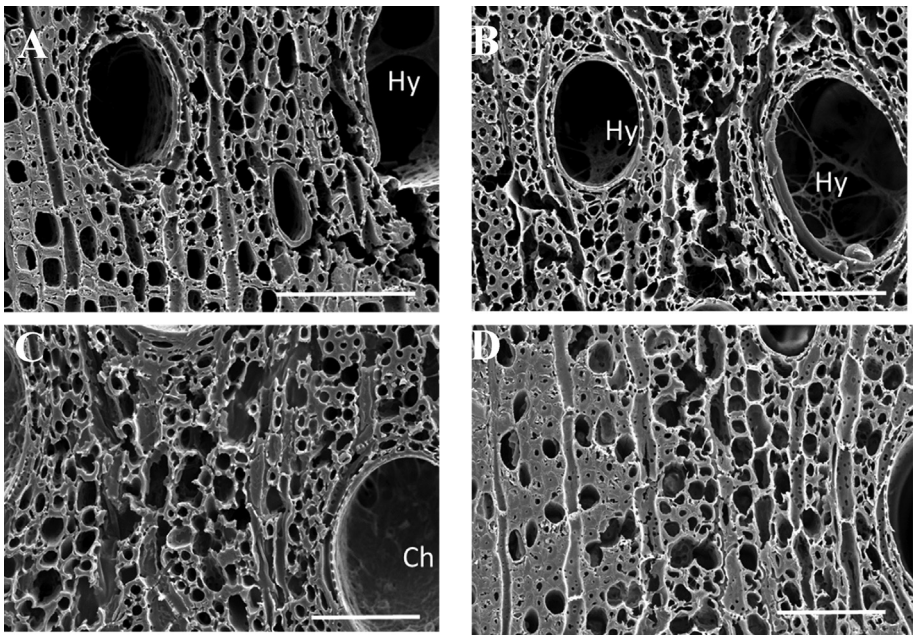

Fig 4. Scanning electron micrographs of white rot decay on live oak (*Quercus virginiana*) wood caused by (A) *G. curtisii* (B) *G. meredithiae* (C) *G. sessile* and (D) *G. zonatum* showing all cell wall components are degraded "Hy" represents hyphae and "Ch" represents chlamydospores. All fungi attacked cells by causing a progressive erosion from the cell lumina toward the middle lamella. Secondary cell walls and middle lamella between cells were degraded. Bars = 100 μm.

palm wood, the phloem cells inside the vascular bundles were preferentially decayed first (Fig 5). All *Ganoderma* species decayed the phloem cells readily. In addition, the thin-walled parenchyma cells adjacent to the xylem vessels were also decayed readily by *G. curtisii* and *G. meredithiae* (Fig 5A and B).

The thick-walled xylem vessels appeared resistant to degradation. Similarly, in wood of *P. taeda*, the late wood was more resistant to degradation, while the large early wood cells were rapidly degraded. Generally, the thick-walled sclerenchyma fibers of cabbage palm wood adjacent to the phloem cells were resistant to decay (Fig 5). However, *G. zonatum* and to some extent *G. sessile*,

