Identification of Potentially Invasive Wood Borers From China Using Sentinel Gardens of Stressed American Trees

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Abstract

Thousands of insect species have been introduced to non-native regions around the world; many have harmlessly integrated into the new ecosystems, while some became agents of mortality of the new plant hosts. To prepare for possible new invasions, scientists and agencies need to identify traits that predispose wood boring species to become tree-killers, and focus on species that possess these traits. Here, we report results of field trials in three different sentinel gardens of North American trees in China, seeking to identify wood borers demonstrating aggressive traits while still in their native range. We also added flooding and partial girdling of the experimental trees to detect wood borers attracted to physiologically stressed but living trees. We identified two species of Cerambycidae (*Anoplophora chinensis*, *Chlorophorus signaticollis*) and 3 species of Curculionidae-Scolytinae (*Cryphalus lipingensis, C. piceus, Hyledius kesiyae*) colonizing living, North American oaks and pines. We have also documented additional seven borer species which colonized our trees only after their vitality index had dropped below recovery, a trait associated with secondary, non-damaging borers. Identifying potential pests and documenting their interactions with the host in sentinel gardens supports risk analysis, and enables trading partners to assess the potential impact of pest-host interactions prior to an invasion.

Introduction

Invasive alien species have caused significant damage to global forest ecosystems and tree industries. The increasing frequency of invasions is attributed to economic globalization, changing land-use, and environmental change (Hulme et al. 2008, Hulme 2009, Chown et al. 2015). In particular, live plant imports and the use of wood as packaging material confer opportunities for colonization and long-distance human-mediated dispersal through international trade networks (Brasier 2008, Liebhold et al. 2012, Meurisse et al. 2019). Additionally, anthropogenic climate change exacerbates host range shifts and expansions, introducing invasive species to novel and naïve hosts (Richardson et al. 2000, Hanewinkel et al. 2013).

Some adventive species have dramatic effects on the newly encountered vegetation, resulting in impacts on ecosystem functioning, alterations of forest community dynamics, and damage to human health and economies (Parker et al. 1999, Binimelis et al. 2007, Kenis and Branco 2010, Sanders et al. 2010, Vilà et al. 2011). However, the damaging species represent only a small percentage of all the newly established species, while the greater majority have only negligible negative effect on native vegetation (Williamson and Fitter 1996). This low probability of high impact is a problem in effective pest risk assessment, focused monitoring, and rapid response to consequential invasions (McGeoch et al. 2012).

Protection against pest invasion typically include pest risk analysis and early detection and rapid response (Baker et al. 2009, Koch et al. 2011, Early et al. 2016, Roy et al. 2018, Rabaglia et al. 2019). Pest risk analysis processes evaluate the known scientific evidence and consider potential ecological, economic, and social consequences of invasion. A decision is then made whether regulation is warranted.

Given the uncertainty of eventual impact, however, regulation of newly detected forest pests is often reactive, not proactive (Keskitalo and Pettersson 2012). Undesirable species are often determined as such only on the basis of being invasive elsewhere (Kulhanek et al. 2011, McGeoch et al. 2012). However, many new forest pests were not known to be invasive before their invasion. Therefore, the risk assessment of potential invasive species should include their intrinsic biological features (Roy et al. 2014, Groom et al. 2017). Without baseline biological data, risk assessment and invasion responses are over-reliant on assumptions, which reduces their effectiveness (Roy et al. 2018).

The research presented here aims to proactively identify potentially invasive and damaging wood borers in Asia that may threaten trees in North America. Among the most destructive forestry invasive species belong to the longhorn beetles (Cerambycidae), jewel beetles (Buprestidae), and wood boring weevils (Curculionidae), particularly the bark and ambrosia beetles in the subfamilies Scolytinae and Platypodinae (Evans et al. 2007, Vega and Hofstetter 2014). Woodborers have caused mass mortality events in natural and planted forest ecosystems (Hoffmeister et al. 2005, Mayfield et al. 2008, Haack et al. 2010). Some species can act as primary pests, which can attack and kill trees that are healthy or under minor stress. Woodborers can also vector tree pathogens(Harrington et al. 2005).

Woodborers have traits and characteristics advantageous in dispersal. Many are small, have cryptic lifestyles, and can take a long time to emerge (Cherepanov et al. 1988, Liebhold et al. 2012). Wood-packaging material, live plants, and firewood can all harbor invasive wood borers (Meurisse et al. 2019). While inside the wood, the insect is protected from pesticide application methods (Frank and Sadof 2011). This habit also confers protection from inspection by predators and biosecurity agents alike.

This project employed the principle of sentinel gardens to identify Asian wood borers that present the potential to be damage important tree species in North America. Sentinel gardens are a versatile method that uses host plants planted in a non-native range (*ex-patria* planting) to document the local, potentially invasive damaging agents (Britton K.O. 2010, Roques et al. 2015, Vettraino et al. 2015, Eschen et al. 2019). Native plants rarely suffer mass-mortality events by native pests and pathogens, and their potential impacts of these as invasive species are hard to predict(Liebhold et al. 2012, Roques et al. 2015). Using non-native trees reduces the uncertainty in pest risk assessments. Pest-host associations documented in this way are more demonstrative of invasive behavior in the invaded region and align with recommendations outlined by Roy et al. (2018) for determining invasive species.

In this project we amend the sentinel garden approach by adding a second key component: tree stress. Some woodborers target trees in their native area that are weakened or stressed, and this trait is often manifested as the attraction to, and killing of, healthy but susceptible species in the invaded areas (Hulcr et al. 2017). This scenario has been shown in some of the most damaging wood borers, including the Asian longhorn beetle (Haack et al. 2010), the redbay ambrosia beetle (*Xyleborus glabratus* Eichoff) (Mayfield et al. 2008) and the Emerald ash borer (*Agrilus planipennis*) when introduced into North America. All three demonstrated the aggressive trait of colonization of living trees in their native range, indicating that damage to susceptible tree species in invaded regions may have been predictable (Hulcr et al. 2017). Therefore, we added the stress component in order to identify wood boring species with preference for stressed but living trees in their native range.

