Diagnosis and Management of the Invasive Shot Hole Borers *Euwallacea fornicatus*, *E. kuroshio*, and *E. perbrevis* (Coleoptera: Curculionidae: Scolytinae)[1]

Demian F. Gomez, Jiri Hulcr, and Daniel Carrillo[2]

Introduction

Invasive species, those that are nonnative and cause economic damage, are one of the main threats to ecosystems around the world. Ambrosia beetles in the tribe Xyleborini (Coleoptera: Curculionidae: Scolytinae) are some of the most common invasive insects, representing half of the nonnative scolytines (bark and ambrosia beetles) established in United States (Gomez et al. 2018a). Several biological features, such as mating with siblings (inbreeding) increase significantly the chances of successful establishment in new regions (Brockerhoff and Liebhold 2017).

Some of the most common invaders within the Xyleborini are the species previously known as the *Euwallacea fornicatus* species complex. The Tea shot hole borer *Euwallacea perbrevis* (Schedl 1951) (TSHB) is considered an important pest of tea in India and Sri Lanka (Danthanarayana 1968). This species was introduced into Florida (United States) in 2002, and, since 2010, increasing damage has been recorded in avocado plantations and even more so on native species (Carrillo et al. 2012, Carrillo et al. 2016, Owens et al. 2018). In 2012, beetles matching the same morphology were introduced in Israel but were later found to be genetically different and named the Polyphagous shot hole borer *Euwallacea fornicatus* (Eichhoff 1868) (PSHB) (Mendel et al. 2012, Stouthamer et al. 2017). The PSHB was recorded in 2003 in California (United States) reporting severe damage in 2012 to the avocado industry (Eskalen et al. 2012). In 2018, it was also reported in South Africa, causing significant damage to trees in urban and natural areas, including avocado orchards (Paap et al. 2018). Another species, commonly known as the Kuroshio shot hole borer *Euwallacea kuroshio* Gomez and Hulcr 2018 (KSHB), was also introduced in California and Mexico, causing severe damage to various native willows in riparian habitats (Boland 2016, García-Avila et al. 2016).

The taxonomic status of the *E. fornicatus* species complex was under discussion until recently. Based on morphological and molecular data, the existence of four species was revealed: *E. fornicatior* (Eggers 1923) (part of the TSHB, not found outside its native range), *E. fornicatus* (PSHB), *E. perbrevis* (part of the TSHB and the species in Florida), and *E. kuroshio* (KSHB) (Stouthamer et al. 2017, Gomez et al. 2018b, Smith et al. 2019). Currently, severe economic impacts have been increasingly reported for all the invasive shot hole borers in South Africa, California, Israel, and throughout Asia. The name *E. whitfordiodendrus* was recently applied to PSHB but was shown to be a synonym (Smith et al. 2019).

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2. Demian F. Gomez, School of Forest Resources and Conservation; Jiri Hulcr, School of Forest Resources and Conservation and the Entomology and Nematology Department; and Daniel Carrillo, UF/IFAS Tropical Research and Education Center; UF/IFAS Extension, Gainesville, FL 32611.

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Distribution

All four species are native to southeastern Asia (Smith et al. 2019). In the United States, *Euwallacea perbrevis* has been introduced into Florida and Hawaii, whereas *E. fornicatus* and *E. kuroshio* have been introduced into California.

Euwallacea fornicatus is considered to be native in China, Japan, Malaysia, Samoa, Sri Lanka, Taiwan, Thailand, and Vietnam, and introduced into Israel, South Africa, and the United States (California). *Euwallacea kuroshio* is native in Indonesia, Japan, and Taiwan, and introduced into Mexico and the United States (California). *Euwallacea perbrevis* is considered to be native in American Samoa, Australia, China, Fiji, India, Indonesia, Japan, Malaysia, Palau, Papua New Guinea, Philippines, Réunion, Singapore, Sri Lanka, Taiwan, Thailand, Timor Leste, and Vietnam, and introduced into the United States (Florida and Hawaii).

