Fungal Biology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Fungal Biology

journal homepage: www.elsevier.com/locate/funbio

Fungi associated with galleries of the emerald ash borer

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ARTICLE INFO

Article history:
Received 14 September 2020
Received in revised form
16 December 2020
Accepted 14 February 2021
Available online xxx

Corresponding Editor: Nabla Kennedy

Keywords:
Fungal diversity
Emerald ash borer
Ash
Canker
Decay
Entomopathogen

ABSTRACT

The emerald ash borer (EAB) is an exotic forest pest that has killed millions of ash trees in the United States and Canada, resulting in an ecological disaster and billions of dollars in economic losses of urban landscape and forest trees. The beetle was first detected in Michigan in 2002 and has spread through much of the Eastern and Midwestern U.S., reaching Minnesota in 2009. Since then, it has spread across the state and poses a great risk to the more than 1 billion ash trees in Minnesota. The larval stage of EAB creates wounds on trees as they feed on the inner bark, causing disruption of water and sap flow that results in tree death. The fungal community associated with EAB larval galleries is poorly understood and the role these fungi may play in tree death is not known. This study describes fungi isolated from EAB larval galleries sampled throughout the main geographic areas of Minnesota where ash is affected by EAB. Fungal cultures were identified by extracting genomic DNA and sequencing the ITS region of the rDNA. Results from 1126 isolates reveal a diverse assemblage of fungi and three functional guilds comprised of canker pathogens, wood decay, and entomopathogenic fungi. The most common canker-associated genera were *Cytospora* followed by *Phaeoacremonium, Paraconiothyrium, Coniothyrium, Nectria, Diplodia,* and *Botryosphaeria.* Fungi in the Basidiomycota were nearly all wood decay causing fungi and many were species of pioneer colonizing genera including *Sistotrema*, *Irpex*, *Peniophora, Phlebia* and *Ganoderma*. Some of these fungi seriously affect urban trees, having the potential to cause rapid wood decay resulting in hazardous tree situations. Several entomopathogenic genera with the potential for biological control of EAB were also isolated from galleries. *Purpureocillium* was the most commonly isolated genus, followed by *Beauveria*, *Clonostachys*, *Lecanicillium, Akanthomyces*, *Cordyceps*, *Microcera*, *Tolypocladium*, and *Pochonia*. The results identify important fungal functional guilds that are occupying a new niche in ash trees resulting from EAB and include fungi that may accelerate decline in tree health, increase hazard tree situations, or may provide options for biological control of this destructive invasive insect.

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1. Introduction

The introduction and invasion of the emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) has been devastating for ash (*Fraxinus* spp.) in the United States and Canada. Since it was first detected in 2002 in southeast Michigan, it has killed hundreds of millions of trees (Herms and McCullough, 2014; Poland and McCullough, 2006) and continues to spread. It has already reached all northeastern states and most midwestern and southern states, as well as five Canadian provinces. Due to its low genetic diversity, invasion by EAB is thought to result from a single introduction from China (Bray et al., 2011). In its native forest ecosystem of northeastern China, the Korean peninsula and eastern Russia, EAB normally attacks native Asian ash species that are stressed, declining, or dying (Baranchikov et al., 2008; Liu et al., 2007; Wei et al., 2004). However, EAB will also attack healthy trees of North American ash species planted in its native range, as well as in its invaded ranges in North America (Cappaert et al., 2005; Duan et al., 2015; Wang et al., 2010). Mortality in ash stands in Michigan and Ohio have exceeded 99% and strategies for controlling EAB are limited and currently under study. Recent research has shown limited resistance in the endemic ash species in North America (Cipollini et al., 2011; Koch et al., 2015; Rebek et al., 2008; Rigsby et al., 2015). The disruption of vascular tissue from the larval

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https://doi.org/10.1016/j.funbio.2021.02.004
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Please cite this article as: B.W. Held, S. Simeto, N.N. Rajtar *et al.*, Fungi associated with galleries of the emerald ash borer, Fungal Biology, https://doi.org/10.1016/j.funbio.2021.02.004

stage feeding in a serpentine pattern on the phloem–xylem interface, leaves wounds on branches and stems (Fig. 1.). Shortly thereafter, canopy decline takes place as the density of larvae and subsequent damage increases and branch and stem dieback occurs (Cappaert et al., 2005; Herms and McCullough, 2014), followed by tree mortality in a 2–4 year period (Herms and McCullough, 2013). In between initial feeding and tree death, many of the larval galleries become exposed and canker lesions adjacent to the larval galleries can occur in affected trees. Woodpeckers commonly feed on larvae and aid in exposing larval galleries. Often these cankered sites become visibly discolored as vascular tissue is disrupted and as a result, dieback and tree mortality occurs (Chamorro et al., 2015). Currently, nothing is known about fungi associated with EAB larval galleries and whether they play a role in tree mortality or structural failure of wood. Unlike bark beetles and ambrosia beetles, which carry fungi on their exoskeleton or have specialized body structures (mycangia) that carry fungal symbionts that often cause tree diseases (e.g. Dutch elm disease and Laurel wilt), wood borers (including *Agrilus* sp.) do not have mycangia and are not currently known to vector plant-pathogenic fungal associates. However, it has been shown that EAB may harbor gut symbionts that potentially degrade lignocellulose (Vasanthakumar et al., 2008), and it is possible that some of these fungi may enter galleries through larval excrement (e.g. frass). Although several entomopathogenic fungi have previously been isolated from mycosed EAB larvae and adults their prevalence and role in EAB galleries remains unknown and many species have not been fully explored for biological control (Bauer et al., 2004; Castrillo et al., 2010a; Johny et al, 2012a, 2012b). The objective of this study was to describe the fungal diversity associated with EAB galleries from trees sampled in several diverse locations in Minnesota, to better understand the functional role(s) of this fungal community, and to determine if exotic fungal species are associated with the infestation of EAB.

2. Materials and methods

Log samples of green ash (*Fraxinus pennsylvanica* Marshall) were obtained from three different regions in Minnesota: South (Rochester and Winona), Central (Minneapolis/St. Paul), and North (Duluth) where EAB was causing ash mortality. Most samples were obtained from city forestry departments that were removing infested ash trees. Samples from infested trees were taken from sections of the main stem and larger branches (sizes ranged from 10 to 40 cm). Logs were stored at −20 °C until processed. Samples from asymptomatic ash were collected from 10 trees in Afton State Park, MN. A sterilized hatchet was used to remove the outer bark after which the blade was sterilized again with 70% ETOH and a section of inner bark/phloem from the main stem was cut, placed in a sterile bag and kept cool while transported to the laboratory and aseptically isolated with the same media that was used for infested samples. Infested logs were processed by using a draw knife sterilized with 70% ETOH to strip the bark and expose larval galleries. Larval galleries included zones of discolored xylem and, unstained wood surrounding galleries. A sterile chisel was used to obtain small segments of wood tissue beneath the cleared off xylem. On average, two areas from each log were sampled if multiple galleries were present. Wood chips were sampled from each area and plated in groups of four to five segments on three different media: 1.5% malt extract agar amended with lindane (M + L) for mite control (15 g of Difco Bacto-agar, 15 g of Difco Bacto malt extract, 0.01 g of Aldrich lindane per L of deinonized water with 0.1 g streptomycin sulphate added after autoclaving); modified Sabourad Dextrose Agar (adapted from Harrington, 1981) amended with lindane (SDA + L) (15 g of Difco Bacto-agar, 15 g of Difco Bacto malt extract, 0.01 g of Aldrich lindane per L of deinonized water, with 0.1 g Aldrich cycloheximide, 0.1 g streptomycin sulphate added after autoclaving), and Basidiomycota Selective Agar (adapted from Worrall, 1991) amended with lindane (BSA + L) (15 g of Difco Bacto-