China and the United States are the world's largest business partners, which increases the risk of invasive species exchange. Many of the invasive woodborers established in the USA are native to East Asia (Poland and McCullough 2006, Aukema et al. 2010, Kenis et al. 2017). In addition, China encompasses many regions of high biological diversity (Mattson et al. 2007). Lastly, the flora of China is phylogenetically similar to that of North America. Herbivorous insects, often specialized at a plant genus or family, benefit from the presence of congeners in invaded regions (Mech et al. 2019).

Methods

Field sites

Three field sites in mainland China were selected (Table 1, Figure 1). Sites were chosen based on availability and suitability for the experiment, all locations being >1000km apart. A wider geographic distribution was selected to survey diverse climates and forest ecosystems. At a local scale, sites were selected where a congener to the introduced trees was present (typically oaks and/or pines).

During these experiments, site selection, tree planting, and garden maintenance involved cooperation with local landowners and land managers. Permission was sought and granted prior to all research being undertaken.

Table 1
The experiment locations in China

Site Name and Location	Co-ordinates, Elevation and Climate Type	Date of Experiment	Vegetation type and local land use
Fuan, Ningde, Fujian Province	27°09'42.0"N 119°40'35.1"E; 260-300m Humid subtropical climate	Trial 09/18/18 – 10/01/18 Full Experiment 05/18/19 – 06/02/19	Local managed forests - Chinese fir (*Cunninghamia lanceolata*) and native pine (*Pinus massoniana*). Understory species -bamboo and native Lauraceae. Surrounding agricultural land -rice at lower elevations and tea on the mountainsides.
Botanical Gardens, Southwest Forestry University, Kunming, Yunnan Province	25°03'58.0"N 102°46'08.3"E; 1948-1969m Subtropical highland climate	Full Experiment 06/06/19 – 06/21/19	Ecological reservoir with protected status – native pine (*P. yunnnanensis* Franchet and *P. armandii* Franchet). Understory - willow (*Salix* spp.), elm (*Ulmus* spp.), firethorn (*Pyracantha fortuneana* (Maxim.) Li) and bamboo Experimental forestry plots - Sabina (*Juniperus chinensis*) and Eucalyptus (*E. globulus* Labill).
Dasi illage, Mountain, Tai'an, Shandong Province	36°02'34.5"N 117°14'25.9"E; 210-258m Subtropical to temperate monsoon climate	Full Experiment 07/29/19 – 08/12/19	Native pine forest (*P. tabuliformis, P. densiflora, P. koraiensis, P. thunbergii*). Low frequency - Maidenhair tree (*Gingko biloba*) and *Wisteria spp.* Cultivated areas – chestnut (*Castanea mollissima* Blume) and poplar (*Poplar* spp.).

Trap tree species

Four tree species were chosen that are native to the Southeastern U.S. and also available in the Chinese nursery network. Slash pine (*P. elliottii*) was chosen because of its ecological and economic importance. Not only it is an important timber species in the U.S., but it is also increasingly important in China, where it is being considered for reforestation efforts where the pine wood nematode has killed native pines (Pan 2000, Lai et al. 2020). Nuttal's oak (*Quercus texana*) was chosen to represent the "red oak" species group that is widespread in the southeastern U.S. Our target region in China contains many species of Fagaceae in several genera, and a number of native woodborers specialized in Fagaceae. Roques et al. (2015) already observed several Asian pests on European oaks in their sentinel garden in China

In addition, two specialty crop tree commodities, not native to North America but economically important in the SE U.S., were used: avocado (*Persea americana*) and mandarin orange (*Citrus reticulata*). Citrus such as oranges, limes, and lemons (Rutaceae) are important commercial crops in China as well as in the USA (Liu et al. 2012). The U.S. citrus industry alone is worth $3.4 billion (NASS 2020). Mandarin orange (*Citrus reticulata*) is native to southeast Asia, but the recent outbreak of Huanglongbing, or citrus greening, around the world as well as in the native China highlighted the industry's vulnerability to unknown diseases and pests (Bové 2006, Zhou 2020). Avocado production has been explored in areas of China (Papademetriou et al. 2000) with several pests identified on Hainan island (Yongming 1995).

Treatments

Two treatments were chosen to attract a wider range of live-tree colonizers: flooding and wounding (Fig. 2). The flood stress treatment is based on the pot-in-pot system by Ranger et al. (2015). Flood-stressed trees are attractive to many invasive woodborers attacking living trees, particularly ambrosia beetles (Ranger et al. 2013). A 15L pot is lined with 3mm plastic sheeting, and the pot with the tree is placed inside the first. Once planted, these pots were filled with water until soil saturation to the root flare. Flood stress was maintained throughout the experiment, approximately one month.

The second treatment involved removing 30% of the perimeter of the bark to mimic injury (Dunn et al. 1986, Dunn and Lorio 1992, McCullough et al. 2009, Reed et al. 2015). This partial girdle began at 15cm above root flare and extended 15cm up the trunk. Girdled trees were watered as needed.

Trap tree sizes and spacing

Root ball trees measuring between 1.6m and 4m in height (DBH ~3cm) were delivered at each site. Twelve of each tree species were planted. Avocado of the correct dimensions could not be sourced, so smaller (~50cm tall) saplings were used and planted in 3L pots. Each site consisted of 12 groups with each group containing one of each of the four species. Six groups were flooded, six were partially girdled. Minimum distances between groups were reliant on the available area. In the Fujian trial and full experiment, groups were > 5m apart. In the Yunnan and Shandong experiments, the groups were >10m apart.

