

Effect of semiochemicals and trap height on catch of *Neocerambyx raddei* in Jilin province, China

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Abstract

We conducted experiments in Jilin, China, in 2011 and 2014 in forest stands dominated by mature *Quercus mongolica* Fisch. ex Ledeb. (Fagaceae) to test the effects of longhorn beetle pheromones, plant volatiles, and trap height on catch of *Neocerambyx raddei* (Blessig & Solsky) (formerly *Massicus raddei*) (Coleoptera: Cerambycidae) in traps. Traps captured 276 specimens of *N. raddei* in 2011 and 379 specimens in 2014 (384 females, 271 males). Ethanol was attractive to female but not male *N. raddei*. However, *N. raddei* was not attracted to any of the longhorn beetle pheromones tested, which included racemic 3-hydroxyhexan-2-one, racemic 3-hydroxyoctan-2-one, *syn*-2,3-hexanediols, *anti*-2,3-hexanediols, racemic *E,Z*-fusicumol, racemic *E,Z*-fusicumol acetate, and monochamol, nor was it attracted to 2-methyl-3-buten-2-ol. Traps placed in the tree canopy captured significantly more beetles than did traps in the understorey. Our results suggest that surveys for *N. raddei* should use ethanol-baited traps placed in the tree canopy. If sex or aggregation pheromones are identified for *N. raddei* in the future, we predict that attraction to them will be enhanced by the presence of ethanol.

Introduction

Neocerambyx raddei (Blessig & Solsky) (previously placed in the genera *Massicus* and *Mallambyx*) (Coleoptera: Cerambycidae) (Löbl & Smetana, 2010) is a pest of oak forests (*Quercus mongolica* Fisch. ex Ledeb. in Jilin, *Quercus wutaishanica* Mayr in Liaoning, Fagaceae) in northeast China (Chen, 1959; Luo et al., 2005). Larvae bore into the lower bole, creating large galleries that greatly reduce the value of the wood for commercial purposes and also cause crown dieback. It is distributed throughout much of the oak forests in China from Heilongjiang in the north to Taiwan in the south. It is also recorded from Vietnam, Korea, Japan, east Siberia, and the Russian Far East (Wang, 2003).

Neocerambyx raddei has a 3-year life cycle in northeast China. Adults emerge over a 40-day period in July and early August, with a 0.97:1 (female:male) sex ratio (Jiang,

2011). After adults emerge, they feed on sap that exudes from wounds they make on the trunk with their mandibles (Tang et al., 2011), but do not feed directly on leaves, bark, or phloem (Zheng et al., 2014). On average, adult females live 22 days and males 19 days (Jiang, 2011). Adults are positively phototactic and fly and crawl in the middle and upper crown of oak trees, with peak activity between 19:00 and 22:00 hours (Tang et al., 2011). After 2–3 days feeding, adults begin mating. Females and males mate more than once, and females begin laying eggs 2–3 days after mating (Hou et al., 2000; Jiang, 2011). Females prefer to infest mature or stressed oak trees. They lay eggs in bark cracks and crevices on the main stem and secrete a white sticky liquid that helps the eggs adhere to the bark; they do not lay eggs in the wounds they chew in the bark (Xie et al., 1999; Jiang, 2011). Eggs hatch in 10–12 days. Larvae feed in the phloem for the first two instars and then feed in both the sapwood and heartwood, taking more than 3 years and five instars to completely develop. For example, larvae hatched from eggs laid in 2014 fed in late summer or early fall of 2014 and from late spring to early fall of 2015 and 2016, overwintered as mature pre-pupal larvae in 2016, and pupated and emerged as adults in July 2017

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(Hou et al., 2000). At a given site, adult emergence peaks every 3 years (Jiang, 2011), but small numbers of adults may be observed in intervening years in Jilin.