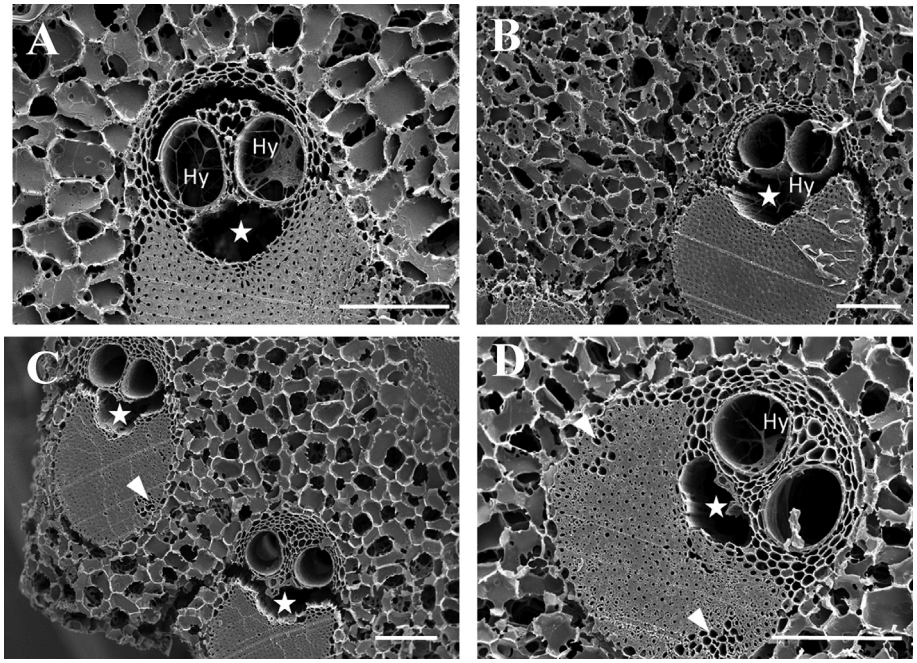

Fig 5. Scanning electron micrographs of cabbage palm (*Sabal palmetto*) wood decayed by (A) *G. curtisii* (B) *G. meredithiae* (C) *G. sessile* and (D) *G. zonatum*. Stars indicate areas where all phloem cells were completely removed, "Hy" indicates hyphal strands, and arrows point to sclerenchyma fiber cells that were decayed. Bars = 250 μm.

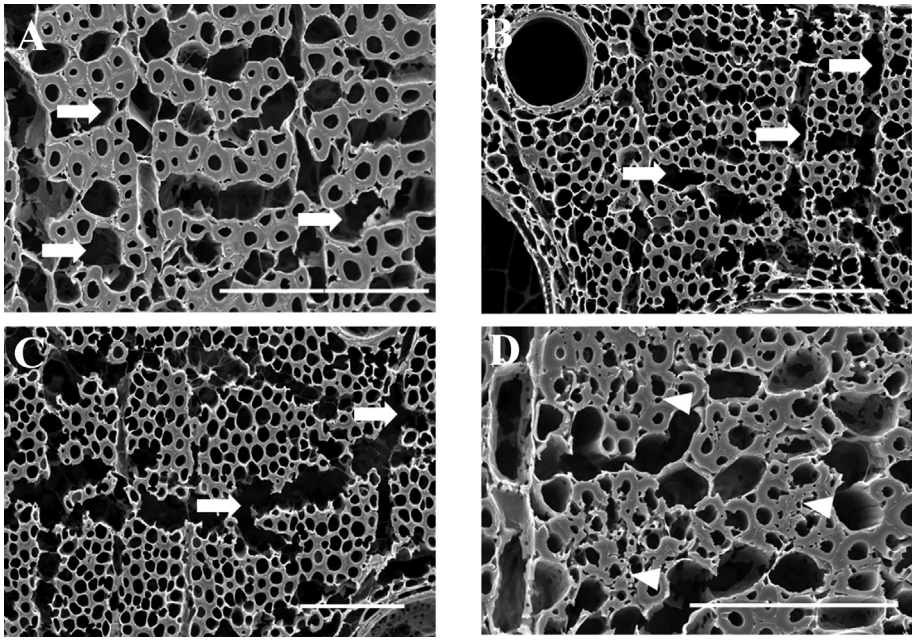

Fig 6. Scanning electron micrographs of white rot decay of water oak (*Quercus nigra*) wood caused by (A) *G. curtisii* (B) *G. meredithiae* (C) *G. sessile* and (D) *G. zonatum*. Simultaneous white rot of fibers completed removed some cells while adjacent fibers were not degraded (arrows). Some fibers in oak decayed by *G. zonatum* had cavities formed within secondary wall layers (arrowheads). Bars = 100 μm.

gradually degraded these thick-walled fibers (Fig 7A and B). The secondary walls of these cells were gradually decayed by these two species. The sclerenchyma fibers of the palm wood were degraded by *G. zonatum* gradually, where initially the type of decay appeared similar to decay caused by a soft rot fungus, where cavities were made in the secondary walls. In later stages the cavities would coalesce and the secondary walls would be completely removed leaving behind only the middle lamella between cells.

3.3. Colony growth effects from water-soluble wood extracts

There were differences in average colony diameter for each *Ganoderma* taxon across the different water-soluble wood extract amended media relative to the standard growing medium 2 % MEA (Fig 8). On 2 % MEA (control), growth rates of isolates of *G. sessile, G. zonatum, G. curtisii* and *G. meredithiae* were different. The *G. sessile* (UMNFL10) isolate grew the fastest on MEA after 5 d of growth (80.2 mm), followed by *G. zonatum* (UMNFL85) (54.3 mm),

