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CAPTURE OF *CNESTUS MUTILATUS*, *XYLOSANDRUS CRASSIUSCULUS*, AND OTHER SCOLYTINAE (COLEOPTERA: CURCULIONIDAE) IN RESPONSE TO GREEN LIGHT EMITTING DIODES, ETHANOL, AND CONOPHTHORIN

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The camphor shot borer, *Cnestus mutilatus* (Blandford) (Coleoptera: Curculionidae: Scolytinae), is an ambrosia beetle of Asian origin (Schiefer & Bright 2004). It was first detected in the United States within Oktibbeha County, Mississippi in 1999 (Schiefer & Bright 2004). In Asia, *C. mutilatus* is known to attack many hardwood species, including *Acer*, *Albizzia*, *Carpinus*, *Castanea*, *Cornus*, *Fagus*, *Lindera*, *Osmanthus*, and *Swietenia* spp. (Wood & Bright 1992, Schiefer & Bright 2004). In the United States, *C. mutilatus* has undetermined pest potential, but has been found in Alabama, Arkansas, Florida, Georgia, Louisiana, North Carolina, Ohio, Tennessee, Texas, and West Virginia (Gandhi et al. 2009; Oliver et al. 2012). The granulate ambrosia beetle, *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae: Scolytinae), was first detected in the United States near Charleston, South Carolina (Anderson 1974). *Xylosandrus crassiusculus* is now found within the northeastern, mid-Atlantic, southeastern, southern, midwestern, and northwestern United States (Ree & Hunter 1995), and can attack more than 120 hosts, ranging from hardwood to pine (Hudson & Mizell 1999; Oliver & Mannion 2001).

Olfaction plays an important role during host-location by ambrosia beetles. Due to its emission from living, but weakened trees, ethanol acts as an important attractant for *X. crassiusculus* and a number of other ambrosia beetles (Oliver & Mannion 2001; Ranger et al. 2010, 2012, 2013; Kelsey et al. 2013). Ethanol-baited traps are commonly used for detecting and monitoring ambrosia beetles, and efforts are underway to identify compounds that enhance trap attractiveness. One promising compound, conophthorin, is associated with the bark of a variety of angiosperm trees and is also produced by several Scolytinae (Huber et al. 2001). Conophthorin disrupts the response of several bark beetles (Huber et al. 2001), but recent studies by Dodds & Miller (2010) found it increased the attractiveness of trap trees to *Xylosandrus germanus* (Blandford).

In addition to olfactory cues, visual cues play a role in host location by various Scolytinae (Campbell & Borden 2009) and may be useful for improving trap attractiveness to ambrosia beetles. For example, Mathieu et al. (1997) demonstrated the preference of *Hypothenemus hampei* for red or white traps depending on the release rate of trap volatiles, and recently Mayfield & Brownie (2013) documented that *Xyleborus glabratus* uses stem silhouette diam as a host-seeking cue. Gorzlancky et al. (2013) demonstrated that ethanol-baited traps supplemented with green (525 nm) light-emitting diode (LED) bulbs were more attractive to Scolytinae than traps baited only with ethanol.

We conducted a field-based trapping study to test the ability of conophthorin and green LEDs to enhance the attractiveness of ethanol-baited traps to *X. crassiusculus* and *C. mutilatus*. Three trap treatments were tested, namely: (1) ethanol alone, (2) ethanol plus conophthorin, and (3) ethanol, conophthorin, and green LEDs. The test used Lindgren-type traps (Contech Inc. Victoria, British Columbia, Canada) consisting of 8 black opaque funnels with a detachable white plastic collection cup at the base. Traps were suspended from shepherd's hooks, resulting in a height of ~1.2 m above ground. A mixture of water and liquid dish detergent (100:1; v:v) (Joy, Procter & Gamble, Cincinnati, Ohio) was added to the bottom of the collection cup to subdue and kill the entering beetles.

An 8 dram (29.5 mL) clear glass vial containing 28 mL of ethanol (95%) (EMD Chemicals Inc. Darmstadt, Germany) and a cotton wick (8 cm × 2 cm, Cotton American Fiber and Finishing Inc., St. Albemarle, North Carolina) held in place with parafilm (Bemis Flexible Packaging, Neenah, Wisconsin) was fastened to the top funnel of each Lindgren trap. Average release rate for ethanol was 3.8 g per day at 25 °C. Ethanol test solutions were replaced every 3 days during the experiment.

Conophthorin was emitted from eppendorf tubes sealed with a permeable cap containing

250 μL of test substance (Contech Inc. Victoria, British Columbia, Canada), which were fastened adjacent to the ethanol vial. The release rate of conophthorin was about 0.5 mg per day at 25 °C and replenishment was not necessary.

Four, 1.5 watt green LEDs (Boesch Built LLC, Waterford Township, Michigan) were spaced equally along the top rim of the Lindgren funnel traps. LEDs were powered continuously throughout the entire duration of the experiment by a 6 volt battery (McMaster-Carr ® Elmhurst, Illinois) that was fastened directly underneath the trap.

Traps were deployed in a randomized complete block design in Tuskegee National Forest, Macon County in Alabama ($N 32^{\circ} 29' 19'' W 085^{\circ} 35' 39''$) from 14-22-Apr-2012. Traps within the block were 6 m apart, and the trap site was characterized by a mixture of partially-shaded pine and hardwood trees. Traps were returned to the laboratory at the end of the field experiment for specimen identification. *Xylosandrus crassiusculus* and *C. mutilatus* were identified to species, but the remaining Scolytinae specimens were grouped as 'other Scolytinae'. There were 5 replicates per treatment with each collection counting as a replicate.