Description and Diagnosis

The genus *Euwallacea* can be usually distinguished from other Xyleborini by the dark color, the small and robust body, and the posterior edge of the elytra, which is more flattened, extended sideways, and has a sharp ridge (Gomez et al. 2018a).

The three species have small differences in a few morphological characters, mostly different body proportions and size (Figure 1). A recent exhaustive morphological analysis revealed differences in elytra length, pronotum length, and number of protibial socketed denticles, but with a considerable degree of overlap between the species (Gomez et al. 2018b). Therefore, DNA sequencing is required for definitive identification.

Figure 1. Dorsal view of *Euwallacea perbrevis*, *E. fornicatus*, and *E. kuroshio*. Bar corresponds to 1.0 mm.
Credits: Demian F. Gomez, UF/IFAS

The species complex includes the four species discussed herein. Morphological characters for species diagnosis are from Gomez et al. (2018a) and Smith et al. (2019). Many features are shared by *E. fornicatus*, *E. kuroshio*, and *E. perbrevis.* The eggs are white, partly translucent, and 0.3 mm long, hatching in four to six days. The larvae are white with a reddish head, have a C-shaped body and are legless. The larval stage is complete in 16–18 days. Pupae are white and have the same size as adult beetles, with beetles emerging after seven to nine days. Adults females have a body length of 2.3–2.8 mm, with a dark brown to almost black color. Segments 2 and 3 in the posterior face of the antennal are only partially visible. The pronotum is rounded anteriorly, with several serrations. The punctures in the elytral are in defined rows. The elytral declivity is convex and gradually sloped, with a sharp edge in the posterolateral margin. Males are smaller, around 1.5 mm, with fused elytra, minute eyes, and non-functional wings. To identify individual species, comparative measures for the three species introduced in the United States can be used as described in Table 1. Elytra length and pronotum length are measured from base to apex in lateral view. Pronotum and elytral width are both measured at the widest point. Identification of ambrosia beetles can be difficult for non-specialists, and, therefore, we recommend consulting with a trained entomologist, such as the UF|IFAS Forest Entomology lab or the UF|IFAS Tropical Research and Education Center (TREC).

Biology

Most ambrosia beetle species attack only freshly dead or dying plants. However, a few species, including *E. fornicatus*, *E. kuroshio*, and *E. perbrevis*, colonize healthy trees and cause damage through mass accumulation (Hulcr and Stelinski 2017), where tree pathogens causing localized necrosis become a significant agent of damage when inoculated by a large number of beetles (Smith and Hulcr 2015). Adult females will usually disperse during the day, attacking hosts in a range of tens of meters (hundreds of feet), but have the ability to travel 400m (a quarter of a mile) (Owens et al. 2019). Females tend to colonize at the base of secondary branches, resulting in localized branch dieback. In avocado, for example, the initial infestation is characterized by white dripping fluid from the beetle entrance holes (Owens et al. 2019a). Females will typically make a divided or simple gallery encircling the stem, with a few longitudinal tunnels in small branches (Browne 1961). Eggs are laid in small clusters once the entrance tunnel has been completed. Like the rest of the ambrosia beetles, diploid females produce haploid male offspring from unfertilized eggs. Because there is no need to find mates, brother-sister mating increases the chances of colonization of new regions (Brockerhoff and Liebhold 2017), and therefore, one or a few individuals are sufficient to establish a new population (Hughes et al. 2017, Storer et al. 2017). The larvae, with typically three instars, feed entirely on symbiotic ambrosia fungi cultivated in longitudinal galleries of twigs and transverse galleries of thicker branches (Gadd 1941). Pupation takes place inside the same communal gallery. Newly emerged females stay in the galleries for several days where they are fertilized by the few brothers present. Once mating occurs, females leave the gallery through the original entrance hole (Browne 1961). Adults develop in 22 days at 24°C (75°F), producing 57 and 68 female adults in 6 weeks, for *E. fornicatus* and *E. perbrevis* respectively, 7% of which are males (Cooperband et al. 2016).

