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Differential flight responses of two ambrosia beetles to ethanol as indicators of invasion biology: the case with Kuroshio shot hole borer (*Euwallacea kuroshio*) and fruit-tree pinhole borer (*Xyleborinus saxesenii*)

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Abstract. 1. Host selection and utilization by ambrosia beetles ranges from healthy trees to weakened, dying, or dead trees. Compared to weakened trees, healthy trees emit little to no ethanol, which is stress-induced and a by-product of anaerobic respiration. The current study tested the attraction of two invasive ambrosia beetles, Kuroshio shot hole borer (KSHB), one member of the species complex, *Euwallacea* nr. *fornicatus*, and fruit-tree pinhole borer, *Xyleborinus saxesenii*, to four treatments with or without ethanol.

2. GC–MS analyses confirmed the release of ethanol from the ethanol lure and boxelder log filled with ethanol and not from the other two treatments. Kuroshio shot hole borer was not preferentially attracted to any ethanol treatment, whereas *X. saxesenii* responded positively to the ethanol treatments. The boxelder bolt infused with ethanol was the most attractive treatment to *X. saxesenii*.

3. We propose that the differential behavioural responses of these two ambrosia beetles to ethanol correspond to their differential aggressiveness for attacking healthy trees: KSHB attacks healthy trees, whereas *X. saxesenii* attacks weakened trees. The proposal is further substantiated by a literature survey on bark and ambrosia beetles from Scolytinae. The survey also indicates that the majority of bark and ambrosia beetles from Scolytinae respond positively to ethanol.

4. Although ethanol is widely used and is an effective lure for attraction of many bark and ambrosia beetles, findings from this current study and many others necessitate research and development of alternative lures for more aggressive species which are ecologically and economically more devastating.

Key words. Ambrosia beetle, Coleoptera, Curculionidae, *Euwallacea*, Scolytinae.

Introduction

Since its first detection around 2013 in San Diego County, California, U.S.A., the ambrosia beetle Kuroshio shot hole borer (KSHB), *Euwallacea kuroshio* (Coleoptera: Curculionidae: Scolytinae), has now spread to Los Angeles, Orange, and Santa Barbara Counties, California (https://ucanr.edu/sites/pshb/pest-overview/ishb-fd-distribution-in-california/; last accessed 22 September 2020). Kuroshio shot hole borer was first thought to be the invasive polyphagous shot hole borer (PSHB) which was first detected in Los Angeles Co., California, U.S.A., but genetic and morphological data indicate they are two of the three members of a species complex, *Euwallacea* nr. *fornicatus* (Eichhoff) (Coleoptera: Curculionidae: Scolytinae) (Gomez *et al.*, 2018; Smith *et al.*, 2019). The third member of the species

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complex is tea shot hole borer, which is morphologically and chemically different from PSHB (Chen *et al.*, 2017). Field observations indicate that KSHB and PSHB exhibit similar host ranges and selection behaviours, with both attacking apparently healthy trees (Coleman *et al.*, 2019) and a wide range of ornamental, and agriculturally important hardwood species hosts and reproducing successfully in approximately 40 native tree species (Eskalen *et al.*, 2013; O'Donnell *et al.*, 2015; Carrillo *et al.*, 2016; Chen *et al.*, 2020).

The fruit-tree pinhole borer (FTPB), *Xyleborinus saxesenii* (Ratzeburg) (Coleoptera: Curculionidae: Scolytinae), is another invasive ambrosia beetle that was detected in California in 1904 and is now widely distributed in the U.S.A. (Seybold *et al.*, 2016). It colonises a broad range of trees, but is of less economic importance and only attacks weakened and dying trees (Seybold *et al.*, 2016).

Ethanol is widely released in declining or decaying trees as a by-product of anaerobic respiration and microbial degradation (Kimmerer & Kozlowski, 1982; Kimmerer & MacDonald, 1987). A variety of abiotic and biotic stressors can induce the production of ethanol, thereby predisposing trees to attack by ambrosia beetles (Kelsey, 1994b; Ranger *et al.*, 2013). Many species of ambrosia beetles including FTPB use the emission of ethanol to locate and attack weakened or dying trees (Kelsey & Joseph, 2001, 2003; Miller & Rabaglia, 2009; Kelsey *et al.*, 2013; Ranger *et al.*, 2015).

In this study, our first objective was to test the attraction of KSHB and FTPB to ethanol under field conditions. We hypothesised that KSHB is not, whereas FTPB is, attracted to ethanol due to differences in their host selection and utilization. Our second objective was to conduct a meta-analysis of the Scolytinae literature to assess the relationship between beetle preference for healthy or weakened trees and responsiveness to ethanol. The majority of bark beetles in Scolytinae are known to colonise weakened trees, whereas comparatively few species attack and colonise healthy trees (Lindgren & Raffa, 2013), but no quantitative results are available regarding how widespread ambrosia beetles positively respond to ethanol and how the two different attacking behaviours correspond to their responses to ethanol.

Materials and Methods

Trapping experiments

The experiment was conducted at Sycuan Golf Resort (GPS: 32°47′ 41.183″N, 116°57′ 45.096″W) located east of El Cajon, San Diego Co., California (Fig. 1). The resort contains diverse tree species including California sycamore (*Platanus racemosa* Nutt.), castor bean (*Ricinus communis* L.), coast live oak (*Quercus agrifolia* Née), and *Eucalyptus* L'Hér. trees. Some large diameter (>25 cm) California sycamore trees have been killed by KSHB.

The experiment was a randomised complete block design with four blocks and four treatments per block. The four treatments (i.e. lures) were (i) unbaited control, (ii) ethanol (120 mg day^{-1} release rate/device, product # 200000082, 4 devices, Contech Enterprises Inc., Delta, British Columbia, Canada; now ScottsMiracle-Gro Corporate, Marysville, Ohio), (iii) boxelder bolt + water, and (iv) boxelder bolt + ethanol. The treatments were attached to 12-unit black Lindgren funnel traps (Contech Enterprises Inc.), which were installed on 167 cm tall metal poles (6 June 2014). We tested boxelder because it is one of the preferred host plants (e.g. high infestation rate and mortality in field surveys) of PSHB (Coleman *et al.*, 2013; Coleman *et al.*, 2019). To simulate fermentation, 28 cm-long hollowed bolts of boxelder were filled with deionised water and 90% ethanol in the hollow core (for boxelder + water and boxelder + ethanol treatments, respectively). Specifically, for the log treatments, boxelder bolts were hollowed along the central axis with an approx. 3 cm drill bit to a depth of approx. 23 cm and filled with approx. 150 ml deionised water or 90% ethanol in deionised water. The boxelder bolts were cut from small live trees growing in a riparian area outside of the areas infested with KSHB and PSHB [Sutter Co., California, approx. 10 km north of Robbins (GPS: 38°58′ 21.0216″N, 121°40′ 52.9422″W)]. The ends of the log sections were sealed with melted paraffin wax (Gulf brand, Product # 203-060-005, Roswell, Georgia). Two eyehole screws were attached to the top of each piece and the central hole in each log section was fitted with a No. 9 LabPure silicone stopper PX 15D-10PK (product number 097041P, D1069819, Batch number174731, Saint-Gobain Performance Plastics, www.LabPure.com, ordered through Fisher Scientific). The log sections were paired by approximate size (treatment vs. control) and stored in boxes in a cold room (4 °C) until they were used.

