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Mycobiota associated with insect galleries in walnut with thousand cankers disease reveals a potential natural enemy against *Geosmithia morbida*

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ABSTRACT

Thousand Cankers Disease (TCD) affects *Juglans* and *Pterocarya* species. This disease poses not only a major threat to the nut and timber industries but also to native stands of walnut trees. Galleries created by *Pityophthorus juglandis* (vector) are colonized by the fungus *Geosmithia morbida* (causal agent of necrosis). It is unknown if other fungi colonizing these galleries might act antagonistically towards *G. morbida*. The objectives of this study were to: (1) characterize the fungal community associated with TCD-infected trees and (2) develop a pilot study addressing their potential antagonism towards *G. morbida*. We collected non-*Geosmithia* fungi from ten TCD-infected walnut trees from California and Tennessee. Four hundred and fifty-seven isolates, representing sixty-five Operational Taxonomic Units (99 % ITS similarity) were obtained. Fungal communities were found to be highly diverse. *Ophiostoma* dominated the communities associated with TCD-compromised trees from California, whereas *Trichoderma* dominated TCD-compromised trees in Tennessee. Six *Trichoderma* isolates showed varying levels of antagonism against three isolates of *G. morbida*, suggesting potential applications for the biological control of TCD. Furthermore, results from this study contribute to the growing knowledge about the observed differential disease development between the western and eastern USA and could overall impact our understanding of TCD etiology.

1. Introduction

Increasing recognition about the importance of the "bark beetle holobiont," defined as the insect and all of its associated microbes, is expected to provide integrative approaches to enhance understanding of bark beetle-associated diseases (Six, 2013). Microbial symbionts can facilitate or limit the establishment of invasive beetle species, thereby influencing the severity of the damage that infestations inflict on trees and ecosystems (Bennett, 2013). For instance, microbial symbionts can act synergistically to overcome host plant defenses (McPherson et al., 2013). The economic and ecological significance of conifer-infesting ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) and associated host plant losses has led to research emphasis and effort in understanding the mycobiota associated with these beetles (Raffa et al., 2008; Kirisits, 2010; Giordano et al., 2013). Still, the complete community of associated fungi has been characterized for only few of the ca. 3500 ambrosia beetle species described worldwide (Biedermann et al., 2013). Even less is known about the fungal community associated with bark-inhabiting beetle species, which in most cases have little to no economic impact due to their preference towards weakened, stressed and recently killed trees (Paine et al., 1997). Many studies have taken a "vector-centric" approach, in which insects are screened for fungi through culture-dependent or culture-independent methods (Six and Bentz, 2003; Giordano et al., 2013; Reed et al., 2013). An alternative approach is to characterize the

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fungi living within insect-created galleries and associated lesions. These fungi may be more metabolically active because they would have access to a constant supply of nutrients such as decaying wood (Stokland et al., 2012) and therefore would better represent the cohort of fungi that beetles interact with during their life cycle. Consequently, beetle galleries and bark-associated lesions represent a largely unexplored niche, especially those systems associated with hardwood host species. These niches could disclose novel fungal species and symbiotic associations. For instance, Endoh et al. (2011) found up to 18 potentially novel species while studying the fungi inhabiting the galleries of the oak ambrosia beetle *Platypus quercivorus* Murayama (Coleoptera: Curculionidae, Platypodinae). Similarly, Kolařík et al. (2017) reported 13 potentially new species of *Geosmithia* from a diverse array of bark beetle and woodborer galleries on hardwoods in California and Colorado, USA.

Thousand Cankers Disease (TCD) affects walnut and butternut trees (*Juglans*) and several wingnut tree (*Pterocarya*) species (Utley et al., 2013; Hishinuma et al., 2016). The insect vector, *Pityophthorus juglandis* Blackman (walnut twig beetle, WTB) (Coleoptera: Curculionidae, Scolytinae) creates galleries in the host phloem, inoculating the fungal pathogen, *Geosmithia morbida* (Ascomycota: Hypocreales, Bionectriaceae) M. Kolařík, E. Freeland, C. Utley, & N. Tisserat. *G. morbida* causes localized phloem necrosis resulting in small cankers that coalesce and eventually induce stem dieback and ultimately tree mortality (Kolařík et al., 2011). *Juglans* species, especially black walnut, *Juglans nigra*, are highly valuable economically and ecologically, due to their lumber quality, nut production, and importance in urban and natural landscapes (Vander Wall, 2001; Victory et al., 2006; USDA-FS-PPQ, 2017). Black walnut is highly susceptible to TCD resulting in death a few years after the first symptoms or signs appear (Kolařík et al., 2011; Tisserat et al., 2011; Seybold et al., 2013; Griffin, 2015). Originating in the western USA, TCD has expanded into the eastern USA, as well as into Europe (Italy) (Montecchio et al., 2015b, 2016; Daniels et al., 2016; Seybold et al., 2016).

Also native to the western USA, adult WTB are approximately 1.5–2 mm long, phloem-feeding bark beetles that reproduce within species of *Juglans* and *Pterocarya* (Seybold et al., 2013, 2016; Hishinuma et al., 2016). To gain access to host phloem, beetles penetrate the outer bark of the branches and main stem, creating push-pin-sized holes. Upon entering the tree, male beetles produce an aggregation pheromone that elicits a mass attack on the host tree. Female WTB join male colonizers in the phloem where together they construct a transversely oriented gallery system used for feeding, mating, oviposition, and overwintering (Seybold et al., 2016). Adult WTB may mate or overwinter in portions of excavated tunnels other than their natal galleries, so WTB populations interact with different fungal communities inhabiting the galleries that they visit throughout their life cycle.

Wood offers poor nutrition to insects because they are generally unable to digest the lignin, cellulose, and hemicellulose components that make up xylem tissues (Dadd, 1970; Geib et al., 2008). In contrast, the richer nutrition source presented by the phloem means that phloem-feeding bark beetles may be less dependent on their fungal associates (Ayres et al., 2000; Kirisits, 2004). Nevertheless, for some phloem-feeding beetles, phloem tissues remain comparatively low in nitrogen and usable sterols, and therefore fungi can serve as a complementary source of nutrients (Six, 2013). Symbiotic fungi are able to access the sapwood, where nitrogen is stored, and translocate it to the phloem where larval- and adult beetles feed (Stokland et al., 2012). Beetles with this feeding habit usually possess mycangia (specialized fungal carrying structures) and exhibit specific associations with a limited set of fungi (Six, 2012). WTB are not known to have mycangia, but they have been associated consistently with *G. morbida* (Kolařík et al., 2011, 2017), although the details of this association in terms of exchange of benefits remains unclear (Zerillo et al., 2014). It is also unknown if WTB has facultative associations with other fungi, which can affect the population fitness of the beetles and consequently TCD incidence. So far, a limited number of studies looking at fungi carried by WTB have reported a limited fungal diversity. Daniels et al. (2014) reported four lineages of *Fusarium solani*; Nguyen (2015) reported two non-pathogenic *Geosmithia* species; and Kolařík et al. (2017) reported four *Geosmithia* species, besides *G. morbida*, from WTB galleries.

The U.S. National Fungus Collections Fungus-Host Database includes 1646 fungal records associated with *Juglans* spp. and 238 associated with *J. nigra* alone (Farr and Rossman, 2017). However, only one study has looked at the fungal communities in TCD-infected black walnut. Results from this survey showed a relative high diversity of fungi co-inhabiting WTB-galleries, with 70 fungal species reported from TCD-infected trees in Tennessee, USA (McDermott-Kubeczko, 2016). In addition to the importance of identifying which fungi co-inhabit WTB galleries and their potential involvement in disease development, this knowledge can also serve as a framework to understand *G. morbida* virulence itself. Lateral transfer of virulence-related genes have been reported between *Ophiostoma novo-ulmi* and *Geosmithia* species (Bettini et al., 2014). Furthermore, lateral genetic exchange is frequently reported among *Fusarium* species (fungal genus frequently isolated from TCD-associated lesions) (Montecchio et al., 2015a; Sitz et al., 2017) and other fungal genera (Khaldi and Wolfe, 2011; Mehrabi et al., 2011; Gluck-Thaler and Slot, 2015). Therefore, having a better knowledge of which fungal species *G. morbida* come in close contact with is a vital variable to take into account when investigating the emergence of new and more virulent strains.

Several fungal lineages that have antagonistic properties towards plant pathogens and/or insect pests, may inhabit galleries of pest vectors of plant diseases (Kirisits, 2010; Endoh et al., 2011; McPherson et al., 2013). However, few studies have evaluated the use of associated fungi as biological control agents. For instance, Díaz et al. (2013) reported a strong *in vitro* antagonistic interaction between *O. novo-ulmi* (causal agent of Dutch elm disease) and an endophytic *Trichoderma* strain isolated from an infected- but relative healthy-elm tree. Approaches to screen candidate biological control agents may be optimized by emphasizing fungal associations that occur within the native range of the host plant or pest. For example, species of endophytic *Trichoderma* isolated from wild *Theobroma* spp. (cacao and relatives), *Coffea arabica* (coffee), and *Hevea* spp. (rubber tree and relatives) have been shown to have biocontrol properties against common diseases found in plantations of these plant species (Evans et al., 2003; Bailey et al., 2008; Mulaw et al., 2013; Gazis and Chaverri, 2015). Despite the devastating impact already caused by TCD in the western USA and the high susceptibility of *J. nigra* (Tisserat et al., 2009, 2011), no studies so far have tested indigenous or specialized fungi to control this disease.

To increase our understanding of long-recognized and recently discovered disease outbreaks on hardwood species, such as in elms (Dutch elm disease), oaks (sudden oak death and foamy bark canker disease), and walnut/wingnut (TCD), we need to acknowledge the fungal communities residing in the galleries created and inhabited by the disease vectors. Understanding the community complexity might provide a more holistic view of disease etiology and development, and can potentially hold the key to disease management. Therefore, the objectives of this study were 1) characterize the fungal community associated with TCD-infected trees in the eastern and western USA, and 2) to assay selected indigenous and specialized fungi to assess preliminary interactions between these candidate biological control agents and *G. morbida* isolates.

We did not attempt to evaluate the factors influencing the differences in fungal assemblages, but we characterized these differences from a taxonomic and ecological perspective.

2. Materials and methods

2.1. Collection of TCD-symptomatic branches and isolation of fungal isolates

Ten TCD-symptomatic walnut trees from California (CA, n = 5) and Tennessee (TN, n = 5) were used to obtain fungal cultures (Table 1). Samples were obtained, handled and shipped following regulations in USDA permits P526P-14-04158 and P526P-15-03500. One to three branches (ca. 5–10 cm diameter and 15–45 cm long) were cut from each tree and stored in a refrigerator (4 °C) until processed. Branches were peeled with a sterilized scalpel to expose galleries and lesions. For each tree, forty subsamples of branch tissue (inner wood) consisting of an approx. 5 × 2 mm section of both healthy and necrotic phloem and cambium tissue were excised from the margins of lesions and insect galleries. Morphology of the necrotic lesions and galleries matched descriptions by Kolarik et al. (2011) and Nix (2013). Subsamples were recorded and maintained by location and by tree, with inner wood pieces plated directly in Petri dishes containing ½ strength Difco™ Potato Dextrose Agar (PDA) amended with antibiotics (1 % chlorotetracycline-streptomycin sulfate solution). Plates were incubated at 25 °C under 12 h fluorescent light and observed daily for fungal growth. Fungal colonies growing out of the wood pieces were further isolated until axenic cultures were obtained.