Tools for survey and monitoring of *N. raddei* would be very useful for its management in China as well as for surveys in parts of the world where it is not present but where it could be accidentally introduced and potentially become invasive. Several species of non-native ambrosia beetles and wood-boring beetles have been introduced to new continents and countries via solid-wood packaging or dunnage on vessels (Haack, 2006; Haack & Rabaglia, 2013). Due to the risk it might pose to oaks if accidentally introduced to Europe, *N. raddei* was recently added to the 'alert list' of the European Plant Protection Organization (EPPO, 2015). Black-light traps are very effective at capturing *N. raddei* (Jiang et al., 2010; Yang et al., 2010) but are expensive and not very practical for surveys in remote areas. Traps baited with semiochemicals attractive to *N. raddei* could provide an alternative survey tool to light traps. Many species of longhorn beetles are attracted to host plant volatiles such as α -pinene and ethanol (Chénier & Philogène, 1989; Allison et al., 2004; Miller, 2006), and traps baited with these attractants are commonly used in bark- and wood-boring beetle surveys (Rabaglia et al., 2008). Sex and/or aggregation pheromones have been discovered in many species of longhorn beetles (Allison et al., 2004; Hanks & Millar, 2016), and certain pheromone motifs are commonly shared within subfamilies such that traps baited with 2,3-hexanediols and hydroxyketones catch many species of Cerambycinae (Hanks & Millar, 2013, 2016), and traps baited with fuscumol and fuscumol acetate catch several species of Lamiinae (Mitchell et al., 2011; Hughes et al., 2013) and Spondylidinae (Sweeney et al., 2010). Furthermore, combining pheromones and host plant volatiles on the same traps often enhances or synergizes catches of longhorn beetles (Sweeney et al., 2010; Allison et al., 2012; Hanks & Millar, 2013; Ryall et al., 2015; Collignon et al., 2016). Trap placement is also an important consideration. The species composition of longhorn beetles collected in traps placed in the tree canopy in temperate forests often differs from that in traps placed in the understorey (Graham et al., 2012; Dodds, 2014; Maguire et al., 2014).

We conducted three field-trapping experiments in mature oak forests of northeast China to test the efficacy of various semiochemical lure combinations and the effect of trap height on detection of *N. raddei* in traps. No pheromones have been identified in *N. raddei*, but we reasoned that the hydroxyketones and hexanediols used by so many other species of Cerambycinae (Hanks & Millar, 2013, 2016) might be attractive and certainly warranted screening in field-trapping bioassays. We also screened the

response of *N. raddei* to combinations of pheromones emitted by longhorn species in other subfamilies, e.g., fuscumol and monochamol, because some cerambycids are attracted to pheromones they do not produce, possibly using them as kairomones that indicate suitable hosts (Mitchell et al., 2011; Wong et al., 2012; Hanks & Millar, 2013; Wickham et al., 2014). We specifically tested the effect of ethanol on attraction of *N. raddei*, alone and in combination with different cerambycid pheromones. We also tested response to 2-methyl-3-buten-2-ol (= MB), a volatile emitted by some hardwood species (Zhang et al., 2012) as well as some pine species (Goldan et al., 1993) and a component of the aggregation pheromone of *Ips typographus* L. (Bakke et al., 1977); MB significantly enhances attraction of *Monochamus galloprovincialis* (Olivier) to traps baited with ipsenol, α -pinene, and ethanol (Pajares et al., 2004; Ibeas et al., 2007). We included trap height (canopy vs. understorey) as a factor in one of our bioassays and predicted that traps placed in the crown would catch more beetles than traps placed in the understorey because the adults are known to spend time flying and crawling in crowns of their hosts. Our overall goal was to develop an effective trap lure for survey and detection of *N. raddei*.

Materials and methods

Three field-trapping experiments were conducted in forest stands dominated by mature *Q. mongolica* at the Wangqi Forestry Station near Dashi village (126°51'34"E, 43°33'45"N) (experiments 1 & 2) and at the Dabei Forestry Station, Shulan city (126°56'4"E, 44°16'55"N) (experiment 3) in Jilin, China (Figure 1A). Black panel intercept traps (Alpha-Scents, Portland, OR, USA) (Figure 1B) with 0.5 l of saturated salt solution as a killing agent and preservative in the collecting cups were used in all experiments. Treatments were replicated in randomized complete blocks with 20–30 m spacing between traps and blocks arranged linearly along trails in the forest. Traps were checked every 1–2 weeks, and the total number of female and male *N. raddei* collected over the entire trapping period summed per trap. Voucher specimens of *N. raddei* are deposited in the Forestry College insect collection of Beihua University, Jilin, China.

