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Atmospheric Chemistry and Physics Discussions
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# *Interactive comment on* "Role of needle surface waxes in dynamic exchange of mono- and sesquiterpenes" *by* J. Joensuu et al.

**J. Joensuu et al.**

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Dear Referee, We thank you for your thorough work and valuable comments. We have made of the suggested changes, as we feel they clearly improved the quality of the manuscript.

Comments from Referee

General comments: Authors have measured whole shoot-level mono- and sesquiterpene emissions of Scots pine seedlings and analysed the needle surface waxes for the same compounds. The aim of the work was to determine if the same terpenes can be found on the epicuticles as in shoot emissions. This approach is needed to better understand the mechanisms how plant release BVOCs in the atmosphere and if there is a temporal storage of BVOCs on plant surfaces.

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Discussion paper

Main observations were that shoot emissions and wax extracts were dominated by monoterpenes and the proportion of some sesquiterpenes was higher in the wax extracts than in whole shoot emissions. Authors have discussed about the pathways of mono- and sesquiterpenes to needle cuticle also considering external sources. Their conclusion was the “any BVOCs found in the extract were most likely not a result of stomatal emissions but rather compounds that had been associated to the epicuticle”. However, whole discussion is based on the assumption that needle emissions are the only source of needle epicuticular mono- and sesquiterpenes. External redeposition is mentioned, but other possible external sources are not discussed. These might include e.g. emissions from the bark of studied branches or other branches and stem, but also emission from neighboring plants. Authors should mention these other pathways of needle deposition of BVOCs.

Specific comments: P 4, L24. The analysis is based only on four seedlings, so crafted shoots representing he same genotype was a good choice. P5, L12. Air flow in the shoot chambers was rather high. How much this may stimulate monoterpene and sesquiterpene emission from bark? P 6, L 22-23. Three replicate samples were reported. How they were collected? Was each of those composed of 20 needles or were these 20 needles divided to 3 subsamples? P10, L9. Redeposition plant’s own BVOCs on epicuticular waxes might not be the only pathway. Adsorption of sesquiterpenes on epicuticular wax layer from external plant sources and their emission back to atmosphere is reported (Li & Blande 2015). As Scots pine bark is important monoterpene and sesquiterpene emitter (e.g. Ghirardoet al. 2012, Heijari et al. 2011). There could be a possibility that part of detected sesquiterpenes on epicuticular wax may originate from earlier sesquiterpene emission from bark of the focal plant and neighboring plants and adsorbed on needles? P 14. Appendix B. Authors should discuss about potential reason for high variation in monoterpene content in replicate samples within each tree. As the same sample has high emission of all common resin monoterpenes (e.g. tree 2 s3 and tree 4 s2), it may suggest e.g. high bark emission from micro cracks near these needles. Together with high sesquiterpene content in some of the needle samples localized biotic stress by e.g. fungal pathogen or mites might also explain these.

References

Ghirardo et al. (2010) Plant, Cell and Environ. 33, 781–792 Li, T & Blande JD, (2015) Global Change Biology 21, 1993–2004 Heijari et al. (2011) Environ. Exp. Bot. 71, 390–398.

Response to comments

Main observations were that shoot emissions and wax extracts were dominated by monoterpenes and the proportion of some sesquiterpenes was higher in the wax extracts than in whole shoot emissions. Authors have discussed about the pathways of mono- and sesquiterpenes to needle cuticle also considering external sources. Their conclusion was the “any BVOCs found in the extract were most likely not a result of stomatal emissions but rather compounds that had been associated to the epicuticle”. However, whole discussion is based on the assumption that needle emissions are the only source of needle epicuticular mono- and sesquiterpenes. External redeposition is mentioned, but other possible external sources are not discussed. These might include e.g. emissions from the bark of studied branches or other branches and stem, but also emission from neighboring plants. Authors should mention these other pathways of needle deposition of BVOCs.

It is true that the possible sources of the redeposited terpenes are not discussed; this is indeed a valuable remark. We have added a mention of the possible pathways in the discussion.

P5, L12. Air flow in the shoot chambers was rather high. How much this may stimulate monoterpene and sesquiterpene emission from bark?

