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Volatiles from the symbiotic fungus *Raffaelea lauricola* are synergistic with Manuka lures for increased capture of the Redbay ambrosia beetle *Xyleborus glabratus*

Emily H. Kuhns*, Yolani Tribuiani*, Xavier Martini*, Wendy L. Meyer*, Jorge Peña†, Jiri Hulcr‡§ and Lukasz L. Stelinski*

**Department of Entomology and Nematology, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL, 33850, U.S.A.,* †*Department of Entomology and Nematology, Tropical Research and Education Center, University of Florida, 18905 S.W. 280 Street, Homestead, FL, 33031, U.S.A.,* ‡*School of Forest Resources and Conservation, University of Florida, 1745 McCarty Drive, Gainesville, FL, 32611, U.S.A., and* §*USDA Forest Service, 160 Zillicoa Street, Asheville, NC, 28801, U.S.A.*

Abstract

1 Redbay ambrosia beetle *Xyleborus glabratus* is an invasive wood boring beetle that has become established in the southeastern U.S.A. and transmits a fungus *Raffaelea lauricola* that causes lethal laurel wilt. Among susceptible Lauraceae hosts are redbay *Persea borbonia* and avocado *Persea americana*.

2 There is a crucial need for detection of this pest as it moves into new areas. Consequently, our goal was to create a better lure for the monitoring and control of redbay ambrosia beetle.

3 We analyzed volatile emissions of *R. lauricola*, created a synthetic odour blend based on this analysis and tested this odour blend as a potential attractant in a redbay forest infested with *X. glabratus*. The synthetic *Raffaelea* odour blend was not attractive to the beetles by itself. However, it synergistically increased attraction to host-mimic volatiles.

4 We tested four commercial release devices for dispensing *Raffaelea* odour at various release rates. Two prototypes with the highest release rate, when paired with commercial manuka oil lures, captured more beetles than manuka oil lures alone. These results indicate that a synthetic blend of volatiles based on the odour of the symbiotic fungus of *X. glabratus* may be useful for the development of more sensitive monitoring lures for this invasive pathogen vector.

Keywords Avocado, isoamyl acetate, isoamyl alcohol, laurel wilt, *Persea americana*, *Persea borbonia*, Scolytinae.

Introduction

The exotic redbay ambrosia beetle *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae) has recently become established in the southeastern U.S.A. (Rabaglia *et al.*, 2006; Fraedrich *et al.*, 2008). Typically, ambrosia beetles use dead or dying trees as hosts in which they inoculate and cultivate a symbiotic fungus for food. The physical holes in the tree combined with inoculation of these fungi plays a valuable role in biomass turnover in forest ecosystems (Edmonds & Eglitis, 1989). However, *X. glabratus* appears to attack healthy trees in the U.S. (Fraedrich *et al.*, 2008) and introduces the fungus *Raffaelea lauricola* that is lethal to trees in the family Lauraceae (Harrington *et al.*, 2008; Harrington & Fraedrich, 2010). This disease, called laurel wilt, results in similar symptoms to other diseases transmitted by wood boring beetles, such as Dutch elm and oak wilt (Fraedrich *et al.*, 2008; Mayfield *et al.*, 2008).

Although redbay *Persea borbonia* (L.) Spreng. and the related species swampbay *Persea palustris* (Raf.) Sarg. are considered the primary hosts, several other Lauraceae are acceptable hosts, including avocado *Persea americana* Mill., a major fruit crop in southern Florida and California, and two threatened or endangered tree species: pondspice *Litsea aestivalis* (L.) Fernald and pondberry *Lindera melissifolia* (Walter) (Fraedrich *et al.*, 2011). The U.S. Avocado industry is valued at $US 30 million per year in Florida alone; therefore, this disease has the potential to negatively impact local farmers and economies, as well as create shortages of a favorite food

Correspondence: Lukasz L. Stelinski. Tel.: 863-956-8851; fax: 863-956-4631; e-mail: stelinski@ufl.edu

crop (Evans *et al.*, 2010). In addition, the destruction of redbay trees is devastating to forests and could alter ecosystem composition, leading to abnormal forest succession (Shields *et al.*, 2011), as shown in other systems (Coleman *et al.*, 2008; Spaulding & Rieske, 2010). Because of the imminent threat to the avocado industry in southern Florida, there is a critical need for more efficient and less expensive traps to monitor and control this insect pest.

