Phytopathology® • 2022 • 112:261-270 • https://doi.org/10.1094/PHYTO-01-21-0041-R

Ecology and Epidemiology

e-Xtra*

Preinvasion Assessment of Exotic Bark Beetle-Vectored Fungi to Detect Tree-Killing Pathogens

You Li,[1,2] Craig Bateman,[3] James Skelton,[4] Bo Wang,[5] Adam Black,[6] Yin-Tse Huang,[1] Allan Gonzalez,[1] Michelle A. Jusino,[4] Zachary J. Nolen,[7] Stanley Freeman,[8] Zvi Mendel,[8] Miroslav Kolařík,[9] Miloš Knížek,[10] Ji-Hyun Park,[11] Wisut Sittichaya,[12] Thai-Hong Pham,[13] Shin-ichiro Ito,[14] Masato Torii,[15] Lei Gao,[16] Andrew J. Johnson,[1] Min Lu,[17] Jianghua Sun,[18] Zhen Zhang,[19] Damian C. Adams,[1] and Jiri Hulcr[1,†]

[1] School of Forest, Fisheries and Geomatics Sciences, University of Florida, Gainesville 32611, U.S.A.
[2] Fujian Province Key Laboratory of Plant Virology, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[3] Florida Museum of Natural History, University of Florida, Gainesville 32611, U.S.A.
[4] Department of Biology, William and Mary, Williamsburg 23185, U.S.A.
[5] CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China
[6] Peckerwood Garden Conservation Foundation, Hempstead 77445, U.S.A.
[7] Department of Biology, Lund University, Lund, Sweden
[8] Plant Protection Institute, The Volcani Center, Rishon LeZion, Israel
[9] Institute of Microbiology, Czech Academy of Sciences, Prague, Czech Republic
[10] Forestry and Game Management Research Institute, 156 04 Prague 5–Zbraslav, Czech Republic
[11] National Institute of Forest Science, Seoul, South Korea
[12] Department of Pest Management, Faculty of Natural Resources, Prince of Songkla University, Songkhla 90110, Thailand
[13] Mientrung Institute for Scientific Research, VNMN and Graduate School of Science and Technology, Vietnam Academy of Science and Technology, Hue, Vietnam
[14] Graduate School of Bioresources, Mie University, Tsu, Japan
[15] Department of Mushroom Science and Forest Microbiology, Forestry and Forest Products Research Institute, Tsukuba 305-8687, Japan
[16] Shanghai Academy of Landscape Architecture Science and Planning, Shanghai Engineering Research Center of Landscaping on Challenging Urban Sites, Shanghai, China
[17] School of Life Sciences, Hubei University, Wuhan, China
[18] Institute of Zoology, Chinese Academy of Sciences, Beijing, China
[19] Key Laboratory of Forest Protection of National Forestry and Grassland Administration, Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing, China

Accepted for publication 10 July 2021.

ABSTRACT

Exotic diseases and pests of trees have caused continental-scale disturbances in forest ecosystems and industries, and their invasions are considered largely unpredictable. We tested the concept of preinvasion assessment of not yet invasive organisms, which enables empirical risk assessment of potential invasion and impact. Our example assesses fungi associated with Old World bark and ambrosia beetles and their potential to impact North American trees. We selected 55 Asian and European scolytine beetle species using host use, economic, and regulatory criteria. We isolated 111 of their most consistent fungal associates and tested their effect on four important southeastern American pine and oak species. Our test dataset found no highly virulent pathogens that should be classified as an imminent threat. Twenty-two fungal species were minor pathogens, which may require context-dependent response for their vectors at North American borders, while most of the tested fungi displayed no significant impact. Our results are significant in three ways; they ease the concerns over multiple overseas fungus vectors suspected of heightened potential risk, they provide a basis for the focus on the prevention of introduction and establishment of species that may be of consequence, and they demonstrate that preinvasion assessment, if scaled up, can support practical risk assessment of exotic pathogens.

Keywords: epidemiology, forest pathology, fungal pathogens, host–parasite interactions

Tree-killing fungi and insects are major contributors to the global decline of the health of forests (Bradshaw et al. 2016; Hyde et al. 2018). In North America, inadvertently introduced fungal pathogens vectored by wood-boring insects devastated entire tree species, including native elms (Brasier 1991), laurels (Rodgers et al. 2014), avocados (Carrillo et al. 2016), and urban trees (O'Donnell et al. 2016). Such invasions are rare, but they cause continental-scale disturbances (Millar and Stephenson 2015) followed by economic and ecological burden (Kovacs et al. 2010), degradation of ecosystem services (Walsh et al. 2016), and loss of biomass and carbon storage (Fei et al. 2019). Should a future tree epidemic impact a major component of North American forests or the urban landscape, such as pines or oaks, it would result in economic and ecological disaster, fundamentally disturbing the socioecological systems of North America (Morris et al. 2018; Potter et al. 2019). Arrivals of new

---

[†] Corresponding author: J. Hulcr; hulcr@ufl.edu

Y. Li and C. Bateman contributed equally to this work.

Funding: C. Bateman, J. Hulcr, M. A. Jusino, Y. Li, and J. Sun were partially supported by the U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service, the U.S. Forest Service Forest Health Protection, the USDA-FS Southern Research Station, the National Science Foundation (grant 1414801 to C. Bateman and grants 1834264 and 1556283 to J. Hulcr), the Florida Department of Agriculture and Consumer Services–Division of Plant Industry, the University of Florida Institute of Food and Agricultural Sciences, and the University of Florida Emerging Pathogens Institute. M. Kolařík was partly supported by Ministry of Agriculture of the Czech Republic institutional support grant MZE-RO0118.

*The ***e*-Xtra** logo stands for "electronic extra" and indicates that supplementary materials are published online.

The author(s) declare no conflict of interest.

exotic pathogens will continue as trade and travel increase, while resources for trade regulation remain limited, and compliance is difficult to enforce (Barbier et al. 2013; Lodge et al. 2006).

The risk associated with international pathogen pathways is lessened by existing regulatory barriers (e.g., Wood Packaging Materials International Standards for Phytosanitary Measures No. 15) and international treaties (e.g., International Plant Protection Convention, North American Plant Protection Organization). The implementation of the actual response to a novel alien invasive species relies on decision-making within each country. For newly introduced pathogens, the optimal approach to damage prevention is early detection and eradication at the onset of the invasion (Luchi et al. 2020; Myers et al. 2000). Such an incident command system has been established, for example, within the U.S. Department of Agriculture (USDA), and it relies on species risk assessment via the Objective Prioritization of Exotic Pests model (U.S. Department of Agriculture Animal and Plant Health Inspection Service 2019) and on expert consensus (e.g., the Plant Protection Act of 2000, the Agricultural Bioterrorism Protection Act of 2002, and the 2008 Amendment to the Lacey Act of 1900). In Europe, the EU 2016/2031 Plant Health Law also stipulates a list of foreign organisms posing unacceptable risk to the Union, albeit without a prioritization algorithm. In both cases, the algorithms and the determination of an appropriate response require reliable data.

Unfortunately, the lack of preinvasion data on species-specific damage potential, combined with limited resources, results in the mostly reactive regulatory approach to new species arrivals (Lodge et al. 2006; Seebens et al. 2018). A more proactive policy stance has been advocated for a robust biosecurity continuum in North America (Early et al. 2016; Eschen et al. 2015; Tilman et al. 2017). Proactive preinvasion decision-making is also needed to meet the Aichi Biodiversity Target 9 of the Convention on Biological Diversity (per the 2011 United Nations Environment Programme), which requires identification and prioritization of management targets for invasive species (McGeoch et al. 2016).

To lessen the uncertainty about the threat of Eurasian wood borer-associated fungi in North America, we use the concept of preinvasion assessment (Fig. 1). We are testing in vivo specific interactions between individual exotic organisms and a particular host that may be impacted. Specific preinvasion tests of eventual damage offer empirical data to risk analysts who would otherwise rely on assumptions, models, experiences from other countries, or expert opinions, especially for species that have not yet expanded beyond their home ranges. Furthermore, empirical risk associated with specific exotic organisms can be scaled up to assess the risk from traded commodities with which these organisms may be imported, or the potential pathways of introduction. Preinvasion assessment data also facilitate "horizon scans" for emerging threats, which typically rely on expert consensus or published information on species that are already invasive somewhere (Roy et al. 2014, 2019).

The goal of this research is to classify various beetle-associated fungi as actionable or not actionable. The key element was the separation of the virulent, systemic pathogens from other pathogens, not a documentation of all potential pathogens from overseas or their interactions with trees. The justification for this triage approach is a pragmatic one: systemic tree-killing pathogens and their vectors have been subject to quarantine before (e.g., *Ophiostoma ulmi*, *Ophiostoma novo-ulmi*, *Raffaelea lauricola*). Many less pathogenic bark beetle-associated fungi are capable of forming lesions or cankers, but their ultimate, landscape-scale impact is dependent on their vector and the susceptibility of the host tree, and, as such, is much more difficult to predict before introduction.

This study develops empirical proactive preinvasion assessment for fungi vectored by bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae). There are >6,000 species of bark and ambrosia beetles, and they are among the most commonly intercepted exotic forest pests on the U.S. border (Haack 2006). True bark beetles develop under bark of trees, feeding mostly on the phloem tissues, and are typically associated with commensal fungi as inadvertent vectors. Ambrosia beetles represent 14 lineages of bark beetles that live inside the xylem, where they introduce specific, coevolved mutualistic symbiotic fungi on which they feed. The fungi associated with bark and ambrosia beetles belong to many taxonomic groups, most commonly Ophiostomatales, Hypocreales, and Ceratocystidaceae (Hulcr and Stelinski 2017; Seifert et al. 2013).

