



# Pre-invasion assessment of potential invasive wood borers on North American tree species in Chinese sentinel gardens

Yiyi Dong<sup>1</sup>, Christopher Marais<sup>1</sup>, Bo Wang<sup>2</sup>, Wei Lin<sup>3</sup>, Youcheng Chen<sup>2</sup>, You Li<sup>4</sup>, Andrew J. Johnson<sup>1</sup>, Jiri Hulcr<sup>1,\*</sup>

<sup>1</sup> School of Forest, Fisheries, & Geomatics Sciences, University of Florida, Gainesville 32603, USA

<sup>2</sup> CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan 666303, China

<sup>3</sup> Technical Center of Gongbei Customs District People's Republic of China, Zhuhai 519001, China

<sup>4</sup> Vector-Borne Virus Research Center, Fujian Province Key Laboratory of Plant Virology, Fujian Agriculture and Forestry University, Fuzhou 350002, China

\* Corresponding author: hulcr@ufl.edu

With 3 figures and 1 table

**Abstract:** Sentinel gardens are effective in assessing the risk of insect or pathogen invasions on plants from offshore regions. Limited knowledge exists on wood borers' potential impact on expatriate plants and host stress levels. We surveyed wood borers colonizing 11 North American tree species planted in two sentinel gardens in China, recording 38 species from 9 families, mainly Curculionidae (Scolytinae), Cerambycidae, and Cossidae. *Quercus texana* was the most attacked host. Wood borers typically infest unhealthy or dead tissues, regardless of overall tree vitality. We identified species capable of colonizing healthy trees: *Anoplophora chinensis* (Forster) (Cerambycidae), *Sinoxylon* sp. (Bostrichidae), and *Zeuzera coffeae* (Nietner) (Cossidae). Several insects colonizing stressed trees included: *Xylosandrus crassiusculus* (Motschulsky), *Xyleborus affinis* (Eichhoff), *Xylosandrus germanus* (Blandford) (all Curculionidae-Scolytinae), and *Chlorophorus* sp. (Cerambycidae). Some, such as *A. chinensis* and *Z. coffeae*, are under-documented but potentially invasive pests with considerable regulatory importance. Most recorded species are already recognized as pest of live trees or invasive elsewhere, indicating a limited number of potentially invasive wood borers rather than an inexhaustible pool. This suggests that sentinel gardens are effective for pest prevention prioritization. We recommend three methodological improvements for pre-invasion assessments: 1) documenting the vitality of specific colonized tissues to avoid misrepresenting secondary colonizers as primary pests, 2) using fully digital data-management from tree planting to insect identification, and 3) routinely identifying pests using DNA.

**Keywords:** invasive species; pest; risk assessment; Scolytinae; Cerambycidae; phytosanitary measures

## 1 Introduction

Sentinel gardens are a concept of pre-invasion risk assessment for overseas pests and diseases. Originally proposed by the United States National Research Council in 2002 (Council 2002), they can yield valuable information about the potential impact of insect pests and pathogens prior to their incursions, provide data for risk assessment (Fagan et al. 2008; Mansfield et al. 2019) and facilitate biosecurity preparedness (Mansfield et al. 2019; Nahrung et al. 2023). The first academic paper on sentinel gardens, documented 91 potential invasive pests and pathogens on New Zealand plants in 110 overseas gardens (Fagan et al. 2008). Since then, sentinel gardens for pre-invasion risk assessment

have been expanded through multiple international research efforts (Tomoshevich et al. 2013; Roques et al. 2015; Redlich et al. 2019; Bouget et al. 2021).

Establishing and monitoring sentinel gardens present several challenges. Environmental conditions can facilitate insect colonization, especially when insects exploit plant stress (Ranger et al. 2015; Hulcr & Skelton 2023). Such stress-induced interactions may mislead the inference in two ways: 1) insect colonization of stressed plants may overestimation of damage to healthy plants, and 2) maintaining stress-free sentinel plants may disguise herbivore impact under suboptimal conditions like intensive management or climate change. A significant issue is that the presence of an insect presence on a tree does not equal to tree mortality. For

instance, Li et al. (2021) found that of 111 fungal species isolated from bark beetles colonizing oaks and pines trees, only 26 were mild pathogens, and none were systemic pathogen of regulatory concern. This highlights the need for rigorous experimentation to assess the actual mortality caused by potential insects and pathogens.

Botanical gardens offer a valuable opportunity for monitoring plant pathogens and pests before their invasion. The Botanic Gardens Conservation International United States and the United States Department of Agriculture Forest Service collaboratively established the Global Sentinel Plant Network. This network unites botanical gardens globally to predict and prevent incursions of potential insect pests, plant pathogens, and invasive plants. This framework enhances the sentinel garden concept, as recording damaging insects is challenging in institutions designed to maintain healthy plants.

Sentinel gardens have rarely been used for pre-invasion assessment of wood borers, despite their ability to vector tree pathogens and cause severe damage. For example, longhorned beetle species in China and Korea have damaged non-native trees before becoming invasive in Europe and North America, where they caused economic losses exceeding \$600 billion (Hérard & Maspero 2019; Dong et al. 2023). The invasive ambrosia beetle *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae) and its symbiotic fungus *Harringtonia lauricola* (Ascomycota: Ophiostomataceae) have caused significant economic and ecological damage in the United States (Mosquera et al. 2015). Most sentinel garden projects have focused on folivorous insects and plant pathogens (Roques et al. 2015), or sap-sucking insects (Redlich et al. 2019). Only one study has examined wood borers, specifically beetles in dead wood (Bouget et al. 2021).

In this work, we used sentinel gardens in China to detect wood borers that could become invasive in North America and colonize living trees, posing threats to forests and tree-based industries. Our study incorporated both healthy and artificially stressed trees to comprehensively assess potential invasion, spread, and damage. The ultimate goal is to generate data to inform strategies for mitigating the impact of these insects in case of invasion.

