Influence of Semiochemical Cues on Mountain Pine Beetle Flight and Subsequent Effect of Flight on Host Colonisation Processes

by

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Abstract

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), is an aggressive pine pest in western North America. This beetle undergoes cyclical population outbreaks, and in the most recent outbreak the beetle expanded its range to the north and east. During this range expansion, beetles breached the Rocky Mountains and entered North-Central Alberta. The spread of the mountain pine beetle into Alberta resulted in colonization of a new pine host, jack pine (*Pinus banksiana*). Dispersal is an extremely important part of mountain pine beetle ecology, yet it is the least understood part of the life cycle. Mountain pine beetle exhibit stratified dispersal, this dispersal type is characterized by large variation in dispersal distance among individuals within the population. Some of this variation can be explained by beetle physiological condition, stand density or abiotic environmental factors; however, these factors do not explain all the variation exhibited. Using computer-linked flight mills I tested how olfactory cues that beetles encounter in the environment contribute to flight variation through an influence on beetle flight propensity and capacity. Further, I tested how this large degree of variation in flight distance impacts subsequent life history events in the mountain pin beetle. These experiments led to six major findings: (1) Exposure to host volatiles before flight interacts with beetle pre-flight weight to influence flight of female but not male beetles; (2) Female beetles fly shorter distances when exposed to volatiles from non-host angiosperms during flight than in clean air; (3) Exposure to aggregation pheromone prior to flight results in increased flight distance and velocity in female beetles; (4) There is a trade-off between host colonization and flight, as beetles that lose more weight during flight are less likely to enter a host; (5) Percent weight lost during flight and distance flown by female beetles influences subsequent production of the aggregation pheromone, *trans*-verbenol; and (6) Male beetle condition prior to flight but not energy used during flight influences *exo*-brevicomin production. This study is the first to show that semiochemical cues not only influence flight orientation, but also flight capacity of the mountain pine beetle. Further, the distance flown by individual beetles influences the subsequent host colonization processes of mountain pine beetle, which could potentially reveal mechanisms behind the well-documented flight polyphenisms in this species. These novel results give insight into the highly variable flight behaviour of the mountain pine beetle.

Preface

This document presents two studies (Chapter 2 and Chapter 3) intended for publication. These studies are collaborative work between myself, Dr. Maya Evenden (Chapter 2 and Chapter 3) and Dr. Nadir Erbilgin (Chapter 3) at the University of Alberta. My responsibilities included method development, data collection, analyses, interpretation and manuscript composition. Dr. Maya Evenden was involved with concept formation and manuscript composition for Chapter 2. Dr. Maya Evenden and Dr. Nadir Erbilgin were involved with concept formation and manuscript composition for Chapter 3. The method development for chemical analyses was completed by Rahmatollah Rajabzadeh and Guncha Ishangulyyeva in the Erbilgin Lab. All research presented in this thesis was conducted in accordance with all applicable laws and rules set forth by provincial and federal governments and the University of Alberta. All necessary training and permits were obtained prior to data collection.

Note: Chapter 2 is formatted for submission to Journal of Chemical Ecology and Chapter 3 is formatted for submission to Journal of Animal Ecology

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Chapter 1: Introduction

Dispersal is an important part of the life cycle of many insects and is defined by the movement to search for resources, find suitable mates, acquire and defend territories, and escape local competition (Matthysen, 2012). Insects move throughout the environment by flying, walking, crawling, swimming or by other environment-specific locomotory adaptations (Roff & Fairbairn, 2007). Adult insects can efficiently move through the environment using flight (Danthanarayana, 1986), as it permits relatively greater dispersal distance compared to other methods of movement (Roff & Fairbairn, 2007). Insects undergo migratory or non-migratory flight depending on the behaviour that drives the movement. Migratory flight involves movement outside of the habitat to colonise new or recolonise old habitats, whereas non-migratory flight is movement within the habitat for feeding, mating and oviposition (Danthanarayana, 1986).

Movement from one habitat to another does not only have implications for individual fitness but also may affect the population dynamics of a species (Bowler & Benton, 2005). Dispersal flight can be both beneficial and detrimental to the overall population. Individuals that disperse are at a higher risk of predation, movement into unsuitable habitats or deterioration of physiological condition (Roff & Fairbairn, 2007; Bowler & Benton, 2009). Although some individuals may not disperse to a suitable habitat, those that do may benefit from a lack of competition, higher quality resources, and genetic outbreeding (Bowler & Benton, 2005; Matthysen, 2012). Population density at these new habitats, however, can affect the relationship between resource quality and individual fitness (Matthysen, 2012). Understanding the drivers of dispersal and the factors that influence dispersal of an individual may aid in managing populations and predicting movement in a changing environment (Bowler & Benton, 2005).

Many species of bark beetles (Coleoptera: Curculionidae, Scolytinae), exhibit cyclical changes in population density. The irruptive nature of some species within the genera *Ips* and *Dendroctonus* make these insects ecologically (Eidmann, 1992; Paine et al., 1997) and economically (Franklin et al., 1987; Grégorie et al., 2015) important. Colonisation of host trees by mass attack during outbreaks can result in tree death over vast areas of forested landscapes (Grégorie et al., 2015). Bark beetles are a species rich sub-family that specialise feeding on subcortical tissue of the host tree (Raffa et al., 2015). Feeding damage combined with host colonisation by symbiotic fungi can result in the death of the host tree (Paine et al., 1997;

Harrington, 2005). Bark beetles spend most of their life in the subcortical environment of the host tree and only leave the hosts for a short period of time to disperse to find a new host for brood production (Raffa et al., 2015). A dispersal phase is obligatory for all bark beetle species but does not necessarily occur in each generation if recolonisation of the same host occurs (Raffa et al., 2015). Beetles need to disperse as the resources provided by the natal host become depleted.

Bark beetles emerge from the natal host in the spring and summer when environmental conditions are suitable for flight (Raffa et al., 2015). Many species of bark beetles exhibit extreme polyphenic flight behaviour. Most individuals disperse only a few hundred metres from the natal tree (Zumr, 1992), however, some beetles undergo dispersal flights of tens of kilometres (Furniss & Furniss, 1972; Jackson et al., 2008). These dichotomous dispersal patterns can be categorised as short and long distance dispersal, however, a large amount of variation occurs within each dispersal type. Short distance dispersal occurs when beetles attack a tree within the same stand as the natal host (Safranyik et al., 1992; Robertson et al., 2007). Long distance dispersal is aided by the wind, and results in the transport of beetles above the canopy. Long distance dispersal results in displacement of beetles over greater distances than would be physiologically possible using energy stores alone (Jackson et al., 2008). Beetles often initiate dispersal flight downwind until they encounter an attractive semiochemical signal that initiates upwind orientation behaviour (Gray et al., 1972; Safranyik et al., 1992). Not all bark beetles are responsive to semiochemical signals immediately following emergence from the natal host. Some species need to undergo a period of non-oriented flight before orientation to host volatiles or conspecific pheromones is possible (Atkins, 1966; Bennett & Borden, 1971; Choudhury & Kennedy, 1980).

Pioneers of some bark beetle species are the first to reach new hosts and initiate colonisation. Pioneers respond to attractive semiochemical signals produced by the tree (Moeck & Simmons, 1991). Plants emit a variety of volatile organic compounds (VOCs), in specific ratios that make up the chemical profile of the plant. Some bark beetles use these chemical signals to discriminate between host and non-host trees (Kohnle, 2004). It is unknown at what range bark beetles discriminate host trees. Chemical signals direct some bark beetles in long-range orientation toward a stand of suitable hosts, and in short range orientation for discrimination between potential hosts within the stand (Kohnle, 2004; Jactel et al., 2001). Other hypotheses suggest that beetles will randomly land on hosts (Hynum & Berryman, 1980) and directly assess host quality post-landing (Pureswaran & Borden, 2003). In contrast, volatile organic compounds in combination with visual cues provided by the host are used for short-range host discrimination by some bark beetles (Campbell & Borden, 2006a, 2006b).

Host VOCs can be exploited for use in management of bark beetles. Traps baited with VOCs produced by hosts of the black pine bark beetle (*Hylastes ater* (Paykull)) and the red-haired pine bark beetle (*Hylurgus ligniperda* (Fabricius)) increase trap catch up to 100 fold, over unbaited control traps (Kerr, 2010). The semiochemical dose mediates attraction, for example, attraction of the European spruce bark beetle (*Ips typographus* Linnaeus) is dose dependent to the host monoterpene, (–)-α-pinene (Erbilgin et al., 2007). Trap capture of the European spruce bark beetle is higher in traps baited with high (–)-α-pinene concentrations, compared to that in traps baited with pheromone alone. At low (–)-α-pinene concentrations, however, trap capture is similar to that in traps baited with pheromone alone. For some bark beetle species, the relative or absolute concentration of host-produced VOCs can govern orientation behaviour. The North American pine engraver (*Ips pini* (Say)) is more attracted to intermediate levels of (–)-α-pinene than to pheromone alone, but is less attracted to high levels of (–)-α-pinene (Erbilgin et al., 2003). Not all volatiles produced by hosts stimulate host finding behaviour in bark beetles. For example, the monoterpene 3-carene attracts *Dendroctonus rhizophagus* Thomas and Bright but other host monoterpenes do not promote orientation behaviour (Cano-Ramírez et al., 2012).

Semiochemicals released by non-host species in the environment can also influence bark beetle behaviour through disruption of orientation (Huber & Borden, 2001a, 2001b), presumably for avoidance of flight toward non-host species. The semiochemical diversity hypothesis states that host location during flight may be more difficult in mixed stands due to the interference of beetle orientation by non-host volatiles (Zhang & Schlyter, 2003). Movement towards aggregation pheromones of the Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopkins) (Huber & Borden, 2001a), the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (Borden et al., 1998), and the stenographer bark beetle (*Ips sexdentatus* (Boerner)) (Jactel et al., 2001) can be disrupted when signals are combined with non-host angiosperm volatiles. Non-host volatiles can interrupt oriented flight as effectively as anti-aggregation pheromones; these are signals that bark beetles produce to end the host colonisation process. Bark beetle orientation to attractive semiochemical-baited traps is equally disrupted by the addition of non-host volatiles or anti-aggregation pheromone (Borden et al., 1998; Huber & Borden, 2001b; Campbell & Borden, 2006a, 2006b). Non-host angiosperm material placed in host stands decreases within-stand attack by the pine shoot beetle, however, when the same is done with non-host conifers, attack is unaffected (Kohnle, 2004). Potentially non-host angiosperm VOCs influence long distance orientation and conifer chemical profiles influence short distance orientation, as non-host and host conifers have similar VOC profiles (Kohnle, 2004). European spruce bark beetles are repelled by several antennally active non-host volatiles (Zhang & Schlyter, 2003). Repellence occurs in response to individual compounds and combinations of non-host volatiles. The effect of some non-host volatiles are redundant to mountain pine beetle (Borden et al., 1998).

Host colonisation is dictated through pheromone-based communication initiated by the pioneering sex, females in the genus *Dendroctonus* and males in the genus *Ips.* Pioneering beetles locate a suitable host for reproduction and initiate feeding (Raffa et al., 2015) prior to the release of aggregation pheromones. Many bark beetle species use aggregation pheromones to orchestrate the host colonisation process (Raffa et al., 2015). Non-pioneering beetles respond to conspecific aggregation pheromones that act in synergy with host semiochemicals (Byers et al., 1988). Although redundancy to some individual compounds occurs, aggregation pheromone blends are species-specific signals. Aggregation pheromones attract both sexes of non-pioneering conspecifics. Responders of the opposite sex to the pioneer beetle enter a gallery of an already established beetle, and those of the same sex initiate a new gallery on the host tree.

The distance flown by individual beetles in response to semiochemical cues to initiate or join a mass attack may be largely based on beetle physiological condition. Lipid content is often used as a metric for beetle physiological condition. Lipid content is positively related to flight capacity in the genus *Dendroctonus*; beetles with high lipid content fly further (Kinn et al., 1994; Williams & Robertson, 2008; Chen et al., 2011; Evenden et al., 2014) and for longer (Hodges & Barras, 1974; Kinn et al., 1994; Williams & Robertson, 2008; Chen et al., 2011) than beetles with low lipid content. Lipid fuels flight in the Chinese white pine beetle (*Dendroctonus armandi* Tsai & Li) (Chen et al., 2011), the Douglas-fir beetle (Atkins, 1969; Thompson & Bennett, 1971; Williams & Robertson, 2008), the mountain pine beetle (Evenden et al., 2014;

Wijerathna & Evenden, 2019), and the southern pine beetle (*Dendroctonus frontalis* Zimmermann) (Barras & Hodges, 1974; Hedden & Billings, 1977). Physiological condition can also dictate host colonisation behaviours of bark beetles following dispersal. Lipid used during flight can influence host colonisation behaviour, as beetles that fly may be more vulnerable to tree defenses, as beetles need fat to overcome exposure to monoterpenes (Reid et al., 2017). Beetles with low-lipid reserves are less selective and accept poorer quality host trees sooner than do individuals with high-lipid reserves that are capable of continued dispersal (Latty & Reid, 2010; Chubaty et al., 2014). There is also some evidence for a trade-off between flight and reproduction in bark beetles (Wijerathna et al., 2019).

The mountain pine beetle is an example of an ecologically and economically important bark beetle species that specialises on *Pinus* species. Distribution of the mountain pine beetle in Canada was historically restricted to British Columbia (Safranyik et al., 2010). In British Columbia, mountain pine beetle hosts include lodgepole pine (*Pinus contorta* var. *latifolia* Douglas ex Loudon), ponderosa pine (*Pinus ponderosa* Douglas ex Lawson & C.), western white pine (*Pinus monticola* Douglas ex D. Don), whitebark pine (*Pinus albicaulis* Engelmann), and limber pine (*Pinus flexilis* James) (Natural Resources Canada, 2017). Mountain pine beetles experience population outbreaks and naturally go through four distinct population phases; endemic, incipient-endemic, epidemic and post-epidemic (Safranyik & Carroll, 2006). The most recent outbreak of the mountain pine beetle reached epidemic levels in the early 2000s (Safranyik et al., 2010). During this outbreak, the beetle expanded its native range north and east killing over an area of 18 million hectares of pine forests in British Columbia. Due to this range expansion, the mountain pine beetle entered North-Central Alberta in the mid-2000s (Government of Alberta, 2006; Natural Resources Canada, 2017). In the expanded range, the mountain pine beetle has encountered a novel host, jack pine (*Pinus banksiana* Lambert), a species that comprises much of Canada's boreal forest (Natural Resources Canada, 2017).

The mountain pine beetle has an obligatory dispersal phase; adult beetles must leave the dead natal host in search of a living host in which to reproduce (Safranyik & Carroll, 2006). Abiotic conditions govern the initial emergence and flight of the mountain pine beetle. Beetle movement through the environment occurs only during optimal climatic conditions. Emergence of adult mountain pine beetles begins when ambient temperatures reach 20°C (Shepherd, 1966;

Gray et al., 1972). Optimal flight temperatures for the mountain pine beetle range from 25-30°C. Mountain pine beetle often initiate flight in the downwind direction (Gray et al., 1972; Safranyik et al., 1989). These beetles fly at velocities of 1.55-1.93 km/hr in lab settings (Evenden et al., 2014), and fly against winds up to 7.2 km/hr in both field and lab settings (Safranyik et al. 1989; Wijerathna, 2016).

The mountain pine beetle exhibits three distinct patterns of population spread as a result of flight dispersal; the first two occur within the stand. Spot growth occurs when beetles attack a tree near the natal host, while spot proliferation occurs when beetles fly past many suitable hosts before selection of a host further away but within the same stand (Safranyik et al., 1992; Robertson et al., 2007). Short distance dispersal flights are extremely variable and may range from only a few metres to tens of kilometres (Safranyik et al., 1992; Evenden et al., 2014). The third type of dispersal is long distance, wind-aided dispersal; wind currents carry beetles above the canopy further downwind than would be physiologically possible using direct flight alone (Jackson et al., 2008).