The majority of introduced fungi associated with bark beetles have had minimal impacts; however, a few exceptional species

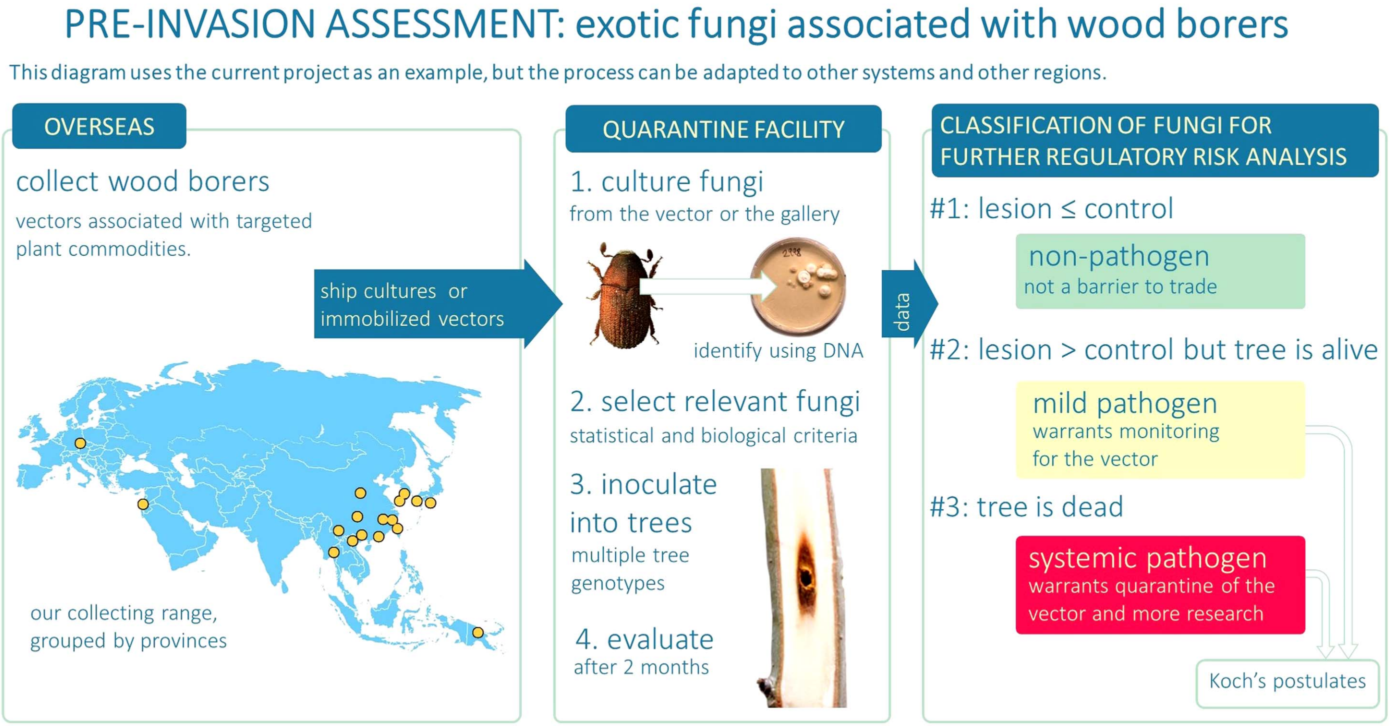

Fig. 1. Preinvasion assessment of exotic fungi associated with bark and ambrosia beetles.

have caused extensive damage to native ecosystems and economies. In the most dramatic cases, the fungus is the actual agent of tree mortality, while the beetle only serves as a vector (e.g., *R. lauricola* with *Xyleborus glabratus* and *O. novo-ulmi* with *Scolytus* spp.) (Brasier 1991; Fraedrich et al. 2008). These two pathosystems are used here as benchmarks, or the "biological positive controls," against which pathogenicity of the assessed fungi is compared.

It is important to reiterate that the goal of this research is to detect highly virulent systems, analogous to the two examples above, not to document all potential pathogens or document pathogenicity mechanisms of all the tested fungi. Distinguishing the categories "virulent pathogen" and "mild pathogen" may conceal the fact that some "mild pathogens" may exhibit tree-killing capacity in certain conditions or in large inoculum loads, but it offers a greater degree of certainty to the regulatory decision-making. Regulatory response toward an organism can be deployed only in the case of certainty of damage, not in the case of the degree of probability of damage. Therefore, highlighting all possible "potential" pathogens, including the mild ones, may further diffuse the agency's capacity for regulatory action.

For example, in North America, newly arriving forest pests are being monitored via the Early Detection and Rapid Response program (Rabaglia et al. 2019) and by the USDA Cooperative Agricultural Pest Survey (CAPS). One aim of these monitoring schemes is to intercept a large number of species to rapidly determine the occurrence of a newly detected species, but they cannot determine its impact. Only if a pest is carrying a pathogen that presents a known, high-certainty threat, the detection can lead to containment investment or eradication attempts. On the contrary, uncertainty of impact prevents the detections to be followed up by a management action. That is why we separate highly virulent pathogens from mildly virulent ones.

In our preinvasion assessment of the threat of fungi associated with exotic bark and wood-boring insects, we separate the effect of the beetle vectors from that of the associated fungi, and we test whether the fungi pose an existential threat to major tree commodities in the corresponding latitudes of eastern North America: pines (*Pinus* spp.) and oaks (*Quercus* spp.). We focus on potential pathogens of North American tree commodities, but this framework can be implemented elsewhere. Ultimately, the goal of the assessment is to demonstrate that the current reactive approach to the national-level invasive species management can be developed into a more focused, effective, and proactive strategy.

MATERIALS AND METHODS

Our screening consisted of the following steps (Fig. 1). First, we sought to locate and collect scolytine and platypodine species that commonly infest key commodities (Pinaceae and Fagaceae) in Asia, Europe, and the Mediterranean region via an international network of collaborating institutions (China, Czech Republic, Israel, Japan, Papua New Guinea, South Korea, Taiwan [China], Thailand, and Vietnam). Beetle species were selected for fungus isolation if they met any of three criteria: regulatory (included on the USDA Animal and Plant Health Inspection Service [APHIS] Prioritized Offshore Pest List), ecological (specific to the commodity in question, i.e., Fagaceae and Pinaceae, and common in the sampled region, thus with high export potential), or economic (a known local pest of the commodity in question). In other words, the source beetles were not sampled randomly, but to maximize our focus on the most likely invasive or impactful pest.

The next step was to isolate, purify, and DNA-barcode fungi consistently associated with the specific vector beetles. Bark and ambrosia beetles span a range of relationships with fungi, from beetles with only stochastic, incidental fungi, to consistent vectors without benefits, to mutually dependent symbionts. Only systems in which the fungus–beetle association is nonrandom were of interest: phoretic fungi isolated from more than half of bark beetle individuals of a given species and mutualistic ambrosia fungi.

The third step was to transport the fungi to a quarantine greenhouse in the United States and emulate a bark beetle entry with subsequent fungus inoculation into pines and oaks that represent most commonly planted species in the southeastern United States: *Pinus taeda*, *Pinus elliottii* var. *elliottii*, *Quercus shumardii*, and *Quercus virginiana*. For the pines, three different genotypes were included. The genotypes were selected by the supplier as originating from three different regions across the southern United States.

The final step was to classify pathogenicity of fungi by comparing tree responses (lesions, mortality) to previously known pathosystems. Fungal effects were classified as (i) insignificant (no regulatory response needed in case of future detection), (ii) minor pathogen (close monitoring warranted), and (iii) lethal pathogen (eradication recommended upon detection).

Vector collection and fungal isolation. In total, 258 bark and ambrosia beetle species were collected in mainland China, Taiwan, Thailand, Vietnam, Japan, South Korea, Papua New Guinea, the Czech Republic, and Israel between 2013 and 2018 (Supplementary Table S2).

Our criteria for species selection retained 55 species. In some cases, even substantial local pests were not tested because they did not yield any specific fungal associates (e.g., *Ips sexdentatus*), because they are pests on unrelated tree commodities (i.e., *Scolytus scolytus*), or because their impact on trees is not mediated by fungi (e.g., *Ips typographus*). We focused also on the target species on the USDA APHIS CAPS list. The list changes frequently, so we included species that appeared on the list within the duration of the experiment, even if temporarily (e.g., *Scolytus intricatus*).

Beetles of species that passed the criteria for selection (regulatory, ecological, and economic) and yielded consistent fungal associates were stored at 10 to 15°C for as long as 48 h before fungal isolation. To corroborate the association of each fungal strain, fungi were isolated from adult, pupal, and larval life stages or from galleries; sampling effort varied across species because it depended on availability. Beetle galleries were sampled by surface-sterilizing the wood piece with ethanol and scraping the gallery walls into phosphate-buffered saline (PBS) solution before serial dilution.

Beetle dissections and fungal culturing took place in the laboratories of the hosting national institutions (see author affiliations). External fungal load was harvested from beetle larvae and adults by surface-wash in a 1-ml sterile solution of 1% Tween 80 (Sigma Chemical) and PBS solution, which was then serially diluted for plating. For internal fungi, larvae and pupae of all species were individually macerated in PBS solution before serial dilution, and adult bark beetles were sectioned into three parts before maceration and dilution: the head, thorax, and abdomen. For ambrosia beetles (beetles obligately associated with mutualistic fungi), the body segments containing mycangia (fungus transport organs) were aseptically removed and processed separately. Serial dilutions of 1:10, 1:100, and 1:1,000 were plated on potato dextrose agar (BD Biosciences) amended with 1.4% additional agar (for extra hardness to facilitate the growth of hyphomycetes over yeasts) and 2% streptomycin. Plates were stored in an incubator in the dark at 25°C for as long as 2 weeks.