## 2 Materials and methods

### 2.1 Experiment sites and tree species in sentinel gardens

To mimic the latitudinal and climatic conditions of the Southeastern United States, we established one sentinel garden in tropical Yunnan province and another in subtropical Fujian, China. The experimental lands were leased from local farmers. Both gardens were situated near natural protected areas to maximize encounters between our planted trees and the indigenous wood borer fauna.

Site 1 is located in Menglun town, Yunnan Province. This area is known for its tropical wet and dry climate, characterized by a rainy season from May to October and a dry season from November to April. Site 2, situated in Shuyang village, Fujian Province, has a subtropical humid climate with cool and humid spring and hot and dry summer, with peak temperatures observed in July and August. The sites, approximately 2600 kilometers apart, are in distinct geographic and climatic zones, and each is expected to host different wood borer communities.

Many American tree species were introduced into China around the early 20<sup>th</sup> century for ornamental and afforestation purposes (Pan & You 1994). These include important landscape trees such as various oaks, pines, and fruit trees, which hold significant commercial and environmental value in their native North America. Our experiment focused on tree species native to or economically important in the Southeastern US, selected for their ecological significance, economic value, and availability in Chinese nurseries. From April to May 2021, 20 or 10 individuals per species were planted (Table 1), starting with a diameter of 3–5 cm (1 cm for avocado). Most trees grew vigorously, achieving substantial trunk diameter suitable for wood borers. Trees were sourced from Chinese nursery markets (they were not imported from North America). To ensure sufficient numbers, we replanted any species with over 50% mortality.

### 2.2 Tree vitality score

We adapted and modified a protocol to visually assess tree vitality based on Callow et al. (2018) and Johnstone et al. (2012). Our simplified scale ranged from 1 (totally dead) to 10 (completely healthy), based on crown density and dead branch proportion (Table S1).

**Table 1.** Tree species originating from Southeastern US planted in the sentinel gardens. The numbers of last two columns represented the total number of trees for per species in each experimental garden. MT: Menglun town, SV: Shuyang village.

	Tree species	MT	SV
1	<i>Quercus texana</i> Buckley	20	20
2	<i>Quercus virginiana</i> Mill	20	20
3	<i>Quercus rubra</i> Linnaeus	20	20
4	<i>Liquidambar styraciflua</i> Linnaeus	20	20
5	<i>Carya illinoensis</i> (Wangenh) K.	20	20
6	<i>Prunus amygdalus</i> Linnaeus	20	20
7	<i>Persea americana</i> Mill	20	20
8	<i>Citrus reticulata</i> Linnaeus	20	20
9	<i>Acer saccharum</i> Marsh	10	10
10	<i>Liriodendron tulipifera</i> Linnaeus	10	10
11	<i>Juglans nigra</i> Linnaeus	10	10

Additionally, we categorized the colonized tissue as dead, unhealthy, or healthy. Dead: tissues showing no signs of life, completely necrotic with discoloration and structural decay. Unhealthy: tissues displaying signs of distress such as discoloration, reduced turgor, partial defoliation, or partial infection by microbes, but not completely dead. Healthy: tissues that are free from any visible signs of distress or disease. Our assessment focused on tissues surrounding wood borer activity to differentiate between wood borers attracted to stressed versus healthy tissues. Distinguishing pre-existing symptoms from insect-induced signs can be difficult, as seemingly healthy tissue might be internally compromised. We noted symptoms like foliage wilting or cankers, avoided assessments based on insect boring to prevent circular reasoning. Tree vitality data were collected over three years, from April 2021 to August 2023. Observations began the day after planting, monitoring at least weekly throughout the year, and almost daily in late spring and early summer when wood borers are most abundant.

### 2.3 Wood borer data collection

To reduce errors from handwritten labels and facilitate data management, we developed a custom mobile application for sentinel garden data collection (Marais 2023).

We systematically recorded both the presence or absence of pests, documenting each tree's vitality, the species colonized, and signs of oviposition or feeding behavior. We noted the insect's specific locations within the trees, the condition of colonized tissues, and the presence of different life stages, including eggs, larvae, pupae, and adults. Wood borers were carefully collected and preserved in 75% ethanol.

Wherever there was evidence of internal feeding (active entry holes, frass, etc.), we carefully sampled affected tree parts, typically branches or trunk sections. These samples were placed in rearing containers and regularly monitored to maintain suitable conditions – such as humidity and temperature – for insect development. If the tree part decayed or larvae were excised during field assessment, larvae were sampled before reaching adulthood. All specimens were documented and preserved for further identification.

### 2.4 Wood borer identification

Adult wood borers were identified based on morphological features using microscope photographs (Olympus BX43 system or ZEISS Smart zoom 5) at the Xishuangbanna Tropical Botanical Garden. These photographs were shared with specialists for identification to the most precise taxon. (Table S2), with photographs available in the supplementary data (<https://doi.org/10.6084/m9.figshare.25050014>). Vouchers are deposited at the Technical Center of Gongbei Customs District People's Republic of China.

DNA analysis confirmed morphological identifications and identified immature stages such as larvae. Genomic DNA was extracted using the Qiagen DNeasy® Blood

& Tissue kit, and mitochondrial cytochrome oxidase c subunit I (COI) and partial nuclear large subunit ribosomal (28S) genes were amplified using the primers COI 1495b 5'-AACAAATCAATTAGATATTGGRAC-3' and COI r750 5'-GAAATTATNCCAATTCCTGG-3'; 28S S3690F 5'-GAGAGTTMAASAGTACGTGAAAC-3' and 28S A4285R 5'-CCTGACTTCGTCCTGACCAGGC-3' (Gomez et al. 2020). Then PCR products were sequenced (BENAGEN Corporation, China), and DNA sequences were identified by blasting against the NCBI genome database (<https://www.ncbi.nlm.nih.gov/>). All sequences from this study were deposited in GenBank (accession numbers OR593387-OR593398 and OR605330-OR605430).

