



Effect of linalool and camphene on capture of *Tetropium* spp. (Coleoptera: Cerambycidae) in traps

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ABSTRACT

Linalool and camphene were tested in field trials to determine their effect on capture of the exotic brown spruce longhorn beetle, *Tetropium fuscum* (F.) and the native *Tetropium cinnamopterum* Kirby (Coleoptera: Cerambycidae) in intercept panel traps. Neither compound, by itself or in combination with spruce volatiles and the aggregation pheromone fuscumol, significantly increased mean catch of either species.

RÉSUMÉ

Le linalool et le camphène ont été testés lors d'essais sur le terrain pour déterminer leur effet sur la capture à l'aide de pièges à interception de l'espèce exotique *Tetropium fuscum* (F.), le longicorne brun de l'épinette, et de l'espèce indigène *Tetropium cinnamopterum* Kirby (Coleoptera : Cerambycidae). Aucun des composés, seuls ou en combinaison avec les substances volatiles émises par l'épinette et la phéromone d'agrégation fuscumol, n'a augmenté de façon significative la capture moyenne des deux espèces.

INTRODUCTION

Conifers produce many different compounds as defense mechanisms against phytophagous insects, many of which are up-regulated during periods of stress. These compounds can act as kairomones, attracting species of bark- and wood-boring insects that exploit stressed trees, and may act synergistically with other semiochemicals such as pheromones (Visser 1986; Allison et al. 2004; Miller et al. 2005; Kännaste et al. 2008). Many species of Cerambycidae are attracted to monoterpenes and other host volatiles (Chénier and Philogène 1989), and *Tetropium fuscum* (F.) and *Tetropium cinnamopterum* Kirby are no exception: both sexes are attracted to a synthetic blend of five monoterpenes (racemic α -pinene, (-)- β -pinene, (+)-3-carene, (+)-limonene, α -terpinolene) that simulates those emitted by red spruce trees (spruce blend) (Sweeney et al. 2004; 2006). *Tetropium fuscum* is an invasive species that has become established in Nova Scotia, Canada, infesting predominantly stressed red spruce; *Tetropium cinnamopterum* a Nearctic congener that infests stressed spruce and occasionally pine (Sweeney et al. 2010). Males of *Tetropium fuscum* and *Tetropium cinnamopterum* emit an aggregation pheromone, fuscumol, that by itself is only weakly attractive but when combined with spruce blend and ethanol, synergizes attraction of males and females of *Tetropium fuscum*, *Tetropium cinnamopterum*, and *Tetropium castaneum* (L.) (Silk et al. 2007; Sweeney et al. 2010). *Tetropium fuscum* likely use volatile cues to select a host, as they land more often on stressed spruce than healthy spruce, and make this choice before landing (Flaherty et al. 2013a).

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Silk et al. (2010) found that the plant volatile (*R*)-(-)-linalool stimulated the antennae (measured by electroantennography) and attracted (measured in y-tube olfactometer assays) both sexes of *Tetropium fuscum*. Linalool also increased the proportion of *Tetropium fuscum* males that assumed the calling posture associated with pheromone emission but did not increase mean catches of *Tetropium fuscum* when tested in combination with fuscumol and ethanol in a field trapping experiment (Silk et al. 2010). Negative results for their field experiment may have been because it was conducted comparatively late in the flight season (27 July to 27 August 2009) (Rhainds et al. 2010) when catches were too low to detect treatment effects. Camphene is also emitted from many conifers (Chénier and Philogène 1989) and is antennally active in *Tetropium fuscum* (Silk et al. 2010) but has not been tested in behavioral bioassays with *Tetropium* spp. Both (*R*)-(-)-linalool and camphene are present in spruce essential oil (hereafter called spruce oil), which is distilled from Norway spruce (*Picea abies* (L.) H. Karst. (Pinaceae)) needles. Silk et al. (2010) used spruce oil as an inexpensive surrogate for red spruce volatiles in GC/EAD assays, as the monoterpene and sesquiterpene blends were comparable to those of red spruce foliar volatiles.

