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# Species Dependent Influence of (–)- $\alpha$ -Pinene on Attraction of Ambrosia Beetles (Coleoptera: Curculionidae: Scolytinae) to Ethanol-Baited Traps in Nursery Agroecosystems

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**ABSTRACT** Field-based trapping experiments were conducted in Ohio in 2003, 2004, and 2008 to determine the influence of (–)- $\alpha$ -pinene on the attraction of exotic and native ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) to ethanol-baited traps. In 2003 and 2004, we determined the effect of adding an (–)- $\alpha$ -pinene ultrahigh release lure (UHR; 2 g/d at 20°C) to traps baited with an ethanol UHR lure (0.39 g/d). Fewer *Anisandrus* (*Xyleborus*) *sayi* (Hopkins) and *Xyleborinus saxeseni* (Ratzeburg) were collected in 2003 and 2004 from traps baited with ethanol UHR plus (–)- $\alpha$ -pinene UHR compared with ethanol UHR. (–)- $\alpha$ -Pinene also reduced the attraction of *Xyloterinus politus* (Say) to ethanol-baited traps in 2004. Total captures of *Xylosandrus germanus* (Blandford) in 2003 were higher in traps baited with ethanol UHR plus (–)- $\alpha$ -pinene UHR than in traps with ethanol UHR alone but not in 2004. In 2008, captures were compared among traps baited with eight combinations of ethanol and (–)- $\alpha$ -pinene at both UHR and low release (LR) rates. Release rates for ethanol LR and (–)- $\alpha$ -pinene LR were 0.027 and 0.0015 g/d, respectively. (–)- $\alpha$ -Pinene UHR and (–)- $\alpha$ -pinene LR reduced the attractiveness of ethanol UHR to *A. sayi* and *X. saxeseni*. Ethanol UHR was also more attractive than ethanol LR to *A. sayi* and *X. germanus*. These findings demonstrate traps baited with ethanol alone are more effective than ethanol plus (–)- $\alpha$ -pinene for monitoring ambrosia beetle flight activity in ornamental nurseries. Ethanol release rate is also an important consideration for monitoring purposes.

**KEY WORDS** ethanol,  $\alpha$ -pinene, ambrosia beetles, Scolytinae

Exotic and native ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) are increasingly being recognized as key pests of ornamental nursery stock (Oliver and Mannion 2001). *Xylosandrus germanus* (Blandford) is native to eastern Asia and among the most economically important exotic ambrosia beetle pests in United States nurseries, particularly in Ohio (Ranger et al. 2010). Additional ambrosia beetle species occurring within Ohio nurseries and elsewhere in the United States include the exotic species *Xylebo-*

*rinus alni* Wood & Bright and *Xyleborinus saxeseni* (Ratzeburg), along with the native species *Anisandrus* (*Xyleborus*) *sayi* (Hopkins) and *Xyloterinus politus* (Say) (Ranger et al. 2010, Reding et al. 2010).

The host range for *X. germanus* includes >200 plant species in 52 families, and although deciduous broadleaf trees and shrubs are preferred, some conifers also are attacked (Weber and McPherson 1983). *X. saxeseni* also has a wide host range that includes both broadleaf deciduous and coniferous species (Kovach and Gorsuch 1985). *X. politus* rarely attacks conifers, and *Anisandrus* (*Xyleborus*) *sayi* favors maples, sassafras, and other deciduous hosts (Solomon 1995). Hosts commonly attacked by ambrosia beetles in ornamental nurseries in Ohio include *Acer*, *Cercis*, *Cornus*, *Magnolia*, *Quercus*, and *Styrax* species (C.M.R., personal observation).

Ambrosia beetles have traditionally been viewed as secondary pests that choose physiologically stressed hosts for colonization (Byers 1995). Compared with healthy specimens, physiologically stressed plants produce greater concentrations of several volatile organic compounds, including ethanol (Kimmerer and Kozłowski 1982, Kelsey and Joseph 2001). Decaying woody tissue also emits ethanol as a by-product of

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fungal growth (Moeck 1970, Gong et al. 1981). Ethanol acts as a primary attractant for numerous ambrosia beetle species, and ethanol-baited traps are commonly used to monitor flight activity (Byers 1995, Oliver and Mannion 2001).