### 2.5 Flooding and wounding treatments

The likelihood of trees being colonized by wood-boring insects increases under injury or flooding, even if the tree is alive (Goldman et al. 2014; Adesso et al. 2018). To attract a broader range of colonizers, in April 2021, we implemented two stress treatments – wounding and flooding – over 28 days on three tree species: *Quercus nuttallii*, *Persea americana*, and *Citrus reticulata*, known for frequent wood borer attacks (Dong et al. 2023). An additional 12 trees per species per treatment were purchased. The initial condition of the trees was documented at planting, followed by daily observations for 28 days.

For flooding, we placed a planter covered with a plastic bag inside another planter, and planted the nursery tree in the inner planter (15 cm wide × 20 cm high) (Fig. S1). For injury treatment, trees were half-girdled by removing a 10 cm × 3 cm section of bark from the trunk, 5 cm above the ground, with a knife sterilized with 75% ethanol, except for avocado, which had a 1 cm × 3 cm window. Methods for observing wood borers and evaluating tree vitality were the same as for non-stressed trees, with all tissues assumed to be unhealthy or stressed due to the treatment.

### 2.6 Data analysis and visualization

Associations between tree vitality scores and wood borer colonization were analyzed and visualized using the ggplot2 and aplot packages. To avoid bias from varying tree species counts, colonization percentage was used, calculated as the number of colonized trees divided by the total trees of that species. To evaluate the relationship between wood borers and tree condition, vitality scores were grouped into three categories (excluding stress treatment data in order for this analysis to reflect the natural pattern): dead (score 1), stressed (scores 2–7), and healthy (scores of 8–10). A Monte Carlo test with 10,000 permutations assessed whether colonization events were randomly distributed among these categories. A Kruskal-Wallis test, followed by a post hoc Dunn's test with false discovery rate (fdr) adjustment, was used to determine which tree health levels attracted different insect abundances.

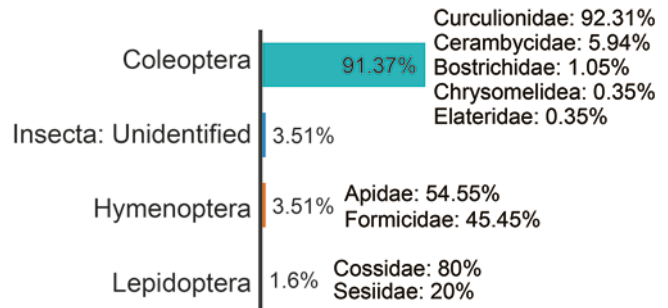
### 3 Results

#### 3.1 Overall results

A total of 142 wood borer colonization events were recorded, involving 38 unique insect species across 9 families within 3 orders on 13 North American tree species (Fig. 1). In the tropical garden at Menglun, 27 species were observed, compared to 20 in the subtropical garden at Shuyang. Five species were found at both sites – *Monochamus adamitus* (Thomson), *Hypothenemus seriatus* (Eichhoff), *Sueus niisimai* (Eggers), *Ceratina cyanea* (Kirby), and *Zeuzera coffeae* (Nietner). The probability of a tree being colonized at least once was 23.16% in the tropical location and 20% in the subtropical garden. The complete dataset is available online as Table S2.

Coleoptera, dominated by the bark and ambrosia beetles (Scolytinae; 92.31% of Coleoptera), accounted for the majority (91.37%) of all wood borer incidents. This order encompassed five different families: Curculionidae, Cerambycidae, Bostrichidae, Chrysomelidae, and Elateridae. The remaining Hymenoptera and Lepidoptera orders were less frequent, constituting 3.51% and 1.6% of the wood borers, respectively. Hymenoptera was primarily represented by carpenter bees (Apidae), while Lepidoptera involved the Cossidae and Sesiidae. Insects that were not identified constituted 3.51% of the observed wood borers and were excluded from the statistical analysis.

To clarify potential impact and regulatory significance of these newly observed pest-tree interactions, it is essen-



**Fig. 1.** Taxonomic proportions of wood borers colonizing on the host plants in this survey.

tial to differentiate whether the insects colonize healthy, dying, or dead tissues. A substantial portion of the colonization events (77.93%) were observed in dead tissues of unhealthy or dead trees (Fig. 2). Incidents on stressed but living trees accounted for 12.76% of the total, while only 3.10% occurred in healthy tissues. These species include *Anoplophora chinensis*, Sesiidae sp., *Sinoxylon* sp., and *Z. coffeae*. The association between wood borers and the conditions of the trees they attacked, as well as between wood borers and the tissues attacked, was found to be non-random (tree conditions: Monte Carlo test, chi-square test = 162.16, p-value < 0.0001; tree tissues: Monte Carlo test, chi-square test = 77.187, p-value < 0.0002). Tree in stressed and dead states were subject to significantly higher colonization by wood borers compared to those in healthy conditions

