



Density-dependent responses of mountain pine beetle to its pheromones and host volatiles in naïve lodgepole pine stands

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ABSTRACT

Mountain pine beetle has expanded its host range into naïve lodgepole pine forests in western Canada and killed a large number of pine trees. Synthetic attractants mainly bark beetle pheromones and host monoterpenes attached to traps are typically used to monitor beetle populations within its range. Despite well-established differences in beetle host selection behavior between endemic and outbreak population densities, the influence of population density on the response of mountain pine beetle to the synthetic attractants is poorly understood. Over three years, we tested the effects of different release rates of female mountain pine beetle aggregation pheromone (*trans*-verbenol) and two host monoterpenes (terpinolene and myrcene) and local beetle population density on trap catches of mountain pine beetle. We estimated local mountain pine beetle population densities using the number of dead trees observed in the previous year's aerial detection survey. During the first three weeks of the flight period, the lures with low release rates of all components captured more mountain pine beetle with increasing beetle population density and the lures with a high release rate of *trans*-verbenol plus a low release rate of host monoterpenes were preferred by beetles in low population density sites. We also found a temporal shift in attraction patterns. As the flight period progressed, the preference for low *trans*-verbenol release rate lures was less pronounced at high mountain pine beetle densities. No sex-specific responses of mountain pine beetles, along with number of competitor and predatory beetles of mountain pine beetle, were found for a particular attractant. These results provide evidence that mountain pine beetle has a density dependent response to attractants and can assess available semiochemicals cues to ensure successful host colonization depending on their density and the flight period. Overall, this study can improve the efficacy of current semiochemical-based monitoring tools, which are essential to implementing informed mitigation strategies for mountain pine beetle especially while they are in a sub-outbreak stage in the expanded distribution.

1. Introduction

Semiochemicals provide external cues for herbivorous insects to locate suitable host plants (Gitau et al. 2013). However, host selection by insect herbivores may also vary with insect population size, especially for eruptive bark beetle species (Coleoptera: Curculionidae, Scolytinae) that utilize a mass attack strategy to overwhelm host tree defenses (Raffa et al., 2008; Safranyik et al., 2010; Lindgren and Raffa, 2013). Tree-killing bark beetle species rely on trees with weakened defenses at low population densities as high concentrations of host defenses can have a negative feedback on beetle aggregation to host trees; in contrast individuals in high density populations are not as limited by such host defense chemicals (Boone et al., 2011; Lindgren and Raffa, 2013). Therefore, what constitutes a suitable host tree for

tree-killing bark beetles really depends on the density of beetles and ability to mass attack trees, and consequently the influence of semiochemical cues used in host selection may also vary with bark beetle density, providing a positive feedback mechanism that can contribute to the promotion of population density-dependent host selection behavior (Wallin and Raffa, 2004).

Mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins) is an eruptive bark beetle that has impacted millions of hectares of mainly lodgepole pine (*Pinus contorta* var. *latifolia*) forests in western North America during a recent and ongoing outbreak (Bentz et al., 2010; Safranyik et al., 2010). It forms a mass attack on a host tree by responding to a combination of pheromones and host tree volatiles, mainly monoterpenes, thereby overwhelming host defense chemicals (Pitman and Vité, 1969; Billings et al., 1976; Borden et al., 1987;

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Erbilgin et al., 2017). However, MPB exhibits an optimal attack density during host colonization that is mediated by the production of aggregation and anti-aggregation pheromones (Raffa and Berryman, 1983; Borden et al., 2008). Specifically, upon arrival to a new host, female MPB produces *trans*-verbenol, which preferentially attracts males (Billings et al., 1976). Males produce *exo*-brevicomin, which is attractive to females (Pitman and Vité, 1969; Pureswaran et al., 2000). The mixture of both female and male pheromones serves as a powerful aggregation pheromone, which usually results in mass aggregation on the host trees (Borden et al., 1987). During this early stage of host colonization, host tree volatiles, mainly monoterpenes such as myrcene and terpinolene can synergize MPB aggregation pheromones (Billings et al., 1976; Borden et al., 2008; Klutsch et al., 2017). Upon reaching an optimal attack density, MPB reduce *trans*-verbenol and *exo*-brevicomin production and both sexes produce anti-aggregation pheromones, which prevent MPB exceeding the optimal attack density (Pureswaran et al., 2000). This aggregation process is required for depletion of host chemical defenses, successful host colonization, and reproduction (Raffa and Berryman, 1983; Raffa et al., 2008; Safranyik et al., 2010).

In western Canada, MPB outbreaks began in central British Columbia and expanded into the eastern edge of the lodgepole pine forests and western edge of the jack pine (*P. banksiana*) forests in western Alberta in 2006 (Cudmore et al., 2010; Cullingham et al., 2011; Erbilgin et al., 2014, 2017). In the expanded range, overall MPB population levels are low and generally cause isolated tree mortality in small patches (Government of Alberta, 2019). Synthetic lures made of a combination of beetle pheromones and host monoterpenes attached to traps are commonly used to detect bark beetle presence and monitor flight (Weslien, 1992; Borden et al., 2008; Billings, 2011; Gitau et al., 2013; Seybold et al., 2018). In particular, flight intercept traps baited with semiochemicals are used to monitor MPB flight throughout its expanded distribution in mature pine forests in Alberta (Government of Alberta, 2016). However, bark beetle attraction, including MPB, to the lures shows variation depending on, in part, beetle population densities in adjacent stands (Miller et al., 1997, 2005; Miller and Borden, 2000). Given that MPB population densities vary across the landscape, especially in the expanded range, land managers may need to consider MPB population density when monitoring beetles using synthetic attractants. Currently, commercially available attractants are not specific to a given MPB density, which may result in less effective monitoring efforts.