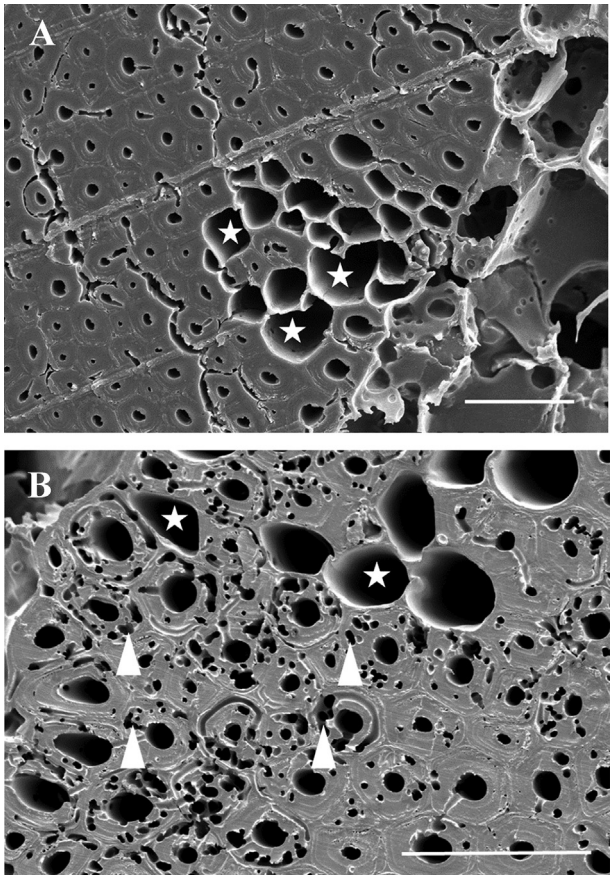

Fig 7. Scanning electron micrographs of white rot decay of cabbage palm (*Sabal palmetto*) wood fibers caused by (A) *G. sessile* and (B) *G. zonatum*. *G. sessile* caused a simultaneous attack on the cells resulting in complete removal of some fibers. "Stars" indicate areas with decayed fibers. *G. zonatum* caused cavities within secondary cell walls (arrows) Cavities enlarge and coalesce to degrade the fiber wall.

G. curtisii (UMNFL60) (44.0 mm), and *G. meredithiae* (UMNFL64) (16.5 mm).

Average colony diameter of *G. curtisii* isolates grown on water oak, live oak and palm amended media was statistically greater than the 2 % MEA control medium, while the average colony diameter of *G. curtisii* isolates on loblolly-amended medium was statistically less than the 2 % MEA control medium (Fig 8). The average colony diameter of *G. meredithiae* isolates was enhanced by all water-soluble wood extract amended media. Isolates of *G. meredithiae* had the largest average colony diameter on water oak amended medium followed by live oak amended medium. Average colony diameter of *G. meredithiae* on loblolly pine and palm-amended media were similar, and both had larger average diameters than the 2 % MEA control medium. Average colony diameters of *G. sessile* and *G. zonatum* were similar. There was no significant difference ($P > 0.05$) between the 2 % MEA, water oak amended medium and palm amended medium on the average colony diameters of *G. sessile* and *G. zonatum*. In contrast, the average colony diameters were less when isolates of *G. sessile* and *G. zonatum* were grown on live oak and loblolly-amended media.

Generally, the average colony diameter of all isolates was the greatest on the water oak amended medium. However, the average colony diameter of *G. sessile* and *G. zonatum* isolates on water oak amended medium was not statistically different than the 2 % MEA control. In comparison, *G. curtisii* and *G. meredithiae* had average colony diameters that were statistically ($P < 0.05$) the largest on the water oak amended medium. Overall, the average colony diameter of *Ganoderma* isolates grown on loblolly pine-amended medium was less, relative to 2 % MEA control, with the exception of *G. meredithiae* (Fig 8), which had a larger diameter on all of the water-soluble wood extract amended media relative to the control.

4. Discussion

Species of *Ganoderma* are primary decay fungi that can be found on trees ranging from healthy to dead, and some species are more effective at decaying certain species of trees or types of wood (Hong and Jung, 2004; Sinclair and Lyon, 2005). One possible explanation for a tightly linked host affinity is that certain fungal species have coevolved with certain types of tree species, and have become more efficient at competing with other potential colonizers of a given host. For example, although water oak trees and fallen water oak logs occur in ecosystems where you can find *G. zonatum* decaying palm wood, and isolates of *G. zonatum* can successfully decay water oak wood *in vitro*, in nature *G. zonatum* has not been observed decaying water oak substrates. Water oak was the most susceptible wood to decay in this study, and there are likely other more competitive primary decay fungi *in situ* that are more efficient at early establishment and utilization of the substrate. Ottosson et al. (2014) showed that primary decay fungi or early successional wood decay species can select, to some degree, the secondary and tertiary fungal species linked to the initial degradation and residual nutrient quality retained (Ottosson et al., 2014).