Morphotypes were designated based on macromorphology for fungal morphologies found in more than one sample within samples from a given beetle species (in other words, singletons were discarded). To ensure accurate and consistent assignment of morphotypes, plates were examined using a binocular microscope and monitored every 2 to 3 days during the 2-week incubation period. Plates were also photographed so morphotype designations could be confirmed by retrospective comparisons of pure cultures and by sequencing portions of the nuclear ribosomal DNA (rDNA). Only specific associates were used here, defined as components of the mycangial or body-surface community recovered from >50% of independent beetle samples (individuals from different galleries, typically from different trees).

Isolates selected for tree inoculations were imported into the United States under USDA APHIS Plant Protection and Quarantine permit P526P-16-02872.

Molecular identification and phylogenetic analysis. One- to two-week-old pure cultures were used for DNA extraction. Morphotype designations were corroborated by Sanger sequencing of the large subunit (LSU) and internal transcribed spacer (ITS) rDNA sequences. DNA was extracted using the Extract-N-Amp Plant PCR kit (Sigma-Aldrich) with the modification of using 3% bovine serum albumin in place of a dilution solution. Primer combinations used for PCR amplification were as follows: (i) LR0R/LR5 (Hopple and Vilgalys 1994; Vilgalys and Hester 1990) for nuclear LSU rDNA and (ii) ITS1F/ITS4 (Gardes and Bruns 1993) for portions of the ITS1-5.8S-ITS2 (ITS) rDNA locus. PCR reactions contained the following: 1 µl of template DNA, 1 µl of forward and reverse primers (10 mM), 12.5 µl of Premix Taq polymerase (Takara Ex Taq), 1 µl of dimethyl sulfoxide, and 9.5 µl of molecular-grade DNA-free water. The PCR conditions for LSU and ITS gene were following Li et al. (2021). Amplified products were purified and Sanger-sequenced by GENEWIZ.

Many fungal isolates were unknown to science, and their closest species identity was assigned by phylogenetic comparisons with congeneric sequences available in NCBI GenBank. Sequence alignments were conducted using MAFFT (Katoh et al. 2019). The most suitable nucleotide substitution model was selected using the Akaike information criterion in jModeltest 2.1.10 (Guindon and Gascuel 2003; Posada 2008). Maximum likelihood analyses were performed using the RAxML2.0 plugin (Stamatakis 2014) in Geneious.

Pathogenicity test. Inoculum suspensions in sterile water were made from 10- to 14-day-old fungal cultures plated on potato dextrose agar (Difco). The inoculation was intended to emulate beetle infestation; therefore, the inoculum concentrations (estimated using a hemocytometer) reflected the highest total yield from each respective beetle species, calculated from the CFU counts from serial dilutions. The CFU counts were estimated using a hemocytometer. Other projects focused on tree pathogens may use much higher inoculum loads, as much as three orders of magnitude higher (Fraedrich et al. 2008), or mycelial plugs with no quantification (Eskalen et al. 2012; Inácio et al. 2012; Takashina et al. 2019). In those projects, the goal is typically to elicit a response in the host tree with an arbitrary spore concentration, or there is no obvious biological justification for the concentration. Our goal was a test with realistic natural spore concentrations.

An initial bioassay included a higher number of replicates (eight) and pine genotypes (five) to determine the effect of genotype variability on pathogen response. For the pines, the following Rayonier genotypes were used: Slash3055 and SlashMix for *P. elliottii* and genotypes L2, L3, and L4 for *P. taeda*. Because all genotypes within a species yielded essentially identical responses, subsequent bioassays included only three replicates per tree species and only two pine species, *P. taeda* and *P. elliottii*, and two oak species, *Q. shumardii* and *Q. virginiana*. *P. elliottii* showed significantly more extensive stains than *P. taeda* (Fig. 2), but, otherwise, the two pine species did not differ in the response pattern. Pathogenicity bioassays were conducted in a USDA APHIS-approved Biosafety Level 2 quarantine greenhouse facility of the Florida Department of Agriculture and Consumer Services in Gainesville, FL. The trees (120 to 220 cm tall, in 18-liter pots) were maintained under natural light conditions, watered as necessary, and kept under a night/day temperature regimen averaging 27°C. Treatments were arranged in a completely randomized design, with five trees per fungus treatment and pine genotype combination. Each tree stem was ≥40 cm away from neighboring trees.

The trees were kept in good health before and during the test to allow the search for fungi pathogenic to healthy trees and to avoid complications caused by additional pathogens. Many fungi can cause pathological symptoms in stressed or dying trees, but such fungi are unlikely to be subject to regulation.

To imitate a bark beetle entry, a single hole was drilled at a 45° angle into the xylem of each tree using a 1.98-mm (5/64-inch) drill bit. Holes were made within the basal 30 cm of the stem and were, on average, 2 cm deep. Diameters of the stems at the inoculation sites were, on average, 1.4 cm for oaks and 1.9 cm for pines. Inoculum suspensions were pipetted into the xylem in 50-µl aliquots. Wound sites were wrapped in parafilm following inoculation. Although bioassays on large trees would more accurately represent a hypothetical beetle attack, small trees are a suitable substitute as demonstrated by our benchmark models, *R. lauricola* and *O. novo-ulmi*, both of which kill small seedlings as reliably as larger trees (Martín et al. 2015).

Trees were monitored weekly for signs of mortality and disease development (including foliar discoloration, wilting, resinosis, and mortality). After 10 weeks, inoculation sites were destructively sampled. Bark was scraped away to allow for measurement of phloem necrosis or cankers, and the stem was dissected for the measurement of stain in the xylem (Fig. 1). To complete Koch's postulates in the eventual case of systemic pathogens, stem sections were stored for as long as 2 days at 10°C beforehand to allow for reisolation and reinoculation of the pathogen. Stained and unstained portions of the xylem above and below the inoculation point were surface-disinfested, plated on potato dextrose agar (Difco), and incubated at 25°C. Reisolated fungi were compared with the original inoculum by morphological features. Absolute lesion dimensions were used for statistical analysis, but Figure 2 displays lesion length relative to tree height to compare local pathogens with the systemic pathogens. The entire dataset is available as Supplementary Table S1.

True positive controls of systemic pathogenicity were not available for pines and oaks. Therefore, we included conceptual controls to different hosts (*O. novo-ulmi* in *Ulmus*, *R. lauricola* in *Persea*). We also included technical controls (i.e., fungi that reliably cause lesions and staining, albeit not systemic, in the target hosts and are established in the United States: *Diplodia sapinea* in pines and *Diplodia quercivora* in oaks).

Response analysis. Details of the statistical analysis are included as the full R code in Supplementary Material. Lesion dimension data conformed to normal distribution, tested by the Shapiro test. The tree species within a genus (different pines or oaks) and the collecting event did not exert any main or interactive effect on the pathogenicity result (tested by a linear model with those variables) and were therefore not included as factors in the final test of pathogenicity.

To test for significant responses from each fungal isolate from all its replicates in the stats R package, linear models were implemented by the glm() function using Gaussian distribution, which is analogous to the lm() function. We compared the proportion of the vertical length of xylem stained as an estimate of the degree of systemic colonization. The statistic was calculated as the vertical length of staining divided by the median tree height for each trial between negative control (baseline) and trees inoculated with fungal isolates. The R "summary()" function was used to contrast all levels of the categorical independent variable of fungal treatment in the linear model (Zuur et al. 2009). This function provides coefficients and their standard errors for each level of the categorical factor from the difference between the mean for that level and the mean of the reference group (water controls in our case). The significance of each level is determined by t tests; the provided P values are the probability of observing a difference at least as great as the observed one assuming that there is no difference between that particular level and the water control.

Lesion width, area, and phloem staining were also measured; the complete dataset is reported in Supplementary Material. We assessed the effect in oaks and pines separately. For each model, the response variable was modeled as a function of tree species and fungal isolate, with the negative control group used as the baseline for the effect of fungal inoculations.

RESULTS

Two hundred fifty-one species of bark and ambrosia beetles were collected from pines and oaks across Eurasia, with emphasis on latitudes and ecosystems analogous to the southeastern United States. Fifty-five species were selected for assessment and yielded 111 fungal associates considered specific based on the criteria listed above. The beetle set included most scolytine and platypodine vectors responsible for major tree mortality events in Eurasia: *Dendroctonus* spp., *Platypus koryoensis* (Korean oak wilt), *Platypus quercivorus* (Japanese oak wilt), and *Tomicus* spp.

None of the tested fungi triggered mortality of the experimental trees similar to the highly virulent pathogen models *R. lauricola* and *O. novo-ulmi* in their respective hosts (category 3, tree-killing systemic pathogen; Table 1). Of the tested fungal isolates from pine-infesting beetles, 38% (22 of 55) were classified as category 2: weak/minor localized pathogens (Fig. 2; Table 1). Of the USDA Prioritized Offshore Pest List species, all three pine specialist beetles carry category 2 pathogens. The red turpentine beetle *Dendroctonus valens* is an American species invasive in China, where its impact has been reportedly enhanced by a novel association with local *Leptographium* (Lu et al. 2011). This *Leptographium* (CMW25626) was tested and was not found to be any more pathogenic to American pines than water control ($t = 0.183$, $P = 0.076$).