One of the goals of our research is to develop improved tools for the detection and survey of bark- and wood-boring beetles, especially invasive species. The objective of this study was to test the effect of linalool and camphene on mean catch of *Tetropium fuscum* and *Tetropium cinnamopterum* in traps.

METHODS

Lures

Racemic linalool and camphene were purchased from Sigma-Aldrich (Oakville, ON). Each linalool lure consisted of 1 g of racemic linalool soaked into three layers of a piece of kraft board (i.e., cardboard; 1 mm thick x 30 mm x 70 mm), covered in a mesh slip and sealed in a 6-mm thick polyethylene pouch. Each camphene lure consisted of 1 g of camphene loaded into a 1.5 mL plastic polymerase chain reaction tube (Axygen Scientific, CA) with a 1 mm diameter hole in the cap. Release rates of linalool and camphene were 14 mg/d and 10–20 mg/d, respectively, as determined by weight loss at 20°C. A total of 50 mg of fuscumol was loaded into a 0.2 mL thin wall polymerase chain reaction sample tube with a 1 mm hole drilled in the cap. It was released at an initial rate of 800 µg/d, dropping to about 80 µg/d after 30 d at 23°C (Silk et al. 2007; Sweeney et al. 2010). The release rates of the spruce blend and ethanol lures

(ConTech, 7572 Progress Way, Delta, BC) were ca. 2000 mg/d and 275 mg/d respectively (Sweeney et al. 2006).

Field trapping experiments – linalool

The effect of racemic linalool, either by itself or combined with fuscumol and spruce volatiles, on attraction of *Tetropium* spp. was tested in a trapping experiment conducted at Juniper Lake (44.72°N, 63.57°W), near Halifax, NS, 11 July–25 August 2011. Treatments were: 1) linalool (L); 2) fuscumol (F) + spruce blend (SB) + ethanol (E); 3) L + F + SB + E; and 4) unbaited control. Treatments were replicated eight times in a randomized block design using black panel intercept traps (Alpha Scents Inc., West Linn, OR). Traps and blocks were spaced at least 30 m apart. Each trap was suspended from rope tied between two trees so that it was about 1 m from either tree, and the collecting cup was 30–50 cm above the ground (Sweeney et al. 2010). Collecting cups contained a saturated solution of table salt in water with a drop or two of unscented dish detergent added to break the surface tension. Lures were not changed. Traps were checked every 2 weeks, and all *Tetropium* spp. were identified to species and sexed.

The same experiment was repeated 23 May–18 July 2012, except that pure (*R*)-(-)-linalool was tested instead of racemic linalool, as (*S*)-linalool is not present in spruce oil, and treatments were replicated eight times in a completely randomized design rather than in blocks. The experiment was conducted in a red spruce-dominated stand along Highway 102 near Stanfield International Airport, Halifax, NS (44.85°N, 63.57°W).

Field trapping experiments – camphene

The attraction of *Tetropium* spp. to camphene was tested at Jack Lake, near Bedford, NS (44.74°N, 63.67°W) 6 June–15 August 2011 using very similar methods as in the linalool experiment. There were six different treatments: 1) camphene (C); 2) C + SB + E; 3) C + F + SB + E; 4) F + SB + E; 5) SB + E; and 6) unbaited control. All *Tetropium* spp. were identified to species and sexed.

Data analysis

Data on the effect of lure treatment and block on total catch of *Tetropium fuscum* or *Tetropium cinnamopterum* per trap over the full length of the experiments were analyzed using R (R Development Core Team 2011). Generalized linear models using Poisson, negative binomial, zero-inflated Poisson (ZIP), and zero-inflated negative binomial (ZINB) distributions were created and tested for best fit using the Akaike information criterion (AIC) test. Zero-inflated

models were created using package *pscl* (Zeileis et al. 2008). The model with the lowest AIC value (best fit) was then plotted against the original data set for comparison, and a general linear model hypothesis test (*glht*) using Tukey's contrast for multiple means was run using the *multcomp* package (Hothorn et al. 2008). Chi-square goodness of fit tests were used to test whether sex ratio departed from 1:1 for total catch per experiment and lure treatment, so long as ten or more specimens were captured.