Fine mesh screen bags were fashioned for each log section [100 × 100 mesh, 0.0045″ T-316 stainless steel (P/N: 100X100-0-0045-T-316-PW), Screen Technology Group Inc., Washougal, Washington], and the openings and seams in the bags were sealed with a hot glue gun (Ace Hardware, Chicago, Illinois) for exclusion of KSHB and other bark beetles. The bolts were replaced on 30 July 2014 with a second batch of freshly prepared bolts, which remained in the study until the end of the experiment (22 October 2014). Bolts were checked for damage at the end of the experiment and no visible damage of KSHB and other bark beetles was observed.

A collection cup with about 100 ml of ethanol-free, propylene glycol-based antifreeze (Pure Oceans®, model # 499848, West Marine Products, Inc., Watsonville, California), was attached to each funnel trap. Traps were emptied and re-randomised once every 2 weeks. Each treatment was therefore replicated both in space (i.e. four blocks) and time (i.e. eight sampling periods).

Time-course emission of ethanol

Ethanol release rates were analysed from cut boxelder bolts filled with ethanol or water and also from ethanol lures. Five cored stem sections (i.e. bolts) of boxelder were filled with either 75 ml of ethanol or water on June 2 2015 and immediately capped with a Teflon stopper. Ethanol-infused and water-infused stems were placed in separate growth chambers to minimise the risk of volatile contaminations. To simulate temperature condition in the experiment site, the temperature between 07.00 and 18.00 hours was set at 22.9 °C and that between

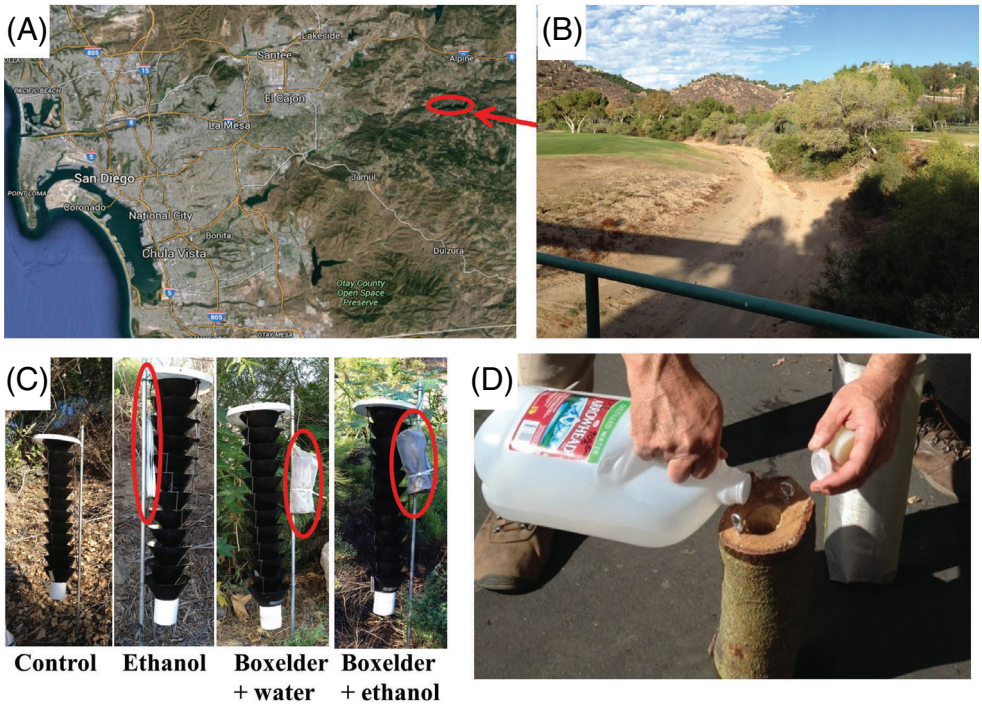

Fig. 1. Study site in San Diego Co., California, U.S.A., and treatments. (A) General location; (B) Riparian area at Sycuan Golf Couse, El Cajon; (C) Four treatments attached to 12-unit Lindgren funnel traps; and (D) Preparation of treatments (e.g. boxelder stem section + water and boxelder stem section + ethanol). [Colour figure can be viewed at wileyonlinelibrary.com].

19.00 and 06.00 hour was set at 18 °C (temperature data under field conditions between 6 June and 24 September 2014 were obtained from the Otay Lake weather station, San Diego, California ID # 147, ca. 40 km south of the study site; http://www.cimis.water.ca.gov/). The photoperiod within the growth chambers was set at 16 : 8 L : D. Each stem was kept vertical with the stoppered-ends upright throughout the entire experiment using metal ring stands.

Volatile emissions were sampled from the bolts by solid phase microextraction (SPME) at 1, 7, 15, 27, 42, and 71 days after infusing with ethanol or water. For sampling purposes, individual bolts were placed vertically inside poly(tetrafluoroethylene-co-perfluoro-(propyl vinyl ether)) (Teflon® PFA) bags with the stoppered-ends upright. A 25 mm band of Teflon tape was then wrapped five to six times around the circumference of the upper end of the bolt. A syringe containing a retracted SPME fibre was temporarily held in parallel with the stem while a cable tie was used to tighten the open end of the sampling bag around the upper circumference of the stem. The cable tie was positioned over the stem section containing the band of Teflon tape to aid with the compression seal among the stem, Teflon tape, and PFA bag created by cable tie. The SPME fibre was then immediately exposed within the sampling bag for 30 min. A SPME coating of carboxen-polydimethylsiloxane (CAR/PDMS; 75 µm coating; Sigma-Aldrich, St. Louis, Missouri) was used because it is ideal for gases and low-molecular-weight compounds (MW 30–225). Fibres were retracted after sampling, capped with Teflon tubing to prevent contamination, and then immediately transferred to −20 °C for temporary storage until analysis on the same day as sampling by gas chromatography–mass spectrometry. Sampling bags were cleaned using a kimwipe moistened with distilled water and then baked in an oven at 100 °C for 3 h before each use.