Experiment 1. Effect of cerambycine pheromones and ethanol on trap capture

The objective of this experiment was to determine the response of *N. raddei* to pheromones reported for other species of Cerambycinae, as well as ethanol, and ethanol-pheromone combinations. The experiment followed a pheromone*plant volatile factorial setup with five levels of

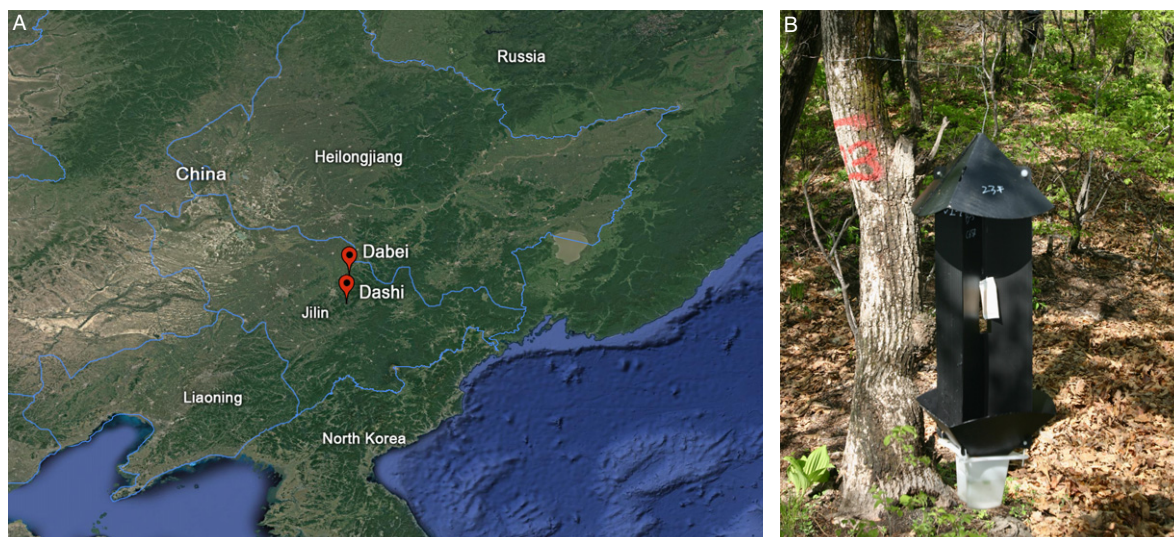


Figure 1 (A) Location of field sites (Dashi, Dabei) in Jilin, China. (B) Black panel intercept trap.

pheromone (four compounds + blank) and two levels of plant volatiles (ethanol present or absent) for a total of 10 lure treatments: (1) racemic 3-hydroxyhexan-2-one (K6), (2) racemic 3-hydroxyoctan-2-one (K8), (3) *syn*-2,3-hexanediols (a 1:1 blend of *R,R*- and *S,S*-2,3-hexanediols) (*syn* D6), (4) *anti*-2,3-hexanediols (a 1:1 blend of *R,S*- and *S,R*-2,3-hexanediols) (*anti* D6), (5) ethanol emitted at ultra-high release (UHR) rate (ET), (6) K6 + ET, (7) K8 + ET, (8) *syn* D6 + ET, (9) *anti* D6 + ET, and (10) an unbaited control (blank). The release devices for hydroxyketones and hexanediols consisted of a cellulose sponge loaded with a given chemical and sealed within a polyethylene pouch (7.0 × 5.0 × 0.5 cm). Source of pheromones, purity, and release rates of lures are indicated in Table 1. Traps were suspended from wire fixed between two trees such that the collecting cup was 30–50 cm above the forest floor and the trap was about 1 m from either tree. Treatments were replicated 8×, and the experiment ran from 9 July to 25 August 2011.

Experiment 2. Effect of lure combination and trap height on capture

This 2 × 3 factorial experiment tested the effects of trap height (middle to upper tree canopy vs. understorey) and three lure combinations: (1) K6 + K8 + ET, (2) *E/Z*-fusicumol + *E/Z*-fusicumol acetate + ET, (3) monochamol + ipsenol + α -pinene + ET (Table 1) on detection of *N. raddei*. Each of the six treatments was replicated 8×, and the experiment ran from 9 July to 28 September 2014. All lures but ethanol and ipsenol were replaced after 8 weeks. Traps were placed either in the understorey with collecting cups 30–50 cm above the ground (as described above) or were suspended from rope over branches in the

middle to upper canopy of trees, 10–15 m above the ground, using methods described in Hughes et al. (2014).

Experiment 3. Effect of pheromone lures and plant volatiles on detection

This was a 2 × 2 × 2 factorial experiment testing three lures: (1) K8, (2) ET, and (3) 2-methyl-3-buten-2-ol (MB) (Table 1) in all combinations, replicated 6× in a randomized complete block design. All traps were placed in the understorey of oak forests at the Dabei Forestry Station, Shulan, from 6 July to 21 September 2014. The K8 and MB lures were replaced after 8 weeks.

