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Chemical similarity between historical and novel host plants promotes range and host expansion of the mountain pine beetle in a naïve host ecosystem

Nadir Erbilgin[1], Cary Ma[1], Caroline Whitehouse[2], Bin Shan[1], Ahmed Najar[1] and Maya Evenden[3]

[1]Department of Renewable Resources, University of Alberta, 442 Earth Sciences Building, Edmonton, AB T6G 2E3, Canada; [2]Environment and Sustainable Resources Development, Forest Management Branch, Peace River, AB T8S 1T4, Canada; [3]Department of Biological Sciences, University of Alberta, 214A Earth Sciences Building, Edmonton, AB T6G 2E3, Canada

Author for correspondence:
Nadir Erbilgin
Tel: +1 780 492 8693
Email: erbilgin@ualberta.ca

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Summary

- Host plant secondary chemistry can have cascading impacts on host and range expansion of herbivorous insect populations.
- We investigated the role of host secondary compounds on pheromone production by the mountain pine beetle (*Dendroctonus ponderosae*) (MPB) and beetle attraction in response to a historical (lodgepole pine, *Pinus contorta* var. *latifolia*) and a novel (jack pine, *Pinus banksiana*) hosts, as pheromones regulate the host colonization process.
- Beetles emit the same pheromones from both hosts, but more *trans*-verbenol, the primary aggregation pheromone, was emitted by female beetles on the novel host. The phloem of the novel host contains more α-pinene, a secondary compound that is the precursor for *trans*-verbenol production in beetle, than the historical host. Beetle-induced emission of 3-carene, another secondary compound found in both hosts, was also higher from the novel host. Field tests showed that the addition of 3-carene to the pheromone mixture mimicking the aggregation pheromones produced from the two host species increased beetle capture.
- We conclude that chemical similarity between historical and novel hosts has facilitated host expansion of MPB in jack pine forests through the exploitation of common host secondary compounds for pheromone production and aggregation on the hosts. Furthermore, broods emerging from the novel host were larger in terms of body size.

Introduction

Understanding novel host plant–herbivore interactions is one of the most challenging issues in invasion biology (Bertheau *et al.*, 2010) as host plant characteristics, such as host secondary compounds, can have cascading consequences for the establishment success of new herbivore populations, their population dynamics and the herbivore's invasion potential in the invaded range (Roques *et al.*, 2006; Futuyma, 2008; Ammunét *et al.*, 2011; Kausrud *et al.*, 2012). Although herbivores are not always successful in exploiting their novel hosts (Bertheau *et al.*, 2010; Økland *et al.*, 2011), they may capitalize on the 'evolutionary naïvety' of novel host plants and exploit them more effectively than hosts with which they have co-evolved (Walther *et al.*, 2009; Mooney & Cleland, 2010).

Several overlapping hypotheses have been proposed to explain the role of plant secondary compounds during an insect range and host expansion (Ehrlich & Raven, 1964; Jermy, 1984; Feeny, 1991). Although these hypotheses are not mutually exclusive, they all emphasize that novel host plants are suitable for colonization by herbivorous insects if their secondary compounds are related to those of the historical hosts of the invading herbivorous insects. Several studies have provided empirical evidence to support these hypotheses (Futuyma & McCafferty, 1990; Feeny, 1991; Berenbaum, 1995; Becerra, 1997; Lopez-Vaamonde *et al.*, 2003; Murphy & Feeny, 2006). Few studies have focused on the role of plant secondary compounds in range and host expansion of forest insects.

Bark beetles (Coleoptera: Curculionidae) contain some of the most ecologically and economically important forest insect species in North America (Safranyik *et al.*, 2010). Three features of the relationship of bark beetles with their host plants are particularly relevant to their interaction with host secondary compounds (Raffa *et al.*, 2005). First, they must kill their hosts to reproduce and complete their development (eggs, larvae, pupae, adult) within the phloem of the host trees. Failure to kill the host tree usually results in failed reproduction caused by unsuccessful gallery establishment as a result of adult and brood mortality caused by toxic host secondary compounds. Second, beetle broods emerging from the parental host disperse and must locate and kill live trees in which to breed. Some debate exists as to the mechanism of initial host selection by bark beetles. Although some species, such as pine engraver beetles, can utilize volatile plant secondary chemicals as long-distance cues to locate their hosts

(i.e. Erbilgin & Raffa, 2000), for others, the role of host secondary compounds in host location is less clear. For example, the mountain pine beetle (*Dendroctonus ponderosae*, MPB) utilizes a combination of random landings and visual orientation for host location (Safranyik *et al.*, 2010). Third, pheromonal communication among conifer-infesting bark beetles has been closely linked to host secondary compounds, particularly monoterpenes (Blomquist *et al.*, 2010). The plant secondary compounds may influence the production and release of aggregation pheromones. For example, some bark beetles may convert plant monoterpenes to oxygenated products, which serve as aggregation pheromones. Exposure to host monoterpenes may also stimulate *de novo* synthesis of pheromones (Blomquist *et al.*, 2010). Bark beetle pheromones function in mating, habitat location, counteraction of host defenses and resource partitioning.

Mountain pine beetle (MPB) is the most damaging forest insect species in North America (Safranyik *et al.*, 2010). The natural range of the beetle extends from northern Mexico, through the western USA to central British Columbia in Canada, affecting a considerable portion of the western conifer forests (Wood, 1982). Within its native range, MPB colonizes numerous pine species, including lodgepole pine (*Pinus contorta*). Lodgepole pine is a dominant tree species throughout western North America, including British Columbia and western and central Alberta (Canada) (Fig. 1). Beetles share a long co-evolutionary history with lodgepole pine (Kelley & Farrell, 1998) and thus have adapted to utilize secondary compounds of this host species (Keeling & Bohlmann, 2006; Raffa *et al.*, 2013). Recent large-scale climatic shifts have allowed the current MPB epidemic to expand beyond the beetle's historical range in lodgepole pine forests, and beetle populations have reached epidemic levels in areas previously thought to be unsuitable for beetle survival in northern British Columbia (Cudmore *et al.*, 2010).