2.2. DNA extraction, amplification, and sequencing

Individual isolates were grown in Difco™ Potato Dextrose Broth (PDB) at 25 °C for up to 2 weeks. Mycelium was harvested from each liquid culture, dried, and lysed with a Bead Mill 24 homogenizer (Fisher Scientific, Pittsburgh, PA, USA). Genomic DNA was extracted by using Thermo Scientific Gene Jet Genomic Purification Kit (Fisher Scientific), according to manufacturer's protocol with few modifications, which included an increase in proteinase K (20 mg/ml) up to 40 μL/sample and an extended overnight incubation at 56 °C.

The Internal Transcribed Spacer (ITS) region of the ribosomal RNA operon was amplified by using the ITS primers ITS1F (Gardes and Bruns, 1993) and ITS4R (White et al., 1990). PCR reactions were assembled as follows: 12.5 μL GoTaq®G2 Hot Start Master Mix (Promega Corp., Madison, WI, USA), 1.25 μL 10 mM reverse primer, 1.25 μL 10 mM forward primer, 1 μL dimethyl sulfoxide (DMSO, Sigma–Aldrich, St Louis, MO, USA), 1 μL of genomic DNA (~25 ng/μL) and double-distilled water to complete the total volume of 25 μL. The thermocycler protocol for the amplification of the ITS region was as follows: 94 °C for 2 min followed by 15 cycles of denaturation at 94 °C for 30 s, annealing at 65 °C for 30 s and primer extension at 74 °C for 1 min; followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s and primer extension at 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. PCR products were cleaned and sequenced at MCLAB laboratories (www.mclab.com). Sequencher TM 4.9 (Gene Codes Corp., Ann Arbor, MI, USA) was used to assess the quality of the chromatograms and assemble the strands into contigs. DNA sequences of representative isolates were deposited in GenBank (Table S1).

2.3. Identification of fungal isolates and phylogenetic analysis of noteworthy taxa

ITS sequences were assigned to taxa based on the results of the Basic Local Alignment Search Tool (BLAST), using the NCBI nucleotide database (www.ncbi.nlm.nih.gov/BLAST). BLAST searches were conducted by using the following settings: exclude uncultured/environmental sample sequences and limit the search to sequences from type material. For queries in which less than 98 % identity were obtained under these parameters, a non-restricted BLAST search was conducted. Additionally, *Fusarium* sequences were compared against FUSARIUM-ID (http://www.fusariumdb.org/) and basidiomycetes against UNITE (https://unite.ut.ee/) nucleotide databases. Due to the importance of the following genera: *Fusarium*, *Sporothrix* (=*Ophiostoma*), and *Trichoderma*, phylogenetic analyses were conducted to determine a more accurate placement of these isolates and their relationship to known pathogenic or mycoparasitic species.

For the phylogenetic analysis, individual datasets were built for each of the genera (Table S2, including GenBank accession numbers and references). Sequences generated from type specimens or from voucher cultures were used as references and these sequences were chosen to include as many representatives of closely-related fungal species as possible. In the case of *Trichoderma*, we also included sequences from strains used in biological control of plant pathogens, some of which are commercially available. Sequences in each dataset were aligned with MAFFT 7 online (http://mafft.cbrc.jp/alignment/server/ (Katoh and Standley, 2013), by using the E-INS-I strategy (Katoh and Toh, 2008). Flanking regions were trimmed in Mesquite 3.2 (Maddison and Maddison, 2017) and phylogenies were estimated under maximum likelihood by using raxmlGUI 1.3.1 (Silvestro and Michalak, 2012) with 1000 bootstrap replicates and the GTR + GAMMA model.

2.4. Ecological analyses

To estimate species diversity, Operational Taxonomic Units (OTUs) were delimited with the average neighbor algorithm implemented in MOTHUR 1.39.1 (Schloss et al., 2009). OTUs used in downstream analyses were assigned with a 99 % ITS sequence similarity threshold, which has been recommended for widespread

Table 1
Collection data for infected *Juglans* spp. branch sections used in this study.

Tree code	Location	GPS coordinates	Collector	Walnut species or hybrid	Number of isolates recovered
TN1	Blount Co., TN	35°44′25.6″N, 83°58′32.8″W	W. Klingeman	*J. nigra*	69
TN2	Knox Co., TN	36°04′25.2″N, 83°51′33.0″W	W. Klingeman	*J. nigra*	64
TN3	Rhea Co., TN	35°48′66″N, 85°02′05.4″W	E. Ören	*J. nigra*	71
TN4	Marion Co., TN	35°25′62.6″N, 85°44′90.3″W	E. Ören	*J. nigra*	53
TN5	Sequatchie Co., TN	35°19′04″N, 85°26′14″W	E. Ören	*J. nigra*	51
CA33	Tehama Co., CA	39°823′7.63″N, 122°63′01.93″W	S. Seybold	*J. hindsii*	13
CA34	Humboldt Co., CA	40°97′62.1″N, 123°64′10.92″W	S. Seybold	*J. hindsii* x *nigra*	35
CA35	Tehama Co., CA	40°25′94.1″N, 122°47′37.27″W	S. Seybold	*J. hindsii*	24
CA36	Sonoma Co., CA	38°27′82.08″N, 122°46′13.74″W	S. Seybold	*J. hindsii*	21
CA37	Los Angeles Co., CA	34°19′17.5728″N, 118°30′01.27″W	S. Seybold	*J. californica*	56

and highly diverse fungal genera as the ones recovered in this study (Gazis et al., 2011). Due to the difference in the number of isolates obtained from CA vs. TN (149 vs. 308, respectively), individual-based (=isolates) species accumulation curves were charted to compare their diversity. Curves were also extrapolated to determine the sample size at which the accumulation of species would reach an asymptote. To accomplish both tasks, EstimateS 9.1 (Colwell, 2013) was used with a basis of MOTHUR 99 % ITS sequence similarity OTU grouping and 1000 randomizations performed without replacement.

2.5. Antagonism assays

The antagonistic activity of selected *Trichoderma* isolates obtained from TN samples against *G. morbida* isolates from both states was assessed in our pilot study. Antibiosis assays followed a standard "dual plate" design (Gazis and Chaverri, 2015) and were conducted twice, once using PDA and once using Difco™ Malt Extract Agar (MEA) because biocontrol activity has been shown to depend on the medium used (Naher et al., 2015). The following *G. morbida* isolates were tested: GM10 (TN), GM17 (TN), and GM34-7 (CA) against the following *Trichoderma* isolates: TN1-15, TN1-66, TN3-46, TN4-40, TN4-47, and TN5-34. In both dual plate assays, a 5 mm^2 hyphal plug was cut from the edges of actively growing colonies of selected 1 week-old *Trichoderma* cultures. The hyphal plug was then inoculated equidistantly at the margin of PDA or MEA Petri dishes (90 mm) containing 6 week-old *G. morbida* (Fig. 1). Older *G. morbida* cultures were used to account for its slow growth rate in comparison to *Trichoderma*. Plates containing only *G. morbida* isolates were maintained as controls. Plates were incubated at 25 °C, observed and photographed after 8 and 15 d, and final data collected and scored 15 d after inoculation. The slow, irregular, and stunted growth of *G. morbida* prevented a measurement of percent growth inhibition, therefore the degree of antagonism was scored based on a scale of 1–4 to reflect the challenges imposed by the *in vitro* growth of *G. morbida* (Fisher et al., 2013; Ören et al., 2018). The scale is a modification of the one proposed by Bell et al. (1982): *Trichoderma* completely overgrew the pathogen and covered the entire Petri dish, resulting in sporulation over the *G. morbida* colony (1); *Trichoderma* completely overgrew the pathogen and covered the entire Petri dish but no sporulation was observed (2); *Trichoderma* grew over most of the Petri dish, colonies came into contact (3); and *Trichoderma* grew over at least two-thirds of the Petri dish, but there was a "halo" not colonized by either organism (4). An isolate of *Trichoderma* was considered to be antagonistic to the pathogen if its final score (=median) across the interaction assays at d 15 was ≤2, but not highly antagonistic if it was >2 (Fig. 2).

3. Results

3.1. Diversity and composition of the fungal community associated with TCD-symptomatic walnut trees

Four hundred subsamples (40 sub-samples/tree; ca. 5 × 2 mm wood pieces) from 10 TCD-symptomatic CA and TN trees were evaluated, and 457 non-*G. morbida* fungal isolates were recovered. The 457 isolates collected were classified into 65 OTUs, based on 99 % ITS nuclear ribosomal DNA sequence similarity clustering, which here are treated as putative species. California samples yielded 27 OTUs, whereas TN samples yielded 41 OTUs (Table S1). Because the number of isolates recovered was disproportional, with 149 isolates from CA and 308 from TN, further comparisons of diversity are derived from species accumulation curves based on the number of individuals (= isolates). Curve comparisons revealed that the sampling was incomplete at both locations (i.e., curves did not reach an asymptote). Extrapolation of these curves, based on the observed data, suggests that at least 850 (CA) and 800 (TN) isolates would have been needed to yield complete sampling cohorts. However, based on this restricted sample set, the surveyed communities contain comparable diversity (95 % confidence intervals overlapped) in terms of number of OTUs (Figure S1). Both communities present a common species distribution pattern that can be found in highly diverse communities (Fig. 3), in which a small set of the taxa can be considered relatively abundant (abundance ≥ 10), whereas the majority of the others are present in low abundance or are even singletons (12 in CA/11 in TN) and doubletons (13/3).

Although community diversity seems to be comparable at both locations, the taxonomic composition and abundance of the

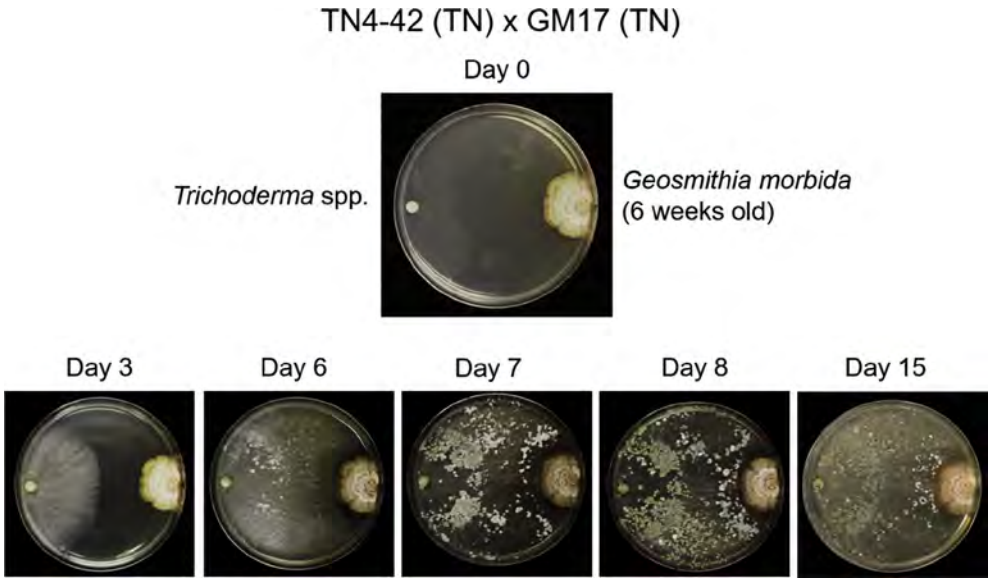


Fig. 1. Protocol followed in antagonistic assays of various *Trichoderma* spp. with *Geosmithia morbida* cultures obtained from Tennessee and California infested trees.

