Variation in Manuka Oil Lure Efficacy for Capturing *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae), and Cubeb Oil as an Alternative Attractant

JAMES L. HANULA,[1,2] BRIAN T. SULLIVAN,[3] AND DAVID WAKARCHUK[4]

ABSTRACT Redbay ambrosia beetle, *Xyleborus glabratus* Eichoff, is an exotic species to North America vectoring a deadly vascular wilt disease of redbay [*Persea borbonia* (L.) Spreng], swampbay [*P. palustris* (Raf.) Sarg.], avocado (*P. americana* Mill.), and sassafras [*Sassafras albidum* (Nutt.) Nees]. *Xyleborus glabratus* is attracted to manuka oil lures, which are commercially available, and phoebe oil. Variable efficacy of manuka oil lures and insufficient availability of phoebe oil prompted us to investigate the reasons behind changes in manuka oil lure efficacy and to test cubeb oil, a readily available essential oil from *Piper cubeba* L. seeds, as an alternative attractant. Attraction, release rates and durations, and volatile composition of manuka oil lures manufactured in 2008 were compared with manuka oil lures manufactured in 2012, and to whole and a distilled fraction of cubeb oil. Manuka oil lures from 2008 were more attractive to *X. glabratus* than controls for 8 wk, whereas lures from 2012 were attractive for only 2 wk. Cubeb oil and the distilled fraction of it were as attractive as or more attractive than manuka oil in three trials. In gravimetric studies, manuka oil lures from 2008 and cubeb oil lures continued to release volatiles for 57 d, whereas lures from 2012 stopped after 16 d. The chemical composition of volatiles released from new manuka oil lures from 2008 was similar to 2012; however, a preservative (butylated hydroxytoluene) was detected in the 2008 lures. Cubeb oil was an effective attractant for *X. glabratus* that lasted 8–9 wk when released from bubble lures.

KEY WORDS manuka oil, laurel wilt, *Piper cubeba*, α-copaene, essential oils

Redbay ambrosia beetle, *Xyleborus glabratus* Eichoff, is an exotic species to North America that vectors the fungus, *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva, causing a deadly vascular wilt disease of redbay [*Persea borbonia* (L.) Spreng], swampbay [*P. palustris* (Raf.) Sarg.], avocado (*P. americana* Mill.), and sassafras [*Sassafras albidum* (Nutt.) Nees] (Fraedrich et al. 2008, Hanula et al. 2008, Harrington et al. 2008, Mayfield et al. 2008). Since its discovery in 2002 near Savannah, GA (Rabaglia et al. 2006), it has spread rapidly through redbay populations and its range now extends from North Carolina to southern Florida and westward to Mississippi (USDA FS 2012). Upon entering a new area the beetle and its symbiotic fungus kills most redbay trees within 2 yr (Fraedrich et al. 2008) and all trees larger than 5 cm in diameter within ≈6–8 yr (J.L.H., unpublished data).

Both manuka oil, an extract of *Leptospermum scoparium* Forst. & Forst. (family Myrtaceae) from New Zealand, and phoebe oil, extracted from *Ocotea porosa* (Nees & Martius) Barroso (family Lauraceae) trees in Brazil, are attractive to the beetles and have performed well in trapping trials in Georgia and South Carolina (Hanula and Sullivan 2008, Hanula et al. 2011). Recently, questions about the efficacy of manuka oil have been raised and phoebe oil has been suggested as an alternative (Kendra et al. 2012). However, the supply of phoebe oil is limited both by insufficient production facilities and the rarity of phoebe trees in natural areas of Brazil where it is considered vulnerable to overharvesting (International Union for Conservation of Nature 2012). In light of the difficulties surrounding manuka and phoebe oil lures, an alternative that is effective and readily available is clearly needed.

An effective, economical, and reliable bait for *X. glabratus* is a critical tool for monitoring the beetle as it spreads outside of the range of redbay into areas where sassafras is the only available host. To date, monitoring the spread of laurel wilt in areas where redbay is present has been accomplished by scouting for dead trees that are easily recognized by their reddish brown foliage, which remains on the tree for a year or more. Conversely, sassafras trees die and lose their leaves quickly making detection more difficult. In addition, a lure more attractive to *X. glabratus* than those currently available is highly desirable for use in

potential control strategies and for detection of new arrivals at ports of entry. The latter is particularly important in warm tropical and temperate areas of the world where Lauraceous trees are more diverse and an important component of the forests (Rohwer 2000).