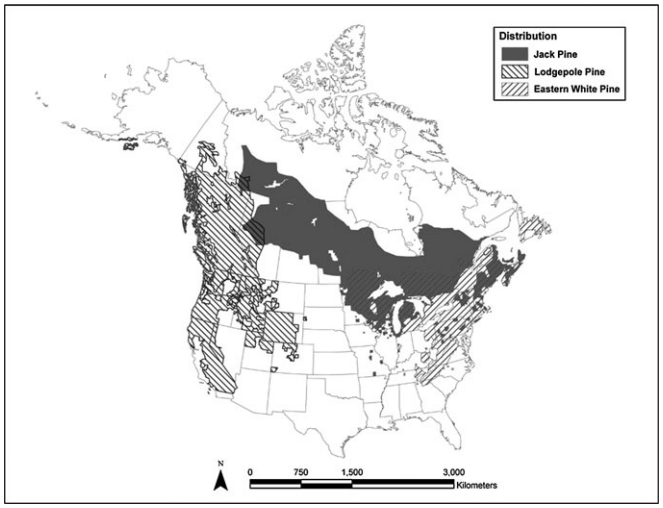

Fig. 1 Distribution of lodgepole pine (*Pinus contorta*), jack pine (*Pinus banksiana*) and eastern white pine (*Pinus strobus*) in North America. The lodgepole–jack pine hybrid zone is illustrated by the overlap of lodgepole and jack pine ranges.

As MPB has expanded eastward from British Columbia into Alberta it has spread across the lodgepole pine × jack pine hybrid zone, and into boreal jack pine (*Pinus banksiana*) forests in northern Alberta (Fig. 1) (Cullingham *et al.*, 2011). Jack pine is a common conifer species throughout the Canadian boreal forest and extends from the Northwest Territories and Alberta to the east coast of Canada, eventually overlapping with eastern white pine (*Pinus strobus*) in the upper Midwest, Middle Atlantic States and New England of the USA (Fig. 1). Thus, further range expansion of beetles from the jack pine forests in Alberta to eastern Canada could potentially affect a number of ecologically important tree species in eastern North America (Logan *et al.*, 2003). Unlike lodgepole pine, jack pine is a novel host and thus is considered 'naïve' in terms of encounters with MPB (Safranyik *et al.*, 2010).

In the MPB–lodgepole pine system, mass attack involves close interactions between volatile chemicals produced by host (monoterpenes) and beetle (pheromones) (Borden *et al.*, 2008). When a female beetle initiates an attack on a tree, it releases *trans*-verbenol that is attractive to both males and females. Arriving males mate with females and release *exo*-brevicomin that attracts mainly females (Pureswaran *et al.*, 2000). The mixture of pheromone components from female and male beetles serves as a powerful aggregation pheromone, which usually results in mass attacks of the host trees. At this stage, host chemicals released with beetle aggregation pheromones from the attacked trees can improve beetle attraction (Borden *et al.*, 2008). This aggregation process is required for the depletion of host defenses, successful host colonization and reproduction (Safranyik *et al.*, 2010). At the later stages of host colonization, female and male beetles reduce *trans*-verbenol and *exo*-brevicomin production, respectively, and instead produce frontalin (male only) and verbenone as anti-aggregation pheromones to mediate the number of beetles arriving to the host (Pureswaran *et al.*, 2000). Frontalin can be attractant or repellent depending on its concentration (Borden *et al.*, 1987).

Pheromone components produced by MPB are synthesized either by modifying host precursors or *de novo* (reviewed by Blomquist *et al.*, 2010). The female aggregation pheromone, *trans*-verbenol, is a bicyclic monoterpenoid alcohol and can be induced by feeding. It is most probably produced via cytochrome P450-mediated hydroxylation of α-pinene. The male aggregation pheromone, *exo*-brevicomin, is synthesized *de novo* by epoxidation and cyclization of its precursor long-chain fatty acids. Frontalin, a male-specific anti-aggregation pheromone component, is believed to be synthesized *de novo* from its precursors derived from either monoterpenoid or longer chain fatty acids. Verbenone, an anti-aggregation pheromone produced by either sex, is thought to be an auto-oxidation product of the host monoterpene α-pinene or a result of microbial conversion of *trans*-verbenol.

We investigated the role of host secondary compounds on pheromone production by beetles and beetle attraction in response to a historical and a novel host species. We first examined whether MPB can produce its pheromones in the novel host and whether the quality and/or quantity of the pheromones produced differ from those produced in the beetle's historical host.

We then tested whether the pheromone mixture produced in jack pine is attractive to beetles in a field experiment. As plant chemistry alone cannot explain host suitability, we further evaluated whether the novel host is suitable for beetle reproduction, and whether beetle brood quality is similar between the historical and novel hosts, by measuring the dispersal capacity of brood beetles reared in different hosts. The resulting information will reveal the adaptation potential of MPB and thus the potential for the spread of this species in the novel environment.

Materials and Methods

Pheromone production by MPB on lodgepole pine and jack pine

To determine whether MPB (*Dendroctonus ponderosae* Hopkins) can produce pheromones on jack pine (*Pinus banksiana* Lamb), the volatile chemicals emitted from individual beetle entrance tunnels on bolts from jack and lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) trees were characterized. We cut five trees of each species, taking one bolt (40 cm long) from each tree. Lodgepole pine trees were cut in Hinton (53°25.42′N, 117°34.13′W) and jack pine trees were cut in Lac La Biche (55°02.32′N, 114°02.97′W) on 19 and 21 July 2012, respectively. Both ends of the bolts were sealed with melted wax to minimize the moisture and secondary metabolite loss. Bolts were brought to the laboratory and each was inoculated with four pairs (one male, one female) of beetles. We first opened four 5-mm holes on the bark (equidistant around the circumference of each bolt) using a cork borer (5 mm in diameter), and then introduced a female to each hole. When female introduction was successful, that is, boring dust was observed near the entrance hole, we introduced a male 24 h later into the same hole. We used beetles of the same age (2–3 d post-emergence) to inoculate bolts. These beetles had emerged from jack and lodgepole pine bolts that had been artificially infested and reared in the laboratory before this experiment. Broods from jack or lodgepole pine were used to inoculate bolts of the same species.

We collected volatile chemicals from two holes on each bolt as follows. A small Teflon funnel was placed above each hole and the gap between the cork bark and the base of the funnel was sealed by a charcoal filter (Honeywell, Southborough, MA, USA). Volatiles emitted from individual holes were continuously collected for 4 h using a vacuum pump (Cole-Parmer Canada Inc., Montreal, QC, Canada). Each funnel was attached to a pump with a Teflon tube, and an adsorbent tube (Porapak Q (OD, 6 mm; length, 110 mm; adsorbent: front layer, 150 mg; back up layer, 75 mg; separated by glass wool), SKC Inc., Eighty Four, PA, USA) was inserted in the tube between the pump and the funnel. Volatile chemicals emitted from individual beetle entrance holes were trapped in the adsorbent tubes. The flow rate (100 ml min^{-1}) was kept constant during volatile collection. The same collection protocol from the same entrance hole was repeated with a new adsorbent tube at 12, 24, 36, 48, 72, 96 and 120 h after female beetle introduction. After each collection, the adsorbent tubes were capped and stored at −40°C before extraction. All bolts were kept at room temperature for the duration of the experiment.