RESULTS

Field trapping experiments – linalool

Neither racemic linalool nor (*R*)-(-)-linalool significantly affected mean catch of *Tetropium fuscum*, whether presented alone or in combination with fuscumol plus spruce blend and ethanol (Figure 1a, b). Too few *Tetropium cinnamopterum* were caught to merit statistical analysis in 2011, but its response to (*R*)-(-)-linalool in 2012 was similar to that of *Tetropium fuscum*, i.e., no effect (data not shown). Males and females of both species responded similarly to the lure treatments; sex ratio did not differ significantly from 1:1 in any experiment except for *Tetropium fuscum* in 2011 in which there was a female bias (Table 1).

Field trapping experiments – camphene

Adding camphene to traps baited with either spruce blend plus ethanol or spruce blend, ethanol and fuscumol did not significantly affect catches of either *Tetropium fuscum* (Figure 2a) or *Tetropium cinnamopterum* (Figure 2b). However, adding a camphene lure to traps baited with F + SB + E resulted in mean catches that were no longer significantly different from those in unbaited traps, suggesting a slight deterrent effect (Figure 2b).

DISCUSSION

These results are similar to those of Silk et al. (2010), who found that (*R*)-(-)-linalool did not affect trap catch of *Tetropium fuscum* when combined with ethanol or ethanol plus fuscumol, despite eliciting attraction in two-choice lab olfactometer bioassays. (*R*)-(-)-Linalool is found naturally in spruce oil, whereas (*S*)-linalool is not; therefore, it is possible that (*S*)-linalool might have inhibited attraction to the racemate. However, results were the same in 2012, when only (*R*)-(-)-linalool isomer was used. It is possible that our release rate was too high or too low to elicit attraction or that linalool elicits attraction of *Tetropium fuscum* only at close range (Silk et al. 2010) and this is insufficient to affect capture in intercept traps. We did not test fuscumol in the absence of spruce blend

Table 1. Total catches and sex ratios of *Tetropium* spp. in trapping experiments conducted near Halifax, Nova Scotia in 2011 and 2012. Chi-squared tests were performed to test departures from a 1:1 sex ratio when more than five males and females were caught.

Species	Experiment	Total Catch		Ratio M/F	Goodness of Fit	
		Male	Female		χ^2	p
<i>T. fuscum</i>	linalool	77	117	0.66	8.2	<0.01
<i>T. fuscum</i>	(<i>R</i>)-linalool	194	206	0.94	0.36	0.55
<i>T. fuscum</i>	camphene	84	78	1.08	0.22	0.64
<i>T. cinnamopterum</i>	linalool	6	5	1.20	0.09	0.76
<i>T. cinnamopterum</i>	(<i>R</i>)-linalool	99	92	1.08	0.26	0.61
<i>T. cinnamopterum</i>	camphene	23	36	0.64	2.86	0.09

and ethanol because previous studies had demonstrated it was not attractive in the absence of spruce volatiles (Silk et al. 2007; Sweeney et al. 2010). However, it is possible that the 100 fold greater release rate of monoterpenes from the spruce blend lures masked any effects the linalool lures may have had. These results contrast with those of Nehme et al. (2009), who found that sleeve cages baited with (*R*)-(-)-linalool had the highest mean weekly capture of another cerambycid beetle, *Anoplophora glabripennis* (Motschulsky) in a greenhouse study.