Earlier work showed that increased release rates of pheromones altered MPB catches in baited traps, depending on the population density of beetles (Miller and Borden, 2000; Miller et al., 2005). At low population density, MPB catches initially increased with increasing release rates of *trans*-verbenol but eventually catches declined at high release rates. Conversely, at high population density, MPB trap catch was positively dose-dependent with release rates of *trans*-verbenol (Miller et al., 2005). Apparently increased release rate of *exo*-brevicomin had no effect on count of MPB caught at either population density (Miller et al., 2005). These studies suggest that MPB responses depend on their population density as predicted by the optimal density hypothesis (Raffa and Berryman, 1983; Miller et al., 2005). According to this hypothesis, mechanisms behind density dependent response to semiochemicals in bark beetles can range from selection pressures on beetles in low populations favoring strong responses to cues (Raffa and Berryman, 1987; Miller et al., 2005) that are mediated by natal experience (Burke and Carroll, 2017) to environmentally induced feedback adjusting host selection behavior through assessing adult population numbers (Wallin and Raffa, 2004). However, there is a knowledge gap as to whether population density affects MPB attraction to attractants in the naïve lodgepole pine forests.

Along with the potential for semiochemical cue preference to change with population level, there may be a shift in bark beetle response to the same cues throughout the flight period (Birch, 1974; Roberts et al., 1982; Aukema et al., 2000; Steed and Wagner, 2008). Changes in host condition (e.g., defense chemistry) and environmental

factors (e.g., temperature, precipitation, and wind speed) over the flight period of MPB have been associated with changes in behavior (Safranyik and Carroll, 2006). Furthermore, there may be changes in pheromone production throughout the flight period as bark beetles shift from aggregation to anti-aggregation behaviors and a corresponding responsiveness to pheromones (Birch, 1974, Teale and Lanier 1991). A shift in preference for available cues can potentially impact effectiveness of coordinated attack on host trees throughout the flight period. Thus, there could be consequences of seasonality of monitoring MPB activities using synthetic attractants.

The goal of this study was to identify whether MPB attraction to pheromone and host tree monoterpenes in the sub-outbreak expanded distribution of MPB in north-central Alberta varies with local population density and across the flight period. We used four types of attractant treatments, which varied in release rates of *trans*-verbenol, terpinolene, and myrcene. We kept the release rate of *exo*-brevicomin constant. Specifically, our hypotheses are that (1) increasing release rates of *trans*-verbenol and/or terpinolene, and myrcene increase MPB trap catch, (2) MPB population density affects attraction to the pheromones/host monoterpene combinations, and (3) MPB attraction varies across the flight period. Results from this study could inform whether the density-dependent responses to MPB pheromone (*trans*-verbenol) and host monoterpenes (terpinolene and myrcene) underlie larger population dynamics in MPB. This will aid in the improvement of current MPB semiochemical-based monitoring tools and timing of deployment, which are vital for detecting MPB expansion into naïve habitats.

2. Materials and methods

2.1. Study area

We conducted our study in the Swan Hills and Whitecourt areas in Alberta (54.5° N, –115.3° W), which is made up of approximately 50% lodgepole pine-dominated forest with the remaining forest cover consisting of deciduous tree species and white spruce (*Picea glauca*) (Government of Alberta, 2007). Mountain pine beetle recently expanded its range into Alberta and has been infesting trees in the study region at below outbreak population levels since at least 2008 (Government of Alberta, 2019). During the three summers in 2017 through 2019, we established multiple trapping sites that were in mature lodgepole pine-dominated forests with a recent record of MPB activity. The previous year's aerial detection survey (ADS) of trees killed by MPB from the Government of Alberta (2019) was used for each sampling year to locate trapping sites across a range of MPB population densities (Table 1). Aerial surveys are conducted in late summer to detect groups of three or more red-crowned pine trees, which is an indicator of successful mass-attacks by MPB during the previous summer. The number and location of pines killed by MPB are recorded from a helicopter using a touchscreen tablet equipped with an external global positioning system, mapping software (ESRI ArcPAD 7.1.1), and high-resolution imagery. Ground surveys are conducted following ADS to confirm the presence of MPB. We define local MPB populations as the number of MPB-killed trees within a 7 km radius, based on an earlier study which used a similar neighborhood size to predict points of outbreaking MPB and found high local synchrony at 6 km or less (Aukema et al., 2008). Trapping sites were at least 1 km apart.

2.2. Mountain pine beetle trapping and attractant treatments

In 2017 and 2018, we tested the efficacy of four different combinations of attractants: (1) standard commercially available lure consisting of standard release rate (1x) of MPB pheromones, *trans*-verbenol and *exo*-brevicomin, plus 1x host monoterpenes, terpinolene and myrcene (STD); (2) high *trans*-verbenol release lure consisting of double (2x) *trans*-verbenol and 1x for *exo*-brevicomin and 1x for both host

Table 1

Site location, stand basal area ($\text{m}^2 \text{ha}^{-1}$), and *Dendroctonus ponderosae* (MPB) population density information for 2017–2019 trapping years in north-central Alberta, Canada.