Chemical analysis

The volatiles trapped inside the adsorbent tubes were extracted with 1 ml of dichloromethane (Sigma-Aldrich, St Louis, MO, USA) mixed with 1 ng μl^{-1} heptyl acetate (Sigma-Aldrich) as internal standard. Extracts were transferred into 1-ml vials (Agilent Tech, Santa Clara, CA, USA) and subsequently stored at −40°C. Extracts (1 μl) were injected at a split ratio of 20 : 1 in a Gas Chromatograph/Mass Spectrometer (GC/MS) (Agilent 7890A/5062C, Agilent Tech) equipped with an HP Innowax column (ID, 0.32 mm; length, 30 m; Agilent Tech), with a helium carrier gas flow of 1.8 ml min^{-1}, temperature of 50°C for 2 min, increased to 160°C by 5°C min^{-1} and then ramped up to 250°C by 20°C min^{-1}.

To determine the enantiomeric composition of *trans*-verbenol and α-pinene, a GC/MS equipped with a chiral column (HP Innowax-20B column; ID, 0.25 mm; length, 30 m; Agilent Tech), helium carrier gas flow at 1.1 ml min^{-1}, temperature of 75°C for 15 min, increased to 230°C by 5°C min^{-1}, was used. We focused on the chiral analysis of these two chemicals because α-pinene chirality may affect the production of *trans*-verbenol in female beetles (Blomquist *et al.*, 2010).

Peaks were identified using the following standards. Pheromones: racemic *trans*-verbenol, (−)-*trans*-verbenol (enantiomeric composition, 82%(−)/18%(+)), racemic *exo*-brevicomin, frontalin and verbenone. The chemical purity of these pheromones, except for (−)-*trans*-verbenol which had 75%(−)/25%(+) chemical purity, was higher than 95%. All pheromones were obtained from Contech-Inc (Delta, BC, Canada). Monoterpene standards were: borneol, pulegone, α-terpinene, γ-terpinene, α-terpineol, camphor, 3-carene, α-humulene, terpinolene, α- and β-thujone, (−)-α-pinene, (+)-α-pinene, racemic α-pinene, (−)-β-pinene, (*S*)-(−)-limonene, sabinene hydrate, myrcene, (−)-camphene, *p*-cymene (Sigma-Aldrich), bornyl acetate, *cis*-ocimene (SAFC Supply Solutions, St. Louis, MO, USA) and β-phellandrene (Glidco Inc., Jacksonville, FL, USA). The chemical purity of all of these compounds was > 99%. Compounds were identified by comparing retention times and mass spectra with those of the standard chemicals. The quantity of chemicals was calculated using response curves generated from analyses of a dilution sequence of known quantities of standards. Calibration with these standards allowed for the analysis of quantitative differences on volatile samples among treatments. The amount (ng μl^{-1}) of pheromones per pair beetle (Fig. 2) and the major monoterpenes (ng μl^{-1}) (Table 1) emitted from each entrance hole were reported.

Field experiment

In order to test the attractiveness of beetle aggregation pheromones and a host monoterpene, 3-carene, emitted from beetle entrance holes on each host species, we conducted a field experiment in Grande Prairie, Alberta (55°20.16′N, 118°18.9′W). As

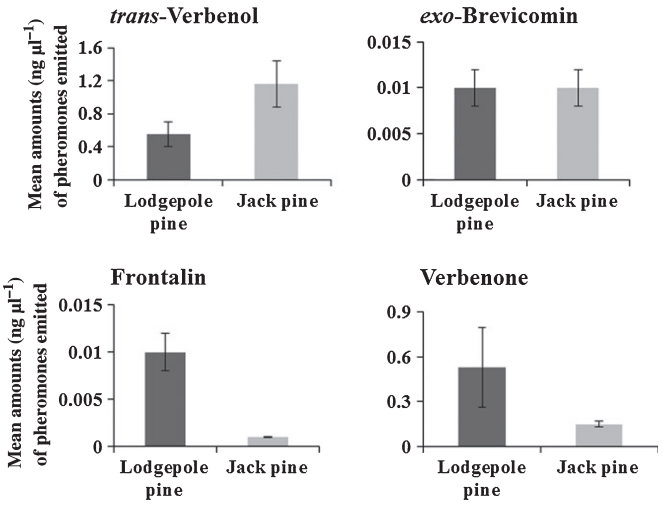

Fig. 2 Mean amounts (± SE) (ng μl^{-1}) of pheromones emitted by mountain pine beetle (*Dendroctonus ponderosae*) on jack pine (*Pinus banksiana*) vs lodgepole pine (*Pinus contorta*) over 120 h ($n = 5$). Volatiles were collected continuously for 4 h at 12, 24, 36, 48, 72, 96 and 120 h after female beetle introduction. Male beetles were introduced after 24 h. The mean amount for each compound for each tree species was calculated by dividing the total cumulative values collected over 120 h by the number of collections (7).

the amount of pheromones and monoterpenes emitted varied quantitatively between beetles from each tree species (Fig. 2, Table 1), the possible effects of such differences on beetle attraction were incorporated in the field experiment. We used various combinations of Eppendorf and PCR tubes with varying wall thicknesses and densities to reach the target release rates (Table 2). As the amounts emitted from individual beetles were quite low compared with the volatiles coming from trees under mass attack, we increased the amount of pheromones emitted by *c.* 100 times and 3-carene by *c.* 50 times to simulate a real beetle colonization on trees in nature. We kept the multiplication rate for 3-carene lower than that of pheromones, because our earlier studies in other bark beetle–host systems (i.e. Erbilgin *et al.*, 2007) showed that monoterpene : pheromone ratios of *c.* 25 : 1 can be highly attractive to tree-killing bark beetle species. Thus, in our experiments, we kept the 3-carene : *trans*-verbenol ratio to *c.* 25 : 1. Further, the release amounts tested were not unrealistic considering the amount of volatile chemicals emitted during the mass aggregation of beetles and the several-fold increase in emission of monoterpenes after bark beetle attacks on conifers (Raffa & Smalley, 1995; Erbilgin & Raffa, 2000). For this experiment, pheromones were obtained from Contech Inc. and 3-carene was obtained from Sigma-Aldrich. The chemical purity of pheromones, except for (−)-*trans*-verbenol, which had 75% chemical purity, was > 95%. The chemical purity of 3-carene was > 95%.