Fig. 1. **(A)** The main stem of green ash showing woodpecker damage from feeding on larvae under the bark **(B)** Bark removed around woodpecker damage showing EAB larval galleries **(C)** EAB larvae feeding on phloem/outer sapwood interface in a serpentine pattern and has not yet caused phloem necrosis **(D)** An older EAB gallery showing killed sapwood tissue leading to a disruption of vascular function and discoloration and necrosis.

agar, 15 g of Difco Bacto malt extract, 2 g of Difco yeast extract, 0.06 g, Aldrich benomyl, 0.03 g of Aldrich lindane per L of deionized water, and 0.1 g streptomycin sulphate, 2 ml lactic acid added after autoclaving). Plates were sealed with Parafilm® and incubated at room temperature for one to two weeks. After initial inoculation and incubation on M + L, SDA + L and BSA + L pure cultures were obtained from samples that showed fungal growth by subculturing colonies to malt extract agar (MEA) (15 g Difco Bacto malt extract, 15 g Difco Bacto agar and 1 L deinonized water) plates and were incubated at room temperature for one to two weeks. DNA extraction was performed using mycelia from pure cultures using a CTAB procedure according to Blanchette et al. (2016), or a NaOH protocol according to Osmundson et al. (2013). Polymerase chain reaction (PCR) was performed to amplify the rDNA internal transcribed spacer region (ITS) using ITS1F and ITS4 primers (Gardes and Bruns, 1993) according to Blanchette et al. (2016). Amplicons were visualized via 1% agarose gel electrophoresis with SYBR green I pre-stain and visualized with trans-illumination on a Dark Reader DR45. Sanger sequencing was done with PCR primers on an ABI 3730xl DNA sequencer (Applied Biosystems – Foster City, CA. Consensus sequences were assembled using Geneious 9.0 (Kearse et al., 2012) and were subjected to the BLASTn algorithm (Zhang et al., 2000) using the megablast option in NCBI GenBank. Identification of cultures was based on the highest BLAST score matching a species level accession from a published study or type specimen. Sequences representative of each taxa were deposited in GenBank and given genera or higher level classification. Values of less than 97% best BLAST match to a verified species (originating from type species or published study) were considered as possible new species (noted with a "*" in Table S1) (Kõljalg et al., 2013; Schoch et al., 2012).

Rarefaction and extrapolation sampling curves of all pooled samples were computed and plotted to estimate sample completeness (sample coverage) based on abundance (individual-based = isolates) in the R iNEXT package (Chao et al, 2014, 2020; Hsieh et al., 2016). A 95% confidence interval was generated by applying 1000 bootstrap iterations. Diversity order q = 0 (species richness) was set for the analysis. Due to uneven sampling at the three different geographic areas, diversity indices to compare sites were not calculated.

3. Results

Wood adjacent to EAB galleries at the time of initial gallery formation was usually not discolored but became brown or black with necrosis as feeding from larvae continues (Fig. 1). After several years, galleries became exposed as more phloem and xylem tissue was affected and woodpecker feeding took place. As trees declined, phloem tissue sometimes became stained by fungi and lesions extended out from the galleries.

A total of 1126 fungal cultures were isolated from EAB galleries from three geographical areas of infestation in Minnesota (North, Central, and South). The total number of isolates comprised of 173 taxa identified to the genus or species level; 138 taxa belonging to 84 genera in 23 different orders in Ascomycota (90%), 25 taxa belonging to 17 genera from five orders in Basidiomycota (9%) and eight taxa belonging to four genera in two orders in the Mucoromycota (2%), and two taxa from 7 isolates belonging to *Mortierella* in the Mortierellomycota (1%) (Figs. 2–4; Table S1). Thirty-two taxa had less than a 97% match to previously described taxa in GenBank and may represent new species. Some of the most abundant Ascomycota species included *Alternaria* sp. (161 isolates), *Cytospora pruinosa* (114), *Epicoccum nigrum* (64), *Paraconiothryium brasiliense* (51), *Purpureocillium lilacinum* (47), *Phaeoacremonium croatiense* (46) (Table S1). *Sistotrema brinkmannii* (30) and *Irpex lacteus* (17)

Division	Class	Order	Family	Genus	Count
Ascomycota	Arthoniomycetes	Arthoniales	Roccellaceae		
				Bactrospora	1
	Dothideomycetes	Botryosphaeriales	Botryosphaeriaceae		
				Botryosphaeria	25
				Diplodia	13
				Lasiodiplodia	1
				Phaeobotryon	1
		Capnodiales	Cladosporiaceae		
				Cladosporium	32
		Dothideales	Dothioraceae		
				Aureobasidium	3
				Kabatiella	1
		Insertae sedis	Pseudeurotiaceae		
				Pseudogymnoascus	1
		Pleosporales	Astrosphaeriellaceae		
				Pithomyces	7
			Cucurbitariaceae		
				Pyrenochaeta	15
				Seltsamia	1
			Didymellaceae		
				Didymella	4
				Epicoccum	63
				Neoascochyta	1
				Phoma	1
				Stagonosporopsis	1
			Didymosphaeriaceae		
				Dendrothyrium	4
				Paraconiothyrium	51
			Leptosphaeriaceae		
				Coniothyrium	38
				Paraleptosphaeria	1
			Periconiaceae		
				Periconia	1
			Phaeosphaeriaceae		
				Banksiophoma	1
				Neosetophoma	2
				Sclerostagonospora	17
				Setophaeosphaeria	2
			Pleosporaceae		
				Alternaria	161
				Curvularia	3
				Pleaospora	2
			Thyridariaceae		
				Pararoussoella	2
				Thyridaria	1
		Pleosporomycetidae	Camarosporiaceae		
				Camarosporium	14
			Pleosporales		
				Ascochyta	8
	Eurotiomycetes	Chaetothyriales	Cyphellophoraceae		
				Cyphellophora	1
			Herpotrichiellaceae		
				Capronia	2
				Exophiala	1
				Phialophora	3
				Veronaea	4
		Eurotiales	Aspergillaceae		
				Penicillium	13
			Trichocomaceae		
				Talaromyces	3
	Leotiomycetes	Leotiomycetidae	Helotiales		
				Cadophora	2
		Helotiales	Dermateaceae		
				Pirottaea	1
			Porodiplodiaceae		
				Porodiplodia	4
			Vibrisseaceae		
				Phialocephala	2
			Tympanidaceae		
				Tympanis	1
	Orbiliomycetes	Orbiliales	Orbiliaceae		
				Arthrobotrys	3
	Sordariomycetes	Amphisphaeriales	Sporocadaceae		
				Monochaetia	1
		Atractosporales	Pseudoproboscisporaceae		
				Pseudoproboscispora	1
		Calosphaeriales	Calosphaeriaceae		
				Jattaea	11
		Diaporthales	Cytosporaceae		
				Cytospora	114
			Diaporthaceae		
				Diaporthe	1
			Valsaceae		
				Valsa	1
		Glomerellales	Glomerellaceae		
				Colletotrichum	2
		Hypocreales	Cordycipitaceae		
				Akanthomyces	3
				Beauveria	22
				Cordyceps	2
				Lecanicillium	4
			Clavicipitaceae		
				Pochonia	1
			Bionectriaceae		
				Clonostachys	13
			Nectriaceae		
				Cosmospora	1
				Fusarium	75
				Microcera	3
				Nectria	23
				Neonectria	1
				Thyronectria	26
				Xenoacremonium	2
			Ophiocordycipitaceae		
				Purpureocillium	47
				Tolypocladium	2
			Niessliaceae		
				Monocillium	4
			Hypocreaceae		
				Trichoderma	3
		Microascales	Ceratocystidaceae		
				Ceratocystiopsis	1
			Microascaceae		
				Graphium	1
		Ophiostomatales	Ophiostomataceae		
				Leptographium	3
				Ophiostoma	1
		Saccharomycetales	Dipodascaceae		
				Geotrichum	1
		Togniniales	Togniniaceae		
				Phaeoacremonium	103
		Xylariales	Aplosporaceae		
				Arthrinium	1
				Nigrospora	3
			Diatrypaceae		
				Cryptosphaeria	6
				Diatrypella	1
				Eutypa	2
			Hypoxylaceae		
				Daldinia	2
				Hypoxylon	1

Fig. 2. List of fungi in the Ascomycota isolated from EAB galleries, their taxonomic placement, and abundance.