To date, *R. lauricola* has been detected in dead avocado trees in southern Florida but has not yet been proven as the cause of tree death. Furthermore, the insect vector *X. glabratus* has seldom been detected within those groves. There are some factors that may explain the presence of the fungus and the virtual absence of the vector. First, avocado is not preferred over redbay or swampbay (*Persea palustris*) (Mayfield & Hanula, 2012) and it is possible that the beetle is present, although the population is below the detection limit of current trapping methods. Second, until recently, manuka oil (essential oil from the leaves of *Leptospermum scoparium* J. R. Forst et G. Forst that contains similar chemicals to redbay) lures have been the only commercially available lure for monitoring *X. glabratus* (Hanula & Sullivan, 2008; Kendra *et al.*, 2012); however, the effectiveness of manuka oil lure for trapping *X. glabratus* has been variable and short lived (Kendra *et al.*, 2012; Hanula *et al.*, 2013). Third, it is possible that the avocado trees have succumbed to other causes, and *R. lauricola* has been introduced into them by other secondary ambrosia beetle species (Carrillo *et al.*, 2012). It is critical to distinguish why the fungus is detected 'ahead' of the vector to enable prediction of the likely movement of the vector through the area in the immediate future. This is another example of why a more efficient monitoring approach is needed for detection of the vector at low population densities.

Xyleborus glabratus is attracted to volatiles produced by cultures of their fungal symbiont in laboratory bioassays (Hulcr *et al.*, 2011). Therefore, we tested the hypothesis that the attractiveness of manuka oil lures could be enhanced by adding the odour of the symbiotic fungus, thereby more closely imitating the odour of a host tree infected with *R. lauricola*. We first analyzed the volatiles from *R. lauricola* and two other common ambrosia fungi and identified the major components of the odour blends via gas chromatography-mass spectrometry (GC-MS). Subsequently, we tested bioactivity of a synthetic reconstitution of this blend and its field efficiency in prototype, commercial release devices using sticky traps.

Materials and methods

Chemicals and fungal cultures

Isoamyl acetate ($\geq$ 97% purity; #W205532), isoamyl alcohol ($\geq$ 98% purity; #W205710) and ethyl acetate ($\geq$ 99.7% purity; #34972) were purchased from Sigma Aldrich (St Louis, Missouri). Ethanol (200 proof; #20701) was purchased from Decon Laboratories (King of Prussia, PA). Pure cultures of ambrosia beetle symbionts *R. lauricola*, *Ambrosiozyma* sp. and *Ambrosiella* sp. were prepared as described by Hulcr *et al.* (2011) and Kolarik and Hulcr (2009). The cultures were maintained on potato dextrose agar (PDA) and were subcultured every 2–3 weeks. Manuka oil lures (#3083) were purchased from Synergy Semiochemicals Corp. (Canada). *Raffaelea lauricola* odour blend (ROB) release devices A, B, C and D and ethanol release devices were purchased from Alpha Scents (West Linn, Oregon).

GC-MS sample preparation

We examined the headspace volatiles of the symbiotic fungus of *X. glabratus* and *R. lauricola*, as well as *Ambrosiella* sp. and *Ambrosiozyma* sp., which are two other ambrosia fungi that are not the primary symbionts of *X. glabratus* (Hulcr *et al.*, 2011). Fungi were grown on slants of PDA for the sampling. Fifteen microlitres each of PDA medium was autoclaved in 40-mL glass vials with tin foil as a lid. The media were allowed to solidify at a 45° angle and two replicate vials were inoculated for each species of fungi. The fungi were allowed to grow for 4 days at 21 °C in the dark until approximately 75–100% of the surface area was covered with fungus. A triphase 50/30 µm DVB/Carboxen/PDMS StableFlex™ solid-phase microextraction (SPME) for volatiles and semivolatiles with molecular weight between 40 and 275 (Supelco, Bellefonte, Pennsylvania) fibre was inserted through the tin foil lid and exposed to the fungal odours for 5 min. The SPME fibre was desorbed for 5 min at 240 °C under splitless conditions and the odour constituents were separated over 40 min on a Stabilwax (Restek, Bellefonte, Pennsylvania) capillary column (60 m × 0.25 mm inner diameter; 0.5 µm film thickness) using a temperature gradient from 40 to 240 °C at 7 °C/min. Helium was used as a carrier gas at 2 mL/min. Identification of the compounds was performed using a Clarus 500 quadrupole mass spectrometer and TURBO MASS software (Perkin Elmer, Shelton, Connecticut). Linear retention times of authentic standards, when available, and mass spectra from the NIST database were used to identify compounds.