Monitoring tree vitality

Treatments were intended to simulate stress factors and daily phytosanitary checks were completed to record changing tree conditions. Our goal was to identify wood borer species that target weakened but living trees. The time and date of colonization events were recorded and physically highlighted on the tree using permanent marker pens. In this way, colonization events and ensuing changes in tree condition could be attributed to the colonizer at the end of the experiment. Trees were inspected upon arrival and during planting. Any evidence of prior disease or colonization was recorded with photographs. Once treatments were established, each tree was systematically inspected between 2 and 4 times per day and any colonization documented. Each tree and any new damage were photographed daily.

Tree health was assessed daily using a visual tree survey method adapted from Callow et al.'s (2018) visual assessment of urban street trees. Our adapted simplified method is a simple scale measuring crown density between 0 (leafless) and 10 (full canopy) (Figure 2 below). Treatments were expected to steadily reduce tree vitality but not kill the trees. Wilting, dieback, and similar disorders would reduce the total healthy foliage resulting in a lower visual assessment score. Conversely, trees could improve, and the visual assessment score increase. Similar treatments implemented by Ranger et al. (2013, 2015) did not lead to tree mortality in the absence of wood borers.

Trees that received visual tree assessment scores of zero for two subsequent days were considered dead. In this event, the above ground biomass was visually inspected thoroughly, and any symptoms were recorded. The tree was then destructively sampled using hand tools by cutting section into 2-20cm pieces, stripping bark, and documenting any observations or collections. This process was also used on all trees at the end of the experiments irrespective of tree health status.

Identifying damaging agents

Woodborers were the target of the experiment and visual surveys. Symptoms attributed to other types of herbivory by a variety of insects were recorded, but they are not presented here.

Collected specimens were stored in 95% ethanol and identified by comparison to morphological features in literature (Yin et al. 1984, Wood 1986, Cherepanov 1988, Hovore 1992, Lingafelter and Hoebeke 2002, Liu et al. 2006, Johnson et al. 2020). The species of *Cryphalus* were identified by Dr. Andrew J. Johnson, and *Hyledius kesiyae* was identified by Dr. Roger A. Beaver.

Site restoration and phytosanitary considerations

At all sites, the above and below ground biomass of each tree was removed from site and burned. Fungicide and insecticides were then applied to the soil surrounding each experiment tree. At each site, local land managers approved and supervised site restoration activities.

Results

Woodborers observed in pest-host interactions

In total, twelve wood boring species were observed interacting with trap trees (Table 2). Two Cerambycidae species displayed oviposition and feeding. Nine Scolytinae species were recovered from wood after successfully initiating galleries. Two Bostrichidae species were recovered from an initiated gallery. No overlap of species was observed between sites and experiments.

Table 2
Summary of woodborers that colonized trap trees during the experiment. The first column indicates how many hosts were attacked; in parentheses is the number of individual adults recovered. Legend – A – adult recovered (foundress), O – oviposition observed, L – larvae recovered, F – feeding observed.

Host Occurrences	Woodborer		Tree Species				Experiment
	Species	Family	Oak	Pine	Citrus	Avocado	
1 (1)	*Chlorophorous signaticollis*	Cerambycidae			A/O		Fuan 2018
1 (2)	*Sueus niisimai*	Scolytinae	A				Fuan 2018
2 (7)	*Xylopsocus bicuspis*	Bostrychidae	A				Fuan 2018
1 (4)	*Hypothenemus* sp.1	Scolytinae	A				Fuan 2018
1 (1)	*Hypothenemus* sp.2	Scolytinae	A				Fuan 2019
1 (1)	*Anoplophora chinensis*	Cerambycidae			F		Kunming 2019
2 (29)	*Cryphalus lipingensis*	Scolytinae		A/L			Kunming 2019
1 (1)	*Dinoderus ocellaris*	Bostrichidae		A			Kunming 2019
3 (25)	*Hyledius kesiyae*	Scolytinae		A/L			Kunming 2019
8 (136)	*Cryphalus piceus*	Scolytinae		A/L			Dasi 2019
1 (1)	*Cyrtogenius luteus*	Scolytinae		A			Dasi 2019
1 (1)	*Hypothenemus* sp.3	Scolytinae		A			Dasi 2019

Tree condition during colonization

For each insect specimen recovered, tree vitality was recorded (Figure 5). Five wood borer species were intercepted attacking trees scored as healthy, with Tree Vitality (VTA) score over 5. The two Cerambycidae *Anoplophora chinensis* and *Chlorophorus signaticollis* laid eggs and the larvae were successfully developing when the trees were dissected. Three bark beetle species colonized trees with VTA>5 repeatedly: *Cryphalus lipingensis*, *C. piceus*, and *Hyledius kesiyae*. The evidence for repeated colonization by the five species suggests that these may be considered as species of concern, and subjected to further risk assessment. An additional borer - *Dinoderus ocellaris* - attacked a healthy tree, but only as a singleton, and egg laying could not be determined, which renders this record as preliminary.

Cryphalus piceus

136 adult specimens of *Cryphalus piceus* (Eggers) were recovered from a formerly living slash pine *P. elliottii* at the Dasi Village field site (Fig. 6). Most beetles were recovered as mated pairs in individual galleries. Two thirds (8/12) of the pine trees yielded adults, eggs, and larvae in established galleries of *C. piceae*. All incidences of colonization occurred on trees with a VTA score between 3 and 6. Of these, 70% (96/136) of colonizing adults attacked trees at VTA score 4 indicating a pattern of attacking weakened, but still living healthy trees.

Cryphalus lipingensis

29 adult specimens of *Cryphalus lipingensis* (Tsai & Li) were recovered from slash pine at the Southwest Forestry University (Kunming, Yunnan Province) field site (Figure 7). Two out of twelve trees were colonized, both trees had partially-girdled treatments. No galleries were directly associated with the girdles themselves. The colonized trees had VTA scores of 5 and 6 when initially attacked.

Very little biology is known about *C. lipingensis.* The beetle was also recovered from branches of living *Pinus yunnanensis* in the immediate vicinity of both colonized experimental trees.

