Effect of semiochemical exposure on flight propensity and flight capacity of *Dendroctonus ponderosae* in laboratory bioassays

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Abstract

Insect herbivores respond to volatile organic compounds (VOCs) released by host and non-host plants or conspecifics, during flight. *Dendroctonus ponderosae* uses chemical cues including host and non-host VOCs, as well as aggregation pheromones to navigate through the environment and find a suitable reproductive host. The dispersal flight distance of *D. ponderosae* from its natal host to its reproductive host varies widely, even within populations of beetles. Beetle energetics do not entirely explain this flight variation. In this study, we test the effect of beetle exposure to semiochemical cues before and during flight on subsequent flight propensity (the likelihood of flight initiation) and flight capacity (the distance and velocity of flight) using computer-linked flight mills. Exposure to host volatiles before flight interacts with beetle pre-flight weight to influence subsequent flight capacity of female but not male beetles. Female beetles flew further and faster following exposure to their aggregation pheromone, *trans*-verbenol. Flight of female beetles was reduced when they were exposed to volatiles from the non-host, *Populus tremuloides,* during flight. This study is the first to indicate that semiochemical cues not only influence beetle orientation during flight, but also flight capacity of *D. ponderosae*. These results provide baseline information on the effect of environmental cues on dispersal by flight of *D. ponderosae*.

Key Words: flight, bark beetle, volatile organic compounds, angiosperm, *Pinus*

Declarations: Not applicable

Introduction

Dispersal is a key life history component of many plants and animals. Insects disperse for a variety of reasons, including to find resources, escape competition and find mates (Matthysen

2012). Some bark beetles (Curculionidae: Scolytinae) deplete resources in the natal environment within a single generation (Raffa et al. 2015). For these insects to reproduce, they need to disperse to find mates and colonize new host trees. Flight is the main mode of dispersal in bark beetles. Many species exhibit extreme and unexplained interindividual variance in dispersal distance, ranging from short to long distance flights (Zumr 1992; Jackson et al., 2008).

Host tree colonization by bark beetles is mediated through response to plant-produced chemical signals. Plant volatile organic compounds (VOCs) help bark beetles discriminate between host and non-host trees and assess host quality (Kohnle 2004). Bark beetles may use host VOCs for long-range orientation towards a stand of suitable hosts, for short-range orientation and to discriminate between potential hosts within a stand (Jactel et al. 2001; Kohnle 2004). In the absence of pheromone signals, several species of bark beetle are still attracted to host trees through host VOC orientation cues alone. Response to host VOCs in the absence of attractive pheromones, occurs in several species including *Dendroctonus ponderosae* (Erbilgin et al. 2014), *Monarthrum scutellare* (Noseworthy et al. 2012), *Hylastes ater*, and *Hylurgus ligniperda* (Kerr 2010).

After host location, pheromone-based communication initiated by pioneer bark beetles (those that are the first to reach and successfully enter a tree) dictates the host colonization process (Wyatt 2014; Raffa et al. 2015). Later-arriving beetles respond to conspecific aggregation pheromones that act in synergy with host VOCs (Byers et al. 1988). Trap catch of *Ips typographus* is greater in traps baited with high concentrations of the host compound (–)-α-pinene in combination with aggregation pheromone than in traps baited with pheromone alone. Low to intermediate concentrations of (–)-α-pinene, however, do not increase trap catch in pheromone-baited traps (Erbilgin et al. 2007). In contrast, *Ips pini* is mostly responsive to intermediate levels of (–)-α-pinene in combination with pheromone cues, and attraction decreases at low and high (–)-α-pinene concentrations (Erbilgin et al. 2003). Pheromone or host terpenes alone attract only a small number of *Dendroctonus rufipennis* to baited traps, but a combination of pheromone and host terpenes enhances attraction over pheromone alone (Ryall et al. 2013). Individual pheromone components of *Pityogenes chalcographus* attract few beetles to baited traps, but a blend of pheromone components is attractive and the combination of host monoterpenes and pheromone components attracts the most beetles (Byers et al. 1988).

Bark beetles are also sensitive to VOCs released by non-host trees and use this information to avoid colonization of unsuitable hosts. Non-host VOCs disrupt attractiveness of host VOCs and aggregation pheromones. Orientation of *Dendroctonus pseudotsugae* (Huber and Borden 2001a), *D. ponderosae* (Borden et al. 1998), and *Ips sexdentatus* (Jactel et al. 2001) toward aggregation pheromone signals is disrupted by the addition of non-host angiosperm VOCs. Non-host VOCs disrupt orientation to pheromone signals as effectively as anti-aggregation pheromones, which are signals that terminate the host colonization process (Huber and Borden 2001a, 2001b; Borden et al. 1998; Campbell and Borden 2006a, 2006b). The number of attacks by *Tomicus piniperda* within host stands decreases with addition of non-host angiosperm but not non-host conifer material to the stand (Kohnle 2004). Individual non-host VOCs are equally repellent as the complete non-host volatile profile to *I. typographus* (Zhang and Schlyter 2003) and *D. ponderosae* (Borden et al. 1998). Non-host volatiles have an additive disruptive effect (Jactel et al. 2001).

Dendroctonus ponderosae is a destructive bark beetle pest of mature pines in Western North America. Beetles spend most of their life in the sub-cortical environment of the host tree. Adult beetles undergo an obligatory dispersal phase during which they leave the natal host and fly in search of new reproductive hosts (Safranyik and Carroll 2006). The host finding behavior of *D. ponderosae* has been hotly debated in the literature. One hypothesis states that host selection occurs through a combination of visual orientation and random landing (Carroll and Safranyik 2003), followed by assessment of host suitability after landing (Pureswaran and Borden 2003). This hypothesis does not account for in-flight olfactory cues that play a role in host finding. Pioneer beetles likely orient to host VOCs and away from non-host VOCs to find suitable host stands. A second hypothesis suggests that once beetles are within a host stand, a combination of visual and close range, in-flight olfactory cues aid in finding a suitable host (Campbell and Borden 2006a).

In the most recent population outbreak, *D. ponderosae* expanded its native range and crossed the Rocky Mountains, establishing in North-Central Alberta (Safranyik et al. 2010). Movement across the Rocky Mountains resulted in colonization of hybrid trees between the historic host (*Pinus contorta*) and the novel host (*Pinus banksiana*), which aided continued eastward range expansion (Lusebrink et al. 2013). Range expansion by *D. ponderosae* was

facilitated by successful attack of and reproduction within *P. banksiana* hosts (Cullingham et al. 2011), a tree that comprises much of the boreal forest and can potentially act as a pathway for the beetle across Canada (Safranyik et al. 2010).

The dispersal behavior of *D. ponderosae* is an understudied aspect of its ecology (Chen and Walton 2011). Evidence indicates that semiochemicals, including pheromones, and both host and non-host VOCs, are exploited by *D. ponderosae* during flight orientation to new hosts (Borden et al. 1987; Huber and Borden 2001b, Miller et al. 2005; Campbell and Borden 2006b). Although it is known that host and non-host VOCs stimulate receptors on the antennae of *D. ponderosae* (Borden et al. 1998; Huber et al. 2000; Pureswaran et al. 2004) and help mediate host colonization (Pitman 1971; Moeck and Simmons 1991; Huber and Borden 2001; Erbilgin et al. 2014; Erbilgin 2019), it is unclear if these compounds affect flight propensity (the likelihood of flight initiation) and capacity (the distance and velocity of flight) during dispersal.

Here we test the effect of beetle exposure to VOCs from the historic host, *Pinus contorta*; the naïve host, *Pinus banksiana*; the non-host *Populus tremuloides*; and the female-produced aggregation pheromone, *trans*-verbenol, on flight propensity and capacity using a laboratory flight mill bioassay. We first tested the hypothesis that beetle exposure to host VOCs prior to flight would influence subsequent flight activity. We predicted that beetles would have reduced flight propensity and capacity following exposure to host tree VOCs, as chemical cues would indicate suitable hosts in the environment. The second experiment tested the hypothesis that beetle exposure to host and non-host VOCs during flight would influence beetle flight. We predicted that flight distance would be the greatest when beetles were exposed to non-host volatiles that indicate an unsuitable environment for host colonization. The third experiment tested the hypothesis that beetle flight would be modified by exposure to aggregation pheromone prior to flight. We predicted that beetles exposed to aggregation pheromone would have increased flight capacity, as aggregation pheromone indicates high beetle density in nearby trees during the early stages of host colonization.