Volatiles were analysed on the same day as sampling using an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass spectrometer (Wilmington, Delware). Fibres were held in the injection port and thermally desorbed for 2 min at 250 °C. The injection port was operated in splitless mode from 0 to 2 min and then split at a ratio of 1 : 20 for the remainder of the run. A capillary nonpolar DB-5 column (0.25 µm × 30 m × 0.25 µm; cross-linked/surface bonded 5% phenyl, 95% methylpolysiloxane; Agilent J&W, Santa Clara, California) was used for analysis. The mass spectrometer was operated in electron impact mode with a scan range of 32–415 *m/z*. Fibres were conditioned before each analysis by exposure within an injection port for 20 min at 250 °C.

The external standard method was used for determining concentrations of ethanol within the headspace of the sampling bags. Serial dilutions of ethanol ranging from 0.0789 to 789 mg ml^{-1} were made in distilled and deionised water. A 500 µl aliquot containing a known amount of ethanol was then added to the empty PFA bags and fibres were immediately exposed for 30 min. A standard concentration curve was prepared using a logarithmic fit [y = 2102552.31ln(x) + 19164895.16] to determine concentrations of ethanol associated with the infused stems.

Literature survey

The objective of the literature survey was to summarise available knowledge on attraction of ambrosia and bark beetles in the two subfamilies Platypodinae and Scolytinae to ethanol and to examine if the response corresponds to the beetle's invasion biology (i.e. 'aggressiveness:' attacking healthy or weakened trees). The scientific articles were obtained by searching Google Scholar using keywords such as 'ethanol', 'bark beetle', 'ambrosia beetle', 'flight', and 'attraction' alone or in various combinations. The references cited in each article were further examined using the aforementioned criteria and a total 63 articles provided relevant information (see Table 1 for all the articles).

Statistical analyses

Kuroshio shot hole borer and FTPB flight response and ethanol release data were analysed in SAS v. 9.4 (SAS Institute Inc., Cary, North Carolina). A critical level of $\alpha = 0.05$ was used for all analyses. The Kolmogorov–Smirnov test and Levene's test were used to test for normality of the data and homogeneity of variances, respectively. Kuroshio shot hole borer and FTPB catches in the experiment were analysed by a generalised linear mixed model (Proc GLIMMIX in SAS). Treatment (i.e. unbaited, ethanol, boxelder + water, and boxelder + ethanol) and block were fixed factors and the sampling period was a random factor. The distributions of the KSHB and FTPB were modelled as Poisson. The autocorrelation among the sampling periods was addressed by trying various variance–covariance structures (e.g. unstructured, compound symmetry, and autoregressive) provided in the SAS and the structure with the lowest Akaike Information Criterion (AIC) (i.e. unstructured variance–covariance structure) was selected. The Pearson's χ^2 statistic and the ratio of the χ^2 statistic to the degree of freedom were used to determine any dispersion in the trap catches. A *P*-value less than 0.05 and a ratio close to one indicated no over- or under-dispersion of the data. The estimation method is maximum likelihood with adaptive quadrature (method = quad in SAS), and the method for computing the degrees of freedom of the denominator for the fixed effects was BETWITHIN (ddfm = bw in SAS). Multiple mean comparisons were conducted by using LSMEANS in SAS if overall null hypothesis of no significant differences among treatments was rejected.

Because no ethanol was detected from water-infused boxelder stems on each sampling date, ethanol emission from only ethanol lure and ethanol-infused boxelder stems was included in the statistical analysis. Data were analysed by a repeated measure ANOVA (Proc GLIMMIX in SAS). Treatment and sampling period were the two fixed factors. The covariance structure among repeated measurements of the same sample was the default simple diagonal (TYPE = VC in the RANDOM statement). Variance of ethanol emission from ethanol lure was different from that from ethanol-infused boxelder stem and this variance heterogeneity was addressed by the GROUP = treatment in the RANDOM statement. Because the interaction between treatment and sampling period on ethanol emission was statistically significant ($P < 0.001$), effect of treatment on ethanol emission was analysed separately for each sampling period and effect of sampling period was analysed separately for each treatment (by either an ANOVA or a Kruskal-Wallis test).

For data gathered from the literature, the numbers of insect species respond positively and neutral to ethanol in Table 2 were compared by the function prop.test (likelihood ratio X^2) in the package 'stats' in R (R Core Team, 2019), separately for tribe and the total of the two subfamilies. The association between attraction to ethanol and aggressiveness for tribes Hylesinini and total across the three tribes in Table 3 was tested by the function assocstats (likelihood ratio X^2) in the package 'vcd' in R. The association between attraction to ethanol and aggressiveness for the tribe Scolytini was tested by Fisher's exact test (the function fisher.test in the package stats) since some cells in the contingency tables were less than 5% of the total count. The numbers of matches and mismatches between response to ethanol and aggressiveness for tribe Xyleborini was tested by the function prop.test as described above. We assume that the neutral response to ethanol in the literature is not due to low beetle flight activity or low populations. In testing the alternative hypothesis that more beetles respond positively than neutrally to ethanol, an apparent neutral response to ethanol due to low beetle flight activity or low population will skew the analysis to be more conservative (i.e. less likely to detect a significant difference). The effects of a neutral response to ethanol due to potential low beetle flight activity or low population on comparing the numbers of matches and mismatches are difficult to assess because species responding neutrally to ethanol can be either aggressive or non-aggressive.

Results

Trapping experiments

Kuroshio shot hole borer catches were consistently low in the study with most samplings having fewer than two KSHB per trap in 2 weeks (Fig. 2a). The maximum catch (five KSHB per trap) was in unbaited traps during 16 July and 30 July 2014. Kuroshio shot hole borer catches were neither affected by block ($F_{3,121} = 2.45$, $P = 0.07$) nor by treatment ($F_{3,121} = 2.06$, $P = 0.11$; Fig. 2b).

Fruit-tree pinhole borer catches were consistently higher than those of KSHB, in particular, in traps with ethanol (Fig. 3a). Fruit-tree pinhole borer catches were affected by block ($F_{3,121} = 5.00$, $P = 0.003$) and treatment ($F_{3,121} = 68.46$, $P < 0.001$). Traps baited with boxelder + ethanol caught more FTPB than traps baited with ethanol alone, which further caught more beetles than the traps baited with boxelder + water (Fig. 3b). Unbaited traps caught the least beetles.