Site	Year	Latitude/Longitude	Elevation (m)	Live basal area ^a		No trees killed by MPB ^b	Density category ^c
				Pine	Total		
19	2017	54.535/–115.119	843	–	–	17	Low
11	2017	54.746/–115.414	1264	4.6	25.3	18	Low
12	2017	54.763/–115.352	1232	–	–	65	Low
10	2017	54.533/–115.427	924	34.4	48.2	103	High
13	2017	54.764/–115.312	1220	–	–	107	High
14	2017	54.685/–115.305	1091	29.8	43.6	133	High
10	2018	54.223/–116.058	795	34.4	34.4	3	Low
12	2018	54.219/–116.054	801	29.8	64.3	3	Low
28	2018	54.682/–115.404	1051	23.0	45.9	3	Low
29	2018	54.685/–115.305	1091	29.8	43.6	12	Low
27	2018	54.756/–115.323	1181	6.9	16.1	32	Low
26	2018	54.748/–115.416	1264	4.6	25.3	33	Low
23	2018	54.599/–115.384	913	9.2	34.4	56	High
25	2018	54.532/–115.427	924	34.4	48.2	62	High
22	2018	54.604/–115.381	916	18.4	32.1	63	High
24	2018	54.576/–115.400	872	4.6	20.7	63	High
21	2018	54.624/–115.233	920	16.1	34.4	97	High
25	2019	54.532/–115.427	924	34.4	48.2	93	Low
21	2019	54.624/–115.233	920	16.1	34.4	162	High

^a Dashes represent basal area not recorded.

^b Number of killed trees within a 7 km radius of trap site in 2017–2019 were counted from previous year's aerial detection surveys (Government of Alberta, 2019).

^c Density categories used in Fig. 1.

monoterpenes (2MPB); (3) high tree volatiles lure consisting of 2 × host monoterpenes and 1 × MPB pheromones (2TREE); and (4) high release rate (2 ×) of both *trans*-verbenol and host monoterpenes plus 1 × *exo*-brevicomin (2BOTH). In treatments 2–4, release rates of *trans*-verbenol or host monoterpenes, or both, were doubled (2 ×). The standard (1 ×) release rates of *trans*-verbenol, *exo*-brevicomin, terpinolene, and myrcene per day at 20 °C were about 1.4 mg, 120 µg, 175 mg and 175 mg, respectively (Synergy Semiochemicals Inc., Burnaby, BC, CAN). All baits are formulated to last 40–50 days in the field (Synergy Semiochemicals Inc.).

Attractant-baited flight intercept traps (Advanced Pheromone Technologies, Marylhurst, Oregon, U.S.A.) were set up in six trapping sites arranged in two blocks (i.e., linear transects) in 2017 ($n = 48$ traps). In 2018, we expanded the number of sites ($n = 11$ sites) to capture more variability in the hypothesized ADS and attractant treatment interaction but we were only able to have one transect of lured traps due to limitations in number available traps ($n = 44$ traps). To test and refine the results from the previous two years, the two attractant treatments that showed the most difference in MPB response between high and low MPB densities were used in 2019 (STD and 2MPB) in two sites, with 10 and 12 pairs (blocks) of lured traps established 50 m apart in site 25 and 21, respectively ($n = 44$ traps). For all trapping years, traps were spaced 15 m apart within each transect or pair, which were spaced at 50 m. Traps were at least 2 m from the nearest tree with the height of trap tops at 2 m from the ground. Collection cups contained dichlorvos (19.2%) imbedded insecticide strips. Each attractant treatment was randomly assigned to a trap within each transect or pair within a site and all components of the attractants were attached together to the middle of the trap. We rotated the position of each lure (in 2018) or trap (in 2017 and 2019) each week by incrementally advancing the position in the same direction along the transect or pair. We recorded basal area and species composition at the first and third trap along each transect in 2017 and 2018 (metric basal area factor = 4.6).

In all years, trapped insects were collected every seven days, placed in vials, and stored at –40 °C until preserved in 70% ethanol. We collected insects over a five-week period for the 48 total traps in 2017 (17 July – 21 August 2017). In 2018, we collected insects in 44 total traps over six weeks from 11 July to 22 August. In 2019, we set up 24

traps in site 25 on 13 July and 20 traps in site 21 a week later. The last collection date for the 2019 trapping year was 20 August. All MPB were confirmed to species and tallied by sex (except for 15 August 2018 where a maximum of 50 subsampled MPB per trap were tallied by sex due to very large number of MPB caught). All Coleoptera specimens from the 2017 and 2018 (but not 2019) trapping years were identified to family or subfamily. They were assigned functional groups of either competitors or predators for MPB (de Leon, 1934; Amman and Cole, 1983; Moeck and Safranyik, 1984) in the following collection dates: one block in each site from the 31 July and 14 August 2017 collection dates (two weeks with the most MPB catches) and all traps from 18 July through 1 August 2018 collection dates (first three weeks with consistent MPB catches) (Tables S1 and S2).

