

Olfactory Cues, Visual Cues, and Semiochemical Diversity Interact During Host Location by Invasive Forest Beetles

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Abstract Plant-feeding insects use visual and olfactory cues (shape, color, plant volatiles) for host location, but the relative importance of different cues and interactions with non-host-plant volatiles in ecosystems of varying plant biodiversity is unclear for most species. We studied invasive bark beetles and wood borers associated with pine trees to characterize interactions among color, host and non-host volatiles, by employing traps that mimic tree trunks. Cross-vane flight intercept traps (black, green, red, white, yellow, clear) and black funnel traps were used with and without attractants (α -pinene + ethanol), repellents (non-host green leaf volatiles, ‘GLV’), and attractant/repellent combinations in four pine forests in New Zealand. We trapped 274,594 *Hylurgus ligniperda*, 7842 *Hylastes ater*, and 16,301 *Arhopalus fesus*. Trap color, attractant, and color \times attractant effects were highly significant. Overall, black and red traps had the highest catches, irrespective of the presence of attractants. Alpha-pinene plus ethanol increased trap catch of *H. ligniperda* 200-fold but only 6-fold for *H. ater* and 2-fold for *A. fesus*. Green leaf volatiles had a substantial repellent effect on trap catch of *H. ligniperda* but less on *H. ater* and *A. fesus*. Attack by *H. ligniperda* was halved when logs were treated with GLV, and a similar effect was

observed when logs were placed among broadleaved understorey shrubs emitting GLV. Overall, *H. ligniperda* was most strongly affected by the olfactory cues used, whereas *H. ater* and *A. fesus* were more strongly affected by visual cues. Collectively, the results support the semiochemical diversity hypothesis, indicating that non-host plant volatiles from diverse plant communities or artificial dispensers can contribute to resistance against herbivores by partly disrupting host location.

Keywords Attractant · Cerambycidae · Curculionidae · *Pinus* · Repellent · Scolytinae

Introduction

Detecting and finding host plants is critically important for host-specific plant-feeding insects (Bernays and Chapman 1994). Plant volatiles are composed of a wide range of secondary metabolites characteristic of particular plant species (e.g., Baldwin 2010; Pichersky *et al.* 2006), which can be used by insects as olfactory signals for host recognition and location (Bruce and Pickett 2011). Non-host volatiles also are important during host searching; for example, volatiles from broadleaved trees can act as non-host cues for conifer-infesting insects (Huber and Borden 2001a; Jactel *et al.* 2011; Zhang and Schlyter 2004). In addition to olfactory cues, insects use visual cues such as host color and shape for host finding (e.g., Doering and Chittka 2007; Goyer *et al.* 2004) and for discrimination of host and non-host plants (e.g., Campbell and Borden 2009). However, the relative importance of olfactory and visual cues for host plant location (i.e., the process of locating host plants), and to what extent these cues are used synergistically, is largely unknown for most insect taxa (Hanks *et al.* 2012).

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Interactions between olfactory and visual host and non-host cues are likely to play an important role in the dynamics of food webs. Impoverished plant communities such as plantation forests, often grown as ‘monocultures’, may be at an increased risk of insect outbreaks (Jactel and Brockerhoff 2007). The lack of non-host volatiles that could disrupt host location processes may contribute to this. According to the semiochemical diversity hypothesis (Zhang and Schlyter 2003), mixed plant volatiles from diverse plant communities can disrupt olfactory host location by several mechanisms including chemical disguise of host plants, masking insect attractants, and repelling effects (Jactel *et al.* 2011; Jactel and Brockerhoff 2007; Unsicker *et al.* 2009; Zhang and Schlyter 2004).

Bark beetles (Curculionidae: Scolytinae) and longhorn beetles (Cerambycidae) are among the best-studied forest insects because they can cause substantial tree damage and mortality, and play important roles in forest stand renewal, decomposition of woody plants, and forest food webs (Drever *et al.* 2009; Edburg *et al.* 2012; Kurz *et al.* 2008). The ability of adults to find suitable host material may be a limiting factor (Knížek and Beaver 2004), and selection for primary attraction and efficient searching mechanisms to find suitable host material are, therefore, important for individual fitness (De Jong and Sabelis 1988; Tunset *et al.* 1993). Many bark beetles and longhorn beetles associated with coniferous trees find host material by using host-specific volatiles for orientation and host recognition (Brockerhoff *et al.* 2006b; Byers and Zhang 2012; Pureswaran and Borden 2005). The occurrence of α -pinene, a common conifer monoterpene, and ethanol, a decomposition product of phloem and sapwood tissues, is characteristic of conifers that are dying or have died recently (Kelsey 1994). These compounds are important attractants or components of attractant blends especially for beetles colonizing conifer bark and wood (Hanks *et al.* 2012; Miller 2006; Miller and Rabaglia 2009; Schroeder and Lindelöw 1989).