Frass indicated the presence of newly established galleries that were often hidden in the furrows between bark plates. The same pattern of gallery establishment was observed on the native pine. Once the bark was peeled, typical areas of discoloration of vascular tissue were observed. Many galleries intersected with *Hyledius kesiyae* (detailed below) suggesting similar host preference and behavior in both beetle species.

Hyledius kesiyae

Twenty five adults of *H. kesiyae* were recovered from slash pine at the Kunming field site (Figure 8). Three of the 12 experimental trees were colonized. Two of these trees were also girdled and colonized by *C. lipignensis* as described earlier. The third tree was a flood treatment specimen and had a high VTA score (8) when first colonized. Dieback of the tree was noticeable by day 6 of the experiment, but it was not observed whether *Hyledius* colonization preceded or followed the onset of dieback. The established gallery contained multiple larvae. The extent of the larval gallery followed the border of living tree tissue.

Chlorophorus signaticollis

One adult female *Chlorophorous signaticollis* (Castelnau & Gory) was observed during oviposition on a citrus plant during the 2018 field trial in Shuyang Village (Figure 9). The adult remained on the plant for six minutes, slowly traversing and inspecting the main stem. The beetle was recorded ovipositing in between healthy tissue of the main stem and a small remnant of a dead branch, which is a typical oviposition target for *Chlorophorus* (Seunghyun Lee, pers. comm.). Other than the small residue of a dead branch, the tree was in a healthy condition (VTA score 8).

Anoplophora chinensis

Citrus longhorn beetle, *Anoplophora chinensis* (Forster) (Cerambycidae: Lamiinae) is a well-documented wood borer with a wide host range, capable of developing in living trees. We observed multiple beetles feeding on the bark of our citrus trees. Beetles were often disturbed, falling to the ground when the tree was touched, before flying off. The association of *A. chinensis* with citrus is not surprising, but the successful colonization of a living American oak suggests that the species warrants further risk assessment.

Observed species of minor concern

Several species were observed colonizing trees that are either already well studied, or present in trees that are already in terminal decline. Often, the behavior demonstrated in these examples conforms to our current understanding of these species. Despite not being able to highlight new species of concern, these observations can continue to expand our knowledge of pest behavior.

Sueus niisimai

Two adult *Sueus niisimai* (Scolytinae: Hyorrhynchini) were recovered from a single gallery of a dead experimental oak tree (flood treatment) during the test trial at Shuyang Village in 2018. *S. niisimai* mostly colonizes dead twigs (Kabe 1960); it is considered to have high invasion potential, but low damage potential (Li et al. 2020).

Cyrtogenius luteus

A single adult *Cyrtogenius luteus* was recovered from a partially girdled slash pine at the Dasi Village Site in 2019. The tree VTA was 4 when the gallery was first identified on the final day of the experiment. The date of colonization is not known, though interruptions from the cyclone, and the small newly established gallery suggest the colonization had been recent. *C. luteus* has been recorded as a secondary pest of non-native pine in South America and Europe (Faccoli M. 2012, Gómez et al. 2012, Rainho et al. 2019). Our records also corroborate weakened pine trees as potential hosts for this species.

Xylopsocus bicuspis

Eight adults of *Xylopsocus bicuspis* (Bostrichidae: Xyloperthini) were recovered from 5 galleries on two experimental oak trees from Shuyang Village field trial in 2018. One of the flooded trees was already dead when the insects were recovered. The second, partially girdled tree, had a VTA score of 3 when it was harvested and the insects extracted. Galleries were in the topmost apical branches which experienced early dieback. This species appears to be a non-damaging colonizer of dying trees (Liu et al. 2008).

Hypothenemus **spp.**

Six individual species of *Hypothenemus* were recovered: *Hypothenemus brunneus*, *Hypothenemus birmanus*, and *Hypothenmus* sp. similar to *H. birmanus*. Each of them was recovered from dead or dying trees with VTA score between 0 and 3. *Hypothenemus* are minute boring beetles that are common in many forested environments, known for attacking only dead or dying twigs. Our observations conform to this and suggest low potential for damage to living trees.

Dinoderus ocellaris

A single adult of *Dinoderus ocellaris* (Bostrichidae: Dinoderinae) (Stephens, 1830) was recovered from slash pine at the Southwest Forestry University (Kunming, Yunnan Province) field site. The visual tree vitality score of 6 indicate a healthy, though stressed tree. From this single observation we are unable to determine in the species can complete its lifecycle on this host or presents a threat as a potential pest. Along with other Dinoderinae species, this species is predominantly found on bamboo and is commonly intercepted at ports of entry in bamboo products (Walker 2007).

Avocado

In our study, no observations or collections were made from avocado. Due to supply limitations, we were only able to secure smaller plants to use in the experiments. This factor is a likely cause of the lack of infestation by wood-boring beetles, which typically require a greater diameter before initiating a gallery (Ranger et al. 2013, Raffa et al. 2015). Previous studies suggest that avocado is a viable and attractive host to many wood boring beetles. Kendra et al (2011) successfully attracted 17 species of wood boring beetles during field trials using avocado lures in Northern Florida where avocado is not native. Future research efforts should ensure that larger diameter trees are used.

Discussion

Our research supplies a list of wood-boring species that have demonstrated the ability to colonize American commodity tree species in mainland China. Evidence from direct observations of potential pests within their native environments can reduce the need for theoretical assumptions when predicting consequences of invasion (Groom et al. 2017, Roy et al. 2018). Direct observation allows us to identify those potentially invasive wood boring beetles that attack living trees and are more likely to cause damage or increase mortality, and separate them from wood borers limited to dead or dying hosts whose impact is often negligible.

Species that have been previously studied (e.g., Bostrichidae and *Anoplophora*) performed as expected from prior research and observation. In the case of *Cryphalus* spp. and *Hyledius*, however, we report previously unknown interactions with living trees. Two species of *Cryphalus* had the greatest impact of all the recorded borers. *Cryphalus* is a speciose genus of minute bark beetles that is particularly diverse and abundant in Asia. Most species colonize dead or dying hosts, but several are known to attack living, stressed parts of the tree and cause significant tree mortality in conjunction with environmental stress and/or associated fungal pathogens (Faccoli M. 2012, Johnson et al. 2017).