Stressed tree		Healthy tree	
Dead tissues (91, 31.38%)	<i>Ambrosiodmus rubricollis</i> <i>Beaverium</i> sp. <i>Camponotus vitiosus</i> <i>Cerambycidae</i> sp. <i>Ceratina cyanea</i> <i>Dinoplatypus</i> sp. <i>Euwallacea fornicatus</i> <i>Hypothenemus seriatus</i> <i>Hypothenemus</i> sp. <i>Hypothenemus</i> sp.1 <i>Xyleborinus saxesenii</i>	Unhealthy tissues (37, 12.76%) <i>Ceratina cyanea</i> <i>Chlorophorus</i> sp. <i>Coccotrypes</i> sp. <i>Euwallacea fornicatus</i> <i>Insecta</i> sp. <i>Monochamus adamitus</i> <i>Sueus niisimai</i> <i>Xyleborinus saxesenii</i>	Dead tissues (48, 16.55%) <i>Coccotrypes</i> sp. <i>Crematogaster</i> sp. <i>Euwallacea</i> sp. <i>Hypothenemus seriatus</i> <i>Hypothenemus</i> sp.
Dead tree		Healthy tissues (9, 3.10%)	
Dead tissues (87, 30.00%)	<i>Ceratina cyanea</i> <i>Crematogaster</i> cf. <i>discinodis</i> <i>Dryocoetops</i> sp. <i>Hypothenemus seriatus</i> <i>Hypothenemus</i> sp. <i>Hypothenemus</i> sp.1 <i>Insecta</i> sp. <i>Monochamus adamitus</i> <i>Sueus niisimai</i>	Unhealthy tissues (18, 6.21%) <i>Cerambycidae</i> sp. <i>Cnestus mutilatus</i> <i>Elateridae</i> sp. <i>Hypothenemus seriatus</i> <i>Hypothenemus</i> sp. <i>Insecta</i> sp. <i>Monochamus adamitus</i> <i>Sagra</i> sp. <i>Zeuzera coffeae</i>	<i>Anoplophora chinensis</i> <i>Sesiidae</i> sp. <i>Sinoxylon</i> sp.

**Fig. 2.** Relative proportion of observations of colonization of tissues at a specific health level. Healthy trees represent those with vitality scores of 8, 9, and 10; stressed trees represent those with vitality scores ranging from 2 to 7; dead trees are those with a vitality score of 1. The numbers in the bracket are the absolute number of observed wood borers, and the percentage proportions over the entire dataset.



(Kruskal-Wallis:  $H_{(2)} = 6.48$ ,  $p$ -value = 0.0392; stressed vs. healthy trees: fdr adjusted  $p$ -value = 0.042; dead vs healthy trees: fdr adjusted  $p$ -value = 0.042). Colonization frequency was not significantly different between stressed and dead trees (fdr adjusted  $p$ -value = 0.80).

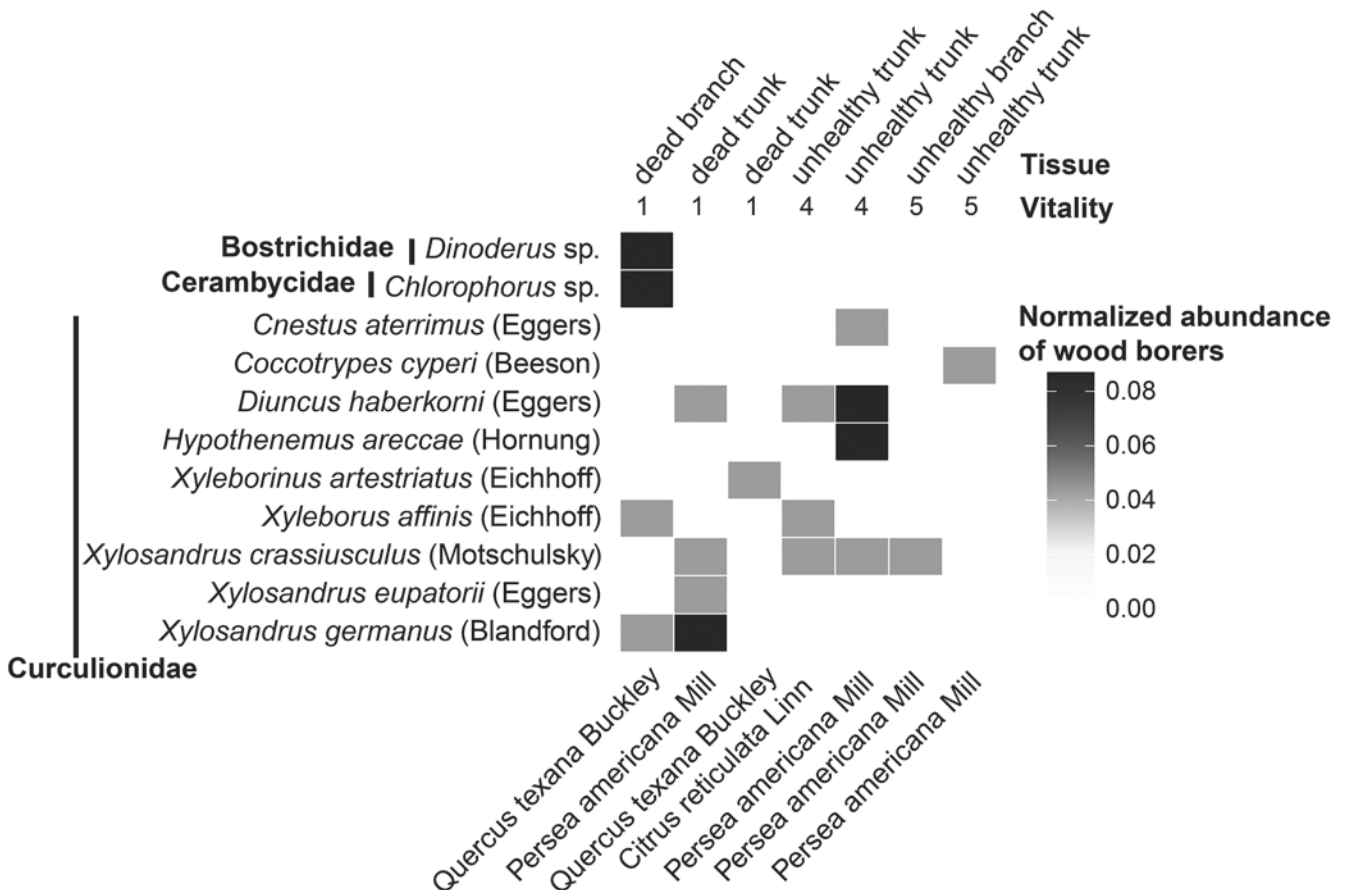
Of the borers on unhealthy tissues, the most common were bark beetles from the genus *Hypothenemus* (Coleoptera: Curculionidae), especially *H. seriatus*. Several other species were observed in the unhealthy branches or trunks of stressed or healthy trees, such as *Chlorophorus* sp., *Euwallacea fornicatus* (Eichhoff) (the polyphagous shot-hole borer *sensu stricto*, as defined in Smith et al. 2019), *Xylosandrus crassiusculus* (Motschulsky), *Xylosandrus germanus* (Blandford), and *Xyleborus affinis* (Eichhoff).

