

Response to Anonymous Referee #1

We thank the reviewer for her/his supportive and constructive comments. We have revised the manuscript in several instances to address the reviewer's concerns, and believe the paper is stronger as a result. This response will address the concerns in the order they were raised. Reviewer comments are in bold italics, with the authors' responses indented below.

General Comments

This manuscript is timely and it has important approach to elucidate the role of biotic stresses for induced emissions of volatile terpenes from conifers. Introduction is covering rather comprehensive the current knowledge of different type of stresses on plant VOC emissions and their potential as precursors of secondary organic aerosols. Elicitor compound methyl jasmonate (MeJA) which affects very efficiently the biosynthesis of terpenoids was selected as to simulate herbivore impact on five conifer species. This manuscript could also have value for environmental impact assessment of modern preventive pest control methods where plant defences are activated with elicitors before pest insect attack. Earlier observations of MeJA treatments on conifers have demonstrated that climate-relevant sesquiterpenes and GLV compounds can be even more responsive to elicitor than monoterpenes (Semiz et al. 2012).

Thank you for this positive feedback and the relevant reference. We will take this into consideration for designing future projects.

Selected GC-MS-FID methodology to assess VOC emissions in different time point is excellent and gives valuable data of monoterpene emission profiles of studied conifer species. Unfortunately, the experimental set up has some serious flaws and does not meet e.g. the requirements of ecological or plant science journals of genuine biological replicates. Experiment with each species is run only once and in VOC studies three out of five species did not even have the control group of plants where to compare the effect elicitor treatment.

We agree that limited replicates for each tree type would be a major limitation of this study if the primary goal were to derive detailed mechanistic algorithms describing plant emission responses to herbivore treatment. However, the larger objective of this project was to investigate effects of herbivory stress on the composition of secondary organic aerosol from biogenic volatile organic compound emissions. With that objective in mind, we chose to prioritize diversity of represented tree species rather than maximize replicates from each tree type. We made this decision because the published data on this topic is severely limited, and we wanted to identify "key" tree species that might potentially demonstrate a large SOA response to herbivory treatment. This information is

needed to help inform future research directions in this field. To clarify this objective, we have added this statement to the introduction to clarify the rationale of this experimental design:

“This study was a component of a project that investigated the effects of herbivory stress on the composition of biogenic secondary organic aerosol generated from BVOC emissions. Published data on this topic is extremely limited, so one goal of this work was to identify “key” tree species that could produce a large herbivore-treatment effect on SOA composition.”

An additional sentence was added to the end of section 2.1:

“Emphasis in the experimental design was on the diversity of representative tree species included, which limited the number of replications that were possible.”

Despite having limited replicates, the detailed on-line GC-MS-FID results published in this paper provide valuable continuous monitoring of speciated monoterpene emission rates. Many of the previous post-herbivory BVOC measurements have provided much lower time resolution of speciated monoterpene emission rates and substantially lower number of measurements due to limitations involved in other sampling and analytical techniques—such as cartridge sampling for example. Where more highly time-resolved measurements are given, the chemical detail is often reduced—such as analytical approaches using PTR-MS for example. We believe the continuous monitoring results presented here are a highly valuable addition to the current literature despite limitations in replicates of the same tree types.

In the case of control treatment, it was not run at the same time as elicitor treatment. Therefore the main approach to compare VOC emission before and after elicitor treatment does not allow estimating the impact of elicitor treatment on VOC emission rates and separate the time depended fluctuation of VOC emission rates from elicitor depended fluctuation.

We agree that an ideal set-up would include two plant chambers: one with a set of treatment trees and one with a set of control trees with continuous BVOC monitoring in both chambers simultaneously. However, as the reviewer describes in a later comment, genotypic variation between plants can result in substantial differences between constitutive emission profiles creating significant complications when comparing emissions between two different sets of trees. As a result, we decided the significant addition of time and resources to simultaneously run a second control chamber would not be justified here. Instead, when a negative control experiment was performed, a methyl jasmonate treatment experiment was always performed with the same tree species within

two weeks (see Table 1). Any season-dependence on elicitor response thus should have been minimized when comparing the two experiments.

It is explained that this study is actually aimed for studies of stress effects on the composition of subsequently formed secondary organic aerosols and results will be published in a separate paper. This nearly unexplored area of biotic stress effect on atmospheric SOA formation in a companion paper will definitely add the value of this manuscript.

Thank you for this comment. We also think these two papers together will be a valuable contribution. We have improved the cross-referencing between the manuscripts to make it easier for readers to compare the particle composition results with the matching BVOC profiles presented in this paper. We did this by adding more detailed references to the companion paper in the experiment summary table, Table 1, where we direct readers to the SOA composition experiments in the companion paper that correspond to the BVOC profiles presented in this paper.