Methods

Collection of D. ponderosae

In November 2017, *D. ponderosae* were collected from three sites in Hinton, Alberta, Canada (53.342167, -117.586800; 53.380417, -117.542683; 53.275450, -117.665267) and two sites in Slave Lake, Alberta, Canada (54.862517, -115.162517; 54.897367, -115.145133). In Hinton, five infested lodgepole pine trees were felled at each site. In Slave Lake, two infested lodgepole pine trees were felled at the first site and seven infested trees were felled at the second site. From the felled trees, bolts were cut one meter above the ground. Bolts were 50 cm in length and two bolts were taken from each tree. To reduce desiccation, cut ends of the bolts were sealed with Paraffin wax (parowax®). Bolts were stored for 2-7 months at 5°C until beetles were needed for bioassays.

Bolts were removed from cold storage when needed and placed in 121 L emergence bins fitted with a glass jar to collect emerging *D. ponderosae*. Emergence bins were housed at 21°C under a 16:8 hr light:dark cycle. As *D. ponderosae* emerged from bolts, they were collected daily, separated by sex, labelled, and placed in 1.5 ml microcentrifuge tubes with a small strip of paper (Evenden et al. 2014). *Dendroctonus ponderosae* were stored at 4°C for 2-5 days post emergence prior to use in the bioassay.

Exposure material

Phloem samples used as VOC exposure material were collected from four trees at a single site for each tree species in July 2017. Phloem samples of *P. contorta*, were obtained from a site near Grande Prairie, Alberta (54.464163, -118.635325). *Pinus banksiana*, phloem samples were collected at a site near Lac La Biche, Alberta (55.157817, -112.019033). Phloem samples from the non-host *P. tremuloides*, were collected at a site just west of Elk Island National Park, Alberta (53.635808, -112.927324). The bark was peeled away from four live, standing trees at each site to expose the phloem. Fifty phloem discs were collected from each tree using a 1.27 cm diameter leather punch. Cut discs were immediately wrapped in aluminum foil (Alcan Plus Heavy Duty Aluminum Foil, ITM/ART 50125, Canada) and submerged in liquid nitrogen for transport back to the laboratory where they were stored at -80°C. Release rate of VOCs from phloem discs are listed in Table 1.

Flight bioassay

Prior to use in the flight bioassay, *D. ponderosae* (2-5 days old) were weighed to the nearest 0.01 mg (Mettler Toledo XPE205 Microbalance, Columbus, Ohio). *Dendroctonus ponderosae* were then randomly separated into two treatment groups, flown and control. Flown *D. ponderosae* were tethered to a 2 cm-long tether made from 32-gauge (0.02 mm) aluminum wire with a small loop at the end which was attached to the pronotum of each beetle using Press-Tite Contact Cement (LePage, Mississauga, Ontario) and placed on flight mills (Fig 1). Control *D. ponderosae* were placed in perforated 1.5 ml microcentrifuge tubes and kept in the flight mill room during the flight bioassay. Bioassays were conducted for 23 hr at 23°C with a 16:8 hr light to dark cycle. The flight assay was initiated 1 hr after the beginning of the photophase. Tethered beetles were attached to flight mills through insertion of the tether into a small piece of wire insulation at the distal end of each flight mill arm. The angle between the tether and wire insulation was ~100°. During the photophase, light was provided by high flicker frequency fluorescent bulbs (550 lux).

As flight propelled the flight mill arm, each rotation was detected by a small magnetic transmitter. A receiver attached to the mill directed the signal to the computer. The computer recorded each revolution of the flight mill arm (~94.4 cm) (LabView software, National Instruments Corporation, Austin, TX). Output included the duration and number of revolutions for each flight burst throughout the 23 hr bioassay.

After the flight bioassay, *D. ponderosae* and tethers were weighed to the nearest 0.01 mg. *Dendroctonus ponderosae* were stored at -20°C until body length and pronotum width measurements were taken using a dissecting microscope fitted with a micrometer (15 x magnification).

Experiment 1: Exposure to host VOCs prior to flight

Experiment 1 tested the hypothesis that *D. ponderosae* pre-exposure to host VOCs before flight would influence subsequent flight propensity and capacity. *Dendroctonus ponderosae* were exposed to host VOCs in an apparatus (Fig. 2) (Mori, 2014) providing a constant stream of humidified air for 3 hr at 23°C in complete darkness prior to flight. Air pushed through a charcoal filter (Flow Activated Carbon Filter, #ADS-STD-C2F, Analytical Research Systems Inc., Florida), and was humidified in a 250 ml Erlenmeyer flask filled with 125 ml of distilled water. The air was channeled into each of three 250 ml flasks at 500 $ml \cdot min^{-1}$. Each flask

contained one 1.27 cm^2 piece of phloem of either *P. banksiana* or *P. contorta*, or no phloem, as a clean air control. The headspace surrounding the phloem was channeled into an exposure chamber housing ~15 *D. ponderosae* in individual perforated 1.5 ml microcentrifuge tubes. Air exiting the apparatus was vented through a fume hood. After the 3 hr exposure, beetles were weighed and prepared for flight as described above. This experiment was completed on both sexes (Flown treatment *D. ponderosae*, females: *clean air* n=76, *jack pine phloem* n=77, *lodgepole pine phloem* n=77; males *clean air* n=84, *jack pine phloem* n=82, *lodgepole pine phloem*n=79).

Experiment 2: Exposure to trans-*verbenol prior to flight*

Experiment 2 tested the hypothesis that beetle pre-exposure to the female-produced aggregation pheromone, *trans*-verbenol, before flight would influence subsequent flight capacity. *Dendroctonus ponderosae* were exposed to *trans*-verbenol for 5 min at 23°C in complete darkness prior to flight. Pre-exposure to *trans*-verbenol occurred in the apparatus (Fig. 2), as described previously. In this experiment, only two treatment chambers were used to expose *D. ponderosae* to either *trans*-verbenol or clean air. The *trans*-verbenol source was a single Mountain Pine Beetle Tree Bait (Contech Enterprises Inc., 300000228, Lot #13014, purchased in 2015) without *exo*-brevicomin that released *trans*-verbenol at ~1 mg·day. The approximate content of the Mountain Pine Beetle Tree Bait included 90-95% pure *trans*-verbenol with 5-10% *cis*-verbenol with an optical purity of 50-60% (1*S*)-(-) Vebenol *EE*. Baits contained <0.5% Verbenone and 0.5% Ethanox 705, as a stabilizer. Post-exposure, *D. ponderosae* were weighed and prepared for flight as described above. In this experiment, 236 female *D. ponderosae* were placed on flight mills (n=118 for both clean air & *trans*-verbenol) and 176 male *D. ponderosae* were placed on flight mills (n=90 clean air and n=86 for *trans*-verbenol).

Experiment 3: Exposure to host and non-host VOCs during flight

Experiment 3 tested the hypothesis that beetle flight would be affected through exposure to host and non-host volatiles during flight. *Dendroctonus ponderosae* were weighed and prepared for flight as described above, but without any pre-exposure period. In the flight mill room, shelves containing flight mills were separated into four sections of equal volume (0.32 m^3), each containing four mills. Three of the four open sides of the shelves were sealed with transparent oven bags (Poly Pan Liners, Elkay Plastics, PTL205285, California) and secured with foil tape

(Naushua®, 322 Multi-purpose HVAC foil tape). *Dendroctonus ponderosae* were then attached to mills, as described above. A 1.27 cm^2 piece of phloem was positioned above each of the four flight mills in each treated section. Phloem was attached to a paper clip that was tied to a transparent string (Beadalon SuppleMax™ Monofilament Illusion Cord, 0.25 mm), and suspended above the mill. Once phloem treatments were applied and *D. ponderosae* were positioned on the mills, the fourth side was enclosed with a cut oven bag and aluminum foil tape. This experiment was completed using female (Flown treatment *D. ponderosae*: n=86 beetles attached to mills for each treatment) and a small number of male (Flown treatment *D. ponderosae* attached to mills: *Aspen* n=46; *Clean* n=44; *Jack* n=42; *Lodgepole* n=48) beetles.

