



1 **Role of needle surface waxes in dynamic exchange of**
2 **mono- and sesquiterpenes**

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11

12 **Abstract**

13 Biogenic volatile organic compounds (BVOCs) produced by plants have a major role in
14 atmospheric chemistry. The different physicochemical properties of BVOCs affect their
15 transport within and out of the plant as well as their reactions along the way. Some of these
16 compounds may accumulate in or on the waxy surface layer of conifer needles and participate
17 in chemical reactions on or near the foliage surface. The aim of this work was to determine
18 whether terpenes, a key category of BVOCs produced by trees, can be found on the
19 epicuticles of Scots pine (*Pinus sylvestris* L.) and, if so, how they compare with the terpenes
20 found in shoot emissions of the same tree. We measured shoot-level emissions of pine
21 seedlings at a remote outdoor location in Central Finland and subsequently analysed the
22 needle surface waxes for the same compounds. Both emissions and wax extracts were clearly
23 dominated by monoterpenes, but the proportion of sesquiterpenes was higher in the wax
24 extracts. There were also differences in the terpene spectra of the emissions and the wax
25 extracts. The results, therefore, support the existence of BVOC associated to the epicuticular
26 waxes. We briefly discuss the different pathways for terpenes to reach the needle surfaces and
27 the implications for air chemistry.

28



1 1 Introduction

2 At the border of the atmosphere and Earth's ecosystems, the living layer of vegetation is an
3 active player interacting with its surroundings in multiple ways. Plants absorb, transmit and
4 produce compounds like water, oxygen and carbon, as well as a myriad of more complex
5 molecules such as volatile organic compounds (VOCs). In addition to this biological activity,
6 plant surfaces provide area for adsorption, desorption and chemical reactions. These
7 phenomena are affected by both environmental conditions and the structure (species, canopy
8 layers etc.) of the vegetation – in turn shaping itself in response to the environment it grows
9 in. The result of these interactions is an extremely complex and dynamic network of
10 simultaneous processes.

11 Biogenic VOCs (BVOCs) produced by plants have a major role in atmospheric chemistry.
12 They affect the formation and destruction of ozone in the troposphere and participate in
13 aerosol formation processes (e.g. Kulmala et al., 2004, Tunved et al., 2006). Despite
14 considerable progress in recent years, aerosol-related processes are a major source of
15 uncertainty in climate estimates (IPCC 2014). Biogenic VOC emissions dominate over those
16 of anthropogenic origin both globally (Guenther et al., 1995) and in the sparsely populated
17 regions of Northern Europe, especially in the summertime (Simpson et al., 1999, Lindfors et
18 al., 2000).

19 Terpenes (monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$)) represent a reactive subgroup
20 of BVOCs that are produced in different plant tissues and during various physiological
21 processes (e.g. Loreto and Schnitzler, 2010). Plants are known to use these compounds in
22 their interactions with insects and other plants, and they may help the plant to adapt to abiotic
23 stress (see Holopainen and Gershenson, 2010 for a review). BVOC emissions in the Eurasian
24 taiga are dominated by monoterpenes (Guenther et al., 1995, Tarvainen et al., 2007, Rinne et
25 al., 2009), but boreal forest trees also produce significant amounts of e.g. sesquiterpenes
26 (Hakola et al., 2006, Holzke et al., 2006, Ruuskanen et al., 2007), which are generally more
27 reactive than monoterpenes (Atkinson and Arey, 2003, Appendix A). Many terpenes are
28 produced constitutively, but synthesis can also be induced by biotic and abiotic stresses such
29 as herbivory or heat (Holopainen and Gershenson, 2010, Loreto and Schnitzler, 2010). Plants
30 store terpenes either in specialised storage structures like the resin canals of conifers on
31 nonspecifically in the mesophyll tissue (Niinemets et al., 2004).



1 On their way from the plant interior to the atmosphere, the terpenes, mostly rather lipophilic
2 in nature (Niinemets and Reichstein, 2003, Appendix A), must first cross the lipophilic cell
3 membranes and then the hydrophilic apoplast before evaporating into the air spaces inside the
4 leaf. It was long assumed that this transfer happens purely by diffusion, but new evidence
5 suggests active transport out of the cells (Widhalm et al., 2015). Finally, emission into the
6 atmosphere occurs first by gas-phase diffusion through the stomata and the leaf boundary
7 layer, where the conditions are significantly affected by the leaf (Schuepp, 1993), and then by
8 turbulent transport. The driving force of diffusion is the concentration gradient between the
9 leaf interior and the atmosphere. The leaf cuticle is generally considered an effective barrier
10 for plant-produced volatiles, preventing direct emission (Niinemets and Reichstein, 2003).