Since the chamber encloses the whole shoot, some of the emissions measured come from the bark/stem of the shoot, not only the needles. This is true of any shoot measurement done with a similar chamber setup. The biomass inside such a chamber is typically 10-25 % wood material (including bark). The needles are more active terpene emitters than the wood/bark, but there is some evidence of compound-specific variation (Anni Vanhatalo, personal communication). It is also likely that the needles are more susceptible to any air current induced disturbance than the bark.

P 6, L 22-23. Three replicate samples were reported. How they were collected? Was each of those composed of 20 needles or were these 20 needles divided to 3 subsamples?

We took three separate samples of 20 needle pairs each. This information has been added to the text.

P10, L9. Redeposition plant's own BVOCs on epicuticular waxes might not be the only pathway. Adsorption of sesquiterpenes on epicuticular wax layer from external plant sources and their emission back to atmosphere is reported (Li & Blande 2015). As Scots pine bark is important monoterpene and sesquiterpene emitter (e.g. Ghirardoet al. 2012, Heijari et al. 2011). There could be a possibility that part of detected sesquiterpenes on epicuticular wax may originate from earlier sesquiterpene emission from bark of the focal plant and neighboring plants and adsorbed on needles?

It is true that the possible sources of the redeposited terpenes are not discussed; this is indeed a valuable remark. We have added a mention of the possible pathways in the discussion.

P 14. Appendix B. Authors should discuss about potential reason for high variation in monoterpene content in replicate samples within each tree. As the same sample has high emission of all common resin monoterpenes (e.g. tree 2 s3 and tree 4 s2), it may suggest e.g. high bark emission from micro cracks near these needles. Together with high sesquiterpene content in some of the needle samples localized biotic stress by e.g. fungal pathogen or mites might also explain these.

This is a very valuable comment. The variation in the terpene content of the epicutic-

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ular waxes cannot be explained by variation in wax yield (i.e. solvent effectiveness). Even though there is variation in wax yield (per needle area), this variation does not correspond to the variation observed in the terpenes. We do not know of previous studies with similar methodology, so there is nothing to compare to. It is possible that some of the variation was caused by the sampling procedure. Despite the short sampling time, it is possible that the emissions caused by plucking needles had sufficient time to adsorb onto other needles that were subsequently picked into a sample. Other possible causes of variation do indeed include small cracks, insect bites or pathogens, in the bark near some of the needles. Some of these may well have escaped visual inspection. One very likely source is true natural variation between needles grown in different parts of the branch/canopy. Very little is known on this topic, but since terpene synthesis is light-dependent, it is very likely that there are differences (Juho Aalto, personal communication). The wax yields have been added to Appendix B and the possible causes of variation have been discussed more thoroughly.

References

Ghirardo et al. (2010) Plant, Cell and Environ. 33, 781–792 Li, T & Blande JD, (2015) Global Change Biology 21, 1993–2004 Heijari et al. (2011) Environ. Exp. Bot. 71, 390–398.

These references are an excellent addition to the manuscript. They have been used to improve the text and added in the references list.

Changes made in the manuscript based on these comments

P6 L 23: Changed to "...and then took needle samples (three separate samples of 20 needle pairs each) in darkness for the wax analysis."

P9 L26 New paragraph: "The is remarkable variation observed in the terpene content of the epicuticular waxes, and this variation cannot be explained by variation in the amount of extracted wax. Possible natural causes of variation include small cracks, insect bites or pathogens in the bark near some of the needles. E.g. insect bites are known to induce both local and systemic terpene emissions (Heijari et al., 2011). Some of these may well have escaped visual inspection. One feasible source is true natural variation between needles grown in different parts of the branch or canopy, due to the light-delendent nature of terpene synthesis. Very little is known on this topic, but it is very likely that there are notable differences (Juho Aalto, personal communication). Some of the variation, however, may have been caused by the sampling procedure itself. Despite the short sampling time, it is possible that the emissions caused by plucking needles had sufficient time to adsorb onto other needles that were subsequently picked into a sample. "

P11 L8: Rewritten start to first chapter: "In theory, there are three mechanisms for the terpenes produced by a plant to end up on the needle surface. The first one is (dry) redeposition after emission from either the tree itself (needles, bark or other parts) or neighbouring trees. Terpene emission from one plant individual and redeposition onto another has been reported, more markedly for sesqui- than monoterpenes (Himanen et al., 2010, Li and Blande, 2015)."