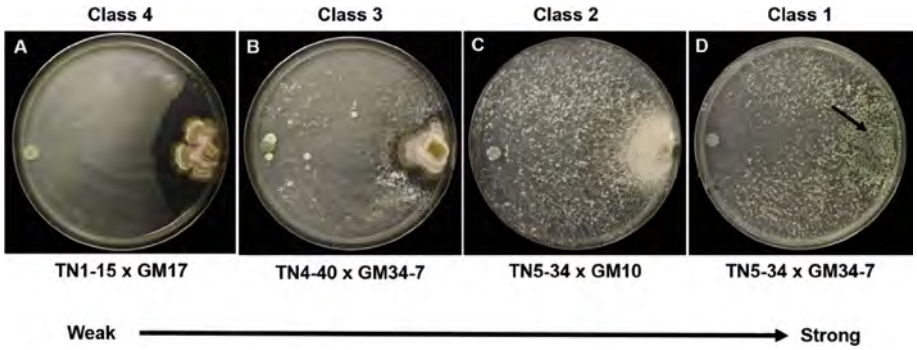


Fig. 2. Antagonism scale (1–4), from weak (4) to strong interaction (1) between *Trichoderma* spp. and *Geosmithia morbida*. Arrow indicates sporulation of *Trichoderma* isolate over *G. morbida* colony.

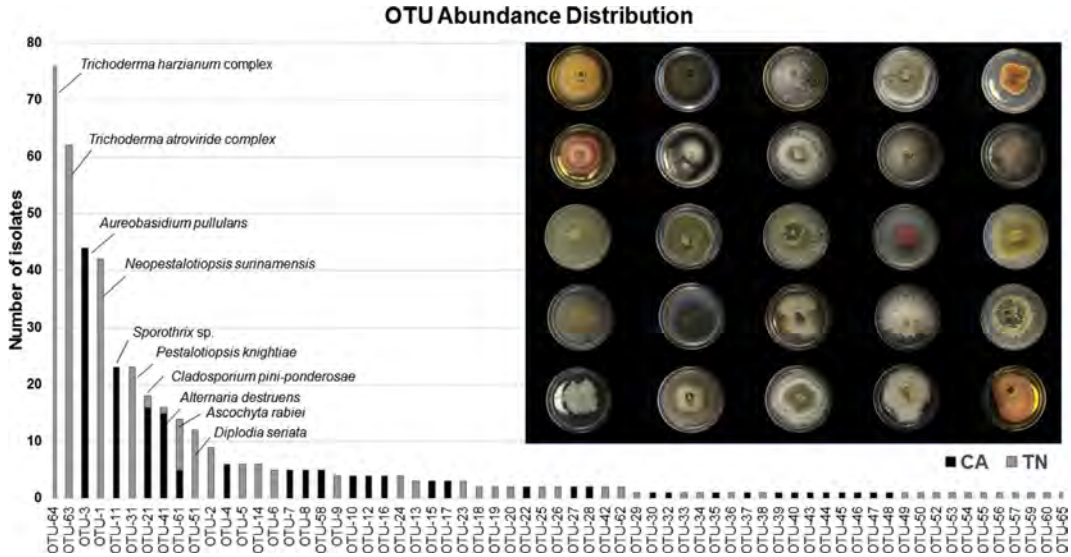


Fig. 3. Bar graph showing the Operational Taxonomic Unit (OTU) abundance in California (black) and Tennessee (grey) samples from TCD-infected *Juglans* spp. branch sections. OTUs with an abundance of 10 or more isolates are labeled with the most likely species name. Inset picture shows a variety of isolates collected in this study.

assemblages varied. The majority of the isolates (abundance ≥ 10) recovered from CA samples were tentatively identified as the following: *Aureobasidium pullulans* (44), *Sporothrix* sp. (23), *Cladosporium pini-ponderosae* (16), and *Alternaria destruens* (15) (Fig. 3). On the other hand, the majority of the isolates (abundance ≥ 10) recovered from TN samples were tentatively identified as: *Trichoderma harzianum* complex (76), *Trichoderma atroviride* complex (62), *Neopestalotiopsis surinamensis* (42), *Pestalotiopsis knightiae* (23), and *Diplodia seriata* (12). Low community similarity was found between CA and TN, with only 3 OTUs overlapping (*C. pini-ponderosae*, *A. destruens*, and *Ascochyta rabiei*) (Fig. 3).

Phylogenetic analysis showed that *Trichoderma* isolates recovered from TN samples belong to at least four clades, with many isolates clustering within the *T. harzianum* and *T. atroviride* complex (Fig. 4). On the contrary, the three *Trichoderma* isolates collected from CA samples clustered together along with strains in the *Trichoderma viridescens* complex. In addition, this analysis revealed the similarity of some of our sequences to the ones generated from commercially available biological control strains and from isolates with demonstrated antagonistic properties towards fungal plant pathogens (Fig. 4, Table S2). Similarly, the phylogenetic analysis of *Fusarium* isolates showed that these belong to different clades, and revealed the genetic similarity among some of the isolates collected in this study and other *Fusarium* isolates associated with disease symptoms in walnut trees in different geographic areas (Fig. 5, Table S2). On the other hand, all *Sporothrix* isolates clustered as one group within the *S. stenoceras* complex-but distinct to other described species within this complex- and showed little to no variation in their ITS sequence (Fig. 6, Table S2).

3.2. Antagonism assays

Challenges while conducting the antagonism assays occurred in relation to extremely slow growth of *G. morbida* in comparison to *Trichoderma* isolates. Growth rate disparities were overcome, in part, by growing *G. morbida* cultures for 6 weeks before exposing cultures to *Trichoderma* isolates (Fig. 1). Besides slow *G. morbida* growth, lobulated colony margins resulting from asymmetrical growth, precluded direct measurements of growth inhibition. To address this challenge, an alternative classification system was developed to evaluate the observed interaction. Six *Trichoderma* isolates selected from our diversity survey of TN samples were tested against three *G. morbida* isolates. Five *Trichoderma* isolates (TN1-15, TN3-46, TN4-40, TN4-47, and TN5-34) were classified in the *T. harzianum* complex (Fig. 4), and TN1-66 was classified in the *T. atroviride* complex. *G. morbida* isolates, GM10 and GM17, were collected from trees in TN (Blount County) as part of our research group's previous study (Hadziabdic et al., 2014) and GM34-7 was

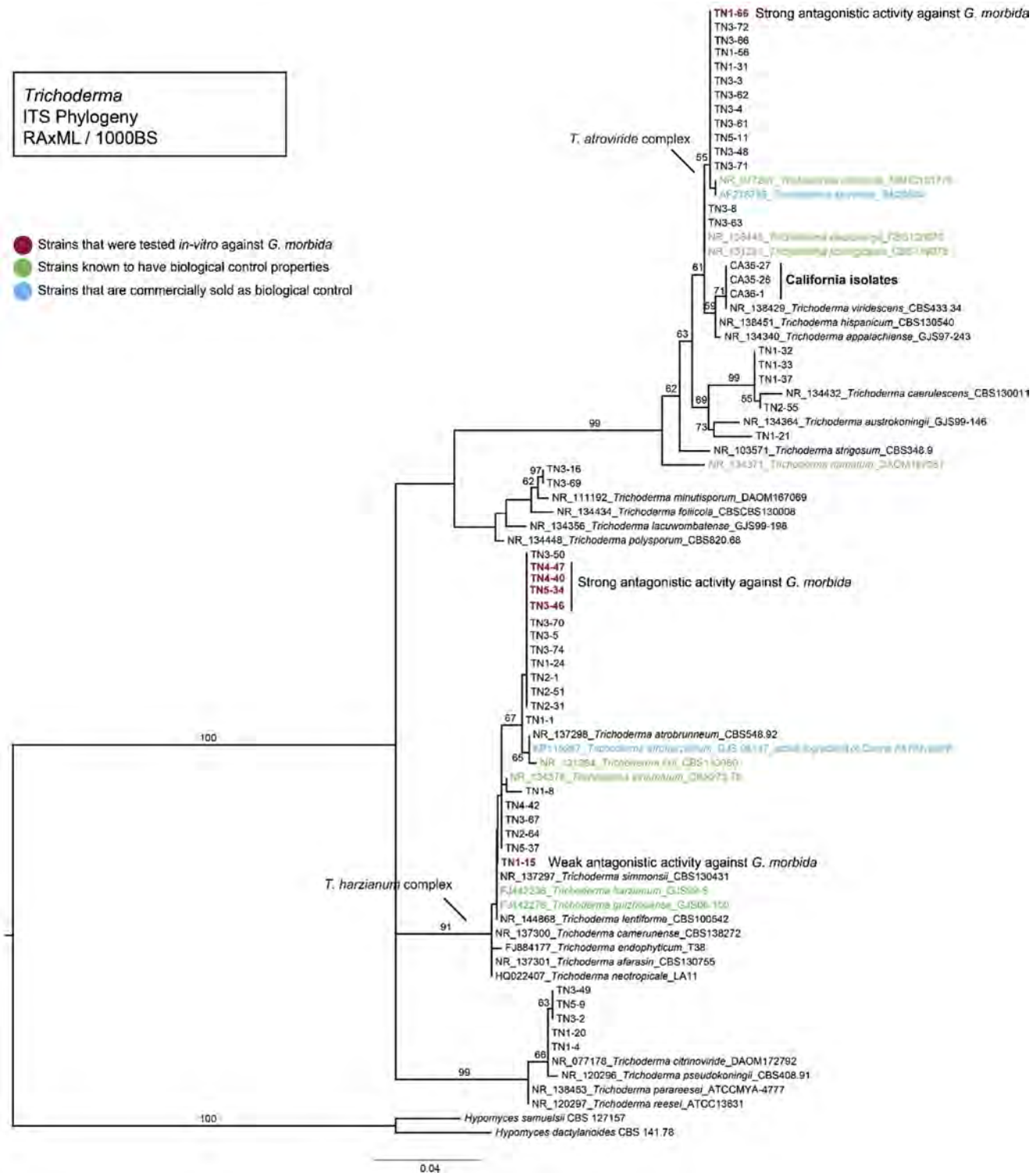


Fig. 4. *Trichoderma* phylogeny of representative isolates collected from TCD-infected *Juglans* spp. branch sections from Tennessee and California and reference sequences. Phylogenetic tree is based on the Internal Transcribed Spacer (ITS) and built under the Maximum Likelihood algorithm with 1000 Bootstraps. Sequences with codes starting with TN indicate isolates from Tennessee and CA indicate isolates from California. *Hypomyces samuelsii* and *H. dactylarioides* were used as outgroup.

isolated from tree CA34 in CA (Humboldt County). Interactions between the *Trichoderma* isolates and *G. morbida* varied when using PDA or MEA as growth medium (Table 2, Figure S2). Antagonistic interactions were stronger in MEA. From the six *Trichoderma* isolates, only one (TN1-15) showed weak antagonistic interaction towards all of the *G. morbida* isolates (average of 3.7 in the antagonism scale, Table 2). The rest of the isolates showed strong antagonistic activity towards most of the *G. morbida* isolates (final score ≤ 2.0). In particular, two of these isolates TN3-46 and TN5-34, showed very strong antagonistic activity (final score ≤ 1, and individual scores of ≤ 1.5) towards all *G. morbida* isolates (Table 2, Fig. 7, S2).