Bark beetles can be broadly categorized as *primary* (species that usually attack live trees), *secondary* (species attacking trees that are weakened or died recently), or *saprophytic* (species breeding exclusively in dead trees) (Paine *et al.* 1997). Among the latter, especially, are numerous species that are successful invaders (Brockerhoff *et al.* 2006a, 2014), probably because they are prevalent in transport pathways such as wood packaging materials used in international trade, and because they may encounter fewer obstacles to establishment as they are not required to overcome defenses of live trees (Liebhold and Tobin 2008). Examples of this group are the pine bark beetles *Hylastes ater* (Paykull) and *Hylurgus ligniperda* (F.) (Curculionidae: Scolytinae), which have invaded two and four continents, respectively (Brockerhoff *et al.* 2006a). The pine longhorn beetle *Arhopalus ferus* (Mulsant) (Cerambycidae), has similar characteristics and is another successful invader that exploits trees that died recently (Brockerhoff and Hosking 2001). These three species often are highly abundant in pine forest regions in New

Zealand (Brockerhoff *et al.* 2006b, Chase *et al.* 2017), particularly following tree harvesting, which provides pulses of deadwood resources for breeding. Although these particular species do not damage live trees, they can cause quarantine issues, may vector fungi colonizing wood (McCarthy *et al.* 2012, 2013), and *H. ater* may damage seedlings (Sopow *et al.* 2015). There is no indication of pheromone use in these species, but previous research has confirmed that pine-specific host volatiles play a role in host location and selection (Brockerhoff *et al.* 2006b; Suckling *et al.* 2001). However, for these and other saprophytic bark beetles and longhorn beetles, there is little information about the role of visual cues and interactions between olfactory and visual cues during host location, and whether non-host volatiles can interfere with host location processes as suggested by the semiochemical diversity hypothesis (Zhang and Schlyter 2003). Few studies on these topics have focussed on saprophytic species, despite their considerable species richness and their importance in biogeochemical cycles and forest food webs.

The underlying aims of the current study were to investigate the mechanisms that are thought to explain the semiochemical diversity hypothesis and its potential application. We conducted a suite of experiments to determine the relative importance of olfactory cues, visual cues, and their interactions in host finding of *H. ater*, *H. ligniperda*, and *A. ferus*, and whether non-host volatiles can disrupt the process. Specifically, our objectives were (i) to test the effects of a range of trap colors (that are similar or dissimilar to host material) in the presence or absence of host volatiles, and (ii) to investigate whether non-host volatiles characteristic of broadleaved, non-host trees can disrupt host location processes when emitted either by non-host plants, topically applied green leaf volatiles, or dispensers containing green leaf volatiles. Ultimately, the aims were to understand the host selection behavior of saprophytic bark beetles and longhorn beetles, and to enable the development of management strategies based on manipulation of host attraction and disruption.

Methods and Materials

Effects of Visual and Olfactory Cues on Trap Catch Ten sites were selected in four *Pinus radiata* forests in the Nelson region, New Zealand: Golden Downs (NZTM E1592524; N5401801), Kainui (E1595200; N5405404), Moutere (E1602469; N5427872), Lee Valley (E1613407; N5417323). All sites had been harvested within the previous 12 mo. We used 200 flight intercept traps (20 treatments \times 10 replicates, see Supplementary Material 1) in different colors (black, green, red, white, yellow, clear), mostly of a cross-vane design, made from Mulflute™ polypropylene sheets (600 \times 210 mm; Mulford International, Christchurch, New Zealand), topped with a piece of Mulflute™ (210 \times 210 mm), and a funnel and catch-cup attached to the bottom for collecting insects. Clear

traps of the same design and dimensions were made from Lexan polycarbonate sheets (SABIC Innovation Plastics, Pittsfield, MA, USA). Black funnel traps were standard eight-funnel traps (PheroTech, Delta, BC, Canada) of the same height and width as the cross-vane traps. To describe the colors of the traps we tested, spectral reflectance measurements were made for the panel and funnel traps as well as *Pinus radiata* bark (see below and Supplementary Material 2).

Ethanol (Nuplex Specialties NZ, Mt. Wellington, New Zealand) and α -pinene (PINECHEM 500, Lawter (N.Z.), Mt. Maunganui, New Zealand) were provided in separate 150 ml dispensers (450 × 50 mm, 150 μ m polyethylene tubing with felt wick strips inside, otherwise as described in Brockerhoff *et al.* (2006b)). Ethanol and α -pinene were applied at release rates of ca. 0.02 g/day and 0.76 g/day, respectively. The non-host volatiles used were two green leaf volatiles (GLV), (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol (both from Bedoukian, Danbury, USA), applied in separate 20 ml polyethylene tubing dispensers (200 × 50 mm, otherwise as described above). Release rates from GLV dispensers were ca. 0.01 g/day and 0.008 g/day, respectively.

