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Atmospheric Chemistry and Physics
Discussions

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

J. Joensuu et al.

johanna.joensuu@helsinki.fi

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Dear Referee, We thank you for your thorough work and valuable comments. We have made most of the suggested changes; where this was not possible, a more thorough explanation is given below.

Comments from Referee

General comments: One of the motivations for this study is non-stomatal ozone deposition. Significant non-stomatal ozone fluxes have been frequently observed, but a general explanation has not been given yet. While there are no obvious reaction sites for ozone reaction in leaf surface waxes, dissolved or attached terpenes could react effectively with ozone. This idea has been around for a while, but although it was not entirely supported by first experiments, a thorough characterization of the system is

still missing and might enable more successful experiments in the future. From this background, the present contribution adds importantly to the knowledge of the fate of terpenes shortly after synthesis and their possible role in ozone deposition.

The authors compare the composition of mono- and sesquiterpenes emitted from pine shoots with their abundance in needle waxes. There are common compounds but also some compounds which appear only in one of the compartments. Alternative ways of transport are discussed. Although the general message of the manuscript is clear, the presentation of the results doesn't seem appropriate to me. Fig. 1 C shows results for compounds where no detection limits are given (a-humulene, aromadendrene). Especially the part with missing standards for some of the compounds measured remains weak and the high amount of sesquiterpenes claimed (up to 50%) doesn't seem to be sufficiently corroborated. Which of the three mentioned compounds (cadinene, cubebene, murolene) would be most abundant?

There were also very high differences between repetitions of the same tree (e.g., Tree 2, myrcene: 9, 6, and 372g m-2; Tree 4,limonene: 15, 355, and 60g m-2). While notable variations between the emissions are mentioned in the discussion, these differences are not discussed. Have similarly large differences been reported before or how could they be explained? Could this be an indication that the solvent was not equally effective?

Specific comments: Due to the indicated artifact and some other unexplained structures, Figure 1C is not very fortunate. It should be possible to find a better series of photographs, or sketch to illustrate the relevant features.

P, 5, L. 21: Were the 'handheld pumps' operated by persons and how could they do this evenly for 30 minutes? If they were machine controlled, why were they handheld?

Technical corrections: P. 2, l. 30: 'or', not 'on'

Response to comments

The authors compare the composition of mono- and sesquiterpenes emitted from pine shoots with their abundance in needle waxes. There are common compounds but also some compounds which appear only in one of the compartments. Alternative ways of transport are discussed. Although the general message of the manuscript is clear, the presentation of the results doesn't seem appropriate to me. Fig. 1 C shows results for compounds where no detection limits are given (a-humulene, aromadendrene). Especially the part with missing standards for some of the compounds measured remains weak and the high amount of sesquiterpenes claimed (up to 50%) doesn't seem to be sufficiently corroborated. Which of the three mentioned compounds (cadinene, cubebene, murolene) would be most abundant?

This comment points to Fig 1 C, but judged by the content it is meant to be 2 C, and our response is based on this assumption. There are essentially two points in the comment: 1) We have now added the missing detection limits for a-humulene and aromadendrene. These compounds do not exist in blank samples, but we calculated the blank levels by integrating background noise of the chromatogram. 2) In addition to quantifying compounds known to be emitted from pine shoots, we wanted to search for any indication of possible additional compounds in the waxes. For this reason, we searched the library for candidate compounds for all unidentified large peaks we observed. However, since we did not have the standards, we do not know the actual responses, and an analysis of the possible relative abundances of these compounds is not possible.

There were also very high differences between repetitions of the same tree (e.g., Tree 2, myrcene: 9, 6, and 372g m-2; Tree 4,limonene: 15, 355, and 60g m-2). While notable variations between the emissions are mentioned in the discussion, these differences are not discussed. Have similarly large differences been reported before or how could they be explained? Could this be an indication that the solvent was not equally effective?

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

Specific comments: Due to the indicated artifact and some other unexplained structures, Figure 1C is not very fortunate. It should be possible to find a better series of photographs, or sketch to illustrate the relevant features.

The photographs collaged to produce 1C are quite old, and we know their quality could be better. However, we feel that the image illustrates an important feature not often discussed in literature (the fact that the epicuticular waxes are actually present not just on the surface but all around the epicuticular cell). Unfortunately it is not possible for us to acquire a better photograph, and a drawing would not be sufficiently credible for this purpose.

P, 5, L. 21: Were the ‘handheld pumps’ operated by persons and how could they do this evenly for 30 minutes? If they were machine controlled, why were they handheld?

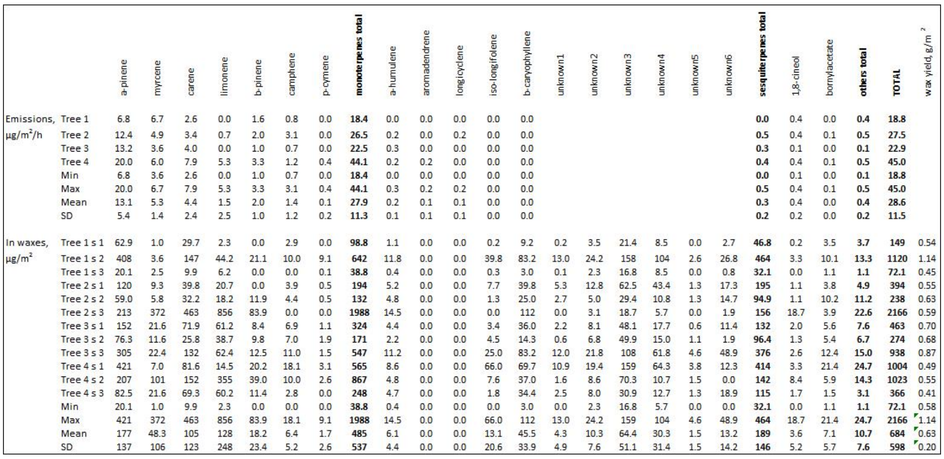
“Handheld” referred actually only to the small size of the pumps; they were batteryoperated. The wording has been changes to “small pumps” to avoid unnecessary confusion.

Technical corrections: P. 2, l. 30: ‘or’, not ‘on’ These mistakes have been corrected, thank you for noticing them!

Changes made in the manuscript based on these comments

P2 L 30: “on” corrected to “or” P5 L 21: “Small pumps were used to pull the sample through the tube (70 ml/min).” (instead of “handheld pumps” P6 L7-8: Added: “The detection limits were . . . 0.05 ng/sample for α-humulene and aromadendrene, . . .” P7 L22: Added: “The limits of detection . . . were 0.15-0.30 ng/sample for . . ., α-humulene, aromadendrene. . .” 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. “ APPENDIX B: Added: Wax yields References: Added: 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.

Interactive comment on Atmos. Chem. Phys. Discuss., doi:10.5194/acp-2015-1024, 2016.



		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