$\alpha$ -Pinene is a type of monoterpene emitted to varying degrees from a wide variety of coniferous and deciduous trees (Geron et al. 2000). Emission levels of  $\alpha$ -pinene and other terpenes increase dramatically after tissue wounding and to a lesser extent in response to physiological stress (Bertin and Staudt 1996, Loreto et al. 2000). Studies have demonstrated  $\alpha$ -pinene increases the attraction of numerous bark beetle species to ethanol (Byers 1995), but the influence on ambrosia beetles is not well documented.  $\alpha$ -Pinene increased the attraction of *Trypodendron lineatum* (Olivier) to ethanol-baited traps but reduced attraction by *Anisandrus dispar* (F.) and *Dryoxylon onoharaensum* (Murayama) (Schroeder and Lindelöw 1989, Miller and Rabaglia 2009). To optimize monitoring the flight activity of exotic and native ambrosia beetles within the nursery agroecosystem, our objective was to assess the effects of (–)- $\alpha$ -pinene on the attractiveness of ethanol-baited traps to common ambrosia beetles.

### Materials and Methods

**Ambrosia Beetle Trapping.** Trapping experiments were conducted under field conditions in 2003, 2004, and 2008. In 2003 and 2004, treatments included 1) ethanol ultrahigh release (UHR) and 2) ethanol UHR plus (–)- $\alpha$ -pinene UHR. Lures were obtained from Phero Tech Inc. (Delta, BC, Canada). Release rates at 20°C for ethanol UHR and (–)- $\alpha$ -pinene UHR were 0.39 and 2 g/d, respectively (Phero Tech, personal communication). Chemical purities for ethanol UHR and (–)- $\alpha$ -pinene UHR were >95%, respectively.

Lures were hung from the lids of eight-unit funnel traps, which resulted in the lures being adjacent to the uppermost funnels. Traps were positioned  $\approx$ 50 m apart along the edge of woodlots at four commercial nurseries in Lake Co., OH. One replicate for each lure treatment was placed at each nursery, resulting in a total of four replicates for each treatment. Traps were emptied and rotated weekly at each location. A 2.54-by-2.54-by-0.9-cm (length by width by height) piece of dichlorvos-impregnated plastic (Vapona NO-Pest Strip, Green Cross, Monsanto, Mississauga, ON, Canada) was placed in the trap collection cups to kill captured insects. Traps were in place from 6 May 2003 to 2 September 2003 and from 5 May 2004 to 1 September 2004.

The following nine lure treatment combinations were tested in 2008: 1) ethanol low release (LR), 2) ethanol UHR, 3) (–)- $\alpha$ -pinene LR, 4) (–)- $\alpha$ -pinene UHR, 5) ethanol LR plus (–)- $\alpha$ -pinene LR, 6) ethanol UHR plus (–)- $\alpha$ -pinene LR, 7) ethanol LR plus (–)- $\alpha$ -pinene UHR, 8) ethanol UHR plus (–)- $\alpha$ -pinene UHR, and 9) an unbaited control. Release rates for ethanol LR and (–)- $\alpha$ -pinene LR were 0.027 and 0.0015 g/d, respectively (Phero Tech Inc., personal communication). Release rates for ethanol UHR and

(–)- $\alpha$ -pinene UHR lures were the same as previously noted. Lures were attached to the traps as previously noted and positioned  $\approx$ 10 m apart along the edge of woodlots at six cooperating nurseries in Lake Co., OH. One replicate of each trap treatment was placed at each nursery, resulting in a total of six nursery site replicates for each treatment. Dichlorvos-impregnated plastic was placed in the trap collection cups to kill collected specimens. Traps were emptied and rotated weekly within each block and were in place from 9 April 2008 to 26 June 2008.