P11 L26: Changed to: "The second option is transport in the aqueous layer . . . This route is naturally only available to terpenes produced by the needle itself, and the effectiveness of the route depends on the existence of such a continuous water film. . ."

APPENDIX B: Added: Wax yields

References: Added: Ghirardo, A., Koch, K., Taipale, R., Zimmer, I., Schnitzler, J.-P. And Rinne, J. Determination of de novo and pool emissions of terpenes from four common boreal/alpine trees by 13CO2 labelling and PTR-MS analysis, Plant Cell Environ, 33, 781-792, 2010. Heijari, J., Blande, J.D. and Holopainen, J.K. Feeding of large pine weevil on Scots pine stem triggers localised bark and systemic shoot emission of volatile organic compounds, Environ Exp Bot, 71, 390-398, 2011. Himanen, S.J., Blande, J.D., Klemola, T., Pulkkinen, J., Heijari, J., and Holopainen, J.K. Birch (Betula

spp.) leaves adsorb and re-release volatiles specific to neighbouring plants – a mechanism for associational herbivore resistance? New Phytol, 186, 722-732, 2010. Li, T. and Blande, J. Associational susceptibility in broccoli: mediated by plant volatiles, impeded by ozone, Global Change Biol, 21, 1993-2004, 2015.

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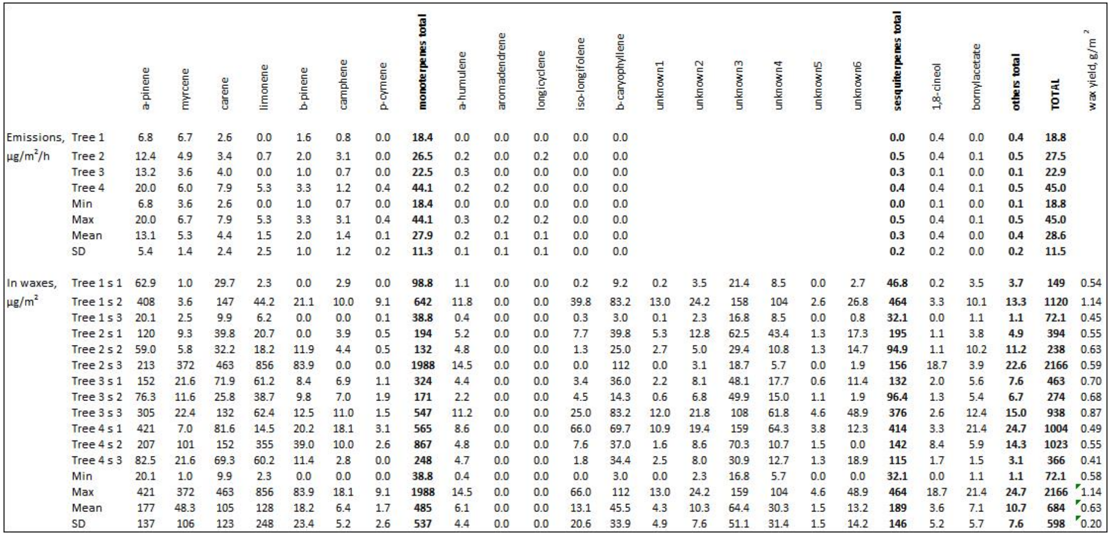