Division	Class	Order	Family	Genus	Count
Basidiomycota	Agaricomycetes	Agaricales	Amylocorticiaceae		
				Plicatura	1
		Cantharellales	Hydnaceae		
				Sistotrema	30
		Polyporales	Fomitopsidaceae		
				Antrodia	1
			Meruliaceae		
				Ceriporiopsis	1
			Ganodermataceae		
				Ganoderma	3
			Phanerochaetaceae		
				Hyphodermella	2
				Irpex	17
			Fomitopsidaceae		
				Ischnoderma	1
			Phanerochaetaceae		
				Meruliopsis	1
			Meruliaceae		
				Bjerkandera	1
				Phlebia	10
			Polyporaceae		
				Lentinus	1
				Trametes	5
		Russulales	Stereaceae		
				Aleurodiscus	1
			Peniophoraceae		
				Peniophora	12
			Stereaceae		
				Stereum	1
	Tremellomycetes	Tremellomycetidae	Tramellales		
				Kwoniella	1

Fig. 3. List of fungi in the Basidiomycota isolated from EAB galleries, their taxonomic placement, and abundance.

Division	Class	Order	Family	Genus	Count
Mucoromycota	Mucoromycetes	Mucorales	Cunninghamellaceae		
				Absidia	1
				Cunninghamella	1
			Mucoraceae		
				Mucor	14
	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae		
				Umbelopsis	6
Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae		
				Mortierella	7

Fig. 4. List of fungi in the Mucoromycota and Mortierellomycota isolated from EAB galleries, their taxonomic placement, and abundance.

were the most abundant in the Basidiomycota and *Mucor circinelloides* (10) and *Mortierella camargensis* (6) were the most prevalent in the Mucoromycota and Mortierellomycota, respectively (Table S1).

Three ecological guilds were identified: canker pathogens (30% of total isolates), decay (8%) and entomopathogens (8%), and other (54%), for fungi that did not fit into a specific guild (Fig. 5) (Nguyen et al., 2016). Within each functional guild, there were taxa that were isolated more frequently. The canker guild had the highest frequency of fungi associated with it (403 isolates) represented by the genera: *Cytospora* (30% out of all canker fungi), *Phaeoacremonium* (26%), *Paraconiothyrium* (13%), *Coniothyrium* (7%), *Thyronectria* (7%), *Nectria* (6%), *Botryosphaeria* (6%), *Diplodia* (species in this genera may also be considered stain fungi) (3%), *Cadophora*, (<1%), *Lasiodiplodia* (<1%), and *Neonectria* (<1%) (Fig. 2). These genera belong to six orders within the phylum Ascomycota: Botryosphaeriales (*Botryosphaeria*, *Diplodia*, and *Lasiodiplodia* within *Botryosphaeriaceae*), Diaporthales (*Cytospora* within *Cytosporaceae*), Hypocreales (*Nectria*, *Neonectria* and *Thyronectria* within *Nectriaceae*), Leotiomycetidae (*Cadophora* within *Helotiales*), Pleosporales (*Paraconiothyrium* within *Didymosphaeriaceae* and *Coniothyrium* within *Leptosphaeriaceae*), and Togniniales (*Phaeoacremonium* within *Togniniaceae*) (Fig. 2).

The second functional guild was composed of entomopathogenic fungi (93 isolates) represented by the genera: *Purpureocillium* (48% of all entomopathogenic fungal isolates), *Beauveria* (23%), *Clonostachys* (13%), *Lecanicillium* (4%), *Akanthomyces* (3%), *Cordyceps* (2%), *Microcera* (3%), *Tolypocladium* (2%), and *Pochonia* (1%). These genera belong to only one order within the phylum Ascomycota: Hypocreales (*Akanthomyces*, *Beauveria*, *Cordyceps* and *Lecanicillium* within the *Cordicypitaceae*, *Pochonia* within Clavicipitaceae, *Clonostachys* within *Bionectriaceae*, *Microcera* within *Nectriaceae*, *Purpureocillium and Tolypocladium* within *Ophiocordycipitaceae*) (Fig. 2).

The third functional guild identified was represented by genera of decay fungi within the Basidiomycota: *Sistotrema* (34% out of all decay fungi), *Irpex* (19%), *Peniophora* (14%), *Phlebia* (11%), *Trametes* (6%), *Ganoderma* (3%), *Hyphodermella* (2%), *Antrodia* (*Resinoporia*)

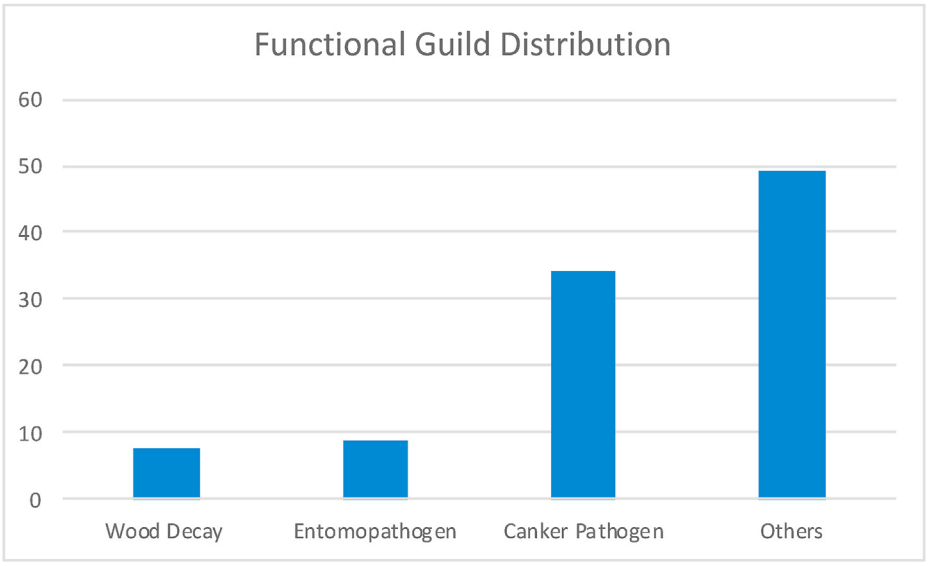

Fig. 5. Graph showing functional guild distribution of fungi isolated from EAB galleries.

(2%), *Acanthopysellum* (*Aleurodiscus*) (1%), *Bjerkandera* (1%), *Ceriporiopsis* (1%), *Ischnoderma* (1%), *Lentinus* (1%), *Meruliopsis* (1%), *Plicatura* (1%), *Stereum* (1%), and most abundant in the orders; Cantharellales (*Sistotrema*), Polyporales (*Irpex*) (Fig. 3).