Time-course emission of ethanol

The interaction between treatment and sampling period significantly affected ethanol emission ($F_{5,48} = 5.40$, $P < 0.001$). Sampling period significantly affected ethanol emission from

Table 1. Bark and ambrosia beetles flight responses to ethanol in the field condition and their aggressiveness.[†]

Taxonomic placement			Preferred host condition for attack and/or breeding	Aggressiveness[‡]		Response to ethanol[§]	References[¶]
Tribe	Genus	Species		Category	References		
Subfamily: Platypodinae							
	Myoplatypus	*flavicornis*	Dead or weakened trees	Non-aggressive	Hulcr *et al.*, 2011	+	Miller & Rabaglia, 2009 (U.S. state: SC)
						0	Miller & Rabaglia, 2009 (U.S. states: AL, FL, GA, NC)
Subfamily: Scolytinae							
Hylesinini	*Dendroctonus*	*brevicomis*	Stressed to healthy trees	Intermediate to aggressive	Kelsey & Joseph, 2003; Hulcr *et al.*, 2011; Lindgren & Raffa, 2013	0	Kelsey & Joseph, 2003
		ponderosae	Stressed to healthy trees	Aggressive	Kelsey & Joseph, 2003; Lindgdren and Raffa, 2013	0	Kelsey & Joseph, 2003
		pseudotsugae	Stressed to healthy, recently dead, compromised (windthrown) trees	Intermediate	Lindgren & Raffa, 2013	+	Kelsey & Manter, 2004
		terebrans	Healthy trees or felled trees	Intermediate	Rane & Tattar, 1987; Flechtmann *et al.*, 1999; Hulcr *et al.*, 2011	+	Fatzinger, 1985
						0	Phillips *et al.*, 1988; Miller & Rabaglia, 2009 (U.S. states: FL site 1; FL site 2; FL site 3; GA, NC, SC)
		valens	Healthy or stressed trees	Intermediate	Kelsey & Joseph, 2003; Hulcr *et al.*, 2011	+	Joseph *et al.*, 2001; Kelsey & Joseph, 2003
	Hylastes	*ater*	Stumps, bolts and roots of harvested pine species	Non-aggressive	Reay & Walsh, 2001	0	Reay & Walsh, 2002
		brunneus	Dead or dying trees	Non-aggressive	Schroeder & Lindelöw, 1989	+	Schroeder & Lindelöw, 1989
		cunicularius	Dead or dying trees	Non-aggressive	Schroeder & Lindelöw, 1989	+	Schroeder & Lindelöw, 1989
		longicollis	Bark on the ground	Non-aggressive	Lindgren & Miller, 2002	+	Joseph *et al.*, 2001
		macer	Dying and dead trees	Non-aggressive	Furniss & Carolin, 1977; Wood, 1982	+	Joseph *et al.*, 2001
		nigrinus	Roots and barks of insect-killed trees	Non-aggressive	Rudinsky & Zethner-MØller, 1967	+	Joseph *et al.*, 2001
		opacus	Dead or dying trees	Non-aggressive	Schroeder & Lindelöw, 1989	+	Schroeder & Lindelöw, 1989

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Table 1. Continued

Taxonomic placement			Preferred host condition for attack and/or breeding	Aggressiveness‡		Response to ethanol§	References¶
Tribe	Genus	Species		Category	References		
		porculus	Healthy or weakened trees	Intermediate	Hulcr *et al.*, 2011	+	Miller & Rabaglia, 2009 (U.S. state (AL)
						0	Miller & Rabaglia, 2009 (U.S. states: GA, NC site 1; NC site 2; SC)
		salebrosus	Recently killed tree	Non-aggressive	Wood, 1982	+	Miller & Rabaglia, 2009 (U.S. state: SC)
						0	Miller & Rabaglia, 2009 (U.S. states: AL, FL, GA, NC site1, NC site 2)
		tenuis	Weakened to healthy hosts	Intermediate	Eckhardt *et al.*, 2007	+	Miller & Rabaglia, 2009 (U.S. state: SC)
						0	Miller & Rabaglia, 2009 (U.S. states: AL, FL, GA, NC site1, NC site 2)
	Hylurgops	*palliatus*	Dead or dying trees	Non-aggressive	Schroeder & Lindelöw, 1989; Byers, 1992	+	Moeck, 1970, 1971; Klimetzek *et al.*, 1986; Schroeder, 1988; Schroeder & Lindelöw, 1989; Byers, 1992
		porosus	Bark on the ground	Non-aggressive	Lindgren & Miller, 2002	+	Joseph *et al.*, 2001; Kelsey & Joseph, 2003
		reticulatus	Dying and dead trees	Non-aggressive	Wood, 1982	+	Joseph *et al.*, 2001
		subcostulatus	Dead trees	Non-aggressive	Hulcr *et al.*, 2011	+	Joseph *et al.*, 2001
	Hylurgus	*ligniperda*	Stumps, bolts and roots of harvested pine	Non-aggressive	Reay & Walsh, 2001	0	Reay & Walsh, 2002;
	Leperisinus	*varius*	Fallen, felled or heavily stressed ash trees	Non-aggressive	Mills, 1991	0	Klimetzek et a., 1986
	Tomicus	*destruens*	Weakened trees	Non-aggressive	Kelsey *et al.*, 2014	+	Kelsey *et al.*, 2014
		piniperda	Newly windbroken/windthrown trees, recently cut bolts	Non-aggressive	Schroeder & Lindelöw, 1989	+	Schroeder & Lindelöw, 1989; Byers, 1992
						0	Klimetzek *et al.*, 1986
Scolytini	*Anisandrus*	*apicalis*	Unknown	Non-aggressive	–	+	Sweeney *et al.*, 2016
		dispar	Dead or dying trees	Non-aggressive	Schroeder & Lindelöw, 1989; Saruhan & Akyol, 2012	+	Montgomery & Wargo, 1983; Klimetzek *et al.*, 1986; Schroeder & Lindelöw, 1989; Sweeney *et al.*, 2016