The genus *Chlorophorus* (Chevrolat) has 280 species, several of which are known invasive pests (Zong et al. 2012, Suma and Bella 2018), and some have wide host ranges (Ernstsons et al. 2021). The only recorded hosts of. *C. signaticollis* are *Punica granatum*, *Pyrus malus*, and various Salicaceae (Hua 2002);

our records from citrus appears to be new. The short experiment time meant that full development of larvae could not be observed. However, *C. signaticollis* may represent a threat to citrus production and warrants further study.

A. chinensis is a common tree pest in China, and is already established in Italy (Van der Gaag et al. 2010). Even more invasive is a closely related congener, *A. glabripennis* (colloquially termed the "Asian long horned beetle" in western literature), which has established in several non-native regions in Europe and North America and triggered large and expensive eradication responses (Lingafelter and Hoebeke 2002, Hérard 2009, Haack et al. 2010, Faccoli and Gatto 2015).

Similar studies using trap trees and sentinel gardens identified novel Chinese pests and pathogens for many planted European host species (Roques et al. 2015), but most were sap sucking or leaf chewing herbivores. Most sentinel gardens are started from small tree individuals, not ready to serve as a reporting tool for wood borers. Small trees can attract high-impact wood borers if physiological stress is imposed on them (Ranger et al. 2013, Ranger et al. 2015). Our methods integrate aspects from each of these methods to identify invasive traits.

Strengths of the study

Physical specimens recovered from hosts trees indicate both the capability and the desire to colonize a host, and also expand the known host range for the insect (Branco et al. 2015). Observations allow damage to be characterized. Similarly, extraction of wood boring beetles from their hosts determines initiation of galleries and evidence of successful development. All these biological trains can aid in the determination of the species' likely impact (Whitney and Gabler 2008).

Identification of pests should not be viewed as a barrier to trade, rather, as an opportunity to increase international partnerships. The identification of injurious forest pests can benefit the international community, including the region of study. Non-native trees are globally established as commodity crops. Damages to tree crops, whether by native beetles to planted exotic trees, or by non-native insects to native trees, impact communities at all economic levels. Decision-support tools support forestation by using potential pest information (Varma et al. 2000, Garrett K.A. 2013, Segura et al. 2014, Li et al. 2021).

Limitations of the study

Our approach is limited to observations made in a manipulated research environment. The plant species used into this environment are native to the Southeast USA; therefore, factors not accounted for in this study may be important in their performance, including weather, tree phenology, humidity, soil type or soil organisms.

In all the 2019 experiments we witnessed extreme weather events. Fujian Province, Shuyang Village site, experienced flooding and extended rainfall. In contrast, the Kunming site in Yunnan Province experienced a drought that extended into the traditional rain season affecting the first 8 days of the experiment. Shandong Province, Dasi Village, was hit by Typhoon Lekima on the 12th day of the experiment. In each incidence, abundance of species, emergence and flight behavior, and ease of observations are likely to have been affected(Hansen and Bentz 2003, Bancroft and Smith 2005, Ainslie and Jackson 2011).

Further notable limitations concern the short duration of the experiments. Experiments were designed for 14 days though start dates were delayed due to practical limitations with delivery. Initial tree health was noticeably reduced in the third experiment at Dasi Village compared to the two earlier experiments. Short durations also reduce the time that colonizing insects can establish. As such, we are only able to determine if a species attempts to colonize a host and then begins its lifecycle.

Fourteen days is not enough to categorize the threat posed by all members of the local wood borer community, due to their diversity and niche partitioning (Gilbert M. 2005). Half of the observed species occurred only once. While these observations are valid, additional observations are needed to strengthen the inference of impact potential.

The longer-term health impacts on individual trees can only be alluded to. The study does not measure survivorship of trees that have been colonized. Measuring tree vitality has no defined and accepted methodology (Franklin et al. 1987, Johnstone et al. 2013, Sepúlveda and M Johnstone 2019) and impacts of colonization are often obscured by other stressors that may have predisposed the host to attack by a woodborer (Shigo 1985, Dunn et al. 1986, Holopainen 2011).

The final caveat is that this method provides limited ability to scale up our observations to the dimension of forest stands in new regions. Colonization of an exotic tree in a sentinel garden demonstrates attraction to the host, and initial stages of reproduction in it, but it does not measure the ultimate success in reproduction, semiochemical ecology, novel natural enemies, etc. The potential for a species to have irruptive growth is not determined using this method.

This initial set of gardens has successfully identified several species with potential tree-killing traits, but it is still only a pilot study. Gardens in additional regions, or in the same regions during different seasons, and gardens with larger trees, may attract new species. Longer-term plantings should therefore be established in these regions, to fulfill the potential of the sentinel garden model.

Conclusion

Our novel trap-tree system allowed us to identify twelve new host interactions of wood boring insect species. By demonstrating invasive traits such as the ability to colonize American hosts, as well as attack weakened but still living trees, we can highlight species that pose a risk of increasing mortality should they invade new regions. Species whose biology is already understood performed as expected and highlight the relevance of this new method. We conclude that this stressed-tree sentinel garden model can be used to inform the risk assessment of potentially destructive invasive forestry species.