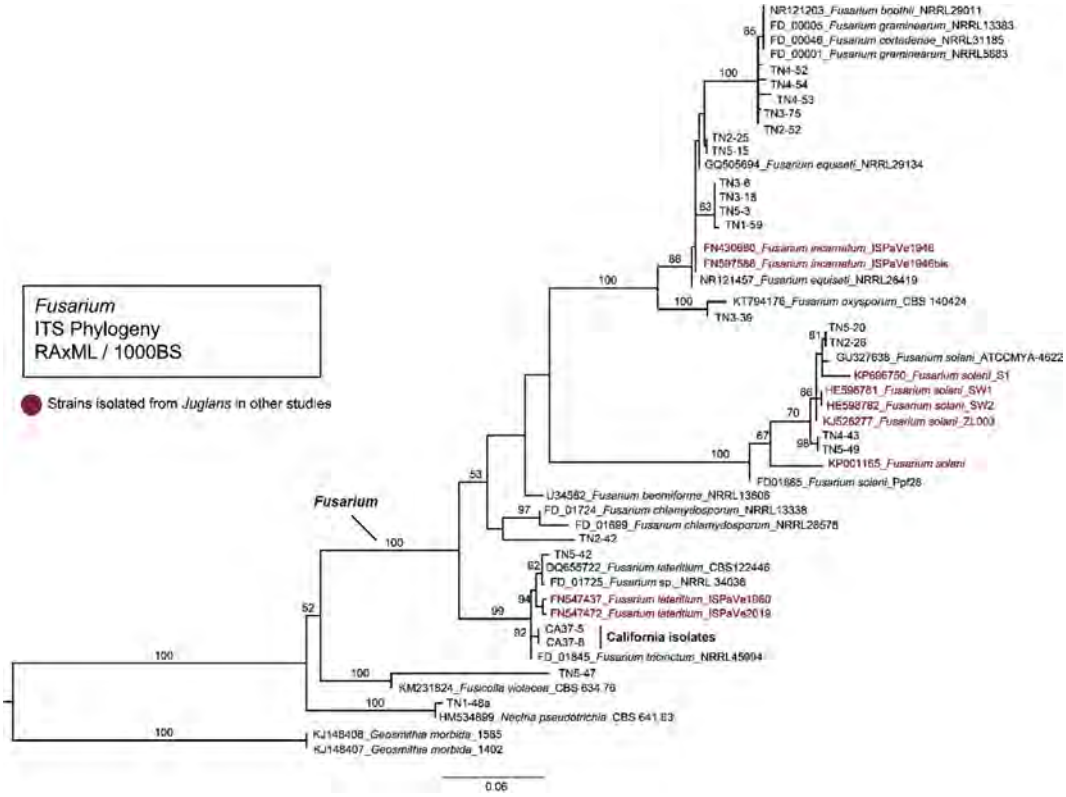


Fig. 5. *Fusarium* phylogeny of the isolates collected from TCD-infected *Juglans* spp. branch sections from Tennessee and California and reference sequences. Phylogenetic tree is based on the Internal Transcribed Spacer (ITS) and built under the Maximum Likelihood algorithm, with 1000 Bootstraps. Sequences with codes starting with TN indicate isolates from Tennessee and CA indicate isolates from California. Two isolates of *Geosmithia morbida* were used as outgroup.

4. Discussion

Fungal community members associated with bark and ambrosia beetles and beetle galleries may on occasion become causal agents of host plant disease, can act synergistically to lower host plant defenses allowing aggressive colonization, and may provide primary or secondary nutritional sources for adult beetles and their larvae (Kirisits, 2004; Six and Wingfield, 2011). These fungal communities associated with beetle galleries have been best characterized for economically important coniferous host plants (Raffa et al., 2008; Kirisits, 2010; Giordano et al., 2013), yet are less thoroughly understood among bark and ambrosia beetles that exploit hardwood species. The community complexity within the galleries that bark and ambrosia beetle species inhabit is poorly understood, as are interactions among the many phloem-feeding bark beetle species and their fungal symbionts, and interactions among fungi that may co-occur within beetle galleries (Ploetz et al., 2013).

The taxonomic spectrum of fungi identified from TCD-infected walnut trees in CA and TN was consistent with that reported by (McDermott-Kubeczko, 2016). There were also overlaps, at the generic and species level, with fungi categorized by studies of beetle galleries in conifers (Kirisits, 2010; Giordano et al., 2013; Strid et al., 2014) and hardwood species (Endoh et al., 2011; McPherson et al., 2013; Ceriani-Nakamurakare et al., 2016). McDermott-Kubeczko (2016) reported a greater number of fungal species associated with TCD-affected trees in TN compared to our study (70 vs. 41). This difference can be explained by a number of factors such as variation in occurrence between sampling sites and time of sampling. However, the most plausible explanations are their larger sample size (12 vs. 5 trees) and the girdling treatment imposed to the majority of their sample trees (9 out of 12), which could increase fungal colonization. Most of the isolates recovered in our samples were *Alternaria*, *Aureobasidium*, *Cladosporium*, and *Epicoccum*, which are ubiquitous endophytes of hardwood trees, including *Juglans regia* (Pardatscher and Schweigkofler, 2009). These genera have also been reported as secondary or weak plant pathogens across an extensive array of host plants (Farr and Rossman, 2017). Although the presence of these fungi can be explained both by their air-dispersal mechanisms and frequently reported endophytic habits, these genera have also been recovered from the exoskeleton of a diverse array of beetles (Six and Wingfield, 2011; Reed et al., 2013; Ceriani-Nakamurakare et al., 2016). It is likely that these fungi are vectored opportunistically by beetles, including WTB. Both our study and that of McDermott-Kubeczko (2016) report isolating *Botryosphaeria dothidea*, *F. solani*, and *Diplodia seriata* from trees in TN, and these species are fungal pathogens of walnut and other hardwood trees in the eastern USA (Farr and Rossman, 2017).

This study was restricted to fungi that could be cultured, so the characterized fungal diversity reported here likely underestimates the full diversity of fungi present in the galleries and lesions associated with TCD-symptomatic trees. Accumulation curves did not stabilize, indicating that more sampling is needed to properly compare the diversity between locations. Only when extrapolated data were expanded to 800–850 individual isolates did the curves start to reach a projected asymptote. The scale of this sampling challenge is typical of highly diverse niches, and is consistent with observations of the endophytic communities inhabiting trunk or leaf tissues of tropical trees (Gazis and Chaverri, 2015). Although accumulation curves suggest that robust comparisons will require more extensive sampling, both the CA and TN communities presented comparable fungal diversity on the basis of observed OTUs

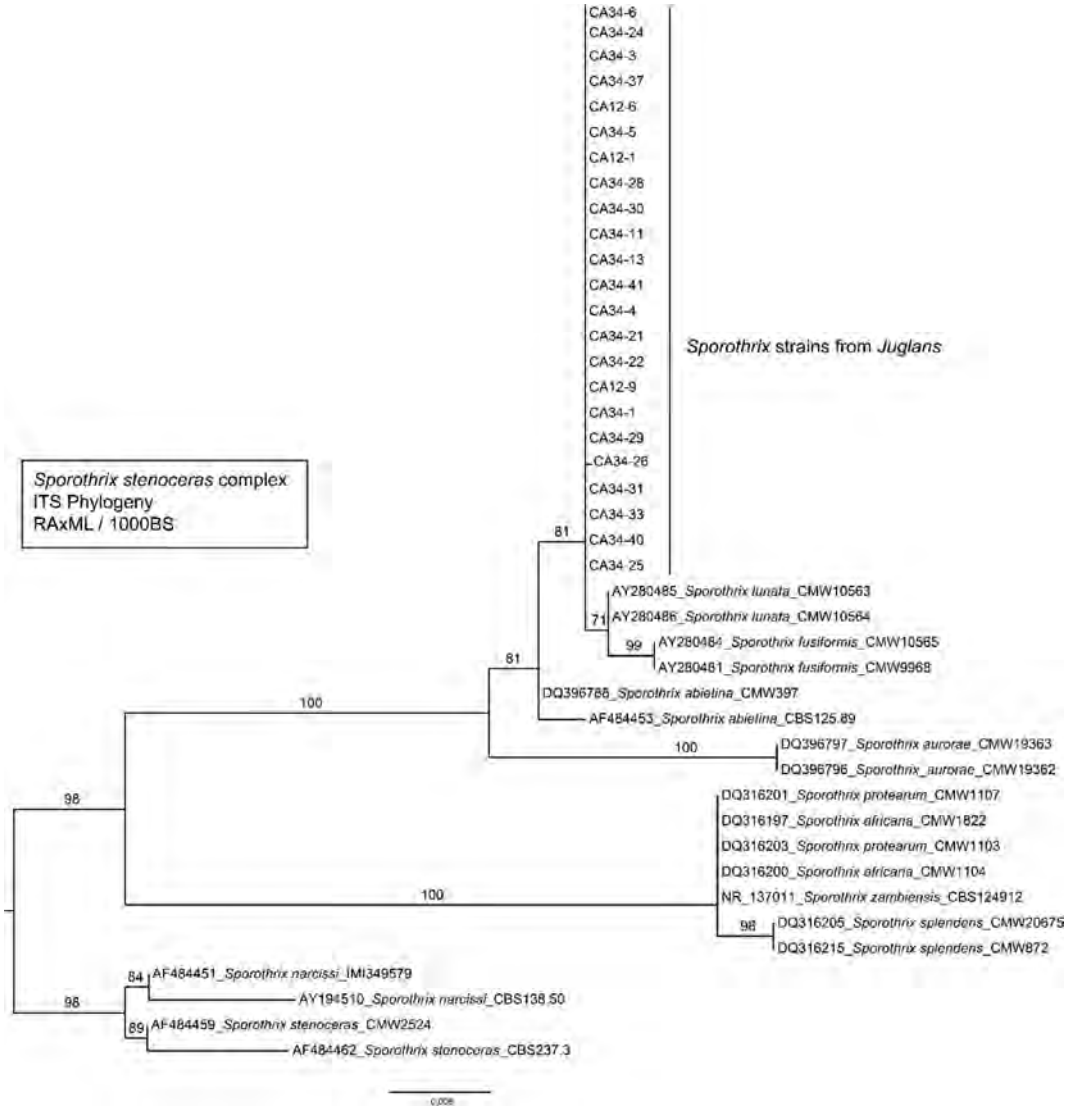
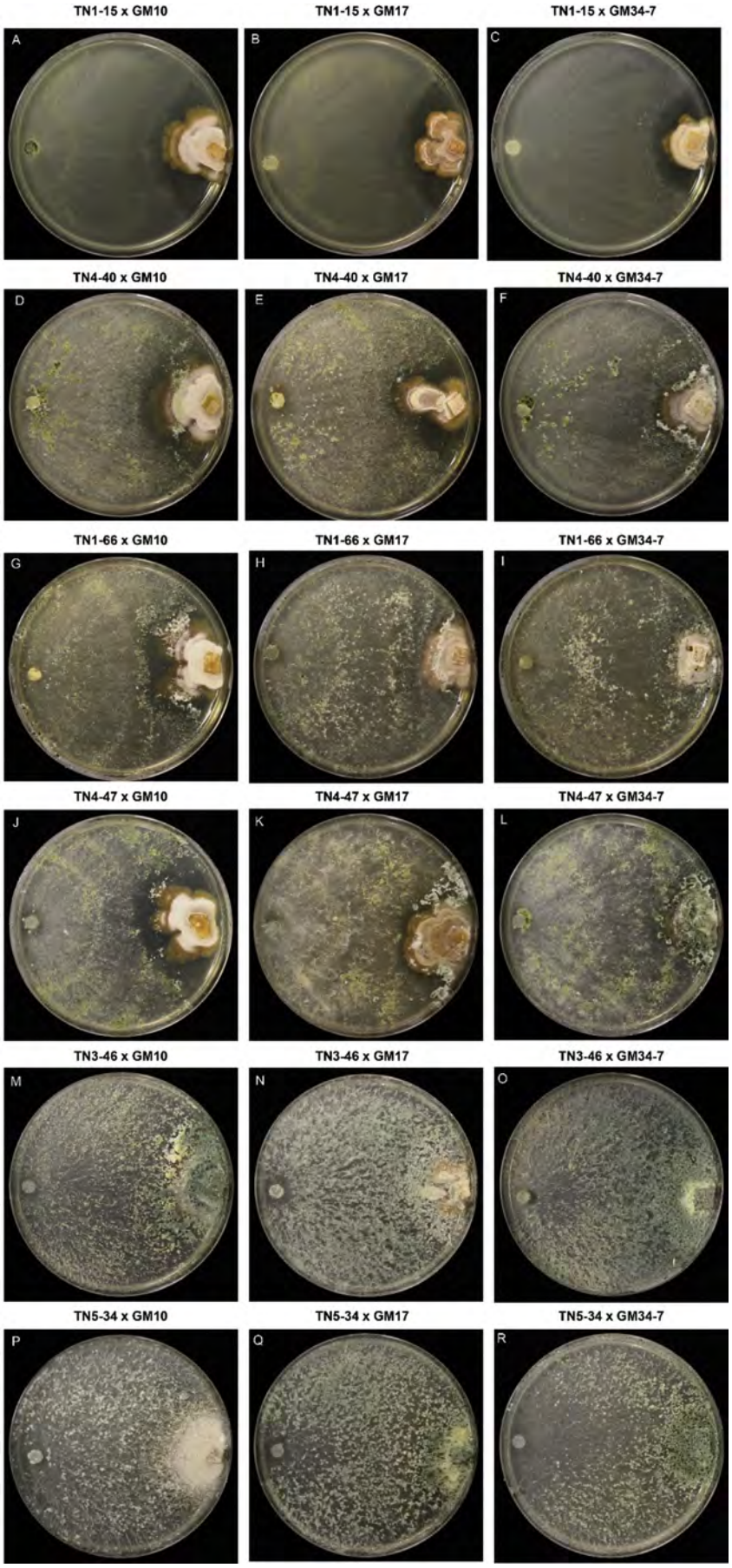