The mechanisms driving the different dispersal strategies of the mountain pine beetle remain unknown. It has been hypothesized that the mountain pine beetle requires an obligatory flight period before becoming responsive to conspecific pheromones and host kairomones (Shepherd, 1966; Borden et al., 1987; Robertson et al., 2007). Flight and physiological condition govern the responsiveness to host volatiles in many scolytid species (Atkins, 1966; Bennett & Borden, 1971; Hagen & Atkins, 1975; Williams & Robertson, 2008). These species either have an obligatory dispersal period before response to chemical signals or respond based on the amount of remaining lipid stores. Although it has been hypothesized that response to attractive chemical cues may be driven by physiological state in the mountain pine beetle (Shepherd, 1966; Borden et al., 1987; Robertson et al., 2007), this hypothesis remains untested.

Host selection behaviour elicited by mountain pine beetle has been a topic of debate in the literature. One hypothesis supports a combination of random landing behaviour and visual orientation to the tree, with assessment of host suitability occurring post-landing (Pureswaran & Borden, 2003; Safranyik & Carroll, 2006). A second hypothesis suggests host volatiles likely aid pioneer beetles in long distance orientation to a suitable stand of host trees, and once in the stand, visual and short-range in-flight olfactory cues allow the beetle to choose a suitable host

(Campbell & Borden, 2006a). The mountain pine beetle perceives and responds to a variety of compounds emitted by host trees, including α-pinene, β-phellandrene, limonene, 3-carene, and myrcene (Whitehead, 1986; Moeck & Simmons, 1991; Pureswaran et al., 2004). These VOCs likely indicate host quality to pioneering beetles.

Non-host volatiles produced by angiosperms also elicit antennal response from the mountain pine beetle (Huber et al., 2000). When exposed to both host and non-host volatiles simultaneously, beetles are repelled by the non-host cues and orient towards the pine host (Gray et al., 2015). The presence of non-host volatiles disrupts host selection by the mountain pine beetle. Host trees baited with non-host volatiles receive fewer attacks than unbaited host trees (Borden et al., 1998; Huber & Borden, 2001b). Non-host volatiles cause avoidance behaviour by flying beetles, even to trees baited with attractive conspecific pheromone (Campbell & Borden, 2006b). Non-host volatiles often have an additive, disruptive effect that makes it easier for the mountain pine beetle to discriminate between hosts and non-hosts as the beetle approaches the stand (Campbell & Borden, 2006b).

Once a suitable host is located, female mountain pine beetles initiate host colonisation and aggregation. Females release the aggregation pheromone *trans*-verbenol upon initiation of gallery construction and feeding (Pitman & Vité, 1969; Pureswaran et al., 2000). *trans*-Verbenol production requires the oxidation of the precursor α-pinene (Hughes, 1975) obtained from the natal host (Chiu et al., 2018). *trans*-Verbenol production varies with the concentration of α-pinene present in the reproductive host (Taft et al., 2015), suggesting that the α-pinene precursor can be acquired from either the natal or reproductive host tree. Mountain pine beetle accumulate α-pinene in the form of monoterpenyl esters during larval feeding in the natal host (Chiu et al., 2018). These monoterpenyl fatty acid esters are stored in the fat body for subsequent *trans*-verbenol production. The release of *trans*-verbenol acts to attract both sexes of mountain pine beetle (Borden et al., 1987). As females land on the tree, they initiate the construction of new galleries and males enter galleries already constructed by females (Safranyik & Carroll, 2006).

Male beetles release the pheromone *exo*-brevicomin upon emergence from the natal host (Song et al., 2014). The complete biosynthetic pathway of *exo*-brevicomin remains unknown, however, this pheromone is synthesized *de novo* from fatty acyl-CoA precursors within the fat body (Vanderwel, 1994; Song et al., 2014). Males release *exo*-brevicomin during flight and subsequently have lower pheromone titres when they land on the reproductive host and join the female galleries. Low levels of *exo*-brevicomin act in synergism with *trans*-verbenol to attract more females to help overwhelm host defenses (Borden et al., 1983).

Once the host tree contains enough parent beetles to overcome the host defenses, the host colonisation process stops. The cessation of attack is signalled by anti-aggregation pheromones (Berryman et al., 1985; Zhang et al., 1992). Both male and female mountain pine beetles produce verbenone as an anti-aggregation pheromone, but male mountain pine beetles also produce frontalin (Libbey et al., 1985). Verbenone is produced through the auto-oxidation of *trans*-verbenol, which is mediated through microorganisms and fungi associated with the beetle (Hunt & Borden, 1989; Cale et al., 2019). Frontalin is synthesized *de novo* in the midgut of males, from the 20-carbon precursor, geranylgeranyl diphosphate (Barkawi et al., 2003; Keeling et al., 2013). Both pheromones signal that the tree is full which discourages additional attacks by conspecifics. Beetles use anti-aggregation pheromones to obtain optimal attack density, roughly 62 attacks/m^2 in the historic range (Raffa & Berryman, 1983). The optimal attack density occurs when the number of attacking pairs is high enough to kill the tree but not too high to result in offspring competition.

It is a benefit to the mountain pine beetle to arrive at the reproductive host in good physiological condition. Physiological condition influences tree entry, as mountain pine beetle in good body condition are more likely than beetles in poor condition to enter a host tree (Latty & Reid, 2010; Chubaty et al., 2014). Of the beetles that enter the host during a mass attack, the beetles in the poorest condition enter the soonest. These beetles are less choosey compared to beetles in good condition (Chubaty et al., 2014). Physiological condition influences the beetles' ability to overcome host monoterpene defenses (Reid & Purcell, 2011). Beetles in better condition are more likely to survive exposure to monoterpenes, and larger beetles proportionally lose less mass during the exposure period (Reid et al., 2017). Beetle physiological condition also influences reproductive traits such as egg size that reduces with body condition of the parent beetle (Elkin & Reid, 2005). Further, a flight period, which directly influences beetle energy reserves (Evenden et al., 2014; Wijerathna & Evenden, 2019), also influences reproduction (Wijerathna et al., 2019). Beetles that undergo a flight period produce fewer offspring compared to beetles not given the opportunity to fly (Wijerathna et al., 2019).

The sub-cortical life history stages of irruptive bark beetle species are well understood, but less is known about dispersal by flight (Jones et al., 2019), despite its importance to the ecology of these aggressive pest species. In particular, the research effort on mountain pine beetle biology is substantial, yet much remains to be understood regarding environmental influences governing the obligatory dispersal phase, and how this dispersal phase influences subsequent life history processes.

Objectives

My thesis assesses how semiochemicals influence flight propensity and capacity, and the subsequent effect of flight on host colonisation. In Chapter 2, I test the influence of beetle exposure to aggregation pheromones, host and non-host VOCs prior to and during flight on flight propensity and capacity. In Chapter 3, I test the effect of flight on host colonisation and aggregation pheromone production after the initiation of attack. This research provides information on the interactions between flight and semiochemical response in the mountain pine beetle. An understanding of this interaction will provide insight into beetle population dynamics and spread across the landscape.

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Chapter 2: Effect of semiochemical exposure on flight propensity and capacity of *Dendroctonus ponderosae* in laboratory bioassays

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Abstract

Insect herbivores respond to volatile organic compounds (VOCs) produced 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 during flight and find a suitable reproductive host. The distance and duration of dispersal flight of *D. ponderosae* varies even within populations of beetles. Beetle energetics do not 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 and capacity using computer-linked flight mills. Exposure to host volatiles before flight interacts with pre-flight weight to influence flight of female but not male beetles. Female beetles exposed to volatiles from the non-host, *Populus tremuloides,* during flight flew shorter distances than in clean air. Female beetles flew further and faster when exposed to *trans*-verbenol prior to flight. This study is the first to indicate that semiochemical cues not only influence flight orientation, but also flight capacity of *D. ponderosae*. These results provide baseline information on the effect of environmental cues on flight dispersal of *D. ponderosae*.

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

Introduction

Volatile organic compounds (VOCs) are compounds released by organisms that help mediate inter- and intraspecific interactions within an ecosystem (Tumlinson 2014). Plant-produced VOCs attract pollinating insect species (Pichersky and Gershenzon 2002), defend against phytophagous insects (Song and Ryu 2013) and attract natural enemies to herbivore hosts (Heil 2008). Phytophagous insects can also use plant-produced VOCs to locate and determine the condition of host plants and to choose an appropriate host (Mukherjee et al. 2015). Plants emit blends of various VOCs that make up the chemical profile of the plant. Species-specific VOC profiles help herbivores locate suitable host plants (Grison-Pigé et al. 2002). Blend composition of plant VOCs changes with phenological stage (Matile and Altenburger 1988) or plant stress (Gouinguené and Turlings 2002; Lusebrink et al. 2011). Herbivorous insects can detect changes in the host VOC profile to determine host condition, which permits discrimination between potential hosts in the same species based on host plant quality (Webster 2012). Concentrations of VOC emissions can indicate plant stress such as drought (Lusebrink et al. 2011; Simpraga et al. 2011) or previous herbivory (Mukherjee et al. 2015) to herbivores.

Pheromones are chemical signals that govern intraspecific interactions between individuals and are instrumental in the biology of many insect species (Wyatt 2014b). Insects can have multiple pheromone signals comprised of different pheromone components that relay different messages (Tumlinson 2014). Pheromones can signal mate attraction, aggregation, alarm and foraging trails, especially in gregarious or social insects. Aggregation pheromones attract both sexes of a species to mediate group living (Cardé 2014; Wyatt 2014a). Bark beetles (Coleoptera: Curculionidae: Scolytinae) use aggregation pheromones to concentrate conspecifics for host colonisation of chemically defended host trees.

Bark beetles pioneer mass attacks of host trees through response to plant-produced chemical signals. Plant VOCs help beetles to discriminate between host and non-host trees and assess host quality (Kohnle 2004). Bark beetle may use host VOCs for long-range orientation towards a stand of suitable hosts, and short-range orientation to discriminate between potential hosts within a stand (Jactel et al. 2001; Kohnle 2004). Host VOCs can attract many bark beetles to hosts in the absence of attractive pheromones, 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 beetles dictates the host colonisation process (Wyatt 2014a; Raffa et al. 2015). Non-pioneering 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, does 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 concentrations (Erbilgin et al. 2003). Pheromone alone or host terpenes alone attract a small number of *Dendroctonus rufipennis* to baited traps, but a combination of pheromone and host terpenes enhances attraction (Ryall et al. 2013). Pheromone components of *Pityogenes chalcographus* presented individually in baited traps attract few beetles (Byers et al. 1988). A blend of components enhances beetle response and the addition of host monoterpenes to pheromone blends attracts the most beetles (Byers et al. 1988).

Bark beetles are also sensitive to VOCs produced by non-host trees and use this information to avoid colonisation of an unsuitable host. Non-host VOCs disrupt attractiveness of host VOCs and aggregation pheromones. Orientation of *Dendroctonus pseudotsugae* (Huber and Borden 2001), *D. ponderosae* (Borden et al. 1998), and *Ips sexdentatus* (Jactel et al. 2001) toward aggregation pheromone signals can be disrupted through the addition of non-host angiosperm VOCs. Non-host VOCs can disrupt orientation to pheromone signals as effectively as anti-aggregation pheromones, which are signals that end the host colonisation process (Huber and Borden 2001; 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). The effects of different non-host VOCs on bark beetles can be redundant. 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).

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 dead natal host and fly in search of new reproductive hosts (Safranyik and Carroll 2006). The host finding behaviour 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 selection. Host VOCs and non-host VOCs likely play a role in orienting pioneer beetles to 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 dictate the choice of a suitable host (Campbell and Borden 2006a).

In the most recent population outbreak, *D. ponderosae* has expanded its native range and crossed the Rocky Mountains, establishing in North-Central Alberta (Safranyik et al. 2010). Movement across the Rocky Mountains has resulted in colonisation of trees in a hybrid zone between the historic host (*Pinus contorta*) and the novel host (*Pinus banksiana*), which has acted as a stepping stone for the beetle to continue range expansion eastwards (Lusebrink et al. 2013). With the help of this hybrid zone, *D. ponderosae* has encountered, successfully attacked and reproduced within *P. banksiana* hosts (Cullingham et al. 2011). This range expansion has given *D. ponderosae* a novel habitat to exploit. *Pinus banksiana* comprises much of the boreal forest and can potentially act as a pathway across Canada (Safranyik et al. 2010).

The dispersal behaviour 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 mediate host colonisation (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 and capacity. 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 aggregation pheromone, *trans*-verbenol, on flight propensity and capacity.

Methods

Collection of beetles

In November 2017, beetles were collected from three sites in Hinton, Alberta (53.342167, -117.586800; 53.380417, -117.542683; 53.275450, -117.665267) and two sites in Slave Lake, Alberta (54.862517, -115.162517; 54.897367, -115.145133). In Hinton, five infested trees were felled at each site. In Slave Lake, two infested 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 prevent 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 from cold storage when needed and placed in 121 L emergence bins fitted with a glass jar to collect emerging beetles. Emergence bins were housed at 21°C under a 16:8 hr light:dark cycle. As beetles 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). Beetles were stored at 4°C before use in the bioassay at 2-5 days post emergence from the bolt.

Exposure material

Phloem samples used as exposure material were collected from four trees at a single site for each tree species in July 2017. Phloem samples of lodgepole pine, *P. contorta*, were obtained from a site near Grande Prairie, Alberta (54.464163, -118.635325). Jack pine, *P. banksiana*, phloem samples were collected at a site near Lac La Biche, Alberta (55.157817, -112.019033). Phloem samples from the non-host trembling aspen, *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.

Flight bioassay

Prior to use in the flight bioassay, beetles (2-5 days old) were weighed to the nearest 0.01 mg (Mettler Toledo XPE205 Microbalance, Columbus, Ohio). Beetles were then randomly separated into two groups, flown and control. Flown beetles 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).

Control beetles in perforated 1.5 ml microcentrifuge tubes were placed in the flight mill room. Bioassays were conducted for 23 hr in the flight mill room kept at 23°C with a 16:8 hr light to dark cycle. The flight assay was initiated 4 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 at a ~100° angle. During the photophase, light was provided by high flicker frequency fluorescent bulbs (550 lux).

As beetle flight propelled the flight mill arm in a circular direction 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 occurring for each flight burst throughout the 23 hr bioassay.

After the flight bioassay, beetles and tethers were weighed to the nearest 0.01 mg. Beetles were stored at -20°C until body length and pronotum width measurements were taken. Beetles found dead or detached from tethers during flight were not included in the subsequent statistical analyses.

Experiment 1: Exposure to host VOCs prior to flight

Experiment 1 tested the hypothesis that beetle exposure to host VOCs before flight would influence subsequent flight propensity and capacity. Beetles were exposed to host VOCs for 3 hr at 23°C in complete darkness prior to flight. Beetles were exposed to VOCs through a constant stream of air. The VOCs exposure occurred in an apparatus (Mori, 2014) (Fig. 1) positioned in a fumehood, and connected to the air input. 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 three 250 ml flasks at 500 ml·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 the beetles in perforated 1.5 ml microcentrifuge tubes. Air exiting the apparatus was vented to the fumehood exhaust. After the 3 hr exposure, beetles were weighed and prepared for flight as described above. This experiment was completed on both sexes (Flown treatment beetles, females: *Clean* n=76, *Jack* n=77, *Lodgepole* n=77; males *Clean* n=84, *Jack* n=82, *Lodgepole* n=79).