**Statistical Analyses.** For the 2003 and 2004 data sets, total trap captures were compared between ethanol alone and ethanol plus (–)- $\alpha$ -pinene by using an unpaired *t*-test ( $\alpha = 0.05$ ; PROC GLM, SAS Institute 2001). The effects of lure composition and release rate, and interactions among these main effects, were analyzed for 2008 total cumulative trap captures using two-way analysis of variance (ANOVA) ( $\alpha = 0.05$ ; PROC GLM, SAS Institute 2001). When the interaction or main effects were significant ( $P < 0.05$ ), differences between means were separated using Tukey's honestly significant difference (HSD) multiple comparisons test. Data were  $\log_{10}(x + 1)$  transformed for all data sets, but untransformed data are presented.

### Results

Analysis of the total number of *A. sayi* captured from 6 May 2003 to 2 September 2003 determined that significantly fewer beetles were attracted to traps baited with ethanol UHR plus (–)- $\alpha$ -pinene UHR compared with ethanol UHR alone (Fig. 1A;  $t = 3.99$ ,  $df = 6$ ,  $P = 0.004$ ). Significantly fewer *X. saxeseni* also were collected from traps baited with ethanol plus (–)- $\alpha$ -pinene compared with ethanol alone (Fig. 1B;  $t = 2.03$ ,  $df = 6$ ,  $P = 0.044$ ). In contrast, significantly more *X. germanus* were attracted to traps baited with ethanol plus (–)- $\alpha$ -pinene compared with ethanol alone (Fig. 1C;  $t = 2.37$ ,  $df = 6$ ,  $P = 0.038$ ). A significant difference between treatments was not detected for *X. politus* in 2003 (Fig. 1D;  $t = 0.97$ ,  $df = 6$ ,  $P = 0.185$ ).

Analysis of total captures from 5 May 2004 to 1 September 2004 determined significantly fewer *A. sayi* (Fig. 2A;  $t = 2.19$ ,  $df = 6$ ,  $P = 0.035$ ), *X. saxeseni* (Fig. 2B;  $t = 4.61$ ,  $df = 6$ ,  $P = 0.005$ ), and *X. politus* (Fig. 2D;  $t = 1.99$ ,  $df = 6$ ,  $P = 0.043$ ) were collected from traps baited with ethanol plus (–)- $\alpha$ -pinene compared with ethanol alone. A significant difference was not detected between these treatments for *X. germanus* in 2004 (Fig. 2C;  $t = 1.07$ ,  $df = 6$ ,  $P = 0.162$ ).

A significant interaction effect was associated with total trap captures of *A. sayi* from 9 April 2008 to 26 June 2008 (Fig. 3A;  $F = 8.29$ ,  $df = 4$ ,  $P < 0.0001$ ). Total captures were significantly higher in traps baited with ethanol UHR alone compared with all other treatments, including ethanol UHR plus (–)- $\alpha$ -pinene LR and ethanol UHR plus (–)- $\alpha$ -pinene UHR. A significant difference was not detected between ethanol LR and ethanol LR plus (–)- $\alpha$ -