Table 1. Continued

Taxonomic placement			Preferred host condition for attack and/or breeding	Aggressiveness‡		Response to ethanol§	References¶
Tribe	Genus	Species		Category	References		
	(syn. *Xyleborus dispar*)						
		maiche	Weakened and unhealthy hosts	Non-aggressive	Rabaglia *et al.*, 2009	+	Sweeney *et al.*, 2016
		sayi	Physiologically stressed or dying trees	Non-aggressive	Kelsey & Joseph, 2001; Ranger *et al.*, 2011	+	Ranger *et al.*, 2010, 2011; Reding *et al.*, 2011; Ranger *et al.*, 2014
	Cryptocarenus	*heveae*	Small cut or stressed hosts	Non-aggressive	Wood, 1982	+	Miller & Rabaglia, 2009 (U.S. state: FL)
	Cyclorhipidion (syn. *Xylleborus pelliculosus*)	*pelliculosum*				+	Ranger *et al.*, 2014
	Euwallacea	*nr. fornicatus*	Healthy trees	Aggressive	Seybold *et al.*, 2016	0	Carrillo *et al.*, 2015
		validus	Injured, weakened, or dying hosts	Non-aggressive	Haack & Rabaglia, 2013	+	Ranger *et al.*, 2014
						0	Ranger *et al.*, 2010
	Gnathotrichus	*materiarus*	Dead trees	Non-aggressive	Hulcr *et al.*, 2011	+	Miller & Rabaglia, 2009 (U.S. state: NC)
						0	Miller & Rabaglia, 2009 (U.S. states: AL, FL, GA, SC)
		retusus	Stressed	Non-aggressive	Borden *et al.*, 1980	+	Borden *et al.*, 1980; Liu & McLean, 1989; Kelsey, 1994a,b; Joseph *et al.*, 2001
		sulcatus	Dead trees	Non-aggressive	Hulcr *et al.*, 2011	+	Liu & McLean, 1989
	Hypothenemus	*dissimilis*	Damaged or weakend hosts	Non-aggressive	Wood, 1982; Solomon, 1995	+	Ranger *et al.*, 2010; Reding *et al.*, 2011
		eruditus	Damaged or weakend hosts	Non-aggressive	Wood, 1982; Solomon, 1995	+	Reding *et al.*, 2011
	Ips	*grandicollis*	Stressed trees	Non-aggressive	Flechtmann *et al.*, 1999	0	Miller & Rabaglia, 2009 (U.S. states: AL, GA, FL site 1, FL site 2, NC, SC)
		latidens	Stressed trees	Non-aggressive	Miller *et al.*, 1986	0	Joseph *et al.*, 2001
		pini	Healthy, fallen, and weakened trees	Intermediate	Lieutier *et al.*, 2009	+	Joseph *et al.*, 2001
		typographus	Healthy trees	Aggressive	Lieutier *et al.*, 2009	0	Klimetzek *et al.*, 1986
	Monarthrum	*fasciatum*	Stressed, injured or dying trees	Non-aggressive	Solomon, 1995	+	Miller & Rabaglia, 2009 (U.S. states: FL)
						0	Miller & Rabaglia, 2009 (U.S. states: AL, GA, NC, SC)

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Table 1. Continued

Taxonomic placement			Preferred host condition for attack and/or breeding	Aggressiveness‡		Response to ethanol§	References¶
Tribe	Genus	Species		Category	References		
		mali	Stressed, injured or dying trees	Non-aggressive	Solomon, 1995	+	Montgomery & Wargo, 1983
						0	Ranger *et al.*, 2011
		scutellare	Weakend, dying, diseased trees	Non-aggressive	Bright, 1976	+	Noseworthy *et al.*, 2012
	Pityophthorus	*cariniceps*	Weakened or dying twigs	Non-aggressive	Bright, 1981	0	Miller & Rabaglia, 2009 (U.S. state: NC)
		juglandis	Weakened or dying twigs	Non-aggressive	Seybold *et al.*, 2016	+	Seybold *et al.*, 2015
	Pseudopityophthorus	*minutissimus*	Wilting or weakened hosts	Non-aggressive	Ambourn *et al.*, 2006	+	Montgomery & Wargo, 1983
	Scolytus	*unispinosus*	Weakened, dying, or recently dead hosts	Non-aggressive	Furniss & Carolin, 1977; Kelsey & Joseph, 2001	+	Kelsey & Joseph, 2001
	Trypodendron	*domesticum*	Weakened hosts	Non-aggressive	Byers, 1992	+	Byers, 1992; Sweeney *et al.*, 2016
		lineatum	Dead or dying trees	Non-aggressive	Schroeder & Lindelöw, 1989	+	Moeck, 1970; Klimetzek *et al.*, 1986; Schroeder, 1988; Schroeder & Lindelöw, 1989; Kelsey, 1994a; Shore & Lindgren, 1996; Sweeney *et al.*, 2016
	Xyleborinus	*attenuatus* (*syn. X. alni*)	Dying hosts	Non-aggressive	Borowski *et al.*, 2012	+	Ranger *et al.*, 2011; Sweeney *et al.*, 2016
		saxesenii	Physiologically stressed or dying trees	Non-aggressive	Furniss & Carolin, 1977; Solomon, 1995; Flechtmann *et al.*, 1999; Kelsey & Joseph, 2001; Ranger *et al.*, 2011	+	Montgomery & Wargo, 1983; Klimetzek *et al.*, 1986; Miller & Rabaglia, 2009 (U.S. states: AL, FL, GA, NC, SC); Ranger *et al.*, 2011; Reding *et al.*, 2011 (U.S. states: OH and VA); Ranger *et al.*, 2014; Sweeney *et al.*, 2016
						0	Ranger *et al.*, 2010
	Xyleborus	*affinis*	Decomposing xylem	Non-aggressive	Phillips *et al.*, 1988	+	Phillips *et al.*, 1988; Miller & Rabaglia, 2009 (U.S. state: FL)
						0	Miller & Rabaglia, 2009 (U.S. states: AL, GA, NC, SC)

Table 1. Continued

Taxonomic placement			Preferred host condition for attack and/or breeding	Aggressiveness‡		Response to ethanol§	References¶
Tribe	Genus	Species		Category	References		
		ferrugineus	Dead trees	Non-aggressive	Hulcr *et al.*, 2011	+	Miller & Rabaglia, 2009 (U.S. states: AL, FL, GA, NC)
						0	Miller & Rabaglia, 2009 (U.S. state: AL, SC)
		glabratus	Healthy trees	Aggressive	Hanula & Sullivan, 2008; Kendra *et al.*, 2014a	0	Kendra *et al.*, 2014b
		pubescens	Weakened trees	Non-aggressive	Dixon & Payne, 1979	+	Phillips *et al.*, 1988; Miller & Rabaglia, 2009 (U.S. states: FL, NC, SC)
						0	Miller & Rabaglia, 2009 (U.S. states: AL, GA)
	Xyloterinus	*politus*	Physiologically stressed or dying trees	Non-aggressive	Kelsey & Joseph, 2001; Ranger *et al.*, 2011	0	Ranger *et al.*, 2010, 2011, 2014
Scolytoplatypodini	*Scolytoplatypus*	*tycoon*	Second borer	Non-aggressive	Beaver & Gebhardt, 2006	+	Sweeney *et al.*, 2016
Xyleborini	*Dryoxylon*	*onoharaensum*	Little is known	Little is known	Bright & Rabaglia, 1999	+	Miller & Rabaglia, 2009 (U.S. states: FL, GA, NC, SC)
						0	Miller & Rabaglia, 2009 (U.S. state: AL)
	Xylosandrus	*compactus*	Healthy trees	Aggressive	Hara & Beardsley Jr, 1979; Beaver, 1988; Solomon, 1995; Oliveira *et al.*, 2008	+	Miller & Rabaglia, 2009 (U.S. state: FL); Burbano *et al.*, 2012
						0	Miller & Rabaglia, 2009 (U.S. states: AL, GA, NC, SC)
		crassiusculus	Healthy or weakened trees	Intermediate	Beaver, 1988; Atkinson *et al.*, 1988; Oliver & Mannion, 2001	+	Miller & Rabaglia, 2009 (U.S. states: AL, FL site 1, FL site2, GA, NC); Reding *et al.*, 2011; Burbano *et al.*, 2012
						0	Miller & Rabaglia, 2009 (U.S. state: SC); Ranger *et al.*, 2014