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

Experiment 2 tested the hypothesis that beetle exposure to the female-produced aggregation pheromone, *trans*-verbenol, before flight would influence subsequent flight capacity. Beetles were exposed to *trans*-verbenol for 5 min at 23°C in complete darkness prior to flight. Exposure to *trans*-verbenol occurred in the apparatus (Fig. 1), as described previously. In this experiment, only two treatment chambers were used to expose beetles to either *trans*-verbenol or clean air. The *trans*-verbenol source was a single Mountain Pine Beetle Tree Bait (Phero Tech Inc., 300000228, Lot #13014) without *exo*-brevicomin that released trans-verbenol at ~1 mg·day. Post-exposure, beetles were weighed and prepared for flight as described above. This experiment was completed for females only (Flown treatment beetles: n=118 for both clean & *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. Beetles were weighed and prepared for flight as described above, without any pre-exposure period. In the flight mill room, shelves containing flight mills were separated into four sections of equal volume, 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). Beetles 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 beetles were positioned on the mills, the fourth side was enclosed with oven bags and aluminum foil tape. This experiment was completed on female (Flown treatment beetles: n=86 for each treatment) and a small sample of male (Flown treatment beetles: *Aspen* n=46; *Clean* n=44; *Jack* n=42; *Lodgepole* n=46) beetles. The male data is not presented.

Treatment position was randomized between days of flight. Oven bags were removed from shelves and disposed. Shelves housing the 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 contamination.

Chemical analysis of exposure materials

Materials that beetles 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 for aeration of materials. Two holes were cut into the lid of the glass jar, 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). When aerations were conducted, a single phloem disc or a single *trans*-verbenol bubble pack was placed into the glass jar. The lid was sealed against the glass jar using PTFE Teflon tape with parafilm overtop. The laboratory bench vacuum was set to pull air at 100 $\mathrm{ml{\cdot}min^{-1}}$ for 5 min for the *trans*-verbenol bubble packs and 3 hr for the phloem discs.

Each Porapak Q tube from each aeration sample 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) with 5 µl of heptyl acetate to act 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. 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 *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·min^{-1}. Two µl samples of each extract 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·min^{-1}, increased to 200°C by 10°C·min^{-1}, and then increased to 250°C by 25°C·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·min^{-1}. Two µl samples of each extract 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·min^{-1} and held for 2 min, increased to 130°C by 2°C·min^{-1}, and then increased to 250°C by 25°C·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

All data analyses were performed in R version 3.4.1 (R Core Team, 2018). For all experiments, data were tested for normality and heteroscedasticity using visual techniques and the Shapiro-Wilks test. Response variables tested in linear mixed effects models were flight distance, duration, and velocity. Distance flown and duration of flight were cube-root transformed in Experiments 1 and 3. Distance flown and duration of flight were transformed to the fourth root in Experiment 2. 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 flight mill were used as random factors in all models (Tables 1-4).

P-values above $\alpha = 0.05$ but below 0.07 are considered marginally significant (Burnham & Anderson 2014) due to the high variation in *D. ponderosae* flight, as well as the high variation in VOCs released by the phloem and pheromone sources.

Results

Experiment 1: Exposure to host VOCs prior to flight

Of the 230 female beetles placed on flight mills, 164 beetles initiated flight. Exposure to host VOCs did not influence flight propensity of female beetles (X^2=0.009, p=0.9957). An interaction between pre-flight weight and pre-flight exposure treatment affected female flight distance (Table 1). There was a positive relationship between pre-flight weight and distance flown in beetles exposed to clean air. There was no relationship between pre-flight weight and distance flown in females exposed to host VOCs, from either *P. banksiana* or *P. contorta* phloem (Figure 2). The same interaction dictated flight duration. Pre-flight weight of females positively influenced flight velocity. The other two measures of body size, pronotum width and body length, did not significantly affect flight distance, duration or velocity.

Of the 248 male beetles placed on flight mills, 166 beetles initiated flight. Flight propensity of male beetles did not differ between exposure treatments (X^2=0.2916, p=0.8643). All three body size measurements significantly influence male flight distance (Table 2, Figure 3). Larger beetles flew farther distances, for longer durations, and faster velocities than smaller beetles.

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

Of the 236 female beetles placed on flight mills, 172 beetles initiated flight. Female beetles show no difference in flight propensity as a result of exposure to *trans*-verbenol before flight (X^2=0.343, p=0.558). Pre-flight weight positively affected flight distance, however, there was no relationship between flight distance and pre-flight exposure treatment in this model (Table 3). There was no relationship between flight distance and pronotum width or body length, however, pre-flight exposure treatment significantly affected flight distance and duration in these models. Beetles exposed to *trans*-verbenol flew further distances for longer durations than beetles exposed to clean air (Figure 4). Body size measurements and pre-flight exposure treatment affected flight velocity. Heavy or large beetles flew faster than light or small beetles. Beetles exposed to *trans*-verbenol flew faster than those exposed to clean air (Figure 5).

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

Of the 345 female beetles placed on flight mills, 252 beetles initiated flight. Exposure to VOCs during flight did not influence the flight propensity of female beetles (X^2=0.009, p=0.9998). Flight distance was affected by pre-flight weight, pronotum width, body length and exposure treatment during flight (Table 4). Beetles exposed to clean air during flight flew further than those exposed to volatiles released from *P. tremuloides* phloem (Figure 6). Fight duration was not affected by any of the beetle body size measures. Flight duration was impacted by exposure treatment during flight in the model that included pre-flight weight as an independent variable. A post-hoc test showed that beetles exposed to clean air during flight flew longer than beetles exposed to *P. tremuloides* during flight. Flight velocity was affected by pre-flight weight, pronotum width and body length, but not exposure treatment.

Chemical analysis of exposure material

Quantities of antennally active volatile organic compounds released from *P. banksiana* and *P. contorta* phloem discs were variable (Table 5). *Populous tremuloides* produced an average of 0.51 ± 0.11 $\mu g \cdot ml^{-1}$ of 1-hexanol and 1.55 ± 0.30 $\mu g \cdot ml^{-1}$ of nonanol. Benzyl alcohol was not produced in detectable levels from *P. tremuloides* phloem. The *trans*-verbenol bubble pack used in Experiment 2 released 12.19 $\mu g \cdot ml^{-1}$ 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 a strong positive orientation response 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, these host VOCs also influence beetle flight distance and duration. Pre-flight weight positively affects flight distance of beetles exposed to clean air before flight. 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 beetles are exposed to both *P. banksiana* and *P. contorta* VOCs prior to flight. Large beetles may undergo shorter flights in the presence of potential hosts. Exposure to host VOCs could prime female beetles for host colonisation instead of flight behaviours.

Undergoing short dispersal flights is a safe strategy, as female beetles arrive at the host with high lipid stores (Chubaty et al. 2014) or expend less energy during flight (Jones et al. 2019), and are more likely to successfully colonise the host (Latty and Reid 2009, 2010).

Pre-exposure to host VOCs does not influence male *D. ponderosae* flight capacity. Male beetles exposed to clean air, *P. banksiana* or *P. contorta* VOCs prior to flight had equal flight capacity. 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 colonisation process. As some female beetles 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 beetles do not initiate colonisation but join a mass attack in progress and likely rely more heavily on aggregation pheromones than host VOCs for orientation. Differential response to VOCs by male and female beetles also occurs in *Monarthrum scutellare*. Traps baited with host VOCs, capture more male *M. scutellare* suggesting that males, the pioneering sex, are more receptive to host VOCs than females (Noseworthy et al. 2012).

Interestingly, exposure to host VOCs during flight did not have the same influence on *D. ponderosae* flight behaviour as the pre-exposure experiment. Behavioural responses to specific olfactory signals are highly dependent on external and internal factors (Deisig et al. 2014). Response to olfactory signals may be dependent on other cues such as gustatory, visual or auditory. Thus, the differences in behavioural response in the current study could be linked to the differences in visual cues between the two experiments. The pre-exposure treatment was completed in complete darkness, while the exposure during flight treatments were completed in 16:8 L:D cycle. *Dendroctonus ponderosae* may require both visual and olfactory cues to respond to hosts while flying. Dispersing *D. ponderosae* are less likely to attack chemically attractive traps that visually resemble non-hosts compared to those that resemble hosts (Campbell and Borden 2006b). Several other species respond similarly including *Dendroctonus autographus, D. pseudotsugae* and *Dryocoetes confusus,* but response of *D. ponderosae* is the most consistent (Campbell and Borden 2006b). Similar results of avoidance of chemically attractive traps that visually resemble non-hosts occurs in *Ips typographus, Dendroctonus frontalis* and *Trypodendron lineatum* (Strom et al. 1999). The absence of visual stimuli representing hosts while in the presence of attractive olfactory cues in the exposure during flight may send a conflicting signal to the central nervous system, resulting in no behaviour change. When an olfactory stimulus occurs in constant concentration beetles may rely more heavily on visual stimulus (Campbell and Borden 2006a). The pre-exposure experiment does not deliver a constant olfactory stimulus to the beetles and all visual stimuli are removed during this exposure period. Comparatively, the exposure during flight experiment delivers a constant olfactory stimulus with visual cues that do not resemble hosts. These differences in visual stimuli may explain the conflicting results between the pre-exposure and exposure during flight experiments.

Alternatively, the conflicting results between the pre-exposure and exposure during flight experiments can be explained by habituation and sensory adaptation, respectively. Habituation occurs when response to an odorant declines with continuous exposure, due to interference at the central nervous system (Twick et al. 2014). In the pre-exposure to host VOCs beetles are exposed over a 3 hr period and then removed from the stimulus. It is possible that this prolonged stimulus exposure at a relatively constant concentration could result in habituation. Interference at the central nervous system causes a decrease in response behaviour to the stimulus in habituated individuals (Vinaguer et al. 2013) which could explain the flight pattern exhibited by female beetles exposed to host VOCs. It cannot be known for certain if female *D. ponderosae* in the current experiment are habituated to host VOCs as beetles were not presented with the stimulus after initial exposure. In contrast to the pre-exposure experiment, *D. ponderosae* show no change in flight capacity when exposed to host VOCs during flight. Sensory adaptation occurrs in the peripheral nervous system, and is characterized by antennae adaption to odours under constant exposure, which results in no signal sent to the central nervous system (Csiro 1982). Adaptation of the antennal receptors to host VOCs can explain the lack of response in the exposure during flight experiment, as adapted antennal receptors would fail to send a signal to the central nervous system.

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 hr flight bioassay compared to 6.80 ± 0.69 km in clean air. Non-host VOCs elicit antennal activity (Huber et al. 2000) and behavioural responses from bark beetles (Borden et al. 1998; Huber and Borden 2001a, 2001b). Exposure to non-host VOCs 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). The current study is the first to show that non-host VOCs also negatively influence flight capacity of *D. ponderosae*.

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 colonisation 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). However, male *D. pseudotsugae* and *D. ponderosae* respond negatively only to the visual non-host cue, and addition of non-host VOCs in the trap does not 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 (Zang and Schlyter 2003). The effect of non-host VOCs or visual cues likely impacts the pioneering sex to a greater extent, as colonisation costs are higher for pioneers (Zhang and Schlyter 2003).

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 beetles. Female beetles exposed to *trans*-verbenol prior to flight fly an average of 7.32 ± 0.72 km compared to female beetles exposed to clean air 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 colonisation process (Miller et al. 2005). Mass attack occurs on trees baited with *trans*-verbenol alone, while no other *D. ponderosae* pheromone can elicit high attack densities (Borden et al. 1990). Further, the olfactory signal *trans*-verbenol elicits may dominate other sensory modes in the host selection process. *Dendroctonus ponderosae* will attack trees with a small diameter, which would otherwise indicate a poor quality host, when baited with *trans*-verbenol (Rasmussen 1972). The effect of exposure to *trans*-verbenol but not host VOCs on flight velocity is interesting, as previous work shows consistent flight velocity of *D. ponderosae* regardless of beetle age, sex, or size (Evenden et al. 2014). Further, abiotic factors like light and temperature do not alter flight velocity of *D. ponderosae* (Wijerathna 2016). The influence of *trans*-verbenol on subsequent flight distance and velocity could be due to the importance of this semiochemical in host colonisation.

Processing of pheromone and host volatiles occurs in separate neurological pathways, the accessory and the main pathway, respectively (Galizia and Rössler 2010). Aggregation pheromones are generally more attractive to beetles than host VOCs, as these pheromones indicate the presence of a host and conspecifics (Lee et al. 2010). 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). Odorant binding proteins transport odour molecules to olfactory receptor neurons, which are tuned to specific ligands for semiochemical detection (Gadenne et al. 2016). The binding of the ligand to the olfactory receptor neuron initiates an action potential which is transmitted to the corresponding glomerulus in the antennal lobe (Deisig et al. 2014). In *Heliothis virescens* (Lepidoptera: Noctuidae), the glomeruli associated with the accessory pathway are situated in the antennal lobe closest to the antennal nerve (Hillier and Vickers 2007). It is hypothesized that this spatial organization can reflect the way semiochemical cues are processed and transmitted into behaviour (Hillier and Vickers 2007). The glomeruli associated with odour cues that require rapid processing, like aggregation pheromones, may be situated close to the antennal nerve to allow for rapid relay of information to projection neurons. If the central nervous system is similarly arranged in bark beetles, this spatial organization could explain the different flight response as a result of exposure to attractive host VOCs and pheromones in the current study. Increased flight distance and velocity could be a behavioural indication of a stronger response to the pheromone exposure compared to the host VOCs.

Here we provide evidence that 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 beetles. Exposure to host VOCs, however, does not influence male *D. ponderosae* flight capacity. This differential response between the sexes could be due to the difference in semiochemical cues used by male beetles during host colonisation. Exposure to the volatiles of *Populus tremuloides* phloem during flight decreases the distance flown by female beetles, 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 to flight dispersal of *D. ponderosae* will add to our understanding of beetle movement in the environment.

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Figures & Tables

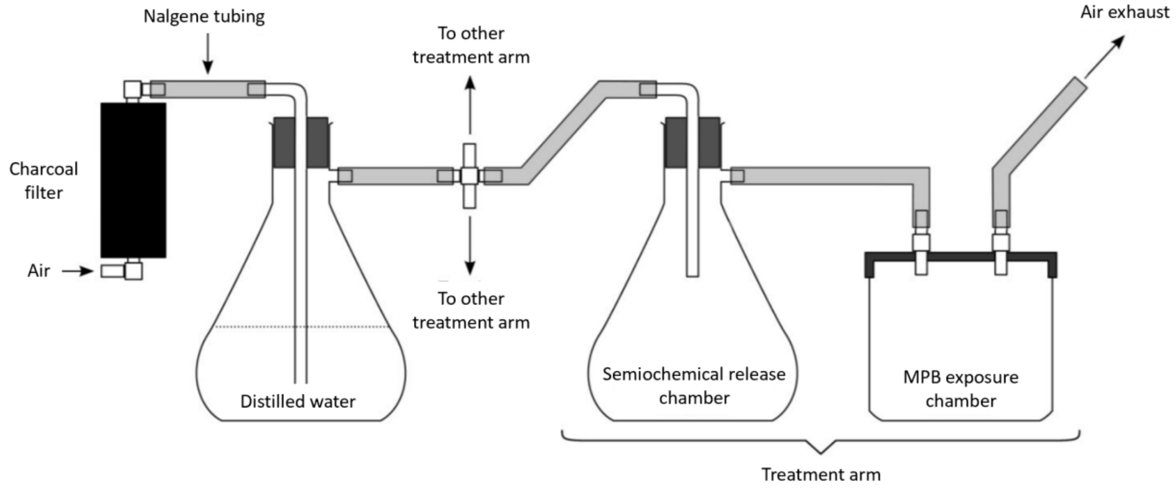

Fig 1 Apparatus and diagram modified from Mori (2014). The apparatus sits in a fumehood, connected to the air input, air is pushed through a charcoal filter, and humidified in a 250 ml Erlenmeyer flask filled with 125 ml of distilled water. From the humidifying flask, air is channeled into three directions, 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 fumehood exhaust vent.