2.3. Statistical analyses

To analyze the impact of attractant treatment, local MPB population density and an interaction effect on the count of MPB per trap, we performed a generalized linear mixed model with a first-order autoregressive correlation structure for successive collection dates (PROC GLIMMIX in SAS 9.4) on each year's data separately. The error structure for the 2017 and 2018 catch counts was negative binomial and Poisson for 2019 data (link = natural log). To account for experimental design, random effects for 2017 data were site and block nested within site, site for 2018 data, and block for 2019 data. Since 2019 had only two sites, Tukey's HSD tests were performed for multiple comparisons among attractant treatment and sites. Furthermore, three blocks (i.e. pairs of traps) in site 25 and two blocks in site 21 in 2019 did not catch MPB during the trapping period and were removed from analysis. We ran the above models on the number of total, female, and male MPB caught for each year. The same analyses for all years were conducted to identify whether the proportion of females caught was dependent on attractant treatment, local MPB population density and their interaction but models used a beta distribution (link = logit).

We also visualized the effect of local population density on the weekly number of MPB caught by plotting weekly averages for each attractant treatment for sites with the highest (Fig. 1A, C, E) and lowest (Fig. 1B, D, F) previous year's ADS separately (Table 1). To then analyze whether there was seasonality of timing in catch patterns, we looked at

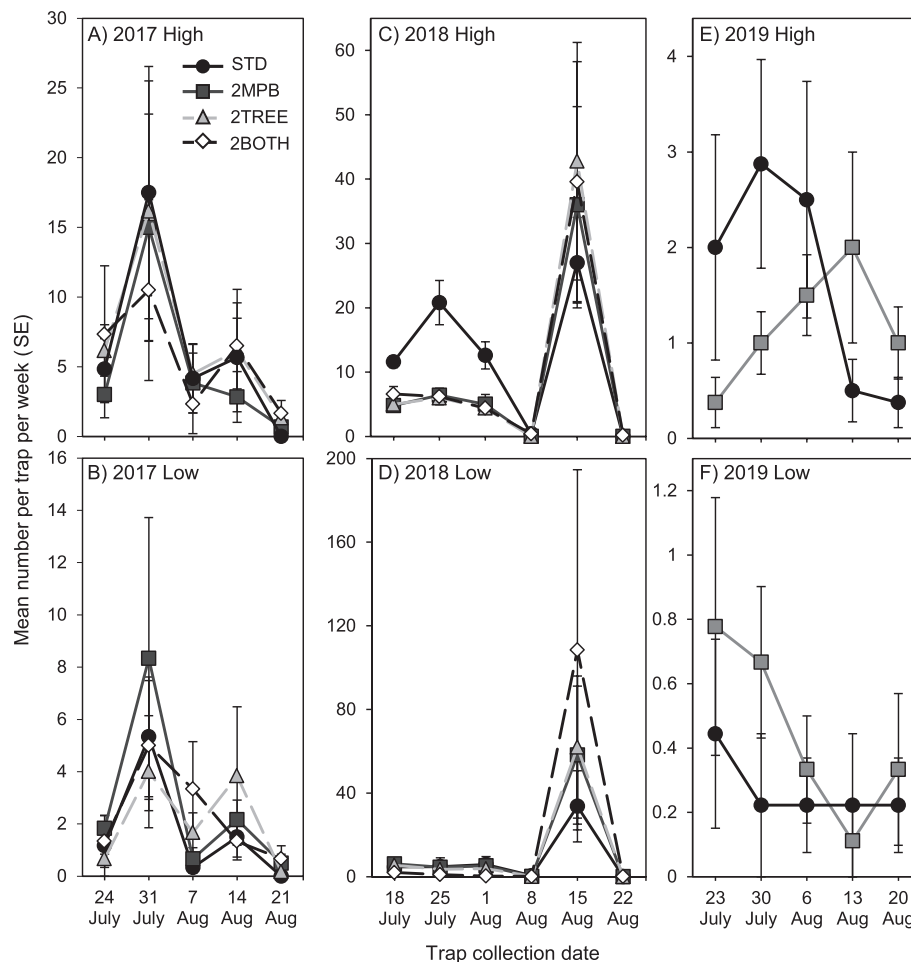


Fig. 1. Weekly average trap catches of *Dendroctonus ponderosae* (MPB) across treatments in 2017 (A and B), 2018 (C and D) and 2019 (E and F) in high (A, C, E) and low (B, D, F) population densities (See Table 1). The release ratios based on commercially available release rates for MPB pheromones *trans*-verbenol, *exo*-brevicomin and host monoterpenes in lures are: 1:1:1 for standard (STD), 2:1:1 for high *trans*-verbenol (2MPB), 1:1:2 for high host monoterpenes (2TREE), and 2:1:2 for high *trans*-verbenol and host monoterpenes (2BOTH). Only STD and 2MPB tested in 2019. Error bars are SE.

the number of MPB caught from the first three weeks and the subsequent weeks in separate analyses. For 2017 and 2019, the last two weeks were considered the second half of the flight period, while for 2018, only the fifth week was analyzed due to very low number of MPB in the fourth and sixth week (80% and 95% traps did not catch MPB, respectively). Furthermore, we did not catch any MPB the first week of collection (13–20 July 2019) from site 25 and this week was therefore disregarded. A chi square test was used to determine if MPB sex counts differed between attractant treatment during the entire sampling period, first three weeks, and last two weeks (PROC FREQ).