11 The different physicochemical properties of terpenes affect their transport within and out of
12 the needle as well as their reactions along the way (Atkinson and Arey, 2003, Niinemets and
13 Reichstein, 2003, Appendix A). For example volatility (described by Henry's law constant H ;
14 $\text{Pa m}^3 \text{mol}^{-1}$) and partitioning between the lipid and aqueous phases (octanol-water partition
15 coefficient K_{OW}) vary between compounds, as do reaction rates with oxidants such as O_3 .

16 Terpenes participate in many chemical reactions at and near the needle surfaces. For example,
17 terpenes can protect the plant from oxidative stressors such as ozone (O_3) by reacting with it
18 before it reaches the sensitive tissues inside the leaves (Loreto and Schnitzler, 2010). BVOC
19 reactions are known to be a major factor in non-stomatal O_3 deposition in forests (Goldstein et
20 al., 2004, Bouvier-Brown et al., 2009). The terpene- O_3 reactions can occur in the atmosphere
21 after terpene emission, but they can also take place in the leaf boundary layer, in the air spaces
22 or aqueous phase inside the leaf – or on the leaf surface (Altimir et al., 2006). In addition to
23 gas-phase reactions, heterogeneous reactions are known to play a key role in BVOC
24 chemistry (Shen et al., 2013). It has been suggested that some of the BVOCs produced by
25 foliage could be attached to the epicuticular waxes (Sabljić et al., 1990, Welke et al., 1998),
26 providing additional protection against oxidants, but scientific knowledge on this issue is
27 currently very limited. At least in theory BVOCs also affect the formation of water films on
28 leaf surfaces (Rudich et al., 2000, Sumner et al., 2004), thereby enhancing O_3 deposition
29 mediated by surface wetness.

30 The surfaces of conifer needles are both complex and dynamic in nature. As they grow,
31 needles are covered with a waxy layer secreted by the epicuticular cells (Fig. 1). This layer is
32 lipophilic and hydrocarbons are known to be taken up in it (Binnie et al., 2002, Brown et al.,



1 1998, Welke et al., 1998). With time and weathering, the surfaces undergo chemical and
2 structural changes (Barnes and Brown, 1990, Huttunen and Laine, 1983). Irregularities in the
3 surface provide sites for water adsorption (Rudich et al., 2000). As a result, the originally
4 water-repellent surface becomes more wettable as it wears down. Compounds accumulating
5 on the surface change the characteristics of both the surface and the water film that forms on it
6 (Neinhuis and Barthlott, 1997, Burkhardt and Eiden, 1994). Such water films are ubiquitous
7 when the ambient relative humidity is above 70 % – a common condition in boreal areas –
8 and can even extend through the stomata, creating a pathway for water-soluble compounds
9 between the leaf inside and the surface (Burkhardt et al., 2012).

10 Thus it is plausible that plant-derived terpenes with varying chemical properties could
11 accumulate on foliage surfaces in amounts and proportions difficult to predict and participate
12 in reactions with other compounds. Because of their importance for both atmospheric
13 chemistry and the plant's adaptation to stress, it is necessary to analyze how the surface
14 processes might change the composition of terpenes reaching the free atmosphere.

15 The aim of this work was to determine whether terpenes can be found on the epicuticles of
16 Scots pine (*Pinus sylvestris* L.) and, if so, to compare the spectra of the terpenes with those
17 found in shoot emissions. To our knowledge this is the first time shoot terpene emissions are
18 compared with terpenes on needle surfaces of the same tree.

19

20 **2 Materials and methods**

21 We measured shoot-level emissions of pine seedlings at a remote outdoor location in Central
22 Finland (Hyytiälä, 61°51'N, 24°17'E). The subsequent needle surface wax analysis was
23 performed in the laboratory of the Finnish Meteorological Institute in Helsinki.