Trap catches were compared with different combinations of trap type, host-based attractants, and non-host repellents (control, attractant, repellent, and attractant+repellent) as shown in Supplementary Material 1. Effects of repellents were tested only for the subset of black, white, and clear panel traps. Traps were placed 20 m apart, suspended from steel Y-posts at about 1.4 m height, and positioned such that no two traps of similar color or treatment were adjacent. Traps were monitored fortnightly from November 2008 until May 2009, and moved one position clockwise at each monitoring to avoid position effects. In addition, traps in Kainui Forest were monitored at 3 hr intervals to record daily activity patterns during part of the peak flight and to assess whether flights occur more during light or dark, as this is relevant to the understanding of the role of vision and responses to colors. This was done over six 24 hr periods from 2 February 2009 (1:30 pm) until 5 February 2009 (10:30 am) and from 18 January 2010 (10:30 am) until 21 January 2010 (1:30 pm).

Effects of Synthetic Non-Host Volatiles on Attack of Pine Logs

Effects of topically applied, non-host green leaf volatiles (GLVs) on attack of *P. radiata* log billets by *H. ater* and *H. ligniperda* were assessed in Chaney's Forest, NE Christchurch (NZTM E1573380; N5192655). This trial was set up on 23 September 2009 during the spring flight of *H. ater* and *H. ligniperda*. Trees of the same age, diameter and similar bark thickness were felled and cut into 0.5 m log sections of about 200 mm diam. Fifty logs were placed in a recently harvested part of the forest from which most larger-diameter logging debris had been cleared. Two eight-funnel traps with ethanol and α -pinene (see above) were installed nearby to monitor beetle activity while the logs were in the

field. Traps were cleared weekly, and beetles caught were recorded. Five replicates of ten logs were set out in parallel lines (about 50 m between lines) with logs ca. 20 m apart. Each trap log was placed in an east–west direction to standardize log positions with respect to insolation.

GLVs, (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol in silicon oil solution (50 ml, 30 % GLV, 70 % oil), were applied with a paint brush to every second log within each replicate, covering the logs entirely. The remaining logs were left untreated as controls. Bark beetle attacks of logs were assessed weekly by counting the number of beetle gallery entrances and marking them with mapping pins. For the final assessment, all logs were taken to the laboratory on 16 November 2009. At that time, each log was bagged individually and placed in a freezer (-20 °C) to stop further beetle activity. The bark of each log was removed later with a chisel, and the number of colonizing adult beetles present was counted. No teneral adults from the next generation were present.

Effects of Natural Non-Host Volatiles on Attack of Pine Logs

To test the effects of naturally occurring green leaf volatiles on bark beetle attack of pine logs, five sites in Chaney's Forest were selected that all contained areas with patches of understory vegetation of broadleaved fabaceous shrubs, mainly *Cytisus scoparius*, *Ulex europaeus*, and *Lupinus arboreus* in varying proportions, as well as open areas with no understory vegetation. As related Fabaceae are known to emit (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol (Thöming *et al.* 2014), these shrubs are likely to emit green leaf volatiles; and as these are not emitted by pines, they can be considered non-host volatiles for pine bark beetles. Fifty 0.5 m-long logs were placed at the five study sites (10 logs per site). At each site, five pairs of logs were distributed such that each pair consisted of one log placed in an area without understory, and another log placed nearby among understory shrubs, with at least 50 m distance between pairs. Assessment of beetle attack of logs was as for the topically applied GLV trial (above), weekly in the field, and a final assessment in the laboratory. Six logs were lost over the trial period.

Measurement of Spectral Reflectance

We measured the spectral reflectance of *Pinus radiata* bark from a freshly cut tree and of the materials from which panel traps and Lindgren funnel trap were made, using samples measuring 100 × 80 mm. Samples were housed individually within a box lined with black-out cloth, which absorbs scatter light, prevents reflection from the box walls, and ensures that only the light reflected from the material was measured. Natural sun light was focused onto the samples through a N-BK7 lens (25.4 mm diam, focal length $f = 100$ mm) into an anodized aluminium lens tube (30 mm long, 25.4 mm diam). The spectral quality of reflected light was measured as the average of 20 scans with a 5 ms scanning speed using an Ocean Optics USB2000-NIR-VIS fibre spectrometer (OceanOptics, FL, USA). The relative

intensity of reflected light was calculated as a ratio of the sampled wavelength and the peak reflected wavelength for each material tested. The spectral composition of the clear trap material (Lexan polycarbonate sheet) could not be determined as it transmits all wavelengths in the visible region.