Of the 31 isolates from oak-infesting beetles, only one induced symptoms significantly more severe than water controls: *Ophiostoma* sp. 7736 from *Cyclorhipidion distinguendum*. The three oak specialist beetles on the USDA Prioritized Offshore Pest List did not prove to carry pathogens (Fig. 1; Table 1). The overall lack of systemic pathogenicity and the low pathogenicity in oaks is not a methodological issue, as inoculations of positive technical controls caused significant staining of the xylem (*D. sapinea* in pines and *D. quercivora* in oaks, known pathogens established in the United States).

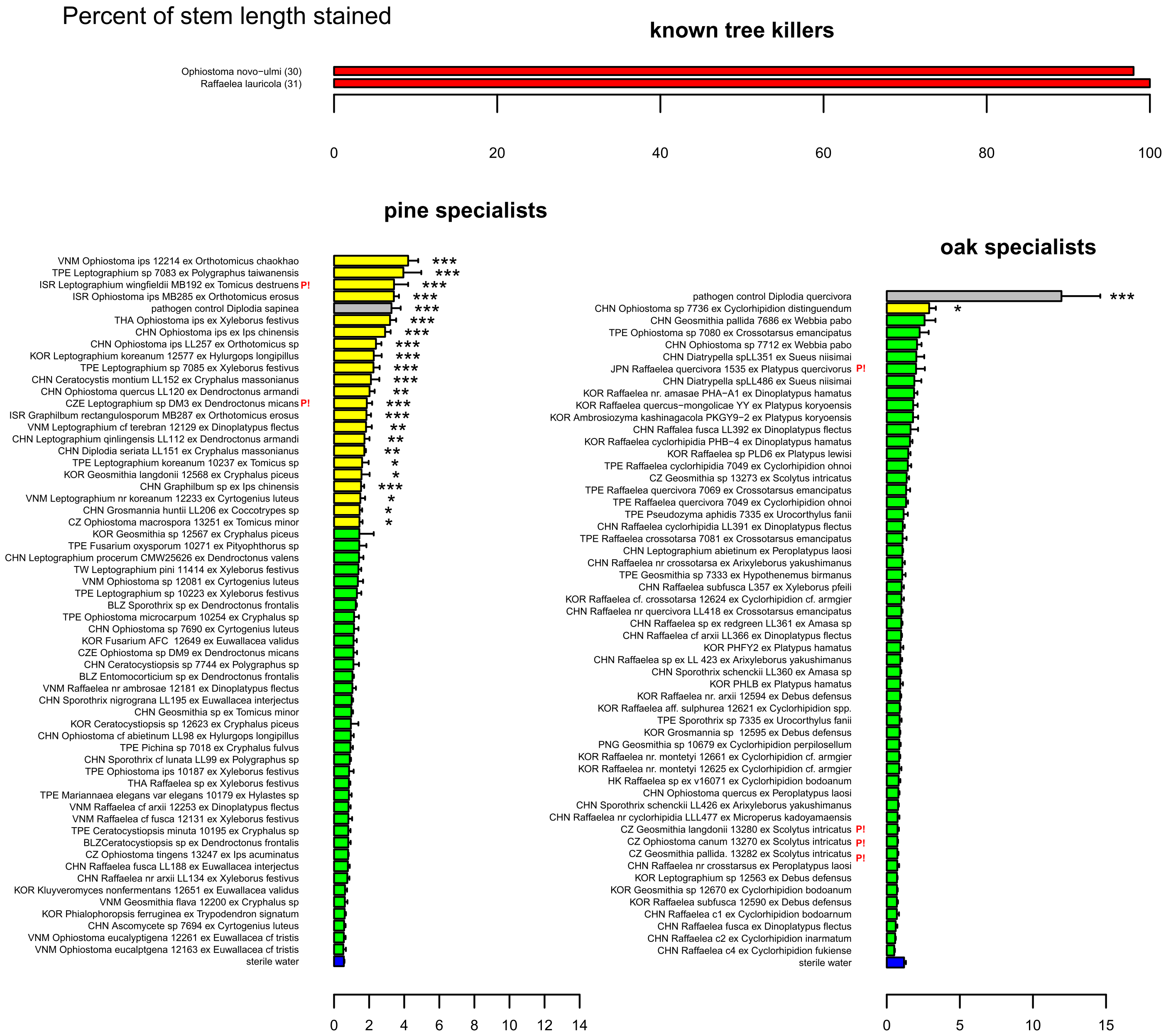

Fig. 2. Fungal pathogenicity as mean length of xylem staining as percentage of total stem length (bars indicate standard error) observed in experimentally inoculated pines and oaks. Yellow bars indicate significantly greater pathogenicity than sterile water negative controls (blue bars). Green bars represent fungi classified here as nonpathogens because they produced lesions not different from the control. Red bars represent previously published data for known tree-killing fungi: *Ophiostoma novo-ulmi* inoculated in elm (Smalley and Guries 1993) and *Raffaelea lauricola* in redbay (Dreaden et al. 2017). Gray bars represent positive controls consisting of known tree pathogens not typically vectored by beetles. Asterisks indicate significance for comparison with negative controls: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. “P!” denotes the U.S. Department of Agriculture Animal and Plant Health Inspection Service Prioritized Offshore Pests within the duration of the experiment (the most recent list may differ).

TABLE 1. Sampling design overview, lesion dimensions, and results of the linear model test of significance of tree response to respective fungal isolate

Region	Fungus	Vector	Xylem lesion length (%)[a]	*P* value	Pathogenicity
Pine					
China mainland	**_Grosmannia huntii_ LL206**	**_Coccotrypes_ sp.**	**1.46**	**0.046510193[b]**	**Mild, warrants monitoring**
	Ceratocystis montium LL152	**_Cryphalus massonianus_**	**2.10**	**0.000583444[b]**	**Mild, warrants monitoring**
	Diplodia seriata LL151	**_Cryphalus massonianus_**	**1.73**	**0.00946419[b]**	**Mild, warrants monitoring**
	Ascomycete sp. 7694	*Cyrtogenius luteus*	0.57	0.971284101	Not significant
	Ophiostoma sp. 7690	*Cyrtogenius luteus*	1.14	0.116114098	Not significant
	Leptographium qinlingensis LL112	**_Dendroctonus armandi_**	**1.74**	**0.008958115[b]**	**Mild, warrants monitoring**
	Ophiostoma quercus LL120	**_Dendroctonus armandi_**	**2.03**	**0.001086454[b]**	**Mild, warrants monitoring**
	Leptographium procerum CMW25626	*Dendroctonus valens*	1.42	0.056458495	Not significant
	Raffaelea fusca LL188	*Euwallacea interjectus*	0.79	0.642301307	Not significant
	Sporothrix nigrograna LL195	*Euwallacea interjectus*	1.01	0.339502029	Not significant
	Ophiostoma cf. *abietinum* LL98	*Hylurgops longipillus*	0.96	0.40029658	Not significant
	Graphilbum sp.	**_Ips chinensis_**	**1.54**	**8.26E-05[b]**	**Mild, warrants monitoring**
	Ophiostoma ips	**_Ips chinensis_**	**2.91**	**7.77E-20[b]**	**Mild, warrants monitoring**
	Ophiostoma ips LL257	**_Orthotomicus_ sp.**	**2.39**	**4.44E-05[b]**	**Mild, warrants monitoring**
	Ceratocystiopsis sp. 7744	*Polygraphus* sp.	1.10	0.140312051	Not significant
	Sporothrix cf. *lunata* LL99	*Polygraphus* sp.	0.90	0.481322228	Not significant
	Geosmithia sp. 12567	*Tomicus minor*	0.99	0.074408637	Not significant
	Raffaelea nr. *arxii* LL134	*Xyleborus festivus*	0.76	0.694927674	Not significant
Czech Republic	**_Leptographium_ sp. DM3**	**_Dendroctonus micans_**	**1.87**	**0.000282016[b]**	**Mild, warrants monitoring**
	Ophiostoma sp. DM9	*Dendroctonus micans*	1.11	0.136527344	Not significant
	Ophiostoma tingens 13247	*Ips acuminatus*	0.80	0.620652058	Not significant
	Ophiostoma macrospora 13251	**_Tomicus minor_**	**1.45**	**0.049064343[b]**	**Mild, warrants monitoring**
Israel	**_Graphilbum rectangulosporum_ MB287**	**_Orthotomicus erosus_**	**1.85**	**0.000349751[b]**	**Mild, warrants monitoring**
	Ophiostoma ips MB285	**_Orthotomicus erosus_**	**3.41**	**6.05E-15[b]**	**Mild, warrants monitoring**
	Leptographium wingfieldii MB192	**_Tomicus destruens_**	**3.42**	**5.13E-15[b]**	**Mild, warrants monitoring**
Korea	*Ceratocystiopsis* sp. 12623	*Cryphalus piceus*	0.96	0.39393715	Not significant
	Geosmithia langdonii 12568	**_Cryphalus piceus_**	**1.57**	**0.025820654[b]**	**Mild, warrants monitoring**
	Geosmithia sp. 12567	*Cryphalus piceus*	1.44	0.050831909	Not significant
	Fusarium AFC 12649	*Euwallacea validus*	1.11	0.230785373	Not significant
	Kluyveromyces nonfermentans 12651	*Euwallacea validus*	0.63	0.927113646	Not significant
	Leptographium koreanum 12577	**_Hylurgops longipillus_**	**2.26**	**0.000144121[b]**	**Mild, warrants monitoring**
	Phialophoropsis ferruginea	*Trypodendron signatum*	0.61	0.963472324	Not significant
Taiwan	*Pichina* sp. 7018	*Cryphalus fulvus*	0.94	0.424138721	Not significant
	Ceratocystiopsis minuta 10195	*Cryphalus* sp.	0.81	0.607285223	Not significant
	Ophiostoma microcarpum 10254	*Cryphalus* sp.	1.14	0.203908583	Not significant
	Mariannaea elegans var. *elegans* 10179	*Hylastes* sp.	0.85	0.552608911	Not significant
	Fusarium oxysporum 10271	*Pityophthorus* sp.	1.44	0.052192429	Not significant
	Leptographium sp. 7083	**_Polygraphus taiwanensis_**	**3.95**	**1.81E-18[b]**	**Mild, warrants monitoring**
	Leptographium koreanum 10237	**_Tomicus_ sp.**	**1.60**	**0.020987925[b]**	**Mild, warrants monitoring**
	Leptographium pini 11414	*Xyleborus festivus*	1.39	0.066987384	Not significant
	Leptographium sp. 10223	*Xyleborus festivus*	1.30	0.10210479	Not significant
	Leptographium sp. 7085	**_Xyleborus festivus_**	**2.25**	**9.20E-06[b]**	**Mild, warrants monitoring**
	Ophiostoma ips 10187	*Xyleborus festivus*	0.86	0.537444128	Not significant
Thailand	**_Ophiostoma ips_**	**_Xyleborus festivus_**	**3.19**	**7.98E-23[b]**	**Mild, warrants monitoring**
	Raffaelea sp.	*Xyleborus festivus*	0.85	0.200995677	Not significant
Vietnam	*Geosmithia flava* 12200	*Cryphalus* sp.	0.62	0.945278692	Not significant
	Leptographium nr. _koreanum_ 12233	**_Cyrtogenius luteus_**	**1.50**	**0.037387543[b]**	**Mild, warrants monitoring**
	Ophiostoma sp. 12081	*Cyrtogenius luteus*	1.36	0.122277239	Not significant
	Leptographium cf. _terebrantis_ 12129	**_Dinoplatypus flectus_**	**1.82**	**0.004906573[b]**	**Mild, warrants monitoring**
	Raffaelea cf. *arxii* 12253	*Dinoplatypus flectus*	0.82	0.588791623	Not significant
	Raffaelea nr. *ambrosiae* 12181	*Dinoplatypus flectus*	1.05	0.291897224	Not significant
	Ophiostoma eucalyptigena 12163	*Euwallacea* cf. *tristis*	0.53	0.897111429	Not significant
	Ophiostoma eucalyptigena 12261	*Euwallacea* cf. *tristis*	0.56	0.951525446	Not significant
	Ophiostoma ips 12214	**_Orthotomicus chaokhao_**	**4.22**	**9.75E-16[b]**	**Mild, warrants monitoring**
	Raffaelea cf. *fusca* 12131	*Xyleborus festivus*	0.82	0.596685522	Not significant
Oak					
Czechia	*Geosmithia langdonii* 13280	*Scolytus intricatus*	0.74	0.672976328	Not significant
	Geosmithia pallida 13282	*Scolytus intricatus*	0.73	0.664950182	Not significant
	Geosmithia sp. 13273	*Scolytus intricatus*	1.37	0.866112473	Not significant
	Ophiostoma canum 13270	*Scolytus intricatus*	0.73	0.664950182	Not significant
China/Hong Kong	*Raffaelea* sp. v16071	*Cyclorhipidion bodoanum*	0.80	0.725151447	Not significant
China mainland	*Raffaelea* sp. *redgreen* LL361	*Amasa* sp.	0.68	0.854607786	Not significant
	Sporothrix schenckii LL360	*Amasa* sp.	0.52	0.808432111	Not significant
	Raffaelea nr. *crossotarsa*	*Arixyleborus yakushimanus*	0.65	0.910520242	Not significant
	Raffaelea sp. LL *423*	*Arixyleborus yakushimanus*	0.60	0.840882927	Not significant
	Sporothrix schenckii LL426	*Arixyleborus yakushimanus*	0.47	0.79429875	Not significant
	Raffaelea nr. *quercivora* LL418	*Crossotarsus emancipatus*	0.62	0.860630288	Not significant