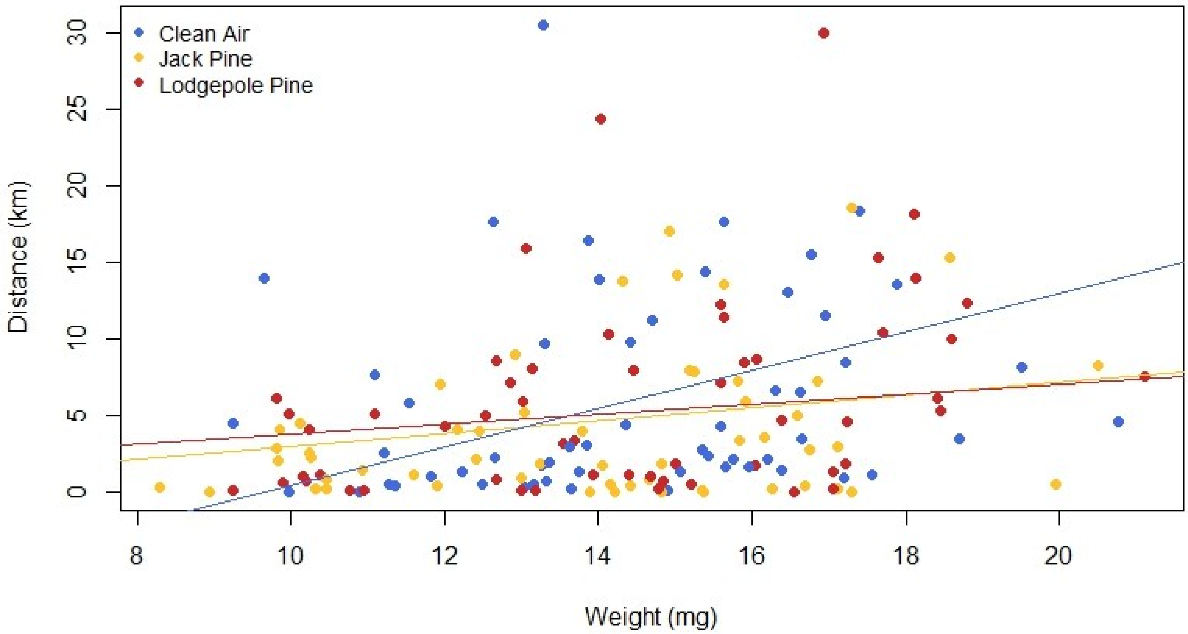

Fig 2 Interaction of exposure prior to flight and pre-flight weight for female beetles (X^2=9.5565, p=0.0588). Flight distance for beetles exposed to *Pinus banksiana* (jack pine) or *Pinus contorta* (lodgepole pine) prior to flight did not vary with pre-flight weight. Flight distance for beetles exposed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

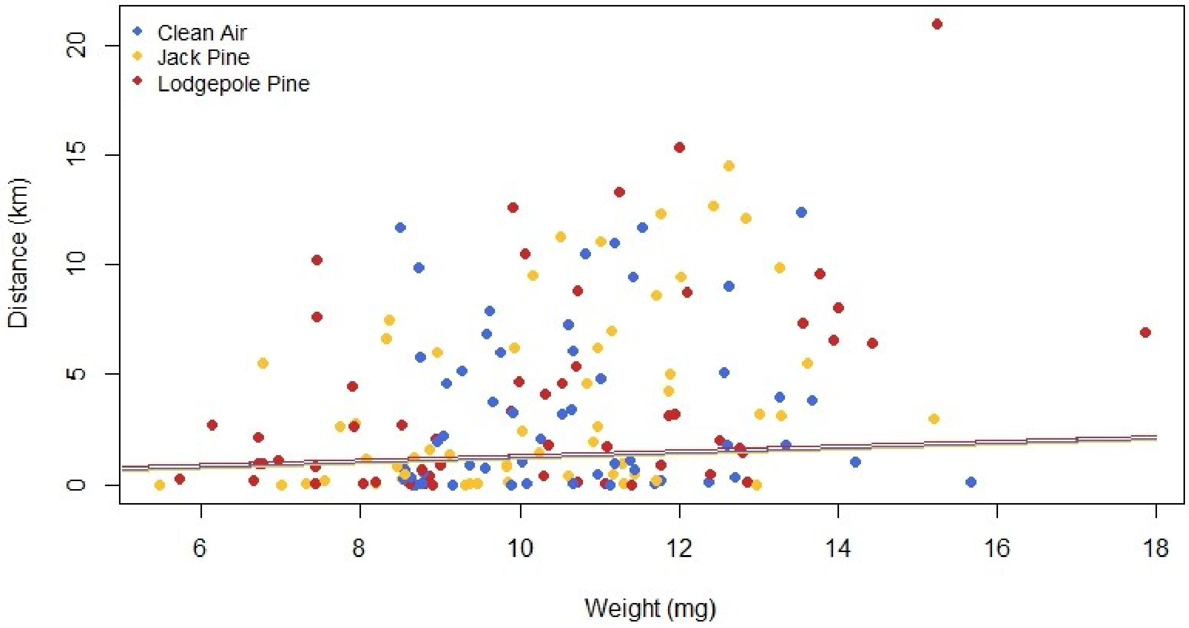

Fig 3 Flight distances (km) of male beetles exposed to clean air, *Pinus banksiana* (jack pine) or *Pinus contorta* (lodgepole pine) prior to flight. Flight distance increased with increasing pre-flight weight (X^2=14.8453, p=0.0001) for all exposure treatments. No difference in flight distance between exposure treatments occurred (X^2=0.5609, p=0.7555).

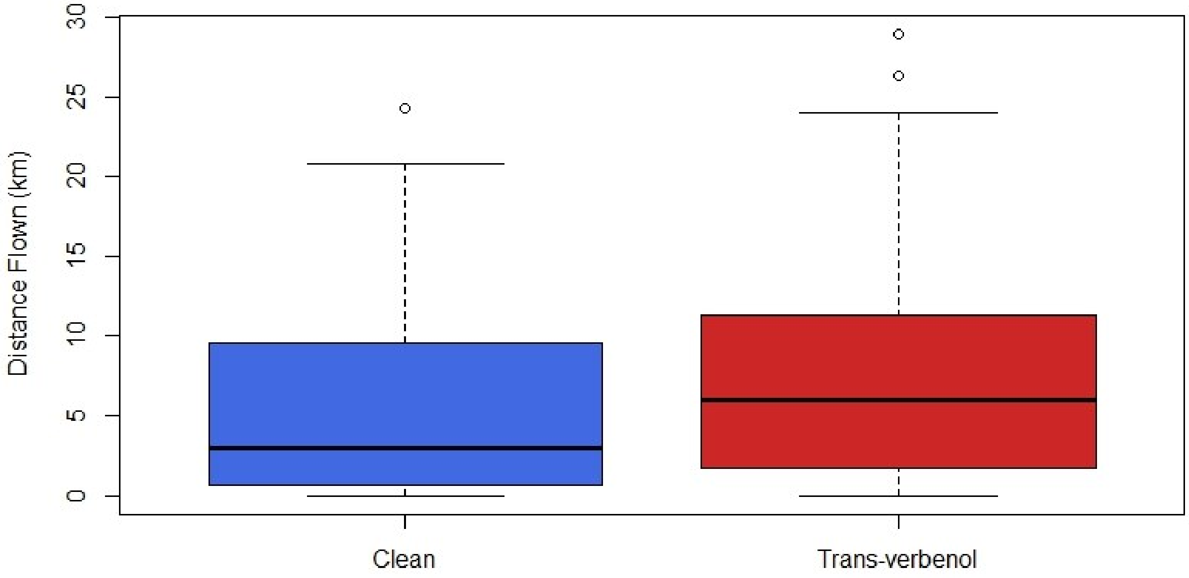

Fig 4 Box plots of flight distance (km) of female beetles exposed to clean air or *trans*-verbenol prior to flight. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values and circles represent outliers. Beetles exposed to *trans*-verbenol prior to flight flew further distances than those exposed to clean air (X^2=5.6578, p=0.0173).

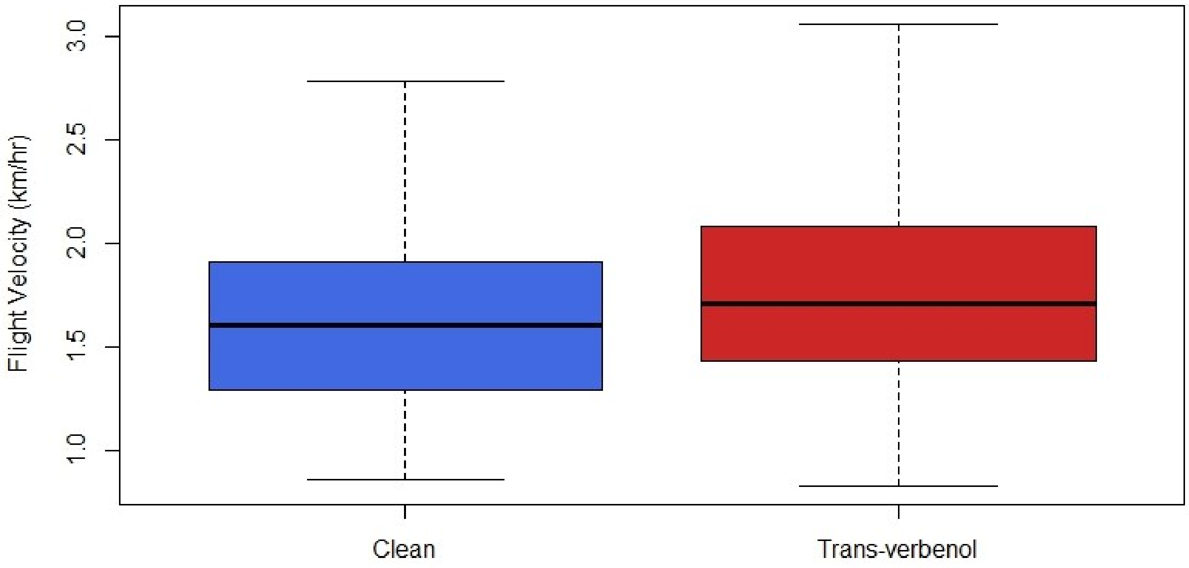

Fig 5 Box plots of flight velocity (km·hr^{-1}) of female beetles exposed to clean air or *trans*-verbenol prior to flight. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. Beetles exposed to *trans*-verbenol prior to flight flew at higher velocities than those exposed to clean air (X^2=4.0895, p=0.0431).

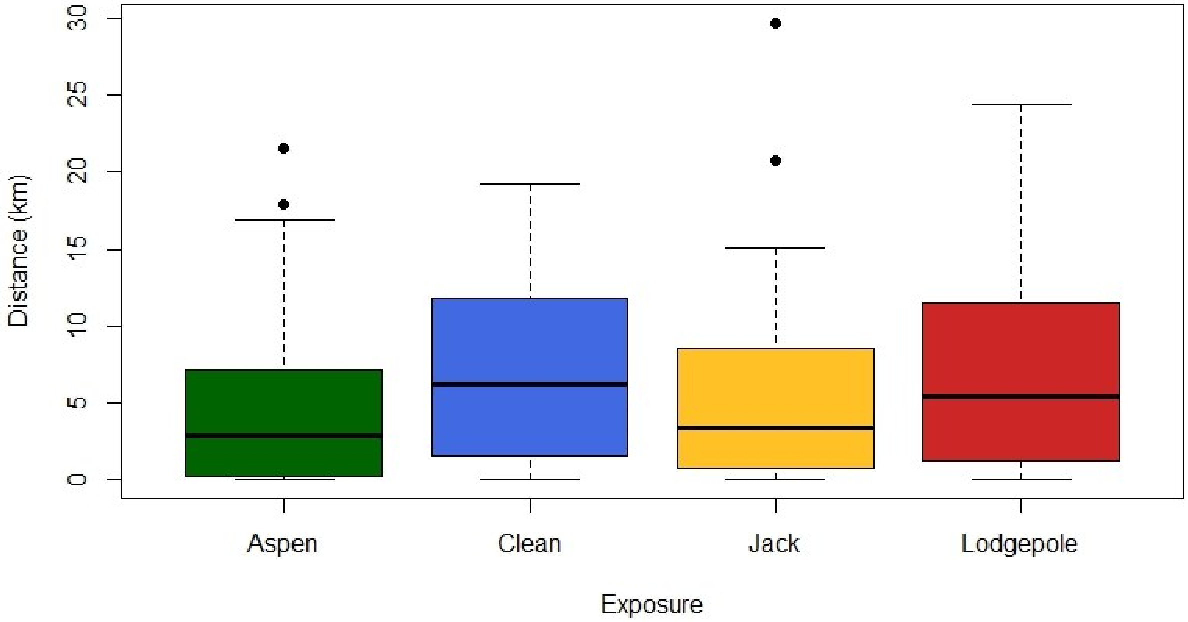

Fig 6 Box plots of flight distance (km) of female beetles exposed to *Populous tremuloides* (trembling aspen), clean air, *Pinus banksiana* (jack pine) or *Pinus contorta* (lodgepole pine) during flight. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles,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

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: X^2=16.6214, p=4.563x10^{-5}
		EXP: X^2=0.5831, p=0.7471
		PFW*EXP: X^2=9.5565, p=0.0588
	lmer($\sqrt[3]{Distance}$ ~ EXP + PW, random = bolt + mill)	PW: X^2=2.7016, p=0.1002
		EXP: X^2=1.2798, p=0.5273
	lmer($\sqrt[3]{Distance}$ ~ EXP + BL, random = bolt + mill)	BL: X^2=2.7074, p=0.0999
		EXP: X^2=1.4138, p=0.4932
Duration	lmer($\sqrt[3]{Duration}$ ~ EXP * PFW, random = bolt + mill)	PFW: X^2=16.6214, p=0.0588
		EXP: X^2=0.5831, p=0.7471
		PFW*EXP: X^2=5.6658, p=0.0588
	lmer($\sqrt[3]{Duration}$ ~ EXP + PW, random = bolt + mill)	PW: X^2=2.7016, p=0.1002
		EXP: X^2=1.2798, p=0.5273
	lmer($\sqrt[3]{Duration}$ ~ EXP + BL, random = bolt + mill)	BL: X^2=2.7074, p=0.0999
		EXP: X^2=1.4138, p=0.4932
Velocity	lmer(velocity ~ EXP + PFW, random = bolt + mill)	PFW: X^2=6.8077, p=0.0091
		EXP: X^2=1.2421, p=0.5374
	lmer(velocity ~ EXP + PW, random = bolt + mill)	PW: X^2=3.0074, p=0.0829
		EXP: X^2=4.3460, p=0.1138
	lmer(velocity ~ EXP + BL, random = bolt + mill)	BL: X^2=3.1235, p=0.0772
		EXP: X^2=4.5043, p=0.1052

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: X^2=14.8453, p=0.0001
		EXP: X^2=0.5609, p=0.7555
	lmer($\sqrt[3]{Distance}$ ~ EXP + PW, random = bolt + mill)	PW: X^2=7.4333, p=0.0064
		EXP: X^2=0.2455, p=0.8845
	lmer($\sqrt[3]{Distance}$ ~ EXP + BL, random = bolt + mill)	BL: X^2=7.9440, p=0.0048
		EXP: X^2=0.1705, p=0.9183
Duration	lmer($\sqrt[3]{Duration}$ ~ EXP + PFW, random = bolt + mill)	PFW: X^2=10.783, p=0.0010
		EXP: X^2=0.495, p=0.7808
	lmer($\sqrt[3]{Duration}$ ~ EXP + PW, random = bolt + mill)	PW: X^2=4.3133, p=0.0378
		EXP: X^2=0.1623, p=0.92205
	lmer($\sqrt[3]{Duration}$ ~ EXP + BL, random = bolt + mill)	BL: X^2=4.5148, p=0.0336
		EXP: X^2=0.1138, p=0.9447
Velocity	lmer($\sqrt[4]{velocity}$ ~ EXP + PFW, random = bolt + mill)	PFW: X^2=4.6565, p=0.0309
		EXP: X^2=0.1223, p=0.9407
	lmer($\sqrt[4]{velocity}$ ~ EXP + PW, random = bolt + mill)	PW: X^2=6.4124, p=0.0113
		EXP: X^2=0.0945, p=0.9539
	lmer($\sqrt[4]{velocity}$ ~ EXP + BL, random = bolt + mill)	BL: X^2=7.3081, p=0.0069
		EXP: X^2=0.0709, p=0.9652