The forest stand in which this experiment was conducted is dominated by lodgepole pine trees and has experienced extensive beetle infestations over the last 7 yr. We also wanted to test the same treatments in a jack pine forest in eastern Alberta; however, this was not possible because the jack pine forest did not have an active beetle infestation at the time of the experiment (all jack pine trees attacked by beetles were removed to lower the beetle population).

We tested the following five treatments in the field: (1) pheromones mimicking emission on jack pine (*trans*-verbenol (2×), *exo*-brevicomin (1×), frontalin (1×)); (2) pheromones mimicking emission on lodgepole pine (*trans*-verbenol (1×), *exo*-brevicomin (1×), frontalin (10×)); (3) pheromones mimicking emission on jack pine plus 3-carene (2×); (4) pheromones mimicking emission on lodgepole pine plus 3-carene (1×); (5) blank control. Numbers next to '×', that is, 1×, 2×, etc., represent the amount of chemicals released from dispensers in one treatment relative to the same chemical released from another treatment. For example, 2× indicates that two times more *trans*-verbenol was released from dispensers mimicking beetle pheromone on jack pine relative to the *trans*-verbenol released on lodgepole pine. We included three of the four major beetle pheromones identified (*trans*-verbenol, *exo*-brecomin, frontalin) in the field experiment. These three pheromones are part of an aggregation pheromone blend that elicits colonization behavior on host trees (Borden, 1985). Verbenone was not included as it inhibits beetle attraction to aggregation pheromones. 3-Carene was included because our earlier field experiment found that 3-carene increased beetle attraction to pheromone more than any other monoterpene, including α-pinene or myrcene (N. Erbilgin, unpublished data).

There were eight blocks of five flight intercept panel traps (Advanced Pheromone Technologies, Marylhurst, OR, USA) each. One trap representing each of the five treatments was included in each block and treatments were randomly distributed among traps in each block. We collected the trapped beetles every

Table 1 Mean amounts of monoterpenes emitted from lodgepole pine (*Pinus contorta*) (Lp) vs jack pine (*Pinus banksiana*) (Jp) over 120 h

	Mean amounts (ng μl^{-1}) (± SE) of monoterpenes released									
Tree species	Total monoterpenes	α-Pinene	β-Pinene	β-Phellandrene	3-Carene	Myrcene	Limonene	Camphene	α-Phellandrene	Terpinolene
Lp	344.3 (83.6)	80.1 (21.7)	87.1 (25.4)	123.8 (29.9)	32.2 (4.1)	6.9 (1.6)	10.6 (2.2)	3.7 (1.1)	5.6 (1.5)	8.4 (2.4)
Jp	228.5 (23.7)	132.8 (17.3)	18.1 (2.8)	0.0 (0.0)	62.4 (7.5)	2.5 (0.5)	1.9 (0.4)	5.1 (1.5)	1.7 (0.4)	0.2 (0.0)
$F_{1,136}$	6.14	4.59	7.11	16.82	5.63	6.88	14.99	0.53	5.42	11.6
P	0.02	0.04	<0.01	<0.0001	0.02	<0.01	<0.001	0.47	0.02	<0.001

Volatiles were collected for 4 h at 12, 24, 36, 48, 72, 96 and 120 h after female mountain pine beetle (*Dendroctonus ponderosae*) introduction. Male beetles were introduced after 24 h. The mean amount of each compound for each tree species was calculated by dividing the total cumulative values collected over 120 h by the number of collections (7).

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Table 2 Desired release amount and type of release device used and actual release rate tested in the field experiment

	Desired release amount (ng d^{-1}) (×100)		Type (and number) of release devices (ng d^{-1})		Release rate achieved/tested in the field experiments (ng d^{-1}) (×100)	
Chemicals	Lodgepole pine	Jack pine	Lodgepole pine	Jack pine	Lodgepole pine	Jack pine
trans-Verbenol	55	116	0.2 ml PPFC[a] (3)	0.2 ml PPFC (6) + 0.5 ml BPCR[b] (1)	54.21	116.69
exo-Brevicomin	1	1	0.2 ml PPFC[c] (1)	0.2 ml PPFC[c] (1)	1.83	1.83
Frontalin	1	0.1	0.5 ml EPCR[d](1)	0.5 ml EPCR[d](1)	1.7	0.3
3-Carene	1600	3100	15 ml UHRB[e] (1)	15 ml UHRB (2)	1550.33	3100.66

The amounts of *trans*-verbenol, *exo*-brevicomin and frontalin reported in Fig. 1 were multiplied by 100 and the amount of 3-carene reported in Table 1 was multiplied by *c*. 50 to make the amounts originating from our release devices biologically active in the field experiment. All release devices with active compounds were tested in the fume hood at 23°C for 30 d, and the amounts reported represent the average at the end of 30 d.
[a]0.2 ml PPFC. Polypropylene PCR tube. We added 3 μl of *trans*-verbenol in each tube. Each tube released 18.07 ng d^{-1} over a 30-d period at 23°C.
[b]0.5 ml BPCR. Brand Tech Thin Wall PCR tube. We added 3 μl of *trans*-verbenol in each tube. Each tube released 8.27 ng d^{-1} over a 30-d period at 23°C.
[c]0.2 ml PPFC. Polypropylene PCR tube. We added 3 μl of *exo*-brevicomin in each tube. Each tube released 5.83 ng d^{-1} over a 30-d period at 23°C. In order to reduce the amount, we covered the outer surface of the tube with duct tape.
[d]0.5 ml EPCR. Eppendorf Thick-Walled PRC tube. We added 3 μl of frontalin in each tube. Each tube released 1.7 ng d^{-1} over a 30-d period at 23°C. This is the smallest amount determined. In order to reduce the amount for jack pine, we covered the outer surface of the tube with duct tape.
[e]15 ml UHRB. Polyethylene ultrahigh release bottle. We added 8 ml of 3-carene to each bottle. Each tube released 1550 ng d^{-1} over a 30-d period at 23°C.

4 d for a total of eight collections. At each collection, traps in each block were re-randomized. Captured beetles were brought to the laboratory and separated by sex. As a result of rain, four collections contained few or no beetles in traps, and so they were removed from the analysis. The experiment started in mid-July and continued until the end of August during the peak flight period of MPB.