The location of each treatment in the flight mill room was randomized between days of flight. Oven bags were removed from shelves and disposed. The shelves and flight mills were cleaned with three washes of hexane followed by three washes of acetone. Paperclips securing phloem and the paper that lined the flight mill shelves were transported to the next similarly treated shelf to avoid treatment cross contamination.

Chemical analysis of exposure materials

Materials that *D. ponderosae* were exposed to were aerated to determine the release rates of VOCs from host and non-host phloem discs and the synthetic aggregation pheromone bait. A 250 ml glass jar with a screw top tin lid was modified to aerate materials. Two holes were cut into the lid of the jar, and brass hose connectors were fitted to the holes and sealed with a soldering iron. PTFE tubing (Cole-Parmer, 3/16" x 1/4", RK-06605-32) was connected to the jar, and subsequently connected to the laboratory bench vacuum. A split in the PTFE tubing allowed for the connection of a Porapak Q tube (6 x 110-mm, 2 sections: 75/150 mg sorbent, 20/40 mesh). A single phloem disc or a single *trans*-verbenol bubble pack was placed into the glass jar, and the lid was sealed using PTFE Teflon tape with parafilm overtop. The laboratory bench vacuum was set to pull air at 100 $ml \cdot min^{-1}$ for 5 min for the *trans*-verbenol bubble packs and 3 hr for the phloem discs.

Each Porapak Q tube from each aeration sample (n=3) was scored with a glass cutter to remove the adsorbent beads. The beads from the tube were placed into a 2 ml Axygen microtube that was placed onto dry ice. The stock solution of the extraction solvent contained 500 ml DCM (methyl chloride) (Fisher Scientific, HPLC Grade) with 5 µl of heptyl acetate (Fisher Scientific,

GC Grade) as an internal standard. One ml of the stock solution was dispensed (0.5-5 ml dispenser, Dispensette Organic) into each 2 ml microtube containing adsorbent material from each sample. Microtubes containing adsorbent material and stock solution were vortexed for 30 sec at maximum speed (3000) (VWR Pulsing Vortex Mixer) and then placed into a sonicator (Symphony) for 10 min. Microtubes were centrifuged for 15 min at 0°C at 16100 rcf (Eppendorf AG 2231 Hamburg, Germany).

To filter the extract, the solvent solution was pipetted into a modified pipette (Fisher, borosilicate glass, 13-67-20A) containing a small amount of glass wool to act as a filter. The filtered extract was collected in 2 ml Autosampler vials (Fisher, 9 mm/Amber-ID, 03-391-9) that were capped (Autosampler caps, 9 mm screw thread/PTFE/Silicone, 03-391-14) and stored at -40°C until chemical analyses.

Quantification of monoterpenes (3-carene, α-pinene and myrcene) released from phloem discs of the two pine species, *P. tremuloides* volatiles (1-hexanol, benzyl alcohol and nonanol) and the aggregation pheromone, *trans*-verbenol, were performed using a Gas Chromatograph/Mass Spectrometer (GC/MS, Agilent 7890A/5975C, Agilent Tech., Santa Clara, CA, USA) with a DB-5MS UI (I.D. 0.25 mm, length 30 m) column. Helium was the carrier gas with a flow rate of 1 $ml \cdot min^{-1}$. Two µl samples of each extract (N=12) were injected in a Pulsed Splitless mode. The oven temperature started at 40°C and held for 2 min, it was then increased to 70°C by $3°C \cdot min^{-1}$, increased to 200°C by $10°C \cdot min^{-1}$, and then increased to 250°C by $25°C \cdot min^{-1}$ and held for 1 min. The data for the monoterpenes and *trans*-verbenol was acquired using SCAN mode. The data for the *P. tremuloides* volatiles was acquired using SIM mode. The quantified compounds were based on standards: monoterpenes (Sigma, 3-carene >98.5% purity, α-pinene >98.5% purity and myrcene >94% purity), *P. tremuloides* volatiles (Alpha-Scents, >97% purity), *trans*-verbenol (Contech Enterprises Inc., >99% purity).

To quantify the monoterpenes β-phellandrene and limonene, a HP-CHIRAL-20β column was installed. Helium was the carrier gas with a flow rate of 1 $ml \cdot min^{-1}$. Two µl samples of each extract (N=12) were injected in Pulsed Splitless mode. The oven temperature started from 40°C and held for 1 min, increased to 100°C by $10°C \cdot min^{-1}$ and held for 2 min, increased to 130°C by $2°C \cdot min^{-1}$, and then increased to 250°C by $25°C \cdot min^{-1}$ and held for 3 min. The data was acquired

using SIM mode. The quantified compounds were based on standards from Sigma (β-phellandrene >77.1% purity and limonene >99% purity).

Data analyses

Dendroctonus ponderosae were considered to have initiated flight when flight mill recording indicated the individual completed more than three rotations. Flight distance was calculated by multiplying the total rotation count over the 23 hr assay by 0.944 m. Velocity was calculated by dividing the total distance by the total duration of flight (flight mills stop recording time when the sensor stops being tripped).

All data analyses were performed in R version 3.4.1 (R Core Team, 2018). Lmer models were created with the lme4 package (Bates et al. 2015). Comparison of fixed factors was conducted using the Anova function in the car package which completes a Type II Wald χ^2 test as the test statistic (Fox and Weisberg 2011). Post-hoc comparison between treatments was made using pairwise comparisons with the emmeans package (Speed and Milliken 1980).

For all experiments, data were tested for normality and heteroscedasticity using visual techniques and the Shapiro-Wilks test. When assumptions were not met, the data was transformed for lmer models. For female data, distance flown was cube-root transformed in Experiments 1 and 3, and quarter-root transformed in Experiment 2. For male data, distance flown was cube-root transformed in all experiments. Velocity data from male *D. ponderosae* were quarter-root transformed in Experiments 1 and 2, and square-root transformed in Experiment 3. To avoid confounding factors, body measurements (pre-flight weight, body length and pronotum width) used as explanatory variables were analyzed in separate models. For each model, exposure treatment and a single body measurement (pre-flight weight, body length or pronotum width) were used as the explanatory variables. Natal host and individual flight mill were used as random factors in all models (Supplementary Material Tables 1-6).

Due to the high variation in *D. ponderosae* flight, as well as the high variation in VOCs released by the phloem and pheromone sources $\alpha = 0.06$.

Results

Experiment 1: Exposure to host VOCs prior to flight

Of the 230 female *D. ponderosae* placed on flight mills in Experiment 1, 164 initiated flight. Pre-exposure to host VOCs before flight did not influence flight propensity of female *D. ponderosae* (χ^2=0.009, p=0.9957). An interaction between pre-flight weight and pre-flight VOC pre-exposure treatment affected female flight distance (χ^2=9.5565, p=0.0588). When exposed to clean air before flight, heavier female *D. ponderosae* flew further than lighter female *D. ponderosae* (Fig.3). There was no relationship between pre-flight weight and distance flown, however, in females exposed to host VOCs of either *P. banksiana* or *P. contorta* phloem before flight (Fig. 3). Heavier beetles flew at higher velocities than lighter *D. ponderosae* (χ^2=16.6214, p=4.563x10^{-5}). The other two measures of body size, pronotum width and body length, did not significantly affect flight distance or velocity (Supplementary Table 1).

Of the 248 male *D. ponderosae* placed on flight mills in Experiment 1, 166 initiated flight. Flight propensity of male *D. ponderosae* did not differ with VOC pre-exposure treatments (χ^2=0.2916, p=0.8643). Only body size (pre-flight weight, body length and pronotum width) impacted flight parameters of male *D. ponderosae* (Fig. 4). Larger males flew further and faster than smaller males (Supplementary Table 2).