Statistical Analysis Statistical analyses were performed using R version 3.1.1. We used generalized estimating equations (GEE) with Poisson errors (R-package *geepack*, Halekoh *et al.* (2006)) to test for differences between trap catches of the target beetle species caught with different trap colors with and without olfactory attractants (lures) across the 10 sites. The GEE approach was used instead of generalized linear mixed effects models because it could account for overdispersion and within-site correlation using ‘site’ as cluster identification. We applied species-specific GEE models that contained ‘seasonal trap catch’ (the sum of insects caught within a season) as response variable, and ‘color’ (including funnel traps serving as reference) as well as ‘attractant’ (ethanol and α -pinene *vs.* unbaited control) and their interaction as explanatory variables. The significance of the explanatory variables was assessed *via* backwards selection using Wald tests and the quasi-likelihood criterion QIC_u (Pan 2001; Zuur *et al.* 2009). GEE parameter estimates and variance-covariance matrices were used for multiple comparison tests using Tukey contrasts (Hothorn *et al.* 2008) (R-package *BSagri*; R-package *multcomp*). Resulting *P*-values were adjusted using Benjamini and Yekutieli’s (2001) method that controls the false discovery rate.

Effects of attractant and/or repellent addition on catch numbers of the same beetle species were tested using generalized linear mixed models (GLMMs) with negative binomial error distribution and logit link function [R package *glmmADMB*, (Skaug *et al.* 2013)]. Species-specific models contained ‘trap treatment’ (factor with four levels: ‘control’, ‘repellent’, ‘attractant’, and ‘attractant + repellent’) as explanatory variable and the factor ‘site’ as a random term. The effects of topically applied GLVs or naturally emitted non-host volatiles on bark beetle attack of logs were tested using species-specific GLMMs with negative binomial error distribution, logit link function, and ‘site’ as a random term [R package *glmmADMB*, Skaug *et al.* (2013)]. In all GLMMs, the significance of the fixed terms was determined *via* backwards selection using likelihood-ratio tests and Akaike’s Information Criterion (AIC; Zuur *et al.* 2009).

Results

Effects of Visual and Olfactory Cues on Trap Catch Large numbers of beetles of the three target species were caught during the main experiment of this study, including 274,594 *H. ligniperda*, 7842 *H. ater*, and 16,301 *A. ferus*. The main effects, trap color and attractant, and the color \times attractant interaction were all highly significant (Fig. 1; Wald tests, all

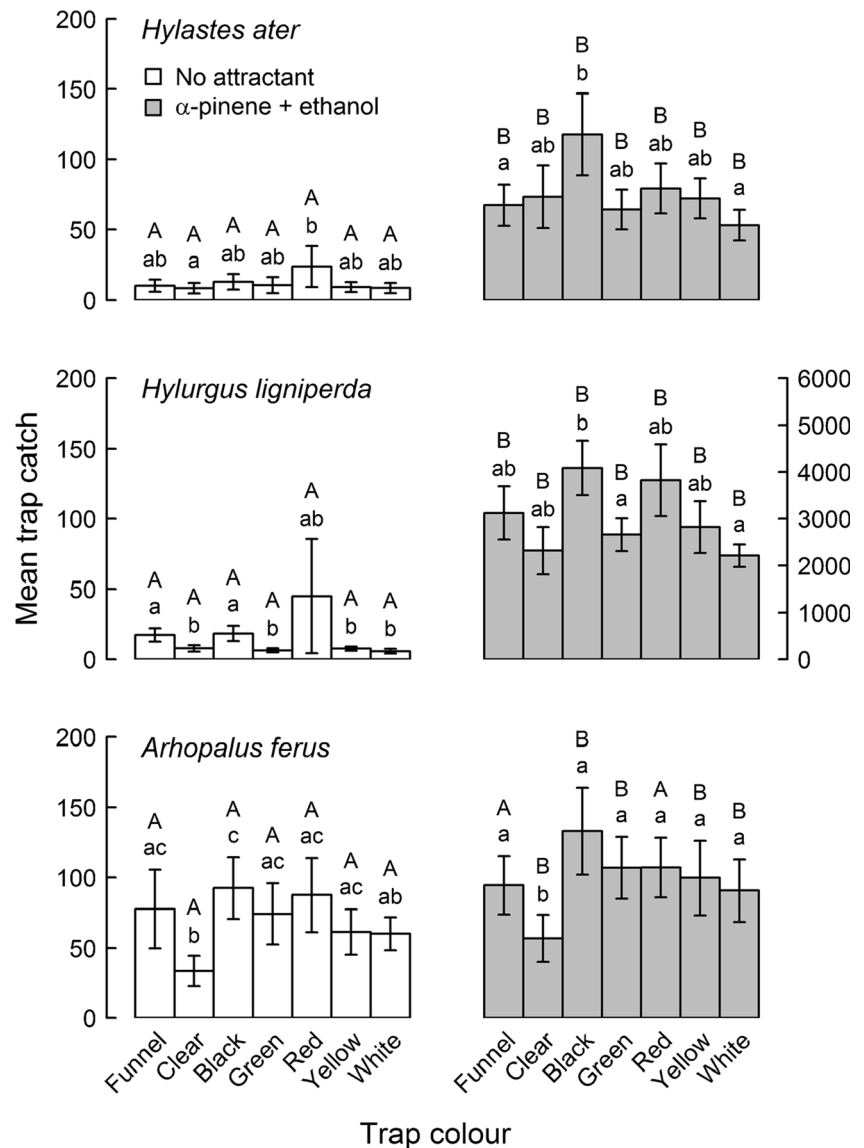
$P < 0.001$, see Supplementary Material 3). For traps without attractants, black and red panel traps tended to have the highest catches, whereas clear and white traps tended to have the lowest, and these were significantly different from some of the other trap colors (Fig. 1). For example, for *H. ligniperda*, catches in black panel traps differed significantly from those in green, yellow, and white panel traps, while for *A. ferus*, catches in black traps differed from those in clear and white traps. Catches in clear traps also differed from those in red, green, and yellow traps, as well as in black funnel traps. For *H. ater*, red panel traps had significantly more catches than clear traps (Fig. 1). This overall preference for black and red traps is consistent with the spectral reflectance of pine bark, which has a similar peak intensity as our red traps and high levels of intensity overlapping with our black traps (Supplementary Material 2).