(*Continued on next page*)

TABLE 1. (*Continued from previous page*)

Region	Fungus	Vector	Xylem lesion length (%)[a]	*P* value	Pathogenicity
	Raffaelea c1	*C. bodoanum*	0.55	0.64109625	Not significant
	***Ophiostoma* sp. 7736**	***Cyclorhipidion distinguendum***	**2.90**	**0.045294739**[b]	**Mild, warrants monitoring**
	Raffaelea c4	*Cyclorhipidion fukiense*	0.52	0.523865493	Not significant
	Raffaelea c2	*Cyclorhipidion inarmatum*	0.62	0.563030209	Not significant
	Raffaelea cf. *arxii* LL366	*Dinoplatypus flectus*	0.49	0.902316542	Not significant
	Raffaelea cyclorhipidia LL391	*Dinoplatypus flectus*	0.60	0.952939717	Not significant
	Raffaelea fusca	*Dinoplatypus flectus*	0.55	0.587901324	Not significant
	Raffaelea fusca LL392	*Dinoplatypus flectus*	1.60	0.713782797	Not significant
	Raffaelea nr. *cyclorhipidia* LLL477	*Microperus kadoyamaensis*	0.53	0.711130385	Not significant
	Leptographium abietinum	*Peroplatypus laosi*	0.83	0.914732502	Not significant
	Ophiostoma quercus	*P. laosi*	0.60	0.713095194	Not significant
	Raffaelea nr. *crosstarsus*	*P. laosi*	0.63	0.660014349	Not significant
	Diatrypella sp. LL351	*Sueus niisimai*	0.41	0.370766789	Not significant
	Diatrypella sp. LL486	*S. niisimai*	0.48	0.453518292	Not significant
	Geosmithia pallida 7686	*Webbia pabo*	2.59	0.101642436	Not significant
	Ophiostoma sp. 7712	*W. pabo*	2.07	0.301179177	Not significant
	Raffaelea subfusca L357	*Xyleborus pfeili*	0.52	0.925980453	Not significant
Japan	*Raffaelea quercivora* 1535	*Platypus quercivorus*	2.01	0.439435559	Not significant
Papua New Guinea	*Geosmithia* sp. 10679	*Cyclorhipidion perpilosellum*	0.86	0.755167783	Not significant
South Korea	*Geosmithia* sp. 12670	*Cyclorhipidion bodoanum*	0.70	0.646750164	Not significant
	Raffaelea cf. *crossotarsa* 12624	*Cyclorhipidion* cf. *armgier*	1.00	0.862472155	Not significant
	Raffaelea nr. *montetyi* 12625	*Cyclorhipidion* cf. *armgier*	0.85	0.750384671	Not significant
	Raffaelea nr. *montetyi* 12661	*Cyclorhipidion* cf. *armgier*	0.85	0.751579554	Not significant
	Raffaelea aff. *sulphurea* 12621	*Cyclorhipidion* spp.	0.89	0.780430116	Not significant
	Grosmannia sp. 12595	*Debus defensus*	0.88	0.768369544	Not significant
	Leptographium sp. 12563	*Debus defensus*	0.70	0.646750164	Not significant
	Raffaelea nr. *arxii* 12594	*Debus defensus*	0.91	0.796188147	Not significant
	Raffaelea subfusca 12590	*Debus defensus*	0.70	0.642232597	Not significant
	Raffaelea cyclorhipidia PHB-4	*Dinoplatypus hamatus*	1.59	0.70187127	Not significant
	Raffaelea nr. *amasae* PHA-A1	*Dinoplatypus hamatus*	1.87	0.517454732	Not significant
	Raffaelea sp. PHFY2	*Platypus hamatus*	0.39	0.877824325	Not significant
	Raffaelea sp. PHLB	*Platypus hamatus*	0.48	0.927042455	Not significant
	Ambrosiozyma kashinagacola PKGY9-*2*	*Platypus koryoensis*	1.80	0.563248794	Not significant
	Raffaelea quercus-mongolicae YY	*Platypus koryoensis*	1.81	0.556890219	Not significant
	Raffaelea sp. PLD6	*Platypus lewisi*	1.47	0.786406813	Not significant
Taiwan	*Ophiostoma* sp. 7080	*Crossotarsus emancipatus*	2.25	0.314485996	Not significant
	Raffaelea crossotarsa 7081	*Crossotarsus emancipatus*	1.08	0.922235411	Not significant
	Raffaelea quercivora 7069	*Crossotarsus emancipatus*	1.33	0.894692337	Not significant
	Raffaelea cyclorhipidia 7049	*Cyclorhipidion ohnoi*	1.44	0.808292862	Not significant
	Raffaelea quercivora 7047	*Cyclorhipidion ohnoi*	1.32	0.897184599	Not significant
	Geosmithia sp. 7333	*Hypothenemus birmanus*	1.06	0.904743381	Not significant
	Pseudozyma aphidis 7335	*Urocorthylus fanii*	1.16	0.976160985	Not significant
	Sporothrix sp. 7335	*Urocorthylus fanii*	0.88	0.771981953	Not significant

[a] Mean xylem lesion length as a percentage of total height.

[b] Mild pathogen ($P < 0.05$) appear in bold. No systemic pathogen was found.

The taxonomic identity of the fungi was determined to the greatest degree possible using the phylogenetic analyses (Table 1). Many isolates could not be named to species. Several Ophiostomatales and Xylariales isolates that did not cluster with any known species were described (Li et al. 2020b; Simmons et al. 2016), and the characterization and description of other isolates is ongoing.

Our first dataset cannot completely exclude the possibility that highly pathogenic nonnative beetle-associated fungi exist; therefore, continued preinvasion assessment and monitoring of invasion pathways is warranted.