Experiment 2: Exposure to trans-*verbenol prior to flight*

Of the 236 female *D. ponderosae* placed on flight mills in Experiment 2, 172 initiated flight. Female *D. ponderosae* showed no difference in flight propensity as a result of exposure to *trans*-verbenol before flight (χ^2=0.343, p=0.558). Heavier females flew further than lighter females (PFW: χ^2=10.6607, p=0.0011), however, there was no relationship between flight distance and pre-flight exposure treatment in the model with beetle weight as the body measurement factor (χ^2=2.5565, p=0.0865). There was no relationship between flight distance and female pronotum width or body length, however, pre-flight exposure treatment did significantly affect flight distance in models in which pronotum width and body length were the body measurement factors. *Dendroctonus ponderosae* pre-exposed to *trans*-verbenol flew further than *D. ponderosae* maintained in clean air before flight (Fig. 5a, Pronotum width model: χ^2=5.6578, p=0.0173; Body length model: χ^2=5.8636, p=0.0553). Body size measurements and pre-flight exposure treatment affected flight velocity. Heavy or large *D. ponderosae* flew faster than light or small *D. ponderosae*. Females pre-exposed to *trans*-verbenol flew faster than those

maintained in clean air flight (Fig. 5b, Pre-flight weight model: EXP: χ^2=3.6461, p=0.0562, Supplementary Table 3).

Of the 176 male *D. ponderosae* placed on flight mills in Experiment 2, 127 initiated flight. Male *D. ponderosae* showed no difference in flight propensity as a result of exposure to *trans*-verbenol before flight (χ^2=0.126, 0.722). Pre-flight weight, pronotum width and body length did not influence flight distance or velocity. Pre-flight exposure to clean air or *trans*-verbenol did not influence flight distance or velocity (Supplementary Table 4).

Experiment 3: Exposure to host and non-host VOCs during flight

Of the 345 female *D. ponderosae* placed on flight mills in Experiment 3, 252 initiated flight. Exposure to VOCs during flight did not influence the flight propensity of female *D. ponderosae* (χ^2=0.009, p=0.9998). Flight distance, however, was affected by pre-flight weight, pronotum width, body length and exposure treatment during flight (Supplementary Table 5). *Dendroctonus ponderosae* exposed volatiles released from *P. tremuloides* phloem flew shorter distances as compared to the clean air control treatment (Fig. 6, t=2.989, p=0.0162). Flight velocity was affected by pre-flight weight, pronotum width and body length, but not exposure treatment (Supplementary Table 5).

Of the 180 male *D. ponderosae* placed on flight mills in Experiment 3, 115 initiated flight. Exposure to VOCs influenced the propensity to fly. Male *D. ponderosae* exposed to jack pine during the flight bioassay showed an increased propensity to initiate flight compared to the clean air control (χ^2=14.0733, p=0.0008). Exposure of males to VOCs during flight did not influence flight distance or velocity (Pre-flight weight model: χ^2=6.1699 p=0.1036). Pre-flight weight, pronotum width and body length all influenced flight distance and velocity (Supplementary Table 6). It should be noted that due to limitations collecting male *D. ponderosae* these results are based on small sample sizes, increasing the chance of a Type II error.

Chemical analysis of exposure material

Quantities of antennally active volatile organic compounds released from *P. banksiana* and *P. contorta* phloem discs were variable (Table 1). *Populus tremuloides* released an average of 0.17 ± 0.04 $\mu g \cdot ml^{-1} \cdot h^{-1}$ of 1-hexanol and 0.52 ± 0.10 $\mu g \cdot ml^{-1} \cdot h^{-1}$ of nonanol. Benzyl alcohol was not

released in detectable levels from *P. tremuloides* phloem. The *trans*-verbenol bubble pack used in Experiment 2 released 2.44 $\mu g \cdot ml^{-1} \cdot min^{-1}$ during the 5 min aeration period.

Discussion

Dendroctonus ponderosae perceives and responds to a variety of compounds emitted by host trees, including but not limited to α-pinene, β-phellandrene, limonene, 3-carene and myrcene (Whitehead 1986; Pureswaran et al. 2004). During flight, *D. ponderosae* exhibits positive orientation to host VOCs (Moeck and Simmons 1991), and experiences greater aggregation on trees with high levels of α-pinene (Burke and Carroll 2016). Results from the current study indicate that in addition to oriented flight, host VOCs also influence the flight capacity of *D. ponderosae*.

When exposed to clean air prior to flight, heavier female *D. ponderosae* flew further than lighter *D. ponderosae*. This relationship is similar to that seen in other *D. ponderosae* flight mill studies (Evenden et al. 2014; Wijerathna and Evenden 2019). Interestingly, this relationship does not exist when female *D. ponderosae* are exposed to either *P. banksiana* or *P. contorta* phloem VOCs prior to flight. Large *D. ponderosae* may undergo shorter flights in the presence of potential hosts. Exposure to host VOCs could prime female *D. ponderosae* for host colonization instead of flight behaviors. Undergoing short dispersal flights is a safe strategy, as female *D. ponderosae* arrive at the host with high lipid stores (Chubaty et al. 2014) or expend less energy during flight (Jones et al. 2020), and are more likely to successfully colonize the host (Latty and Reid 2009, 2010).

Pre-exposure to host VOCs and exposure during flight does not influence male *D. ponderosae* flight capacity. Male *D. ponderosae* had similar flight capacity after pre-exposure to clean air, *P. banksiana* or *P. contorta* phloem VOCs prior to flight. Further, flight capacity of males did not vary with exposure treatment during flight. The difference in effect of exposure to host cues on the flight of male and female beetles could be due to the different roles each sex takes in the host colonization process. As pioneer female *D. ponderosae* need to initiate a mass attack, females may use host VOCs in long-range orientation to a stand and in close-range orientation to suitable host trees (Jactel et al. 2001; Kohnle 2004). Male *D. ponderosae* do not initiate colonization but join a mass attack in progress and likely rely more heavily on

aggregation pheromones than host VOCs for orientation during flight. Differential response to VOCs by male and female *D. ponderosae* also occurs in *Monarthrum scutellare* (Coleoptera: Scolytinae). Traps baited with host VOCs, capture more male than female *M. scutellare* suggesting that the pioneering males are more receptive to host VOCs than females (Noseworthy et al. 2012). In the current study, flight of male *D. ponderosae* was not affected by pre-exposure to *trans*-verbenol prior to flight. The lack of effect of body size on flight of male *D. ponderosae* exposed to clean air did not support previous findings on flight (Evenden et al. 2014; Wijerathna and Evenden 2019). There was no relationship between flight capacity and pre-flight weight or other body measurements on flight capacity of male *D. ponderosae* exposed to either clean air or *trans*-verbenol before flight.

Pre-exposure to a synthetic copy of the female-produced aggregation pheromone, *trans*-verbenol, prior to flight increases flight speed and enhances the distance flown by female *D. ponderosae*. Female *D. ponderosae* pre-exposed to *trans*-verbenol prior to flight fly an average of 7.32 ± 0.72 km compared to female *D. ponderosae* maintained in clean air before flight that fly an average of 5.56 ± 0.64 km. *trans*-Verbenol is highly attractive to *D. ponderosae*, as it mediates the initial mass attack in the host colonization process (Miller et al. 2005). Attraction of *Ips grandicollis* to aggregation pheromone, ipsendiol, is greater than to the host monoterpene (–)-α-pinene. Similarly, *Scolytus multistriatus* aggregate to a greater degree in response to aggregation pheromones than host volatiles alone (Lee et al. 2010). Increased flight distance and velocity could be a behavioral indication of a stronger effect of pheromone exposure on the beetle nervous system processing compared to the host VOCs. Exposure to aggregation pheromone post-emergence but prior to flight could indicate to *D. ponderosae* that they are already in a densely populated area and that further flight is needed to avoid offspring competition.