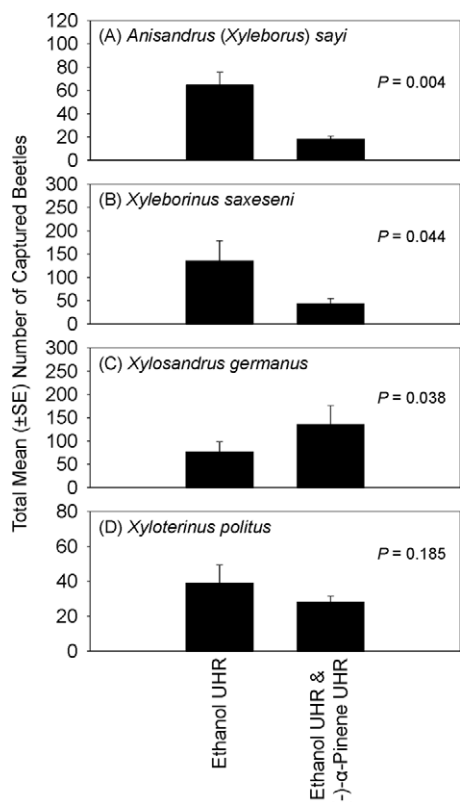


Fig. 1. Total mean  $\pm$  SE trap captures of *A. sayi* (A), *X. saxeseni* (B), *X. germanus* (C), and *X. politus* (D) during 2003 in response to traps baited with either ethanol UHR alone or a combination of ethanol UHR plus  $(-)$ - $\alpha$ -pinene UHR (unpaired *t*-test).

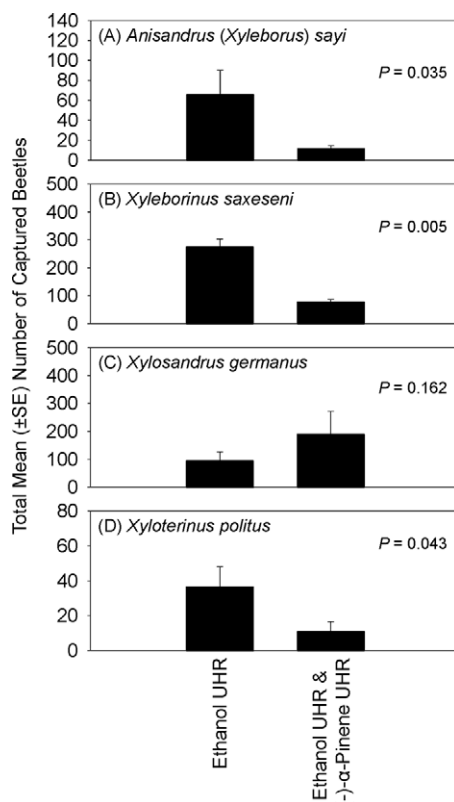


Fig. 2. Total mean  $\pm$  SE trap captures of *A. sayi* (A), *X. saxeseni* (B), *X. germanus* (C), and *X. politus* (D) during 2004 in response to traps baited with either ethanol UHR alone or a combination of ethanol UHR plus  $(-)$ - $\alpha$ -pinene UHR (unpaired *t*-test).

pinene LR or UHR. Ethanol UHR was more attractive than ethanol LR to *A. sayi*.

With respect to *X. alni*, a significant interaction effect was not detected in 2008 (Fig. 3B;  $F = 0.21$ ,  $df = 4$ ,  $P = 0.932$ ). A significant effect was also not associated with  $(-)$ - $\alpha$ -pinene ( $F = 2.69$ ,  $df = 2$ ,  $P = 0.077$ ). A significant effect was associated with ethanol ( $F = 17.43$ ,  $df = 2$ ,  $P < 0.0001$ ), but differences in *X. alni* trap counts were not detected between ethanol UHR and LR.

A significant interaction effect was associated with total trap captures of *X. saxeseni* in 2008 (Fig. 3C;  $F = 2.80$ ,  $df = 4$ ,  $P = 0.040$ ). Ethanol UHR was associated with significantly more total trap captures of *X. saxeseni* compared with all other treatments, except ethanol LR. A significant difference was not detected between ethanol LR and ethanol LR plus  $(-)$ - $\alpha$ -pinene LR or UHR.

A significant interaction effect was not associated with total trap captures of *X. germanus* in 2008 (Fig. 3D;  $F = 0.58$ ,  $df = 4$ ,  $P = 0.681$ ). A significant effect also was not associated with  $(-)$ - $\alpha$ -pinene ( $F = 0.31$ ,  $df = 2$ ,  $P = 0.734$ ). However, ethanol exhibited a significant influence on *X. germanus* trap captures ( $F = 76.84$ ,  $df = 2$ ,  $P < 0.0001$ ). Traps baited with ethanol UHR

alone were significantly more attractive to *X. germanus* than traps baited with ethanol LR alone ( $F = 9.81$ ;  $df = 1, 10$ ;  $P = 0.011$ ).