DISCUSSION

A proactive management of environmental risks, including invasive species, is essential to sustaining our socioecological systems in the globalized world and to meeting international policy targets. A decision-aid dataset on individual exotic pathogen and pest species, such as the one presented here, can be used by agencies as part of an empirical and reliable risk analysis of exotic pests and their invasion pathways. An example is the possibility of including our data on the effect of fungi vectored by wood borers on commodity trees in the USDA APHIS Objective Prioritization for Exotic Pests decision-making model. In analyses of invasion pathways of wood borers and tree pathogens, one of the main variables is the traded tree species; this dataset can improve the accuracy of models that are otherwise mostly focused on trade routes or habitat matching (Lantschner et al. 2020). This concept of empirical preinvasion assessment can also be extended to other systems in which potentially pathogenic symbionts are poorly known (e.g., bacterial pathogens transmitted by sap-sucking insects). The approach presented here is practical for the fungi associated with wood borers; organisms with different biology may require complementary or different approaches for their preinvasion assessment, such as offshore sentinel gardens.

The primary response variable in our test was whether the tree died, and the secondary variable was the extent of lesion. Admittedly, tree responses to pathogens are more continuous and variable than the three categories used here. Tree responses also depend on environmental conditions, stress level, and the insect vector action. Methodologically, the degree of pathogen impact on the tree can differ among inoculation methods, inoculum doses, the pathogenicity mechanism of a fungus, and the level and type of tree stress. For example, a single-point inoculation into tree bark may not reveal pathogenicity of a rust fungus.

However, the goal of this proof of concept was not to describe interactions between the trees and fungi. Pathogens acting locally, such as canker-forming fungi, can become important forest health issues (e.g., *Geosmithia morbida*; Zerillo et al. 2014) and are biologically interesting, but require a context-dependent understanding of the system which is often only discernible after introduction. Introductions of such weak pathogens are unlikely to trigger the incident command system for costly monitoring schemes and attempts at eradication.

In this project, we sought to develop decision-aid triage that places fungal isolates into categories supporting certain regulatory responses: only fungi in category 3 are likely to justify the effort to monitor for, quarantine, and/or or eradicate the vector. Therefore, only a fungus in which a single-point inoculation kills an experimental tree would be classified into category 3, as its effect is comparable to the two benchmark pathosystems that are, or were, regulated in the United States: laurel wilt and Dutch elm disease. The fungi that created lesions greater than the water control but did not kill the trees would be classified in category 2. Additional experiments are needed to clarify the pathogenesis mechanisms in these weaker pathogens.

Similar limitation applies with respect to the number of fungi tested per beetle species. This methodology did not aim to test all possible fungi associated with each beetle, but only those that are consistent associates and, as such, are likely to be introduced to North America in case of the beetle introduction. Even though any bark or ambrosia beetle individual can serve as a vector to many different fungal isolates, only a few of them, often only one, are statistically consistent among multiple individuals (Harrington 2005; Huang et al. 2019; Skelton et al. 2018). Both of our biological positive control pathosystems are consistent associates of their respective vectors.

In some cases of tree-killing beetle–fungus pathosystems, the tree death is a result of a beetle mass accumulation (Carrillo et al. 2016; Kolařík et al. 2011). For instance, the pseudopathogenic ambrosial *Fusarium* spp. (Ambrosia Fusarium clade; Geiser et al. 2021) does not cause substantial mortality on its own and requires the vector beetle *Euwallacea* spp. penetration into the wood tissue for establishment (Freeman et al. 2019). Similarly, the thousand cankers disease of walnuts develops only if the fungus *Geosmithia morbida* is inoculated by a mass attack of the vector *Pityophthorus juglandis*. The threat of a pest that causes damage via mass accumulation was not tested by our methodology. The potential impact of such symbioses would be more appropriately tested via sentinel gardens or through observations of nonnative trees planted in the insect's habitat (Li et al. 2020a; Morales-Rodríguez et al. 2019).

The lack of response of a tree seedling to an experimental fungus in our experimental settings does not completely preclude the potential for a disease caused by that fungus. The goal of the study was to seek compatible pathogen–tree pairs; in the case of negative results, our data do not distinguish between other plant pathology scenarios, such as nonpathogenic fungus, resistant nonhost, incompatibility between the two genotypes, or a pathogenic fungus that requires a different mode or load of inoculation.

Negative trial result also does not negate invasion potential. Invasion of an exotic wood-boring insect and an associated fungus is a process on an ecological scale; pathogenicity of the fungus is only one of the possible components. It is, however, the component that is most important in the triage for high-risk invaders, relevant to the applied and regulatory uses of tree pathology (Bos and Parlevliet 1995).

Our methodology assumes that the responses to fungal inoculation of young trees reflects the responses in mature trees to a sufficient degree. Indeed, our positive controls indicate that inoculation of virulent pathogens (our category 3) results in systemic infection and death of woody tissues, regardless of whether the tree is young or old. The extent of lesions, discoloration, and wilting may differ between seedlings of early age, but the optimal age for testing was determined to be 4 years in tests of *Ulmus* inoculated with *O. novo-ulmi* and *Persea* inoculated with *R. lauricola* (Dreaden et al. 2017; Martín et al. 2015); our trees were also 4 years old. In many other tree species, using seedlings to test for mortality may in fact be a stronger test than using adult trees, as resistance to pathogens usually increases with age (Vivas et al. 2012).

The concept of assessing the threat potential before an eventual invasion is not new (Li et al. 2020b; Morales-Rodríguez et al. 2019), but assessments of in vivo preinvasion compatibility between trees and pathogens are rare. Most preinvasion assessments have focused on hypothetical invaders or were carried out via models such as environmental matching or economic damage assessment and lack the specificity of insect–plant interaction data (Okabe et al. 2012; Susaeta et al. 2017; Worner 1988). Most models also focus on entire assemblages or on higher taxonomic levels (Worner and Gevrey 2006). Models are discerning at the species level (Fournier et al. 2019; Lantschner et al. 2017). To become a reliable tool for policy decisions on individual species, such models need to be parameterized with biological features and ecological data and be ground-truthed with experiments such as the one presented here.

An increasingly used empirical approach to generate data on pest–plant relationships involves the monitoring of sentinel tree gardens of target commodities in the region of the pathogen origin. These transplant studies are increasingly used by the European and Asian forest pest research and regulatory community (Eschen et al. 2019; Kenis et al. 2018), but no analogous assessment exists for the impact of Eurasian pests on American tree species, with the exception of botanical gardens. These experiments are limited to the naturally occurring pests in a limited number of locations, and do not offer the experimental rigor for comparative responses.

Although this was the largest single sample of fungi ever collected across the diversity of Eurasian scolytine beetles, it was far from comprehensive. Attempting to sample fungi from all Eurasian bark and ambrosia beetle species would be exceedingly difficult. Instead, the goal of this research was to focus on the species with the highest likelihood of invasion and impact in North America. Therefore, rather than a broad selection of species, we aimed to sample as many beetle vectors that display elevated likelihood of introduction and impact because they are (i) common across the Palearctic and thus present higher "propagule pressure" (Brockerhoff et al. 2014) and (ii) specific to the tree families of concern or at least commonly colonize them (Pinaceae and Fagaceae). In addition, we aimed to sample from species that were deemed pestiferous in their native range or are on the target list of pest monitoring projects and agencies. For those reasons, our initial sample is enriched for tribes Ipini, Hylurgini, and Xyleborini (Supplementary Table S2), but also includes species from any other tribe that specializes on pines or oaks. We were able to recover and test all species that appeared on the CAPS list, the great majority of species in the Centre for Agriculture and Bioscience International's Invasive Species Compendium (https://www.cabi.org/isc/search/index?q=Scolytinae), and many species on the European and Mediterranean Plant Protection Organization's risk list (Battisti et al. 2020).

In terms of commodities, this project was focused on trees occurring in the southeastern United States. It may seem that most invasive species from eastern and southeastern Asia would arrive to continental North America through the western ports, and therefore we should have tested western trees. However, with current trade shipping routes, many ships are arriving to eastern U.S. ports directly, such as through the widened Panama Canal. Because of that, and because of the ease of establishment in the heavily wooded east, most Asian invasive bark and ambrosia beetles have been first detected in the eastern United States, not in the west (Haack 2006; Rabaglia et al. 2019).

Conclusions. Our dataset suggests a cautiously optimistic perspective: systemic pathogens that would threaten southeastern American pine- and oak-dominated landscapes and industries are rare in Eurasia or do not exist there or are not routinely associated with common bark and ambrosia beetles.

The results and associated approach highlight the value and low cost of proactive preinvasion assessments designed to support decision-making (Jenkins 2013). In the U.S. context, the categorization can aid in the listing or omission of exotic species on the official Prioritized Offshore Pest List and justify keeping the majority of nonthreatening species off the list. This will economize allocation of federal and state resources to monitoring and responses. Our preinvasion decision support required a fraction of the cost of a blanket response to every detected exotic wood borer or the contemporary wait-and-evaluate approach, which routinely misses the initial lag phase of pest establishment and results in continent-wide damages (Kovacs et al. 2010; Susaeta et al. 2017).

These results also demonstrate that preinvasion assessments do not necessarily impede international trade. Instead, decision-aid datasets such as ours facilitate trade by determining which offshore wood borers are low priority and which are potential threats, thereby "right-sizing" the regulatory response.

ACKNOWLEDGMENTS

We thank Sarah M. Cognato-Smith, Anthony Cognato, Bjarte Jordal, Robert Rabaglia, Sangwook Park, Ching Shan Lin, Roger A. Beaver, and Massimo Faccoli for help with identification of beetles; D. Rabern Simmons for fungus identification support; Mee-Sook Kim and Moo-Sung Kim for help with local arrangements in South Korea; Jason A. Smith for tree maintenance support; and the Florida Department of Agriculture and Consumer Services Division of Plant Industry for managing the quarantine greenhouse. We greatly appreciate the help of Hou-Feng Li and Chi-Yu Chen with collecting material in Taiwan. Additional data on archiving of data and materials are available in Supplementary Material. Insect vouchers are available at the University of Florida Forest Entomology collection. Fungus vouchers are deposited at the Forestry and Agricultural Biotechnology Institute fungus collection at the University of Pretoria.