Data analysis

Numbers of males, females, and total *N. raddei* captured per trap were analysed with generalized linear mixed models (Proc GLIMMIX) using SAS/STAT for Windows v.9.2 software (SAS Institute, Cary, NC, USA) with pheromone treatment, plant volatile lure treatment, and trap height as fixed effects, and blocks as random effects. Models were run with both Poisson and negative binomial distributions (log link) and results reported are from the model and distribution with the lowest value of Akaike's information criterion corrected for small sample sizes (AIC_c). The Laplace method of estimating model parameters was used, as recommended when count means are sometimes <5 (Bolker et al., 2008). For significant treatment effects only, post hoc least-square means were compared with the Tukey-Kramer test. The χ^2 goodness of fit test with Yates continuity correction was used to determine whether sex ratio departed from a 1:1 ratio (Zar, 1999).

Table 1 Specifications of lures used in *Neocerambyx raddei* trapping experiments in Jilin province in 2011 and 2014

Lure		Expt.	Lure	Purity ¹ (%)	Release rate (mg/day) at 20 °C	Source
K6	Racemic 3-hydroxyhexan-2-one	1, 3	Pouch	99	20–25	Bedoukian Research Danbury, CT, USA/Contech Enterprises, Delta, BC, Canada
ET	Ethanol UHR lure	1, 2, 3	Pouch	99	300–400	Contech Enterprises
K8	Racemic 3-hydroxyoctan-2-one	1, 2, 3	Pouch	99	20–25	Bedoukian Research/Contech Enterprises
<i>syn</i> D6	Racemic <i>syn</i> -2,3-hexane diol	1	Pouch	99	1–2	Atlantic Forestry Centre, Fredericton, NB, Canada/Contech Enterprises
<i>anti</i> D6	Racemic <i>anti</i> -2,3-hexane diol	1	Pouch	99	1–2	Atlantic Forestry Centre/Contech Enterprises
	(<i>E/Z</i>)-fusicumol (6,10-dimethyl-5,9-undecadien-2-ol)	2	Rubber septa	99	0.5–2	Sylvar Technologies, Fredericton, NB
	(<i>E/Z</i>)-fusicumol acetate	2	Rubber septa	99	0.5–2	Sylvar Technologies
	Monochamol (2-undecyloxy-ethanol)	2	Pouch	99	0.75	Contech Enterprises
	Ipsenol (+50/–50)	2	Bubble cap	>99	0.4	Contech Enterprises
	α -Pinene (+25/–75)	2	Pouch	>98	2000	Contech Enterprises
MB	2-Methyl-3-buten-2-ol	3	Pouch	>98	36	Contech Enterprises

¹Supplied by manufacturer.

Results

Experiment 1. Effect of cerambycine pheromones and ethanol on trap capture

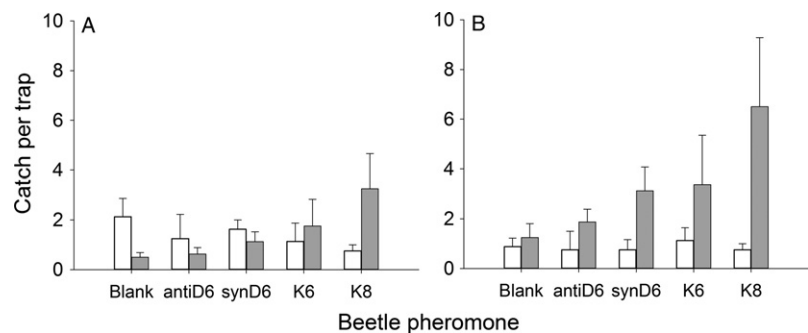
We captured a total of 276 *N. raddei* (163 females and 113 males). The overall sex ratio of 1.44:1 was female biased ($\chi^2 = 8.70$, $P < 0.01$). Mean catch per trap of *N. raddei* was not affected by pheromone ($F_{4,63} = 0.72$, $P = 0.58$) nor by pheromone*ethanol interaction ($F_{4,63} = 2.15$, $P = 0.08$), but was increased by the presence of ethanol ($F_{1,63} = 5.54$, $P = 0.02$). Ethanol increased the mean catch of females ($F_{1,63} = 18.3$, $P < 0.0001$) but not of males ($F_{1,63} = 0.18$, $P = 0.68$) (Figure 2). The sex ratio was female biased in traps baited with ethanol ($\chi^2 = 26.20$, $P < 0.01$) but not in traps without ethanol ($\chi^2 = 1.45$, $P > 0.05$). There was a significant pheromone–ethanol interaction for males