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: X^2=10.6607, p=0.0011 EXP: X^2=2.5565, p=0.0865
	lmer($\sqrt[4]{Distance}$ ~ EXP + PW, random = bolt + mill)	PW: X^2=1.6040, p=0.2053 EXP: X^2=5.6578, p=0.0173
	lmer($\sqrt[4]{Distance}$ ~ EXP + BL, random = bolt + mill)	BL: X^2=1.2373, p=0.2660 EXP: X^2=5.8636, p=0.0553
Duration	lmer($\sqrt[4]{Duration}$ ~ EXP + PFW, random = bolt + mill)	PFW: X^2=6.3259, p=0.0119 EXP: X^2=2.0225, p=0.1550
	lmer($\sqrt[4]{Duration}$ ~ EXP + PW, random = bolt + mill)	PW: X^2=0.5730, p=0.4491 EXP: X^2=4.4669, p=0.0346
	lmer($\sqrt[4]{Duration}$ ~ EXP + BL, random = bolt + mill)	BL: X^2=0.2638, p=0.6075 EXP: X^2=4.6045, p=0.020
Velocity	lmer(velocity ~ EXP + PFW, random = bolt + mill)	PFW: X^2=15.5429, p=8.066x10^{-5} EXP: X^2=3.6461, p=0.0562
	lmer(velocity ~ EXP + PW, random = bolt + mill)	PW: X^2=7.4452, p=0.0006 EXP: X^2=4.0895, p=0.0431
	lmer(velocity ~ EXP + BL, random = bolt + mill)	BL: X^2=10.9373, p=0.0009 EXP: X^2=4.3053, p=0.0380

Table 4 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: $X^2=31.0295$, $p=2.541\text{x}10^{-8}$	A-C: t=2.989, p=0.0162
		EXP: $X^2=9.5565$, p=0.0227	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: $X^2=10.3523$, p=0.0001	A-C: t=2.686, p=0.0385
		EXP: $X^2=9.2082$, p=0.0266	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: $X^2=7.8949$, p=0.0050	A-C: t=2.596, p=0.0489
		EXP: $X^2=8.7058$, 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: $X^2=19.7717$, $p=8.726\text{x}10^{-6}$	A-C: t=2.560, p=0.0536

		EXP: X^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: X^2=6.3148, p=0.0120	
		EXP: X^2=6.9588, p=0.0732	
	lmer($\sqrt[3]{Duration}$ ~ EXP + BL, random = bolt + mill)	BL: X^2=5.3745, p=0.0204	
		EXP: X^2=6.6533, p=0.0838	
Velocity	lmer(velocity ~ EXP + PFW, random = bolt + mill)	PFW: X^2=24.3269, $p=8.129\text{x}10^{-7}$	
		EXP: X^2=0.7734, p=0.8558	
	lmer(velocity ~ EXP + PW, random = bolt + mill)	PW: X^2=8.3488, p=0.0039	
		EXP: X^2=0.4200, p=0.9361	
	lmer(velocity ~ EXP + BL, random = bolt + mill)	BL: X^2=5.2436, p=0.0220	
		EXP: X^2=0.2817, p=0.9643	

Table 5 Volatile organic compounds collected from *Pinus banksiana* and *Pinus contorta* over a 3 hr aeration period. A single phloem sample from each of the four trees was aerated, averages ($\mu g \cdot ml^{-1}$) with standard error reported.

	3-Carene	α-pinene	β-phellandrene	Limonene	Myrcene
P. banksiana	6.18 ± 4.94	16.06 ± 11.75	11.42 ± 7.57	0.87 ± 0.68	0.59 ± 0.37
P. contorta	11.55 ± 9.14	8.46 ± 3.01	67.18 ± 45.65	1.16 ± 0.71	2.68 ± 1.57

Chapter 3: Mechanisms of flight polyphenisms in an outbreaking bark beetle species

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ABSTRACT

1. Flight polyphenisms can exist as discrete or continuous traits. Discrete flight polyphenisms include winged and wingless morphs, whereas continuous flight polyphenisms can take the form of short and long distance fliers. Different morphs that result from discrete flight polyphenisms have varied energy budgets that affect adult life history strategies. However, the mechanisms behind and consequences of continuous insect flight polyphenisms are not completely understood. In insects that go through population outbreaks, understanding these mechanisms may give insight into potential range expansion.

2. The mountain pine beetle (*Dendroctonus ponderosae*) exhibits polyphenic variation in flight distance. This study assessed the effect of flight on subsequent life history strategies revealing potential mechanisms of flight variation in the mountain pine beetle. We tested two hypotheses: (1) that there is an energetic trade-off between flight distance and host tree colonisation capacity; and (2) flight affects subsequent pheromone production by beetles.

3. A 23-h flight treatment was applied to a subset of beetles using computer-linked flight mills. After flight treatment, both flown and unflown control beetles were given the opportunity to colonise bolts of host trees. In addition, to determine if flight influences subsequent pheromone production, beetles from both treatment groups that successfully entered hosts were aerated to quantify pheromones produced by individual beetles.

4. A trade-off occurred between initiation of host colonisation and percent body weight lost during flight. Beetles that lost more weight were less likely to enter a host. Of the beetles that entered, however, those that lost the most weight were the first to enter. Production of the

aggregation pheromone *trans*-verbenol by female beetles was influenced by both percent weight lost during flight and flight distance. While male production of *exo*-brevicomin was affected by beetle condition following flight but not by the energy used during flight.

5. These novel results give insight into the polyphenic flight behaviour of mountain pine beetles. Flight variation is adaptive to ensure aggregation of host trees in this outbreaking species. These findings suggest mechanisms that facilitate the extremities of the continuous flight polyphenism spectrum. Mountain pine beetles that fly short distances are constrained by energy condition. Female beetles that fly long distances produce more aggregation pheromone to attract conspecifics and increase colonization success. These opposing mechanisms maintain the high variation in flight exhibited by this outbreaking species.

1 | INTRODUCTION

Polyphenisms are single genotypes that produce two or more distinct phenotypes in response to environmental conditions. The link between phenotypes and environmental factors promotes individual success under changing environmental conditions (Simpson, Sword & Lo, 2011). The most notable flight polyphenism in insects is the occurrence of winged and flightless morphs within the same species. Although many polyphenisms are discrete, continuous flight polyphenisms also exist (Karlsson & Johansson, 2008; Simpson, Sword & Lo, 2011), and in insects can exist as short *vs*. long distance fliers. Flight is costly, and trade-offs between resource allocation to flight and other life history traits (Karlsson & Johansson, 2008), such as host colonisation (Latty & Reid, 2009; Latty & Reid, 2010) and reproduction (Roff & Fairbairn, 1991) are common. Many studies have focused on understanding the effects of discrete flight polyphenisms on subsequent adult life history strategies (Cisper, Zera & Borst, 2000), but the effects of continuous flight polyphenisms remain less well studied.

In aggressive tree-killing bark beetles (Coleoptera: Curculionidae, Scolytinae), adult beetles must disperse by flight for host colonisation and reproduction (Wood, 1982; Raffa et al., 2005). After flight, the pioneering sex initiates colonisation of a living host through signalling to conspecific male and female beetles with an aggregation pheromone to generate mass attack of the tree (Raffa et al. 2008; Raffa, Grégorie & Lindgren, 2015). Successful host entry that overcomes the host tree defenses is followed by oviposition gallery construction and reproduction (Raffa, Grégorie & Lindgren, 2015). Conifers possess constitutive and induced defenses for both physical and chemical protection from bark beetle attack (Raffa & Berryman, 1982, 1983; Raffa et al., 2005). Sticky resins, mainly composed of toxic terpenes, expel beetles from the tree if too few beetles respond to the pheromone signal (Raffa & Berryman, 1982; Erbilgin 2019). Terpenes at high doses kill both the beetles and their mutualistic fungi that help mediate attack (Raffa & Berryman, 1983; Raffa et al., 2005, 2008; Chiu, Keeling & Bohlman, 2017; Reid, Sekhon & LaFramboise, 2017). A rapid release of terpenes at high concentrations can prevent host colonisation when beetles are present in low numbers (Erbilgin 2019).

Successful attack of a host tree requires the production of aggregation pheromones to attract conspecifics for mass attack (Kane & Kolb, 2010; Boone et al., 2011). The pioneering beetle releases aggregation pheromone that attracts both sexes (Gitau et al., 2013; Raffa et al., 2015), and mediates the mass attack. Beetles of the same sex as the pioneer initiate new attacks along the tree bole, while beetles of the opposite sex enter existing galleries to mate (Gitau et al., 2013). Production of aggregation pheromone has fitness consequences (Raffa, 2001; Pureswaran, Sullivan & Ayres, 2008). If production is low, not enough beetles will aggregate to the focal tree to initiate a mass attack, and pioneering beetles may succumb to host defenses. Pheromone signals for each species is unique in concentration and ratio of chemical components (Blomquist et al., 2010; Tittiger & Blomquist, 2017). These unique signals lead to species specificity in chemical communication (Symonds & Elgar, 2008). Volatile compounds released by the host tree can also synergize the attractiveness of the aggregation pheromone (Seybold et al., 2006; Borden, Pureswaran & Lafontaine, 2008). Bark beetles synthesize pheromone components *de novo* or through the activity of microbial symbionts (Hunt & Borden, 1989; Cale et al. 2019), but also require monoterpene precursors from the host tree for pheromone synthesis (Blomquist et al., 2010).

The host colonisation process is costly and depends on the physiological condition of the adult bark beetles arriving at the host after dispersal (Raffa et al., 2008; Reid, Sekhon & LaFramboise, 2017). Several theories have been put forth to explain the relationships between dispersal behaviour, host choice, and colonisation in bark beetles (Latty & Reid, 2010). The “desperation” hypothesis states that beetles with low energy reserves enter a tree independent of host quality decisions because low energy reserves prohibit further flight (Byers, 1999; Latty &

Reid, 2009). The “safe site” hypothesis posits that beetles enter high quality hosts to promote mate attraction and successful attack (Latty & Reid, 2010). The “condition matching” hypothesis suggests that host colonisation by the beetle should interact with the quality of the host tree; as a result, beetles in good energetic condition can enter well-defended trees (Latty & Reid, 2010; Chubaty, Hart & Rotiberg, 2014).

The mountain pine beetle, (*Dendroctonus ponderosae*), is native to Western North America, and has expanded its range eastward and northward (Cudmore et al., 2010; Cullingham et al., 2011; Raffa et al., 2017) following the most recent population outbreak that started in the early 2000s (Safranyik et al. 2010). The beetle can kill millions of trees while in the epidemic population stage (Safranyik et al. 2010). Dispersal by flight dictates the spread of this species and it is arguably the least understood aspect of mountain pine beetle ecology (Chen & Walton, 2011).

After emerging from the natal host, mountain pine beetle exhibits two patterns of dispersal within the stand – spot growth and spot proliferation (Robertson, Nelson & Boots, 2007). Spot growth involves short distance movements from the natal host to a reproductive host located only a few metres away. Spot proliferation results from beetle flight past suitable hosts followed by host selection much further away from the natal host. Understanding the mechanism underlying this flight polyphenism in the mountain pine beetle and the cascading effects of flight polyphenisms on host selection and colonisation are essential for understanding spatial dynamics of the beetle (Robertson, Nelson & Boots, 2007). Although some variation in flight distance is explained by lipid content (Evenden, Whitehouse & Sykes, 2014), energy reserves alone do not account for the large degree of flight variation exhibited by the mountain pine beetle. A potential explanation behind the varied flight behaviour is that mountain pine beetle may require a flight period before becoming responsive to semiochemicals (Shepherd, 1966; Gray et al., 1972; Safranyik et al., 1992), similar to other bark beetle species. For example, Douglas-fir beetles (*Dendroctonus pseudotsugae*) respond to semiochemicals based on physiological state, and low lipid content following flight promotes host colonisation (Atkins, 1966; Thompson & Bennett, 1971). Beetles with high lipid levels need to expend energy before settling on a host, which could explain flight variation over geographic and temporal scales (Robertson, Nelson & Boots, 2007), although empirical evidence for this hypothesis is lacking.

Although beetle body condition (high lipid to body volume ratio) affects host colonisation behaviour in mountain pine beetle (Elkin & Reid, 2005; Latty & Reid, 2010), it is unknown if the same lipid resources consumed during flight (Evenden, Whitehouse & Sykes, 2014) are also allocated to host colonisation behaviour. Although metabolic costs associated with pheromone production may be insignificant (Pureswaran, Sullivan & Ayres, 2006), mountain pine beetle aggregation pheromones are produced and/ or stored in the fat body (Song et al., 2014; Chiu, Keeling & Bohlmann, 2018). It is unknown whether lipid use during flight influences the production of the male-produced aggregation pheromone *exo*-brevicomin, or the storage and use of *exo*-brevicomin and the female-produced aggregation pheromone, *trans*-verbenol. In addition to these aggregation pheromones, beetles also produce frontalin and verbenone to avoid or minimize brood competition. Both anti-aggregation pheromones are produced *de novo* and by microbial symbionts (Ryker & Libbey, 1982; Hunt & Borden, 1989). Mountain pine beetle reproduction is also linked to body condition. Beetles in poor condition produce smaller eggs (Elkin & Reid, 2005), and there is a trade-off between energy use during flight and offspring production (Wijerathna et al., 2019).

It is important to understand the relationship between energy use during the obligatory dispersal phase of mountain pine beetle and subsequent host colonisation process in order to assess range expansion of this important pine pest. In this study, we test the influence of flight polyphenisms on (1) female beetle host acceptance; and (2) male and female production of aggregation pheromones. Based on previous studies that assess energetics in relation to host colonisation, we predict a trade-off between flight and host colonisation. Since the precursor to *trans*-verbenol is stored within the fat body and lipids are the primary fuel for flight, we predict that flight will negatively affect *trans*-verbenol production by female beetles. Additionally, since *exo*-brevicomin is synthesized and stored within the fat body, we predict that flight will also negatively affect *exo*-brevicomin production by male beetles.

2 | MATERIALS & METHODS

2.1 | Collection of beetles

Beetle-infested lodgepole pine, *Pinus contorta* var. *latifolia* Douglas, bolts were collected from three trees at each of three sites in Hinton, Alberta (53° 20.530 117° 35.208, 53° 22.825 117°

32.561 and 53° 16.527 117° 39.916) in June 2018, and from two trees at each of two sites in Slave Lake, Alberta (54° 51.751 115° 09.751 and 54° 53.842 115° 08.708) in November 2017. Two, 50-cm sections of each tree, removed from 1-2 m above the ground were transported to the University of Alberta. Cut ends of the bolts were sealed with paraffin wax (parowax®) to minimize desiccation and bolts were stored at 5°C until July 2018 when bioassays were conducted.

When beetles were needed for bioassays, bolts were removed from cold storage and placed in 121 L emergence bins fitted with a glass jar. Bins were housed at 21°C under a 16:8 hr light:dark cycle. Emerging beetles caught in the glass jars 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). Beetles were stored at 4°C before use in the bioassay at 3-5 days post emergence from the bolt.