LITERATURE CITED

Barbier, E. B., Knowler, D., Gwatipedza, J., Reichard, S. H., and Hodges, A. R. 2013. Implementing policies to control invasive plant species. Bioscience 63:132-138.

Battisti, A., Benko-Beloglavec, A., Gregoire, J.-C., Hrasovec, B., Hulcr, J., Inward, D., and Jactel, H. 2020. EPPO Study on the risk of bark and ambrosia beetles associated with imported non-coniferous wood, 2020-02-05. EPPO Technical Document No. 1081.

Bos, L., and Parlevliet, J. E. 1995. Concepts and terminology on plant/pest relationships: toward consensus in plant pathology and crop protection. Annu. Rev. Phytopathol. 33:69-102.

Bradshaw, C. J. A., Leroy, B., Bellard, C., Roiz, D., Albert, C., Fournier, A., Barbet-Massin, M., Salles, J. M., Simard, F., and Courchamp, F. 2016. Massive yet grossly underestimated global costs of invasive insects. Nat. Commun. 7:12986.

Brasier, C. M. 1991. *Ophiostoma novo-ulmi* sp. nov., causative agent of current Dutch elm disease pandemics. Mycopathologia 115:151-161.

Brockerhoff, E. G., Kimberley, M., Liebhold, A. M., Haack, R. A., and Cavey, J. F. 2014. Predicting how altering propagule pressure changes establishment rates of biological invaders across species pools. Ecology 95:594-601.

Carrillo, D., Cruz, L. F., Kendra, P. E., Narvaez, T. I., Montgomery, W. S., Monterroso, A., De Grave, C., and Cooperband, M. F. 2016. Distribution, pest status and fungal associates of *Euwallacea nr. fornicatus* in Florida avocado groves. Insects 7:55.

Dreaden, T. J., Campbell, A. S., Gonzalez-Benecke, C. A., Ploetz, R. C., and Smith, J. A. 2017. Response of swamp bay, *Persea palustris*, and redbay, *P. borbonia*, to *Raffaelea* spp. isolated from *Xyleborus glabratus*. For. Pathol. 47:e12288.

Early, R., Bradley, B. A., Dukes, J. S., Lawler, J. J., Olden, J. D., Blumenthal, D. M., Gonzalez, P., Grosholz, E. D., Ibanez, I., Miller, L. P., Sorte, C. J. B., and Tatem, A. J. 2016. Global threats from invasive alien species in the twenty-first century and national response capacities. Nat. Commun. 7:12485.

Eschen, R., Britton, K., Brockerhoff, E., Burgess, T., Dalley, V., Epanchin-Niell, R. S., Gupta, K., Hardy, G., Huang, Y., Kenis, M., Kimani, E., Li, H.-M., Olsen, S., Ormrod, R., Otieno, W., Sadof, C., Tadeu, E., and Theyse, M. 2015. International variation in phytosanitary legislation and regulations governing importation of plants for planting. Environ. Sci. Policy 51:228-237.

Eschen, R., O'hanlon, R., Santini, A., Vannini, A., Roques, A., Kirichenko, N., and Kenis, M. 2019. Safeguarding global plant health: the rise of sentinels. J. Pest Sci. 92:29-36.

Eskalen, A., Gonzalez, A., Wang, D. H., Twizeyimana, M., Mayorquin, J. S., and Lynch, S. C. 2012. First report of a *Fusarium* sp. and its vector tea shot hole borer (*Euwallacea fornicatus*) causing Fusarium dieback on avocado in California. Plant Dis. 96:1070.

Fei, S., Morin, R. S., Oswalt, C. M., and Liebhold, A. M. 2019. Biomass losses resulting from insect and disease invasions in US forests. Proc. Natl. Acad. Sci. 116:17371-17376.

Fournier, A., Penone, C., Pennino, M. G., and Courchamp, F. 2019. Predicting future invaders and future invasions. Proc. Natl. Acad. Sci. 116:7905-7910.

Fraedrich, S. W., Harrington, T. C., Rabaglia, R. J., Ulyshen, M. D., Mayfield, A. E., Hanula, J. L., Eickwort, J. M., and Miller, D. R. 2008. A fungal symbiont of the redbay ambrosia beetle causes a lethal wilt in redbay and other Lauraceae in the southeastern United States. Plant Dis. 92: 215-224.

Freeman, S., Miller, G., Protasov, A., Maymon, M., Elazar David-Schwartz, R., Zhou, J., and Mendel, Z. 2019. Aposymbiotic interactions of three ambrosia beetle fungi with avocado trees. Fungal Ecol. 39:117-130.

Gardes, M., and Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2:113-118.

Geiser, D. M., Al-Hatmi, A., Aoki, T., Arie, T., Balmas, V., Barnes, I., Bergstrom, G. C., Bhattacharyya, M., Blomquist, C. L., Bowden, R., et al. 2021. Phylogenomic analysis of a 55.1 kb 19-gene dataset resolves a monophyletic *Fusarium* that includes the *Fusarium solani* species complex. Phytopathology 111:1064-1079.

Guindon, S., and Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. System. Biol. 52:696-704.

Haack, R. A. 2006. Exotic bark- and wood-boring Coleoptera in the United States: recent establishments and interceptions. Can. J. For. Res. 36:269-288.

Harrington, T. C. 2005. Ecology and evolution of mycophagous bark beetles and their fungal partners. Pages 257-291 in: Insect–Fungal Associations. F. E. Vega and M. Blackwell, eds. Oxford University Press, New York.

Hopple, J. S., Jr., and Vilgalys, R. 1994. Phylogenetic relationships among coprinoid taxa and allies based on data from restriction site mapping of nuclear rDNA. Mycologia 86:96-107.

Huang, Y.-T., Skelton, J., Johnson, A. J., Kolarik, M., and Hulcr, J. 2019. Geosmithia species in southeastern USA and their affinity to beetle vectors and tree hosts. Fungal Ecol. 39:168-183.

Hulcr, J., and Stelinski, L. L. 2017. The ambrosia symbiosis: From evolutionary ecology to practical management. Annu. Rev. Entomol. 62:285-303.

Hyde, K. D., Al-Hatmi, A. M. S., Andersen, B., Boekhout, T., Buzina, W., Jr., Dawson, T. L., Eastwood, D. C., Jones, E. B. G., de Hoog, S., Kang, Y., Longcore, J. E., McKenzie, E. H. C., Meis, J. F., Pinson-Gadais, L., Rathnayaka, A. R., Richard-Forget, F., Stadler, M., Theelen, B., Thongbai, B., Tsui, C. 2018. The world's ten most feared fungi. Fungal Divers. 93: 161-194.

Inácio, M. L., Henriques, J., Lima, A., and Sousa, E. 2012. Ophiostomatoid fungi associated with cork oak mortality in Portugal. IOBC WPRS Bull. 76:89-92.

Jenkins, P. T. 2013. Invasive animals and wildlife pathogens in the United States: the economic case for more risk assessments and regulation. Biol. Invasions 15:243-248.

Katoh, K., Rozewicki, J., and Yamada, K. D. 2019. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Brief. Bioinform. 20:1160-1166.

Kenis, M., Li, H., Fan, J.-T., Courtial, B., Auger-Rozenberg, M.-A., Yart, A., Eschen, R., and Roques, A. 2018. Sentinel nurseries to assess the phytosanitary risks from insect pests on importations of live plants. Sci. Rep. 8:11217.

Kolarík, M., Freeland, E., Utley, C., and Tisserat, N. 2011. *Geosmithia morbida* sp. nov. a new phytopathogenic species living in symbiosis with the walnut twig beetle (*Pityophthorus juglandis*) on *Juglans* in the USA. Mycologia 103:325-332.

Kovacs, K. F., Haight, R. G., Mccullough, D. G., Mercader, R. J., Siegert, N. W., and Liebhold, A. M. 2010. Cost of potential emerald ash borer damage in US communities, 2009-2019. Ecol. Econ. 69:569-578.

Lantschner, M. V., Atkinson, T. H., Corley, J. C., and Liebhold, A. M. 2017. Predicting North American Scolytinae invasions in the Southern Hemisphere. Ecol. Appl. 27:66-77.

Lantschner, M. V., Corley, J. C., and Liebhold, A. M. 2020. Drivers of global Scolytinae invasion patterns. Ecol. Appl. 30:e02103.

Li, H.-P., Wickham, J. D., Bushley, K., Wang, Z.-G., Zhang, B., and Sun, J.-H. 2020a. New approaches in urban forestry to minimize invasive species impacts: The case of Xiongan New Area in China. Insects 11:300.

Li, Y., Skelton, J., Adams, S., Hattori, Y., Smith, M. E., and Hulcr, J. 2020b. The ambrosia beetle *Sueus niisimai* (Scolytinae: Hyorrhynchini) is associated with the canker disease fungus *Diatrypella japonica* (Xylariales). Plant Dis. 104:3143-3150.

Li, Y., Yu, H., Araújo, J. P., Zhang, X., Ji, Y., and Hulcr, J. 2021. *Esteya floridanum* sp. nov.: An ophiostomatalean nematophagous fungus and its potential to control the pine wood nematode. Phytopathology 111:304-311.

Lodge, D. M., Williams, S., Macisaac, H. J., Hayes, K. R., Leung, B., Reichard, S., Mack, R. N., Moyle, P. B., Smith, M., Andow, D. A., Carlton, J. T., and Mcmichael, A. 2006. Biological invasions: Recommendations for US policy and management. Ecol. Appl. 16:2035-2054.