Field sites

Field sites were selected based on monitoring of *X. glabratus* populations in the autumn of 2010, 2011 and 2012 at Lake Kissimmee State Park (LKSP), in Polk County, FL. In the autumn of 2012, monitoring for *X. glabratus* was conducted at Wekiwa Springs State Park (WSSP), Orange County, Florida. To measure *X. glabratus* abundance, full size elm bark beetle traps (Great Lakes IPM, Vestaburg, Michigan) were attached to trees in five locations (between 27°55′11.64″N, 81°22′25.38″W and 27°55′16.98″N, 81°22′39.12″W) in LKSP in 2010, 2011 and 2012 and five locations along the green horse trail in WSSP in 2012 (between 28°43′36.35″N, 81°28′47.72″W and 28°43′35.76″N, 81°29′2.46″W). The trapping locations at both parks were within or along wet flatwood habitats containing declining redbay trees. Each location had one trap baited with a manuka oil lure and one trap with no lure as a control. Beetles were collected from the traps weekly and manuka oil lures were replaced every 2 weeks. The number of *X. glabratus* was observed using a stereomicroscope and recorded for each trap for each week. Based on this monitoring, we chose to conduct

our field trials with ROB lures at WSSP because of the larger proportion of beetles captured (see Results).

Field experiments

Elm bark beetle sticky panel traps were cut in half and one half was stapled to wooden posts (height 1.5 m). The posts were randomly placed in replicate blocks in WSSP with at least 6 m between each trap. The minimum distance between replicate blocks was approximately 60 m. Three field experiments were conducted to test: (i) the attractiveness of ROB and (ii) prototype commercial release devices containing this same odour blend, as well as (iii) a different prototype commercial device characterized by a much higher release rate of the *Raffaelea* odour blend. The intent of these analyses was to determine whether beetle captures with any of the potentially proprietary release devices were sufficiently high to justify further commercial development.

The *Raffealea* odour blend tested (ROB) was a mixture of 36.5 : 29 : 22 : 12.5 of ethyl acetate : ethanol : isoamyl alchohol : isoamyl acetate by volume and was based on GC-MS analysis of *R. lauricola* odour. In the first field experiment, 1 mL of ROB was pipetted into 7-mL polyethylene Beem vials (Thermo Fisher Scientific, Waltham, Massachusetts) for release of odour. The lids were sealed with hot glue (#BSS6-4; Arrow Fastener Co., LLC, Saddle Brook, New Jersey) to prevent the lid from opening in the field. We refer to this initial lure design as device 0. In subsequent field experiments, the same odour blend was presented with other release devices manufactured by Alpha Scents. For each experiment, the lure placement was re-randomized.

In the first experiment, traps were baited with one of four treatments: blank (control), manuka oil lure, manuka oil with device 0, or device 0 alone. The total number of *X. glabratus* was recorded per trap for the 2-week trapping period. Three trials of this experiment were conducted: 25 October to 8 November 2012 ($n = 5$); 8 November to 22 November 2012 ($n = 5$); and 6 December to 20 December 2012 ($n = 12$). The temperature during trapping demonstrated a high of $24.5 \pm 3.3\,^{\circ}$C and a low of $15.1 \pm 3.4\,^{\circ}$C. In addition, the number of beetles from other species of Scolytinae was recorded for the third trial.

In a second field trapping experiment from 6 January to 20 January 2013, three proprietary release devices (Alpha Scents) containing ROB were tested. The lures differed in the release device used; devices A and C were polyethylene vessels differing in volume and wall thickness. Device A had a smaller internal volume compared with device C and device 0. Device C had a higher internal volume than device 0. The third (device B) was a mylar packet containing a cellulose disc and sealed with a layer of polyethylene. The temperature during trapping was a high of $24.3 \pm 4.7\,^{\circ}$C and a low of $12.7 \pm 4.8\,^{\circ}$C. Six replicate blocks of traps were baited with combinations of lures: manuka oil (positive control), blank (negative control), manuka oil with device 0, manuka oil with device A, manuka oil with device B, and manuka oil with device C. The number of *X. glabratus* was observed using a stereomicroscope and recorded for each trap for 2 weeks of trapping.

In a third field trapping experiment from 1 March to 15 March 2013, a new prototype, device D, was evaluated. The purpose of this experiment was to evaluate a higher release of the ROB and to test the effect of a higher release of ethanol on the capture of *X. glabratus*. Additional ethanol release devices were investigated because polyethylene (i.e. the material used for release devices of ROB) is not very permeable to alcohols. Device D comprised a polyethylene bag (10×15 cm) enclosing a foam matrix and the synthetic odour blend. In this experiment, six replicates of nine lure combinations were tested: blank control, manuka oil, ethanol, manuka oil with ethanol, device C with manuka oil, device C with manuka oil and ethanol, device D with manuka oil, device D with manuka oil and ethanol, and device 0 with manuka oil. The temperature during trapping was a high of $21.6 \pm 4.0\,^{\circ}$C and a low of $8.4 \pm 3.1\,^{\circ}$C.