Declarations

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Figures

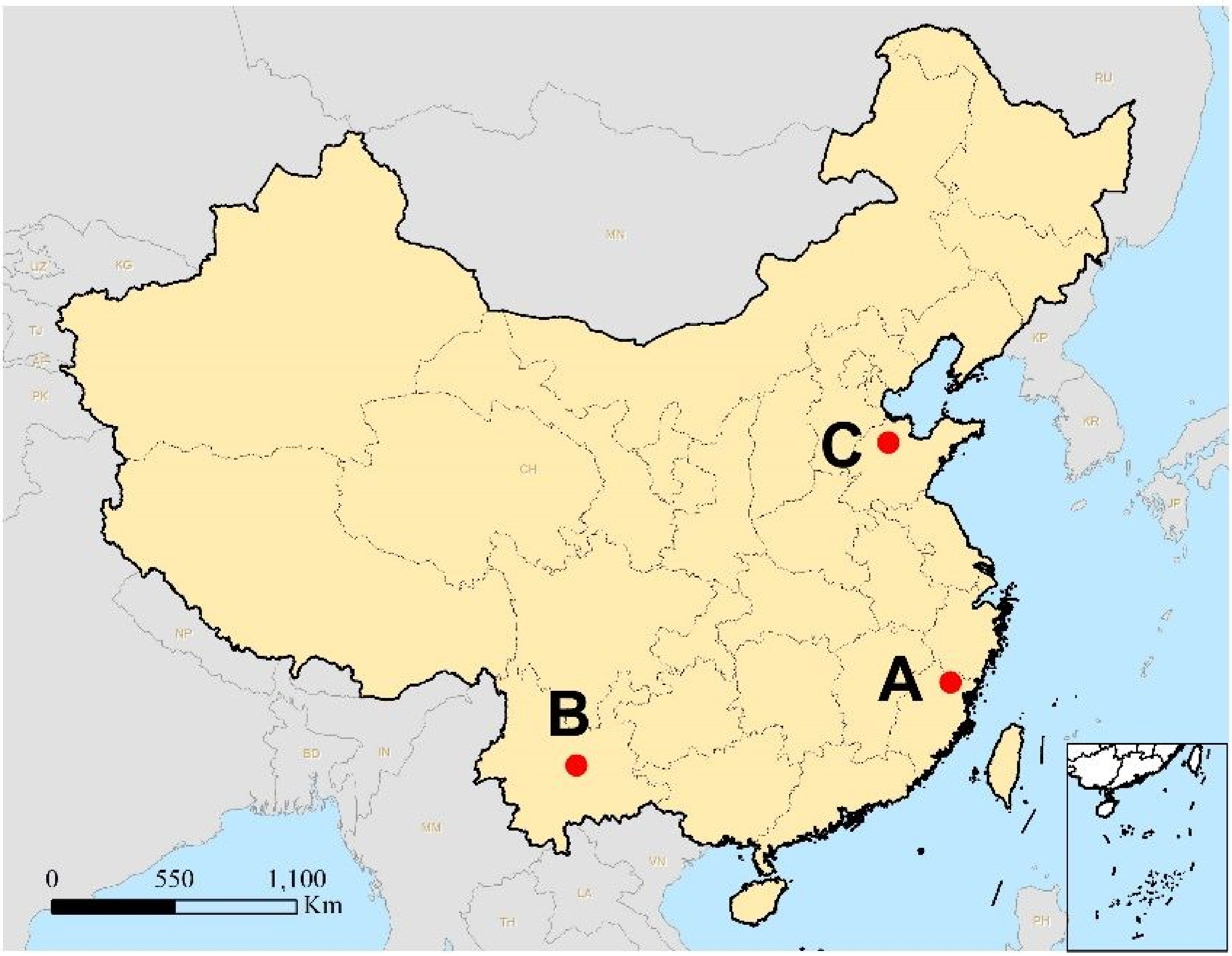

Figure 1

Map of China highlighting the three experiment locations. A - Shuyang Village, Fujian Province. B - Kunming City, Yunnan Province. C – Dasi Village, Shandong Province. Map image: Creative Commons.

Figure 2

A -Partially girdled oak, B - Example of pot-in-pot flooding system on citrus

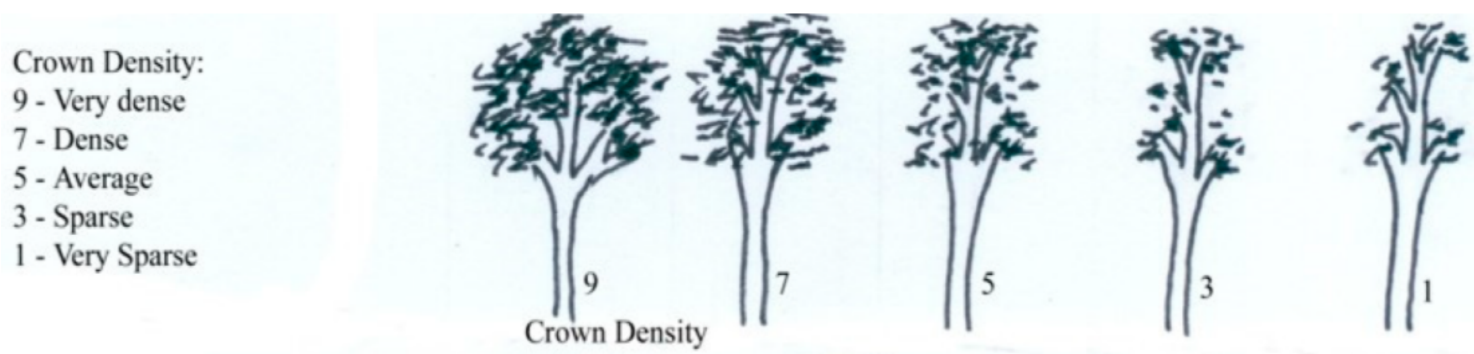

Figure 3

Crown Density visual scale amended from Callow et al. (2018, Figure 1). Reprinted under Creative Commons CC BY license.