Hanula and Sullivan (2008) suggested that α-copaene is the attractive compound in redbay wood and manuka oil and Niogret et al. (2011) supported this finding. Cubeb oil is a readily available essential oil extracted from *Piper cubeba* L. (family Piperaceae) berries that contains a significant proportion of α-copaene (Singh et al. 2007). Therefore, we tested cubeb oil in field trials and compared it to manuka oil lures manufactured in 2008 and 2012 for attraction to redbay ambrosia beetle. These comparisons were conducted to determine if manuka oil lure quality changed recently since Kendra et al. (2011, 2012) and Brar et al. (2012) reported lures were only lasting 2 wk in Florida, whereas trials in Georgia and South Carolina with lures manufactured in 2008 suggested they lasted 6–8 wk (Hanula et al. 2011). In addition, we examined the chemical composition, release rate, and duration of lures containing cubeb and manuka oil.

Methods and Materials

Field trials were conducted in 2011 and 2012 at a forest site near Oak Park, GA composed primarily of redbay and loblolly bay [*Gordonia lasianthus* (L.) Ellis] with occasional remnant loblolly pines (*Pinus taeda* L.). The area contained numerous redbay trees that were dying from laurel wilt. The upland edge of this area had a distinct sharp border with a loblolly pine plantation, and a mixed turkey oak (*Quercus cerris* L.) and longleaf pine (*Pinus palustris* Mill.) stand. A grid of trap positions was established in the upland forest adjacent to the forest containing the redbay. Before setting up traps in 2011, a few small (<5 cm diameter at breast height), dead, and dying redbay were removed from the trapping area so there were no infested trees. The trapping grid consisted of rows of trap positions parallel to the edge of the redbay forest that were spaced 30 m apart within rows that were spaced at 30-m intervals throughout the adjacent forest. The first row was at the boundary between the two forest types. At each trap position a rope was strung between two trees so that the middle funnel of a single 8-unit Lindgren multiple funnel trap suspended from it was supported ≈1–1.5 m above the ground. Collection cups on the traps were filled with antifreeze containing propylene glycol.

All lures were manufactured by Synergy Semiochemicals Corp. (SSC), Burnaby, BC. Manuka oil and Colure were products of Coast Biologicals (Bombay, South Auckland, New Zealand). Colure is a trade name for a manuka oil product that has had much of the triketones removed and therefore is enriched in sesquiterpenes.

Manuka Oil Lure Age and Placement—Trial 1. We tested the attraction of half-size manuka oil lures (Fig. 1; 7.5- by 8-cm plastic pouches with an internal cellulose matrix soaked with 2 ml of manuka oil, SSC
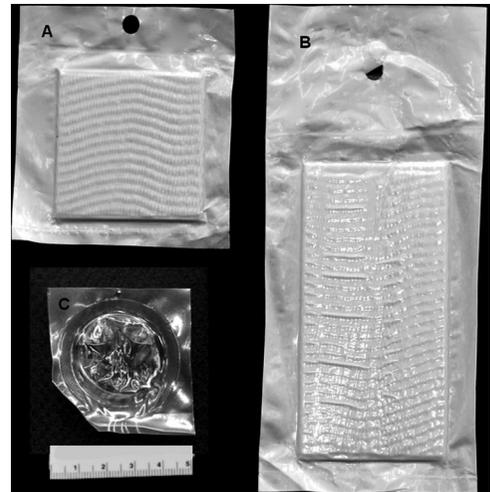

Fig. 1. Examples of lures used in the trials: A) manuka oil half lure, B) manuka oil whole lure, and C) cubeb bubble lure. In all trials, bubble lures were placed in pouches identical to the whole lure pouches (except they were open at the top) to facilitate hanging lures in traps. The scale is 5 cm. (photo by E. Vallery).

product 3165) that had been aged in the field for 30 d from 18 September to 18 October 2011, and compared these to new lures. Field-aged lures were included to determine if they were still attractive because trapping recommendations for *X. glabratus* suggested using manuka oil lures for 2 mo, but Kendra et al. (2011) found they were not attractive that long. In addition, we examined whether altering the site of attachment of a fresh lure on the trap might alter lure effectiveness. Six treatments were tested: 1) a new lure placed inside the middle funnel of the 8-funnel trap, 2) outside the middle funnel, 3) or inside the bottom funnel, 4) an old lure placed inside the middle, 5) or inside the bottom funnel, and 6) a control trap without a lure. Samples were collected biweekly and traps with new lures received a fresh lure at each collection. Traps were rotated one position within rows on each collection date and the experiment was blocked by distance from the source of redbay ambrosia beetles with the first block located 30 m from the redbay forest edge. The experiment consisted of six replicated blocks and ran from 18 October to 30 November 2011.