Release rate

The release rate (mg/day) was measured gravimetrically for each type of device. The devices were hung outdoors at Lake Alfred, Florida, in March 2013. Devices were weighed on subsequent days to determine the weight released during each 24-h period for approximately 30 days. The mean high temperature during this period of time was $22.8\,^{\circ}$C and the mean low temperature was $9.4\,^{\circ}$C. Four replicates of each device were tested, except for device A and device B where three replicates were tested. Gravimetric release rates were calculated based on the reduction in weight between subsequent days. The mean release for each lure was calculated for days 1–3 and 3–7. The data were also fitted with an exponential decay curve. Release of individual components of ROB was not measured for the devices.

Statistical analysis

All statistical analyses were performed using the statistical software package R, version 2.15.3 (http://www.r-project.org). For all trapping experiments of *X. glabratus*, the data were not normally distributed; therefore, we performed a generalized linear model (GLM) with a log link function for Poisson distribution. The data also showed over-dispersion, meaning that, in contrast to a Poisson distribution where the mean is equal to the variance, the variance of the data in the present study was larger than the mean. Consequently, we corrected the standard errors using a quasi-GLM model where the variance is given by $\varphi \times \mu$ and where φ is the dispersion parameter and μ is the mean. The standard errors are corrected by multiplying them with the square root of φ (Zuur *et al.*, 2009). Trapping data for nontarget scolytine beetles were not overdispersed, and therefore we used a Poisson distribution in our GLM. We started with a model that included lure treatment, block number and trial date as fixed variables: $Beetles \sim Lure + Trial + Block$. When trial date or block number did not show a significant effect ($\alpha > 0.05$), they were removed from the model to obtain a minimal adequate model (Crawley, 2009). When a significant effect of the lure treatment was found, a post-hoc Tukey's test (function 'glht', package 'multcomp') was performed to

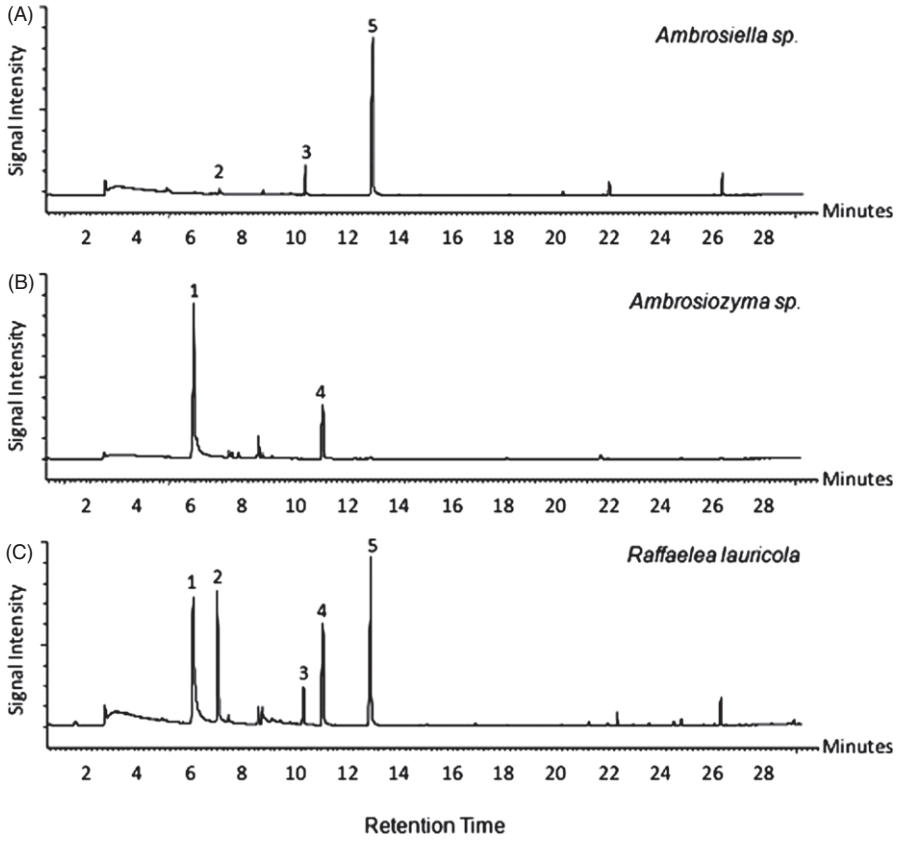

Figure 1 Chromatograms representing odours from three cultured ambrosia beetle fungal symbiont species (A–C). Each species was cultured on potato dextrose agar and volatiles were collected by solid-phase microextraction. The odours were analyzed by gas chromatography-mass spectrometry, and identified by matching mass spectra and the linear retention time of authentic standards. The major constituents are numbered: (1) ethyl acetate, (2) ethanol, (3) isobutyl alcohol, (4) isoamyl acetate and (5) isoamyl alcohol.

determine which treatments (lure combinations) differed with respect to their attraction of beetles.