Lu, M., Wingfield, M. J., Gillette, N., and Sun, J.-H. 2011. Do novel genotypes drive the success of an invasive bark beetle–fungus complex? Implications for potential reinvasion. Ecology 92:2013-2019.

Luchi, N., Ioos, R., and Santini, A. 2020. Fast and reliable molecular methods to detect fungal pathogens in woody plants. Appl. Microbiol. Biotechnol. 104:2453-2468.

Martín, J. A., Solla, A., Venturas, M., Collada, C., Domínguez, J., Miranda, E., Fuentes, P., Burón, M., Iglesias, S., and Gil, L. 2015. Seven *Ulmus minor* clones tolerant to *Ophiostoma novo-ulmi* registered as forest reproductive material in Spain. iForest-Biogeosci. For. 8(2):172.

McGeoch, M. A., Genovesi, P., Bellingham, P. J., Costello, M. J., McGrannachan, C., and Sheppard, A. 2016. Prioritizing species, pathways, and sites to achieve conservation targets for biological invasion. Biol. Invasions 18:299-314.

Millar, C. I., and Stephenson, N. L. 2015. Temperate forest health in an era of emerging megadisturbance. Science 349:823-826.

Morales-Rodríguez, C., Anslan, S., Auger-Rozenberg, M.-A., Augustin, S., Baranchikov, Y., Bellahirech, A., Burokienė, D., Čepukoit, D., Çota, E., Davydenko, K., Doğmuş Lehtijärvi, H., Drenkhan, R., Drenkhan, T., Eschen, R., Franić, I., Glavendekić, M., De Groot, M., Kacprzyk, M., Kenis, M., Kirichenko, N., Matsiakh, I., Musolin, D., Nowakowska, J., O'hanlon, R., Prospero, S., Roques, A., Santini, A., Talgø, V., Tedersoo, L., Uimari, A., Vannini, A., Witzell, J., Woodward, S., Zambounis, A., and Cleary, M. 2019. Forewarned is forearmed: harmonized approaches for early detection of potentially invasive pests and pathogens in sentinel plantings. NeoBiota 47:95-123.

Morris, J. L., Cottrell, S., Fettig, C. J., Derose, R. J., Mattor, K. M., Carter, V. A., Clear, J., Clement, J., Hansen, W. D., Hicke, J. A., Higuera, P. E., Seddon, A. W. R., Seppa, H., Sherriff, R. L., Stednick, J. D., and Seybold, S. J. 2018. Bark beetles as agents of change in social-ecological systems. Front. Ecol. Environ. 16:S34-S43.

Myers, J. H., Simberloff, D., Kuris, A. M., and Carey, J. R. 2000. Eradication revisited: Dealing with exotic species. Trends Ecol. Evol. 15:316-320.

O'Donnell, K., Libeskind-Hadas, R., Hulcr, J., Bateman, C., Kasson, M. T., Ploetz, R. C., Konkol, J. L., Ploetz, J. N., Carrillo, D., Campbell, A., Duncan, R. E., Liyanage, P. N. H., Eskalen, A., Lynch, S. C., Geiser, D. M., Freeman, S., Mendel, Z., Sharon, M., Aoki, T., Cosse, A. A., and Rooney, A. P. 2016. Invasive Asian *Fusarium–Euwallacea* ambrosia beetle mutualists pose a serious threat to forests, urban landscapes and the avocado industry. Phytoparasitica 44:435-442.

Okabe, K., Masuya, H., Kanzaki, N., and Goka, K. 2012. Species risk assessment of microscopic exotic organisms associated with forest-related commodities and goods. Hum. Ecol. Risk Assess. 18:1237-1254.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. Mol. Biol. Evol. 25:1253-1256.

Potter, K. M., Escanferla, M. E., Jetton, R. M., and Man, G. 2019. Important insect and disease threats to United States tree species and geographic patterns of their potential impacts. Forests 10:304.

Rabaglia, R. J., Cognato, A. I., Hoebeke, E. R., Johnson, C. W., Labonte, J. R., Carter, M. E., and Vlach, J. J. 2019. Early detection and rapid response: A ten-year summary of the U.S. Forest Service program of surveillance for non-native bark and ambrosia beetles. Am. Entomologist 65:29-42.

Rodgers, L., Derksen, A., and Pernas, T. 2014. Expansion and impact of laurel wilt in the Florida Everglades. Fla. Entomol. 97:1247-1250.

Roy, H. E., Bacher, S., Essl, F., Adriaens, T., Aldridge, D. C., Bishop, J. D., Blackburn, T. M., Branquart, E., Brodie, J., Carboneras, C., Cottier-Cook, E. J., Copp, G. H., Dean, H. J., Eilenberg, J., Gallardo, B., Garcia, M., García-Berthou, E., Genovesi, P., Hulme, P. E., Kenis, M., Kerckhof, F., Kettunen, M., Minchin, D., Nentwig, W., Nieto, A., Pergl, J., Pescott, O. L., Peyton, J. M., Preda, C., Roques, A., Rorke, S. L., Scalera, R., Schindler, S., Schönrogge, K., Sewell, J., Solarz, W., Stewart, A. J. A., Tricarico, E., Vanderhoeven, S., Velde, G. V. D., Vilà, M., Wood, C. A., Zenetos, A., and Rabitsch, W. 2019. Developing a list of invasive alien species likely to threaten biodiversity and ecosystems in the European Union. Glob. Change Biol. 25:1032-1048.

Roy, H. E., Peyton, J., Aldridge, D. C., Bantock, T., Blackburn, T. M., Britton, R., Clark, P., Cook, E., Dehnen-Schmutz, K., Dines, T., and Dobson, M. 2014. Horizon scanning for invasive alien species with the potential to threaten biodiversity in Great Britain. Glob. Change Biol. 20: 3859-3871.

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pysek, P., Van Kleunen, M., Winter, M., Ansong, M., Arianoutsou, M., Bacher, S., Blasius, B., Brockerhoff, E. G., Brundu, G., Capinha, C., Causton, C. E., Celesti-Grapow, L., Dawson, W., Dullinger, S., Economo, E. P., Fuentes, N., Guenard, B., Jager, H., Kartesz, J., Kenis, M., Kuhn, I., Lenzner, B., Liebhold, A. M., Mosena, A., Moser, D., Nentwig, W., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H. E., Scalera, R., Schindler, S., Stajerova, K., Tokarska-Guzik, B., Walker, K., Ward, D. F., Yamanaka, T., and Essl, F. 2018. Global rise in emerging alien species results from increased accessibility of new source pools. Proc. Natl. Acad. Sci. USA 115:E2264-E2273.

Seifert, K.A., De Beer, Z.W., and Wingfield, M.J. 2013. The ophiostomatoid fungi: expanding frontiers. CBS-KNAW Fungal Biodiversity Centre, de Uithof, The Netherlands.

Simmons, D. R., De Beer, Z. W., Huang, Y.-T., Bateman, C., Campbell, A. S., Dreaden, T. J., Li, Y., Ploetz, R. C., Black, A., Li, H. F., and Chen, C. Y. 2016. New *Raffaelea* species (Ophiostomatales) from the USA and Taiwan associated with ambrosia beetles and plant hosts. IMA Fungus 7: 265-273.

Skelton, J., Jusino, M. A., Li, Y., Bateman, C., Thai, P. H., Wu, C. X., Lindner, D. L., and Hulcr, J. 2018. Detecting symbioses in complex communities: the fungal symbionts of bark and ambrosia beetles within Asian pines. Microb. Ecol. 76:839-850.

Smalley, E. B., and Guries, R. P. 1993. Breeding elms for resistance to Dutch Elm Disease. Annu. Rev. Phytopathol. 31:325-354.

Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312-1313.

Susaeta, A., Soto, J. R., Adams, D. C., and Hulcr, J. 2017. Expected timber-based economic impacts of a wood-boring beetle (*Acanthotomicus* sp.) that kills American sweetgum. J. Econ. Entomol. 110:1942-1945.

Takashina, K., Chuma, I., Kajimura, H., Kameyama, N., Goto, C., and Kuroda, K. 2019. Pathogenicity and distribution of *Fusarium solani* isolates associated with *Erythrina* decline in Japan. Plant Dis. 104:731-742.

Tilman, D., Clark, M., Williams, D. R., Kimmel, K., Polasky, S., and Packer, C. 2017. Future threats to biodiversity and pathways to their prevention. Nature 546:73-81.

U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service. 2019. Cooperative Agricultural Pest Surveys Resources. http://caps.ceris.purdue.edu/

Vilgalys, R., and Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. 172:4238-4246.

Vivas, M., Martín, J. A., Gil, L., and Solla, A. 2012. Evaluating methyl jasmonate for induction of resistance to *Fusarium oxysporum, F. circinatum* and *Ophiostoma novo-ulmi*. For. Syst. 21:289-299.

Walsh, J. R., Carpenter, S. R., and Vander Zanden, M. J. 2016. Invasive species triggers a massive loss of ecosystem services through a trophic cascade. Proc. Natl. Acad. Sci. USA 113:4081-4085.

Worner, S. P. 1988. Ecoclimatic assessment of potential establishment of exotic pests. J. Econ. Entomol. 81:973-983.

Worner, S. P., and Gevrey, M. 2006. Modelling global insect pest species assemblages to determine risk of invasion. J. Appl. Ecol. 43:858-867.

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., and 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:e112847.

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., and Smith, G. M. 2009. Mixed Effects Models And Extensions in Ecology with R. Springer Science & Business Media, Berlin, Germany.