A significant interaction effect was not associated with 2008 total trap captures of *X. politus* (Fig. 3E;  $F = 0.72$ ,  $df = 4$ ,  $P = 0.580$ ). Ethanol and  $(-)$ - $\alpha$ -pinene also were not associated with significant main effects ( $F = 1.18$ ,  $df = 2$ ,  $P = 0.318$  and  $F = 2.85$ ,  $df = 2$ ,  $P = 0.068$ , respectively).

## Discussion

Our study demonstrates that depending on the species of ambrosia beetle,  $(-)$ - $\alpha$ -pinene may enhance or reduce attraction to ethanol-baited traps.  $(-)$ - $\alpha$ -Pinene enhanced the response of *X. germanus* to ethanol in 2003, but the effect was negligible in 2004 and 2008. In contrast,  $\alpha$ -pinene reduced the attractiveness of ethanol to *A. sayi* in 2003, 2004, and 2008; *X. alni* in 2004; and *X. saxeseni* in 2003, 2004 and 2008. Although the effect was negligible in some years, we did not observe  $(-)$ - $\alpha$ -pinene to enhance ambrosia beetle attraction to ethanol in 1 yr and reduce attraction in another year, and vice versa. Miller and Rabaglia (2009) and Gandhi et al. (2010) found  $(-)$ - $\alpha$ -pinene

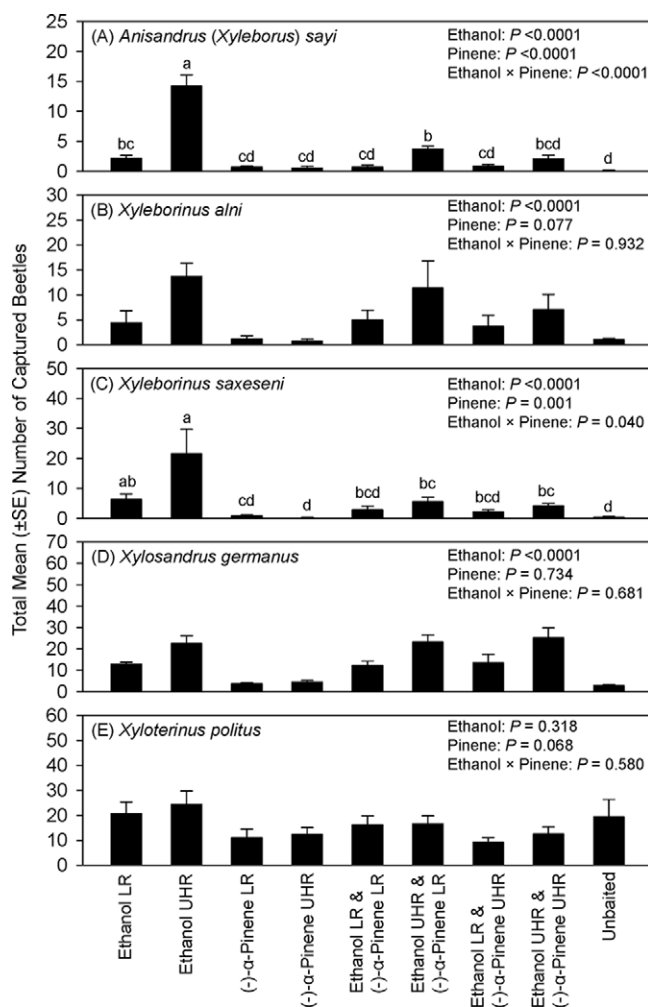


Fig. 3. Effects of ethanol and (–)- $\alpha$ -pinene release rate (LR versus UHR) and lure combinations on total mean  $\pm$  SE trap captures of *A. sayi* (A), *X. alni* (B), *X. saxeseni* (C), *X. germanus* (D), and *X. politus* (E) during 2008. Different letters indicate significant differences for species where a significant interaction effect was detected (Tukey's HSD test). See Results section for interpretation of differences between means for species not associated with a significant interaction effect.

had a negligible effect on *X. germanus* attraction to ethanol. (–)- $\alpha$ -Pinene reduced attraction of *D. onoharaensum* to ethanol, but the reduction was inconsistent for *A. sayi*, *A. tachygraphus*, *X. ferrugineus*, and *X. saxeseni* (Miller and Rabaglia 2009).