All three species, like the rest of the ambrosia beetles, feed on a cultivated fungus within the xylem of woody hosts (Batra 1967). They have a specialized pocket-like structure called mycangia where they transport fungi to newly colonized trees (Batra 1963). The fungi are obligate symbionts of the beetles and serve as their source of nutrition, with severe pathogenic effects for some species. The symbiotic fungus invades the tree vascular tissue, causing cambial necrosis, sugar or gum exudates, branch dieback, and mortality of a broad range of tree hosts (Eskalen et al. 2013). The symbiotic fungus *Fusarium euwallaceae* is associated with *E. fornicatus*, causing branch dieback once introduced (Eskalen et al. 2012, Freeman et al. 2013). Carrillo et al. (2016) studied the fungal symbionts associated with *Euwallacea perbrevis* in Florida, where *Fusarium* sp. and *Graphium* sp. were the most common fungal partners recovered. More recently, Na et al. (2017) recovered two new pathogenic symbionts associated with *E. kuroshio*, *Fusarium kuroshium,* and *Graphium kuroshium*, highlighting the threat of *E. fornicatus* and *E. kuroshio* in California. The pathogenicity tests on healthy avocado plants showed that both fungus species are mildly pathogenic, with shorter lesion lengths for *F. kuroshium* when compared to those caused by *F. euwallaceae*. Despite being closely related species, *E. fornicatus* from Israel and California showed high rates of mortality when fed on the fungus carried by *E. perbrevis* (Freeman et al. 2012). Nevertheless, recent research suggests that fungal symbionts can be switched or shared in the genus *Euwallacea* (Kasson et al. 2013, O'Donnell et al. 2015, Dodge et al. 2017a).

Hosts

The three species treated herein pose a significant threat in their native and introduced ranges. Because the taxonomic identity of the species complex was recently reassessed, all host records, with a broad host range, should be understood as reported for the entire species complex. In the United States (California), the beetles were found on more than 200 hosts, though only 19 of these were shown as "reproductive" (supporting the reproduction of beetle colonies; Eskalen et al. 2013). *Euwallacea perbrevis* in Florida so far shows a narrower host range including avocado, mango, soursop, royal poinciana, swampbay, wild tamarind, and albizia (Carrillo et al. 2012; Owens et al. 2018). More recently, Gomez et al. (2019) updated the list to more than 400 species of plant hosts in 75 families, reporting 110 as breeding hosts. Interestingly, 95 of the plant hosts correspond to commercial timber species, and 43 are classified as relevant to the fruit industry.

Survey and Monitoring

Although ethanol is normally used as a lure in ambrosia beetle monitoring, it does not seem to attract any *Euwallacea*, as shown for *E. perbrevis* in Florida (Carrillo et al. 2015) and *E. fornicatus* and *E. kuroshio* in California (Dodge et al. 2017b). Two components, quercivorol (Carrillo et al. 2015, Dodge et al. 2017b) and *a*-copaene, are attractants, with synergistic effects increasing captures when combined (Kendra et al. 2017). The combination of the compounds provides the best known detection system; results from Florida for *E. perbrevis* suggest a recommended trap spacing of approximately 30 m (100 ft) for surveillance programs (Owens et al. 2019a). Field life of the combination lure is 12 weeks (Owens et al 2019b).

Cooperband et al. (2017) suggested the existence of two pheromones, 2-heneicosanone and 2-tricosanone, for the three species present in North America. Pheromones have rarely been reported for ambrosia beetles within the Scolytinae with just a few known examples (Borden et al. 1976, Borden and Slater 1969, Francke and Heemann 1974); these are probably involved in social behavior inside galleries and the ecological and applied implications are yet to be tested.

Prevention and Control

Preventative strategies for homeowners and forest and agricultural managers include:

- Keep trees as healthy as possible though proper silviculture/horticulture.