Members of the Apidae and Cerambycidae families were recorded on trees across a wide range of vitality scores, including those appeared healthy. However, wood borers generally targeted trees with lower vitality, particularly those with scores from 1 to 6. Wood borer colonization tended to be influenced more by the health condition of specific tree tissues rather than the overall vitality of the whole trees.

Tree species differed markedly in the rate of colonization by wood borers. The highest frequency was observed on *Quercus texana* (90%, the number derived from the number of tree individuals colonized by wood borer(s) divided by the total trees of that species deployed), *Citrus reticulata* (63%), *Quercus virginiana* (43%), and *Persea americana* (43%) (Table S3).

### 3.2 Wood borers attacking artificially stressed trees

The flooding and girdling treatments yielded distinct results: 11 unique wood-boring species were exclusively attracted to, and collected from, trees subjected to the flooding treatment, while no wood-boring colonizers were observed on the trees subjected to the girdling treatment. Among the treated species, *Q. texana* and *P. americana*, followed by *C. reticulata*, were most heavily affected by wood borer infestations under stress conditions (Fig. 3 and Table S4). *X. crassiusculus* was the most commonly recorded wood borer from the flooded plants. Trees with scores higher than 5 showed no signs of wood-borer attack throughout the duration of the stress treat-



**Fig. 3.** The flooding experiment: a heatmap for wood borer preference for different vitality scores of the flooded trees. Rows represent species or taxa observed, and columns represent separate observations on experimental tree individuals. The color differences in cells reflect the normalized abundance of wood borers. The columns are arranged by the tree vitality, then by the specific tree tissue vitality.

ments. Attacks were observed only on trees with scores of 5 or lower, indicating that tree health significantly influences wood borer behavior and colonization patterns, in some cases more than tree identity.

## 4 Discussion

American tree species were colonized by wood borers in China, however, most wood borers tended to colonize stressed trees, or specific unhealthy tree tissues, rather than healthy trees. This shows that, tree health and environmental conditions need to be included in pre-invasion assessment studies. All wood borers found in living trees have previously been documented, and many of them are already classified as pests in the native or invaded regions. Contrary to the common concept of ever-increasing invasions and a large source pool of “unknown unknowns” (Vantarová et al. 2023), our findings demonstrated that the fauna of wood borers is already known to a degree that enables effective international pest prediction.

Monitoring trees planted in non-native regions via sentinel gardens has proven to be an efficient approach to horizon scanning for potentially invasive borers and pathogens. More than 100 insect species were reported on European tree species in sentinel gardens in China (Roques et al. 2015; Kenis et al. 2018). In this study, over 300 wood borer individuals from 38 species on 11 American tree species were collected, many representing new associations between wood borers and their host plants. Previous studies, such as Tomoshevich et al. (2013) and Bouget et al. (2021), also identified new fungal species and beetle-host associations, respectively, demonstrating the utility of sentinel gardens in discovering novel ecological interactions.

The findings from our study contribute to ecological understanding and regulatory implications. By identifying potential pest threats in sentinel gardens, our research can inform and refine biosecurity measures. Pest biology data from native regions can significantly strengthen pest predictions, detection, and response programs. However, it is essential to recognize that a colonization of a tree in sentinel gardens by a particular insect does not automatically imply future invasiveness of that insect in non-native regions. The invasive process is subject to various factors, including matching climate and introduction pathways (Thompson et al. 2021). It is also critical to distinguish between the majority of wood borers that colonize tissues with lower vitality and those attacking healthy tree tissues, since the latter ones are potentially serious pests. Records of wood borers on logs of non-native trees are available (Bouget et al. 2021), however, records from dead wood do not allow for predictions of impact on living trees. Therefore, interpreting these results requires consideration of additional abiotic and biotic factors in a comprehensive risk assessment.

Species that have repeatedly occurred deserve special attention. For instance, *Anoplophora chinensis* has been repeatedly recorded colonizing living trees in our study, and also frequently reported in previous publications as a polyphagous species posing a serious threat to the global citrus industry (Hérard & Maspero 2019; Dong et al. 2023). This study extends of its host range to include an American oaks species, *Q. texana*. Among the observed species, *A. chinensis* and *Z. coffeae* are most likely to become significant concern should they arrive to the US, though any regulatory decisions will require a more complete risk assessment. These species were repeatedly recorded on oak trees with high vitality, indicating a potential threat to healthy trees. If the occurrence of wood borer attacks is infrequent and limited to one or a few instances, it does not necessarily confer a new pest status on this association between the wood borer and host plants. We must exercise caution when interpreting results from sentinel gardens, particularly regarding species with new and low-frequency reports.

In contrast, *Xylosandrus crassiusculus*, an Asian ambrosia beetle, is one of the most invasive forest insects (European and Mediterranean Plant Protection Organization alert list, accessed on October 12, 2023), carrying symbiotic fungus, *Ambrosiella roeperi* Harrington & McNew (Microascales: Ceratocystidaceae), which is widely polyphagous and adaptable to various conditions. This adaptability may contribute to the success of this symbiotic species pair. Our findings corroborate existing observations, which is that it is attracted to dying or over-watered trees. This species is typically non-destructive in most forest environments, but it can become a significant pest in nurseries and other stressed ecosystems affected by frost or flooding (Ranger et al. 2015).