Mixed models with Poisson error structure were performed to identify relationships between attractant and local MPB population and the sum of competitor and predator beetles of MPB from one block from all sites during 31 July and 14 August 2017 and the first three weeks of the 2018 collections.

3. Results

3.1. Mountain pine beetle catches

In 2017, a total of 984 MPB were caught over the entire sampling period across a range of local MPB population levels (number of MPB-killed trees within 7 km radius ranged from 17 to 133; Table 1). In the five-week trapping period, there was a significant effect of attractant (lure type III test $F_{(3,222)} = 2.95$, $p = 0.034$) and interaction between attractant and estimated local population ($F_{(3,222)} = 3.40$, $p = 0.019$),

which indicates that the effect of attractant treatment on the number of MPB varies across local population densities. This interaction effect was also significant in the first three weeks (Fig. 2A), with high *trans*-verbenol (2MPB) performing the best in low populations and with low *trans*-verbenol (2TREE and STD) performing best at high population densities. In comparison, there was no effect of attractant nor local population density on the number of MPB for the last two weeks of the trapping period (Fig. 2B). Furthermore, there was no effect of attractant nor local population density on the number of females or males caught during the entire sampling period, first three weeks, or last two weeks ($p > 0.05$).

In 2018, a total of 3066 MPB were caught over the six sampling weeks across a range of local population densities (number of MPB-killed trees within 7 km radius ranged from 3 to 97; Table 1). The date of 15 August 2018 recorded what we suspect was a major inflight of MPB (total of 2301 MPB caught across all traps) from outside the local population and accounted for 75% of the total MPB caught in 2018 (Fig. 1C, D). Models testing the effect of attractant treatment and local population density on MPB catches over the total trapping period and the fifth week were not significant ($p > 0.05$). However, the effect of attractant treatment (lure type III test $F_{(3,115)} = 10.60$, $p < 0.001$) and the interaction effect with local population density was significant in the first three weeks (Fig. 3A). The performance of the attractant treatment with a combination of both low *trans*-verbenol and low host volatiles (STD) increased with increasing population density and caught the most MPB at highest local population density. In contrast, the

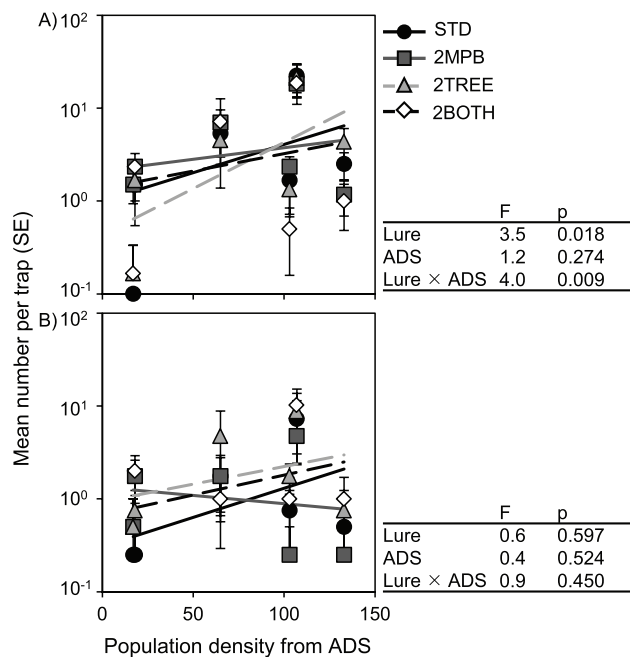


Fig. 2. Relationship between population density from aerial detection survey (ADS) and attractant treatments (lure) on average trap catches of *Dendroctonus ponderosae* (MPB) in 2017 in the first three weeks (A) and last two weeks (B). Release rate details for the attractants are reported in Fig. 1 caption. Error bars are SE. F-values and their associated p-values for the effect of attractant treatment, ADS, and interaction of attractant and ADS are presented.

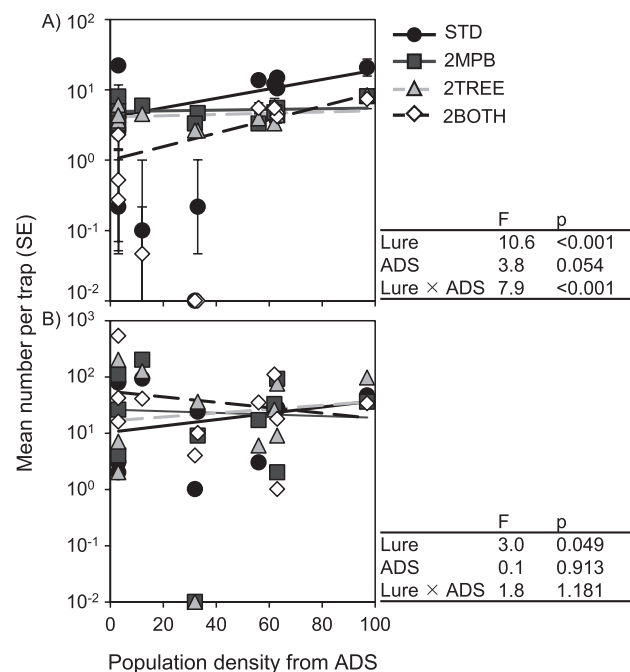


Fig. 3. Relationship between population density from aerial detection survey (ADS) and attractant treatments (lure) on average trap catches of *Dendroctonus ponderosae* (MPB) in 2018 in the first three weeks (A) and fifth week (B). Release rate details for the attractants are reported in Fig. 1 caption. Error bars are SE. F-values and their associated p-values for the effect of attractant treatment, ADS, and interaction of attractant and ADS are presented.