- Avocado growers should periodically survey for trees showing branch dieback and signs of beetle attack at junctions of small and mid-size shaded branches showing the presence of white "sugar volcanoes." Infested branches should be removed and destroyed (chipped, burned, or buried) to prevent further spread of these beetles (Figure 2). Chipping infested material should be to sizes smaller than 5 cm (Jones and Paine 2015).
- Sanitation. Heavily infested trees need to be taken out and destroyed (chipped, burned, or covered by a tarp under direct sun for "solarization"). Solarization works best using polyethylene sheeting during high temperature months, with ambient temperatures at least 35°C (95°F) (Jones & Paine 2015). Trees that are lightly infested often have a branch or two where most of the infestation is concentrated; those branches need to be pruned and destroyed, which has been an effective measure in avocado orchards in Israel and Florida.
- Pheromone deployment may also be a viable control strategy, with push-pull strategies suggested to be more effective compared to mass-trapping alone (Byers et al. 2018). Quercivorol and *a*-copaene can be used as attractants in white sticky traps separated by 30 m (100 ft), whereas verbenone can be used as a deterrent (Kendra et al. 2017, Owens et al. 2019). Contacting an Extension agent or a specialist for advice on installing traps is recommended.
- Homeowners with high-value ornamental trees may consider protecting them *before* any infestation by injecting systemic insecticides. Preventative treatments with emamectin benzoate alone (systemic insecticide) or combined with propiconazole (systemic fungicide), reduced significantly the attack and colonization of *E. fornicatus* in California (Grosman et al. 2019). Injection into trees that are already infested typically does not work. Injection of systemic insecticides is currently not approved for avocado in the United States. Preventative insecticide sprays on bark surface work as well, but need to be re-applied frequently because the effect only lasts between 4 and 8 weeks. Bark penetrant should be used to assure an extended effect.
- The "don't move firewood" awareness campaigns can be successful in slowing the spread of the pest. Ideally, it should be connected with an enforceable quarantine. In Florida, movement of unprocessed wood is regulated by Rule Chapter 5B-65, which restricts both the import of the wood from outside of the state, and the movement of wood more than 50 miles within the state.

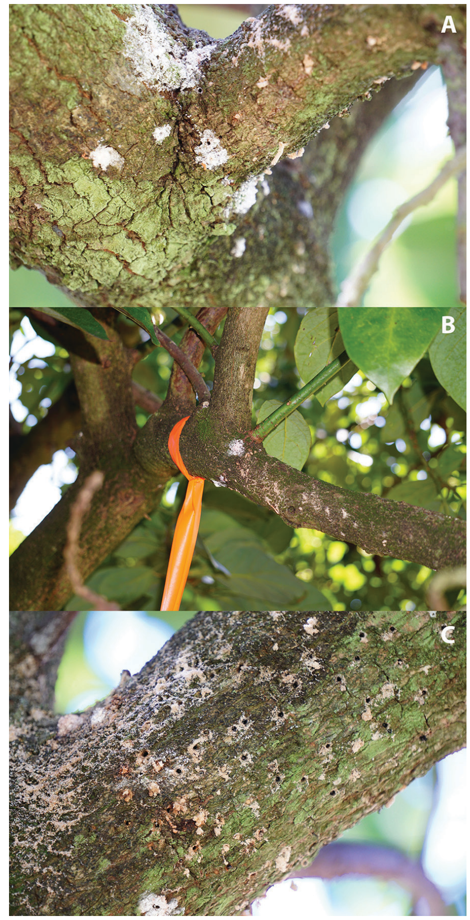

Figure 2. Signs of *Euwallacea perbrevis* infestations in avocado (south Florida, USA). A & B) Fresh "sugar volcanos" in junctions of small shaded branches. C) Entrance holes in a medium-size shaded branch.
Credits: Daniel Carrillo, University of Florida

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Table 1. Table of measurements (mm) for the shot hole borers introduced in the United States: *Euwallacea fornicatus*, *E. kuroshio*, and *E. perbrevis* (modified from Smith et al. 2019).

Species	Total Length	Elytral Length	Pronotal Length	Elytral Width	Pronotal Width	Protibial Denticles
fornicatus	2.60–2.70	1.44–1.72	1.02–1.16	0.48–0.62	1.00–1.14	8–9
kuroshio	2.40–2.80	1.50–1.82	1.08–1.16	0.52–0.56	1.06–1.16	8–11
perbrevis	2.30–2.50	1.42–1.68	1.04–1.16	0.48–0.56	1.02–1.14	7–10