The polyphagous shot hole borer, *Euwallacea fornicatus*, native to South and Southeast Asia, is a well-known invasive pest in multiple regions (Smith et al. 2019). Until recently this species has been confused in literature with *E. perbrevis*, the more tropical tea shothole borer, which precludes assigning host records from older literature to either of the two species (Li et al. 2015). Unlike its typical behavior of infecting healthy plants in the invaded regions of South Africa, California and Israel, in our observations, this species was only found colonizing the unhealthy trunk of *Liquidambar styraciflua* and a dead branch of *Citrus reticulata*. Additionally, another *Euwallacea* sp., not yet identified to the species level, was recorded on a dead branch of *Quercus virginiana* in our garden. Our results suggest that *E. fornicatus* does not always pose a threat to living and healthy trees; instead, it may also infect the dead or unhealthy tissues of living trees.

The longhorn beetle *Chlorophorus* sp. is similar, but not identical to, *Chlorophorus miwai* Gressitt (Coleoptera: Cerambycidae), which has been reported as attacking multiple pine and oak tree species in China (Sun et al. 2000). *Chlorophorus* sp. was found in our stressed *Q. texana* and in unhealthy tissues of living *Citrus reticulata*. Similarly, an unidentified powderpost beetle species similar, but not

identical to *Sinoxylon conigerum* Gerstäcker (Coleoptera: Bostrichidae), a known pest of timber in several countries (Price et al. 2011), was observed in the vicinity of the dead and living parts of the branch of *Q. virginiana* in our garden. While the family Bostrichidae is generally viewed as colonizing dead wood and timber, a few species within this family can also be pests of living trees (Price et al. 2011; Liu et al. 2016).

Closely related insect species often share hosts (Mech et al. 2019) or attack divergent host species that offer similar developmental resources (Endara et al. 2017). Thus, the presence of *Chlorophorus* sp. and *Sinoxylon* sp. in our gardens, especially in unhealthy but live trees, raises concerns about their potential to become pests under suitable environmental conditions.

Environmental stressors of trees like temperature fluctuations, windstorms, wildfires, and droughts are known drivers of insect colonization in non-native regions (Carbonell et al. 2017; Lantschner et al. 2019). We also observed that colonization events took place in living trees that, despite appearing healthy, were in fact under stress from various factors. It is well known that trees with lowered defense capabilities are exploited by a much greater diversity of borers than healthy trees (Cohen et al. 2023).

The rate of colonization by wood borers varied among tree species. The four species with the highest frequencies of wood borer attacks were *Quercus texana* (90%), *Citrus reticulata* (63%), *Quercus virginiana* (43%), and *Persea americana* (43%), compared to the remaining seven species. This disparity in susceptibility may be linked to the various chemical compounds these species release. *Quercus*, *Citrus*, and *Persea* belong to Fagaceae, Rutaceae, and Lauraceae families, respectively, which are known for producing a diverse array of secondary metabolites such as tannins, alkaloids, and sesquiterpenes (Martini et al. 2015). Many wood borers have likely evolved specific detoxification mechanisms to cope with these compounds, or they may be attracted to the chemical signals emitted by these plants.

Our findings indicate a low proportion (3.10%) of colonization of healthy tissues. This suggests that the health status of the tree, rather than its size or identity, plays a more critical role in the susceptibility to wood borer attacks. The low incidence of attacks on specific healthy tissues across different tree sizes further supports this observation.

One limitation of this study is the potential genetic divergence among the tree species that have been cultivated for decades in local nurseries in China (Pan & You 1994). This period of selection under cultivation, however brief, may have led to genetic differences between these trees and their native counterparts in the USA, particularly in traits critical to pest and disease resistance, which are influenced by complex genetic and environmental factors. Such selection pressures in nurseries might favor individuals with traits that are either particularly advantageous or disadvantageous for survival and reproduction in these artificial settings. This highlights the importance of considering the genetic varia-

tions among experimental trees in future research to better assess their susceptibility and resilience to invasive species.

Standardizing protocols for data collection is crucial for comparing data between multiple sentinel garden projects. The heterogeneity in data across different research studies restricts information sharing, result comparison, and ultimately, risk assessment. Providing exhaustive details about the attacks or colonization of wood borers on trees, as well as absence of attacks, is beneficial for accurately assessing impact and helping to avoid overstatement of the damage or pest status. However, there are challenges associated with implementing standardized protocols, particularly concerning the variation in data formats required for different research objectives. This dilemma can be resolved by data recording technologies, such as our smartphone-based interface, which allows for data capture that is both detailed and efficient (Marais 2023). Many teams actively operate sentinel gardens, surveying wood borers, sap-suckers, leaf-chewers as well as pathogens. To cross-reference between these datasets, establishing a universal database will be helpful for future research, ideally including molecular “barcodes” for cross-referencing pest identity. The International Plant Sentinel Network, including plant protection professionals and National Plant Protection Organizations from all over the world, developed a platform to exchange invasive pests and pathogens information, standardize methodologies and materials and incursions monitoring. Comparing datasets from different sources can help identify potential invasive species, and multiple independent observations provide robust evidence of potential threat posed by specific species. In addition, visual monitoring methods may not capture all instances of attack, such as oviposition in rough bark. This could be addressed by improving the fidelity of colonization records, or adoption of advanced technologies (Klouček et al. 2019) and intensive training of human observers, enhancing the accuracy of wood borer detection.

The study confirms the feasibility of extending the sentinel gardens concept onto wood borers. Our observations provide the foundation for risk assessment of the wood borers that have repeatedly shown the capacity to colonize expatriate healthy trees. It is important that, in the case of wood borers, pre-invasion observations differentiate between colonization of unhealthy tissues, colonization of apparently healthy tissues on stressed trees, and attacks on healthy trees. Establishing a universal database for sentinel garden projects around the world would be helpful for future research.