($F_{4,63} = 2.78$, $P = 0.03$) but not for females ($F_{4,63} = 0.96$, $P = 0.44$). Means were not separated by the Tukey-Kramer test, but trendwise, adding an ethanol lure to blank traps reduced male catch, whereas adding ethanol to traps baited with K8 had the opposite effect (Figure 2A). Mean catch of both sexes was greatest in traps baited with K8 + ethanol (Figure 2).

Experiment 2. Effect of lure combination and trap height on capture

A total of 306 *N. raddei* (181 females and 125 males) were captured in experiment 2. Trap height affected mean catch of both male ($F_{1,35} = 31.8$) and female *N. raddei* ($F_{1,35} = 35.2$, both $P < 0.0001$), with much greater catches in the canopy than in the understorey (Figure 3). Catch of *N. raddei* was not affected by lure treatment (males: $F_{2,35} = 0.45$, $P = 0.64$; females: $F_{2,35} = 1.74$, $P = 0.19$),

Figure 2 Mean (+ SE) catch per trap of (A) male and (B) female *Neocerambyx raddei* in intercept traps baited with various longhorn beetle pheromones with (grey) and without (white) ethanol lures, in Dashi, Jilin, China, in 2011. *Anti* D6, racemic *anti*-2,3-hexane diol; *syn* D6, racemic *syn*-2,3-hexane diol; K6, racemic 3-hydroxyhexan-2-one; K8, racemic 3-hydroxyoctan-2-one.



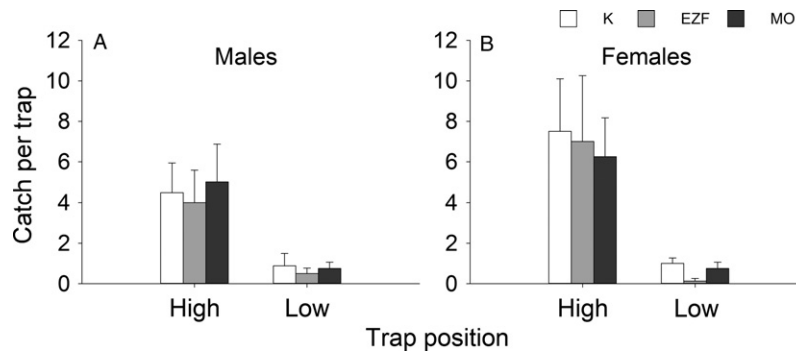


Figure 3 Mean (+ SE) catch per trap of (A) male and (B) female *Neocerambyx raddei* in intercept traps placed in the tree canopy (High) vs. the understorey (Low) and baited with one of three lure blends: racemic 3-hydroxyhexan-2-one + racemic 3-hydroxyoctan-2-one + ethanol (= K), *E/Z*-fusicumol + *E/Z*-fusicumol acetate + ET (= EZF), or monochamol + ipsenol + α -pinene + ET (= MO), in Dashi, Jilin, 2014.

nor by lure*trap height interaction (males: $F_{2,35} = 0.05$, $P = 0.95$; females: $F_{2,35} = 1.15$, $P = 0.33$). Catches were female biased in canopy traps ($\chi^2 = 11.86$, $P < 0.01$) but not in understorey traps ($\chi^2 = 0.28$, $P > 0.05$).

Experiment 3. Effect of pheromone lures and plant volatiles on detection

We captured 73 *N. raddei* (40 females and 33 males) in Shulan during 2014. Traps baited with ethanol captured more total *N. raddei* than traps without ethanol ($F_{1,35} = 9.06$, $P = 0.005$) (Figure 4). By themselves, neither K8 ($F_{1,35} = 0.11$, $P = 0.74$) nor MB ($F_{1,35} = 1.29$, $P = 0.26$) affected trap catches, but their interaction did ($F_{1,35} = 4.39$, $P = 0.04$), suggesting that traps baited with both lures had reduced catches. However, means were not separated in post hoc tests (Figure 4).