Results

GC-MS identification of fungal odours

Raffealea lauricola headspace contained ethyl acetate, ethyl alcohol, isobutyl alcohol, isoamyl acetate and isoamyl alcohol. *Ambrosiozyma* sp. headspace volatiles contained primarily ethyl acetate and isoamyl acetate. *Ambrosiella* sp. headspace contained ethanol, isobutyl alcohol and isoamyl alcohol (Fig. 1). The control vial containing only potato dextrose agar and no fungi had only trace amounts of ethyl acetate (data not shown). The relative abundance of the major constituents of *R. lauricola* headspace was used to create a synthetic odour blend for field trapping (ROB).

Monitoring of beetles in two Florida state parks

In LKSP, 57 *X. glabratus* were captured during 57 days of trapping, and the redbay trees appeared healthy and abundant. However, when the park was re-sampled in 2011, the manuka oil baited traps captured substantially more *X. glabratus* than in the previous year. We captured 4667 *X. glabratus* during 57 days of trapping. In 2011, many trees were dead or dying with visible symptoms of laurel wilt and most trees examined showed evidence of ambrosia beetle boring in the trunks. In 2012, we re-sampled at LKSP and sampled for the first time at WSSP. In 19 days of trapping, 191 *X. glabratus* were captured at LKSP; however, at WSSP, we captured 3985 *X. glabratus*.

Field trials of Raffaelea devices

We tested device 0 by itself and in conjunction with manuka oil lures (Fig. 2A) in three trials. We found a significant difference in trap captures among the four treatments ($\varphi = 15.37$, $F_{3,79} = 82.68$, $P < 0.001$). There was no significant difference between mean capture of *X. glabratus* on traps baited with device 0 alone compared with blank traps ($P = 0.524$) (Fig. 2A). Traps baited with device 0 paired with manuka oil captured more *X. glabratus* on average than traps baited with manuka oil alone ($P = 0.003$) (Fig. 2A).

We also examined the number of other nontarget Scolytinae beetles of various species present on the traps during the December trapping period (Fig. 2B). Catch of nontarget Scolytinae was significantly different among trap types ($F_{3,44} = 14.59$,

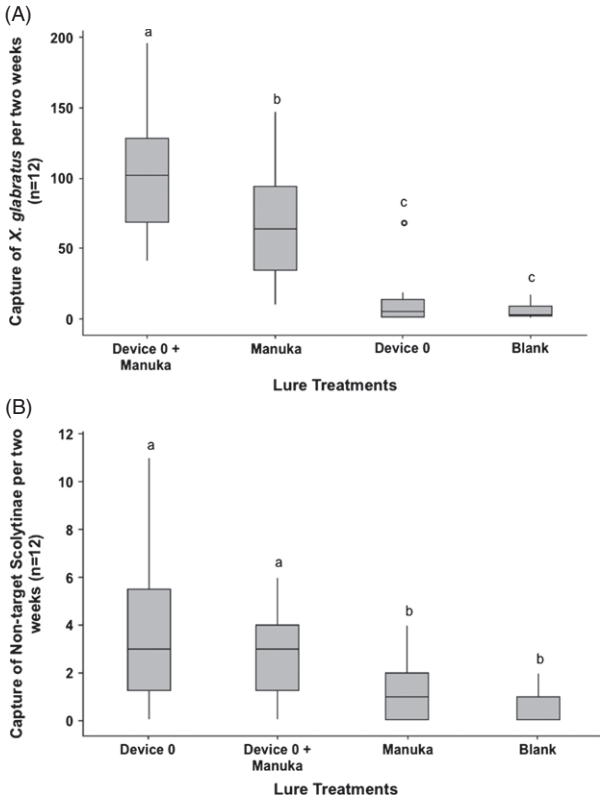

Figure 2 Captures of *Xyleborus glabratus* (A) or nontarget scolytine (B) over 2 weeks on unbaited traps (negative control) or traps baited with manuka lure, device 0, or manuka with device 0. Bars labeled by the same lower case letter are not significantly different.

$P < 0.001$). Beetle capture on device 0 baited traps was significantly higher than on unbaited traps (control = 0.001) and manuka oil-baited traps, although there was no significant difference between capture on traps baited with device 0 and the device 0 + manuka oil combination ($P = 0.512$) (Fig. 2B). Catch of nontarget Scotylinae on manuka oil-baited traps was not significantly different from capture on traps baited with manuka oil paired with device 0 ($P = 0.051$) (Fig. 2B). There was no significant difference between catch of nontarget Scolytinae beetles on manuka oil-baited and unbaited traps ($P = 0.134$) (Fig. 2B).