Fig. 6. *Sporothrix* phylogeny of the isolates collected from TCD-infected *Juglans* spp. branch sections from Tennessee and California and reference sequences. Phylogenetic tree is based on the Internal Transcribed Spacer (ITS) and built under the Maximum Likelihood algorithm, with 1000 Bootstraps. Sequences with codes starting with TN indicate isolates from Tennessee and CA indicate isolates from California.

Table 2
Antagonistic assays between *Trichoderma* isolates from Tennessee (TN) and *Geosmithia morbida* (GM) isolates grown on potato dextrose agar (PDA) and malt extract agar (MEA) scored[a] after 15 d from inoculation. Final score includes Min-Median-Max. An isolate of *Trichoderma* was considered to be antagonistic to the pathogen if their median score across the interaction assays was ≤2, but not highly antagonistic if the score was >2.

Trichoderma		GM10	GM17	GM34-7	Final Score
TN1-15	PDA	4	4	4	3-3.75-4
	MEA	3.5	3	3.5	
TN1-66	PDA	1.5	1.5	2	1-1.25-2
	MEA	1	1	1	
TN4-47	PDA	3.5	2	1	1-1-3.5
	MEA	1	1	1	
TN3-46	PDA	1	1	1	1-1-1
	MEA	1	1	1	
TN4-40	PDA	1.5	3.5	1.5	1-1.5-3.5
	MEA	1	1	2	
TN5-34	PDA	1.5	1	1	1-1-1.5
	MEA	1	1	1	

[a] Scale: 1 = *Trichoderma* completely overgrew the pathogen and covered the entire Petri dish, resulting in sporulation over *G. morbida* colony, 2 = *Trichoderma* completely overgrew the pathogen and covered the entire Petri dish-no sporulation, 3 = *Trichoderma* covered most of the Petri dish, colonies came into contact, 4 = *Trichoderma* covered at least two-thirds of the Petri dish but there was an "halo" not colonized by neither organism. Refer to Fig. 2.


TN1-15 x GM10
TN1-15 x GM17
TN1-15 x GM34-7
A
B
C
TN4-40 x GM10
TN4-40 x GM17
TN4-40 x GM34-7
D
E
F
TN1-66 x GM10
TN1-66 x GM17
TN1-66 x GM34-7
G
H
I
TN4-47 x GM10
TN4-47 x GM17
TN4-47 x GM34-7
J
K
L
TN3-46 x GM10
TN3-46 x GM17
TN3-46 x GM34-7
M
N
O
TN5-34 x GM10
TN5-34 x GM17
TN5-34 x GM34-7
P
Q
R

after normalizing for equal sampling effort. Nevertheless, these communities were very different in their taxonomic composition sharing only three OTUs. The low overlap between CA and TN, as well as among the trees within these locations, may reflect the small sample size. Alternatively, the lack of a core fungal set present across samples, in addition to the widespread nature of most of the fungal species reported here, can also suggest that the majority of the fungi recovered are transient components of WTB galleries and adjacent infected phloem tissues. In other words, many of the community members might be generalists in terms of hosts (plant and beetle) and lifestyle (endophytes, saprotrophs, plant pathogens), and could be associated opportunistically with walnut and WTB galleries. Regardless, these associated fungi are expected to influence the insect-fungus-host symbiosis (Silva et al., 2006; McPherson et al., 2013).

Factors related to the environment, host genetic background, disease stage, beetle population levels and age of the galleries, among others are likely to affect the composition of the fungal communities inhabiting TCD-associated galleries (Kirisits, 2004; Biedermann et al., 2013; McPherson et al., 2013). A combination of all of these factors, acting simultaneously, are likely to be driving the composition of the fungal communities targeted in this study. Our sampling included different *P. juglandis*-infested *Juglans* spp. (*J. californica*, *J. hindsii* and *J. nigra*) collected from different types of management regimes (orchards, urban landscapes with low to no pesticide management, and unmanaged, native trees growing in riparian forests). All of these factors could be contributing to the differences in fungal species that we found; however, all these trees are consistent in their susceptibility to TCD and are competent host plants supporting *P. juglandis* reproduction (Hefty, 2016). Here, we did not attempt to evaluate the factors influencing the differences in fungal assemblages, our objective was to characterize these differences from a taxonomic and ecological perspective.

There was a marked difference in the presence and abundance of beetle-associated yeasts, *Fusarium*, *Sporothrix*, and *Trichoderma* recovered between the CA and TN locations. Yeasts in the Saccharomycetales are isolated frequently from bark and ambrosia beetles and from their galleries (Kirisits, 2004; Giordano et al., 2013; McPherson et al., 2013; Davis, 2015). The functional role of yeasts in the beetle–yeast association is largely unclear, but the relationship may be mutualistic, whether by detoxifying plant defense chemicals (e.g., toxic terpenoids), providing nutritional supplements, or by producing volatiles that can attract or repel beetle conspecifics (Kirisits, 2004; Davis, 2015). Beetle-associated yeasts may also restrict or promote growth of the mutualistic (e.g., ambrosia fungi) or antagonistic (e.g., entomopathogenic) filamentous fungi associated with bark beetles (Six, 2013). Yeast species in this study were isolated only from CA samples. Successful isolation of yeasts may reflect methodological constraints imposed by culture-dependent isolation techniques that are optimized for recovery of filamentous fungi. It is also plausible that CA populations of WTB may be interacting with potentially mutualistic yeast species not present-or less abundant-in the eastern USA. If this hypothesis is true, then it is also possible that this symbiotic relationship may be contributing to the comparatively large population sizes of WTB observed in the western USA (Seybold et al., 2016).

At least seven species of *Fusarium* cause cankers on *Juglans* spp. (Farr and Rossman, 2017). Some of these *Fusarium* species have been isolated from TCD-infected walnut trees, including direct isolations from *G. morbida* cankers and lesions (McDermott-Kubeczko, 2016). Although previous studies have suggested that *Fusarium* strains within the *solani* complex play a role in TCD development, contradictory results have been reported. For instance, Montecchio et al. suggested that *F. solani* may act as an early colonizer contributing synergistically to the early stages of TCD development. Reports by Tisserat et al. (2009) and Sitz et al. (2017) have suggested either a minor role for *F. solani* in tree mortality, and no effect, respectively. We report 20 *Fusarium* isolates, 18 of which were obtained from TN trees. Based on our ITS phylogenetic analysis, and acknowledging the limitations of this marker in *Fusarium* species delimitation, the majority (18) of our sequences clustered with representative strains of the following *Fusarium* species complexes: *graminearum*, *incarnatum-equiseti*, *solani* and *tricintum-lateritium*. Few isolates resulted in indeterminate position. Phylogenetic analysis also revealed that our sequences clustered with other *Fusarium* sequences from *Juglans* spp. generated as part of other studies. For instance, four *F. solani* complex sequences from TN were closely related to isolates reported as *Fusarium* phylogenetic clade 25 in Italy (Montecchio et al., 2015a), as well as with other isolates isolated from cankers on *J. regia* in Spain and China (Gil *unpublished GenBank*). Similarly, another group of our isolates fell within the *tricintum-lateritium* species complex and clustered with isolates recovered from bark and pollen of *J. regia* in Italy (Vitale et al., 2011).