Female *D. ponderosae* fly shorter distances when exposed to the non-host *P. tremuloides* volatiles during flight than in clean air. On average, females exposed to non-host volatiles fly 4.64 ± 0.70 km during the 23 h flight bioassay compared to 6.80 ± 0.69 km in clean air. From other work, it is clear that *D. ponderosae* exhibits avoidance behaviour to non-host VOCs (Huber et al. 2021). In the current study, we were not testing for a behavioral response to the semiochemicals, as orientation and avoidance are not measured using flight mill bioassays. It is

unclear why females completed shorter flights when exposed to *P. tremuloides* volatiles, as longer flights would be expected for *D. ponderosae* to move beyond non-host stands. Under different experimental conditions of mixed exposure to host and non-host VOCs, we might expect to see different flight responses. If decreased flight occurs in a natural setting of non-host VOC exposure, it could have implications for *D. ponderosae* range expansion into the boreal forest. With this range expansion, *D. ponderosae* will encounter more *P. tremuloides* stands intermixed with stands of *P. banksiana* in the central boreal forest (Cavard et al. 2010). Non-host VOCs from *P. tremuloides* elicit antennal activity (Huber et al. 2000) and behavioral repellence in bark beetles (Borden et al. 1998; Huber and Borden 2001a, 2001b). Exposure to non-host VOCs from *P. tremuloides* appears to decrease flight capacity or alter the motivation for *D. ponderosae* to fly. Non-host VOCs also interrupt oriented flight in bark beetles. For example, non-host VOCs applied to the host trees of *D. pseudotsugae* and *D. ponderosae* cause beetles to avoid normally attractive hosts (Borden et al. 1998; Huber and Borden 2001a, 2001b). Non-host volatiles are as repellent to *D. ponderosae* as the anti-aggregation pheromone, verbenone (Borden et al. 1998). Similarly, non-host angiosperm material positioned in host stands decreases the number of host attacks by *I. typographus* within the stand (Kohnle 2004). Future exploration on how non-host volatiles influence *D. ponderosae* flight could help to protect entire stands of host trees (Huber et al. 2020).

Exposure to host and non-host VOCs during flight is unlikely to affect flight of male *D. ponderosae* because males do not initiate the host colonization process. Sex differences in response to non-host cues occurs in other bark beetles. For example, traps that both visually and chemically resemble non-hosts repel female *D. pseudotsugae* and *D. ponderosae* in an additive manner and result in reduced trap capture (Campbell and Borden 2006a, 2006b). Male *D. pseudotsugae* and *D. ponderosae* respond negatively only to the visual non-host cue, and addition of non-host VOCs to the trap does not further affect trap capture (Campbell and Borden 2006b). Similarly, non-host volatiles interrupt oriented flight by pioneering male *I. typographus*, to a greater extent than females (Zhang and Schlyter 2003). The effect of non-host VOCs or visual cues likely impacts the pioneering sex to a greater extent, as colonization costs are higher for pioneers (Zhang and Schlyter 2003).

Here we provide evidence that exposure to host VOCs, non-host VOCs and aggregation pheromones differentially influence *D. ponderosae* flight capacity. Host VOCs interrupt the positive influence of body size on flight distance in female *D. ponderosae*. Pre-exposure to host VOCs, however, does not influence male *D. ponderosae* flight capacity. This differential effect of semiochemcial cues on flight between the sexes could be due to variable importance of these cues between the sexes during host colonization. Exposure to the volatiles of phloem of the non-host, *P. tremuloides,* during flight decreases the distance flown by female *D. ponderosae*, indicating that these repellent compounds not only influence oriented flight but also dispersal in general. Pre-exposure to *trans*-verbenol, on the other hand, increases flight capacity of *D. ponderosae* females. This is the first study to assess the impact of pheromone, host and non-host VOCs on flight capacity in bark beetles. These findings add to previous work on the attractant and repellent response during oriented flight to these VOCs (Huber and Borden 2001b; Miller et al. 2005). Further understanding the impact of semiochemicals on flight dispersal of *D. ponderosae* will add to our understanding of beetle movement in the environment.

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Figures & Tables

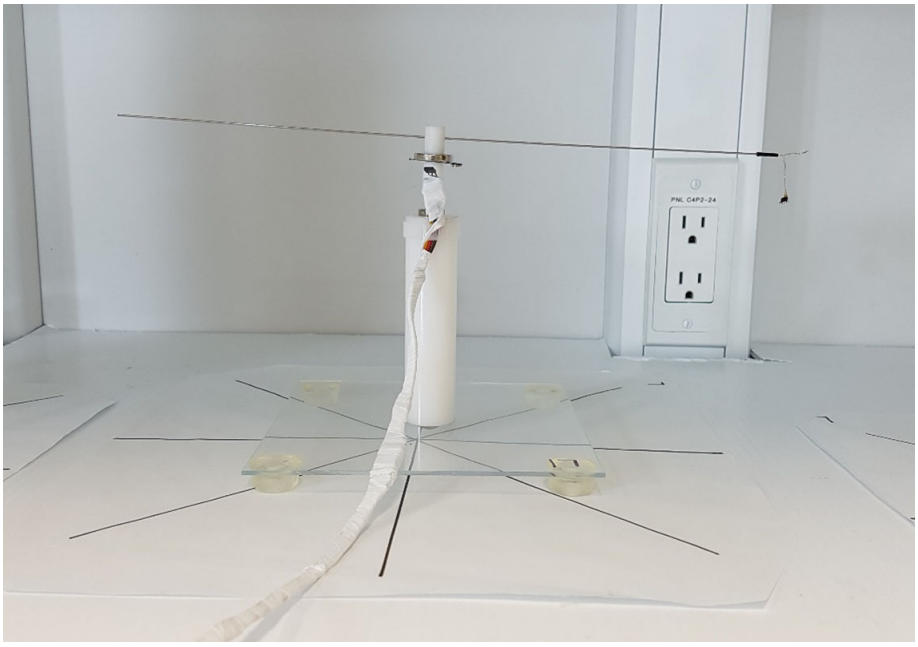


Fig 1 Computer-linked flight mill. A beetle is tethered using a small diameter wire and contact cement. The beetle is then placed on the distal end of the flight mill. The flight mill arm is slightly

raised off of the centre column using the propelling force of two similarly charged magnets. As the beetle flies, the arm of the flight mill is propelled in a circular motion. One full rotation of the flight mill is ~ 94.4 cm. With every full rotation, a magnetic sensor is tripped. This sensor sends flight distance and duration to the computer.

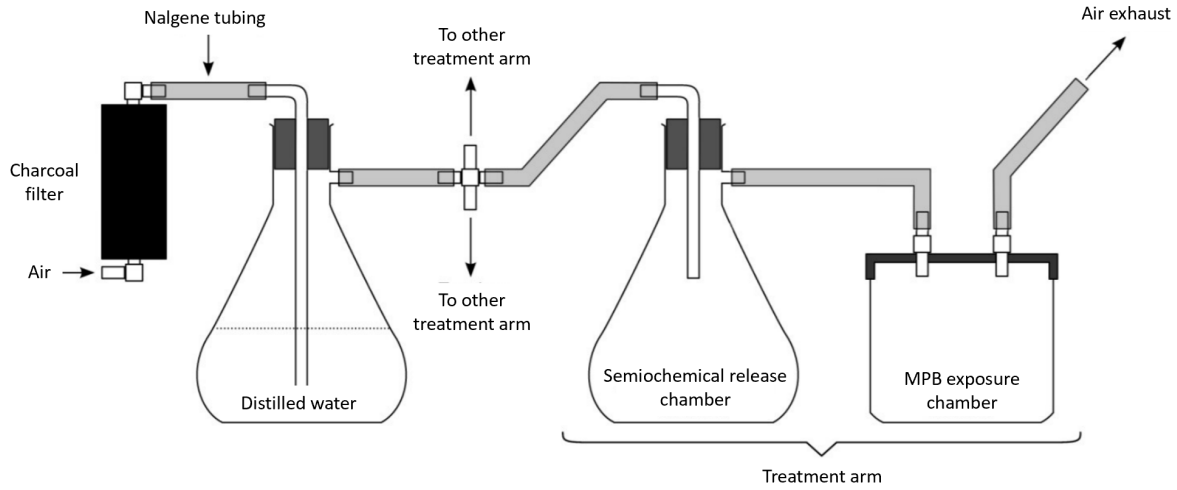
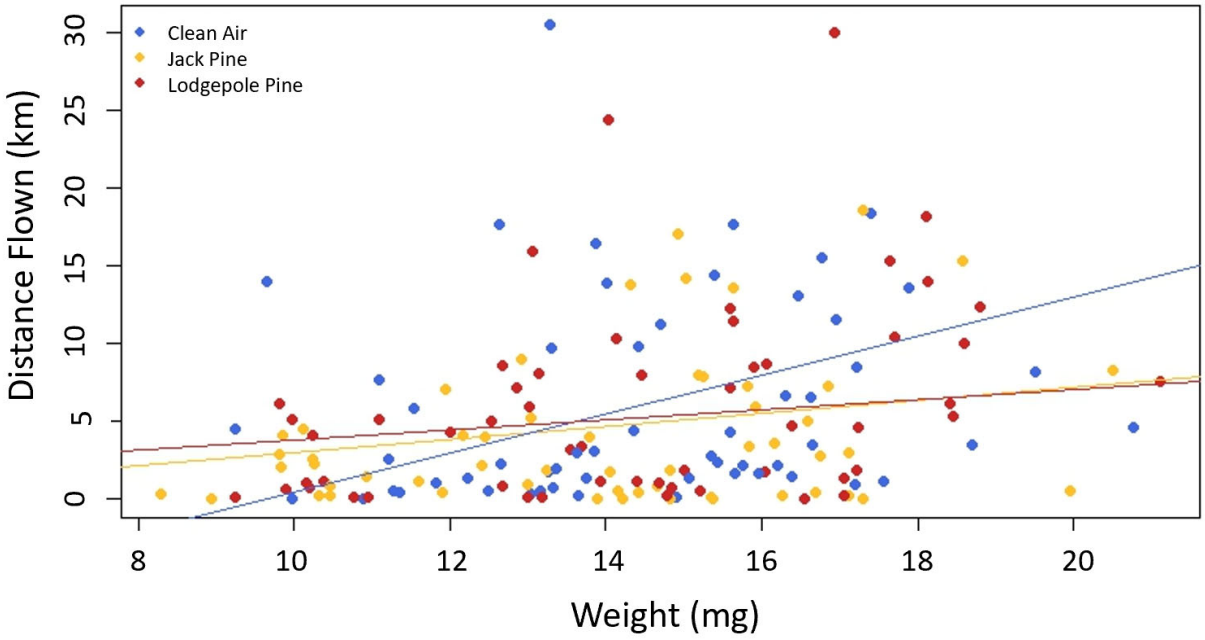