Traps with α -pinene and ethanol as attractants caught considerably more beetles than traps without attractants, although the extent of this varied by species (approximately twice as many *A. ferus*, six times as many *H. ater*, and 200 times as many *H. ligniperda*). These means, along with the respective χ^2 values for color and attractant (Supplementary Material 3), indicate that host attractants were comparatively more important in *H. ligniperda* than in *H. ater* and *A. ferus*. The relative responses to trap color and type differed between traps with and without attractant, given the significant color \times attractant interaction. Attractant-baited black panel traps again tended to have the highest catches, while attractant-baited clear and white traps had the lowest catches for most species. However, black funnel traps caught significantly fewer *H. ater* and *A. ferus* than black panel traps when attractants were used.

Trapping in three-hour intervals showed that there were clear peaks in the daily activity of *H. ater*, *H. ligniperda*, and *A. ferus* (Fig. 2). Trap catches of all three species exhibited two peaks of activity between 07:30 am and 10:30 am and between 7:30 pm and 10:30 pm. During the southern hemisphere summer when the data were gathered, these periods represent morning (post-dawn) and evening (including dusk) when there is typically low wind, low to moderate light, and moderate to warm temperatures (Fig. 2).

Green leaf volatiles (GLVs) had a significant repellent effect on trap catch of *H. ligniperda* (27–55 % reduction in catch), a marginally insignificant effect for *H. ater* ($P = 0.056$), and no effect for *A. ferus* (Fig. 3, Supplementary Material 4). However, there was no significant attractant \times repellent interaction in any of the species (Supplementary Material 4). When we compared the relative effect size of attractants (α -pinene and ethanol) and repellents (GLVs), the effect of attractants exceeded the effect of repellents (as indicated by the larger χ^2 values) in all three species (Supplementary Material 4).

Fig. 1 Mean (\pm SE) trap catch of *Hylastes ater*, *Hylurgus ligniperda*, and *Arhopalus ferus* caught in colored panel traps and black funnel traps without and with attractants (α -pinene and ethanol) ($N = 10$ sites). Different lower case letters indicate statistically significant differences between trap colors within attractant and species, whereas different upper case letters denote statistically significant differences between attractants within trap color and species (multiple comparison tests with Tukey contrasts, $\alpha = 0.05$). Note: Y-axis for *H. ligniperda* plotted on two different scales



Effects of Non-Host Volatiles on Attack of Pine Logs Logs treated with GLVs in silicon oil were attacked by significantly fewer (about half as many) *H. ligniperda* than control logs (Fig. 4, Supplementary Material 5). For *H. ater*, GLV treatment had a similar effect (Fig. 4), but the difference was marginally non-significant ($P = 0.05$, Supplementary Material 5); however, the rate of attack by *H. ater* generally was low reflecting its lower abundance at the study site. Both species were in flight during the trial period, but catches of *H. ligniperda* exceeded those of *H. ater* by about 100:1 (e.g., 46 *H. ligniperda* vs. 0.43 *H. ater* per day in mid-October 2009). No attack of pine logs by *A. ferus* was observed in our trial.

Pine logs placed among an understory of broadleaved shrubs that naturally emit non-host GLVs were attacked considerably less than logs placed in open areas. The number of *H. ligniperda* found on logs that had been placed in areas with understory vegetation was significantly reduced, by about

75 %, compared with logs in the open forest without an understory (Fig. 5, Supplementary Material 6). For *H. ater* no significant treatment effect was found (Fig. 5, Supplementary Material 6); however, attacks by *H. ater* generally were rarer.