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Table 1. Continued

Taxonomic placement			Preferred host condition for attack and/or breeding	Aggressiveness‡		Response to ethanol§	References¶
Tribe	Genus	Species		Category	References		
		germanus	Healthy and stressed trees	Intermediate	Klimetzek *et al.*, 1986; Solomon, 1995; Ranger *et al.*, 2010, 2011	+	Klimetzek *et al.*, 1986; Miller & Rabaglia, 2009 (U.S. state: NC); Ranger *et al.*, 2010, 2011; Reding *et al.*, 2011 (U.S. states: OH and VA); Ranger *et al.*, 2014; Sweeney *et al.*, 2016
						0	Miller & Rabaglia, 2009 (U.S. states: AL, FL, GA, SC)

† Although it is not intended to be inclusive, all species (cases) known to the authors are included. Petrice *et al.* (2004) compared flight responses of *D. valens*, *H. opacus*, *H. ligniperda*, *I. calligraphus*, *I. grandicollis*, *I. pini*, *Orthotomicus caelatus*, *T. piniperda*, and *X. saxesenii* between α-pinene and α-pinene + ethanol. The results in this study are not included in the table because the statistical analyses seem inappropriate.

‡ Aggressive: insect pests that colonise/attack a healthy tree. Intermediate: insect species that are able to attack both healthy and weakened hosts; Non-aggressive: insect pests that only colonise/attack felled, physiologically weakened, dying, or dead trees; The classification is somewhat subjective. For instance, *X. germanus* is considered a secondary pest based on Klimetzek *et al.* (1986), whereas it is considered a primary pest based on Ranger *et al.* (2010, 2011) because it attacks healthy trees.

§ + and 0 denote positive and neutral responses, respectively, compared to unbaited control. Note: The neutral responses for some species might be due to low flight activity or low populations. This potential bias is not accounted in the current analysis.

¶ References followed by one or more U.S. states indicated that the trial was tested in one or more states. Trials from various states were counted as independent studies.

Table 2. Statistical tests on counts of positive to neutral responses to ethanol. See Table 1 for list of bark and ambrosia species surveyed.

Subfamily	Tribe	Response to ethanol (# positive response: # neutral responses; null hypothesis 1 : 1)
Platypodinae	–	1 : 4[†]
Scolytinae	Hylesinini	26 : 27 ($X_1^2 = 0.000$, $P = 1.000$)
	Scolytini	63 : 33 ($X_1^2 = 8.760$, $P = 0.003$)
	Scolytoplatypodini	1 : 0[†]
	Xyleborini	21 : 11 ($X_1^2 = 2.531$, $P = 0.112$)
Total[‡]		112: 75 ($X_1^2 = 6.931$, $P = 0.008$)

[†]Sample sizes too small and tests not meaningful.
[‡]Total species/cases from the two subfamilies.

ethanol-infused boxelder bolts ($X_5^2 = 20.46$, $P = 0.001$) and from ethanol lure ($X_5^2 = 22.16$, $P < 0.001$) (Fig. 4). For ethanol-infused boxelder log, ethanol emission was greatest 7 and 15 days after initiation of sampling and was lowest 1 and 71 days after initiation of sampling. For ethanol lure, on the contrary, emission was the greatest 1 day after initiation of sampling and the level generally declined as time went by. Ethanol emission from ethanol-infused log, however, was greater than that from ethanol lure of the same period for all but the first sampling period (Day 1: $F_{1,8} = 0.17$, $P = 0.69$; Day 7: $X_1^2 = 6.82$, $P = 0.009$; Day 15: $F_{1,8} = 11.00$, $P = 0.01$; Day 27: $X_1^2 = 6.82$, $P = 0.009$; Day 42: $X_1^2 = 6.82$, $P = 0.009$; and Day 71: $F_{1,8} = 14.51$, $P = 0.005$).

Literature survey

Sixty-one bark and ambrosia species from two subfamilies (i.e. Platypodinae and Scolytinae) were categorised according to their aggressiveness for attacking healthy trees and their response to ethanol (Table 1). Species from the subfamily Scolytinae come from four tribes (i.e. Hylesinini, Scolytini, Scolytoplatypodini, and Xyleborini) (Table 1). One case responds positively to ethanol and four cases respond neutrally to ethanol in the subfamily Platypodinae (Table 2). No statistical test was conducted due to the low sample size. For the tribe Hylesinini in the subfamily Scolytinae, 26 respond positively and 27 neutrally to ethanol, and the ratio was statistically not significant ($X_1^2 = 0.000$, $P = 1.000$) (Table 2). For the tribe Scolytini in the subfamily Scolytinae, 63 respond positively and 33 neutrally to ethanol, and the ratio was statistically significant ($X_1^2 = 8.760$, $P = 0.003$) (Table 2). For the tribe Scolytoplatypodini in the subfamily Scolytinae, 1 respond positively and 0 neutrally to ethanol, and no statistical test was conducted due to low sample size (Table 2). For the tribe Xyleborini in the subfamily Scolytinae, 21 respond positively and 11 neutrally to ethanol, and the ratio was statistically not significant ($X_1^2 = 2.531$, $P = 0.112$) (Table 2). Pooling over all cases in the two subfamilies, significantly more cases respond positively (112 cases) than neutrally (i.e. 75 cases) to ethanol ($X_1^2 = 6.931$, $P = 0.008$) (Table 2).

Association or matches and mismatches between response to ethanol and aggressiveness were compared for the three tribes in the subfamily Scolytinae (Table 3). Association between response to ethanol and aggressiveness was significant for tribe Hylesinini and total across all three tribes (Hylesinini: $X^2 = 10.539$, $P = 0.001$; Total: $X^2 = 9.098$, $P = 0.003$). Association between response to ethanol and aggressiveness for tribe Scolytini was not significant ($P = 0.116$). For tribe Xyleborini, numerically more mismatches than matches (10 : 17) were observed, but the ratio was not statistically different from 1 : 1 ($X_1^2 = 1.333$, $P = 0.248$).

Discussion

Species that are closely related phylogenetically typically exhibit more similarities in morphology and feeding behaviour than species that are more distantly related (Bernays, 1998; Gillett *et al.*, 2014). In the family Curculionidae, wood-boring behaviour evolved first in the subfamily Platypodinae, and then in the subfamily Scolytinae (Gillett *et al.*, 2014). The greater likelihood for more recently evolved Scolytinae (111 positive : 71 neutral responses) to positively respond to ethanol compared to the more primitive Platypodinae (1 positive : 4 neutral responses) (Table 2) appears to support the hypothesis that natural selection favours a positive response to ethanol on bark and ambrosia beetles. Yet, this hypothesis is not supported by results from our current study demonstrating variable sensitivity to ethanol among the more recently evolved tribes Xyleborini and Hylesini compared to the tribe Scolytini. This discrepancy could be a function of evolution not being linear, and selection forces such as host plant chemistry being involved in shaping insect host selection behaviour (Bernays, 1998).