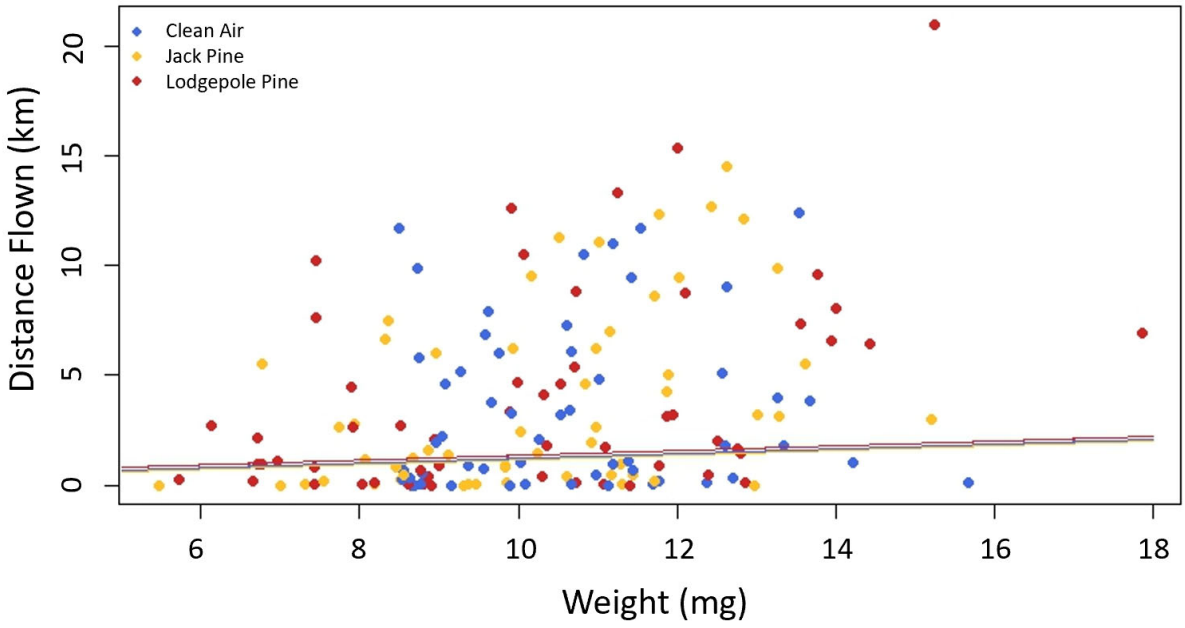
Fig 2 Apparatus and diagram modified from Mori (2014). The apparatus sits in a fume hood, connected to the air input, air is pushed through a charcoal filter, and humidified in a 250 ml Erlenmeyer flask filled with 125 ml of distilled water. From the humidifying flask, air is channeled into three directions, with equal airflow at 500 ml·min^{-1}. Air in each arm enters a 250 ml Erlenmeyer flask containing the exposure material. The headspace air of these flasks containing VOCs is channeled into an exposure chamber. The exposure chamber contained beetles in perforated 1.5 mL microcentrifuge tubes. Air from the exposure chambers is directed to the fume hood exhaust vent.



631

632 **Fig 3** Interaction of pre-exposure treatment prior to flight and pre-flight weight for flight distance
633 of female beetles (χ^2=9.5565, p=0.0588). Flight distance for beetles pre-exposed to *Pinus*
634 *banksiana* (jack pine) or *Pinus contorta* (lodgepole pine) phloem prior to flight did not vary with
635 pre-flight weight. Flight distance for beetles pre-exposed to clean air prior to flight increased
636 with pre-flight weight.

637



638

639 **Fig 4** Flight distances (km) of male beetles pre-exposed to clean air, *Pinus banksiana* (jack pine)
640 or *Pinus contorta* (lodgepole pine) prior to flight. Flight distance increased with increasing pre-
641 flight weight (χ^2=14.8453, p=0.0001) for all exposure treatments. Flight distance did not vary
642 with pre-exposure treatments (χ^2=0.5609, p=0.7555).