Discussion

Host-specific bark beetles and longhorn beetles that feed and reproduce under the bark and in the wood of particular tree species must be able to locate and recognize host material of a suitable species. For saprophytes, the host material also must be of an appropriate condition, as neither live trees nor material that is too decayed can be colonized. For the three pine-infesting beetles we studied, our results indicate that visual and olfactory host and non-host cues together with their interactions are important in the host location process, and that the

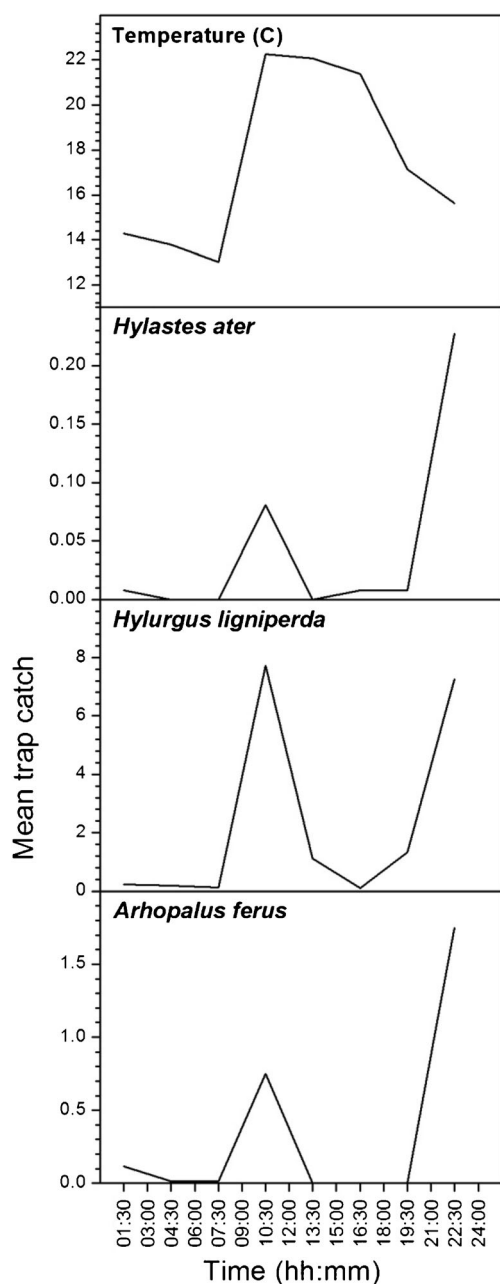


Fig. 2 Fitted mean numbers of *Hylastes ater*, *Hylurgus ligniperda*, and *Arhopalus ferus* caught per trap per 3 hr time period and air temperature from 2 to 5 February 2009 and 18 to 21 January 2010. Note, sunrise and sunset occurred, for example, on 2 February 2009 at 6:34 am and 8:47 pm, respectively

relative importance of these cues is species-specific. While it is well-known that host volatiles such as α -pinene and ethanol play an important role in host location and recognition by bark beetles and longhorn beetles, including the species studied here (Brockerhoff *et al.* 2006b; Miller 2006; Miller and Rabaglia 2009; Schroeder and Lindelöw 1989; Suckling *et al.* 2001), our study contributes new insight into the roles of olfactory and visual cues and their interactions during host location. Although the shape and dark color of funnel traps

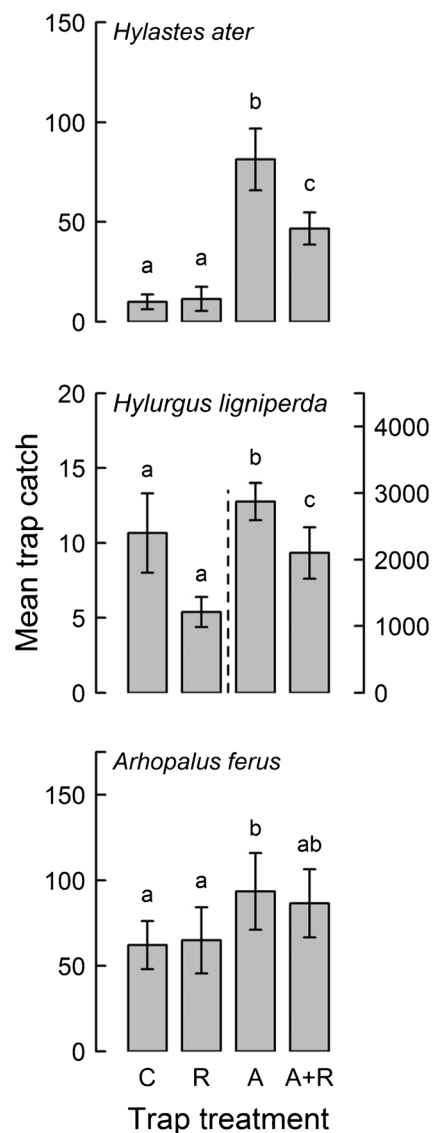


Fig. 3 Mean (\pm SE) trap catch of *Hylastes ater*, *Hylurgus ligniperda*, and *Arhopalus ferus* per trap treatment; C, control; R, repellent; A, attractant; A+R, attractant + repellent. Different lower case letters indicate statistically significant differences between trap treatments (multiple comparison tests with Tukey contrasts, $\alpha=0.05$; see text and [Supplementary Material](#) for details). Note: Y-axis plotted at different scales and with two vertical axes for *H. ligniperda*