| | | a-pinene | myrcene | carene | limonene | b-pinene | camphene | p-cymene | **monoterpenes total** | a-humulene | aromadendrene | longicyclene | iso-longifolene | b-caryophyllene | unknown1 | unknown2 | unknown3 | unknown4 | unknown5 | unknown6 | **sesquiterpenes total** | 1,8-cineol | bornylacetate | **others total** | **TOTAL** | wax yield, g/m$^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Emissions, μg/m$^2$/h | Tree 1 | 6.8 | 6.7 | 2.6 | 0.0 | 1.6 | 0.8 | 0.0 | **18.4** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | **0.0** | 0.4 | 0.0 | **0.4** | **18.8** | |
| | Tree 2 | 12.4 | 4.9 | 3.4 | 0.7 | 2.0 | 3.1 | 0.0 | **26.5** | 0.2 | 0.0 | 0.2 | 0.0 | 0.0 | | | | | | | **0.5** | 0.4 | 0.1 | **0.5** | **27.5** | |
| | Tree 3 | 13.2 | 3.6 | 4.0 | 0.0 | 1.0 | 0.7 | 0.0 | **22.5** | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | **0.3** | 0.1 | 0.0 | **0.1** | **22.9** | |
| | Tree 4 | 20.0 | 6.0 | 7.9 | 5.3 | 3.3 | 1.2 | 0.4 | **44.1** | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 | | | | | | | **0.4** | 0.4 | 0.1 | **0.5** | **45.0** | |
| | Min | 6.8 | 3.6 | 2.6 | 0.0 | 1.0 | 0.7 | 0.0 | **18.4** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | **0.0** | 0.1 | 0.0 | **0.1** | **18.8** | |
| | Max | 20.0 | 6.7 | 7.9 | 5.3 | 3.3 | 3.1 | 0.4 | **44.1** | 0.3 | 0.2 | 0.2 | 0.0 | 0.0 | | | | | | | **0.5** | 0.4 | 0.1 | **0.5** | **45.0** | |
| | Mean | 13.1 | 5.3 | 4.4 | 1.5 | 2.0 | 1.4 | 0.1 | **27.9** | 0.2 | 0.1 | 0.1 | 0.0 | 0.0 | | | | | | | **0.3** | 0.4 | 0.0 | **0.4** | **28.6** | |
| | SD | 5.4 | 1.4 | 2.4 | 2.5 | 1.0 | 1.2 | 0.2 | **11.3** | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 | | | | | | | **0.2** | 0.2 | 0.0 | **0.2** | **11.5** | |
| In waxes, μg/m$^2$ | Tree 1 s 1 | 62.9 | 1.0 | 29.7 | 2.3 | 0.0 | 2.9 | 0.0 | **98.8** | 1.1 | 0.0 | 0.0 | 0.2 | 9.2 | 0.2 | 3.5 | 21.4 | 8.5 | 0.0 | 2.7 | **46.8** | 0.2 | 3.5 | **3.7** | **149** | 0.54 |
| | Tree 1 s 2 | 408 | 3.6 | 147 | 44.2 | 21.1 | 10.0 | 9.1 | **642** | 11.8 | 0.0 | 0.0 | 39.8 | 83.2 | 13.0 | 24.2 | 158 | 104 | 2.6 | 26.8 | **464** | 3.3 | 10.1 | **13.3** | **1120** | 1.14 |
| | Tree 1 s 3 | 20.1 | 2.5 | 9.9 | 6.2 | 0.0 | 0.0 | 0.1 | **38.8** | 0.4 | 0.0 | 0.0 | 0.3 | 3.0 | 0.1 | 2.3 | 16.8 | 8.5 | 0.0 | 0.8 | **32.1** | 0.0 | 1.1 | **1.1** | **72.1** | 0.45 |
| | Tree 2 s 1 | 120 | 9.3 | 39.8 | 20.7 | 0.0 | 3.9 | 0.5 | **194** | 5.2 | 0.0 | 0.0 | 7.7 | 39.8 | 5.3 | 12.8 | 62.5 | 43.4 | 1.3 | 17.3 | **195** | 1.1 | 3.8 | **4.9** | **394** | 0.55 |