Differing Manuka Lures and Cubeb Lure—Trial 2. In 2012 we tested a series of lures containing α-copaene (Fig. 1). Lure treatments were 1) 2-ml distilled cubeb oil in a 29-mm-diameter bubble releaser (SSC product 3087), 2) 2 ml Colure in a 29-mm-diameter bubble releaser (SSC product 3086), 3) 2-ml manuka oil in a 29-mm-diameter bubble releaser (SSC product 3085), 4) a standard full-size manuka oil lure (7.5- by 12.5-cm plastic pouch sealed at the ends containing a cellulose matrix soaked with 4 ml of manuka oil; SSC product 3083), and 5) an unbaited control. Bubble lures were placed in plastic pouches, supplied by the manufacturer, that were identical to those pouches used to manufacture manuka oil lures, but they con-

tained small punctures for water to drain out after rain events and the top of the pouch was left unsealed. The pouches made it easier to hang the lures on the traps. All lures were hung inside the middle funnels of 8-funnel Lindgren traps, which were deployed at the same trapping positions as the previous trial and rotated one position within rows each week when the samples were collected. The experiment consisted of six replicated blocks and was conducted from 21 June to 19 July.

Manuka Oil Lures from 2008 versus 2012—Trial 3. We investigated the longevity of distilled cubeb oil bubble lures in comparison to manuka oil lures and determined if manuka oil lure efficacy had changed over time. *Xyleborus glabratus* does not respond to changes in release rates from 5 to 200 mg/d of essential oils (Hanula and Sullivan 2008, Hanula et al. 2011), so we used the cubeb oil lures from trial 2 to determine how long those lures might last and how they compared with new manuka oil lures. Lure treatments were 1) distilled cubeb oil bubble lures from the previous trial that had already been deployed in the field for 1 mo at the beginning of the current trial; 2) newly opened, unused, full-size manuka oil lures purchased in spring 2012; 3) newly opened, unused, full-size manuka lures purchased in 2008; and 4) an unbaited control. Lures from 2008 were in their original, unopened, aluminum foil pouches that had been stored at −60°C since 2008. The experiment was set up the same as the previous trial by using 8-funnel Lindgren traps. Traps were checked weekly and moved one position within lines each week. The experiment was replicated six times and traps were operated from 19 July to 23 August 2012.

Duration of Lure Activity—Trial 4. We compared magnitude and longevity of attraction of six lure treatments: 1) distilled cubeb oil and 2) whole cubeb oil in bubble releasers, full-size manuka oil lures from 3) 2008 and 4) 2012, 5) half-size manuka oil lures purchased in 2011, and 6) an unbaited control. Distilled cubeb has higher quantities of α-copaene than whole cubeb oil or manuka oil. All lures were stored at −60°C until the trial began on 23 August 2012. The experiment consisted of six replicates and was conducted in the same area by using the same traps as previous trials. Catches were collected weekly for 8 wk to determine the longevity of the various lures.

Chemical Analyses of Lures. A subsample of unused release devices deployed in trials 2–4 were shipped to Pineville, LA and stored separately by lure type in Mylar pouches in a freezer at −13°C for up to 7 wk. Before sampling, lures were placed individually into wide-mouth Erlenmeyer flasks (500 ml) and permitted to equilibrate in a fume hood for ≈15 min. Afterward, an absorbent cartridge (PFA tubing [1.6 mm inside diameter, 5.5 cm in length] containing 0.1-g Porapak Q [50–80 mesh, Grace Chromatography, Columbia, MD] secured with silanized glass wool plugs) attached to a piece of flouropolymer tubing was inserted into the flask so that the opening of the cartridge was <1 cm from the bottom of the flask but not in direct contact with the lure. The mouth of the flask then was stopped with a 3–4-cm-wide piece of acti-

vated charcoal mesh (Universal Replacement Prefilter, HRF-AP1, Honeywell, Southborough, MA) that was rolled repeatedly around the tubing to form a cylindrical plug; this plug allowed air to be replaced inside the flask although limiting the incursion of outside odors. The end of the tubing exiting each flask was attached to a vacuum pump, and air was drawn through the cartridges at ≈30 ml/min for 60 min at 25°C. Twelve lures (as determined by availability: six 2012 manuka lures [two from the manufacturer lot used in trapping trial 2, and four from the lot used in trials 3 and 4], four manuka lures from 2008 [same lot as all trapping trials], and two distilled cubeb lures [same lot as all trapping trials]) were sampled simultaneously. Tests performed by placing sampling cartridges in series indicated >99% absorption of lure volatiles by a single cartridge. Cartridges were stored at −80°C and within 3 d were extracted at room temperature with 1.2-ml redistilled pentane (determined to be a quantity sufficient to desorb >99% of volatiles). In addition, 1 μl of distilled and whole cubeb oil (supplied by Synergy Semiochemicals) was each diluted in 1-ml hexane. Each sample was spiked with 38-μg cycloheptanone as an internal standard and a 1-μl portion of each was analyzed in split mode on a Hewlett–Packard (Palo Alto, CA) G1800C coupled gas chromatograph-mass spectral detector (GC-MS) fitted with an HP-INNOWax (Agilent Technologies; 60-m by 0.25-mm by 0.25-μm film) column. The temperature program was 40°C for 1 min, 16°C/min to 80°C, then 7°C/min to 230°C and held for 7 min; the injector and detector ports were 200 and 240°C, respectively. Compounds were identified by mass-spectral and retention-time matches to identified standards (unless noted otherwise) and the absolute quantity of alpha-copaene in each sample was determined from response curves calculated by analyzing a sequence of dilutions of the compound (Fluka Inc., Buchs, Switzerland) spiked with an identical concentration of internal standard as present in the cartridge extracts. Total ion chromatogram peaks in manuka lure samples were matched by retention time and mass spectrum, and the abundance (relative to the internal standard) of all peaks representing >0.2% the total ion abundance in the 2008 manuka lures were contrasted against the other lures. This quantitative contrast assumed linear dose–response by the GC-MS peak integrator, and thus the results were approximate.