Insect rearing

In order to evaluate how jack pine host substrate affects beetle colonization and brood quality, after volatile collection, inoculated bolts were placed in individual rearing containers for 6 wk at room temperature and were then transferred to a cold room (+ 2°C) to simulate winter temperatures for 10 wk. After overwintering, containers were removed from the cold room and placed at room temperature to allow the offspring of inoculated beetles to complete their development. Bolts were removed from cold storage in late January to early February 2013, and emerging broods were collected daily for the next 3 months and separated by host tree species and sex. Individual beetles were placed in a microcentrifuge tube with a small piece of paper and stored in the dark at 4°C until use in the flight bioassay (below). After all brood beetles had emerged, the impact of host tree species on beetle survival and fecundity was measured. Beetle galleries under the bark were exposed by removing the outer bark, and the number of maternal galleries with or without a mating chamber on each bolt was recorded, and the length of each maternal gallery was measured.

Insect flight on flight mill

The flight capacity of emergent brood beetles from both pine hosts was measured using a flight mill bioassay as a measure of offspring condition. At 3–5 d post-emergence, beetles were removed from cold storage and prepared for flight. Individual beetles emerging from bolts were weighed to the nearest 0.01 mg and then tethered using a 2-cm-long and 0.03-mm-diameter aluminum wire with a 0.14-mm loop at the distal end. The tether loop was dipped in LePage Pres-Tite Contact Cement (Mississauga, ON, Canada) and secured to the center of the pronotum, ensuring that elytra movement was not impeded. After all beetles had been tethered, individuals were transferred to the flight mills to assess flight capacity. Beetles were tethered to the arm of a digital flight mill apparatus linked to a computer. One beetle-powered rotation of the flight mill arm was recorded each time the magnet located under the flight mill arm tripped the sensor attached to the flight mill base. A single rotation of the flight mill arm equaled a distance of 94.2 cm. The flight mills were housed in an environmental chamber set to 16 h : 8 h light : dark at 24°C. Between one and 13 beetles were flown each day. Flight assays were initiated 4 h after the beginning of the flight phase. At the end of the 24-h flight period, beetles were removed from the flight mills, the tether was detached and the beetles were weighed again. The total duration and distance of flights were summed over the first 24 h. Beetles were then killed and stored at −20°C for future lipid extraction. Male and female beetles were flown on alternate days. Mass loss during flight was calculated as the difference in mass before and after flight, and the difference in mass was converted into a proportion by dividing the mass difference by the pre-flight mass. Beetles that did not survive the entire flight period were removed from the dataset.

Lipid extraction

To quantify the energetic condition of male and female beetles after flight, flown beetles were submitted to lipid extraction using petroleum ether (Atkins, 1969). Beetles were dried in an oven at 60°C for 24 h, and then weighed to the nearest 0.01 mg. Beetles were placed individually into perforated 0.2-ml microfuge tubes

and submerged in petroleum ether in a Soxhlet apparatus (45/50 Pyrex; Fisher Scientific, Ottawa, Ontario, Canada). Lipid was extracted for 8 h. After extraction, beetles were again dried at 60°C for 24 h and then weighed. Lipid mass remaining after flight was calculated as the difference in dry mass before and after extraction. Lipid mass after flight was converted into a relative measure by dividing by the pre-extraction beetle dry mass.

Data analysis

All data analyses were performed in R (R Development Core Team, 2012) using the linear model function (glm with a binomial error distribution for dichotomous data, lm for models with fixed effects and lme for models with mixed effects). Bolts were treated as a 'block' (random effect). Differences between tree species in the mean amounts (ng μl^{-1}) of pheromones and monoterpenes emitted by beetles were compared after square root transformation to meet the assumptions of normality and homogeneity of variance. For the field experiments, mean numbers of insects were compared among five treatments after square root transformation. Data were analyzed using 'block' as a random effect. If there was no block effect, the random effect of 'block' was removed from the models and the data were analyzed using only fixed effects, including 'tree species'. Honestly significant difference (HSD) test was conducted for multiple comparisons among treatments. *Post-hoc t*-tests were performed on select pairwise comparisons. In both laboratory and field data, as data were collected over time, we applied repeated measures using the ANOVA function from the 'car' package in R.

Differences in propensity for the flight of beetles reared in the different tree hosts measured on the flight mills were analyzed using a generalized linear model. The response variables of pre-flight mass, total distance flown and total flight duration were log-transformed to meet assumptions of normality. Explanatory variables included sex, pre-flight mass and host species, and appropriate two-way interactions. Bolts were treated as a blocking factor. In all models, the most parsimonious model was chosen by backward model selection achieved by dropping non-significant variables one at a time and hypothesis testing.

Differences between trees in terms of phloem thickness, tree size (diameter at breast height, DBH), number of maternal galleries and maternal gallery length were determined by modeling data as linear models, and analyzing them using ANOVA in a generalized linear model.

Results

Pheromone emission

All four major components were emitted from beetle tunneling on both tree species (Fig. 2), and only the quantity of *trans*-verbenol emitted differed between host species. Female beetles on jack pine emitted about two times more *trans*-verbenol than females on lodgepole pine ($F_{1,136} = 5.16$, $P = 0.02$). Male beetles on lodgepole pine emitted more frontalin than male beetles on jack pine, but the differences were not significant ($F_{1,136} = 2.01$, $P = 0.1$). Likewise, emissions of verbenone ($F_{1,136} = 1.95$, $P = 0.16$) and *exo*-brevicomin ($F_{1,136} = 0.09$, $P = 0.76$) were identical between the hosts.

The mean amount of (−)-*trans*-verbenol emitted by beetles was higher in jack pine (1.03 ± 0.25) than in lodgepole pine (0.49 ± 0.14) ($F_{1,136} = 5.06$, $P = 0.03$). Likewise (+)-*trans*-verbenol release was higher in jack pine (0.14 ± 0.04) than in lodgepole pine (0.06 ± 0.02) ($F_{1,136} = 4.24$, $P = 0.04$). However, the percentage of (−)-*trans*-verbenol emitted by beetles was similar between host species (86.63% in lodgepole pine vs 88.72% in jack pine).

Monoterpene emission

Significantly more monoterpenes were emitted from beetle entrance holes on lodgepole than jack pines over the first 120 h after female beetle introduction (Table 1). The individual compounds reported comprise >95% of the total monoterpenes identified. Among these compounds, higher amounts of α-pinene and 3-carene were released from jack than lodgepole pines and more (−)-β-pinene, β-phellandrene, myrcene, limonene, α-phellandrene and terpinolene were released from lodgepole than jack pines. The amount of camphene released was similar between the hosts.

There were also differences between the enantiomeric ratios of (−)- and (+)-α-pinene released by the two pine species. More (−)-α-pinene ($70.2 \pm 20.8\%$) was released from lodgepole than jack ($21.56 \pm 12.5\%$) pine.