2.2 | Flight mills

Beetles (3-5 days old) were weighed to the nearest 0.01 mg (Mettler Toledo XPE205 Microbalance, Columbus, OH, USA). Beetles were assigned randomly to one of two treatments: 23 hr flight period (flown), or 23 hr without the opportunity to fly (control). Beetles in the flown treatment were tethered using a 2 cm long, 30 guage aluminum wire (0.02 mm diam.) with a small loop at the end. The loop was attached to the pronotum of each beetle using Press-Tite Contact Cement (LePage, Mississauga, ON, CAN) so that elytra movement was not restricted. Twenty-two tethered beetles were positioned on flight mills on each of 13 days, and given the opportunity to fly during the 23 hr treatment period. Control beetles were housed with a piece of paper in perforated 1.5 mL microcentrifuge tubes in the flight mill room during the treatment period. The flight mill room was kept at 23°C with a 16:8 hr light to dark cycle. The distal end of each tether was attached to the flight mill arm at a ~100° angle using a small piece of wire insulation. Light (550 lux) was provided by high flicker frequency fluorescent bulbs (Evenden, Whitehouse & Sykes, 2014).

A small magnetic transmitter positioned on the flight mill arm detected the arm rotation propelled by beetle flight. The transmitter directed the signal to the attached computer. LabView software (National Instruments Corporation, Austin, TX, USA) measured each revolution of the flight mill arm (94.4 cm in circumference). Output included the duration and number of revolutions for each flight burst initiated by the beetle. Total flight distance and duration, as well as flight velocity and number of flight bursts were calculated from this output.

After the 23 hr treatment period, the tether was removed from each flown beetle, and both flown and control beetles were weighed to the nearest 0.01 mg. Beetles that died or became detached from tethers during flight treatment were not included in the subsequent bioassays or statistical analyses.

2.3 | Inoculation material

Three uninfested lodgepole pine trees were felled at each of three sites (53° 20.530 117° 35.208, 53° 22.825 117° 32.561 and 53° 16.527 117° 39.916) in Hinton, Alberta. From each tree, three 50-cm bolts were harvested between 1-2.5 m above the ground. Bolts were transported to the University of Alberta, where the cut ends of each bolt were sealed with paraffin wax and stored at 5°C until needed for bioassays.

2.4 | Host colonisation experiment

The first experiment tested the hypothesis that flight treatment influences subsequent host colonisation behaviour by female mountain pine beetle. Host colonisation was measured as capacity to enter lodgepole pine bolts and the time taken for successful host entry. Uninfested bolts were removed from cold storage 24 hr prior to beetle inoculation. Ten clear plastic cups (30 mL) were positioned 10 cm from the bottom of the bolt and secured with flagging tape. A charcoal filter (Paasche Charcoal Filter, WY, USA) skirt was placed between the bolt and the cup to fill any gaps.

Immediately following flight treatment and measurement of post-treatment weight, each female beetle was introduced to an individual cup positioned on a lodgepole pine bolt. Flown and control beetles were placed in alternating order on each bolt. Beetle activity was monitored for 72 hr following the initial placement in the cup or until host entry or death. Boring dust within the cup indicated host entry. Data for beetles that escaped from the cup were removed.

2.5 | Pheromone production experiment

A second experiment tested the hypothesis that flight treatment affects pheromone production by mountain pine beetles following successful host entry. A subset of female beetles, from both treatment groups flown (n=12) and control (n=9), that entered host material within 24 hr of inoculation were used in aeration bioassays to measure semiochemicals released by the beetles.

A single flown (n=11) and control (n=7) male was introduced into galleries of individual females 24 hr after females were introduced to cups. Males were flown the day after females and introduced to the bolts in a different manner. The bark was peeled back slightly around the female entrance hole and boring dust was blown away to reveal the exact point of entrance. Males were gently pushed into the female entrance hole. Once the male was firmly positioned within the entrance hole, the set-up described below was assembled for aeration.

Aerations were conducted using the methods described in Erbilgin et al., (2014). Once female beetles entered the bolt, the clear plastic cup was removed, and replaced with a glass funnel (DWK Life Sciences Kimble K2895045, 45 mm diameter, 50 mm stem). The glass funnel was positioned over a charcoal filter skirt pressed tightly against the bolt and secured with flagging tape. The stem of the glass funnel was connected to a small, 10 cm portion of PTFE tubing (Cole-Parmer, 3/16" x 1/4", RK-06605-32). A second piece of PTFE tubing was attached to PVC tubing (Fisherbrand, 3/16 inner diameter, 1/16 wall) that was subsequently connected to a laboratory bench vacuum. To collect the semiochemicals released by beetles, Porapak Q tubes (6 x 110-mm, 2 sections: 75/150 mg sorbent, 20/40 mesh) were inserted between the two portions of PTFE tubing. Over a 4 hr duration, the vacuum pulled air (100 $mL \cdot min^{-1}$) over the site of beetle entry to trap semiochemicals produced by the beetle pair into the attached Porapak Q tube. After the 4 hr aeration, Porapak Q tubes were removed from the PTFE tubing and were capped, wrapped in tinfoil, and stored at -80°C until extraction. Repeated aerations measured semiochemical production at 12, 24, 36, 48, 72, 96 and 120 hr after introduction of females to cups. Males were introduced 24 hr after females, so the 12 hr time point contained emissins from females only; the subsequent collections were conducted on beetle pairs.

2.6 | Chemical extraction & analyses

Each Porapak Q tube from each aeration sample was scored with a glass cutter to remove the adsorbent beads from the tube 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) with 5 µl of heptyl acetate to act 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 were 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, GER).

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. 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^{o}C until chemical analyses.

Chemical analyses were performed using a Gas Chromatograph/Mass Spectrometer (GC/MS, Agilent 7890A/5975C, Agilent Tech., Santa Clara, CA, USA) with a HP-CHIRAL-20β column (I.D. 0.25 mm, length 30 m) (Agilent Tech.). Helium was the carrier gas with a flow rate of 1 $mL \cdot min^{-1}$. Two μl samples of each extract were injected in a Pulsed Splitless mode. The oven temperature started at 45°C for 2 min, increased to 70°C by $20°C \cdot min^{-1}$, increased to 90°C by $10°C \cdot min^{-1}$, increased to 120°C by $2°C \cdot min^{-1}$, increased to 150°C by $3°C \cdot min^{-1}$, and then increased up to 230°C by $30°C \cdot min^{-1}$ and held for 1 min. The data was acquired using SIM mode. The quantified compounds included (1) *trans*-verbenol; and (2) *exo*-brevicomin. Compounds were quantified by comparison with commercially available standards with a chemical purity > 99% (Contech Enterprises Inc., Vancouver, BC, CAN).

2.7 | Statistical Analyses

All data analyses were performed in R version 3.4.1 (R Core Team, 2018). The explanatory variable, percent weight lost during the flight treatment, was calculated by dividing the difference between pre- and post-treatment weight by pre-treatment weight, and multiplying this value by 100. Data were tested for normality and heteroscedasticity using visual techniques and Shapiro-Wilks test. Due to the confounding nature of the variables percent weight lost, pre-treatment weight and distance flown, the effects of these independent factors were analyzed in separate models to avoid spurious associations.

The effect of flight treatment on female beetle host acceptance was analyzed using a contingency table. Dichotomous entry data in the host colonisation experiment was analyzed using a binomial distribution in a generalized linear mixed effects model with natal bolt and reproductive bolt defined as random factors in each model (Table 1). The response variable, host entry, was assessed in three separate models, (1) host entry explained by percent weight lost by both flown and control female beetles, during the flight period; (2) host entry explained by distance flown by female beetles during the flight period; and (3) host entry explained by pre-treatment weight of both flown and control female beetles. For model 1, percent weight lost was square-root transformed to meet the assumption of normality; for model 2, distance flown was transformed to the fourth root to meet the assumption of normality. Four cox proportional models were used to analyze entry success and time until host entry (Table 1) in relation to (1) square-root transformed percent weight lost for all beetles; (2) percent weight lost for flown beetles; (3) fourth-root transformed distance flown; and (4) pre-treatment weight for all beetles. Entry success and time of entry were used to define the “Survival” function in the cox proportional models. For the beetles that entered, the relationship between time until entry and percent weight lost was analyzed using a mixed effects linear model separately for flown and control beetles with a Bonferroni correction of $\alpha = 0.025$. Both natal and reproductive hosts were included as random factors in both analyses.

Pheromone production data was analyzed using linear mixed effects models with natal bolt and reproductive bolt defined as random factors in each model (Table 1). The response variable, total *trans*-verbenol production, was assessed in three separate models (1) *trans*-verbenol production as explained by percent weight lost, by both flown and control female beetles, during treatment; (2) *trans*-verbenol production as explained by distance flown by female beetles during the flight period; and (3) *trans*-verbenol production as explained by pre-treatment weight of both flown and control female beetles. For models 1 and 3, total *trans*-verbenol production was cube-root transformed to meet the assumption of normality. The response variable, total *exo*-brevicomin production, was assessed in three separate models (1) *exo*-brevicomin production as explained by percent weight lost, by both flown and control male beetles, during treatment; (2) *exo*-brevicomin production as explained by distance flown by male beetles during the flight period, and (3) *exo*-brevicomin production as explained by pre-treatment weight of both flown and control male beetles.

3 | RESULTS

3.1 | Host colonisation experiment

Of the 267 female beetles used in the host colonisation study, 40% entered the host material within 72 hr. Initiation of host colonisation was influenced by flight treatment. Beetles that flew on flight mills were 13% less likely to initiate host colonisation compared to control beetles that were not given the opportunity to fly (X^2=5.2722, p=0.0216).

Generalized linear models indicated a relationship between host entry and the percent weight lost during the flight treatment (X^2=31.774, p=1.732×10^{-8}). Female beetles that lost less weight during flight treatment were more likely to enter a host (Figure 1). No relationships between host entry and distance flown (X^2=0.0763, p=0.7824) or pre-treatment weight (X^2=0.5286, p=0.4672) were found.

Cox proportional models showed that percent weight lost affected host entry and entry time for all beetles (Z=6.264, p=3.74×10^{-10}) and flown beetles alone (Z=2.184, p=0.029, Figure 2). There was no relationship, however, between distance flown (X^2=0.408, p=0.683) or pre-treatment weight (X^2=0.704, p=0.482) and host entry. Of the beetles that entered the bolts, the time until entry was negatively influenced by the percent weight lost during the treatment in flown (X^2=7.0248, p=0.0080, Figure 2) but not control (X^2=0.0093, p=0.923) beetles.

3.2 | Pheromone production experiment

The production of *trans*-verbenol by female beetles was influenced by the percent weight lost during flight treatment (X^2= 3.8706, p=0.04914) and the distance flown (X^2=5.1584, p=0.0231), but not by pre-flight weight (X^2=1.1417, p=0.2853). Females that lost more weight (Figure 3) and flew further distances (Figure 4) produced more *trans*-verbenol.

The production of *exo*-brevicomin by male beetles was influenced by pre-flight treatment weight (X^2=5.6937, p= 0.0170) and distance flown (X^2=9.5932, p=0.0020), but not by percent weight lost during flight (X^2=0.9912, p=0.3195). Males that weighed more prior to flight treatment produced more *exo*-brevicomin (Figure 5); males that flew further produced less *exo*-brevicomin (Figure 6).

4 | DISCUSSION

The important life history traits of adult mountain pine beetle include dispersal from the natal host, host colonisation, aggregation triggered by pheromone production, and reproduction after overcoming host defenses. All these traits require allocation of energy. The current study uncovers mechanisms by which energy use during flight influences host entry and pheromone production by beetles. Mountain pine beetle acquire resources largely from feeding throughout larval development and feeding on the natal host before flight and at the reproductive host following dispersal (Safranyik & Carroll, 2006). The amount of lipids retained by females following flight dictates the outcome of host colonisation success (Chubaty, Hart & Rotiberg, 2014). Females that arrive at host trees with low energy reserves are less likely to initiate host colonisation (Elkin & Reid, 2005). In the current study, female beetles that lost less than 10% of their body weight during flight were more likely to enter hosts compared to those that lost more than 10%. In mountain pine beetle, weight loss is linked to lipid metabolism during flight (Evenden, Whitehouse & Sykes, 2014). Our findings are in agreement with the results of earlier studies on male pine engraver beetles (*Ips pini*) in which beetles that enter host material have 21% more lipid compared to those that do not enter (Wallin & Raffa, 2000). In fact, mountain pine beetle with high lipid content withstand high concentrations of host monoterpenes in order to colonise a well-defended host tree (Reid & Prucell, 2011; Manning & Reid, 2013; Reid, Sekhon & LaFramboise, 2017).

The timing of host colonisation is also dependent on energy reserves remaining in female beetles after dispersal. For mountain pine beetle that successfully enter hosts, those with low energy reserves initiate colonisation first (Latty & Reid, 2010). In the current study, the fastest beetles to enter the host had lost the most weight during the flight treatment. Our study indicates a trade-off between energy-use during flight and host acceptance in female mountain pine beetle, which likely intensifies the flight-reproduction trade-off previously suggested for this species (Wijerathna et al., 2019). These results lend further support to the "desperation hypothesis" (Latty & Reid, 2010). Host quality was not measured in the current study, so alternative hypotheses could not be tested. In contrast to our findings, time to host entry by pine engraver beetles declined with beetle starvation (Wallin & Raffa, 2002), suggesting that energy-use trade-offs may not be consistent across bark beetle species.

Distant dispersal away from the natal tree may increase the need for effective signalling to attract conspecifics to mount a mass attack. We show that female flight distance and energy use, as measured by percent weight lost, is linked to a subsequent increase in *trans*-verbenol production by females following host entry. Release of high concentrations of *trans*-verbenol should increase the success of pioneer beetles that initiate attack on distant hosts to increase the aggregation of conspecifics (Erbilgin et al., 2014), as attraction of the southern pine beetle (*Dendroctonus frontalis*) increases positively with *trans*-verbenol dose (Shepherd & Sullivan, 2019). Beetles that disperse only a short distance would benefit less from the production of strong pheromone signals.

Female mountain pine beetle begin to release *trans*-verbenol upon initiation of gallery construction and feeding (Pitman & Vité, 1969; Pureswaran et al., 2000). *trans*-Verbenol production requires the oxidation of the precursor, α-pinene (Hughes, 1975), obtained from the natal host (Chiu, Keeling & Bohlmann, 2018). Additionally, *trans*-verbenol production varies with the concentration of α-pinene present in the reproductive host (Erbilgin et al., 2014; Taft, Najar & Erbilgin, 2015), which suggests that the α-pinene necessary for pheromone synthesis could be obtained from both sources. Female mountain pine beetles accumulate α-pinene in the form of monoterpenyl esters which are fatty acid esters stored in the fat body (Chiu, Keeling & Bohlmann, 2018). As we have shown that flight increases *trans*-verbenol production in female mountain pine beetles, the biochemical mechanism dictating this increase may be the result of lipid use during flight (Evenden, Whitehouse & Sykes, 2014) through impact on the stored monoterpenyl esters. High variability in pheromone production, including *trans*-verbenol, occurs in other bark beetles. Variation in pheromone production can be linked to body size (Pureswaran & Borden, 2003), variation in host tree monoterpenes (Erbilgin et al., 2014; Taft, Najar & Erbilgin, 2015) and genetics (Domingue & Teale, 2008), but causes of variation are not consistent across different pheromone components.