Lure Release Rates. Manuka oil lures release an average of ≈50 mg/d in a 37-liter chamber at a constant 20°C with an air flow of 250 liters/min (D. W., unpublished data), but average daily temperatures in the southern United States exceed 20°C for at least 6 mo/yr, with average high temperatures during the summer of 30°C or higher. Therefore, we collected data on manuka and cubeb lure release rates and life expectancies under local conditions to predict an appropriate frequency for lure replacement within traps. We gravimetrically tested release rate and duration of bubble releasers containing cubeb oil or distilled cubeb oil, and 2008 and 2012 full-size manuka lures, and 2011 half-size manuka oil lures in Athens, GA. Two

Table 1. Mean number of redbay ambrosia beetles, *X. glabratus*, captured per trapping period in 8-funnel Lindgren traps baited with either new or 30-d-old lures placed at different positions on the trap

Lure	Position	Oct. 18–Nov. 3		Nov. 3–16		Nov. 16–30	
		No.	$\bar{x} \pm SE^a$	No.	$\bar{x} \pm SE^a$	No.	$\bar{x} \pm SE^a$
None		6	4.2 ± 2.2a	6	0.5 ± 0.2a	6	1.7 ± 0.9a
Old lure	Inside middle funnel	6	8.3 ± 3.1ac	6	0.5 ± 0.3a	6	5.5 ± 2.7a
	Inside bottom funnel	6	3.83 ± 3.1a	6	0.5 ± 0.3a	6	1.7 ± 0.6a
New lure	Inside middle funnel	4	45.2 ± 11.1b	6	12.5 ± 1.9b	6	84 ± 15.9b
	Outside middle funnel	6	27.8 ± 11.3bc	6	8.7 ± 3.3b	6	29.8 ± 5.5bc
	Inside bottom funnel	6	21.7 ± 6.5bc	6	6.7 ± 1.5b	6	20.5 ± 4.0c
		$F_{5,23} = 7.31$		$F_{5,25} = 25.81$		$F_{5,25} = 27.61$	
		$P = 0.0003$		$P < 0.0001$		$P < 0.0001$	

^{a} Means followed by the same letter are not significantly different at $\alpha = 0.05$ according to the REGWQ multiple comparison test. Data were log transformed for analysis but untransformed means are presented.

lures of each type were tested. Bubble lures were placed in plastic pouches that were identical to those pouches used in field trials. Lures were hung by small binder clips, so they were not touching each other or other objects, beneath an insectary porch roof where they were exposed to normal outdoor temperatures but protected from rain and direct sunlight. All lures were weighed before being hung on the porch and then weighed daily for an 8-wk period or until weight loss ceased (i.e., was not detected for 2 wk). Temperature was measured with an Extech datalogger (model RH110, Knoxville, TN) located in the same area as the lures. The weight of each lure was subtracted from its weight the previous day and the difference divided by the time between weighings to obtain mean weight change per hour. Weight change per hour was multiplied by 24 to obtain weight change per day.

Statistical Analyses. Field trials were randomized complete block experiments with treatments blocked by distance from the redbay forest. Trap catch data were log $(x + 1)$ or square root $(x + 0.5)$ transformed to reduce heteroscedasticity and ensure a normal distribution before analysis of variance by using the general linear models procedure in the SAS statistical package (PROC GLM, SAS version 8.1, SAS Institute, Cary, NC). Means were separated using the Ryan–Einot–Gabriel–Welch quotient multiple comparison test (REGWQ, SAS version 8.1, SAS Institute, Cary, NC).

Results

Manuka Oil Lure Age and Placement—Trial 1. During the first trapping period (October 18-November 3), traps with old lures were no more effective at capturing *X. glabratus* than unbaited control traps and this trend continued throughout the experiment (Table 1). Likewise, traps with new lures were more effective than controls in all trapping periods and they were more effective than all old lure treatments in two of the three trapping periods. In the first trapping period, however, old lures placed inside the middle funnel were not significantly different from two of the new lure treatments. The position of new lures on traps had little effect on trap captures, except during the third trapping period, when traps with new lures placed inside the middle funnel captured more beetles than those with lures placed inside the bottom funnel.