Beetle attraction in field experiment

Overall, baited traps caught more beetles than did unbaited control traps (Fig. 3). Among baited traps, the highest number of beetles was found in traps baited with pheromones plus 3-carene mimicking jack pine, although this did not differ significantly from the number caught in the traps baited with pheromones plus 3-carene mimicking lodgepole pine ($F_{4,155} = 20.88$, $P < 0.0001$). Pheromones mimicking lodgepole pine caught the

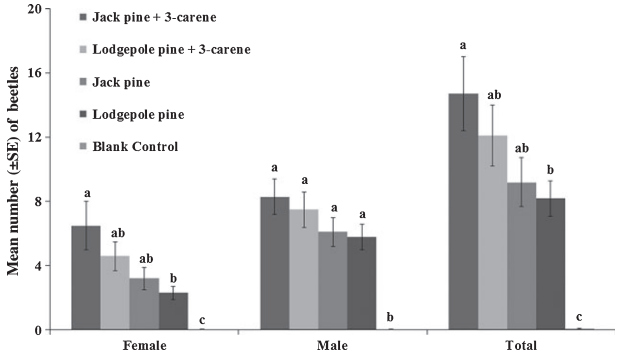

Fig. 3 Mean number (± SE) of mountain pine beetles (*Dendroctonus ponderosae*) attracted to various treatments mimicking jack pine (*Pinus banksiana*) and lodgepole pine (*Pinus contorta*) with or without the host tree monoterpene 3-carene ($n = 32$). Different letters in each category (female, male, total) indicate statistical differences among treatments at $\alpha = 0.05$.

lowest number among the baited traps. Female ($F_{4,155} = 13.22$, $P < 0.0001$) and male ($F_{4,155} = 21.72$, $P < 0.0001$) beetle responses showed differences among treatments.

Comparison of jack pine and lodgepole pine with or without 3-carene indicated significant differences between treatments. Jack pine with 3-carene caught significantly more beetles than jack pine without 3-carene (total beetles: $F_{1,62} = 5.22$, $P < 0.01$). Likewise, traps baited with beetle pheromones emitted from lodgepole pine with the same monoterpene caught more beetles than those baited without 3-carene (total beetles: $F_{1,62} = 4.78$, $P < 0.01$).

Subcortical data

Totals of 90 (57 female, 27 male, 6 undetermined) and 115 (54 female, 43 male, 18 undetermined) beetles emerged from jack pine and lodgepole pine, respectively. We measured the maternal gallery length because it has been linked to pheromone production in some bark beetle species (Erbilgin *et al.*, 2007). The mean number of galleries per bolt was also similar between tree species; however, mean maternal gallery length per beetle pair was higher on lodgepole pine than on jack pine (Table 3). Phloem thickness and mean diameter at 1.3 m height were similar between jack and lodgepole pine (Table 3).

Flight data

Beetle pre-flight weight influenced the probability of flight, as our data indicated that heavier beetles showed greater tendency for flight than lighter beetles ($\chi^2_{1,149} = 27.74$, $P < 0.0001$). Propensity of flight was not influenced by sex ($\chi^2_{1,150} = 1.71$, $P = 0.19$), tree species ($\chi^2_{1,149} = 27.74$, $P = 0.74$) or their interactions ($\chi^2_{1,141} = 3.61$, $P = 0.06$) (Table 4). Although flight distance ($F_{1,83} = 9.91$, $P = 0.01$) and duration ($F_{1,82} = 5.61$, $P = 0.02$) were significantly influenced by pre-flight weight, the total distance ($F_{1,83} = 3.78$, $P = 0.06$) and duration ($F_{1,82} = 3.61$, $P = 0.07$) flown did not differ between the sexes. The total flight distance ($F_{1,83} = 2.78$, $P = 0.09$) and duration ($F_{I,82} = 0.91$, $P = 0.35$) of beetles did not differ with the host tree species in which they were reared (Table 4). The duration of flight was not influenced by interaction between sex and host tree species ($F_{1,82} = 3.76$, $P = 0.06$). Female beetles were heavier than male beetles in the pooled sample from both hosts ($F_{1,142} = 14.75$, $P < 0.0001$), and beetles from jack pine were larger overall than those from lodgepole pine ($F_{1,7} = 12.79$, $P = 0.01$) (Table 4).

Beetles that emerged from lodgepole pine lost more mass during flight than beetles from jack pine ($F_{1,83} = 4.96$, $P = 0.03$) (Fig. 4a); however, neither sex ($F_{1,83} = 1.87$, $P = 0.18$) nor distance flown ($F_{1,83} = 1.01$, $P = 0.95$) affected proportional loss of mass during flight. A significant interaction between host species and sex influenced the proportion of fat remaining after flight ($F_{1,71} = 6.64$, $P < 0.01$) (Fig. 4b), with females from jack pine burning more fat in flight than those from lodgepole pine; however, the opposite was observed in males. Interestingly, distance flown did not predict the proportion of fat remaining post-flight ($F_{1,71} = 0.01$, $P = 0.92$).

Discussion

We demonstrated that MPB can produce the main components of its pheromone on jack pine, a novel host for this species now under attack in the Canadian boreal forest. Some of the beetle-produced pheromone components (*trans*-verbenol, verbenone) are derived from a host monoterpene precursor, whereas others are produced *de novo* (*exo*-brevicomin, frontalin) (Blomquist *et al.*, 2010). Interestingly, pheromone components that are synthesized *de novo* were present in much smaller amounts than those derived from a monoterpene precursor (1% on average in lodgepole pine and 0.1–1% in jack pine), supporting earlier studies in this (Pureswaran *et al.*, 2000) and other (Pureswaran *et al.*, 2006) bark beetle systems. This is the first demonstration of pheromone production by MPB on jack pine, as the beetle has recently expanded its geographic and host range to the naïve jack pine forest ecosystems in Alberta.

In general, the results of the current study showed that chemical similarity between historical and novel hosts has facilitated the range and host expansion of MPB through utilization of host secondary compounds that are common in both hosts for pheromone production and aggregation on the host trees. This conclusion is supported by the results of earlier studies by Ehrlich & Raven (1964) and others, who predicted that chemical similarity among host plants is the most probable basis for the overall pattern of host shifts by herbivorous insects (Futuyma & McCafferty, 1990; Feeny, 1991; Berenbaum, 1995; Becerra, 1997; Lopez-Vaamonde *et al.*, 2003; Murphy & Feeny, 2006). This is the first study to demonstrate that similarity of secondary

Table 3 Mean diameter at breast height (DBH) (cm), phloem thickness (mm), number of maternal galleries and maternal gallery length (cm) of bolts of jack pine (*Pinus banksiana*) and lodgepole pine (*Pinus contorta*) used in pheromone studies

	Mean (± SE) values			
Tree species	DBH (cm)	Phloem thickness (mm)	No. maternal gallery per log	Maternal gallery length (cm)
Lodgepole pine	26.30 ± 0.43	1.53 ± 0.05	3.00 ± 0.32	38.36 ± 10.05
Jack pine	26.65 ± 0.61	1.56 ± 0.07	2.4 ± 0.25	13.34 ± 3.55
F	0.34	0.14	2.25	5.52
P	0.58	0.72	0.17	0.02
df (ndf, ddf)	1,8	1,8	1,8	1,38

(ndf, ddf), numerator and denominator degrees of freedom.