Discussion

Our results indicate that *N. raddei* is attracted to ethanol and that females are more attracted to ethanol than are males. This suggests that *N. raddei* uses ethanol as an olfactory cue when searching for brood hosts. Many cerambycids are attracted to plant volatiles such as ethanol and monoterpenes such as α -pinene (Chénier & Philogène, 1989), and ethanol has been shown to enhance attraction of cerambycids to their pheromones (Hanks & Millar, 2013; Miller et al., 2015). As ethanol is usually emitted in greater concentrations from stressed trees (Kelsey et al., 2014), the results also suggest *N. raddei* preferentially targets stressed oaks for oviposition. This supposition is supported by a study by Tang et al. (2011) that found the risk of infestation by *N. raddei* was greater for trees growing on poor sites that were more subject to moisture stress than for healthy trees. If female *N. raddei*

prefer to oviposit on stressed hosts, then the preference-performance hypothesis (Jaenike, 1978) would predict that survival from egg to adult and/or female size and fecundity would also be greater in stressed oaks than in healthy oaks. To our knowledge, neither oviposition preference nor performance of *N. raddei* on stressed vs. healthy oaks has been tested.

Traps placed in the middle or high canopy of oak trees captured more *N. raddei* than those placed in the understorey, indicating that canopy traps would be more efficacious for surveys and mass trapping than understorey traps. Previous studies have found that the species

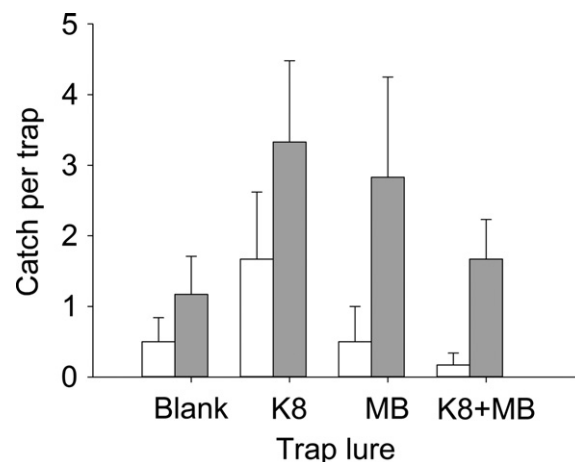


Figure 4 Effect of ethanol lure on mean (+ SE) catch per trap of *Neocerambyx raddei* in intercept traps baited with racemic 3-hydroxyoctan-2-one (K8), 2-methyl-3-buten-2-ol (MB), both (K8 + MB), or neither (Blank) at Shulan, Jilin, China, July-August 2014. Grey bars, ethanol was added; white bars, no ethanol was added.

composition of longhorn beetles in traps differs between the forest canopy and the understorey (Graham et al., 2012; Dodds, 2014; Webster et al., 2016). Feeding habits of larvae and adults may influence whether beetles were captured in canopy or understorey traps. Many Lamiine species that girdle and/or chew on branches before laying eggs were captured in canopy traps (Graham et al., 2012). Greater catches of *N. raddei* in the tree canopy may be because adults are more active in the canopy than in the understorey (Jiang, 2011). It is also possible that host volatiles emitted from oak foliage are attractive to *N. raddei*, and these volatiles may have been present in greater concentrations near canopy traps than near understorey traps.

None of the pheromones identified as attractive to other longhorned beetles that we tested, including those known to be emitted by several species of Cerambycinae (e.g., hydroxyketones, hexanediols), were attractive to *N. raddei*. However, the pheromone compounds that we tested by no means exhausted the many types of pheromone structures recently discovered for species of Cerambycinae, e.g., (2*S*,4*E*)-2-hydroxy-4-octen-3-one (Zou et al., 2015), 1-(1*H*-pyrrol-2-yl)-1,2-propanedione (Zou et al., 2016), and 10-methyldodecanal (Silva et al., 2016). Ginzel & Hanks (2005) hypothesized that mate location in species of Cerambycinae involved three sequential behavioural stages: both sexes are attracted to hosts by plant volatiles, males then attract females over shorter distances with pheromones, and finally males recognize females by contact pheromones in the female's epicuticular wax layer. If male *N. raddei* emit pheromones, we predict that ethanol would enhance attraction of both sexes to the pheromone, as observed in other species of Cerambycinae (Miller et al., 2015; Hanks & Millar, 2016). We have shown traps baited with ethanol and placed in the tree canopy are a fairly effective semiochemical-based means of surveying for *N. raddei*. However, careful chemical analyses of volatiles from adult *N. raddei* and from foliage and cortex of host oak trees may identify pheromones and host volatiles that could greatly improve attraction and trap sensitivity in the future.

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