Differing Manuka Lures and Cubeb Lure—Trial 2. Bubble lures containing a distilled fraction of cubeb oil were more attractive to redbay ambrosia beetle than any other tested lure (Fig. 2; $F_{4,20} = 35.04$, $P < 0.0001$). Bubble caps containing Colure caught significantly more beetles than the control or full size manuka oil lures. Bubble lures containing whole manuka oil trapped more beetles than the controls, but the full-size manuka oil pouch lures were not attractive (i.e., they did not capture significantly more beetles than control traps).

Manuka Oil Lures From 2008 Versus 2012—Trial 3. Failure of the pouch type manuka oil lures in the previous trial was surprising because this lure construction and formulation was highly attractive in previous tests (Hanula and Sullivan 2008, Hanula et al. 2011). Therefore, we tested manuka oil lures purchased in 2008 (same lot used in Hanula and Sullivan 2008) against those purchased in 2012 (as were the unattractive manuka pouch lures of trial 2), and compared these to the distilled cubeb oil lures from the previous trial. Initially, during the first week of the trial ($F_{3,15} = 3.97$, $P = 0.029$), only the distilled cubeb lures caught more than controls, but the 2008 and 2012 manuka oil lures did not catch significantly fewer beetles than distilled cubeb oil lures (Fig. 3). In week 2 all lures caught significantly more than the controls and there was no difference among lure types ($F_{3,15} = 9.7$, $P = 0.0008$). During weeks 3–5, the 2012 but not the 2008 manuka oil lures caught significantly fewer beetles than distilled cubeb oil (week 3, $F_{3,15} = 10.03$, $P = 0.0007$; week 4, $F_{3,15} = 14.94$, $P < 0.0001$; week 5, $F_{3,15} = 8.58$, $P = 0.0015$). Conversely, the 2012 manuka oil lures were not more effective than controls in weeks 4 and 5, and the 2008 manuka oil lures were not significantly different than controls during week 5. Distilled cubeb lures attracted more beetles in week 5 than the controls indicating they were still attractive to beetles after nine weeks in the field because the same lures had been used in the previous trial. When trap captures were summed for the entire study ($F_{3,15} = 22.8$, $P < 0.0001$), traps baited with distilled cubeb oil in bubble releasers caught significantly more beetles than manuka oil lures and all types of lures caught significantly more redbay ambrosia beetles than the control traps.

Duration of Lure Activity—Trial 4. During week 1 of the trial ($F_{5,25} = 12.41$, $P < 0.0001$), traps baited with
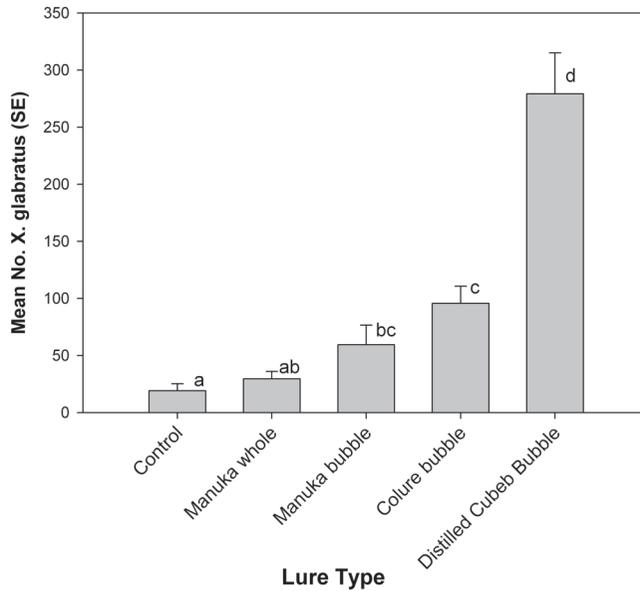
Fig. 2. Mean number of redbay ambrosia beetles, *X. glabratus*, captured in 8-funnel Lindgren traps baited with various lures from 21 June to 19 July 2012 near Oak Park, GA. Bars with the same letter are not significantly different ($\alpha = 0.05$) according to the REGWQ multiple comparison test. Data were log transformed for analysis but untransformed means are presented.

whole cubeb oil and the distilled fraction of cubeb oil in bubble releasers caught significantly more beetles than the 2012 manuka oil lure and unbaited controls but not the half-size manuka oil lure and the 2008 manuka oil lure (Fig. 4). The 2012 manuka oil lures did not catch significantly more than the controls. In week 2 all lure types caught similar numbers of beetles and all caught more than control traps ($F_{5,25} = 21.18$, $P < 0.0001$). However, for the remaining 6 wk of the experiment both the manuka half lure and the 2012 manuka lure failed to catch more beetles than the unbaited control (week 3, $F_{5,25} = 4.24$, $P = 0.0063$;