Based on the evaluation of device 0, we tested three proprietary release devices (A, B and C) developed by Alpha Scents, which varied the release rate of the *Raffaelea* blend described in the present study. We found a significant difference among treatments (dispersion parameter $\varphi = 13.86$, $F_{5,30} = 6.66$, $P < 0.001$). Post-hoc tests indicated that beetle catch on device B ($P = 0.010$), C ($P = 0.004$) and 0 was significantly higher than on unbaited, control traps (Fig. 3). Possibly as a result of lower replication, the number of *X. glabatus* captured with device A and manuka was not significantly different from the control ($P = 0.079$ and $P = 0.059$, respectively).

In a subsequent experiment that included device C, a new prototype device D and ethanol as treatments, we found a significant difference for treatments (dispersion parameter $\varphi = 17.94$, $F_{8,43} = 14.187$, $P < 0.001$) (Fig. 4). More

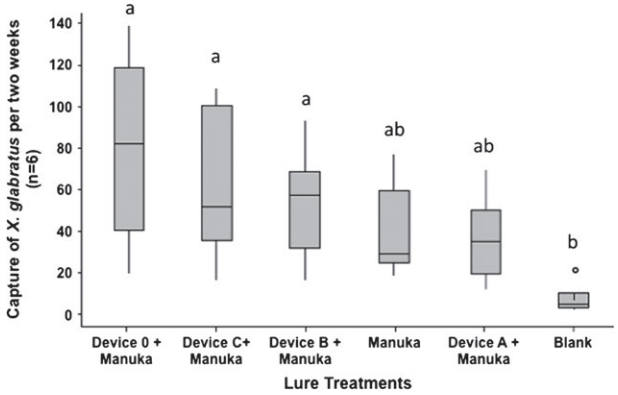

Figure 3 Captures of *Xyleborus glabratus* per 2 weeks with three prototype commercial *Raffaelea*-odour devices: A, B and C. The number of *X. glabratus* captured per 2 weeks with device C was compared with device 0 paired with manuka and the pooled data for the three device types that captured the fewest beetles ($n = 5$). Bars labeled by the same lower case letter are not significantly different.

X. glabratus were captured on traps baited with device C with manuka oil and ethanol ($P = 0.003$), device D with manuka oil ($P < 0.001$), device D with manuka oil and ethanol ($P < 0.001$), and manukia oil and ethanol ($P = 0.006$) (Fig. 4) compared with unbaited control traps. In addition, device D with manuka ($P = 0.001$) and device D with manuka and ethanol ($P = 0.023$) captured more *X. glabratus* than manuka oil baited traps. When examining pairwise effects of additional ethanol lures to lure combinations, there were no significant differences between device D with manuka oil versus device D with manuka oil and ethanol ($P = 0.987$), device C with manuka oil and device C with manuka oil and ethanol ($P = 0.071$) or manuka oil versus manuka oil with ethanol ($P = 0.638$).

Release rates

Dispensers differed greatly in their release rates. Device D had the highest release rate, followed by device C then B, and lure A had the lowest release rate. The release rates were modelled by exponential decay. The decay constants, as well as mean release for days 1–4 and 4–7 are reported in Table 1. The release rates for each lure type over 32 days are shown in Fig. 5(A–D).

Discussion

Our main hypothesis was based on previous data indicating that the volatiles produced by cultured *R. lauricola* were attractants for *X. glabratus* (Hulcr *et al.*, 2011). The hypothesis was supported in field tests. Volatiles from *R. lauricola* synergistically increased the attractiveness of tree volatiles to *X. glabratus*. Previous research suggested that the odours of the fungus were not attractive based on trapping with wounded healthy and infected trees (Hanula *et al.*, 2008); however, the release of volatiles from wounding those trees may have overridden the underlying odours of the fungus. Based on our data using synthetic fungus and host-mimic odours, we conclude that the vector beetle incorporates stimuli from both the host