At a smaller scale, we propose the differential responses of KSHB and FTPB to ethanol in the current study corresponds to their aggressiveness for attacking healthy trees: KSHB is aggressive and is not attracted to stress-induced ethanol, whereas FTPB is non-aggressive and is attracted to ethanol. Our literature review compiled data on the responses of 61 species of bark and ambrosia beetles to ethanol (Table 1), which documented 113 out of 176 instances (64.20%) whereby sensitivity to ethanol matched host selection aggressiveness (Table 3). The number of matches is significantly greater than the number of mismatches based on a 1 : 1 ratio, although the degree of matching differed among tribes (Table 3).

The invasion aggressiveness of pests might be determined by their ability to circumvent host tree defence. To circumvent tree defence, certain species of bark and ambrosia beetles produce aggregation pheromones to aggregate and overwhelm tree defence (Boone *et al.*, 2011). Bark and ambrosia beetles could utilise pheromones released by conspecific insects (Mori, 1975; Seybold *et al.*, 1995; Tillman *et al.*, 1999; Blomquist *et al.*, 2010) or allelochemicals released by heterospecific insects or host trees in response to biotic and abiotic stresses for an aggregation purpose (Byers, 1995; Wallin & Raffa, 2002). A positive response to ethanol and utilization of weakened trees as hosts would likely minimise selection pressure for an aggregation pheromone, as is the case for many species in the Xyleborini. In contrast, a neutral or negative

Table 3. Statistical tests on association (Tribes Hylesinini, Scolytini, and Total) or matches/mismatches (Tribe Xyleborini) between aggressiveness and response to ethanol (subfamily: Scolytinae).[†]

Tribe	Attraction to ethanol (+: Positive; 0: Neutral)	Aggressiveness[‡]		# Matches: # Mismatches (null hypothesis 1 : 1)
		Aggressive[‡]	Non-aggressive	
Hylesinini	+	6	**20**	38: 15 ($X^2 = 10.539$, $P = 0.001$)
	0	**18**	9	
Scolytini	+	1	**62**	65: 31 ($P = 0.116$)
	0	**3**	30	
Xyleborini[§]	+	17	**0**	10: 17 ($X^2 = 1.333$, $P = 0.248$)
	0	**10**	0	
Total	+	24	**82**	113: 63 ($X^2 = 9.098$, $P = 0.003$)
	0	**31**	39	

[†] Positive and Non-aggressive, and Neutral and Aggressive were considered as matches (in bold).
[‡] Aggressive and intermediate in Table 1 were considered aggressive.
[§] Aggressiveness of *Dryoxylon onoharaensum* is unknown and associated five cases (Table 1) were excluded in this table.

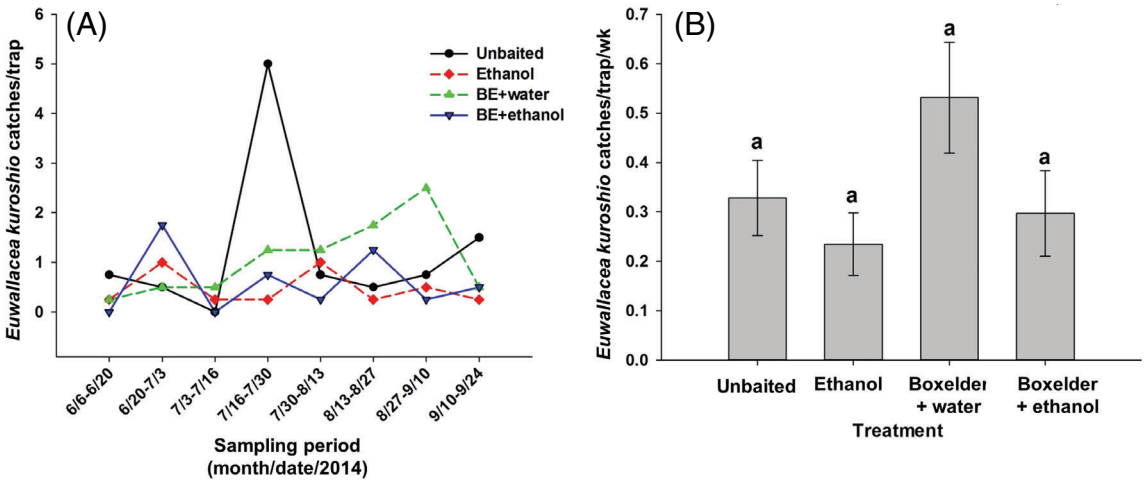

Fig. 2. Flight response of female Kuroshio shot hole borer, *Euwallacea kuroshio*, to the four treatments. (A) Seasonal flight (total trap catch); and (B) Means (± 1 standard error). [Colour figure can be viewed at wileyonlinelibrary.com].

response to ethanol might indicate the active involvement of pheromone or other semiochemicals in aggregation and they can attack healthy trees. This is evidenced by the scarcity of literature on flight response of western pine beetle, *Dendroctonus brevicomis*, and mountain pine beetle, *D. ponderosae* to ethanol and the active utilization of pheromones for aggregation in these two systems. *Dendroctonus brevicomis* females and males produce and release exo-brevicomin and frontalin, respectively (Pitman *et al.*, 1969). *Dendroctonus ponderosae* females and males produce and release trans-verbenol and frontalin, respectively (Hughes, 1973; Tillman *et al.*, 1999; Blomquist *et al.*, 2010). Alternatively, the capability of attacking healthy trees implies that those species possess other strategies such as formation of symbiotic association with fungi (Lieutier *et al.*, 2009) and detoxification of defensive compounds (Lindgren & Raffa, 2013). In its native range, KSHB attacks pruned regions of avocado trees (Eskalen *et al.*, 2013). In its introduced range (San Diego Co., California), the KSHB attacks healthy trees (Coleman *et al.*, 2019), but the mechanisms (strategies) by which it circumvents tree defences remains to be elucidated. The evolution of the capability to attack healthy trees might result from 'intensive interspecific competition' as Lindgren and Raffa (2013) suggested.

Among the fungus farming ambrosia beetles, the capability to establish gardens of nutritional fungal symbionts within healthy versus weakened trees could also influence host selection and utilization mechanisms. Egg laying within host tree galleries tends to be initiated after the fungal gardens are established, thereby posing a critical bottle neck in colonization success, brood production, and survivorship. Ethanol promotes the growth rate of the fungal symbionts of ethanol-responsive ambrosia beetles in the Xyleborini, and the presence of ethanol in host tree tissues benefits the colonization success of foundress beetles (Ranger *et al.*, 2018). The concentration of ethanol within host tree tissues can also impart interspecific differences in beetle colonization success, and perhaps drive niche-partition (Rassati *et al.*, 2020).