The ability of (–)- $\alpha$ -pinene to reduce attraction of selected ambrosia beetles to ethanol may be related to host range and host quality preferences. *A. sayi*, *X. politus*, and *X. saxeseni* are recognized as colonizers of physiologically stressed or dying hardwood trees, which are known emitters of ethanol (Kimmerer and Kozlowski 1982, Solomon 1995, Kelsey and Joseph 2001). (–)- $\alpha$ -Pinene emissions are elevated from physically damaged and recently felled trees, but ethanol emissions would be comparatively low (Bertin and Staudt 1996). *X. germanus* also attacks stressed hosts but has been reported to colonize apparently healthy trees (Weber 1982, Grégoire et al. 2001). The

ability of (–)- $\alpha$ -pinene to enhance or have a negligible effect on *X. germanus* attraction to ethanol may be due to a more aggressive host selection behavior.

It is currently unclear what factors contributed to the year-to-year intraspecific variability in the effects of (–)- $\alpha$ -pinene on ambrosia beetle attraction to ethanol-baited traps. Other studies have documented intraspecific variability across sampling locations in the effects of (–)- $\alpha$ -pinene on attraction to ethanol by ambrosia and other wood-boring beetles (Miller 2006, Miller and Rabaglia 2009). Differences in ambrosia beetle populations between years and trapping locations may have contributed to inconsistent effects. Low sample sizes could also have resulted in nonsignificant differences being detected when significant differences truly existed (Miller and Rabaglia 2009). Ambient air temperature also may have contributed to observed variability in the effects of (–)- $\alpha$ -pinene on



ambrosia beetle attraction to ethanol (Czokajlo and Teale 1999), but these data were not recorded as part of our study.

Our findings also demonstrated a positive concentration response to ethanol by *A. sayi* and *X. germanus*, which has not been reported previously for *A. sayi*. Klimetzek et al. (1986) tested ethanol release rates from 0.024 mg/d to 6 g/d and also demonstrated a positive concentration response by *X. germanus* and *X. saxeseni*. Additional studies are warranted to determine the uppermost limit of ethanol lure emission for attracting ambrosia beetles as part of detection and monitoring programs. However, too high of a release rate could have a repellent effect on ambrosia beetle orientation (Salom and McLean 1990). Montgomery and Wargo (1983) found a 2 g/d ethanol release rate was more attractive to Scolytinae than higher release rates. Similarly, Bakke (1983) demonstrated an  $\alpha$ -pinene release rate of 30 mg/d significantly increased the attraction of *T. lineatum* to its pheromone, but a release rate of 100 mg/d reduced trap captures.

Due to the ability of  $(-)\alpha$ -pinene to reduce ambrosia beetle attraction to ethanol, our study indicates that traps baited with ethanol alone are more effective than ethanol plus  $(-)\alpha$ -pinene for monitoring flight activity in ornamental nurseries. However, recent studies have indicated companion traps baited with ethanol plus  $(-)\alpha$ -pinene could be useful for detecting recent introductions of exotic Scolytinae (Miller and Rabaglia 2009, Gandhi et al. 2010). Ethanol release rates  $\geq 0.39$  g/d also should be used to maximize trap attractiveness. Additional studies are warranted to determine the influence of lower concentrations of  $(-)\alpha$ -pinene ( $<0.0015$  g/d) on ambrosia beetle attraction to ethanol, along with the role of enantiomeric composition.

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