attractant treatment with the combination of both high *trans*-verbenol and high host volatiles (2BOTH) caught the least MPB at low local population densities. The number of female MPB caught during the first three weeks followed the same pattern as total MPB (lure × population type III test $F_{(3,97)} = 3.10$, $p = 0.030$). However, the number of male MPB was greatest in the low *trans*-verbenol and low host volatiles (STD) attractant treatment across all local population densities (lure type III test $F_{(3,98)} = 6.83$, $p < 0.001$) and catches increased for all attractant treatments with increasing population densities (population type III test $F_{(1,98)} = 10.67$, $p = 0.002$).

In 2019, a total of 145 MPB were caught at the two sampling sites. In the five-week trapping period, there was an effect of ADS (type III test $F_{(1,151)} = 8.17$, $p = 0.005$), with 78% of total MPB caught in the site with high population density (162 MPB-killed trees within 7 km radius). There was a similar pattern with number of males but not females caught during the five weeks. The interactions between attractant treatment and ADS were significant in the first three weeks for the total number of MPB (Fig. 1E, F, A), females, and males. The attractant treatment with low *trans*-verbenol (STD) caught significantly more MPB in the high local population site compared to STD in the low-density site (t-value = -3.78 , $p = 0.002$) along with the high *trans*-verbenol attractant (2MPB) in either site (low-density site t-value = -2.96 , $p = 0.020$; high-density site t-value = -3.29 , $p = 0.008$; Fig. 4A). There was no effect of attractant or ADS on number of MPB in the last two sampling weeks (Fig. 4B).

3.2. Sex ratios

In 2017, 472 female and 512 male MPB were caught (damage precluding sex determination of one individual). In the chi-square analysis, sex ratio did not vary with attractant treatment during the entire sampling period, first three weeks, nor last two weeks ($p > 0.05$). However, there was a significant interaction effect of

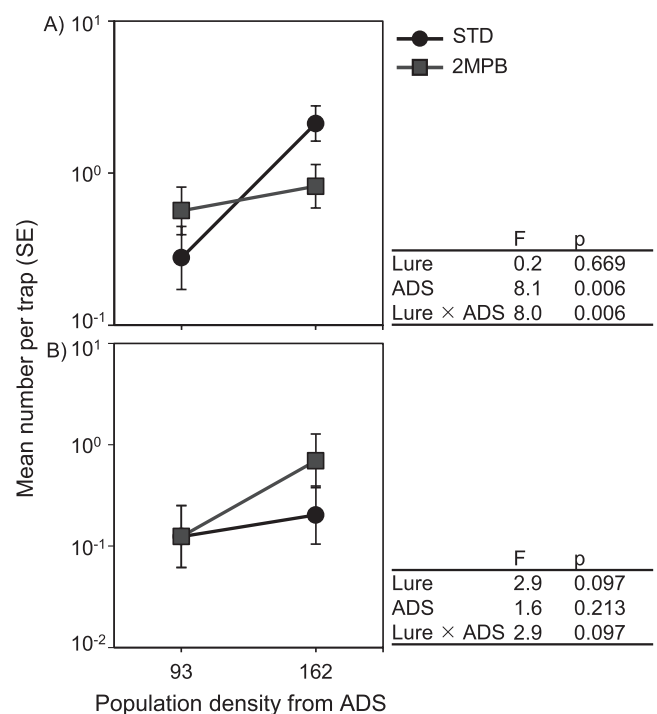


Fig. 4. Relationship between population density from aerial detection survey (ADS) and attractant treatments (lure) on average trap catches of *Dendroctonus ponderosae* (MPB) in 2019 in the first three weeks (A) and last two weeks (B). Release rate details for the lures are reported in Fig. 1 caption. Error bars are SE. F-values and their associated p-values for the effect of attractant treatment, ADS, and interaction of attractant and ADS are presented.

attractant treatment and local population density for the proportion of females caught during the entire sampling period (type III test $F_{(3,72)} = 3.10$, $p = 0.032$) and the first three weeks (type III test $F_{(3,41)} = 4.51$, $p = 0.007$), which showed that the proportion of females dropped with increasing local population density for attractant treatments with high *trans*-verbenol (2MPB and 2BOTH), with no change in the treatments with low *trans*-verbenol (2TREE and STD).

For 2018, we sexed 425 females and 329 males from the first three weeks of sampling (damage precluding sex determination of three). As with 2017, the sex ratio did not vary with attractant treatment for the entire sampling period and the first three weeks ($p > 0.05$). The proportion females caught were also not dependent on attractant treatment nor local population density ($p > 0.05$). We also sexed 892 females and 290 males from the fifth week of trap catches (51% of the 2301 total MPB caught). During the fifth week, STD treatment caught the lowest proportion of females (0.69) compared to 2TREE (0.81), 2MPB (0.77), and 2BOTH (0.75) ($X^2_{(3, 1180)} = 10.32$, $P = 0.016$). The sex ratio differed between the first three weeks and the fifth week ($X^2_{(1, 1934)} = 77.58$, $P < 0.001$), with the fifth week having greater proportion females (0.76) than the first three weeks (0.56).