Fungi isolated from healthy ash samples included the following 12 taxa *Anthostomelloides leucospermi, Camarographium carpini, Camarosporium brabeji, Cladosporium* sp., *Coniothyrium pyrinum, Microcera larvarum, Nigrograna fuscidula, Peniophora cinerea, Phaeobotryon cupressi, Pithomyces chartarum, Pleospora igbalii,* and *Thydridaria broussonetiae* (Table S2). Six of these taxa; *Camarographium brabeji*, *Cladoporium* sp., *Coniothyrium pyrinum*, *Microcera larvum*, *P. chartarum*, and *Peniophora cinereal*, were also isolated from EAB galleries. *A. leucospermi*, *C. carpini* and *P. igbalii* had low best BLAST matches of 83%, 85%, and 85%, respectively.

Rarefaction curves based on abundance (individual-based) show a steep increase initially that did not reach an asymptote (Fig. 6). The estimated sample coverage reached 93% and extrapolation of the curve show that by doubling the number of samples a 97% of sample coverage would have been reached.

4. Discussion

The results of this study show a diverse group of fungi are associated with the new ecological niche resulting from EAB attack of ash in North America and identifies early fungal colonizers. Most importantly, three functional guilds of fungi were recognized; canker, decay, and entomopathogenic fungi (Fig. 5). Canker pathogens were the most abundant and likely invade areas where larvae have wounded phloem and xylem tissue and potentially enlarge the affected area surrounding EAB galleries causing additional necrosis. *Cytospora* was the most abundant genus of the canker fungi (30% of all canker fungi) and has a wide host range on woody plants and some species within the genus are host-specific (Sinclair et al., 1987). Kepley and Jacobi (2000) showed that *C. pruinosa* was host specific to green ash (*F. pennsylvanica*) and also attributed canker formation by this fungus to stress and environmental factors. *Phaeoacremonium* species (26%) were also frequently isolated and are considered plant pathogens with a wide range of woody hosts, including *Fraxinus latifola,* have been also associated with larvae of bark beetles (Di Marco et al., 2004; Kubátová et al., 2004; Mostert et al., 2006b). Of particular interest, *Phaeoacremonium* species are known to cause brown wood staining associated with larval galleries of bark beetles of *F. pennsylvanica* in North Dakota and *Fraxinus excelsior* in Sweden (Hausner et al., 1992; Mostert et al., 2006a). Species in the genera *Paraconiothyrium* and its anamorph *Coniothyrium* (20%) are important plant pathogens and similar to the other genera, they commonly colonize many woody plants and are found associated with pruning cuts, mechanical damage or dieback/decline on hosts (Damm et al., 2008; Elena et al., 2018; McTavish et al., 2018; Schulz et al., 2018). However, this fungus has not yet been reported colonizing *Fraxinus* species (Verkley et al., 2004). Drought stress in trees can result in greater growth of canker causing fungi (Blodgett et al., 1997; Li et al., 2019) and the stress imposed by the larval galleries of EAB likely influences the growth, composition, and effect of these associated fungi in the tree. The association between fungi recovered in this study and adult beetles or larvae remains unclear. However, a recent microbiome study of adult EAB beetles in Montreal, Canada showed Ascomycota dominated the fungal community and two of the most abundant genera were, *Kabatiella* and *Pyrenochatea* (Mogouong et al., 2020). Many species in these genera are plant pathogens. In this study, 12 isolates of *Pyrenochatea corni*, most often found as an endophyte or saprobe, and one isolate of *Kabatiella microsticta*, which causes daylily leaf streak, were found. This may indicate that adult EAB beetles vector some plant pathogenic fungi as well as saprophytes.

Among the entomopathogenic fungi guild, *P. lilacinum* was the most abundant species (48% out of all entomopathogenic fungal isolates). This is a ubiquitous species found in the environment, usually isolated from soil, nematodes, and insects (Luangsa-Ard et al., 2011). *P. lilacinum* may also function as a plant endophyte and has been reported as pathogen of broad insect groups (Lopez et al., 2014; Toledo-Hernández et al., 2019). Further, it is commonly used as a biological control agent against parasitic nematode eggs (Atkins et al., 2005). Additionally, in a study by, Johny et al. (2012b), 2012a, *P. lilacinum* was isolated from mycosed larva and adults of *A. planipennis* and was considered to be established in EAB populations in Canada due to its isolation frequency. The second most frequent entomopathogenic genus was *Beauveria* (22%). *Beauveria bassiana*, the predominant species isolated within the genus, is a cosmopolitan facultative pathogen of a broad range of arthropods (Rehner et al., 2011). It also occurs as a plant endophyte and saprotroph (Mascarin and Jaronski, 2016; McKinnon

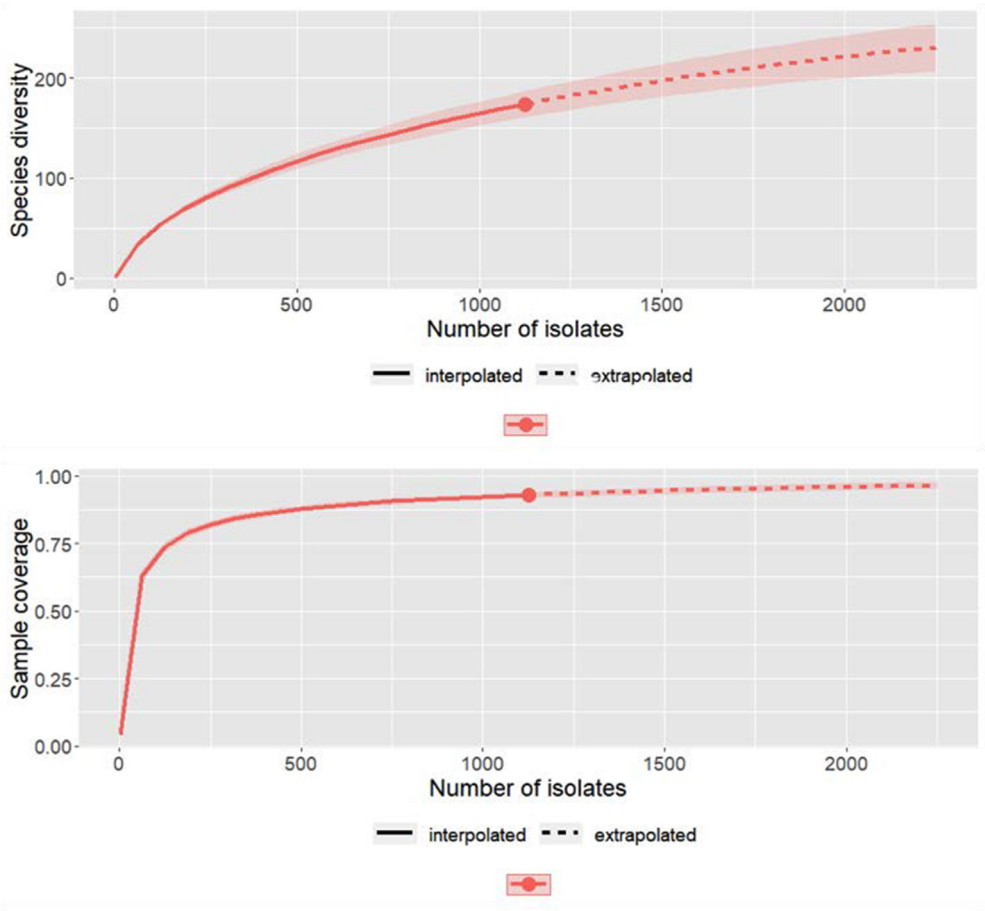

Fig. 6. Sample based rarefaction (solid line) and extrapolation (dashed line) up to double the sample size (top) and coverage based rarefaction (solid line) and extrapolation (dashed line) up to double the sample size (bottom).