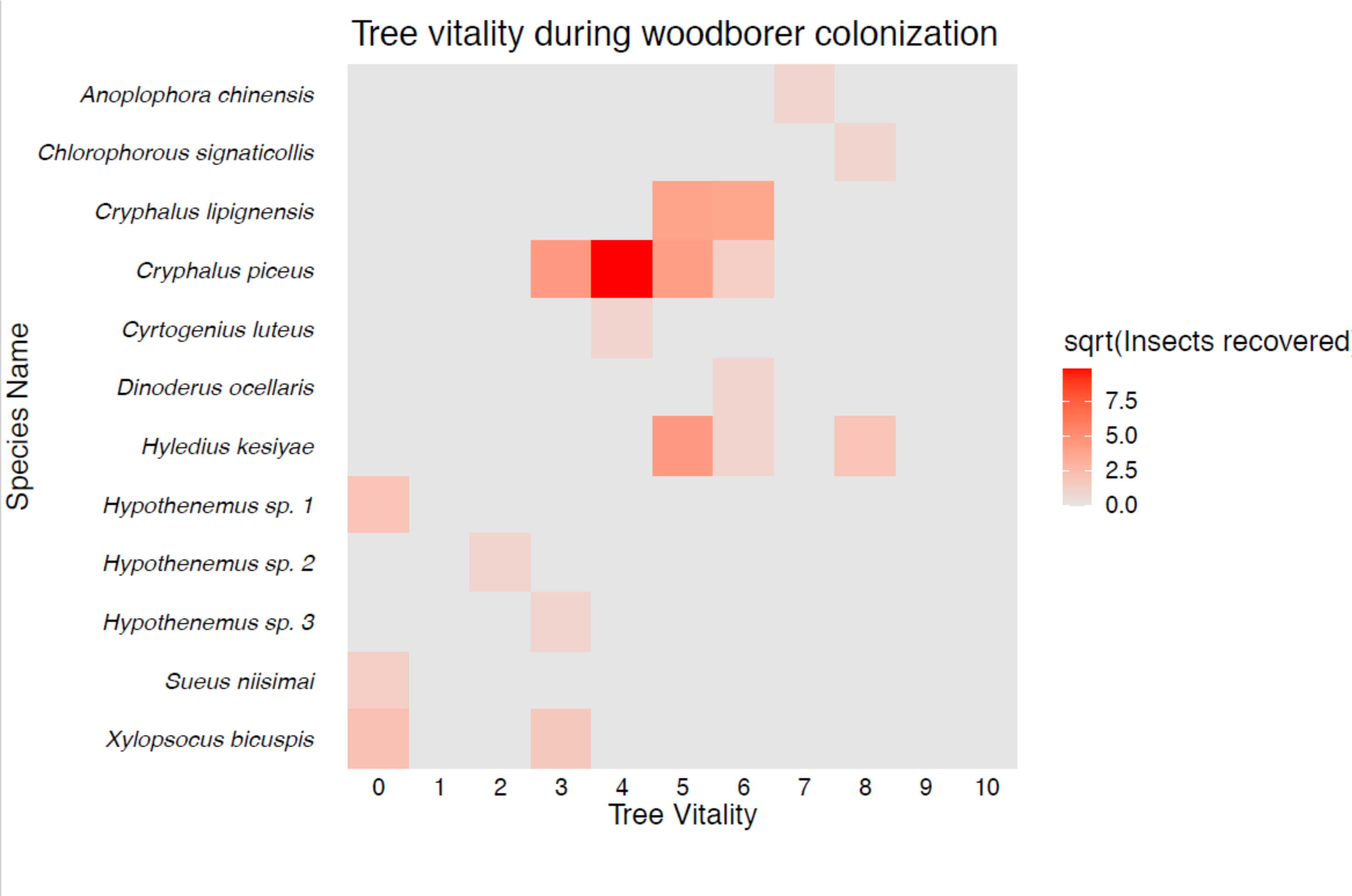

Figure 4

Tree condition during insect-host interaction. Depth of color represents abundance of colonizing insects (square root 0 – 10).

Figure 5

A - Evidence of gallery established by C. piceus, B - Extraction of C. piceus from Slash Pine, staining of vascular tissue occurs around established galleries , C - Field experiment tree colonized by C. piceus on experiment day 7, VTA score 5