In 2019, 80 female and 64 male MPB were caught (damage precluding sex determination of one individual). The sex ratio did not vary with attractant treatment during the entire sampling period, first three weeks, nor last two weeks ($p > 0.05$). Similarly, there was no effect of attractant or ADS on the proportion of females caught ($p > 0.05$).

3.3. Competitors and predators

In 2017, 52 competitor and 29 predator beetles were counted from traps in one block per site in the two peak weeks of MPB trapping (second and fourth weeks) (Table S1). The number of competitor and predator beetles did not vary with attractant treatment nor local population density ($p > 0.05$).

In 2018, a total of 118 competitor and 239 predatory beetles were counted in the first three trapping weeks and the fifth week (Table S2). In the first three weeks, neither attractant nor local MPB population density had an effect on catches of competitors or predators ($p > 0.05$). Despite the large increase in MPB caught in the fifth week (15 August 2018), the average number of competitor and predator beetles caught per trap was 2.5 and 1.9 times greater, respectively, in the first three weeks compared to the fifth week (competitors: $F = 8.20$, $P = 0.005$, predators: $F = 9.36$, $P = 0.003$). Competitor and predatory beetles were not recorded for 2019.

4. Discussion

Our study provides evidence that at populations below outbreak levels the attractiveness of the aggregation pheromone and host volatiles differs with the local MPB density as estimated within a 7 km radius by ADS during the first half of the flight period. As population densities increase, MPB preference during the first portion of the flight period shifts from high release rate to low release rate of *trans*-verbenol. Across densities tested, a low release rate of terpinolene and myrcene seems to be more attractive to MPB than a high release rate of the same monoterpenes during the first half of the flight period. The changes in MPB response between low and high release rates of *trans*-verbenol is likely influencing host colonization of MPB to optimize attack density on trees (Raffa and Berryman, 1983; Berryman et al., 1985; Lindgren and Raffa, 2013). Moreover, MPB preference to the same attractants change during the later portion of the flight period. Overall these results suggest that MPB population density should be factored into monitoring MPB activities using traps baited with synthetic attractants when MPB densities are below outbreak threshold and that trapping activities should concentrate on early season flight.

Endemic vs. incipient-epidemic population phases in the historical distribution of MPB are characterized as having different interactions

with host trees (Amman, 1984; Safranyik and Carroll, 2006). Our results provides additional evidence of population density-dependent behavioral preference between these population phases identified in the historical distribution of MPB (Miller et al., 2005; Burke and Carroll, 2017) and other *Dendroctonus* spp. (Wallin and Raffa, 2004). As population densities increase, the breadth of the suitable hosts also increases to more well-defended trees (Wallin and Raffa, 2004; Safranyik and Carroll, 2006; Burke and Carroll 2017). In low densities and during the first half of the flight period, MPB were proportionally more attracted to a high release rate of *trans*-verbenol (2.8 mg d^{-1}) as a way to overcome host defenses which would likely be more difficult to achieve due to Allee effect in low MPB populations (Liebhold and Tobin, 2008). Similarly, Miller et al. (2005) showed a positive dose-dependent response of endemic phase MPB to increasing release rates of *cis*-/*trans*-verbenol from 0.12 to 2.58 mg d^{-1} . In contrast, in high densities, we observed a shift in MPB preference towards a low release rate of *trans*-verbenol (1.4 mg d^{-1}) during the first portion of the flight period. The preferential shift from high to low release rate of *trans*-verbenol likely promotes colonization of a large number of trees, rather than attacking on few heavily attacked trees. These results reflect a possible switch in MPB behavior from relying on their ability to find individual or small groups of attacked trees to large groups of successfully attacked trees. Thus, the density-dependent attraction to aggregation pheromones, which can facilitate selection of optimal densities on single trees to maximize fitness (Raffa and Berryman, 1983; Berryman et al., 1985), can also be a mechanism that contributes to the control of population dynamics in both the naïve habitat and the historical distribution of MPB.

High release rates of host monoterpenes consistently caught less MPB in traps at low local population densities during the first half of the flight period. This is in agreement with some observed bark beetle responses reported in relation to the host defense chemistry in literature. First, high release rates of host volatiles likely convey to MPB that a tree may be well defended and thus should be avoided (Raffa and Berryman, 1983; Lindgren and Raffa, 2013). In fact, Burke and Carroll (2017) have recently demonstrated that MPB offspring from low density parental populations preferred host trees with low monoterpene concentrations compared to offspring from outbreak parental populations, suggesting that maternal population conditions can reinforce attack behavior across generations. Second, while increased release rates of host volatiles can inhibit attraction of some bark beetle species to their pheromones (Erbilgin et al., 2003, 2007), MPB has either shown no inhibition or increased response to increasing release rates of terpinolene and myrcene (Miller and Borden, 2000; Borden et al., 2008). Our results showed a lack of synergism by host volatiles to MPB pheromones when release rates of host volatiles are high. Further research is needed to understand what pre- and post-dispersal mechanisms are behind this density-dependent responsiveness in MPB to pheromone and host volatiles.