et al., 2017; Vega et al., 2008). Its broad host range and *in vitro* growth characteristics have made *Beauveria* one of the entomopathogenic genera most widely used for commercially produced mycoinsecticides worldwide (Lacey et al., 2015; Mascarin and Jaronski, 2016) and it has been investigated for biological control against EAB (Castrillo et al., 2010a, 2010b; Liu and Bauer, 2008). *Clonostachys rosea* was also a relatively abundant species among the isolated entomopathogenic fungi (13%). This species colonizes living plants as an endophyte and has also been isolated from soil. It has been widely described as a parasite of fungi and nematodes, but has also been reported as an entomopathogen (Anwar et al., 2018; Toledo et al., 2006; Vega et al., 2008). Although recovered in very low frequency in our study, *Cordyceps farinosa* was also previously isolated from EAB as the anamorph *Isaria farinosa* (Johny et al., 2012a, 2012b). While previous studies have isolated entomopathogenic genera such as *Purpureocillium*, *Beauveria* and *Cordyceps* from mycosed larvae and adults of EAB, in this study we report that entomopathogenic species were also isolated from wood surrounding the galleries of EAB, indicating their capacity for colonizing wood.

Fungi isolated that belonged to the wood decay guild included aggressive pioneer white rot genera such as *Irpex, Peniophora* and *Phlebia*. While others, such as *Trametes* and *Ganoderma*, have also been reported as causing dieback of woody plants above ground as well as causing root rot on many tree species (Coetzee et al., 2015; Paterson, 2007). Most of the genera in this guild are white rot fungi with the exception of *Antrodia* and *Sistotrema,* which have been associated with brown rot (Lombard, 1990; Potvin et al., 2012; Spirin et al., 2015). *S. brinkmannii* was the most abundant species (33%) among the decay fungi isolated from EAB galleries. This species has been previously reported to be associated with soil, mosses and wood of gymnosperms and angiosperms and was suggested to produce brown-rot in natural environments and forest products (Ginns and Lefebvre, 1993; Potvin et al., 2012; Zabel and Morrell, 1992). Although *S. brinkmannii* was one of the most abundant taxa, it was only one of two brown rot fungi recovered among the majority of white rot fungi in this guild. Since this fungus does not produce a large macroscopic fruiting body, it likely has been underrepresented in similar environments based on the detection of basidiocarps. This fungus also produces basidiospores directly on mycelium which may also aid in dissemination and explain its high abundance (Hao et al., 2010). Decay fungi cause significant loss of wood strength during the decay process, weakening the affected wood in early stages of decay, posing a potential of hazardous tree situations (Eriksson et al., 1990). Although green ash (*F. pennsylvanica*) is considered intermediate in terms of susceptibility of breakage, EAB infested ash trees have shown a higher risk of tree failure and pose a threat to tree care workers as they remove dead or dying ash (Persad et al., 2013). Future studies on the decay potential of the most prevalent species of Basidiomycota isolated from EAB galleries are necessary to determine their role in rapid decay of EAB infested trees.

The canker pathogen *Coniothyrium pyrinum* and decay fungus *P. cinerea* that were isolated from healthy ash tissue were also

isolated from EAB galleries and may indicate a possible endophytic lifestyle (Wang et al., 2005; Yuan et al., 2011), although additional sampling of healthy ash is needed to determine how prevalent these fungi are in healthy sapwood. While sampling of healthy ash was limited, fewer fungi were recovered than expected compared to other studies of healthy ash (Bakys et al., 2009; Kosawang et al., 2018).

Apart from the functional guilds identified, cosmopolitan genera such as *Alternaria*, *Epicoccum* and *Cladosporium* were among the most commonly isolated. Conversely, there were also many taxa that were isolated infrequently. Of all the taxa, 118 (78%) were isolated five times or less and 66 taxa (42%) were isolated only once (Table S1), indicating these taxa are not widely associated with EAB galleries or amenable to isolation. Species diversity in many fungal communities, such as wood-inhabiting, endophytic, insect galleries-associated, and ectomycorrhizal, are often represented by a few highly abundant taxa and a long "tail" of rare species (with low abundance) (Abrego et al., 2014; Arnold and Lutzoni, 2007; Dickie et al., 2020; Gazis et al., 2018; Taylor, 2002). The abundance - based rarefaction curves in our study show that while the numbers of detected species did not reach an asymptote, sampling was nearing saturation as sampling coverage (sample completeness) reached 93% for the total of 174 species (Fig. 6). This means that the estimated undetected proportion of species is only 9%. Extrapolation indicates that by adding approximately 1000 samples, sample completeness would only increase completeness to 97%. According to Chao et al. (2020, 2014), rarefaction estimation based on abundance (individual-based) with a diversity order $q = 0$ represents the species richness of an assemblage. Species richness refers to the number of (different) species present in an assemblage or sample (Gotelli and Chao, 2013). When considering species richness, all species are weighted equally, thus rare species are weighted the same as common ones (Chao et al., 2014), and species relative abundance does not factor in at all (Gotelli and Chao, 2013). Thus, the estimate of species richness is highly sensitive to rare species that might not be detected, even in relatively large samples (Gotelli and Chao, 2013). This may be the case with the data presented here, due to the high number of rare species and the weight these have on the construction of abundance-based rarefaction curves, saturation of the curves was not reached.

The fungal functional guilds identified give insight into the fungal community and possible functions as the wood resource is made available by EAB. Infection may occur from spores entering through wounds, or they may be already be present as latent endophytes and perform their associated functions once insect attack has taken place (Parfitt et al., 2010). Canker fungi likely invade areas where larvae have wounded phloem and xylem tissue. These fungi may have the potential to attack healthy tissue, potentially enlarging the affected area by the EAB galleries and causing additional necrosis. Some decay fungi are early colonizers of wood and rapidly colonize wounded tissue and cause degradation (Boddy and Heilmann-Clausen, 2008). Entomopathogens, either entering with newly emerged larvae, as spores, or having previously established as endophytes, may survive in galleries as saprotrophs, but also infect larvae and adult beetles. While entomopathogens, primarily *Beauveria* species, have been studied for application as biocontrol agents, additional studies are needed to establish details of their interactions throughout the different stages of EAB attack and gallery formation.

We conclude from our results that a wide range of fungi are associated with EAB larval galleries and many have the potential to cause lesions that expand the necrotic area around EAB galleries, decay ash wood or be pathogenic to the insect. None of the fungi isolated appear to be exotic plant-pathogenic species. The three identified functional guilds of fungi are capturing the resources of the new niche that EAB has created in ash. This first look into the fungal diversity of EAB galleries identifies the major taxa present and raises questions about the priority effects that shape the fungal community and processes of succession of functional groups. Studying other *Agrilus* species, both invasive and native, would aid in understanding these communities across larger scales. Although *B. bassiana* has been shown to be a potential biocontrol agent for EAB (Castrillo et al., 2010a; Liu and Bauer, 2008) the other entomopathogenic species found in this study deserve additional research. Further studies are also warranted on the pathogenicity of canker fungi and the potential of decay fungi to increase hazardous tree conditions in EAB infested trees, as well as to better understand how they influence EAB induced tree mortality.

Acknowledgements

The authors thank Drs. Robert Venette, US Forest Service and Brian Aukuma, University of Minnesota for assistance in sample collection. We also thank the many city and park personnel in Minneapolis, St. Paul, Plymouth, Duluth, Rochester, Winona, MN. We thank Leah Grim, Sam Redford, Connor Lund, Amanda Stear, and Camille Schlegel for assistance in the laboratory. Project funding was provided by the Minnesota Environment and Natural Resources Trust Fund, Minnesota Invasive Terrestrial Plants and Pests Center, and supported by the USDA National Institute of Food and Agriculture, Hatch Project MIN-22-081.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2021.02.004.