and panel traps is presumed to visually mimic tree trunks, few prior studies have systematically examined this, especially for such a comprehensive range of trap colors and interactions with olfactory cues as employed in our study (but see Campbell and Borden 2006, 2009; Strom and Goyer 2001).

Trap color was a significant factor in attraction to traps in all three species we studied, although without the concurrent application of olfactory cues, differences in trap catch among the seven color/design treatments were not as striking. However, clear, white and yellow traps, which least resembled host tree trunks and bark, yielded lower catches overall than traps of darker colors. These observations are consistent with

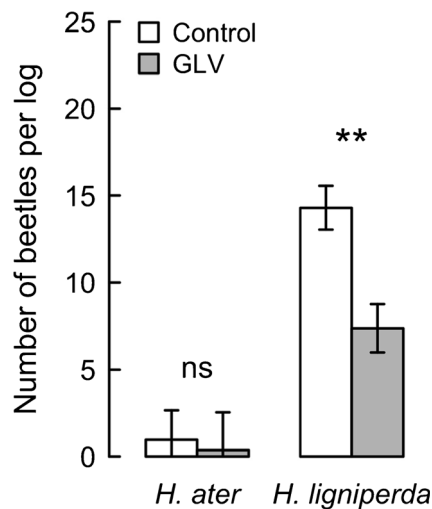


Fig. 4 Mean (\pm SE) number of *Hylastes ater* and *Hylurgus ligniperda* found on logs treated with green leaf volatiles (GLV) and untreated logs (control) ($N=5$ sites, ** $P<0.01$, ns: not significant; see text and Supplementary Material for details)

the similarities in the spectral reflectance of some of our traps and pine bark (Supplementary Material 2), considering that insect vision typically covers the range from about 350 nm (ultraviolet) to 700 nm (red/infrared), with some variation among species (Briscoe and Chittka 2001). The brightness of different colors probably is also perceived by insects. Although *A. ferus* is known for its crepuscular flight and activity behavior (Suckling *et al.* 2001), we observed that all three species in our study fly to some degree during the morning and evening hours of daylight, and they should, therefore, be able to see objects and perceive color to some extent. However, the lack of a strong color response and the more noticeable response difference between darker and lighter-

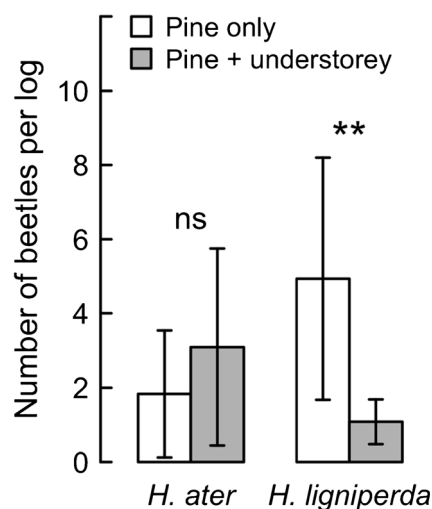


Fig. 5 Mean (\pm SE) number of *Hylastes ater* and *Hylurgus ligniperda* found to attack logs placed in ‘open’ areas of pine stand without understory and in pine stands with broadleaved understory vegetation that emits natural non-host volatiles ($N=5$ sites, ** $P<0.01$, ns: not significant; see text and Supplementary material for details)

colored traps seem to confirm the conclusions of Strom and Goyer (2001) that color is less important in bark beetle host location than the presence of a dark, rather than light-colored, silhouette.