| | Tree 2 s 2 | 59.0 | 5.8 | 32.2 | 18.2 | 11.9 | 4.4 | 0.5 | **132** | 4.8 | 0.0 | 0.0 | 1.3 | 25.0 | 2.7 | 5.0 | 29.4 | 10.8 | 1.3 | 14.7 | **94.9** | 1.1 | 10.2 | **11.2** | **238** | 0.63 |
| | Tree 2 s 3 | 213 | 372 | 463 | 856 | 83.9 | 0.0 | 0.0 | **1988** | 14.5 | 0.0 | 0.0 | 0.0 | 112 | 0.0 | 3.1 | 18.7 | 5.7 | 0.0 | 1.9 | **156** | 18.7 | 3.9 | **22.6** | **2166** | 0.59 |
| | Tree 3 s 1 | 152 | 21.6 | 71.9 | 61.2 | 8.4 | 6.9 | 1.1 | **324** | 4.4 | 0.0 | 0.0 | 3.4 | 36.0 | 2.2 | 8.1 | 48.1 | 17.7 | 0.6 | 11.4 | **132** | 2.0 | 5.6 | **7.6** | **463** | 0.70 |
| | Tree 3 s 2 | 76.3 | 11.6 | 25.8 | 38.7 | 9.8 | 7.0 | 1.9 | **171** | 2.2 | 0.0 | 0.0 | 4.5 | 14.3 | 0.6 | 6.8 | 49.9 | 15.0 | 1.1 | 1.9 | **96.4** | 1.3 | 5.4 | **6.7** | **274** | 0.68 |
| | Tree 3 s 3 | 305 | 22.4 | 132 | 62.4 | 12.5 | 11.0 | 1.5 | **547** | 11.2 | 0.0 | 0.0 | 25.0 | 83.2 | 12.0 | 21.8 | 108 | 61.8 | 4.6 | 48.9 | **376** | 2.6 | 12.4 | **15.0** | **938** | 0.87 |
| | Tree 4 s 1 | 421 | 7.0 | 81.6 | 14.5 | 20.2 | 18.1 | 3.1 | **565** | 8.6 | 0.0 | 0.0 | 66.0 | 69.7 | 10.9 | 19.4 | 159 | 64.3 | 3.8 | 12.3 | **414** | 3.3 | 21.4 | **24.7** | **1004** | 0.49 |
| | Tree 4 s 2 | 207 | 101 | 152 | 355 | 39.0 | 10.0 | 2.6 | **867** | 4.8 | 0.0 | 0.0 | 7.6 | 37.0 | 1.6 | 8.6 | 70.3 | 10.7 | 1.5 | 0.0 | **142** | 8.4 | 5.9 | **14.3** | **1023** | 0.55 |
| | Tree 4 s 3 | 82.5 | 21.6 | 69.3 | 60.2 | 11.4 | 2.8 | 0.0 | **248** | 4.7 | 0.0 | 0.0 | 1.8 | 34.4 | 2.5 | 8.0 | 30.9 | 12.7 | 1.3 | 18.9 | **115** | 1.7 | 1.5 | **3.1** | **366** | 0.41 |
| | Min | 20.1 | 1.0 | 9.9 | 2.3 | 0.0 | 0.0 | 0.0 | **38.8** | 0.4 | 0.0 | 0.0 | 0.0 | 3.0 | 0.0 | 2.3 | 16.8 | 5.7 | 0.0 | 0.0 | **32.1** | 0.0 | 1.1 | **1.1** | **72.1** | 0.58 |
| | Max | 421 | 372 | 463 | 856 | 83.9 | 18.1 | 9.1 | **1988** | 14.5 | 0.0 | 0.0 | 66.0 | 112 | 13.0 | 24.2 | 159 | 104 | 4.6 | 48.9 | **464** | 18.7 | 21.4 | **24.7** | **2166** | 1.14 |
| | Mean | 177 | 48.3 | 105 | 128 | 18.2 | 6.4 | 1.7 | **485** | 6.1 | 0.0 | 0.0 | 13.1 | 45.5 | 4.3 | 10.3 | 64.4 | 30.3 | 1.5 | 13.2 | **189** | 3.6 | 7.1 | **10.7** | **684** | 0.63 |
| | SD | 137 | 106 | 123 | 248 | 23.4 | 5.2 | 2.6 | **537** | 4.4 | 0.0 | 0.0 | 20.6 | 33.9 | 4.9 | 7.6 | 51.1 | 31.4 | 1.5 | 14.2 | **146** | 5.2 | 5.7 | **7.6** | **598** | 0.20 |

**Fig. 1.** Appendix B, new version