24 The plant material consisted of four grafted Scots pine seedlings, grown for five years in an
25 outdoor plant nursery field. Grafted material was selected to reduce variation in the emissions,
26 since it is well known that the spectrum of terpene emissions depends, among other factors,
27 on the genetic background (Bäck et al., 2012). The height of the seedlings was 1.5–2 m. The
28 trees were transplanted in 15 l plastic pots in May 2013. The plants were kept outdoors in
29 light shade and were well watered. Emission measurements were done during the first days of
30 August. Scots pine terpene emissions have an annual and a diurnal pattern (Hakola et al.,
31 2006, Holzke et al., 2006, Ruuskanen et al., 2007, Aalto et al., 2015); the measurement period



1 was selected to capture sesquiterpene emissions that peak in the summer (Hakola et al., 2006,
2 Tarvainen et al., 2005).

3 We aimed to measure the terpene emissions of each seedling once in similar environmental
4 conditions close to noon and to take three needle samples from each seedling for subsequent
5 wax analysis.

6

7 **2.1 Terpene emissions at shoot level**

8 We measured terpene emissions from the seedlings with a dynamic chamber. The chamber
9 consisted of a steel frame, coated with PTFE tubing, and a FEP bag supported by the frame
10 (volume 4.5 l). The chamber was fitted with an inlet and outlet tube made of PTFE. An
11 external pump, with an active carbon filter and an ozone scrubber, pushed air through the
12 chamber (2.5 l/min). The chamber system is described in more detail in Hakola et al. (2006).

13 A healthy mid-crown branch was selected for the emission measurement. Before
14 measurement, the tip of the branch (approximately 30 cm) was gently fitted in the frame. The
15 measured section included needles grown in 2013 and 2012. The growth of the new needles
16 was not quite complete at the time of measurement. The FEP bag was then pulled over the
17 frame, the pump was started and the system was left to stabilize for 30 minutes to minimize
18 the effect of emissions induced by handling.

19 A sample flow was then directed through adsorbent tubes (Tenax-TA and Carboxen-100) attached
20 to the inlet and outlet tubes with a stainless steel T piece. The resin filling of the tube
21 adsorbs terpenes, which can later be desorbed and analyzed. Handheld pumps were used to
22 pull the sample through the tube (70 ml/min). The sampling time was 30 minutes, after which
23 the chamber was removed. The air temperature inside and the PAR above the chamber were
24 measured during chamber closure with thermistors (Philips KTY 80/110) and quantum sensor
25 (LI-190SZ), respectively. During the 60-minute closure, the temperature inside the chamber
26 increased by 1.5–3 degrees Centigrade. The same chamber was used to measure all the
27 seedlings. To minimize the effect of changing light conditions, the measurements were done
28 between 10 AM and 1 PM, which allowed us to measure one tree per day. Each tree was
29 measured once. After emission measurement and needle sampling (as described below), the
30 measured shoot was cut and weighed for fresh and dry mass. A 10 % subsample was taken
31 and weighed separately. For this subsample, we measured needle dimensions (length, width



1 and thickness) and calculated needle area according to Tirén (1927). This needle area was
2 then used to estimate the needle area for the shoot using the respective dry weights of the
3 subsample and main sample.

4 The contents of the adsorbent tubes were analyzed at the Finnish Meteorological Institute
5 with a thermal desorber (Perkin-Elmer TurboMatrix 650 ATD) connected to a gas
6 chromatograph – mass spectrometer (Perkin-Elmer Clarus 600) with HP-1 column (60 m, i.d.
7 0.25 mm). The detection limits were 0.04 ng/sample for camphene, 0.10–0.15 ng/sample for
8 α -pinene, β -pinene and carene, 0.20–0.42 ng/sample for sabinene, limonene, 1,8-cineol,
9 bornylacetate and β -caryophyllene and 0.55–0.64 ng/sample for sesquiterpenes. The measured
10 compounds were identified using authentic standards and NIST library.

11 The observed emission rate (E , $\mu\text{g}/\text{m}^2/\text{h}$) was calculated based on the two concentrations of
12 each compound as

$$13 \quad E = \frac{(C_2 - C_1)}{A} F \quad (1)$$

14 Where C_2 is the concentration in the outlet air ($\mu\text{g}/\text{m}^3$), C_1 is the concentration in the inlet air
15 ($\mu\text{g}/\text{m}^3$), F is the flow rate into the enclosure (m^3/h) and A is the needle area of the measured
16 shoot (m^2). From E , we obtained the spectra of emitted compounds (% of total emissions).