The importance of olfactory host cues differed among the species we studied. For the two bark beetles, host volatiles were more important during the host location process compared with the longhorn beetle. *Hylastes ater* catches with host-characteristic visual and olfactory cues (*i.e.*, traps with a black tree trunk-like silhouette emitting α -pinene and ethanol) were about ten-fold higher than for a similar trap without olfactory cues. This effect was even more striking in *H. ligniperda* where the use of host volatiles increased trap catch by more than 100-fold. By contrast, for *A. ferus*, host volatiles increased trap catch only slightly (less than double), albeit significantly, compared with unbaited black traps (see also Brockerhoff *et al.* 2006b). Furthermore, in this species, visual cues had a slightly larger effect than volatiles: black panel traps caught more than twice as many *A. ferus* as clear panel traps. Collectively, this indicates that for *A. ferus*, visual cues are more important during host location than olfactory cues, contrary to the situation with the two bark beetles. Our results for the two pine bark beetles are consistent with other studies, which documented strong attraction of conifer-infesting species to α -pinene and ethanol (*e.g.*, Miller and Rabaglia 2009). However, *A. ferus* showed only a weak response to α -pinene and ethanol, contrasting with the findings of Miller (2006) who found the combination of these compounds to be a strong attractant for a number of longhorn beetles attacking pine, including the congeneric *Arhopalus rusticus nubilus*. It cannot be ruled out that *A. ferus* requires some additional attractant compounds. For longhorn beetles in particular, it is not uncommon that host volatiles are less effective as attractants than combinations of host attractants and various pheromone compounds (see Hanks *et al.* 2012). Nevertheless, host volatiles can be important synergists with other attractants and beetle pheromones (Hanks *et al.* 2012), and whether this applies to *A. ferus* requires more research.

The addition of olfactory non-host cues [*i.e.*, green leaf volatiles (GLVs)] resulted in a moderate reduction in trap catch of the bark beetles, but had no apparent effect on *A. ferus*. Topical application of these GLVs to fresh pine logs resulted in a similar (moderate) reduction in bark beetle attack of pine logs (no attack by *A. ferus* was observed). These and other GLVs have been shown to repel or reduce attraction for a number of conifer-infesting bark beetles and longhorn beetles (Huber and Borden 2001a; Jactel *et al.* 2011; Suckling *et al.* 2001; Zhang and Schlyter 2004). The lack of a repellent effect of GLVs on *A. ferus* was an unexpected result because a previous study found significant repellent effects of GLVs (Suckling *et al.* 2001); however, that study used walking and oviposition bioassays that tested close-range responses, whereas our trapping study assessed longer-range responses

of flying beetles, which is the probable reason for this difference.

Our comparison of attack of pine logs placed among an understory of broadleaved shrubs and *vs.* open areas aimed to test whether naturally emitted non-host green leaf volatiles can provide a similar effect as artificially applied GLVs, as predicted by the semiochemical diversity hypothesis (Zhang and Schlyter 2003). While attacks of pine logs in the understory treatment were substantially reduced, especially for *H. ligniperda*, this effect is unlikely to be due solely to non-host volatiles, as the understory probably also created physical and visual barriers. However, this combination of non-host volatiles and other barriers to insect colonization is clearly involved in the biodiversity effects described from mixed forests that tend to be less prone to insect attack than forests of lower diversity of plant species. Tree-feeding insects have been shown to cause less damage and to be less abundant in forests composed of several tree species than in single-species forests (Jactel and Brockerhoff 2007). Furthermore, mixtures of tree species that are phylogenetically more distant, such as conifers and broadleaved trees, have been found to regulate populations of tree-feeding insects more effectively than mixtures of trees of more closely related species (Castagneyrol *et al.* 2014; Jactel and Brockerhoff 2007). This effect is known as associational resistance which is defined as “*direct and indirect interactions between plants in close proximity, in which the influence of one plant on another ... decreases the likelihood of detection by, and/or vulnerability of a focal plant to, herbivores (above and beyond its innate ability to avoid detection or damage owing to herbivory)*” (Barbosa *et al.* 2009; see also Castagneyrol *et al.* 2014). Closely-related tree species tend to emit relatively similar volatiles and share other traits, so it is not surprising that the host range of pine bark beetles and borers typically includes many species of pine, irrespective of their geographic origin (Branco *et al.* 2015).

We suggest associational resistance can also occur within single-species forests, such as the pine forests in which our study took place, when an understory of distantly related species is present, broadleaved shrubs in our case. While disruption of host location has been demonstrated by way of experimental application of non-host cues for numerous conifer feeding beetles (Byers *et al.* 2004; Campbell and Borden 2006, 2009; Goyer *et al.* 2004; Huber and Borden 2001a, b; Strom and Goyer 2001; Suckling *et al.* 2001; Zhang and Schlyter 2004), and in the present study, our findings suggest that mixed species planting and the encouragement of a diverse understory vegetation may reduce the impact of pine beetles.

In summary, our study shows that the host-finding behavior of bark beetles and longhorn beetles relies on both olfactory and visual cues from both host and non-host plant species, but to varying extents, with the two bark beetles relying more on olfactory cues, and the longhorn beetle more on visual cues.

Based on our results and previous work on numerous other bark beetles and longhorn beetle, it is clear that such host-finding processes can be complex and show a surprising amount of variation among species. Such research is not only relevant for our understanding of the ecology of these insects, which are prominent biological invaders world-wide, but also for the development of ‘greener’ and more sustainable methods for the management of detrimental species, such as stimulo-deterrent diversion and push-pull techniques (*e.g.*, Pickett *et al.* 2014) by combining attractants and repellents such that insects are diverted away from timber.

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