Ophiostoma (*sensu lato*) species are cosmopolitan and have been associated with many coniferous and hardwood tree species, yet this is the first time it is reported in association with *Juglans*. Based on ITS similarity, we recovered only one genetically distinct and potentially novel lineage of *Sporothrix* (= *Ophiostoma*) within the *stenoceras* species complex (previously referred as the *Sporothrix schenckii–O. stenoceras* species complex) (de Meyer et al., 2008). This *Sporothrix* lineage was represented by 23 isolates and all were collected from a single tree in CA (CA34, Tehama, *J. hindsii*). Our phylogenetic analysis, using de Meyer et al. (2008) and de Beer et al. (2016) studies as framework, showed that the recovered isolates are closely related, but distinctly different to the clade comprised by *S. lunatum* and *S. fusiforme*. Both of these taxa have been isolated from living inner bark tissue and galleries within hardwood and conifer tree species and are also associated in Austria with *Ips cembrae* bark beetles in *Larix* species (Aghayeva et al., 2004). ITS sequences showed no variation, suggesting that this genotype had extensively colonized the host tree. Beyond the sampled fungal isolates, an additional three isolates of the *Sporothrix* lineage, which were taken from a TCD-infected tree (*J. hindsii*) in Butte CA, were also included in the analysis and these isolates yielded almost identical ITS sequences. These findings suggest that this lineage of *Sporothrix* may be distributed widely in Californian walnut populations.

Most evident was the difference in the abundance of *Trichoderma* between CA and TN communities. A high proportion of the isolates from TN (52 %) were *Trichoderma* isolates, whereas in CA *Trichoderma* species represented only 2 % of the community. *Trichoderma* is known to contain species that can act as antagonists towards a broad variety of fungal pathogens. Antagonism may be accomplished through direct mycoparasitism, production of pathogen-inhibiting secondary metabolites, and by promoting plant vigor (Howell, 2003; Harman et al., 2004). In other tree crops (e.g., *Coffea arabica*, *Hevea brasiliensis*, *Theobroma cacao*), indigenous *Trichoderma* have been shown to contribute positively to their host's plant health (Bailey et al., 2008; Mulaw et al., 2013; Gazis and

Fig. 7. Results of the antagonism assays between isolates of *Trichoderma* spp. and *Geosmithia morbida* grown on potato dextrose agar (PDA) 15 d after inoculation. Isolates of *Trichoderma* were from TCD-infected *J. nigra* branch sections from TN; two of the three *G. morbida* isolates were from TCD-infected *J. nigra* branch sections from Tennessee, whereas the third (GM34-7) was from a TCD-infected *J. hindsii* x *nigra* branch section from California.

Chaverri, 2015). Although few studies have supported *in planta* disease reduction after promising results were obtained through *in vitro* studies, studies on biological control of *Mycosphaerella fijiensis* in banana and *Phytophtora palmivora* in cacao have validated *in vitro* preliminary results under field conditions (Cavero et al., 2015; Sriwati et al., 2015). Most *Trichoderma* isolates recovered from TN samples are closely related to clades reported to be mycotrophs, such as *T. harzianum* and *T. atroviride* species complexes (Brunner et al., 2005; Chaverri et al., 2015). On the other hand, the three *Trichoderma* isolates recovered from CA are closely related to species found frequently in soils and decaying wood (Jaklitsch et al., 2013).

Experimental screening assays yielded two major candidate *Trichoderma* isolates that demonstrated competitive interactions with *G. morbida*. These isolates, TN3-46 and TN5-34, not only overgrew all *G. morbida* isolates but completely covered and sporulated over the pathogen's colony. Due to the confounding challenges imposed by the slow and irregular *G. morbida* growth habit, data on percent growth inhibition were not available for statistical comparisons. Viability of the *G. morbida* isolates was not assayed following the inoculation tests due to the intrinsic challenges of *G. morbida* recovery (Fisher et al., 2013; Ören et al., 2018). However, these isolates scored a median of 1 in our antagonism ranking, and below 1.5 across all interactions. Although these *G. morbida* isolates may not be killed outright, *Trichoderma* may be capable of displacing *G. morbida* via competitive exclusion across time. Phylogenetic reconstruction revealed that both isolates belong to the *T. harzianum* species complex, which is known for its mycoparasitic properties and uses in commercial biological control products (Kullnig et al., 2001). Moreover, these isolates are closely related to the strain used in AkTRIvator® (active ingredient strain: GJS 08137), a commercial product that targets fungal pathogens of fruit trees (Woo et al., 2014). On the other hand, *Trichoderma* isolates TN1-15, TN4-40, and TN4-47 presented evidence of low antagonism towards GM. The visible halo surrounding the GM isolate (Fig. 7), suggests that the GM isolates could be producing a diffusible metabolite that appears to be restricting growth of the *Trichoderma* isolate. Future work is needed to repeat and experimentally validate this observation and to investigate the presence of a putative antibiosis of GM towards *Trichoderma*.

Despite their low incidence, which could be due to our culture-based approach, there are other noteworthy taxa that were isolated differentially from CA and TN. Three OTUs recovered from TN trees showed high sequence similarity to species known to have either mycoparasitic or entomopathogenic properties. Two OTUs (OTU60 and OTU34) blasted 99 % similarity with *Microcera rubra* (previously referred as *Fusarium larvarum*) and *Tolypocladium* sp., two fungal taxa with documented entomopathogenic activity (Samson and Soares, 1984; Dao et al., 2015). In addition, BLAST results indicated that OTU33 could be placed in the genus *Fusicolla*, which parasitizes other fungi (Gräfenhan et al., 2011). The use of mycoparasitic or entomopathogenic fungi to ameliorate the impact of bark beetles, by reducing the inoculum of their mutualistic fungi or by directly introducing fungi that can parasitize the beetles, has been proposed to control some bark beetle-associated diseases (Sevim et al., 2010; Popa et al., 2012; Díaz et al., 2013; Carrillo et al., 2015). However, none have been characterized for efficacy as management options for TCD management.

Casual observations and a published study suggest a slow-down of the spread of TCD and apparent recovery of some TCD-infected trees, in TN after their initial decline in 2010 (Griffin, 2015). Reduced mortality of black walnut trees associated with TCD in the eastern vs. the western USA (Randolph et al., 2013) may reflect differences in population pressure from relatively low WTB populations (Wiggins et al., 2014). Other factors limiting disease progression in TN include the spatial distribution and high heterogeneity of the locations where native black walnut grows (Williams et al., 1990) and the effect of quarantine measurements preventing the movement of wood among counties (Haun et al., 2010). Based upon recovery of diverse fungal assemblages associated with TCD-infected inner wood tissue and a differential taxonomic composition and abundance between CA and TN samples, we hypothesize that the fungal community co-existing with WTB and *G. morbida* also has an impact on the development of the disease. This is particularly true for *Trichoderma* isolates, which when present in host trees, could play a critical role limiting pathogen growth. If future research supports this hypothesis, these initial results may help explain why the severity and persistence of TCD in the eastern USA is lower than has been described for the western USA.

This study revealed the presence of diverse fungal assemblages associated with *G. morbida*-infected inner bark tissue within the native ranges of *J. californica*, *J. hindsii*, and *J. nigra*, and a differential taxonomic composition and abundance of fungi between CA (*J. californica*, *J. hindsii*) and TN (*J. nigra*) samples. Although most fungal species recovered are considered non host-specific and have widespread host and geographic distributions, their common association with disease and decay of hardwood species suggests that they may play important roles in the development of TCD. Our future biological control efforts will focus on those fungal species, including the two *Trichoderma* isolates proposed for further biological control testing. *In planta* assays are also needed to verify the antagonistic properties of these *Trichoderma*. Finally, research using culture-independent techniques would complement our findings by categorizing a broader abundance of associated fungi and should yield additional taxa that might have been overlooked.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.funbio.2018.01.005.

References

Aghayeva, D.N., Wingfield, M.J., de Beer, Z.W., Kirisits, T., 2004. Two new *Ophiostoma* species with *Sporothrix* anamorphs from Austria and Azerbaijan. Mycologia 96, 866–878. https://doi.org/10.2307/3762119.

Ayres, M.P., Wilkens, R.T., Ruel, J.J., Lombardero, M.J., Vallery, E., 2000. Nitrogen budgets of phloem-feeding bark beetles with and without symbiotic fungi. Ecology 81, 2198–2210. https://doi.org/10.1890/0012-9658(2000)081 [2198:NBOPFB]2.0.CO;2.

Bailey, B.A., Bae, H., Strem, M.D., Crozier, J., Thomas, S.E., Samuels, G.J., Vinyard, B.T., Holmes, K.A., 2008. Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. Biol. Control 46, 24–35. https://doi.org/10.1016/j.biocontrol.2008.01.003.

Bell, D.K., Wells, H.D., Markham, C.R., 1982. In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology 72, 379–382. https://doi.org/10.1094/Phyto-72-379.

Bennett, A.E., 2013. Can plant–microbe–insect interactions enhance or inhibit the spread of invasive species? Funct. Ecol. 27, 661–671. https://doi.org/10.1111/1365-2435.12099.

Bettini, P.P., Frascella, A., Kolařík, M., Comparini, C., Pepori, A.L., Santini, A., Scala, F., Scala, A., 2014. Widespread horizontal transfer of the cerato-ulmin gene between *Ophiostoma novo-ulmi* and *Geosmithia species*. Fungal Biol. 118, 663–674. https://doi.org/10.1016/j.funbio.2014.04.007.

Biedermann, P.H.W., Klepzig, K.D., Taborsky, M., Six, D.L., 2013. Abundance and dynamics of filamentous fungi in the complex ambrosia gardens of the primitively eusocial beetle *Xyleborinus saxesenii* (Ratzeburg) (Coleoptera: Curculionidae, Scolytinae). FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 83, 711–723. https://doi.org/10.1111/1574-6941.12026.

Brunner, K., Zeilinger, S., Ciliento, R., Woo, S.L., Lorito, M., Kubicek, C.P., Mach, R.L., 2005. Improvement of the fungal biocontrol agent *Trichoderma atroviride* to enhance both antagonism and induction of plant systemic disease resistance. Appl. Environ. Microbiol. 71, 3959–3965. https://doi.org/10.1128/AEM.71.7.3959-3965.2005.

Carrillo, D., Dunlap, C.A., Avery, P.B., Navarrete, J., Duncan, R.E., Jackson, M.A., Behle, R.W., Cave, R.D., Crane, J., Rooney, A.P., Peña, J.E., 2015. Entomopathogenic fungi as biological control agents for the vector of the laurel wilt disease, the redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae). Biol. Control 81, 44–50. https://doi.org/10.1016/j.biocontrol.2014.10.009.

Cavero, P.A.S., Hanada, R.E., Gasparotto, L., Coelho Neto, R.A., Souza, J.T.D., 2015. Biological control of banana black Sigatoka disease with *Trichoderma*. Ciência Rural 45, 951–957.

Ceriani-Nakamurakare, E., Slodowicz, M., Gonzalez-Audino, P., Dolinko, A., Carmarán, C., 2016. Mycobiota associated with the ambrosia beetle *Megaplatypus mutatus*: threat to poplar plantations. Forestry 89, 191–200. https://doi.org/10.1093/forestry/cpw001.

Chaverri, P., Branco-Rocha, F., Jaklitsch, W., Gazis, R., Degenkolb, T., Samuels, G.J., 2015. Systematics of the *Trichoderma harzianum* species complex and the re-identification of commercial biocontrol strains. Mycologia 107, 558–590. https://doi.org/10.3852/14-147.

Colwell, R.K., 2013. EstimateS: statistical estimation of species richness and shared species from samples. User's guide and application available from: http://purl.oclc.org/estimates.

Dadd, R.H., 1970. Digestion in insects. In: Florkin, M., Scheer, B.T. (Eds.), Chemical Zoology. Academic Press, New York, pp. 117–145.