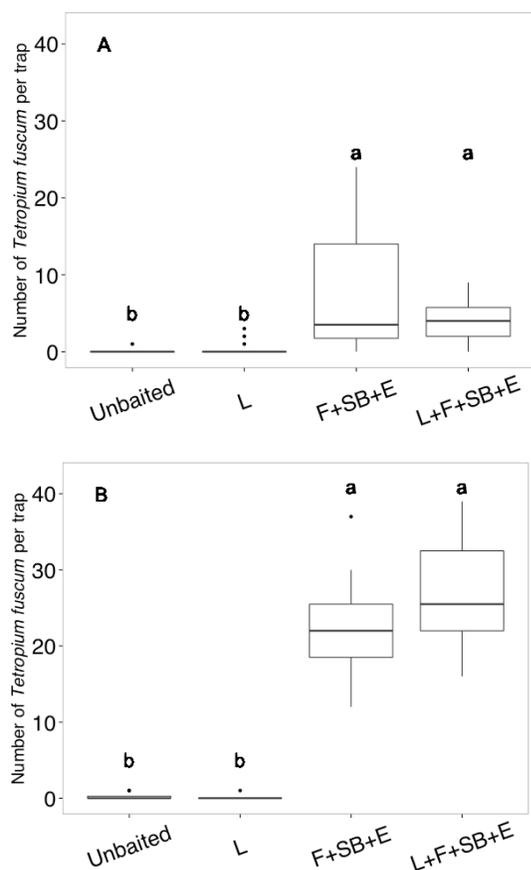
Other than a female bias in catch of *Tetropium fuscum* in one experiment, we observed a 1:1 sex ratio in catch of both species. Similar 1:1 sex ratios in *Tetropium fuscum* were reported for adults reared from field-collected Norway spruce (Juutinen 1955) and those landing on red spruce trees (Flaherty et al. 2013b). Significant female biases have previously been reported for *Tetropium fuscum* and *Tetropium cinnamopterum* captured in traps baited with fuscumol, spruce blend, and ethanol (Sweeney et al. 2010) and for *Tetropium fuscum* adults that emerged from red spruce bolts (Flaherty et al. 2013b).

Camphene consistently stimulated large electroantennogram (EAG) peaks for *Tetropium fuscum* (Silk et al. 2010), but nothing was known about its effect on *Tetropium* behavior. Our results suggest that camphene has little effect on catch of *Tetropium fuscum* or *Tetropium cinnamopterum* in traps.

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Figure 1. Box plots of *Tetropium fuscum* captured per trap in traps baited with different lure combinations (Control = unbaited, F=fuscumol, SB=spruce blend, L= racemic linalool (2011) or (R)-(-)-linalool (2012), E=ethanol) at: A) Juniper Lake, NS 11 July–25 August 2011; and B) Jack Lake, NS 23 May–18 July 2012. The whiskers represent the highest and lowest values; the dark line inside the box represents the median; the upper and lower lines of the box represent the 75th percentile and 25th percentile respectively; and the circles represent outliers. Different letters above boxes denote significant differences (Tukey's test for multiple comparisons of means of the generalized linear model, $p < 0.05$).

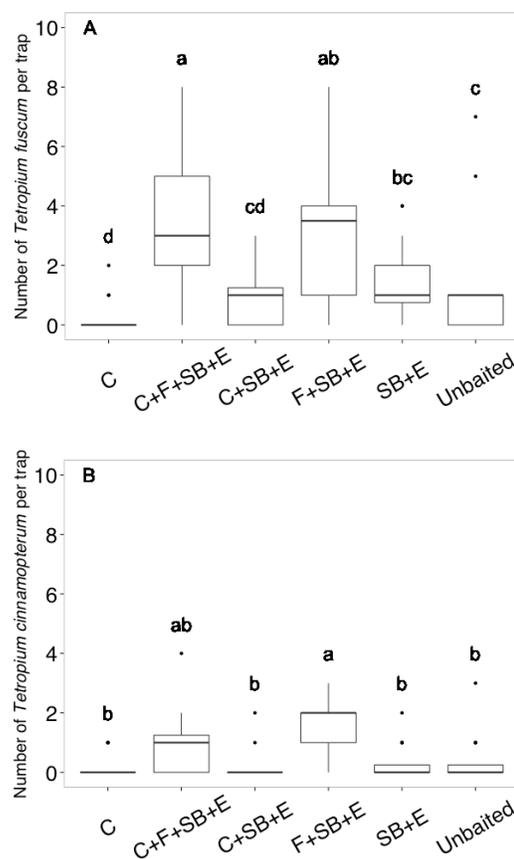


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Figure 2. Box plots of A) *Tetropium fuscum* and B) *Tetropium cinnamopterum* captured per trap in traps baited with different lure combinations (Control = unbaited, F=fuscumol, SB=spruce blend, C=camphene, E=ethanol). Different letters above boxes denote significant differences (Tukey's test for multiple comparisons of means of the generalized linear model, $p < 0.05$).



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