Table 4 Various aspects of mountain pine beetle (*Dendroctonus ponderosae*) flight on the flight mills

Tree species	Proportion flew (*n*)[a]	Total distance flown (SE), km (*n*)	Total duration of flight (SE), h (*n*)	Pre-flight weight ± SE, mg (*n*)
Lodgepole pine				
Male	0.49 (37)	1.3 ± 0.5 (18)	1.5 ± 0.7 (18)	7.9 ± 0.3 (18)
Female	0.74 (46)	1.8 ± 0.6 (34)	1.2 ± 0.4 (34)	11.4 ± 0.4 (34)
Jack pine				
Male	0.67 (21)	1.0 ± 0.5 (14)	0.7 ± 0.3 (14)	8.8 ± 0.6 (14)
Female	0.57 (49)	3.0 ± 0.7 (28)	2.5 ± 0.8 (28)	13.1 ± 0.5 (28)

Beetles used in this experiment were obtained from lodgepole pine (*Pinus contorta*) and jack pine (*Pinus banksiana*) bolts that had been used in the pheromone experiment in Fig. 1 and Table 1.
[a]Proportion flew indicates the proportion of beetles for each tree species that flew on the flight mill.

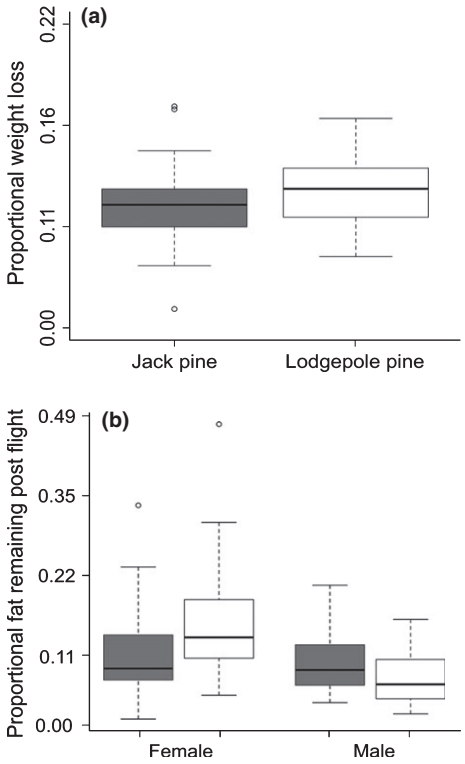

Fig. 4 (a) Proportional mass loss of individual mountain pine beetle (*Dendroctonus ponderosae*) brood emerging from jack pine (*Pinus banksiana*) and lodgepole pine (*Pinus contorta*). (b) Proportional fat remaining post-flight of both sexes of mountain pine beetle brood emerging from jack pine (gray boxes) and lodgepole pine (white boxes). The bottom and top of the box represent the first and third quartiles, respectively, and the band inside the box is the second quartile (the median). Lines extending vertically from the boxes (whiskers) indicate variability outside the upper and lower quartiles. Any data not included between the whiskers (circles) are outliers.

compounds between historical and novel hosts can facilitate host shift in a bark beetle species.

For at least three reasons, secondary compounds of jack pine have promoted the host expansion of beetles in the jack pine forest. First, jack pine phloem contains an essential monoterpene precursor for pheromone production in MPB, as beetles rely on α-pinene to produce *trans*-verbenol and verbenone (Blomquist *et al.*, 2010). Indeed, the emission of the primary aggregation pheromone, *trans*-verbenol, by female beetles was higher on the novel host than on the historical host, indicating that the higher production of *trans*-verbenol is probably a result of the higher α-pinene content in the phloem of jack pine than lodgepole pine (Wallin & Raffa, 1999; Colgan & Erbilgin, 2011; Lusebrink *et al.*, 2011). Likewise, α-pinene is one of the main constituents of phloem of other host species that are commonly colonized by MPB in its natural range, including ponderosa pine (*P. ponderosa*) (Davis & Hofstetter, 2012), limber pine (*P. flexilis*) (Conner *et al.*, 1980) and whitebark pine (*P. albicaulis*) (Raffa *et al.*, 2013).

Furthermore, differences in enantiomeric ratios of (−)-α-pinene in the phloem tissues of the historical and novel hosts (Pureswaran *et al.*, 2004; Erbilgin & Colgan, 2012) apparently do not constrain the synthesis of (−)-*trans*-verbenol by MPB. It is interesting to note that apparently MPB needs only a very small amount of (−)-α-pinene as precursor to produce (−)-*trans*-verbenol compared with the amount of (−)-α-pinene released from hosts (amount of (−)-*trans*-verbenol released is < 1% of (−)-α-pinene released). Although the mechanism of (−)-*trans*-verbenol production in MPB is not clear, Blomquist *et al.* (2010) proposed two distinct α-pinene hydroxylating pathways in female beetles. One pathway is indiscriminate between α-pinene enantiomers, whereas the second appears to be specific for (−)-α-pinene; together, both pathways produce (−)-*trans*-verbenol in beetles.

Second, the production of *trans*-verbenol, *exo*-brevicomin and other pheromone components on jack pine may allow MPB to colonize and mate in the novel host. Given that populations of MPB have probably been constrained by cold winter temperatures in eastern Canada (Régnière & Bentz, 2007) and will probably remain at low population levels until the climate becomes more suitable for rapid expansion, the production of *trans*-verbenol and other pheromone components may allow mate finding and colonization of jack pine by beetles. This was supported by our field experiment, where *trans*-verbenol, together with *exo*-brevicomin and frontalin (mimicking the pheromones emitted by beetles on jack pine), was as attractive as pheromones emitted by beetles on lodgepole pine, and even more so in terms of the total number of beetles attracted (jack vs lodgepole pine treatments in Fig. 3: 385 vs 295). We suspect that the quantity of *trans*-verbenol released caused the difference in beetle attraction between hosts in the current study.