References

Abrego, N., García-Baquero, G., Halme, P., Ovaskainen, O., Salcedo, I., 2014. Community turnover of wood-inhabiting fungi across hierarchical spatial scales. PloS One 9, e103416.

Anwar, W., Ali, S., Nawaz, K., Iftikhar, S., Javed, M.A., Hashem, A., Alqarawi, A.A., Abd Allah, E.F., Akhter, A., 2018. Entomopathogenic fungus Clonostachys rosea as a biocontrol agent against whitefly (Bemisia tabaci). Biocontrol Sci. Technol. 28, 750–760.

Arnold, A.E., Lutzoni, F., 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? Ecology 88, 541–549.

Atkins, S.D., Clark, I.M., Pande, S., Hirsch, P.R., Kerry, B.R., 2005. The use of real-time PCR and species-specific primers for the identification and monitoring of Paecilomyces lilacinus. FEMS Microbiol. Ecol. 51, 257–264.

Bakys, R., Vasaitis, R., Barklund, P., Thomsen, I.M., Stenlid, J., 2009. Occurrence and pathogenicity of fungi in necrotic and non-symptomatic shoots of declining common ash (Fraxinus excelsior) in Sweden. Eur. J. For. Res. 128, 51–60.

Baranchikov, Y., Mozolevskaya, E., Yurchenko, G., Kenis, M., 2008. Occurrence of the emerald ash borer, *Agrilus planipennis* in Russia and its potential impact on European forestry. EPPO Bull. 38, 233–238.

Bauer, L.S., Liu, H.P., Haack, R.A., Gao, R., Zhao, T., Miller, D.L., Petrice, T.R., 2004. Emerald ash borer natural enemy surveys in Michigan and China. In: Proceedings of Emerald Ash Borer Research and Technology Development Meeting. Romulus, Michigan.

Blanchette, R., Held, B., Hellmann, L., Millman, L., Buntgen, U., 2016. Arctic driftwood reveals unexpectedly rich fungal diversity. Fungal Ecol. 23, 58–65.

Blodgett, J.T., Kruger, E.L., Stanosz, G.R., 1997. Effects of moderate water stress on disease development by Sphaeropsis sapinea on red pine. Phytopathology 87, 422–428.

Boddy, L., Heilmann-Clausen, J., 2008. Basidiomycete community development in temperate angiosperm wood. In: Boddy, L., Frankland, J., van West, P. (Eds.), Ecology of Saprotrophic Basidiomycetes. Elsevier Academic Press, UK, pp. 211–237.

Bray, A.M., Bauer, L.S., Poland, T.M., Haack, R.A., Cognato, A.I., Smith, J.J., 2011. Genetic analysis of emerald ash borer (Agrilus planipennis Fairmaire) populations in Asia and North America. Biol. Invasions 13, 2869–2887.

Cappaert, D., McCullough, D.G., Poland, T.M., Siegert, N.W., 2005. Emerald ash borer in North America: a research and regulatory challenge. Am. Entomol. 51, 152–165.

Castrillo, L.A., Bauer, L.S., Liu, H., Griggs, M.H., Vandenberg, J.D., 2010a. Characterization of Beauveria bassiana (Ascomycota: Hypocreales) isolates associated