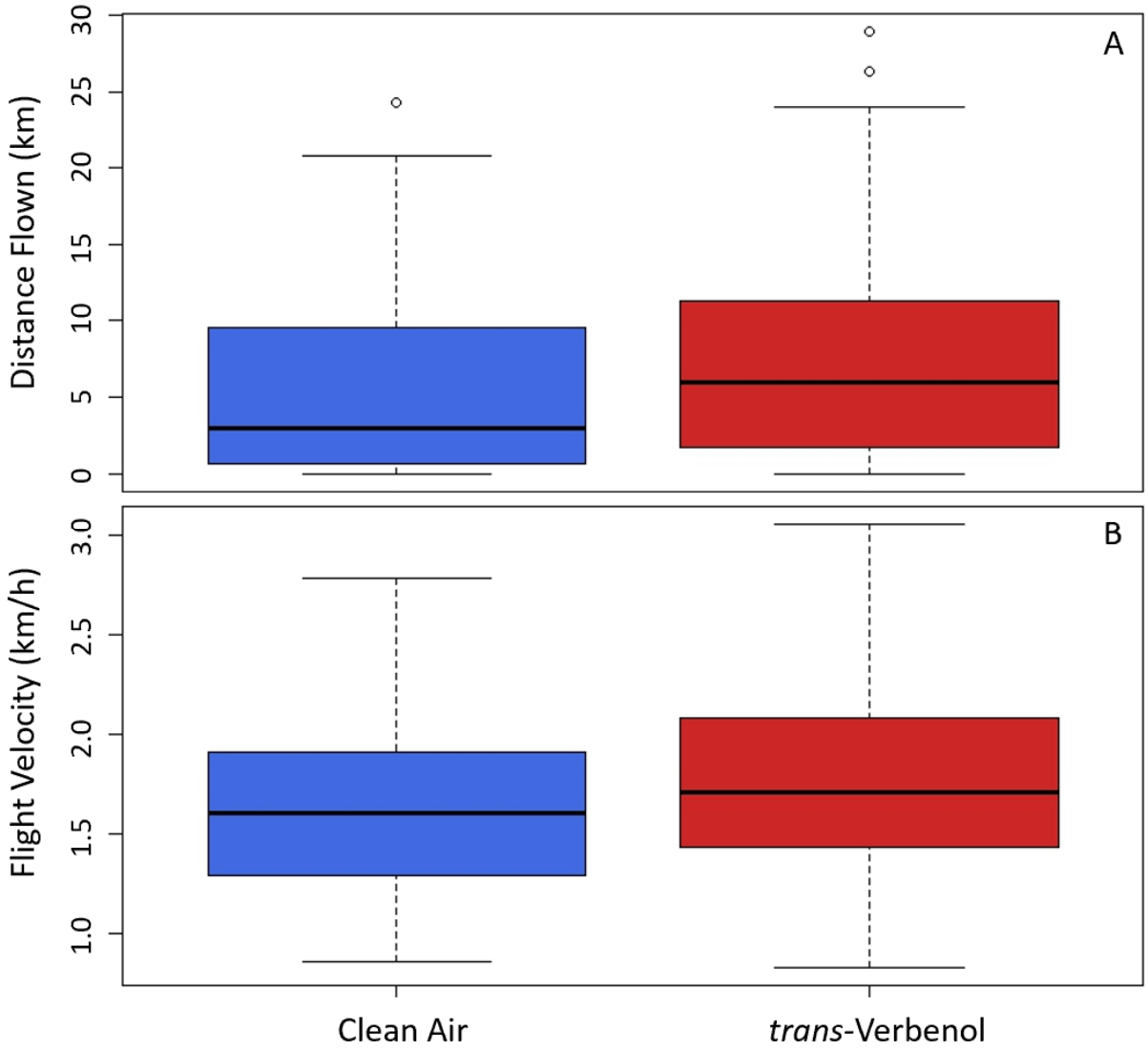


Fig 5 Box plots of (A) flight distance (km) and (B) flight velocity ($km \cdot h^{-1}$) of female beetles pre-exposed to clean air or *trans*-verbenol prior to flight. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values and circles represent outliers. Female beetles pre-exposed to *trans*-verbenol prior to flight flew further than those pre-exposed to clean air (χ^2=5.6578, p=0.0173). Beetles pre-exposed to *trans*-verbenol prior to flight flew at higher velocities than those exposed to clean air (χ^2=4.0895, p=0.0431).

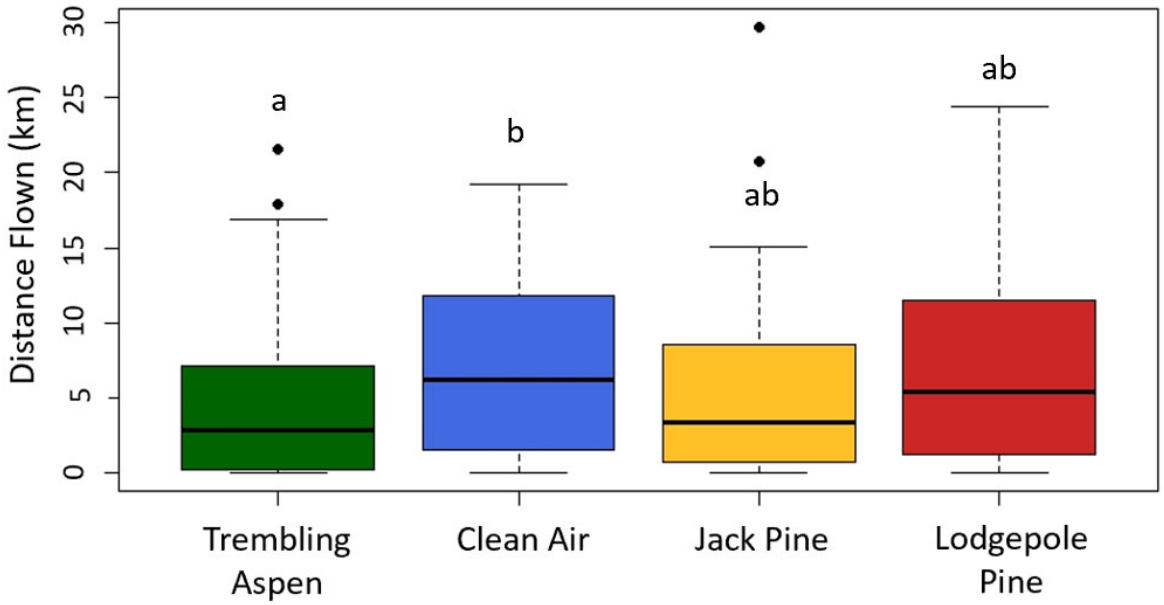


Fig 6 Box plots of flight distance (km) of female beetles exposed to *Populus tremuloides* (trembling aspen), clean air, *Pinus banksiana* (jack pine) or *Pinus contorta* (lodgepole pine) during flight. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values and circles represent outliers. Beetles exposed to *Populus tremuloides* phloem during flight flew shorter distances than those exposed to clean air (t=2.686, p=0.0385).

Table 1 Volatile organic compounds collected from *Pinus banksiana* and *Pinus contorta* over a 3 h aeration period. A single phloem sample from each of the four trees was aerated, averages ($\mu g \cdot ml^{-1} \cdot h^{-1}$) with standard error reported.

	3-Carene	α-pinene	β-phellandrene	Limonene	Myrcene
P. banksiana	2.06 ± 1.65	5.35 ± 3.91	3.80 ± 2.52	0.29 ± 0.23	0.20 ± 0.12
P. contorta	3.85 ± 3.04	2.82 ± 1.00	22.39 ± 15.21	0.39 ± 0.24	0.89 ± 0.52

Supplementary Information

Supplementary Table 1 Statistical models and results for exposure of female beetles to host VOCs prior to flight. EXP: exposure prior to flight, PFW: pre-flight weight, PW: pronotum width, BL: body length, bolt: natal host tree, mill: flight mill.

Response Variable	Model	Statistical Results
Distance	lmer($\sqrt[3]{Distance}$ ~ EXP * PFW, random = bolt + mill)	PFW: χ^2=16.6214, p=4.563x10^{-5}
		EXP: χ^2=0.5831, p=0.7471
		PFW*EXP: χ^2=9.5565, p=0.0588
	lmer($\sqrt[3]{Distance}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=2.7016, p=0.1002
		EXP: χ^2=1.2798, p=0.5273
	lmer($\sqrt[3]{Distance}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=2.7074, p=0.0999
		EXP: χ^2=1.4138, p=0.4932
Duration	lmer($\sqrt[3]{Duration}$ ~ EXP * PFW, random = bolt + mill)	PFW: χ^2=16.6214, p=0.0588
		EXP: χ^2=0.5831, p=0.7471
		PFW*EXP: χ^2=5.6658, p=0.0588
	lmer($\sqrt[3]{Duration}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=2.7016, p=0.1002
		EXP: χ^2=1.2798, p=0.5273

	lmer($\sqrt[3]{Duration}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=2.7074, p=0.0999
		EXP: χ^2=1.4138, p=0.4932
Velocity	lmer(velocity ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=6.8077, p=0.0091
		EXP: χ^2=1.2421, p=0.5374
	lmer(velocity ~ EXP + PW, random = bolt + mill)	PW: χ^2=3.0074, p=0.0829
		EXP: χ^2=4.3460, p=0.1138
	lmer(velocity ~ EXP + BL, random = bolt + mill)	BL: χ^2=3.1235, p=0.0772
		EXP: χ^2=4.5043, p=0.1052

Supplementary Table 2 Statistical models and results for exposure of male beetles to host VOCs prior to flight. EXP: exposure prior to flight, PFW: pre-flight weight, PW: pronotum width, BL: body length, bolt: natal host tree, mill: flight mill.

Response Variable	Model	Statistical Results
Distance	lmer($\sqrt[3]{Distance}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=14.8453, p=0.0001
		EXP: χ^2=0.5609, p=0.7555
	lmer($\sqrt[3]{Distance}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=7.4333, p=0.0064
		EXP: χ^2=0.2455, p=0.8845
	lmer($\sqrt[3]{Distance}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=7.9440, p=0.0048
		EXP: χ^2=0.1705, p=0.9183
Duration	lmer($\sqrt[3]{Duration}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=10.783, p=0.0010
		EXP: χ^2=0.495, p=0.7808
	lmer($\sqrt[3]{Duration}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=4.3133, p=0.0378
		EXP: χ^2=0.1623, p=0.92205

Velocity	lmer($\sqrt[3]{Duration}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=4.5148, p=0.0336
		EXP: χ^2=0.1138, p=0.9447
	lmer($\sqrt[4]{velocity}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=4.6565, p=0.0309
		EXP: χ^2=0.1223, p=0.9407
	lmer($\sqrt[4]{velocity}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=6.4124, p=0.0113
		EXP: χ^2=0.0945, p=0.9539
	lmer($\sqrt[4]{velocity}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=7.3081, p=0.0069
		EXP: χ^2=0.0709, p=0.9652

Supplementary Table 3 Statistical models and results for exposure of female beetles to *trans*-verbenol prior to flight. EXP: exposure prior to flight, PFW: pre-flight weight, PW: pronotum width, BL: body length, bolt: natal host tree, mill: flight mill.