week 4, $F_{5,25} = 4.89$, $P = 0.003$; week 5, $F_{5,25} = 10.12$, $P < 0.0001$; week 6, $F_{5,25} = 10.99$, $P < 0.0001$; week 7, $F_{5,25} = 10.39$, $P < 0.0001$; week 8, $F_{5,25} = 21.08$, $P < 0.0001$). In contrast, the two cubeb lures and the 2008 manuka lure continued to attract significant numbers of beetles through week 8 (with the exception that the distilled cubeb failed to catch significantly more than the control in week 3), and these three lures did not differ significantly from each other during any single week for the entire duration of the experiment. Total catches for the 8 wk study showed that distilled cubeb oil in bubble releasers caught more *X. glabratus* than
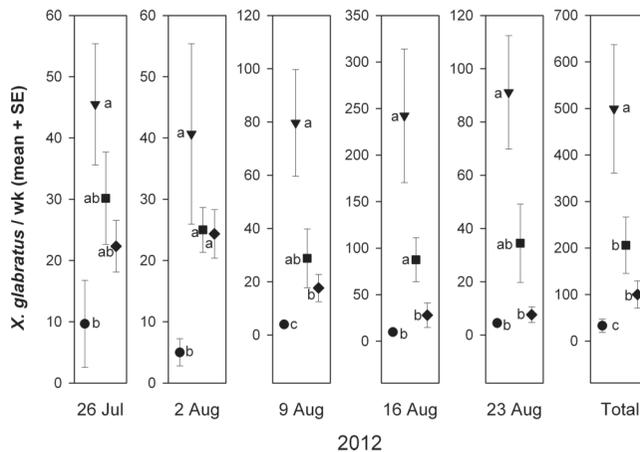

Fig. 3. Mean number of redbay ambrosia beetles, *X. glabratus*, caught per week in 8-funnel Lindgren traps baited with either distilled cubeb oil (▼), manuka oil lures from 2008 (■), manuka oil lures from 2012 (♦), or no lure (●). Traps were operated from 19 July to 23 August 2012. Within graphs, symbols with the same letter beside them are not significantly different ($\alpha = 0.05$) according to the REGWQ multiple comparison test. Data were log transformed for analysis but untransformed means are presented.
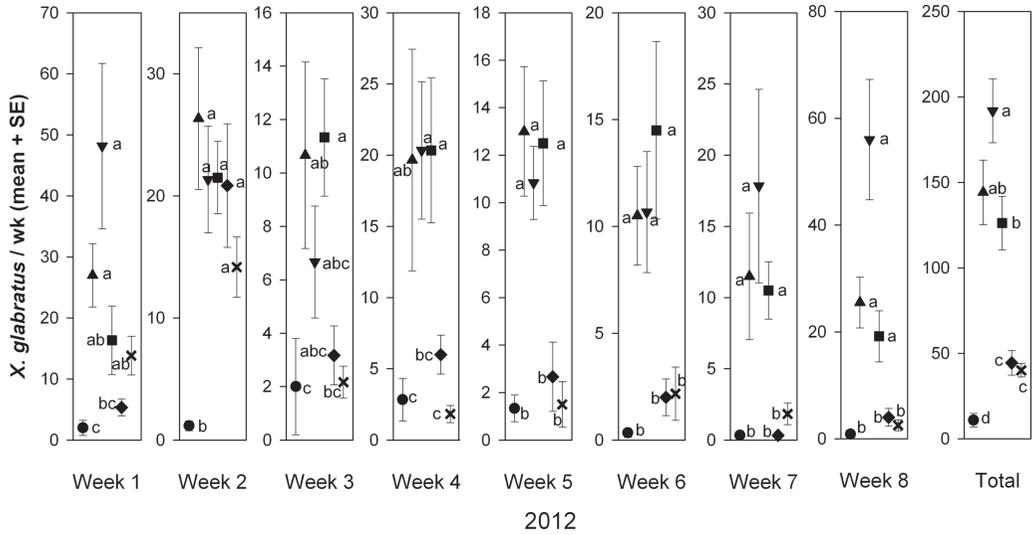
Fig. 4. Mean number of redbay ambrosia beetles, *X. glabratus*, caught per week in 8-funnel Lindgren traps baited with either cubeb oil (▲), Distilled cubeb oil (▼), manuka oil lures from 2008 (■), manuka oil lures from 2012 (◆), half-size manuka oil lures (×), or no lure (●). Traps were operated from 23 August to 17 October 2012. Within graphs, symbols with the same letter beside them are not significantly different ($\alpha = 0.05$) according to the REGWQ multiple comparison test. Data were log transformed for analysis but untransformed means are presented.

2008 manuka oil lures but not significantly more than whole cubeb oil lures, whereas the latter two lure types caught similar numbers of beetles ($F_{5,25} = 57.54$, $P < 0.0001$). Half-sized manuka oil lures and lures from 2012 captured fewer beetles than other lures but both caught more than unbaited control traps.