Furthermore, in a survey of basidiomycete endophytic species within wood of the genus *Hevea,* Martin et al. (2015) found that 75 % of the fungi identified were in the order Polyporales, which includes *Ganoderma* (Martin et al., 2015). It could be possible that *Ganoderma* species, such as *G. zonatum,* can live endophytically in palm trees, and act as a latent saprophyte or pathogen until tissues become weakened and susceptible to decay (Martin et al., 2015; Song et al., 2017). Additional host preference, competition tests and endophyte surveys of *Ganoderma* species are needed to address this issue.

In Study 1 and 2 the percent mass loss of wood across all *Ganoderma* taxa, least to greatest, was: live oak, loblolly pine, sabal palm and water oak. Wood density was not correlated to percent mass loss because water oak was the second densest wood, but consistently was decayed the most. Although density did not correlate, it is possible that other physical properties such as differences in oxygen availability in wood could be causing differences in the amount of decay observed, which can be related to wood density (Boddy and Rayner, 1983; Schwarze, 2007). Previously, authors reported that cultural growth rates of *Ganoderma* taxa were proportional to the decay rates, specifically with isolates of *G. lucidum sensu lato* and *G. tsugae* (Adaskaveg and Gilbertson, 1986a). This pattern was not observed in this study. Isolates of *G. meredithiae* grew the slowest in culture (2–3 mm/day) and the percent mass loss was statistically similar ($P > 0.05$) to the percent mass loss of *G. sessile* on live oak, sabal palm and water oak blocks in Study 1, while *G. sessile* grew 4–5 times as fast as *G. meredithiae* on MEA. Nutritional content of wood, water availability and oxygen content can influence decay rates of wood decay fungi, which could influence the growth rates of *G. meredithiae* (Schwarze et al., 2013).

Adaskaveg and Gilbertson (1986a) showed that different taxa of *Ganoderma* could decay wood types that the fungus rarely or never was observed on in nature For example, they showed *G. tsugae,* a taxon typically found decaying hemlock trees in the temperate Eastern and Midwestern United States, was able to decay grape and silver leaf oak, although *G. tsugae* was never reported on these hosts in nature (Adaskaveg and Gilbertson, 1986a). Similarly in this study, most *Ganoderma* taxa were able to decay all woods tested, although

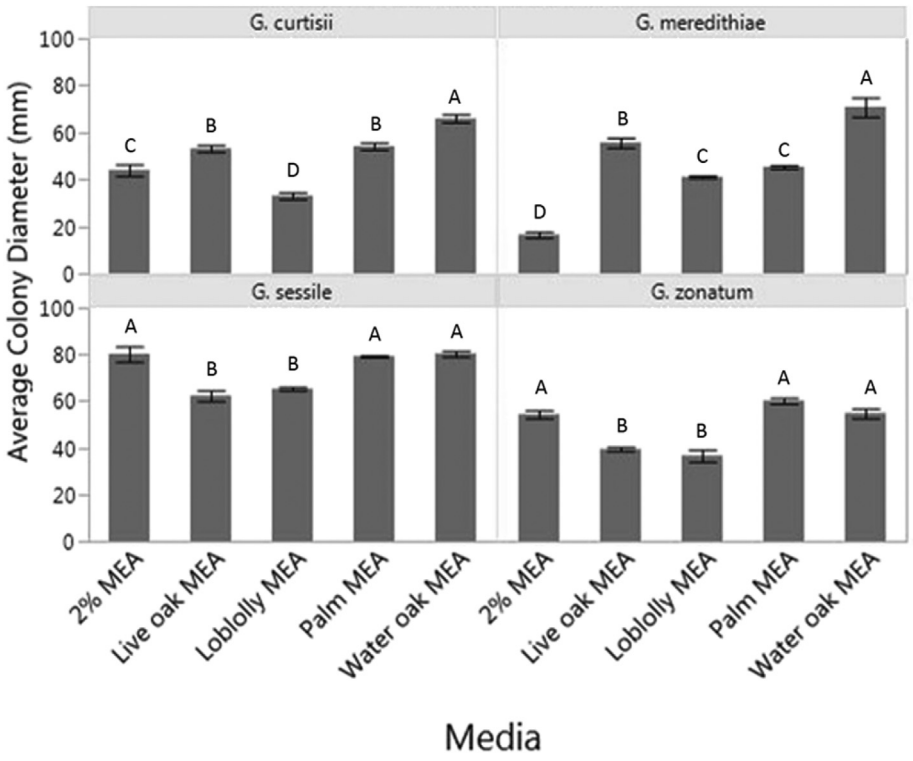

Fig 8. Average colony diameter of *Ganoderma* species grown on different media amended with water-soluble wood extracts from four wood types. Error bars represent the standard error of the mean and different letters represent means separation scores using Tukey's HSD for each *Ganoderma* species independently across the five growth media.