17

18 **2.2 Terpenes in the epicuticular waxes**

19 To detect the presence of terpenes associated to the epicuticular surfaces, we collected the
20 waxy material from the needle surfaces for subsequent terpene analysis.

21 After each emission measurement, we darkened the measured tree for 30 minutes to close the
22 stomata and minimize stomatal terpene emission and then took needle samples (20 needle
23 pairs) in darkness for the wax analysis. The needles were immediately stored in a liquid
24 nitrogen dry shipper until analysis (two weeks later).

25 We collected the epicuticular wax layer by dipping each needle pair in 5 ml dichloromethane
26 for 15 seconds. The dipping time was optimized in a preliminary experiment to remove most
27 of the wax layer but to keep the solvent from reaching the inside of the needle through
28 stomata (visual inspection under a stereo microscope). We took special care to use only intact
29 needles and to not immerse the cut base of the needle in the solvent. This was done to prevent



1 compounds originating inside the needle from getting into the extract. Dipping the needles
2 while they were frozen should also minimize the extraction of compounds from inside the
3 needle. After wax extraction, the needles were weighed for fresh and dry mass and measured
4 for their dimensions (width, length and thickness). From these dimensions, needle surface
5 area was approximated according to Tirén (1927).

6 The obtained extract was evaporated to 1 ml volume with pure nitrogen gas. The reduced
7 extract was then analyzed with a gas chromatograph (Agilent 6890N) with a mass
8 spectrometric detector (Agilent 5973) to identify terpenes. A JandW DB-5MS column (30 m,
9 i.d. 0.25 mm) and a 5 m pre-column (Agilent FS) were used for the chromatography. The
10 limits of detection were estimated from the standard deviations of blank samples and were
11 0.15–0.30 ng/sample for p-cymene, bornyl acetate and iso-longifolene, 0.48–0.72 ng/sample
12 for α -pinene, camphene, myrcene, 1,8-cineol and longicyclene and 1.55–2.29 ng/sample for
13 β -pinene, 3-carene and β -caryophyllene. The analysis method is described in more detail in
14 Vestenius et al. (2011). The compounds to be identified were not predetermined, and hence
15 we did not have calibration standards for all of them. Some of the compounds were therefore
16 identified and quantified only tentatively, using the reference from another compound. After
17 the analysis the extract was left to evaporate, and the solid wax residue left in the vial was
18 weighed (Mettler AT2000).

19 For an estimation of the terpenes lost during the evaporation, we performed a separate
20 evaporation test, letting known concentrations of selected terpenes evaporate as described
21 above. The test gave no indication of any significant loss of terpenes associated with the
22 method.

23

24 **3 Results**

25 The weather conditions during the experiment were slightly variable. The first two days
26 (measuring emissions from trees 1 and 2) were relatively warm (+19–21 °C during the
27 measurements) but partly cloudy. The last two days were sunny and warm, especially the last
28 day (+21–24 °C). This deserves notice, since the amount of terpenes emitted by a plant is
29 affected by temperature, irradiation and humidity that on one hand regulate the biosynthetic
30 processes that produce BVOCs and on the other hand affect volatilization and diffusion rates
31 (Lerdau and Gray, 2003, Niinemets et al., 2004, Tarvainen et al., 2005).



1

2 **3.1 Terpenes in shoot emissions**

3 The shoot emissions were clearly dominated by monoterpenes (96–98 % of total terpene
4 emissions, Fig. 2). Sesquiterpenes amounted to 0–2 % of total emissions. The compounds
5 found in each group and the variation in their emissions are presented in detail in Appendix B
6 and Fig. 2.

7 The most abundant monoterpenes were α -pinene (36–58 % of total emissions), myrcene (13–
8 36 %) and carene (12–18 %). The emitted sesquiterpenes included α -humulene (0–1 % of
9 total emissions), aromadendrene (0–0.5 %) and longicyclene (0–0.8 %). None of the
10 identified sesquiterpenes was detected in the emissions of all four pine seedlings, and one
11 seedling showed no sesquiterpene emission. In addition, 1,8-cineol was observed in the
12 emissions, as was a small percentage of bornyl acetate.

