

Navigating the semiochemical landscape: attraction of subcortical beetle communities to bark beetle pheromones, fungal and host tree volatiles

Response to Reviewer 2 Comments

Thank you very much for taking the time to review this manuscript. Please find the detailed response below and the corresponding revisions/corrections highlighted in red.

Comment 1: Line 49-50: In the sentence “Species-specific pheromones are commonly used to monitor tools economically important bark beetle species” , “tools” should be deleted.

Response 1: Thank you for your suggestion, we removed “tools” from line 49.

Comment 2: In 2.1 Experimental design, why the authors did not test the synergistic effects of MPB pheromones and SVCs ? Also, FVOCs, SVCs and MPB pheromone together? Need explanation.

Response 2: Thank you for your comment, we would like to further clarify our approach. The field testing was conducted based on the amount of information available for us. We had different levels of information for FVOCs and SVCs. Our previous experiment (Zaman et al. 2023b) found MPB were strongly attracted to FVOCs emitted by two fungal symbionts of MPB (*Ophiostoma montium* and *Grosmannia clavigera*) in bioassays. Therefore, it was a logical to further test these FVOCs in the field and determine whether they could synergize the attraction of MPB to pheromone lures. However, our study was the first trial testing the attraction of bark beetles and other subcortical beetles to SVCs of fungal species associated with MPB in the field. It would be premature to assume that SVCs would synergize MPB attraction to its pheromones. In our next phase of the ongoing experiments, we will test fewer SVC compounds with or without MPB pheromone.

Comment 3: Line 143-144, the authors refer that “these traps were placed at a different location from the other two experiments to avoid possible spillover effects from the use of the MPB lure.”, but the premise was the population density of MPB should be similar between different experiment sites, please confirm this.

Response 3: Thank you for your comment, traps with MPB lures were placed at a different site (our Site 1) from the FVOC and SVC alone experiments (Site 2). This was necessary to avoid spillover effects of the lures. However, these two sites were very close (640m) and had very similar characteristics. Due to the proximity of the sites, it is most likely that we were trapping the same population of MPB at both sites, and the density of MPB at each site was determined to be the same (low) before starting our experiments.

Comment 4: Line 146-148, and line 169-170, figure 1d, why used different types of traps? Your aim was to test the difference of chemical signals, so the authors should use the similar type of traps, or you could not compare them theoretically. Also in figure 1c, did the author consider

about the edge effect of trap setting? Why not the author test FVOCs and SVCs with pair wise setting in experiment 2?

Response 4: Overall, we needed 21 traps in each study. Due to the limited number of traps available in either trap type, we decided to use different traps in different experiments. Since we did not compare the results between the two studies, using different traps were reasonable.

Edge effect was not the case in either studies because both studies were conducted in clear cut blocks. We are not really clear what the referee refers to 'FVOCs and SVCs with pairwise setting'. Due to large number of baits used in each test, having too many treatments in the same experiment may not be reasonable.

Comment 5: In Table 2, the author used $\mu\text{l/ml}$ to describe the concentration, maybe the author better used mole quantities.

Response 5: We used the concentrations reported in our earlier study. Also, we are not familiar with the approached suggested by the reviewer.

Comment 6: Line 189-190, line 193-194, and line 199, three traps for each treatment, I know this was a labor costing work in the mountain area, but three replicates may not enough.

Response 6: Thank you for your comment. We had three replicates for each treatment and 4 weeks of collections (5 collections minus collection 2), which brings the spatio-temporal replicates to 12 for each treatment throughout the entire field season. We believe this is sufficient for a field experiment testing these chemicals for the first time since our primary goal was to identify chemicals of interest for further experiments. Any chemicals of interest will require further field testing before being implemented as semiochemical tools. We make sure to highlight this limitation in our discussion, for example: "Therefore, these compounds may be useful semiochemicals when combined with MPB lure to attract low-density MPB populations in the field, though further research is required to confirm their field efficacy" (L: 394).

Comment 7: Line 216: "mixed at different concentrations depending on the stress agent (Table 3, [16])", I could not find any description in table 3. Line 217, what were the seven treatments? May be you should refer it to table 4.

Response 7: Thank you for your comment. The reference to Table 3 is to provide more details about the exact chemicals used in our SVC blends and does not include more details about the SVC profiles themselves. We agree that the exact treatments used for the SVC experiment should be clarified and further highlighted in the methods. We added more details about the SVC profiles in the methods: "**We tested seven treatments; SVC profiles of three symbiotic fungi, two other common phytopathogens, a healthy tree profile (positive control) and mineral oil (negative control) in three blocks for 21 traps**" (L: 220).

Comment 8: In table 4, the description of SVC should be more detailed.

Response 8: Thank you for your comment, we agree that the Table 4 caption should include a better description our treatments and experiment groups. We added more details to the

caption: “Total catches of subcortical beetle families in field traps over the entire collection period in each volatile experiment. We tested three experiments: (1) fungal volatiles (FVOCs) alone, (2) FVOCs with the addition of mountain pine beetle (MPB) lures, and (3) host stress volatile (SVC) profiles. The SVC treatments include the profiles of lodgepole pine infected by *Atropellis piniphila* (AP), *Grosmannia clavigera* (GC), Healthy tree (Healthy), *Leptographium longiclavatum* (LL), *Ophiostoma montium* (OM), and *Endocronartium harknessii* (EH). In the SVC experiment, the healthy tree profile (Healthy) was used as a positive control and mineral oil alone as a negative control. The fungal volatile (FVOC) blend treatment (Mixture) refers to a synthetic blend of FVOCs based on the *O. montium profile*” (L: 256).

Comment 9: In figure 4, the X-axis title and category were lost in a) and b)

Response 9: Thank you for your comment, the figure 4 axes for a) and b) were purposely excluded since the graphs below have the same axes. We believe this reduces the complexity of the figure and makes the results easier to interpret.