observations of some of the taxa on all wood types in nature was rare or never observed. Although SEM analysis showed a simultaneous degradation of live oak cells with a pattern of decay similar to the other *Ganoderma* taxa, there was significantly less mass loss with *G. zonatum*. The transverse sections of the wooden blocks were from the exterior surfaces of the decayed blocks. No degradation of the internal live oak cells was apparent. Overall, *G. zonatum*, a species found predominately decaying palm wood, was unable to decay live oak wood, the densest wood in this study. Southern live oak trees (*Q. virginiana*) are found naturally in bottomland hardwood forests along the Southeastern U.S. and Gulf Coast and have a geographic range that overlaps with the cabbage palm (*S. palmetto*) (Broschat, 1993). Furthermore, the forests that include Southern live oaks and cabbage palms also include water oak (*Q. nigra*), and *G. zonatum* caused on average 45 % mass loss of water oak blocks in the *in vitro* decay assays in Study 1 and 2. Considering this information, it is probable that natural "barriers" prevent *G. zonatum* from decaying non-palm substrates in nature, whether the decay potential is present or not. These "barriers" could include physical and chemical properties of wood that prevent establishment of the fungus in the wood (Wong et al., 1983). For example, the water-soluble wood extracts produced by the live oak wood had a negative effect of growth of *G. zonatum* isolates, and similarly, the high density of the live oak wood could have negatively influenced the ability for *G. zonatum* to decay this wood due to a greater concentration of phenolic compounds and a larger surface area of wood (Scheffer, 1973). Chemical properties and density of wood have been shown to influence decay in previously studies (Scheffer, 1973; Wong et al., 1983; Shigo, 1985).

In addition to physical and chemical properties of wood, there are also likely differences in the competitive ability of *Ganoderma* species to other decay fungi on certain substrates. Competition for allocation of a substrate by wood degrading fungi can occur in several ways including: chemical antagonism, mycoparasitism, and biological incompatibility (Boddy, 2000). All of these strategies can result with the displacement of a "vulnerable" fungus, or the hindrance of normal physiological function of a given fungus (Boddy, 2000).

Ganoderma taxa are capable of causing a similar white rot that simultaneously degrades all cell wall components on different types of wood, and there appears to be different susceptibility levels across wood types. Micromorphological results also demonstrate that certain cell types can affect decomposition. Vessel elements resist attack even when they are colonized by hyphae of *Ganoderma* and after adjacent cells have often been degraded. Differences in the chemical and physical structure of vessel elements appear responsible for this selective degradation (Blanchette et al., 1988). The unusual attack of thick walled fibers in oak and sclerenchyma cells in palm by *G. zonatum* resulting in the penetration of secondary walls to produce cavities is a decay characteristic not observed for other *Ganoderma* species. Cavity formation in cells walls is a characteristic found with soft rot fungi and it is rare to find fungi in the Basidiomycota causing this type of attack (Eriksson et al., 2012; Schwarze et al., 2013). The results presented in this study show cavities that are formed by *G. zonatum* and the progressive stages of cell wall attack appear somewhat similar to those typically produced by Ascomycota that cause a type 1 soft rot. However, in our study, this type of attack occurs only in cells that have very thick secondary walls and likely have extractives in cell lumina that restrict growth and fungal activity. The molecular basis for what triggers different types of decay by fungi is an important area of study that is just beginning to be better understood (Floudas et al., 2012; Riley et al., 2014). *Ganoderma* species such as *G. zonatum*, are unusual among white rot fungi with their ability to use several different mechanisms to decay wood. The information presented here provides new knowledge on the

diverse fungal decomposition strategies produce by *Ganoderma* and provides a framework of information useful to better understand the biology and ecology of these important taxa. Relative differences of decay progression of four *Ganoderma* species across four wood types were observed, but because the studies were *in vitro* and not conducted in the field, some caution is warranted in interpreting the results. Since *Ganoderma* species are primary decay fungi and can be found decaying living trees, pathogenicity tests are needed to determine if *Ganoderma* species occur as pathogens. In addition, further studies focusing on better understanding the life cycle and ecology of *Ganoderma* species are needed, specifically the potential for an endophytic life cycle (Martin et al., 2015).

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