13

14 **3.2 Terpenes in epicuticular waxes**

15 The wax yield from the pine needles was 0.0066–0.0114 g/ g DW (average 0.0075 g/g) or
16 0.43–1.23 g/m² of needles (average 0.76 g/m²). As for the shoot emissions, the epicuticular
17 wax extracts were dominated by monoterpenes (76–93 % of total terpene amount). The
18 proportion of sesquiterpenes, however, was notably higher than in emissions: 5–21 %. Taking
19 into account the six unidentified sesquiterpenes for which we did not have standards for
20 (described below), the proportion of total sesquiterpenes in the waxes rises to 7–50 %
21 (average 34 %).

22 The results for different compounds were highly variable also in the wax analysis (Appendix
23 B). The most abundant monoterpenes in the waxes were α -pinene (10–57 % of total), carene
24 (11–26 %) and limonene (2–40 %) (Fig. 2). For sesquiterpenes, the highest amounts were
25 measured for β -caryophyllene (4–16 % of total), iso-longifolene (0–9 %) and humulene (0.5–
26 3 %). Of the sesquiterpenes seen in shoot emissions, only α -humulene was found in the
27 surface waxes. Iso-longifolene was found in the waxes but not in emissions. In addition to the
28 pre-selected compounds (with standards available), we detected six unidentified
29 sesquiterpenes, some in relatively high proportions. This group is likely to include cadinene,



1 cubebene and murolene. Also 1,8-cineol was found in the waxes, but in much smaller
2 proportion than in emissions.

3

4 **4 Discussion**

5 **4.1 The terpene spectra in emissions and pine epicuticular waxes**

6 The composition of the emitted pine shoot terpenes measured in this study is generally in the
7 range observed by others (Bäck et al., 2012, Hakola et al., 2006, Holzke et al., 2006,
8 Tarvainen et al., 2005), allowing for the natural variation in BVOC emission and the
9 differences in methodology. The pine seedlings in our study emitted more than twice as much
10 α -pinene than carene, thus representing the pinene or intermediate chemotype described in
11 Bäck et al. (2012). The fact that the pine seedlings were grafted (genetically identical
12 canopies) is likely to have reduced the variation in the results. Grafted seedlings have the
13 advantage of providing, at least in theory, identical replicates that should only show variation
14 caused by differences either in the environmental conditions or life histories (mechanical
15 injuries, insect attacks and similar). Nevertheless, notable variation in the emissions was
16 observed, underlining the importance of the effects of varying conditions and life history
17 experienced by individual trees on their terpene emissions.

18 The amount of terpenes found in the epicuticular waxes is the equivalent to 4–84 hours of the
19 measured emissions for the same compound (per m² of needle surface), depending on the
20 compound. For example, it would take the shoot on average 14 h to emit the amount of α -
21 pinene that was present on the needle surfaces. For myrcene the time would be 9 hours, for
22 carene 24 hours and for limonene 84 hours. For most sesquiterpenes this comparison cannot
23 be done, because they were found in either only emissions or only epicuticular waxes, but for
24 α -humulene the equivalent time would be 34 hours.

25 The short exposure to the solvent and the fact that the stomata were virtually closed means
26 that any BVOCs found in the extract were most likely not a result of stomatal emissions but
27 rather compounds that had been associated to the epicuticle. In studies with extracts from
28 crushed needles, the proportion of mono- and sesquiterpenes has been found to be in the same
29 range as observed here for both emissions and epicuticular waxes. For example Manninen et
30 al., (2002) reported a mean total monoterpene ratio of 67 % for a Scots pine provenance from
31 central Finland and listed α -pinene and carene as the major monoterpenes in the needles. In



1 our study, these two were among the main compounds in both emissions and waxes.
2 Achotegui-Castells et al. (2013) reported camphene, α -pinene, β -pinene, β -caryophyllene and
3 germacrene D as the most abundant terpenes in Scots pine needles. Limonene, in our study
4 the third most abundant compound in waxes, was notably less abundant in whole needles
5 (Achotegui-Castells et al., 2013, Manninen et al., 2002). On the other hand camphene was
6 relatively more abundant both in the whole-needle extracts (Achotegui-Castells et al., 2013,
7 Manninen et al., 2002) and in the emissions in our study than in the needle waxes. This is a
8 strong indication that the solvent used in our study did not reach the needle interior during the
9 procedure.