A Kruskal-Wallis non-parametric test ($P < 0.05$; SAS 2003) was used to detect significant differences among treatments with mean separations performed using a Wilcoxon Rank Sum test ($P < 0.05$).

Ethanol-baited traps supplemented with conophthorin and green LEDs captured significantly more *X. crassiusculus* ($Z = 2.3$, $df = 2$, $P = 0.02$) (Wilcoxon) than traps baited with ethanol alone (Fig. 1), but no difference was detected between traps baited with ethanol plus conophthorin. Likewise, no differences in *X. crassiusculus*

were detected between ethanol plus conophthorin and ethanol alone. Similarly, traps integrating ethanol, conophthorin, and green LEDs captured significantly more other Scolytinae ($Z = 2.1$, $df = 2$, $P = 0.036$) than traps baited with ethanol alone (Fig. 1), but no difference was detected between traps baited with ethanol plus conophthorin and traps baited with only ethanol. No difference in *C. mutilatus* counts was detected among any trap treatments ($df = 2$, $P = 0.74$) (Kruskal-Wallis) (Fig. 1). Captures of *X. crassiusculus* ($r = 0.53$, $P = 0.044$) and *C. mutilatus* ($r = 0.61$, $P = 0.016$) were significantly correlated with total Scolytinae captured. Therefore, integrating green LEDs and conophthorin improved overall Scolytinae trap captures, which improved sampling of scolytine beetles, including the two target species. The integration of specific LED wavelengths into other trap configurations could increase captures by providing a potential synergistic visual cue.

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SUMMARY

A field test was conducted using Lindgren traps baited with (1) ethanol, (2) ethanol plus conophthorin, and (3) ethanol, conophthorin, and green LEDs (525 nm) to compare their efficacy for capture of bark and ambrosia beetles. Captures of *Cnestus mutilatus* were similar with all 3 treatments. Captures of *Xylosandrus crassiusculus* and other Scolytinae were significantly higher

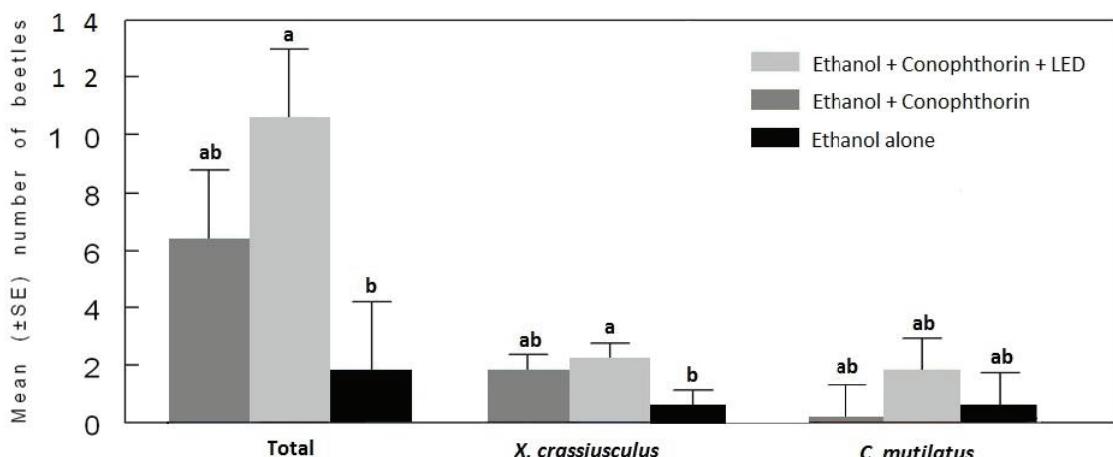


Fig. 1. Mean (\pm SE) ambrosia beetle adults collected with traps baited with ethanol alone, ethanol plus conophthorin, and ethanol, conophthorin, plus green LED lights (525 nm). Traps were deployed from 14-22-Apr-2012 in Tuskegee National Forest, Alabama, USA. Means with different letters indicate significant differences within a species (Wilcoxon; $P < 0.05$).

with ethanol-baited traps supplemented with conophthorin and green LEDs, as compared to traps baited with ethanol alone. Results of this study indicate that detection and monitoring of pest Scolytinae may be improved by incorporating conophthorin and green LEDs into the standard ethanol-baited Lindgren trap.

Key Words: attractants, LED, ethanol, *Cnestus mutilatus*, *Xylosandrus crassiusculus*, Scolytinae, traps.

RESUMEN

Se utilizaron trampas para determinar el atractivo de los 3 diferentes tratamientos sobre la corteza y escarabajos ambrosías (Coleoptera: Curculionidae: Scolytinae): trampas cebedas sólo con etanol, trampas con etanol y conophthorin, y trampas con etanol, conophthorin y LED verde. Las trampas cebedas con etanol integradas con conophthorin y LED verde (525 nm) fueron más atractivas para *X. crassiusculus* y otros Scolytinae que las trampas cebedas con solo etanol. La captura de *Cnestus mutilatus* no fue afectada por la adición de conophthorin, o de conophthorin y LED verde en trampas cebedas con etanol. Este estudio indica que trampas cebedas con etanol y suplementadas con el conophthorin y LED verde pueden ser útiles para mejorar el atractivo de *X. crassiusculus* y otros Scolytinae hacia las trampas cebedas con etanol.

Palabras Clave: atrayentes, LED, etanol, *Cnestus mutilatus*, *Xylosandrus crassiusculus*, Scolytinae, trampas.

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