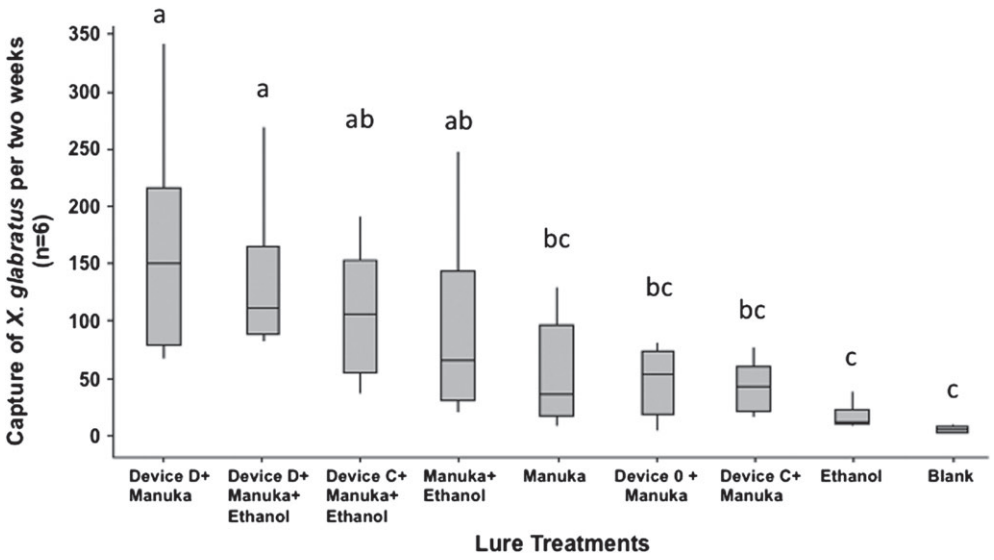

Figure 4 Captures of *Xyleborus glabratus* per 2-week interval with prototype commercial *Raffaelea*-odour device C and D with ethanol against various other lures ($n = 5$). Bars labeled by the same lower case letter are not significantly different.

Table 1 Gravimetric release rate analysis of odor lures tested for attraction of *Xyleborus glabratus*

	Mean release rate (mg/day)		Exponential decay	
Lure/device	Days 1–3	Days 3–7	Coefficient	R^2
Device 0	51.3 ± 8.3	30.7 ± 2.6	−0.002	0.916
Device A	3.3 ± 0.6	2.1 ± 0.3	−0.002	0.998
Device B	117.4 ± 17.7	45.5 ± 8.5	−0.007	0.895
Device C	31.3 ± 3.4	17.4 ± 0.4	−0.004	0.997
Device D	220.8 ± 42.8	520.8 ± 46.2	−0.006	0.991
Manuka oil lure	98.2 ± 25.1	35.2 ± 6.5	−0.001	0.841

tree and its symbiotic fungus in its search behaviour. This trend was reproducible in all of the experiments conducted throughout 1 year. Biologically, it makes sense that odoors from a symbiotic fungus of ambrosia beetles would synergize with plant odours to enhance beetle response. Finding a suitable host is a limiting factor of ambrosia beetle life history; thus, from the beetles' perspective, attraction to fungi indicates the presence of an acceptable host where congeneric beetles may have already succeeded in establishing galleries. This readily available food source would increase the fitness of those beetles that are attracted to it by immediately providing food during gallery excavation and subsequent oviposition.

The ROB blend that we tested is based on GC-MS analysis as sampled by SPME. SPME has limitations when used to quantify volatile release from a source because not all compounds adhere to the solid phase with equal efficiency. In addition, the differing internal volume and wall thickness of our release devices likely affected the permeability of various compounds as our release rate analyses suggest. Device 0 was not completely sealed; therefore, it is possible that ethanol was released from this device at a higher rate compared with the completely heat-sealed proprietary devices. We chose to increase the release rate of ethanol in a subsequent experiment. In this field trapping experiment, we evaluated device 0 and device C paired with manuka oil, as well as device C with manuka oil and ethanol against a new high release prototype, device D. Traps baited with device D with manuka oil and device D with manuka oil and ethanol captured the most *X. glabratus*. Ethanol did not appear to impact catch of *X. glabratus*. When we performed pairwise comparisons between lure combinations with and without ethanol, there were no significant differences. This is congruent with previous studies suggesting that ethanol does not attract *X. glabratus* (Hanula & Sullivan, 2008; Hulcr *et al.*, 2011; Kendra *et al.*, 2012). Future studies may want to examine the role of ethanol for the catch of this species when released with other odours at the appropriate release rate and blend.

Device 0 by itself captured more nontarget Scolytinae than the unbaited control traps, although the catch of these nontarget species was low compared with the catch of *X. glabratus*. The similarity of odours released by various ambrosia fungi may explain some of these nontarget captures; ethanol may be a main driver for the attraction of nontarget beetles because many are known to be attracted to this compound. As a tree declines from *X. glabratus* infestation, it becomes more attractive to other generalist Scotylinae as a result of the release of odours of wood decay. Our lures may be perceived as an indicator of availability of a generalists' host. Therefore, it may not be surprising that, when ROB was present, traps captured more nontarget species of Scolytinae than those without ROB.

Our trapping experiments evaluated the efficacy of devices during the first 14 days of deployment. Gravimetric release rate analysis revealed considerable variability in release between device types during the initial few days of deployment. The best performing ROB lure for capture of *X. glabratus* was device D and this lure also exhibited steady gravimetric release, suggesting that this lure may be effective for long-term, unsupervised deployment.