10 In the epicuticular waxes, we observed six unidentified sesquiterpenes, some in relatively
11 high proportions. Although this group is likely to include cadinene, cubebene and murolene,
12 the exact identification and quantification of these compounds would require a more detailed
13 study. Naturally, the possible role of these compounds in the emissions remains unknown, but
14 their existence in the waxes suggests that the production of sesquiterpenes in Scots pine
15 deserves more attention.

16 It is interesting to note that despite the large variation there is some indication that the most
17 water-soluble compound in our study, 1,8-cineol, (Appendix A) was relatively more abundant
18 in the emissions, while the compounds with a large K_{OW} (more likely to partition into the lipid
19 than the water phase), like α -humulene, β -caryophyllene and iso-longifolene, were relatively
20 more abundant in the surface waxes. This finding is in line with the results of Welke et al.
21 (1998), who found the cuticular matrix to be a much stronger sink for limonene than for
22 isoprene from air. The compounds with the highest reactivities towards ozone (α -humulene
23 and β -caryophyllene; Appendix A) were more abundant in the epicuticular waxes than
24 emissions. Since the inlet air used in our experiment was scrubbed of ozone, the result is not
25 due to O_3 -VOC reactions inside the chamber.

26

27 **4.2 The fate of terpenes on leaf surfaces**

28 In theory, there are three mechanisms for the terpenes produced by a needle to end up on the
29 needle surface. The first one is (dry) redeposition after emission. This route is more likely for
30 the less volatile terpenes like longicyclene and p-cymene (Appendix A). The most lipophilic
31 terpenes, such as β -caryophyllene and α -humulene, are also the most reactive ones. Although



1 they are more likely to bind into or onto the lipophilic wax layer, they are also most unlikely
2 to survive in the air phase long enough for redeposition to happen (Atkinson and Arey, 2003).
3 The observed spectra, with β -caryophyllene observed in the pine epicuticular waxes but not in
4 the shoot emissions and with α -humulene being relatively more abundant in the waxes than
5 the emissions, are an indication that this route can be considered of minor importance. This
6 conclusion is supported by Cape et al. (2009), who observed that α -pinene did not dissolve or
7 adsorb into a wax layer to enhance O_3 removal. Another way for the emitted compounds to
8 bind onto the epicuticular waxes after emission into the air is absorption or adsorption into or
9 onto the layer of water on the surface.

10 The second option is transport in the aqueous layer extending from the outer needle surface
11 through the stoma all the way into the substomatal cavity, as suggested by Burkhardt et al.
12 (2012). The effectiveness of this route depends on the existence of such continuous water
13 film, and also on the water-solubility and diffusion capabilities in water of the compound in
14 question. Because of their low water solubility, it has often been assumed that the reactions of
15 terpenes in the aqueous phase do not contribute significantly to the total reactions. Wang et al.
16 (2012) however propose that the reactions of biogenic unsaturated hydrocarbons happening
17 on wet surfaces, like those of plants growing in nature, can have a significant effect on ozone
18 deposition. In this work, we cannot differentiate between compounds that were in or on the
19 epicuticular waxes from those that may have been bound in the surface water. The most
20 water-soluble of the detected compounds was 1,8-cineol, which was present in greater
21 proportion in shoot emissions than epicuticular waxes. It is then possible that some of the 1,8-
22 cineol emitted from the shoot is redeposited onto the surface.

23 The third alternative is direct transport from the production sites inside the cells through the
24 plant cuticle. In xerophytic plants, such as conifers, the cuticle has a strongly layered
25 structure. The insoluble lipid cutin is partly embedded as intracuticular wax under the cuticle
26 proper, not as an even layer but forming legs towards the epidermal cell wall (Evert, 2007,
27 Fig. 1). The production of surface waxes takes place in epidermal cells during the first few
28 weeks and months of needle growth (Kinnunen et al, 1998), and they are transported via
29 microchannels or diffusion to the surface (Evert, 2007). Despite some reports of terpene
30 emissions through the cuticle (e.g. Guenther et al, 1991), this route is usually considered
31 negligible for terpene emissions (Niinemets and Reichstein, 2003) because of the
32 considerably slower diffusion rate of terpenes within the cuticle than in air or water. It does



1 not, however, contradict the notion that terpenes might be transported into the epicuticle
2 and accumulate there. Theoretically, this mode of transport would be more effective for the
3 most lipophilic compounds like α -humulene and β -caryophyllene (Kirsch et al., 1997,
4 Appendix A). Indeed, these compounds were found in greater proportion in the waxes than in
5 the emissions, suggesting that this may be an important pathway for lipophilic terpenoids.