Boxelder bolts filled with water did not attract KSHB, whereas they attracted FTPB, compared to unbaited traps. The differential attraction of boxelder bolts filled with water, however, is not

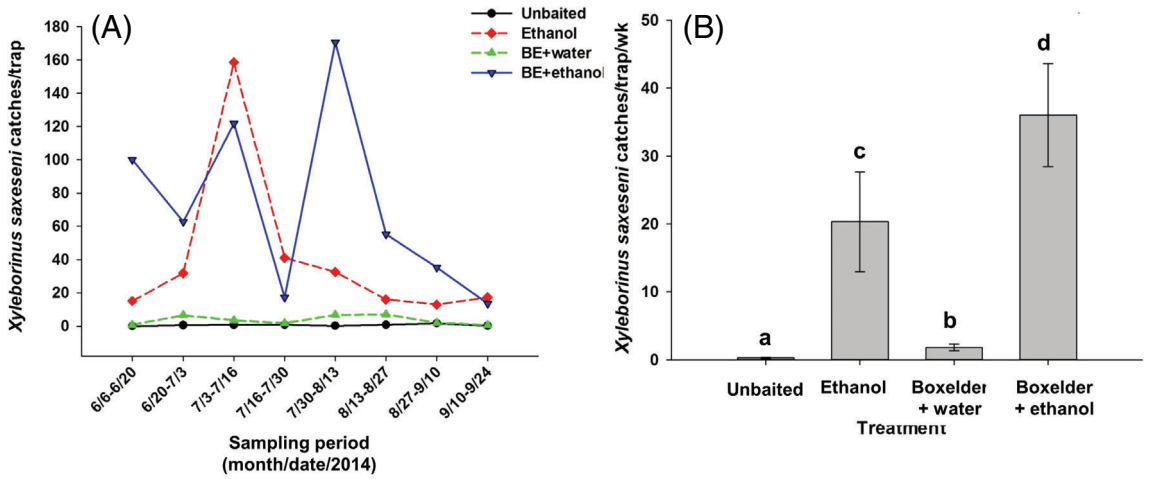

Fig. 3. Flight response of female fruit-tree pinhole borer, *Xyleborius saxesenii*, to the four treatments. (A) Seasonal flight (total trap catch); and (B) Means (± 1 standard error). [Colour figure can be viewed at wileyonlinelibrary.com].

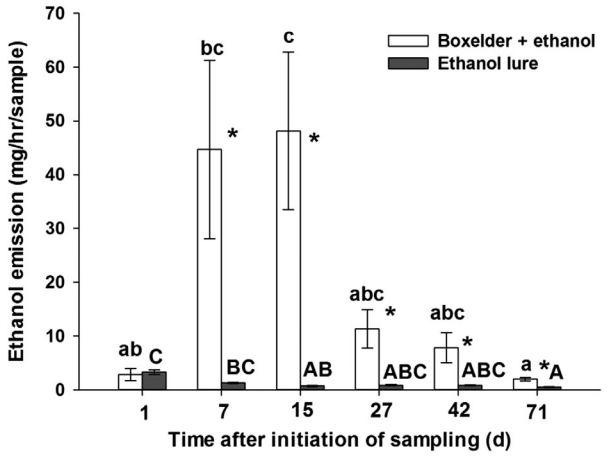

Fig. 4. Time-course emission of ethanol from ethanol-infused boxelder stem sections and commercial lures in the laboratory; No ethanol was detected from water-infused boxelder stems on each sampling date (data not shown). * above bars indicates significant difference between boxelder + ethanol and ethanol lure within the same sampling period; Different lower-case letters indicates significant difference among sampling periods of the boxelder + ethanol treatment; The same upper-case letters indicates no significant difference among sampling periods of ethanol lure treatments.

caused by the differential responses of these two pests to ethanol because ethanol was not detected from boxelder bolts filled with water. The failure of detecting ethanol from boxelder bolts filled with water is unexpected because ethanol is known to be produced from fermenting plant tissues (Gong *et al.*, 1981; Olsson & Hahn-Hägerdal, 1996; Lin & Tanaka, 2006). One explanation is that water added to the stems may have been absorbed and then evaporated before it interacted with fungi due to the dry and sometimes hot climate in the study site. In a previous study, Y. Chen *et al.* (unpublished) found that boxelder bolts were attractive to PSHB. Comparing the response of KSHB to a boxelder bolt filled with water in this study to that in the previous study, and assuming PSHB and KSHB behave similarly, this indicated that some semiochemicals arising from organisms attacking unscreened boxelder bolts or organisms per se are responsible for the attraction of PSHB in the previous study. Results in this study indicates that the attraction of boxelder bolts filled with water to FTPB is most likely attributable to some unknown volatile organic compounds released from boxelder bolts per se or after an interaction with water. Boxelder bolts used in the present study were screened during the study and no insect infestation was observed at the end of the experiment. The greater attraction of boxelder bolts filled with ethanol than ethanol lure could result from the consistently greater concentration from boxelder bolts filled with ethanol than that from ethanol lure (Fig. 4). Alternatively, it might result from an additive or a synergistic interaction between boxelder bolt and ethanol lure.

In summary, FTPB was attracted to ethanol and KSHB was not. The different behavioural responses of these two ambrosia beetles to ethanol correspond to their aggressiveness: KSHB attacks healthy trees and FTPB attacks weakened trees. Future studies will be directed to elucidate the mechanism(s) that KSHB has evolved to circumvent tree defence. The validation or the validity of a generalization of the observed phenomenon by testing responses of a wide range of bark and ambrosia beetles to ethanol is also warranted. Although ethanol is widely used and is an effective lure for detection of many bark and ambrosia beetles (Rabaglia *et al.*, 2019), findings from the current study and many others necessitate research and development of alternative trapping strategies (e.g. trap colours and aggregation pheromones; Y. Chen *et al.*, unpublished) for more aggressive species which are ecologically and economically more devastating. For example, quercivorol, an aggregation pheromone of the oak ambrosia beetle, *Platypus quercivorus*, effectively attracted the aggressive PSHB (Dodge *et al.*, 2017).

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Author contribution

SJS and YC designed the study; SJS, TWC, CMR, and YC contributed to acquisition and analysis of data; CMR performed GC–MS; YC performed statistical analyses and drafted the manuscript; SJS, TWC, and CMR critically revised the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. All vouch specimen collected in the study are stored at the Department of Entomology, University of California, Davis, CA 95618, U.S.A.

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