Daniels, D.A., Lord, R.J., Wadl, P.A., Nix, K.A., Vito, L.M., Merten, P.R., Windham, M.T., Lambdin, P.L., Grant, J.F., Wiggins, G.J., Hadziabdic, D., 2014. To the vector go the spoils: identifying fungal pathogens associated with walnut twig beetle, a vector of thousand cankers disease complex. Phytopathology 104, 30.

Daniels, D.A., Nix, K.A., Wadl, P.A., Vito, L.M., Wiggins, G.J., Windham, M.T., Ownley, B.H., Lambdin, P.L., Grant, J.F., Merten, P., Klingeman, W.E., Hadziabdic, D., 2016. Thousand cankers disease complex: a forest health issue that threatens *Juglans* species across the US. Forests 7, 260. https://doi.org/10.3390/f7110260.

Dao, H.T., Beattie, G.A.C., Rossman, A.Y., Burgess, L.W., Holford, P., 2015. Systematics and biology of two species of *Microcera* associated with armoured scales on citrus in Australia. Mycol. Prog. 14, 17. https://doi.org/10.1007/s11557-015-1044-0.

Davis, T.S., 2015. The ecology of yeasts in the bark beetle holobiont: a century of research revisited. Microb. Ecol. 69, 723–732. https://doi.org/10.1007/s00248-014-0479-1.

de Beer, Z.W., Duong, T.A., Wingfield, M.J., 2016. The divorce of *Sporothrix* and *Ophiostoma*: solution to a problematic relationship. Stud. Mycol. 83, 165–191. https://doi.org/10.1016/j.simyco.2016.07.001.

de Meyer, E.M., De Beer, Z.W., Summerbell, R.C., Moharram, A.M., de Hoog, G.S., Vismer, H.F., Wingfield, M.J., 2008. Taxonomy and phylogeny of new wood-and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 100, 647–661. https://doi.org/10.3852/07-157R.

Díaz, G., Córcoles, A.I., Asencio, A.D., Torres, M.P., 2013. In vitro antagonism of *Trichoderma* and naturally occurring fungi from elms against *Ophiostoma novo-ulmi*. For. Pathol. 43, 51–58. https://doi.org/10.1111/j.1439-0329.2012.00792.x.

Endoh, R., Suzuki, M., Okada, G., Takeuchi, Y., Futai, K., 2011. Fungus symbionts colonizing the galleries of the ambrosia beetle *Platypus quercivorus*. Microb. Ecol. 62, 106–120. https://doi.org/10.1007/s00248-011-9838-3.

Evans, H.C., Holmes, K.A., Thomas, S.E., 2003. Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and a preliminary assessment of their potential as biocontrol agents of cocoa diseases. Mycol. Prog. 2, 149–160. https://doi.org/10.1007/s11557-006-0053-4.

Farr, D.F., Rossman, A.Y., 2017. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/. (Accessed 14 February 2017).

Fisher, J.R., McCann, D.P., Taylor, N.J., 2013. *Geosmithia morbida*, thousand cankers disease of black walnut pathogen, was found for the first time in southwestern Ohio. Plant Health Prog. https://doi.org/10.1094/PHP-2013-1201-01-BR.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x.

Gazis, R., Chaverri, P., 2015. Wild trees in the Amazon basin harbor a great diversity of beneficial endosymbiotic fungi: is this evidence of protective mutualism? Fungal Ecol. 17, 18–29. https://doi.org/10.1016/j.funeco.2015.04.001.

Gazis, R., Rehner, S., Chaverri, P., 2011. Species delimitation in fungal endophyte diversity studies and its implications in ecological and biogeographic inferences. Mol. Ecol. 20, 3001–3013. https://doi.org/10.1111/j.1365-294X.2011.05110.x.

Geib, S.M., Filley, T.R., Hatcher, P.G., Hoover, K., Carlson, J.E., del Mar Jimenez-Gasco, M., Nakagawa-Izumi, A., Sleighter, R.L., Tien, M., 2008. Lignin degradation in wood-feeding insects. Proc. Natl. Acad. Sci. USA 105, 12932–12937. https://doi.org/10.1073/pnas.0805257105.

Giordano, L., Garbelotto, M., Nicolotti, G., Gonthier, P., 2013. Characterization of fungal communities associated with the bark beetle *Ips typographus* varies depending on detection method, location, and beetle population levels. Mycol. Prog. 12, 127–140. https://doi.org/10.1007/s11557-012-0822-1.

Gluck-Thaler, E., Slot, J.C., 2015. Dimensions of horizontal gene transfer in eukaryotic microbial pathogens. PLoS Pathog. 11 https://doi.org/10.1371/journal.ppat.1005156 e1005156.

Gräfenhan, T., Schroers, H.J., Nirenberg, H.I., Seifert, K.A., 2011. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. Stud. Mycol. 68, 79–113. https://doi.org/10.3114/sim.2011.68.04.

Griffin, G.J., 2015. Status of Thousand Cankers Disease on eastern black walnut in the eastern United States at two locations over 3 years. For. Pathol. 45, 203–214. https://doi.org/10.1111/efp.12154.

Hadziabdic, D., Vito, L.M., Windham, M.T., Pscheidt, J.W., Trigiano, R.N., Kolařík, M., 2014. Genetic differentiation and spatial structure of *Geosmithia morbida*, the causal agent of Thousand Cankers Disease in black walnut (*Juglans nigra*). Curr. Genet. 60, 75–87. https://doi.org/10.1007/s00294-013-0414-x.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2, 43–56. https://doi.org/10.1038/nrmicro797.

Haun, G., Powell, S., Strohmeier, C., Kirksey, J., 2010. State of Tennessee Thousand Cankers Disease Action Plan. Tennessee Department of Agriculture. Division of Regulatory Services and Division of Forestry, Nashville, TN, USA. Accessed February 2017. http://www.tn.gov/agriculture/publications/regulatory/TN_TCD_ActionPlan.pdf.

Hefty, A.R., 2016. Risk of Invasion by Walnut Twig Beetle throughout eastern North America (PhD. dissertation). University of Minnesota, 130 pages. Retrieved from the University of Minnesota Digital Conservancy. http://hdl.handle.net/11299/182824.

Hishinuma, S.M., Dallara, P.L., Yaghmour, M.A., Zerillo, M.M., Parker, C.M., Roubtsova, T.V., Nguyen, T.L., Tisserat, N.A., Bostock, R.M., Flint, M.L., Seybold, S.J., 2016. Wingnut (Juglandaceae) as a new generic host for *Pityophthorus juglandis* (Coleoptera: Curculionidae) and the thousand cankers disease pathogen, *Geosmithia morbida* (Ascomycota: Hypocreales). Can. Entomol. 148, 83–91. https://doi.org/10.4039/tce.2015.37.

Howell, C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis. 87, 4–10. https://doi.org/10.1094/PDIS.2003.87.1.4.

Jaklitsch, W.M., Samuels, G.J., Ismaiel, A., Voglmayr, H., 2013. Disentangling the *Trichoderma viridescens* complex. Persoonia Mol. Phylogeny Evol. Fungi 31, 112–146. https://doi.org/10.3767/003158513X672234.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Katoh, K., Toh, H., 2008. Recent developments in the MAFFT multiple sequence alignment program. Briefings Bioinf. 9, 286–298. https://doi.org/10.1093/bib/bbn013.

Khaldi, N., Wolfe, K.H., 2011. Evolutionary origins of the fumonisin secondary metabolite gene cluster in *Fusarium verticillioides* and *Aspergillus niger*. Int. J. Evol. Biol. 11 https://doi.org/10.4061/2011/423821. Article ID 423821.

Kirisits, T., 2004. Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi. In: Lieutier, F., Day, K.R., Battisti, A., Grégoire, J.C., Evans, H.F. (Eds.), Bark and wood Boring Insects in Living Trees in Europe, a Synthesis. Springer, Netherlands, pp. 181–236. https://doi.org/10.1007/978-1-4020-2241-8.

Kirisits, T., 2010. Fungi isolated from *Picea abies* infested by the bark beetle *Ips typographus* in the Białowieża forest in Northeastern Poland. For. Pathol. 40, 100–110. https://doi.org/10.1111/j.1439-0329.2009.00613.x.

Kolařík, M., Freeland, E., Utley, C., Tisserat, N., 2011. *Geosmithia morbida* sp. nov., a new phytopathogenic species living in symbiosis with the walnut twig beetle (*Pityophthorus juglandis*) on *Juglans* in USA. Mycologia 103, 325–332. https://doi.org/10.3852/10-124.

Kolařík, M., Hulcr, J., Tisserat, N., De Beer, W., Kostovčík, M., Kolaříková, Z., Seybold, S.J., Rizzo, D.M., 2017. *Geosmithia* associated with bark beetles and woodborers in the western USA: taxonomic diversity and vector specificity. Mycologia 109, 185–199. https://doi.org/10.1080/00275514.2017.1303861.

Kullnig, C.M., Krupica, T., Woo, S.L., Mach, R.L., Rey, M., Benítez, T., Lorito, M., Kubicek, C.P., 2001. Confusion abounds over identities of *Trichoderma* biocontrol isolates. Mycol. Res. 105, 769–772. https://doi.org/10.1017/S0953756201229967.

Maddison, W.P., Maddison, D.R., 2017. Mesquite: a modular system for evolutionary analysis. Version 3.2. http://mesquiteproject.org.

McDermott-Kubeczko, M., 2016. Fungi Isolated from Black Walnut Branches in Indiana and Tennessee Urban Areas (Master's Thesis). University of Minnesota Digital Conservancy, 76 pages. http://hdl.handle.net/11299/180201.

McPherson, B.A., Erbilgin, N., Bonello, P., Wood, D.L., 2013. Fungal species assemblages associated with *Phytophthora ramorum*-infected coast live oaks following bark and ambrosia beetle colonization in northern California. For. Ecol. Manag. 291, 30–42. https://doi.org/10.1016/j.foreco.2012.11.010.

Mehrabi, R., Bahkali, A.H., Abd-Elsalam, K.A., Moslem, M., M'barek, S.B., Gohari, A.M., Jashni, M.K., Stergiopoulos, I., Kema, G.H., de Wit, P.J., 2011. Horizontal gene and chromosome transfer in plant pathogenic fungi affecting host range. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev. 35, 542–554. https://doi.org/10.1111/j.1574-6976.2010.00263.x.

Montecchio, L., Faccoli, M., Short, D.P.G., Fanchin, G., Geiser, D.M., Kasson, M.T., 2015a. First Report of *Fusarium solani* phylogenetic species 25 associated with early stages of Thousand Cankers Disease on *Juglans nigra* and *Juglans regia* in Italy. Plant Dis. 99, 1183. https://doi.org/10.1094/PDIS-01-15-0103-PDN.

Montecchio, L., Fanchin, G., Simonato, M., Faccoli, M., 2015b. First record of thousand cankers disease fungal pathogen *Geosmithia morbida* and walnut twig beetle *Pityophthorus juglandis* on *Juglans regia* in Europe. Plant Dis. 98, 1445. https://doi.org/10.1094/PDIS-07-14-0719-PDN.