with Agrilus planipennis (Coleoptera: Buprestidae) populations in Michigan. Biol. Contr. 54, 135–140.
Castrillo, L.A., Griggs, M.H., Liu, H., Bauer, L.S., Vandenberg, J.D., 2010b. Assessing deposition and persistence of Beauveria bassiana GHA (Ascomycota: Hypocreales) applied for control of the emerald ash borer, Agrilus planipennis (Coleoptera: Buprestidae), in a commercial tree nursery. Biol. Contr. 54, 61–67.
Chamorro, M.L., Jendek, E., Haack, R.A., Petrice, T., Woodley, N.E., Konstantinov, A.S., Volkovitsh, M.G., Yang, X.-K., Grebennikov, V.V., 2015. Illustrated Guide to the Emerald Ash Borer, Agrilus Planipennis Fairmaire and Related Species (Coleoptera, Buprestidae). Pensoft, Sofia, Bulgaria.
Chao, A., Gotelli, N.J., Hsieh, T.C., Sander, E.L., Ma, K.H., Colwell, R.K., Ellison, A.M., 2014. Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecol. Monogr. 84, 45–67.
Chao, A., Kubota, Y., Zelený, D., Chiu, C., Li, C., Kusumoto, B., Yasuhara, M., Thorn, S., Wei, C., Costello, M.J., Colwell, R.K., 2020. Quantifying sample completeness and comparing diversities among assemblages. Ecol. Res. 35, 292–314.
Cipollini, D., Wang, Q., Whitehill, J.G.A., Powell, J.R., Bonello, P., Herms, D.A., 2011. Distinguishing defensive characteristics in the phloem of ash species resistant and susceptible to emerald ash borer. J. Chem. Ecol. 37, 450–459.
Coetzee, M.P.A., Marincowitz, S., Muthelo, V.G., Wingfield, M.J., 2015. Ganoderma species, including new taxa associated with root rot of the iconic Jacaranda mimosifolia in Pretoria, South Africa. IMA Fungus 6, 249–256.
Damm, U., Verkley, G.J.M., Crous, P.W., Fourie, P.H., Haegi, A., Riccioni, L., 2008. Novel Paraconiothyrium species on stone fruit trees and other woody hosts. Persoonia Mol. Phylogeny Evol. Fungi 20, 9–17.
Di Marco, S., Calzarano, F., Osti, F., Mazzullo, A., 2004. Pathogenicity of fungi associated with a decay of kiwifruit. Australas. Plant Pathol. 33, 337–342.
Dickie, I.A., Wakelin, A., Richardson, S.J., 2020. Rare species of wood inhabiting fungi are not local. Ecol. Appl. 2.
Duan, J.J., Van Driesche, R.G., Bauer, L.S., Kashian, D.M., Herms, D.A., 2015. Risk to ash from emerald ash borer: can biological control prevent the loss of ash stands? In: Van Driesche, R.G., Reardon, R.R. (Eds.), Biology and Control of Emerad Ash Borer. USDA Forest Service, Forest Health Technology Enterprise, Morgantown, WV, pp. 153–163.
Elena, G., Bruez, E., Rey, P., Luque, J., 2018. Microbiota of grapevine woody tissues with or without esca-foliar symptoms in northeast Spain. Phytopathol. Mediterr. 57, 425–438.
Eriksson, K.E., Blanchette, R.A., Ander, P., 1990. Microbial and Enzymatic Degradation of Wood and Wood Components. Springer Verlag, Berlin, Heidelberg.
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.
Gazis, R., Poplawski, L., Klingeman, W., Boggess, S.L., Trigiano, R.N., Graves, A.D., Seybold, S.J., Hadziabdic, D., 2018. Mycobiota associated with insect galleries in walnut with thousand cankers disease reveals a potential natural enemy against Geosmithia morbida. Fungal Biol. 122, 241–253.
Ginns, J.H., Lefebvre, M.N.L., 1993. Lignicolous Corticioid Fungi (Basidiomycota) of North America : Systematics, Distribution, and Ecology. APS Press, St. Paul, Minn.
Gotelli, N.J., Chao, A., 2013. Measuring and estimating species richness, species diversity, and biotic similarity from sampling data. In: Levin, S.A. (Ed.), The Encyclopedia of Biodiversity. Academic Press, Waltham, Massachusetts, pp. 195–211.
Hao, Y., Chen, S.Y., Blanchette, R.A., Liu, X., 2010. Sistotrema brinkmannii, a psychrotolerant fungus from Antarctic soil. Mycosystema 29, 864–868.
Harrington, T.C., 1981. Cycloheximide sensitivity as a taxonomic character in Ceratocystis. Mycologia 73, 1123.
Hausner, G., Eyjólfsdóttir, G.G., Reid, J., Klassen, G.R., 1992. Two additional species of the genus Togninia. Can. J. Bot. 70, 724–734.
Herms, D.A., McCullough, D.G., 2014. Emerald ash borer invasion of north America: history, biology, ecology, impacts, and management. Annu. Rev. Entomol. 59, 13–30.
Herms, D.A., McCullough, D.G., 2013. Emerald ash borer: ecology and management. Encycl. Pest Manag. 1–14.
Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (H ill numbers). Methods Ecol. Evol. 7, 1451–1456.
Johny, S., Kyei-Poku, G., Gauthier, D., Frankenhuyzen, K. van, Krell, P.J., 2012a. Characterization and virulence of Beauveria spp. recovered from emerald ash borer in southwestern Ontario, Canada. J. Invertebr. Pathol. 111, 41–49.
Johny, S., Kyei-Poku, G., Gauthier, D., van Frankenhuyzen, K., 2012b. Isolation and characterisation of Isaria farinosa and Purpureocillium lilacinum associated with emerald ash borer, Agrilus planipennis in Canada. Biocontrol Sci. Technol. 22, 723–732.
Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., Drummond, A., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.
Kepley, J.B., Jacobi, W.R., 2000. Pathogenicity of Cytospora fungi on six hardwood species. J. Arboric. 26, 326–333.
Koch, J.L., Carey, D.W., Mason, M.E., Poland, T.M., Knight, K.S., 2015. Intraspecific variation in Fraxinus pennsylvanica responses to emerald ash borer (Agrilus planipennis). New Times 46, 995–1011.
Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm on fungal identification. Mol. Ecol. 5271–5277.
Kosawang, C., Buchvaldt Amby, D., Bussaban, B., Mckinney, L.V., Xu, J., Kjaer, E.D., Collinge, D.B., Nielsen, R., 2018. Fungal communities associated with species of Fraxinus tolerant to ash dieback, and their potential for biological control. Fungal Biol. 122, 110–120.
Kubátová, A., Kolařík, M., Pažoutová, S., 2004. Phaeoacremonium rubrigenum - hyphomycete associated with bark beetles found in Czechia. Folia Microbiol. 49, 99–104.
Lacey, L.A., Grzywacz, D., Shapiro-Ilan, D.I., Frutos, R., Brownbridge, M., Goettel, M.S., 2015. Insect pathogens as biological control agents: Back to the future. J. Invertebr. Pathol. 132, 1–41.
Li, P., Liu, W., Zhang, Y., Xing, J., Li, J., Feng, J., Su, X., Zhao, J., 2019. Fungal canker pathogens trigger carbon starvation by inhibiting carbon metabolism in poplar stems. Sci. Rep. 9, 10111.
Liu, H., Bauer, L.S., 2008. Microbial control of emerald ash borer, Agrilus planipennis (Coleoptera: Buprestidae) with Beauveria bassiana strain GHA: greenhouse and field trials. Biol. Contr. 45, 124–132.
Liu, H., Bauer, L.S., Miller, D.L., Zhao, T., Gao, R., Song, L., Luan, Q., Jin, R., Gao, C., 2007. Seasonal abundance of Agrilus planipennis (Coleoptera: Buprestidae) and its natural enemies Oobius agrili (hymenoptera: Encyrtidae) and tetrastichus planipennisi (hymenoptera: Eulophidae) in China. Biol. Contr. 42, 61–71. 42.
Lombard, F.F., 1990. A cultural study of several species of Antrodia (polyporaceae, Aphyllophorales). Mycologia 82, 185–191.
Lopez, D.C., Zhu-Salzman, K., Ek-Ramos, M.J., Sword, G.A., 2014. The entomopathogenic fungal endophytes Purpureocillium lilacinum (formerly Paecilomyces lilacinus) and Beauveria bassiana negatively affect cotton aphid reproduction under both greenhouse and field conditions. PloS One 9.
Luangsa-Ard, J., Houbraken, J., van Doorn, T., Hong, S.B., Borman, A.M., Hywel-Jones, N.L., Samson, R.A., 2011. Purpureocillium, a new genus for the medically important Paecilomyces lilacinus. FEMS Microbiol. Lett. 321, 141–149.
Mascarin, G.M., Jaronski, S.T., 2016. The production and uses of Beauveria bassiana as a microbial insecticide. World J. Microbiol. Biotechnol. 32, 1–26.
McKinnon, A.C., Saari, S., Moran-Diez, M.E., Meyling, N.V., Raad, M., Glare, T.R., 2017. Beauveria bassiana as an endophyte: a critical review on associated methodology and biocontrol potential. BioControl 62, 1–17.
McTavish, C.K., Fulbright, D.W., Jarosz, A.M., Jarosz, Andrew M., 2018. Spruce decline and diaporthe: incidence, taxonomy, virulence, and tree susceptibility in Michigan. Plant Dis. 102, 2330–2340.
Mogouong, J., Constant, P., Lavalí Ee, R., Guertin, C., 2020. Gut microbiome of the emerald ash borer, Agrilus planipennis Fairmaire, and its relationship with insect population density. FEMS Microbiol. Ecol. 96, 141.
Mostert, L., Groenewald, J.Z., Summerbell, R.C., Gams, W., Crous, P.W., 2006a. Taxonomy and pathology of togninia (Diaporthales) and its Phaeoacremonium anamorphs. Stud. Mycol. 54, 1–113.
Mostert, L., Halleen, F., Fourie, P., Crous, P.W., 2006b. A review of Phaeoacremonium species involved in Petri disease and esca of grapevines. Phytopathol. Mediterr. 45, S12–S129.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248.
Osmundson, T.W., Eyre, C.A., Hayden, K.M., Dhillon, J., Garbelotto, M.M., 2013. Back to basics: an evaluation of NaOH and alternative rapid DNA extraction protocols for DNA barcoding, genotyping, and disease diagnostics from fungal and oomycete samples. Mol. Ecol. Resour. 13, 66–74.
Parfitt, D., Hunt, J., Dockrell, D., Rogers, H.J., Boddy, L., Griffith, G.W., 2010. Do all trees carry the seeds of their own destruction? PCR reveals numerous wood decay fungi latently present in sapwood of a wide range of angiosperm trees. Fungal Ecol. 3, 338–346.
Paterson, R.R.M., 2007. Ganoderma disease of oil palm-A white rot perspective necessary for integrated control. Crop Protect. 26, 1369–1376.
Persad, A.B., Siefer, J., Montan, R., Kirby, S., Rocha, O.J., Redding, M.E., Ranger, C.M., Jones, A.W., 2013. Effects of emerald ash borer infestation on the structure and material properties of ash Trees. Arboric. Urban For. 39, 11–16.
Poland, T.M., McCullough, D.G., 2006. Emerald ash borer: invasion of the urban forest and the threat to North America's ash resource. J. For. 104, 118–124.
Potvin, L.R., Richter, D.L., Jurgensen, M.F., Dumroese, R.K., 2012. Association of pinus banksiana lamb. And populus tremuloides michx. Seedling fine roots with Sistotrema brinkmannii (Bres.) J. Erikss. (Basidiomycotina). Mycorrhiza 22, 631–638.
Rebek, E.J., Herms, D.A., Smitley, D.R., 2008. Interspecific variation in resistance to emerald ash borer (Coleoptera: Buprestidae) among North American and Asian ash (*Fraxinus* spp.). Environ. Entomol. 37, 242–246.
Rehner, S.A., Minnis, A.M., Sung, G.H., Luangsa-ard, J.J., Devotto, L., Humber, R.A., 2011. Phylogeny and systematics of the anamorphic, entomopathogenic genus Beauveria. Mycologia 103, 1055–1073.
Rigsby, C.M., Showalter, D.N., Herms, D.A., Koch, J.L., Bonello, P., Cipollini, D., 2015. Physiological responses of emerald ash borer larvae to feeding on different ash