Effects of flight on pheromone production were not consistent across pheromone components, as flight distance negatively affected *exo*-brevicomin production by males. This difference in pheromone production in response to flight between sexes could be due to the timing of pheromone production. Males can begin to produce *exo*-brevicomin immediately upon emergence from the natal host (Pureswaran & Borden, 2003; Song et al., 2014). The complete biosynthetic pathway behind the production of *exo*-brevicomin remains unknown, however, it is synthesized *de novo* from fatty acyl-CoA precursors and stored in the fat body (Vanderwel, 1994; Song et al., 2014). Energy use during flight could influence *exo*-brevicomin storage in the fat body, with more pheromone released during periods of flight than rest. This may explain why males produce lower levels of *exo*-brevicomin when they enter the female nuptial galleries to reproduce (Song et al., 2014). These lower levels of *exo*-brevicomin are likely beneficial to mediating aggregation as low concentrations of *exo*-brevicomin are the most attractive (Rudinsky et al., 1974). *exo*-Brevicomin is attractive to female mountain pine beetles at low concentrations but becomes a deterrent at higher concentrations (Rudinsky et al., 1974). Flight may be a mechanism that promotes low, attractive quantities of *exo*-brevicomin. Males potentially have a finite amount of pheromone to release based on the condition of the beetle at the time of pupation. Our finding that heavier males produce more *exo*-brevicomin than lighter males supports this idea. The quality of the natal host likely has a large influence on the amount of *exo*-brevicomin males can produce in a lifetime, as good quality hosts produce larger, more robust offspring (Graf et al., 2012). This supports previous findings indicating a marginal link between mountain pine beetle body weight and length to *exo*-brevicomin production (Pureswaran & Borden, 2003).

Interestingly, weight loss during flight influences pheromone production in females but not males. This is potentially due to differential energy use during flight between the sexes. Females rely heavily upon lipids during long distance flight, while males use both lipids and proteins to power flight (Wijerathna & Evenden 2019). This is likely driven by variation in the energy needed for host colonisation, as females require proteins for reproduction (Pitt et al., 2014). The reliance on lipids by female beetles for flight likely has a direct impact on weight lost during flight (Evenden, Whitehouse & Sykes, 2014), whereas weight lost by male beetles is a combination of the depletion of multiple energy sources (Wijerathna & Evenden 2019). If lipid-use is responsible for differing pheromone titers, the link between weight loss and pheromone production in males would be lost. In the fat body, female beetles store monoterpenyl esters used in the production of *trans*-verbenol (Chiu, Keeling & Bohlmann, 2018), while male beetles store *exo*-brevicomin in its final form in the fat body (Song et al., 2014). Lipid use during flight may allow for the release of pheromone from storage in males and reduce the subsequent pheromone titer available to males calling at the new host. In females, since the entire biosynthetic pathway of *trans*-verbenol remains unknown, all that can be concluded is that flight could aid or promote biosynthesis of this compound.

Here we provide evidence for mechanisms that drive flight polyphenisms in bark beetles. The trade-off between energy-use during flight and host colonisation could select for short distance dispersal so that beetles have enough energy to successfully colonise their reproductive host. Alternatively, long distance dispersal might be adaptive for outbreeding and access to high quality hosts (Raffa, Phillips & Salom, 1993; Croteau, 2010). Energy-use during flight positively impacts subsequent pheromone production in female beetles. Increased *trans*-verbenol production will aid beetles in mediating mass attack at a distant host, this in combination with other benefits at these distant locations will select for long-distance dispersers. These results provide evidence for mechanisms that promote contrasting selection on flight in bark beetles. Selection at both ends of the polyphenism spectrum maintains high dispersal variability within the population. This intraspecific variation in dispersal strategies promotes an evolutionarily stable strategy for bark beetle populations (Kautz et al., 2016). These polyphenic strategies are beneficial in the face of changing environments. Understanding variation in spatial movement of bark beetles across landscapes will help to predict future population spread of these aggressive tree pests.

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AUTHOR CONTRIBUTIONS

KJ, NE and ME developed the idea and designed experimental methodology; KJ collected data for the host colonisation experiment; KJ and GI collected data for the pheromone production experiment; Pheromone analysis was conducted in the Erbilgin lab (https://sites.ualberta.ca/~erbilgin/); RR and GI created methods for and conducted chemical analyses; KJ completed statistical analyses; KJ led the writing of the manuscript, with guidance and edits from ME and NE. All authors contributed critically to the manuscript drafts and gave approval for publication of final product.

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FIGURES & TABLES

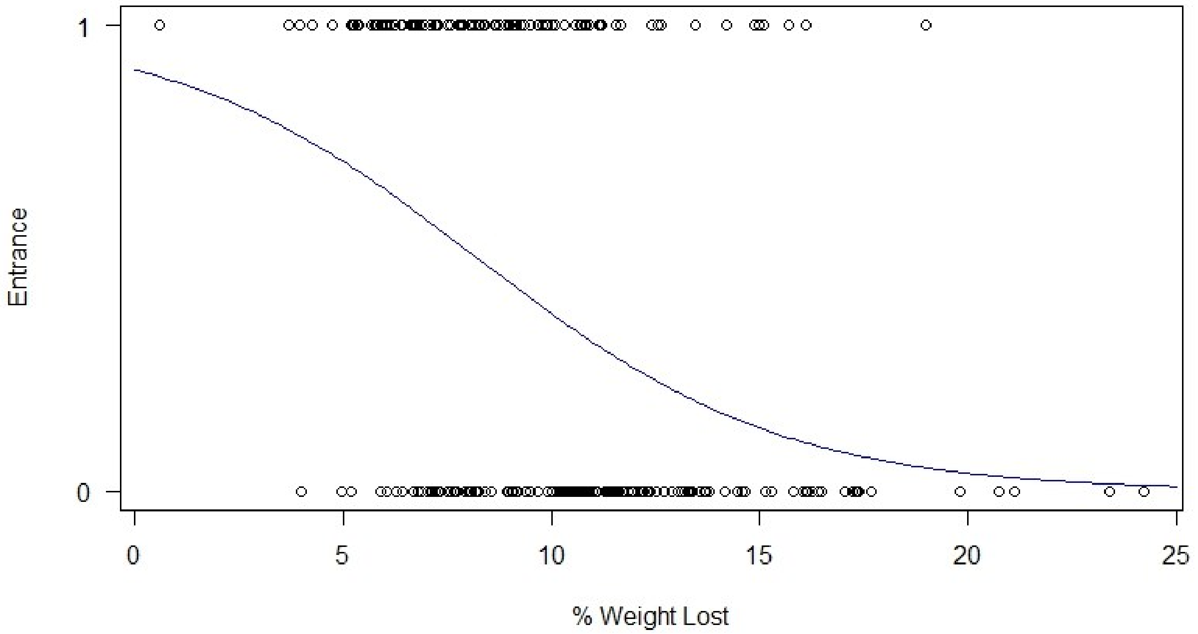

Figure 1. Effect of percent weight lost during flight, for both control and flown female mountain pine beetle on subsequent host colonisation. On the y-axis, 0 represents females that failed to colonise a lodgepole pine host and 1 represents females that successfully colonised a host (X^2=31.774, p=1.732 x 10^{-8}).

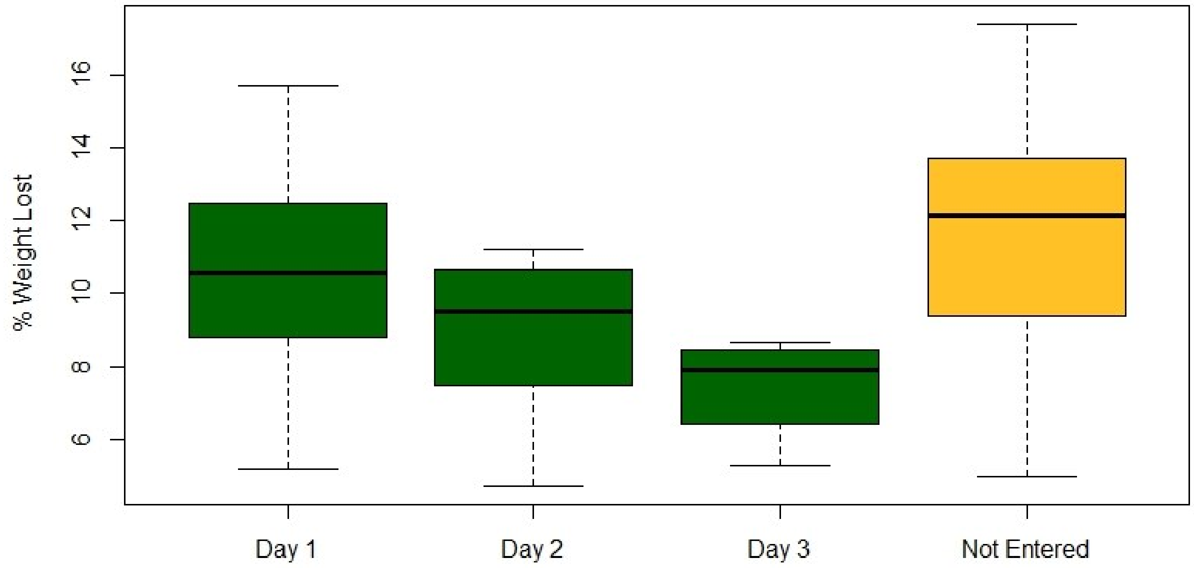

Figure 2. Box plots of percent weight lost during the assay for flown female mountain pine beetle that entered lodgepole pine hosts at different times post inoculation. The midline indicates the median and the bottom and top of the box represent the 25th and 75th percentiles, respectively. Vertical lines extending from the box (whiskers) represent the maximum and minimum values. Beetles that entered host material (green bars) lost less weight during the flight treatment compared to those that subsequently failed to enter hosts (yellow bar) ($Z=2.184$, $p=0.029$). Weight lost after flight influenced the length of time it took beetles to initiate colonisation after flight ($X^2=7.0248$, $p=0.0080$) (green bars).

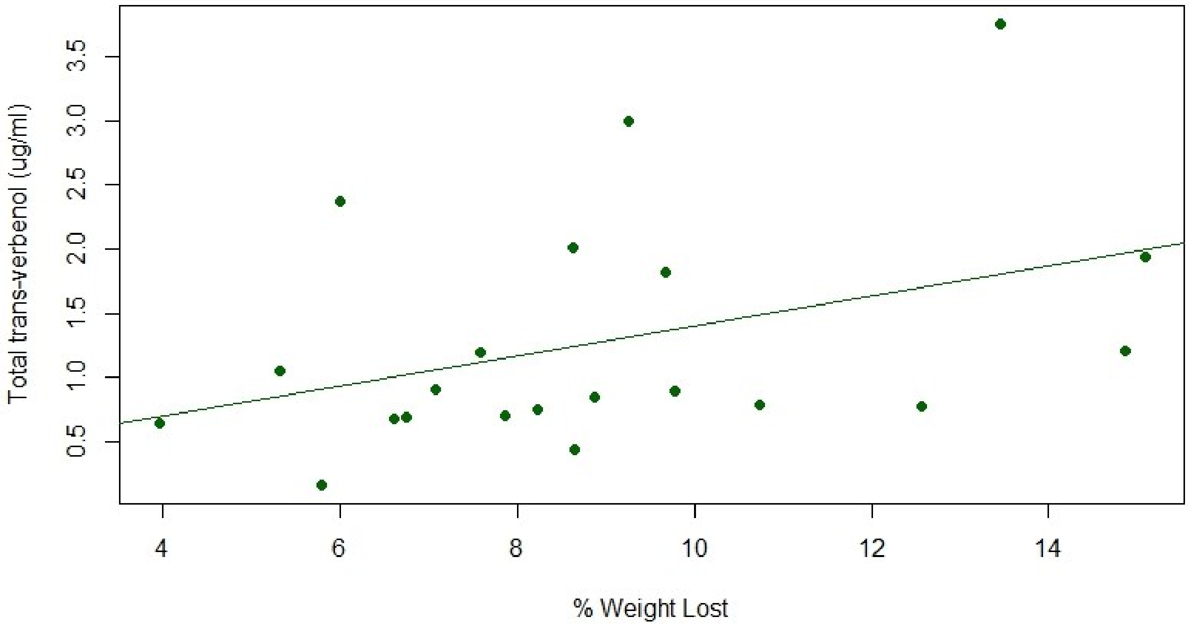

Figure 3. The relationship between percent weight lost during the bioassay and subsequent *trans*-verbenol (µg/ml) production for both flown and control female mountain pine beetle in lodgepole pine bolts. Beetles that lost more body weight during the assay produced higher amounts of *trans*-verbenol (X^2= 3.8706, p=0.0491).

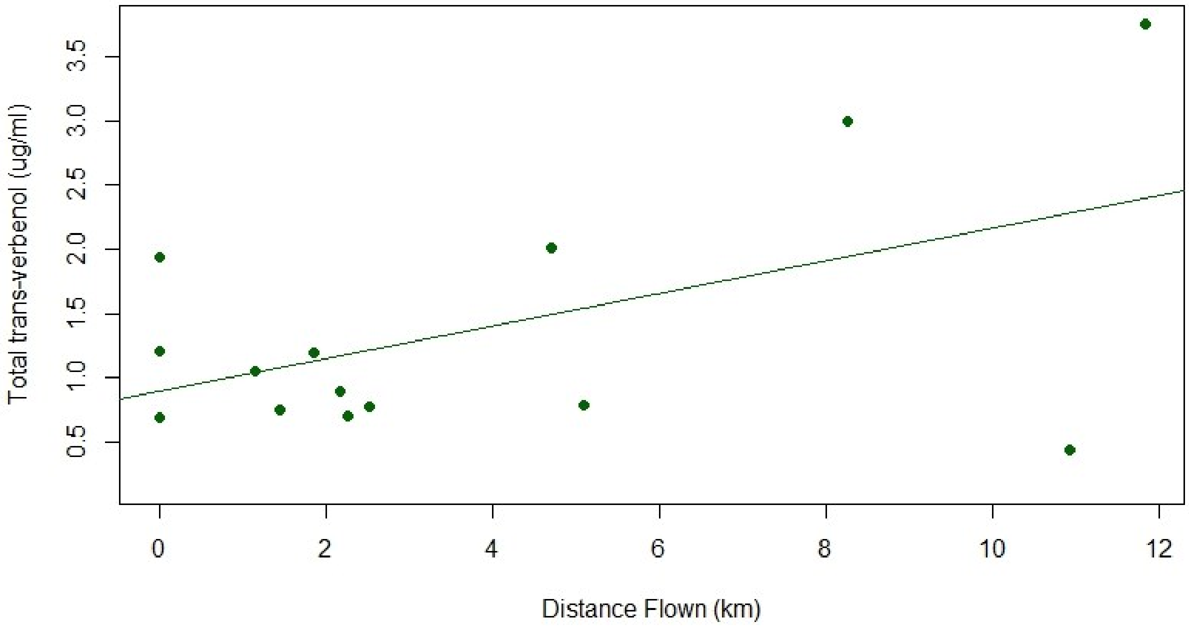

Figure 4. The relationship between distance flown (km) during the assay and subsequent *trans*-verbenol (µg/ml) production for flown female mountain pine beetle in lodgepole pine bolts. Flight promoted *trans*-verbenol production in female beetles (X^2=5.1584, p=0.0231).