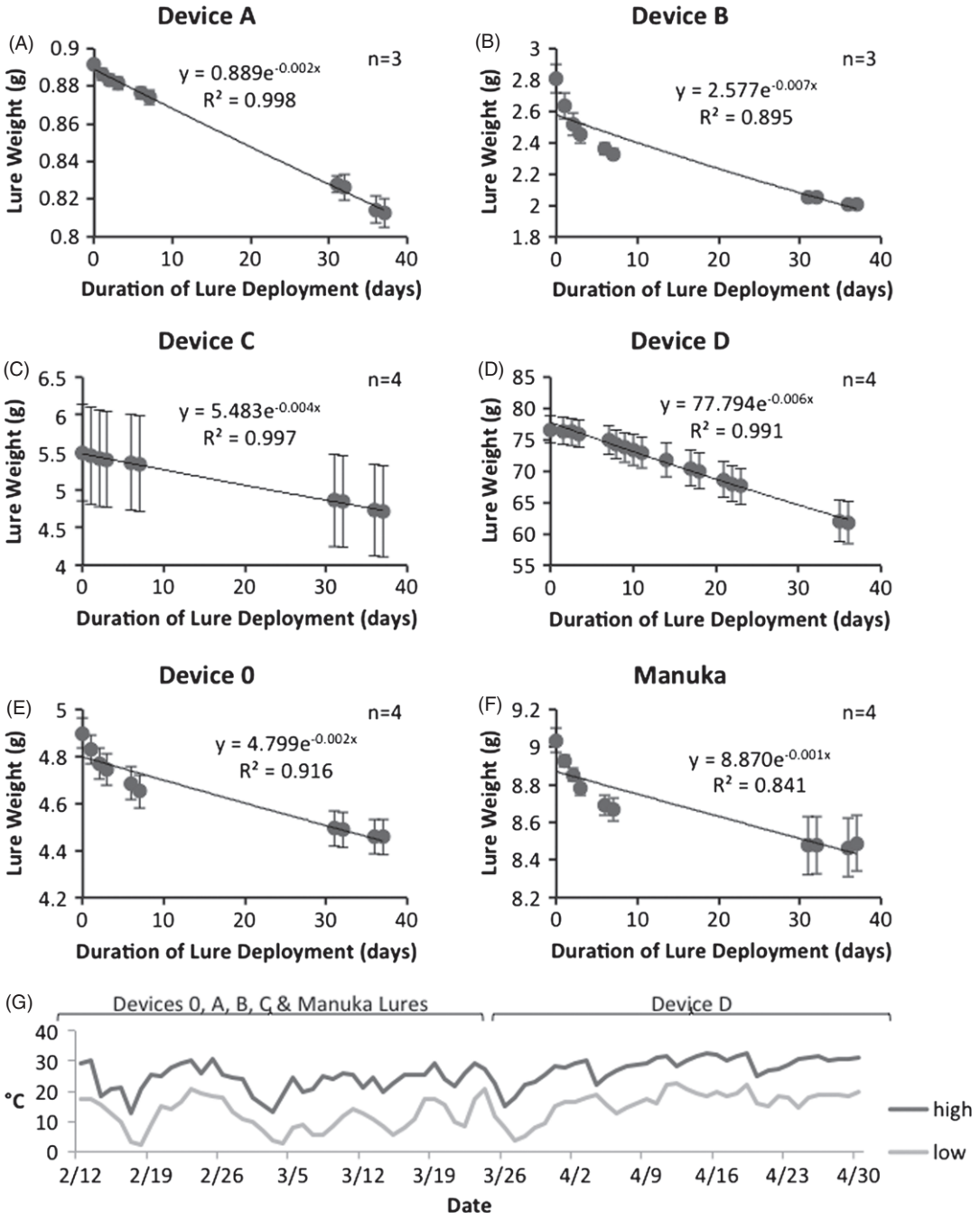

Figure 5 Release rates of six *Raffaelea* odour dispensers and manuka oil lures. Release rates of device 0 (E), device A (A), device B (B), device C (C), device D (D) and manuka (F) are reported (mg/day). Each data set was fitted with an exponential decay curve; equation and R^2 is shown for each device. Temperature highs and lows during gravimetric analysis of the lures are shown in (G).

Based on our findings, it appears that a more effective lure for *X. glabratus* may be possible by combining manuka oil (or other relevant plant odours) with *Raffaelea* associated odours into a single release device or at least when used in combination. The components used in ROB are commercially available and relatively inexpensive because they are common compounds used in the flavor industry. Based on the captures of *X. glabratus* recorded with an initial prototype of a commercial lure, it appears that such a release device should be specifically tuned for the ideal release rate and the blend of the odour constituents to optimize monitoring of this beetle. This combination of fungal and plant odourants may improve monitoring of *X. glabratus* in locations where beetle densities are low and below current detection thresholds, particularly in areas where the beetle is not yet known to be established.

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