Response Variable	Model	Statistical Results
Distance	lmer($\sqrt[4]{Distance}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=10.6607, p=0.0011
		EXP: χ^2=2.5565, p=0.0865
	lmer($\sqrt[4]{Distance}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=1.6040, p=0.2053
		EXP: χ^2=5.6578, p=0.0173
	lmer($\sqrt[4]{Distance}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=1.2373, p=0.2660
		EXP: χ^2=5.8636, p=0.0553
Duration	lmer($\sqrt[4]{Duration}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=6.3259, p=0.0119
		EXP: χ^2=2.0225, p=0.1550
	lmer($\sqrt[4]{Duration}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=0.5730, p=0.4491
		EXP: χ^2=4.4669, p=0.0346

	lmer($\sqrt[4]{Duration}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=0.2638, p=0.6075
		EXP: χ^2=4.6045, p=0.020
Velocity	lmer(velocity ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=15.5429, p=8.066x10^{-5}
		EXP: χ^2=3.6461, p=0.0562
	lmer(velocity ~ EXP + PW, random = bolt + mill)	PW: χ^2=7.4452, p=0.0006
		EXP: χ^2=4.0895, p=0.0431
	lmer(velocity ~ EXP + BL, random = bolt + mill)	BL: χ^2=10.9373, p=0.0009
		EXP: χ^2=4.3053, p=0.0380

Supplementary Table 4 Statistical models and results for exposure of male beetles to *trans*-verbenol prior to flight. EXP: exposure prior to flight, PFW: pre-flight weight, PW: pronotum width, BL: body length, bolt: natal host tree, mill: flight mill.

Response Variable	Model	Statistical Results
Distance	lmer($\sqrt[3]{Distance}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2= 2.0272, p=0.7072
		EXP: χ^2=0.1410, p=0.1545
	lmer($\sqrt[3]{Distance}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=0.2493 p=0.6176
		EXP: χ^2=0.1098, p=0.7404
	lmer($\sqrt[3]{Distance}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=0.0010, p=0.9753
		EXP: χ^2=0.1032, p=0.7480
Duration	lmer($\sqrt[3]{Duration}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=1.1362, p=0.2865
		EXP: χ^2=0.0986, p=0.7535
	lmer($\sqrt[3]{Duration}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=0.0324, p=0.8572
		EXP: χ^2=0.0495, p=0.8240

Velocity	lmer($\sqrt[3]{Duration}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=0.1201, p=0.7289 EXP: χ^2=0.0315, p=0.8592
	lmer($\sqrt[4]{velocity}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=0.9182, p=0.3379 EXP: χ^2=0.0014, p=0.9701
	lmer($\sqrt[4]{velocity}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=1.6760, p=0.1955 EXP: χ^2=0.0178, p=0.8938
	lmer($\sqrt[4]{velocity}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=2.0311, p=0.1541 EXP: χ^2=0.0882, p=0.7665

Supplementary Table 5 Statistical models and results for female beetles exposed to clean air, host and non-host VOCs during flight. EXP: exposure during flight, PFW: pre-flight weight, PW: pronotum width, BL: body length, bolt: natal host tree, mill: flight mill, A: *Populous tremuloides*, C: clean air, J: *Pinus banksiana*, L: *Pinus contorta*

Response Variable	Model	Statistical Results	Post-Hoc Results
Distance	lmer($\sqrt[3]{Distance}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=31.0295, p=2.541x10^{-8} EXP: χ^2=9.5565, p=0.0227	A-C: t=2.989, p=0.0162 A-J: t=1.598, p=0.3819 A-L: t=2.089, p=0.1595 C-J: t=1.356, p=0.5281 C-L: t=0.881, p=0.8149 J-L: t=0.462, p=0.9672
	lmer($\sqrt[3]{Distance}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=10.3523, p=0.0001 EXP: χ^2=9.2082, p=0.0266	A-C: t=2.686, p=0.0385 A-J: t=0.670, p=0.9084 A-L: t=1.954, p=0.2086 C-J: t=2.057, p=0.1705

			C-L: t=0.721, p=0.8887
			J-L: t=1.311, p=0.5570
	lmer($\sqrt[3]{Distance}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=7.8949, p=0.0050	A-C: t=2.596, p=0.0489
		EXP: χ^2=8.7058, p=0.0334	A-J: t=0.650, p=0.9155
			A-L: t=1.935, p=0.2164
			C-J: t=1.982, p=0.1976
			C-L: t=0.647, p=0.9166
			J-L: t=1.311, p=0.5570
Duration	lmer($\sqrt[3]{Duration}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=19.7717, p=8.726x10^{-6}	A-C: t=2.560, p=0.0536
		EXP: χ^2=7.5893, p=0.0553	A-J: t=1.686, p=0.3331
			A-L: t=2.120, p=0.1498
			C-J: t=0.830, p=0.8392
			C-L: t=0.419, p=0.9752
			J-L: t=0.402, p=0.9779
	lmer($\sqrt[3]{Duration}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=6.3148, p=0.0120	
		EXP: χ^2=6.9588, p=0.0732	
	lmer($\sqrt[3]{Duration}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=5.3745, p=0.0204	
		EXP: χ^2=6.6533, p=0.0838	
Velocity	lmer(velocity ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=24.3269, p=8.129x10^{-7}	
		EXP: χ^2=0.7734, p=0.8558	
	lmer(velocity ~ EXP + PW, random = bolt + mill)	PW: χ^2=8.3488, p=0.0039	
		EXP: χ^2=0.4200, p=0.9361	
	lmer(velocity ~ EXP + BL, random = bolt + mill)	BL: χ^2=5.2436, p=0.0220	

		EXP: χ^2=0.2817, p=0.9643

Supplementary Table 6 Statistical models and results for male beetles exposed to clean air, host and non-host VOCs during flight. EXP: exposure during flight, PFW: pre-flight weight, PW: pronotum width, BL: body length, bolt: natal host tree, mill: flight mill, A: *Populous tremuloides*, C: clean air, J: *Pinus banksiana*, L: *Pinus contorta*

Response Variable	Model	Statistical Results
Distance	lmer($\sqrt[3]{Distance}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=17.4288, p=2.9830x10^{-5}
		EXP: χ^2=6.1699 p=0.1036
	lmer($\sqrt[3]{Distance}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=5.5246, p=0.0188
		EXP: χ^2=3.7455, p=0.2903
	lmer($\sqrt[3]{Distance}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=4.0108, p=0.04521
		EXP: χ^2=3.5927, p=0.3089
Duration	lmer($\sqrt[3]{Duration}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=11.6503, p=0.0006
		EXP: χ^2=5.3148, p=0.1501
	lmer($\sqrt[3]{Duration}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=3.6418, p=0.0563
		EXP: χ^2=2.9849, p=0.3940
	lmer($\sqrt[3]{Duration}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=2.4006, p=0.1213
		EXP: χ^2=2.8699, p=0.4121
Velocity	lmer($\sqrt{velocity}$ ~ EXP + PFW, random = bolt + mill)	PFW: χ^2=6.9804, p=0.0082
		EXP: χ^2=4.0297, p=0.2583

lmer($\sqrt{velocity}$ ~ EXP + PW, random = bolt + mill)	PW: χ^2=5.5225, p=0.0188 EXP: χ^2=0.1.3946, p=0.7068
lmer($\sqrt{velocity}$ ~ EXP + BL, random = bolt + mill)	BL: χ^2=7.7517, p=0.0054 EXP: χ^2=1.5682, p=0.6666