species reveal putative resistance mechanisms and insect counter-adaptations. J. Insect Physiol. 78, 47–54.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Bolchacova, E., Voigt, K., Crous, P.W., Miller, A.N., Wingfield, M.J., Aime, M.C., An, K.D., Bai, F.Y., Barreto, R.W., Begerow, D., Bergeron, M.J., Blackwell, M., Boekhout, T., Bogale, M., Boonyuen, N., Burgaz, A.R., Buyck, B., Cai, L., Cai, Q., Cardinali, G., Chaverri, P., Coppins, B.J., Crespo, A., Cubas, P., Cummings, C., Damm, U., de Beer, Z.W., de Hoog, G.S., Del-Prado, R., Dentinger, B., Diéguez-Uribeondo, J., Divakar, P.K., Douglas, B., Dueñas, M., Duong, T.A., Eberhardt, U., Edwards, J.E., Elshahed, M.S., Fliegerova, K., Furtado, M., García, M.A., Ge, Z.W., Griffith, G.W., Griffiths, K., Groenewald, J.Z., Groenewald, M., Grube, M., Gryzenhout, M., Guo, L.D., Hagen, F., Hambleton, S., Hamelin, R.C., Hansen, K., Harrold, P., Heller, G., Herrera, C., Hirayama, K., Hirooka, Y., Ho, H.M., Hoffmann, K., Hofstetter, V., Högnabba, F., Hollingsworth, P.M., Hong, S.B., Hosaka, K., Houbraken, J., Hughes, K., Huhtinen, S., Hyde, K.D., James, T., Johnson, E.M., Johnson, J.E., Johnston, P.R., Jones, E.B.G., Kelly, L.J., Kirk, P.M., Knapp, D.G., Kõljalg, U., Kovács, G.M., Kurtzman, C.P., Landvik, S., Leavitt, S.D., Liggenstoffer, A.S., Liimatainen, K., Lombard, L., Luangsa-ard, J.J., Lumbsch, H.T., Maganti, H., Maharachchikumbura, S.S.N., Martin, M.P., May, T.W., McTaggart, A.R., Methven, A.S., Meyer, W., Moncalvo, J.M., Mongkolsamrit, S., Nagy, L.G., Nilsson, R.H., Niskanen, T., Nyilasi, I., Okada, G., Okane, I., Olariaga, I., Otte, J., Papp, T., Park, D., Petkovits, T., Pino-Bodas, R., Quaedvlieg, W., Raja, H.A., Redecker, D., Rintoul, T.L., Ruibal, C., Sarmiento-Ramírez, J.M., Schmitt, I., Schüßler, A., Shearer, C., Sotome, K., Stefani, F.O.P., Stenroos, S., Stielow, B., Stockinger, H., Suetrong, S., Suh, S.O., Sung, G.H., Suzuki, M., Tanaka, K., Tedersoo, L., Telleria, M.T., Tretter, E., Untereiner, W.A., Urbina, H., Vágvölgyi, C., Vialle, A., Vu, T.D., Walther, G., Wang, Q.M., Wang, Y., Weir, B.S., Weiß, M., White, M.M., Xu, J., Yahr, R., Yang, Z.L., Yurkov, A., Zamora, J.C., Zhang, N., Zhuang, W.Y., Schindel, D., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc. Natl. Acad. Sci. U. S. A. 109, 6241–6246.

Schulz, A.N., Mech, A.M., Cram, M.M., Asaro, C., Coyle, D.R., Lucardi, R.D., Lucas, S., Gandhi, K.J.K., 2018. Association of Caliciopsis pinea Peck and Matsucoccus macrocicatrices Richards with eastern white pine (Pinus strobus L.) seedling dieback. For. Ecol. Manag. 423, 70–83.

Sinclair, W.A., Lyon, H.H., Johnson, W.T., 1987. Diseases of Trees and Shrubs. Cornell University Press, Ithaca, NY.

Spirin, V., Runnel, K., Vlasák, J., Miettinen, O., Põldmaa, K., 2015. Species diversity in the Antrodia crassa group (Polyporales, Basidiomycota). Fungal Biol. 119, 1291–1310.

Taylor, A.F.S., 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. In: Smith, S.E., Smith, F.A. (Eds.), Diversity and Integration in Mycorrhizas. Developments in Plant and Soil Sciences. Springer, Dordrecht, pp. 19–28.

Toledo-Hernández, R.A., Toledo, J., Valle-Mora, J., Holguín-Meléndez, F., Liedo, P., Huerta-Palacios, G., 2019. Pathogenicity and virulence of Purpureocillium lilacinum (Hypocreales: Ophiocordycipitaceae) on Mexican fruit fly adults. Fla. Entomol. 102, 309.

Toledo, A.V., Virla, E., Humber, R.A., Paradell, S.L., Lastra, C.C.L., 2006. First record of Clonostachys rosea (Ascomycota: Hypocreales) as an entomopathogenic fungus of Oncometopia tucumana and sonesimia grossa (Hemiptera: Cicadellidae) in Argentina. J. Invertebr. Pathol. 92, 7–10.

Vasanthakumar, A., Handelsman, J.O., Schloss, P.D., Bauer, L.S., Raffa, K.F., 2008. Gut microbiota of an invasive subcortical beetle, Agrilus planipennis Fairmaire, across various life stages. Environ. Entomol. 37, 1344–1353.

Vega, F.E., Posada, F., Catherine Aime, M., Pava-Ripoll, M., Infante, F., Rehner, S.A., 2008. Entomopathogenic fungal endophytes. Biol. Contr. 46, 72–82.

Verkley, G.J.M., Da Silva, M., Wicklow, D.T., Crous, P.W., 2004. Paraconiothyrium, a new genus to accommodate the mycoparasite Coniothyrium minitans, anamorphs of Paraphaeosphaeria, and four new species. Stud. Mycol. 50, 323–335.

Wang, X.-Y., Yang, Z.-Q., Gould, J.R., Zhang, Y.-N., Liu, G.-J., Liu, E., 2010. The Biology and ecology of the emerald ash borer, Agrilus planipennis , in China. J. Insect Sci. 10, 1–23.

Wang, Y.U., Guo, L.D., Hyde, K.D., 2005. Taxonomic placement of sterile morphotypesof endophytic fungi from Pinus tabulaeformis (Pinaceae) in northeast China based on rDNA sequences. Fungal Divers. 20, 235–260.

Wei, X., Reardon, D., Wu, X., Sun, J.-H., 2004. Emerald ash borer, Agrilus planipennis Fairmaire (Coleoptera: Buprestidae), in China: a review and distribution survey. Acta Entomol. Sin. 47, 679–685.

Worrall, J.J., 1991. Media for selective isolation of hymenomycetes. Mycologia 83, 296–302.

Yuan, Z.L., Rao, L.B., Chen, Y.C., Zhang, C.L., Wu, Y.G., 2011. From pattern to process: species and functional diversity in fungal endophytes of Abies beshanzuensis. Fungal Biol. 115, 197–213.

Zabel, R.A., Morrell, J.J., 1992. Wood Microbiology. Academic Press, San Diego, CA.

Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7, 203–214.