Female MPB initiate the host colonization process and thus their choice drives where the next trees will be colonized; hence their attraction to semiochemical cues is critical for understanding MPB population dynamics. We did not find a statistical difference in the sex ratio of MPB between the high and low release rates tested, suggesting that they can equally be attractive to female MPB across the population densities tested. However, in 2017 we found a decrease in the proportion of females with increasing population density for high release rate of *trans*-verbenol. While Miller et al. (2005) reported females had higher sensitivity to *cis*-/*trans*-verbenol than males in the low density population they tested, we found equal sex ratios which suggests that both sexes have the same attack optimality curves in the sub-outbreak population densities tested in the current study. In addition, we found a greater proportion of females in 15 August 2018, which was a result of a large inflight from the current outbreaking populations in Jasper National Park, about 200 km southwest of our study sites. The high wind speed and southwesterly direction measured by weather stations in the region on 14 August suggest the source of the exceptionally large

MPB catches were not local populations but instead from the large outbreak nearby (<https://agriculture.alberta.ca/acis>).

We show that detecting MPB using the same pheromones may not be equally effective throughout the MPB flight period in a given season. Studies have reported evidence of seasonal shifts in pheromone preference in some bark beetle species (Birch, 1974; Roberts et al., 1982; Teale and Lanier, 1991; Miller et al., 1997; Aukema et al., 2000; Steed and Wagner, 2008). These response shifts may reflect a range of changes in host conditions, insect physiology, or environmental factors throughout the flight period (Roberts et al., 1982). Alternatively, whether parent adults have re-emerged and established a second brood may also impact responses to pheromones. Establishment of second brood was documented in MPB during warmer summers (Reid, 1962). In our study, there was evidence of reduced response to a low release rate of *trans*-verbenol later in the flight period, which also corresponds to lower number of MPB caught across all attractant treatments (3%, < 1%, and 11% of the total catch in 2017, 2018 and 2019 on or after 20 August, respectively). While longevity of attractants could impact catch numbers, they were formulated to last beyond the time tested in the field (Personal comm., David Wakarchuk, Synergy Semiochemicals Inc.). The MPB searching for hosts after a peak flight period are likely responsive to high release rates of aggregation pheromones to locate what suitable host is left. Therefore, reduced responses to low release rate of *trans*-verbenol by MPB late in the flight period may optimize host tree colonization.

The transition of MPB population phases from low to incipient-epidemic densities can also be facilitated or impeded by populations of competitors and natural enemies (Carroll et al., 2006; Safranyik and Carroll, 2006; Tabacaru and Erbilgin, 2015). In this study, the number of beetles from families with species known to be competitors or natural enemies of MPB were low and did not differ among treatments. While our functional categorization of insects by their family is coarse, the woodboring beetles (Cerambycidae) and secondary bark beetle species (Scolytinae) are major competitors and their numbers were similar across attractant treatments and MPB densities. Furthermore, we found few major predators of MPB and other bark beetles, such as clerids (Cleridae) and flies in the family Dolichopodidae (Diptera) (Amman and Cole, 1983; Safranyik et al., 2010). These patterns suggest that the natural enemies in our study area are acting more as generalists in terms of responding to both MPB pheromones and tree monoterpenes.

We would like the readers of the journal to consider one possible shortcoming of the current study. We used ADS to estimate the number of killed trees within a 7 km radius, which may not be an ideal approach to observe MPB responses at small scales (stand level). A recent study conducted in western U.S.A. showed that the identification of MPB-caused mortality was very accurate from aerial surveys due to high host specificity although aerial surveys can under-estimate individual and small patches of attacked trees (Coleman et al., 2018). While the ADS used in the current study was ground checked, there is still the possibility of an under-estimation of MPB local populations. Nevertheless, consistent differences in attraction behavior across sites found over three years suggest that aerial surveys are a good proxy to estimate MPB population densities at the given scale, supporting Coleman et al. (2018).

4.1. Management implications

Our results suggest that MPB population density should be considered when selecting attractants for monitoring and detection purposes and that additional research into bark beetle density-dependence and underlying mechanisms are needed. For example, using attractants with a range of release rates of *trans*-verbenol may be informative to monitor emerging incipient-epidemic populations. Additionally, where populations of MPB are high enough that operational control programs may be useful, our results support that a lure with a low release rate of *trans*-verbenol and standard host volatiles and *exo*-brevicomin could be

effective to concentrate MPB attacks into a defined area as part of a suppression effort (Gray and Borden, 1989). When MPB populations are below outbreak levels, monitoring and detection efforts should also consider focusing on the first half of the flight period due to the higher responses of MPB to *trans*-verbenol earlier in the flight period. Furthermore, for the optimal detection of MPB in areas with populations below outbreak levels, especially in the expanded eastern range, we recommend the standard commercially available attractant in sites with high population densities and a combination of high release of *trans*-verbenol plus standard host volatiles and *exo*-brevicomin at low density sites. Therefore, manipulating the release rate of *trans*-verbenol may become a useful tool to meet a number of different management objectives.

CRediT authorship contribution statement

Jennifer G. Klutsch: Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Gail Classens:** Investigation, Writing - review & editing. **Caroline Whitehouse:** Methodology, Resources. **James F. Cahill:** Writing - review & editing, Supervision. **Nadir Erbilgin:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2020.118257>.

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