**Acknowledgments:** This research was supported by the United States Department of Agriculture Forest Service International Projects, and the USDA Animal and Plant Health Inspection Service. We thank Dr. Mingyong Tang and Xiaocui Shi for their help with DNA extraction. We thank the Central Laboratory of Public Technology Service Center of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, for the guidance and technical support in microscopy.



## References

- Addesso, K., Baysal-Gurel, F., Oliver, J., Ranger, C., & O'Neal, P. (2018). Interaction of a preventative fungicide treatment and root rot pathogen on ambrosia beetle attacks during a simulated flood event. *Insects*, 9(3), 83. <https://doi.org/10.3390/insects9030083>
- Bouget, C., Brin, A., & Larrieu, L. (2021). The use of sentinel logs to assess host shifts in early beetle colonisers of deadwood under climate-and forestry-induced tree species substitutions. *Insect Conservation and Diversity*, 14(1), 117–131. <https://doi.org/10.1111/icad.12434>
- Callow, D., May, P., & Johnstone, D. M. (2018). Tree vitality assessment in urban landscapes. *Forests*, 9(5), 279. <https://doi.org/10.3390/f9050279>
- Carbonell, J. A., Velasco, J., Millán, A., Green, A. J., Coccia, C., Guareschi, S., & Gutiérrez-Cánovas, C. (2017). Biological invasion modifies the co-occurrence patterns of insects along a stress gradient. *Functional Ecology*, 31(10), 1957–1968. <https://doi.org/10.1111/1365-2435.12884>
- Cohen, A., Basu, S., & Crowder, D. W. (2023). Drought stress affects interactions between potato plants, psyllid vectors, and a bacterial pathogen. *FEMS Microbiology Ecology*, 99(1), fiac142. <https://doi.org/10.1093/femsec/fiac142>
- Council, N. R. (2002). Predicting invasions of nonindigenous plants and plant pests. <https://doi.org/10.17226/10259>
- Dong, Y., Gao, J., & Hulcr, J. (2023). Insect wood borers on commercial North American tree species growing in China: Review of Chinese peer-review and grey literature. *Environmental Entomology*, 52(3), 289–300. <https://doi.org/10.1093/ee/nvad039>
- Endara, M. J., Coley, P. D., Ghabash, G., Nicholls, J. A., Dexter, K. G., Donoso, D. A., ... Kursar, T. A. (2017). Coevolutionary arms race versus host defense chase in a tropical herbivore–plant system. *Proceedings of the National Academy of Sciences of the United States of America*, 114(36), E7499–E7505. <https://doi.org/10.1073/pnas.1707727114>
- Fagan, L., Bithell, S., & Dick, M. (2008). Systems for identifying invasive threats to New Zealand flora by using overseas plantings of New Zealand native plants, pp 51–62. (Froud KJ, Popay AI, Zydenbos SM eds). Surveillance for biosecurity: Pre-border to Pest Management. *Proceedings of a symposium held on 11 August 2008 in Paihia, New Zealand*. The New Zealand Plant Protection Society (Incorporated), Hastings, New Zealand.
- Goldman, A., Eggen, B., Golding, B., & Murray, V. (2014). The health impacts of windstorms: A systematic literature review. *Public Health*, 128(1), 3–28. <https://doi.org/10.1016/j.puhe.2013.09.022>
- Gomez, D. F., Li, Y., & Storer, C. (2020). PCR for amplification of bark beetles or fungal DNA. <https://www.protocols.io/>. <https://doi.org/10.17504/protocols.io.bnvbme2n>
- Hérad, F., & Maspero, M. (2019). History of discoveries and management of the citrus longhorned beetle, *Anoplophora chinensis*, in Europe. *Journal of Pest Science*, 92(1), 117–130. <https://doi.org/10.1007/s10340-018-1014-9>
- Hulcr, J., & Skelton, J. (2023). *Ambrosia beetles*. In D. Allison, J., Paine, T.D., Slippers, B., Wingfield, M.J. (eds) Forest Entomology and Pathology: Volume 1 (pp. 339–360). Springer, Cham. [https://doi.org/10.1007/978-3-031-11553-0\\_11](https://doi.org/10.1007/978-3-031-11553-0_11)
- Johnstone, D., Tausz, M., Moore, G., & Nicolas, M. (2012). Chlorophyll fluorescence of the trunk rather than leaves indicates visual vitality in *Eucalyptus saligna*. *Trees (Berlin)*, 26(5), 1565–1576. <https://doi.org/10.1007/s00468-012-0730-7>
- Kenis, M., Li, H., Fan, J. T., Courtial, B., Auger-Rozenberg, M. A., Yart, A., ... Roques, A. (2018). Sentinel nurseries to assess the phytosanitary risks from insect pests on importations of live plants. *Scientific Reports*, 8(1), 11217. <https://doi.org/10.1038/s41598-018-29551-y>
- Klouček, T., Komárek, J., Surový, P., Hrach, K., Janata, P., & Vašíček, B. (2019). The use of uav mounted sensors for precise detection of bark beetle infestation. *Remote Sensing (Basel)*, 11(13), 1561. <https://doi.org/10.3390/rs11131561>
- Lantschner, M. V., Aukema, B. H., & Corley, J. C. (2019). Droughts drive outbreak dynamics of an invasive forest insect on an exotic host. *Forest Ecology and Management*, 433, 762–770. <https://doi.org/10.1016/j.foreco.2018.11.044>
- Li, Y., Bateman, C., Skelton, J., Wang, B., Black, A., Huang, Y.-T., ... Hulcr, J. (2021). Pre-invasion assessment of exotic bark beetle-vectored fungi to detect tree-killing pathogens. *Phytopathology*, 112(2), 261–270. <https://doi.org/10.1094/PHYTO-01-21-0041-R>
- Li, Y., Lucky, A., & Hulcr, J. (2015). Tea shot-hole borer *Euwallacea fornicatus* (Eichhoff, 1868) (Insecta: Coleoptera: Curculionidae: Scolytinae) IFAS Publication, EENY 624. University of Florida Institute of Food and Agricultural Sciences, Gainesville, FL. <https://edis.ifas.ufl.edu/in1090>
- Liu, L., Ghahari, H., & Beaver, R. A. (2016). An annotated synopsis of the powder post beetles of Iran (Coleoptera: Bostrichoidea: Bostrichidae). *Journal of Insect Biodiversity*, 4(14), 1–22. <https://doi.org/10.12976/jib/2016.4.14>
- Mansfield, S., McNeill, M. R., Aalders, L. T., Bell, N. L., Kean, J. M., Barratt, B. I., ... Teulon, D. A. (2019). The value of sentinel plants for risk assessment and surveillance to support biosecurity. *NeoBiota*, 48, 1–24. <https://doi.org/10.3897/neobiota.48.34205>
- Marais, C. (2023). *Digital Monitoring and Automated Identification of Bark and Ambrosia Beetles*. Master thesis, University of Florida.
- Martini, X., Hughes, M. A., Smith, J. A., & Stelinski, L. L. (2015). Attraction of redbay ambrosia beetle, *Xyleborus glabratus*, to leaf volatiles of its host plants in North America. *Journal of Chemical Ecology*, 41(7), 613–621. <https://doi.org/10.1007/s10886-015-0595-5>
- Mech, A. M., Thomas, K. A., Marsico, T. D., Herms, D. A., Allen, C. R., Ayres, M. P., ... Tobin, P. C. (2019). Evolutionary history predicts high-impact invasions by herbivorous insects. *Ecology and Evolution*, 9(21), 12216–12230. <https://doi.org/10.1002/ece3.5709>
- Mosquera, M., Evans, E. A., & Ploetz, R. (2015). Assessing the profitability of avocado production in south Florida in the presence of laurel wilt. *Theoretical Economics Letters*, 5(2), 343–356. <https://doi.org/10.4236/tel.2015.52040>
- Nahrung, H. F., Liebhold, A. M., Brockerhoff, E. G., & Rassati, D. (2023). Forest insect biosecurity: Processes, patterns, predictions, pitfalls. *Annual Review of Entomology*, 68(1), 211–229. <https://doi.org/10.1146/annurev-ento-120220-010854>
- Pan, Z., & You, Y. (1994). *Introduction and Cultivation of Major Exotic Tree Species in China*. Beijing: Beijing Science and Technology Press.
- Price, T., Brownell, K. A., Raines, M., Smith, C. L., & Gandhi, K. J. (2011). Multiple detections of two exotic auger beetles of the genus *Sinoxylon* (Coleoptera: Bostrichidae) in Georgia,