Specific Comments

P. 13461, Line 27. If already published, give a citation here.

We have added a citation to the companion paper that is currently under consideration for publication in Atmospheric Chemistry and Physics:

Faiola, C. L., Wen, M. and VanReken, T. M.: Chemical characterization of biogenic SOA generated from plant emissions under baseline and stressed conditions: inter- and intra-species variability for six coniferous species, Atmos. Chem. Phys. Discuss., 14(18), 25167–25212, doi:10.5194/acpd-14-25167-2014, 2014.

P. 13462, L. 14. If there were clear symptoms of natural stressor in some of the plant where the most influenced plants included in the experiments? If included, it might give some bias in the results.

The plant storage approach could lead to some exposure to natural stressors as the reviewer points out. However, storing plants in an unnatural environment, such as a greenhouse, could also produce unnatural plant behavior not representative of their emissions in a more natural environment. We decided that storing the plants outside was the most appropriate method for the overall objective of the project, despite the possibility that this could lead to an uncontrolled natural stress exposure. Again, the overall objective of this project was to perform the first investigation of the effects of

herbivory on biogenic SOA composition from a wide variety of plants. In the natural environment, exposure to multiple stressors is likely the rule rather than the exception (Holopainen and Gershenson, 2010). Thus, the possibility of uncontrolled stress exposure does not detract from the ultimate objective, which is to understand the effects of herbivory on both BVOC emission profiles and biogenic SOA composition generated from those plant emissions. Furthermore, only one group of plants displayed clear symptoms of uncontrolled stress exposure, *Abies grandis*. This was noted in the manuscript on page 13469 L. 1-2 and was further discussed in detail in Section 3.5 (p. 13477-13480). To further clarify the point, we have revised the wording in the methods section from this (p. 13462, L. 11-15):

“They were stored outside of the greenhouse to be closer to their natural environmental conditions. This also meant the plants could have been exposed to natural stressors (e.g., heat or herbivory). These natural stressors were not controlled but would be representative of conditions encountered by the plants in natural environments.”

To this:

“They were stored outside of the greenhouse to be closer to their natural environmental conditions and prevent unnatural plant emission behavior that could occur within greenhouse conditions. This also meant the plants could have been exposed to natural stressors (e.g., heat or herbivory). These natural stressors were not controlled but would be representative of conditions encountered by the plants in nature because it is likely that exposure to multiple stressors is the rule rather than the exception in a forest environment (Holopainen & Gershenson, 2010).”

P. 13468, L. 23. This is what one should expected, when studying another provenance of the same tree species. Merely the genotypic variation without any elicitor treatment affects the ratio of monoterpenes in conifers.

Thank you for this useful information. We have added a sentence to the paragraph clarifying that genotypic variation between plants of the same tree species can result in this level of variation in BVOC emissions. The text now reads:

*“The profile from *Abies grandis* in this study was dominated by beta-pinene, but no beta-pinene was observed by Ortega et al. (2008). This difference could be explained by natural genotypic variation because Ortega et al., (2008) also observed natural variation in the constitutive BVOC profiles between plants of the same tree species. However, the *Abies grandis* monoterpenoid pre-treatment BER measured in our experiment was $12.67 \mu\text{g-C g}^{-1} \text{ h}^{-1}$, substantially higher than any other pre-treatment monoterpenoid BER observed in this study and more than an order of magnitude*

greater than that reported by Ortega et al. (2008) for the same tree species. These high emission rates could suggest the Abies grandis saplings were likely exhibiting a stress response prior to treatment.”

P. 13470, L.5-15. This is very odd choice of methodology. After stressor treatment exactly the same seedlings were used as water-treated and then again as stress-treated seedlings. Why? During active shoot growth in early season VOC synthesis is found to be more responsive to elicitors in Pinus sylvestris than after ceasing of elongation growth. This might be the case also with Picea pungens.

We tried to minimize the variation in emissions due to genotypic variation between plants by using the same set of plants. This way, any changes in emission profiles and emission rates could be attributed to either a seasonal-dependence on emissions or the possibility of a natural stressor exposure. We waited two months after first stressor treatment before using the plants again to allow the initial treatment response to subside. We agree that one explanation for the differences observed in the *Picea pungens* MeJA response could be attributed to a seasonal effect. Thank you for this valuable information about known elicitor responses of *Pinus sylvestris*. We have added a statement after P. 13472 L. 1-2, which currently reads:

“In contrast to the May experiment, in the July Picea pungens experiment the monoterpenoid average profile did not significantly change after treatment (Figure 3).”

The added statement and citation read as follows:

“This could be due to seasonal differences in the sensitivity of Picea pungens to herbivore-treatment. This has been observed in other coniferous plant species. For example, monoterpene synthesis in Pinus sylvestris is more responsive to plant stressors during the spring when shoots are actively growing (Bäck et al., 2005).”

Reference

Holopainen, J. K. and Gershenzon, J.: Multiple stress factors and the emission of plant VOCs, Trends in plant science, 15(3), 176–184, 2010.