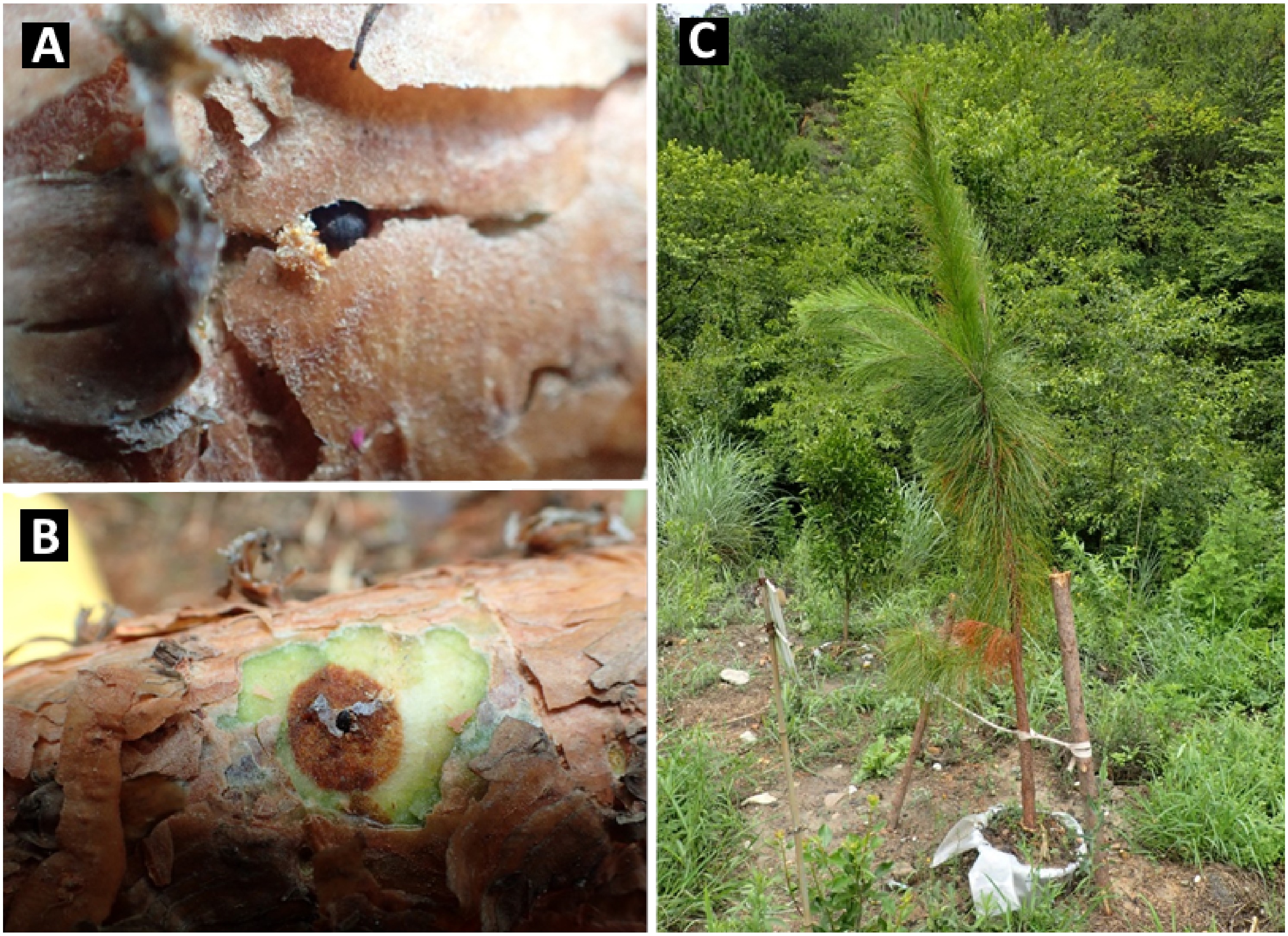

Figure 6

A - C. lipingensis establishing a gallery in a slash pine, B - As "A" but with surrounding bark removed, C – Condition of slash pine when attacked by C. lipingensis, VTA score 6

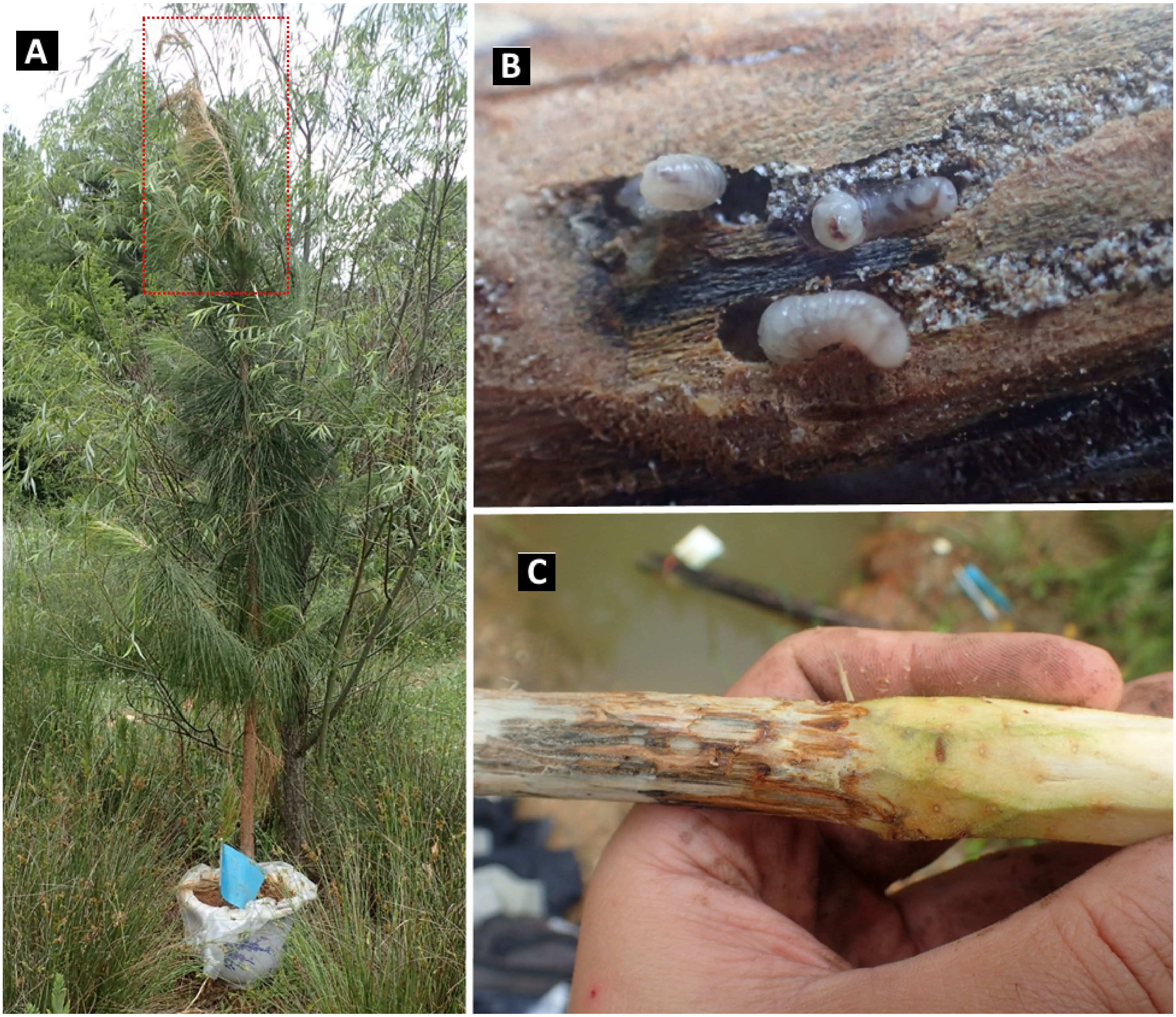

Figure 7

A – Apical tip dieback (highlighted) on slash pine colonized by H. kesiyae, B - Larvae descending the main stem, C - border of live and dead wood on the main stem showing the larval galleries at the base of dieback