- USA. *The Florida Entomologist*, 94(2), 354–355. <https://doi.org/10.1653/024.094.0235>
- Ranger, C. M., Schultz, P. B., Frank, S. D., Chong, J. H., & Reding, M. E. (2015). Non-native ambrosia beetles as opportunistic exploiters of living but weakened trees. *PLoS One*, 10(7), e0131496. <https://doi.org/10.1371/journal.pone.0131496>
- Redlich, S., Clemens, J., Bader, M. K.-F., Pendrigh, D., Perret-Gentil, A., Godsoe, W., ... Brockerhoff, E. G. (2019). Identifying new associations between invasive aphids and Pinaceae trees using plant sentinels in botanic gardens. *Biological Invasions*, 21(1), 217–228. <https://doi.org/10.1007/s10530-018-1817-x>
- Roques, A., Fan, J. T., Courtial, B., Zhang, Y. Z., Yart, A., Auger-Rozenberg, M. A., ... Sun, J. (2015). Planting sentinel European trees in Eastern Asia as a novel method to identify potential insect pest invaders. *PLoS One*, 10(5), e0120864. <https://doi.org/10.1371/journal.pone.0120864>
- Smith, S. M., Gomez, F. D., Beaver, R. A., Hulcr, J., & Cognato, A. I. (2019). Reassessment of the species in the *Eurwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) complex after the rediscovery of the “lost” type specimen. *Insects*, 10(9), 261. <https://doi.org/10.3390/insects10090261>
- Sun, X., Wei, W., Li, Q., & Xia, Y. (2000). Biological characteristics and control of *Chlorophorus miwai* Gressitt. *Journal of Henan Forestry Science and Technology*, 20, 31–32.
- Thompson, L. M., Powers, S. D., Appolon, A., Hafker, P., Milner, L., Parry, D., ... Grayson, K. L. (2021). Climate-related geographical variation in performance traits across the invasion front of a widespread non-native insect. *Journal of Biogeography*, 48(2), 405–414. <https://doi.org/10.1111/jbi.14005>
- Tomoshevich, M., Kirichenko, N., Holmes, K., & Kenis, M. (2013). Foliar fungal pathogens of European woody plants in Siberia: An early warning of potential threats? *Forest Pathology*, 43(5), 345–359. <https://doi.org/10.1111/efp.12036>
- Vantarová, K. H., Eliáš, P., Jr., Jiménez-Ruiz, J., Tokarska-Guzik, B., & Cires, E. (2023). Biological invasions in the twenty-first century: A global risk. *Biologia*, 78(5), 1211. <https://doi.org/10.1007/s11756-023-01394-7>

Manuscript received: January 24, 2024

Revisions requested: April 17, 2024

Revised version received: May 3, 2024

Manuscript accepted: June 12, 2024

The pdf version (Adobe JavaScript must be enabled) of this paper includes an electronic supplement:  
**Supplementary Table S1–S4; Figure S1**