Third, the addition of 3-carene, another monoterpenoid that is common in both hosts, increased MPB attraction to its aggregation pheromone mimicking the historical and novel hosts (based on pair-wise comparisons). Volatile 3-carene constituted *c.* 10% and 27% of chemicals emitted from lodgepole and jack pines, respectively, in the current study, similar to the rates reported earlier (Lusebrink *et al.*, 2011). The influence of host monoterpenes in bark beetle attraction has been commonly reported (i.e. Erbilgin & Raffa, 2000; Erbilgin *et al.*, 2007). Furthermore, jack pine trees commonly emit this compound from their stem and foliage (Lusebrink *et al.*, 2011), making them potentially vulnerable to beetle colonization by increasing the number of beetles arriving during aggregation on host trees. However, non-host tree volatiles can also influence beetle attraction to its pheromones (i.e. Huber & Borden, 2001); thus additional studies are needed to understand the complexity of volatiles emitted from host and non-host trees in beetle ecology in the jack pine forest ecosystem.

Verbenone is a product of the auto-oxidation of the host monoterpene α-pinene or the microbial conversion of *trans*-verbenol (Blomquist *et al.*, 2010). The emission of verbenone was similar between lodgepole pine and jack pine, although the latter contains more α-pinene in its phloem tissue (Lusebrink *et al.*, 2011), suggesting a critical role of microbial gut communities in verbenone production (Hunt & Borden, 1990). Verbenone plays a key function in beetle biology, including the optimization of beetle colonization on host trees by diverting in-flight beetles to nearby host trees (Raffa *et al.*, 2005). This behavior reduces intraspecific competition, whilst maximizing colonization efficiency. Currently, we do not know whether verbenone emission by MPB on jack pine can affect beetle colonization strategy; however, we suspect that beetles would probably have a different colonization strategy on the novel host if the production of verbenone does not impose anti-aggregation behavior to arriving beetles.

Based on beetle reproductive data from bolts of both host species, we corroborated earlier observations that the novel host is suitable for beetle reproduction (Cerezke, 1995; Cullingham *et al.*, 2011), suggesting that differences between historical and novel hosts do not constrain their use by beetles. Furthermore, the fitness of beetles emerging from the novel host was superior in terms of body size relative to those emerging from the historical host. This is an important finding because body size can influence bark beetle biology (Sahota & Thompson, 1979; Graf *et al.*, 2012) and larger female beetles can disperse farther, have better survival ability and lay more eggs than smaller females (Amman & Cole, 1983). In addition, female beetles emerging from jack pine lost proportionally less mass during flight than did those emerging from lodgepole pine, indicating that they could arrive at the host with greater mass, that is, fat reserves that are convertible to eggs. Perhaps the higher amount of *trans*-verbenol released by female beetles on jack pine in the current study is related to the size of the beetles, as larger beetles can potentially produce and release more pheromones, but this contradicts the results of a previous study (Pureswaran & Borden, 2003). Further investigations should test whether greater fat reserves of females will be translated into increased fecundity and/or offspring quality.

Some limitations of this study are important to consider in relation to the main findings. First, we evaluated only four components of the beetle pheromone complex, although they produce additional pheromone components (Pureswaran *et al.*, 2000). We are currently identifying these components and characterizing their roles in beetle ecology. However, given that beetles are attracted to three of the pheromone components in the field, the functions of other compounds appear to be complementary (Pureswaran *et al.*, 2000; Pureswaran & Borden, 2004). Second, variation in jack pine phloem chemistry (chemotypes) in its natural range and how such variation affects beetle pheromone production were not incorporated into the current study. Jack pine is likely to have more than one chemotype throughout its natural range (Lusebrink *et al.*, 2011), which might affect the amount of pheromones, particularly *trans*-verbenol produced by beetles and their ability to establish in certain jack pine forest stands. We are currently investigating the chemotypes of jack pine in its range in Canada and their possible impacts on pheromone production in beetles. Likewise, we only sampled lodgepole pine in one region and volatiles collected may not reflect the chemistry of lodgepole pine trees in other locations (Forrest 1980). Furthermore, Clark *et al.* (2010) reported phenotypic differences among populations of lodgepole pine trees in British Columbia, and suggested that the historical presence or absence of beetle pressure may cause such differences. As there is no record of beetle attacks on lodgepole pine trees in western Alberta, lodgepole pine trees used in the current study may in fact be naïve in the sense that MPB has not encountered these populations. However, as all populations of lodgepole pine contain α-pinene in their resin (Forrest 1980), albeit in different concentrations, the conclusions made about lodgepole pine chemistry do not change the fact that pine trees contain α-pinene, which is a precursor of *trans*-verbenol production. Third, as we explained in the Materials and Methods section, we did not conduct the field experiments in the jack pine forests. Although we do not know whether this had an impact on the results, Erbilgin & Raffa (2001) reported that the forest background usually favors tree species associated with the same forest type. If a similar conclusion is applicable to the results of the current study, we would expect more beetles caught in traps baited with pheromones mimicking jack pine in the jack pine forest. Until we test the same pheromones in jack pine forests with adequate beetle density, we cannot be certain about the possible role of forest background in beetle attraction. Finally, we trapped volatiles from individual beetle galleries on cut bolts. Although we cannot elaborate as to whether this affected our results, our earlier trials to introduce female and male beetles to standing live trees yielded very little successful beetle colonization and did not allow us to test our objectives. Furthermore, we suspect that differences in secondary compounds remaining in the cut bolts can still affect pheromone production by beetles (i.e. Zhao *et al.*, 2011).

In conclusion, Allee effects are critical barriers to the establishment of invasive species (Johnson *et al.*, 2006; Tobin *et al.*, 2007). In bark beetles, Allee effects can result from difficulty in mate location and in sparse populations that subvert the benefits of aggregation (Liebhold & Tobin, 2008). In the current study,

we provided the first evidence that MPB can attract mates necessary for host (jack pine) procurement via pheromones. This aggregation process is particularly important for beetles at the low population level, when they are highly vulnerable to local extinction as a result of Allee effects in the novel habitat. Furthermore, broods emerging from jack pine were able to conduct dispersal flights similar to those from lodgepole pine, further increasing the 'invasion risk' and lowering the probability of 'extinction' of beetle populations in the novel habitat. However, currently, we do not know how regional stochasticity caused by environmental and other demographic processes, such as reproduction or immigration, influences the meta-population dynamics of beetles in the novel habitat (Safranyik *et al.*, 2010). Nevertheless, the current study suggests that beetles will probably survive and sustain their population in the jack pine boreal forest, and that spread can be limited by low winter temperatures and by the speed of adaptation to seasonality in the novel range (Logan *et al.*, 2003; Safranyik *et al.*, 2010).

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