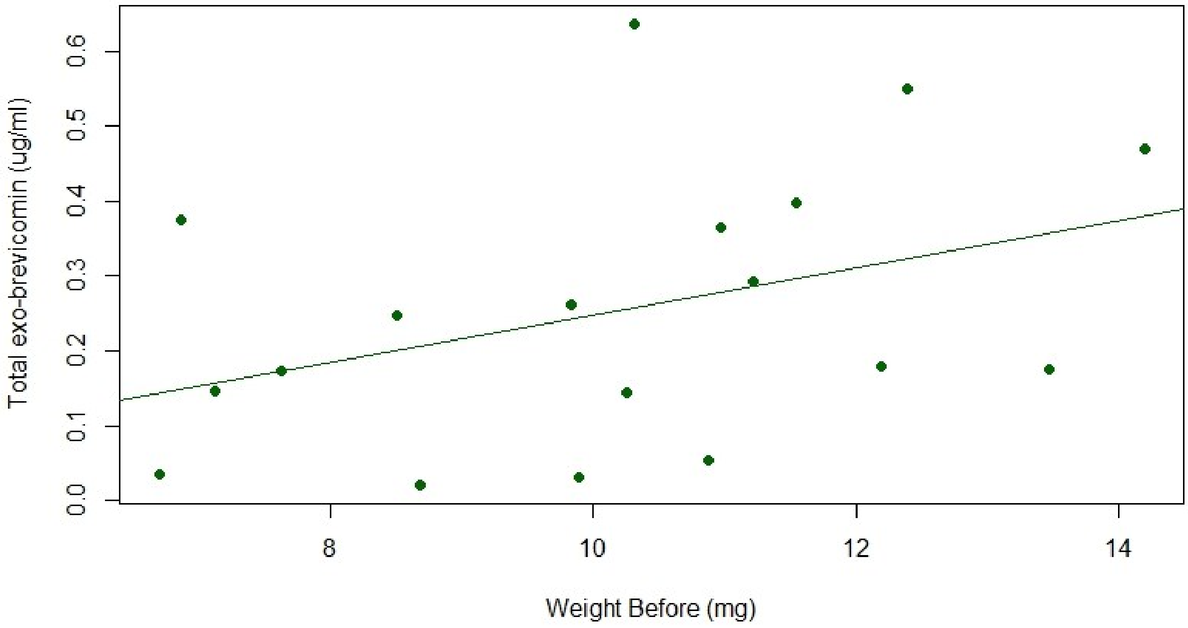

Figure 5. The relationship between pre-bioassay weight and subsequent *exo*-brevicomin production (µg/ml) for flown and control male mountain pine beetle in lodgepole pine bolts. Heavier beetles produced more *exo*-brevicomin (X^2=5.6937, p=0.0170).

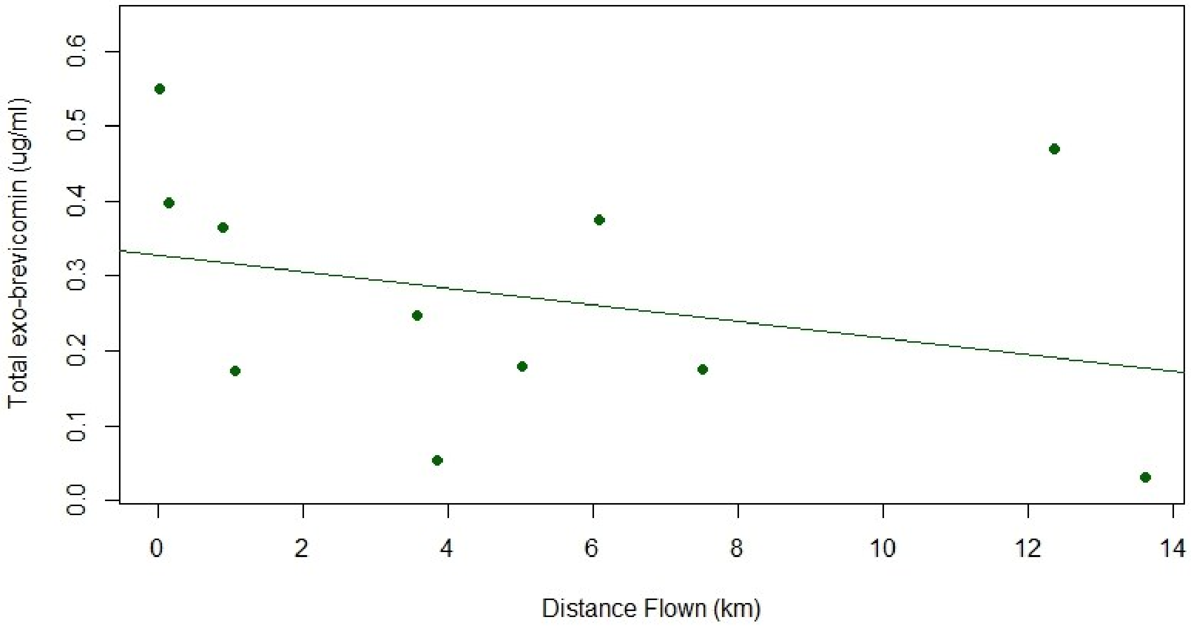

Figure 6. The relationship between distance flown (km) during the assay and subsequent *exo*-brevicomin (µg/ml) production by flown male mountain pine beetle in lodgepole pine bolts. Flight distance is negatively associated with *exo*-brevicomin production in male beetles (X^2=9.5932, p=0.0020).

Table 1. Statistical models used for analysis of female propensity to enter hosts, the time taken to enter hosts, *trans*-verbenol production and male *exo*-brevicomin production. 1. Models including data from both flown and control treatment beetles; 2. Models including data from flown beetles only; and 3. Survival function as defined by the propensity to enter hosts and the time taken to enter hosts.

<table>
<tr><th>Response Variable</th><th>Explanatory Variable</th><th>Model</th></tr>
<tr><td>Host entry</td><td>% Weight lost during treatment[1]</td><td>glmer(host entry ~ $\sqrt[2]{\%\ \text{weight lost}}$ + (1|natal) + (1|reproductive))</td></tr>
<tr><td></td><td>Distance flown[2]</td><td>glmer(host entry ~ $\sqrt[4]{\text{distance flown}}$ + (1|natal) + (1|reproductive))</td></tr>
<tr><td></td><td>Pre-treatment weight[1]</td><td>glmer(host entry ~ pre-treatment weight + (1|natal) + (1|reproductive))</td></tr>
<tr><td>Survival[3]</td><td>% Weight lost during treatment[1]</td><td>coxph(survival ~ $\sqrt[2]{\%\ \text{weight lost}}$)</td></tr>
<tr><td></td><td>% Weight lost during treatment[2]</td><td>coxph(survival ~ % weight lost)</td></tr>
<tr><td></td><td>Distance flown[2]</td><td>coxph(survival ~ $\sqrt[4]{\text{distance flown}}$)</td></tr>
<tr><td></td><td>Pre-treatment weight*</td><td>coxph(survival ~ pre-treatment weight)</td></tr>
<tr><td><i>trans</i>-Verbenol</td><td>% Weight lost during treatment[1]</td><td>lmer($\sqrt[3]{trans\text{-verbenol}}$ ~ % weight lost + (1|natal) + (1|reproductive))</td></tr>
<tr><td></td><td>Distance flown[2]</td><td>lmer(<i>trans</i>-verbenol ~ distance flown + (1|natal) + (1|reproductive))</td></tr>
<tr><td></td><td>Pre-treatment weight[1]</td><td>lmer($\sqrt[3]{trans\text{-verbenol}}$ ~ pre-treatment weight + (1|natal) + (1|reproductive))</td></tr>
<tr><td><i>exo</i>-Brevicomin</td><td>% Weight lost during treatment[1]</td><td>lmer(<i>exo</i>-brevicomin ~ % weight lost + (1|natal) + (1|reproductive))</td></tr>
<tr><td></td><td>Distance flown[2]</td><td>lmer(<i>exo</i>-brevicomin ~ distance flown + (1|natal) + (1|reproductive))</td></tr>
<tr><td></td><td>Pre-treatment weight[1]</td><td>lmer(<i>exo</i>-brevicomin ~ pre-treatment weight + (1|natal) + (1|reproductive))</td></tr>
</table>

Chapter 4: General Conclusion

Mountain pine beetle exhibit great variation in dispersal capabilities. This population variation is known as stratified dispersal and is characterized by a leptokurtic population distribution. Previous work on the mountain pine beetle has shown that some of this variation is attributed to energy content (Evenden et al., 2014; Wijerathna & Evenden 2019), beetle morphology (Shegelski et al. 2019) and environmental conditions (Wijerathna, 2016). In Chapter 2, I add to the known factors that influence mountain pine beetle flight by assessing how aggregation pheromones, host VOCs and non-host VOCs influence flight propensity and capacity. The goal of Chapter 3 was to understand how the large degree of flight variation between beetles influences subsequent host colonisation events. The information presented in this thesis suggests possible factors and mechanisms that cause this dispersal variation.

Mountain pine beetle are both receptive and responsive to a variety of semiochemical cues, including aggregation pheromones, host VOCs and non-host angiosperm VOCs (Borden et al., 1987; Borden et al., 1998; Huber et al., 2000; Huber & Borden, 2001a; Pureswaran et al., 2004; Miller et al. 2005; Campbell & Borden 2006). This study is the first to show that in addition to eliciting antennal activity and flight orientation, these semiochemicals also influence flight capacity of the mountain pine beetle. An interaction occurs between exposure and beetle weight when female beetles are exposed to host volatiles prior to flight. A positive relationship between pre-flight weight and flight distance exists for beetles pre-exposed to clean air; beetles pre-exposed to host volatiles do not exhibit this positive relationship. Exposure to host volatiles during flight may indicate to female beetles that host trees are in close proximity and further host searching is not required. Thus, larger beetles may under go shorter flights in the presence of potential hosts. Exposure to host VOCs could prime female beetles for host colonisation instead of flight behaviours. Exposure to host volatiles does not influence male beetle flight distance. This differential in effect of host volatiles on flight between sexes is likely due to the different roles of male and female beetles during host colonisation. Female beetles initiate gallery construction and mass attack, while males will always join an already constructed gallery of a female. Males will likely have a greater response to aggregation pheromone because of their life history. Sex differences in response to host VOCs occur in other beetles, for example, pioneering male *Monarthrum scutellare* are captured more in host VOCs traps than females (Noseworthy et al., 2012). This difference between sexes suggests that the pioneering sex is more receptive to these chemical cues as they rely on host cues more heavily.

Exposure to trembling aspen during flight decreases female beetle flight distance in comparison to females flown in clean air. Non-host VOCs can be as effective at repelling bark beetles as anti-aggregation pheromones (Huber & Borden, 2001a, 2001b). Disruptive effects of non-host VOCs on orientation occur in the mountain pine beetle (Huber & Borden, 2001a), the Douglas-fir beetle (Huber & Borden, 2001b) and the pine shoot beetle (Kohnle, 2004). This is the first study to show an influence of non-host VOCs on flight capacity. Instead of being inclined to fly further in attempt to escape areas of non-host VOCs, mountain pine beetle exhibit shorter flight distances in the presence of aspen volatiles. This suggests that non-host VOCs not only have a repellent effect on flight orientation, but also a detrimental effect on overall flight motivation of the mountain pine beetle. A potential explanation for this is that female beetles are undergoing short flight bursts. These short flight bursts could help them in orienting through stands of non-hosts. Future experiments should test this hypothesis as the current experimental design does not assess orientation.

Exposure to *trans*-verbenol prior to flight increases flight distance and velocity of female beetles compared to those exposed to clean air only. Aggregation pheromones are important in the host colonisation process as they recruit conspecifics to aid in the mass attack of a host tree (Cardé, 2014; Wyatt, 2014). Without the help of conspecifics, pioneer beetles would not be able to overcome host chemical and physical defenses, and would fail to reproduce (Raffa & Berryman, 1983). Pre-exposure of females to *trans*-verbenol increases flight distance, which potentially aids movement of the beetle through patchy habitats. *trans*-Verbenol is more attractive to mountain pine beetle than host VOCs alone (Borden et al., 1990) and can attract mountain pine beetle to visually unattractive trees (Rasmussen, 1972). Increased flight distances after exposure to *trans*-verbenol, in combination with higher production of *trans*-verbenol after increased flight periods may aid the mountain pine beetle move throughout the expanded range. The greater response of mountain pine beetle to *trans*-verbenol compared to host VOCs may be hard-wired in the spatial organization of the nervous system. Glomeruli that process pheromone information are situated near the antennal nerve in moths (Hillier & Vickers, 2007). It is unknown whether this spatial organization elicits a difference in the onset of behavioural response to odorants.

The large variation in flight exhibited by the mountain pine beetle has implications for subsequent host colonisation processes. Previous work on the mountain pine beetle indicates that body condition upon arrival at the host tree has implications for host colonisation (Latty & Reid, 2010; Chubaty et al., 2014) and reproduction (Elkin & Reid, 2005; Wijerathna et al., 2019). A trade-off exists between energy use during flight and host colonisation propensity in female mountain pine beetle. Female beetles that use more energy during the flight period are less likely to colonise hosts than beetles that use less energy. Of the flown beetles that enter hosts, however, beetles that use the most energy during flight are the first to enter. The same trend does not occur for control beetles, suggesting that flight may exacerbate this trade-off. A positive trend between body condition and host colonisation occurs when energy stores are manipulated through starvation (Latty & Reid, 2010). Lipid reserves also influence time until host entry, as beetles with lower lipid content are the first to enter trees compared to those with higher lipid content (Chubaty et al., 2014). Female beetles rely on lipids heavily during flight (Evenden et al., 2014; Wijerathna & Evenden 2019), the burning of lipids in flight has important implications in host colonisation. The use of lipids during flight needs to be balanced with lipids stores required to complete subsequent host colonisation and reproduction. This results selection pressure for the maintenance of short flight distance, as this safe strategy will increase the likelihood of brood production.

Energy-use during flight also influences aggregation pheromone production in both male and female mountain pine beetle. Females produce the aggregation pheromone *trans*-verbenol, that is highly attractive to conspecifics (Borden et al., 1990; Miller et al., 2005). *trans*-Verbenol is produced from the monoterpene precursor α-pinene, obtained from the natal host (Chiu et al., 2018). α-Pinene is stored as monoterpenyl esters in the fat body and released as *trans*-verbenol when the females begin to feed at the reproductive host (Pitman & Vité, 1969; Pureswaran et al., 2000). Flight distance and energy use during flight promote production of *trans*-verbenol post-flight. Although long-distance flight is risky due to exhaustion and increased risk of predation, it can be greatly beneficial for outbreeding, lower brood competition, higher host quality and higher *trans*-verbenol production (Bowler & Benton, 2005; Matthysen, 2012). High release rates of *trans*-verbenol will aid attraction of conspecifics to distant hosts, and will select for long distance fliers. Female beetles exhibit increased flight distances after exposure to *trans*-verbenol, suggesting that strong signals will successfully recruit conspecifics.

Flight distance impacts the release of male-produced aggregation pheromone, *exo*-brevicomin. *exo*-Brevicomin is produced *de novo* in the fat body and is released immediately upon emergence from the natal host (Vanderwel 1994; Song et al. 2014). Production of *exo*-brevicomin drops when males enter the reproductive host (Song et al., 2014). Low levels of *exo*-brevicomin are attractive to conspecifics (Borden et al., 1983). As more males reach the reproductive host and levels of *exo*-brevicomin released from the whole tree increase, this pheromone becomes an anti-aggregant (Rudinsky et al., 1974). Flight has the opposite effect on *exo*-brevicomin production compared to *trans*-verbenol, as flight distance is negatively related to *exo*-brevicomin production. This differential effect likely benefits aggregation as low levels of *exo*-brevicomin are highly attractive. The different effects of flight on aggregation pheromone production in males and females is likely due to timing of pheromone production in the host colonisation process. Burning lipids during flight may influence storage and release of *exo*-brevicomin leading to a drop in pheromone production at the reproductive host. Comparatively, *trans*-verbenol is not produced until females reach the reproductive host (Pitman & Vité, 1969; Pureswaran et al., 2000). The use of lipids within the fat body where the monoterpenyl esters are stored in females (Chiu et al., 2018) may benefit the subsequent production of this pheromone. The mechanism behind this increased production remains unknown.

This study further adds to current knowledge on the variation of flight behaviour in the mountain pine beetle. A delicate interplay of factors that influence flight capacity, and the subsequent influence of flight on host colonisation will determine the flight behaviour of each individual. The findings in this thesis suggest mechanisms behind both short and long distance fliers. These mechanisms select for opposite ends of the flight polyphenism spectrum, maintaining the high variability in dispersal behaviour of the mountain pine beetle. This thesis reveals internal and external factors that influence flight variation. Pre-determined factors that influence flight capacity include energy stores (Evenden et al. 2014; Wijerathna & Evenden 2019) and phenotypic selection. These factors interact with environmental abiotic (Wijerathna 2016) and biotic factors to govern flight of the mountain pine beetle. Understanding both the internal and external factors that influence flight capacity and subsequent host colonisation processes will help to aid in the management of this destructive pine pest.

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