Lure Release Rates. Full size manuka oil lures from 2008 released nearly 400 mg in the first day as did full size lures from 2012 (Fig. 5). Half size manuka oil lures released ≈170 mg during the first 24 h. Full-size lures from 2012 and half-size manuka oil lures continued to lose weight through day 14, after which both reached zero and remained there for an additional 14 d so they were no longer weighed. In contrast, the 2008 manuka oil lures eluted 113 mg/d on day 14 and continued to lose between 25 and 70 mg/d through day 27; at the end of the experiment (day 58) they released 7.6 mg/d.

Bubble lures containing cubeb oil or a distilled fraction of cubeb oil had release rates of 30–45 mg/d initially and then both exhibited a steadily declining weight loss over time through day 57, when they each eluted ≈5.0 mg/d. Distilled cubeb oil lures lost more weight per day than cubeb oil.

Lure Chemical Composition. We quantified α-copaene in lures specifically because this sesquiterpene is suspected to be a key active constituent of the attractive essential oil baits for *X. glabratus*. The quantities (μg, mean $\pm$ SD) of alpha-copaene recovered from fresh lures during a 1-hr aeration were similar: 49 ± 1 (2012 full-size manuka lure used in trapping trial 2, $n = 2$), 64 ± 8 (2012 full-size manuka lure used in trapping trials 3 and 4, $n = 4$), 56 ± 7 (2008 full-size manuka lure, $n = 4$), and 49 ± 36 (Colure bubble cap, $n = 2$). The two 2012 lots of manuka oil lures did not differ in composition from the 2008 lures qualitatively (i.e., in terms of the presence or absence of any com-

pound that composed >0.2% of total total ion chromatogram peak abundance) except for the preservative butylated hydroxytoluene (BHT), which was detected in small quantities only in the 2008 lures, and small quantities of an unidentified compound present in both the 2008 and 2012 lures of trial 2, but absent from the 2012 lures of trials 3 and 4. Otherwise the two bioassayed lots of fresh 2012 manuka lures did not differ in release rates of any single constituent by >76% (trial 2 lures; mean deviation 36%) and 45% (trials 3 and 4 lures; mean deviation 21%) from the fresh 2008 manuka lures. As used in the cubeb bubble lures, the whole cubeb oil contained 66 $\mu g/\mu l$ and the distilled fraction 255 $\mu g/\mu l$ α-copaene.

Discussion

Bubble lures containing cubeb oil or the distilled fraction of it caught redbay ambrosia beetles as well or better than manuka oil lures in either bubble releasers or pouches. The cubeb oil in the lures contained substantial quantities of α-copaene, which is suspected to be a key attractive constituent in plant essential oils, and their fractions, attractive to *X. glabratus* (Hanula and Sullivan 2008, Niogret et al. 2011). In the initial weeks of deployment, cubeb oil lures typically were not significantly more attractive than fresh manuka oil lures, and chemical analysis indicated that both fresh, full-size manuka in pouches and distilled cubeb bubble lures release α-copaene at similar rates. When trap catches were totaled over five or more weeks, however, cubeb lures outperformed some manuka oil lures (in our study, those manufactured in 2012) apparently because these lures stopped releasing volatiles after just 2 wk. Kendra et al. (2012) also demonstrated that phoebe oil was more effective than manuka oil in long-term trials using commercial baits but that in the
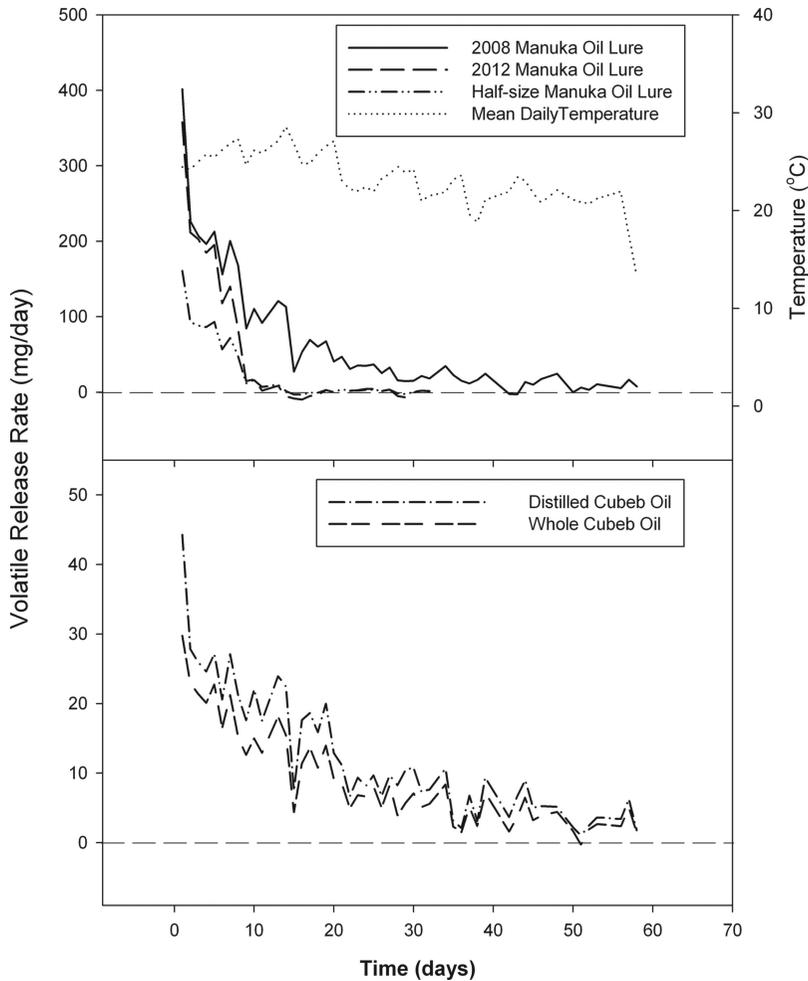
Fig. 5. Average release rate of lures containing essential oils for attraction of redbay ambrosia beetles. Lures were held outdoors in a shaded area in Athens, GA where they were protected from direct sunlight and rain.