Montecchio, L., Vettorazzo, M., Faccoli, M., 2016. Thousand cankers disease in Europe: an overview. Eur. Mediterr. Plant Prot. Organ. Bull. 46, 335–340. https://doi.org/10.1111/epp.12301.

Mulaw, T.B., Druzhinina, I.S., Kubicek, C.P., Atanasova, L., 2013. Novel endophytic *Trichoderma* spp. isolated from healthy *Coffea arabica* roots are capable of controlling coffee tracheomycosis. Diversity 5, 750–766. https://doi.org/10.3390/d5040750.

Naher, L., Mokhtar, S.I., Sidek, N., 2015. *Tricoderma harzianum* T32 growth and antagonistic performance against *Ganoderma boninense* of different culture media. In: 3rd International Conference on Biological, Chemical & Environmental Science (BCES-2015), Kuala Lumpur (Malaysia). http://umkeprints.umk.edu.my/id/eprint/5546.

Nguyen, T.L., 2015. Studies of *Geosmithia morbida*, the causal agent of Thousand Cankers Disease, and nonpathogenic *Geosmithia* species carried by *Pityophthorus juglandis* and their interaction with *Juglans* species of economic and ecological importance in California (Order No. 1604001). Available from ProQuest Dissertations & Theses Global. (1735812038).

Nix, K.A., 2013. The Life History and Control of *Pityophthorus juglandis* Blackman on *Juglans nigra* L. in Eastern Tennessee (Master's Thesis). University of Tennessee, 93 pages. http://trace.tennessee.edu/utk_gradthes/1656/.

Ören, E., Klingeman, W., Gazis, R., Moulton, J., Lambdin, P., Coggeshall, M., Hulcr, J., Seybold, S.J., Hadziabdic, D., 2018. A novel molecular toolkit for rapid detection of the pathogen and primary vector of thousand cankers disease. PLoS One 13, e0185087. https://doi.org/10.1371/journal.pone.0185087.

Paine, T.D., Raffa, K.F., Harrington, T.C., 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annu. Rev. Entomol. 42, 179–206. https://doi.org/10.1146/annurev.ento.42.1.179.

Pardatscher, R., Schweigkofler, W., 2009. Microbial biodiversity associated with walnut *Juglans regia* L. in south Tyrol (Italy). Mitt Klosterneuburg 59, 17–23. http://works.bepress.com/wolfgang_schweigkofler/27/.

Ploetz, R.C., Hulcr, J., Wingfield, M.J., de Beer, Z.W., 2013. Destructive tree diseases associated with ambrosia and bark beetles: black swan events in tree pathology? Plant Dis. 97, 856–872. https://doi.org/10.1094/PDIS-01-13-0056-FE.

Popa, V., Déziel, E., Lavallée, R., Bauce, E., Guertin, C., 2012. The complex symbiotic relationships of bark beetles with microorganisms: a potential practical approach for biological control in forestry. Pest Manag. Sci. 68, 963–975. https://doi.org/10.1002/ps.3307.

Raffa, K.F., Aukema, B.H., Bentz, B.J., Carroll, A.L., Hicke, J.A., Turner, M.G., Romme, W.H., 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. Bioscience 58, 501–517. https://doi.org/10.1641/B580607.

Randolph, K.C., Rose, A.K., Oswalt, C.M., Brown, M.J., 2013. Status of black walnut (*Juglans nigra* L.) in the eastern United States in light of the discovery of Thousand Cankers Disease. Castanea 78, 2–14. https://doi.org/10.2179/12-024.

Reed, S., English, J., Juzwik, J., Ginzel, M., 2013. Bark and ambrosia beetles and their associated fungi colonizing stressed walnut in Missouri and Indiana. Walnut Counc. Bull. 40, 17–19. https://www.treesearch.fs.fed.us/pubs/43963.

Samson, R.A., Soares, G.G., 1984. Entomopathogenic species of the hyphomycete genus *Tolypocladium*. J. Invertebr. Pathol. 43, 133–139. https://doi.org/10.1016/0022-2011(84)90130-7.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.

Sevim, A., Demir, I., Tanyeli, E., Demirbağ, Z., 2010. Screening of entomopathogenic fungi against the European spruce bark beetle, *Dendroctonus micans* (Coleoptera: Scolytidae). Biocontrol Sci. Technol. 20, 3–11. https://doi.org/10.1080/09583150903305737.

Seybold, S.J., Haugen, D., O'Brien, J., Graves, A.D., 2013. Thousand Cankers Disease. USDA Forest Service, Northeastern Area State and Private Forestry Pest Alert. NA-PR-02–10, originally published May 2010. http://www.na.fs.fed.us/pubs/detail.cfm.

Seybold, S.J., Penrose, R.L., Graves, A.D., 2016. Invasive bark and ambrosia beetles in California Mediterranean forest ecosystems. In: Paine, T.D., Lieutier, F. (Eds.), Insects and Diseases of Mediterranean Forest Systems. Springer International Publishing, Gewerbestrasse, Switzerland, pp. 583–662. https://doi.org/10.1007/978-3-319-24744-1_21.

Silva, A., Rodrigues, A., Bacci, M., Pagnocca, F.C., Bueno, O.D.C., 2006. Susceptibility of the ant-cultivated fungus *Leucoagaricus gongylophorus* (Agaricales: Basidiomycota) towards microfungi. Mycopathologia 162, 115–119. https://doi.org/10.1007/s11046-006-0037-6.

Silvestro, D., Michalak, I., 2012. raxmlGUI: a graphical front-end for RAxML. Org. Divers. Evol. 12, 335–337. https://doi.org/10.1007/s13127-011-0056-0.

Sitz, R.A., Luna, E.K., Caballero, J.I., Tisserat, N.A., Cranshaw, W.S., Stewart, J.E., 2017. Virulence of genetically distinct *Geosmithia morbida* isolates to black walnut and their response to coinoculation with *Fusarium solani*. Plant Dis. 101, 116–120. https://doi.org/10.1094/PDIS-04-16-0535-RE.

Six, D.L., 2012. Ecological and evolutionary determinants of bark beetle—fungus symbioses. Insects 3, 339–366. https://doi.org/10.3390/insects3010339.

Six, D.L., 2013. The bark beetle holobiont: why microbes matter. J. Chem. Ecol. 39, 989–1002. https://doi.org/10.1007/s10886-013-0318-8.

Six, D.L., Bentz, B.J., 2003. Fungi associated with the North American spruce beetle, *Dendroctonus rufipennis*. Can. J. For. Res. 33, 1815–1820. https://doi.org/10.1139/x03-107.

Six, D.L., Wingfield, M.J., 2011. The role of phytopathogenicity in bark beetle—fungus symbioses: a challenge to the classic paradigm. Annu. Rev. Entomol. 56, 255–272. https://doi.org/10.1146/annurev-ento-120709-144839.

Sriwati, R., Melnick, R.L., Muarif, R., Strem, M.D., Samuels, G.J., Bailey, B.A., 2015. *Trichoderma* from Aceh Sumatra reduce *Phytophthora* lesions on pods and cacao seedlings. Biol. Control 89, 33–41.

Stokland, J.N., Siitonen, J., Jonsson, B.G., 2012. Biodiversity in Dead Wood. Cambridge University Press, Cambridge.

Strid, Y., Schroeder, M., Lindahl, B., Ihrmark, K., Stenlid, J., 2014. Bark beetles have a decisive impact on fungal communities in Norway spruce stem sections. Fungal Ecol. 7, 47–58. https://doi.org/10.1016/j.funeco.2013.09.003.

Tisserat, N., Cranshaw, W., Leatherman, D., Utley, C., Alexander, K., 2009. Black walnut mortality in Colorado caused by the walnut twig beetle and Thousand Cankers Disease. Plant Health Prog. 11 https://doi.org/10.1094/PHP-2009-0811-01-RS.

Tisserat, N., Cranshaw, W., Putnam, M., Pscheidt, J., Leslie, C.A., Murray, M., Hoffman, J., Barkley, Y., Alexander, K., Seybold, S.J., 2011. Thousand Cankers Disease is widespread on black walnut, *Juglans nigra*, in the western United States. Plant Health Prog. https://doi.org/10.1094/PHP-2011-0630-01-BR.

USDA-FS-PPQ, 2017. Thousand Cankers Disease Survey Guidelines for 2017. http://www.aphis.usda.gov/plant_health/plant_pest_info/tcd/downloads/TCD_Survey_Guidelines.pdf. (Accessed 28 May 2017).

Utley, C., Nguyen, T., Roubtsova, T., Coggeshall, M., Ford, T.M., Grauke, L.J., Graves, A.D., Leslie, C.A., McKenna, J., Woeste, K., Yaghmour, M.A., Cranshaw, W., Seybold, S.J., Bostock, R.M., Tisserat, N., 2013. Susceptibility of walnut and hickory species to *Geosmithia morbida*. Plant Dis. 97, 601–607. https://doi.org/10.1094/PDIS-07-12-0636-RE.

Vander Wall, S.B., 2001. The evolutionary ecology of nut dispersal. Bot. Rev. 67, 74–117. https://doi.org/10.1007/BF02857850.

Victory, E.R., Glaubitz, J.C., Rhodes Jr., O.E., Woeste, K.E., 2006. Genetic homogeneity in *Juglans nigra* (Juglandaceae) at nuclear microsatellites. Am. J. Bot. 93, 118–126. https://doi.org/10.3732/ajb.93.1.118.

Vitale, S., Santori, A., Wajnberg, E., Castagnone-Sereno, P., Luongo, L., Belisario, A., 2011. Morphological and molecular analysis of *Fusarium lateritium*, the cause of gray necrosis of hazelnut fruit in Italy. Phytopathology 101, 679–686. https://doi.org/10.1094/PHYTO-04-10-0120.

White, T.J., Bruns, T., Lee, S., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), PCR Protocols: a Guide to Methods and Applications. Academic Press, New York, pp. 315–322.

Wiggins, G.J., Grant, J.F., Lambdin, P.L., Merten, P., Nix, K.A., Hadziabdic, D., Windham, M.T., 2014. Discovery of walnut twig beetle, *Pityophthorus juglandis*, associated with forested black walnut, *Juglans nigra*, in the eastern US. Forests 5, 1185–1193. https://doi.org/10.3390/f5061185.

Williams, R.D., 1990. *Juglans nigra* L., black walnut. In: Burns, R.M., Honkala, B.H. (Eds.), Silvics of North America, Vol. 2, Hardwoods, Agriculture Handbook U.S. Department of Agriculture Forest Service, Washington, D.C, pp. 391–399.

Woo, S.L., Ruocco, M., Vinale, F., Nigro, M., Marra, R., Lombardi, N., Pascale, A., Lanzuise, S., Manganiello, G., Lorito, M., 2014. *Trichoderma*-based products and their widespread use in agriculture. Open Mycol. J. 8, 71–126. https://doi.org/10.2174/1874437001408010071.

Zerillo, M.M., Caballero, J.I., Woeste, K., Graves, A.D., Hartel, C., Pscheidt, J.W., Tonos, J., Broders, K., Cranshaw, W., Seybold, S.J., Tisserat, N., 2014. Population structure of *Geosmithia morbida*, the causal agent of thousand cankers disease of walnut trees in the United States. PLoS One 9. https://doi.org/10.1371/journal.pone.0112847 e112847.