first 2 wk, when lures were fresh, the two essential oils were equally effective.

Manuka oil lures from 2008 continued to release volatiles (i.e., lose weight) for 57 d, whereas those from 2012 lasted only 14 d when held in the shade in Athens, GA. The short life of 2012 manuka oil lures compared with 2008 lures was unexpected. Manuka oil lures from the 2012 lot used in these trials released much longer when aged indoors in a constant 20°C chamber (D. W., unpublished data). Volatiles released by fresh lures from the two different years of manufacture were similar in concentration of α-copaene and differed relatively little in release of other manuka oil components. Furthermore, lures from 2008 and 2012 did not differ in attractiveness to *X. glabratus* during the first weeks of the trapping experiments. This information suggests that the attractive quality of the manuka oil source had not changed and the only difference between the lures was the premature termination of volatile release by the 2012 devices (Fig. 5). The nature of this change is uncertain; however, we observed that 2008 lures contained BHT, an anti-oxidant used as a preservative not found in 2012 lures. This antioxidant was added during lure manufacture in 2008 but not 2012. In addition, the cellulose matrix used as the core of the 2012 devices differed from the 2008 lures, and potentially this also may have contributed to the short lure longevity observed. Even though factors contributing to the changes in the field life of manuka oil lures have not been elucidated, we found that freshly distilled cubeb oil (lacking preservative) in bubble lures continued to release volatiles and were attractive to *X. glabratus* for over 8 wk.

Regardless of the reason for reduced lifespan of newer lures, it is likely that manuka lure lifespan would be further shortened in tropical or subtropical climates. Kendra et al. (2012) found that manuka oil and phoebe oil lures released similar quantities of α-copaene initially but manuka oil lures released very little α-copaene after 10–12-d exposure in the field in south Florida where the annual average temperature is 4°C warmer than middle Georgia. In contrast, they found phoebe oil lures continued to release α-copaene for up to 26 d or more.

Although manuka oil has been effective in capturing redbay ambrosia beetles in warm temperate climates at both high and low population densities (Hanula and Sullivan 2008, Hanula et al. 2011), evidence suggests that long–term efficacy of lures in tropical and sub-tropical areas is poor (Brar et al. 2012, Kendra et al. 2012) and our data show that lifespan of newer manuka oil lures changed. Cubeb oil offers an effective alternative to manuka oil and it may avoid the problems associated with the limited supply of phoebe oil, i.e., cubeb oil is readily-available from multiple sources and this reduces the likelihood that failure of a single source would disrupt trapping and potential control efforts. In addition, bubble type release devices allow more flexibility for manipulating release rates and increasing lure longevity than pouch style lures (D. W., unpublished data). Lure longevity would be particularly important in trap-out or attract-and-kill efforts to control redbay ambrosia beetle in forested settings where revisiting traps or kill sites to replenish baits would be difficult. Distilled cubeb oil lures in bubble caps were still capturing more beetles than unbaited control traps after 9 wk during summer in central Georgia, and it should be possible to develop lures with even longer life spans.

Manuka oil and phoebe oil are comparable to fresh cut redbay wood in attraction of redbay ambrosia beetles (Hanula and Sullivan 2008). A lure more attractive than the host tree would be ideal for control efforts aimed at this beetle, because competition with attractive, natural odor sources limits the capacity of traps to reduce beetle population densities and damage. We suspect that cubeb oil will perform similarly to phoebe oil based on our current and previously-published trials (Hanula and Sullivan 2008) so, although it is unlikely to be more attractive than redbay wood, it provides an effective alternative to manuka oil and phoebe oil for monitoring traps that is more readily available.

Acknowledgments

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