6

7 **4.3 Implications for gas-phase chemistry**

8 Once in the gas phase, plant-emitted terpenes can react in various ways. They can undergo
9 photolysis or react with hydroxyl or nitrate radicals or ozone (Atkinson and Arey, 2003). The
10 relative importance of the different reaction pathways depends on atmospheric conditions,
11 time of day and the compound in question. Ozone reactions target double bonds in the terpene
12 molecule (Atkinson and Arey, 2003). The most O_3 -reactive compounds have two or three of
13 these double bonds in their structure (Atkinson and Arey, 2003, Appendix A).

14 The available reaction rate coefficients for O_3 -BVOC reactions are almost exclusively for the
15 gas phase (Appendix A). This makes rate calculations of reactions happening on wet plant
16 surfaces challenging. There is evidence that the reaction rates of terpenes on solid and liquid
17 surfaces can be faster than in the gas phase (Shen et al., 2013, Enami et al., 2010), but because
18 of the almost unlimited variation in surface properties and aqueous solutions found in nature,
19 a single coefficient will never cover all circumstances. For a rough estimate of the O_3
20 scavenging potential of the terpenes we found on the needle surfaces, we calculated their
21 “reaction time” or how many hours worth of non-stomatal deposition of O_3 each compound
22 could react with, assuming there were no other sinks, as

23

$$24 \quad \text{Time} = \frac{n_{\text{terp}}}{\text{dep}_{O_3}} \quad (2)$$

25 Where Time is the reaction time (h), n_{terp} is the amount of the terpene in question ($\mu\text{g}/\text{m}^2$) and
26 dep_{O_3} ($\mu\text{g}/\text{m}^2/\text{h}$) is non-stomatal deposition towards the shoot.

27

28 Similarly to Fares et al. (2012), we assumed that each molecule of any terpene can react with
29 one molecule of O_3 , even though some terpenes have more than one double bond available



1 while others have none. Assuming a total O₃ deposition of 30 ng/m²/s towards the shoot with
 2 40 % non-stomatal deposition (realistic values for Scots pine in the area in the summer as
 3 reported by Altimir et al., 2006), the terpenes present on the surfaces could in theory react
 4 with 5 hours of nonstomatal O₃ deposition.

5 Although simple, our calculation shows that the terpenes found in needle surface waxes could
 6 act as a significant O₃ sink. The extent to which this actually happens depends on two factors:
 7 how much of the atmospheric ozone reaches the terpenes within a given time, and how fast
 8 the terpene supply is replenished. The fact that reactive terpenes were present on the needle
 9 surfaces indicates that under the conditions of this study, the terpene supply is renewed at
 10 least at a rate comparable to the amount of O₃ reaching the storage site. Assessing these
 11 factors would present an interesting question for future research.

12

13 Appendix A: Physicochemical properties of BVOCs (at 25 °C)

14 The values for molecular mass (M), water solubility, Henry's law constant (H), saturated
 15 vapor pressure (VP) and octanol-water partition coefficient (KOW) from Copolovici and
 16 Niinemets (2005) unless otherwise marked. Reaction rate constants from Shu and Atkinson
 17 (1995) unless otherwise marked.

	M g/mol	Water sol. mol/m ³	H Pa m ³ /mol	VP Pa	log[KOW] mol/mol	Rate constants for gas phase reactions, cm ³ /molec s		
						OH	O ₃	NO ₃
p-cymene	134.2	0.21	935	197**	4.1	8.5x10 ⁻¹² ***		
α-pinene	136.2	0.0411	13590	558**	4.66	5.4x10 ⁻¹¹ *	8.7x10 ⁻¹⁷ *	6.1x10 ⁻¹² *
β-pinene	136.2	0.0592	6826	404**	4.42	5.7x10 ⁻¹¹ ***	1.2x10 ⁻¹⁷ ***	
camphene	136.2	0.0419	3238	136*	4.56	5.7x10 ⁻¹¹ ***	1.1x10 ⁻¹⁷ ***	
Δ ³ -carene	136.2	0.0214	13640 *	292*	4.61	8.8x10 ⁻¹¹ *	3.7x10 ⁻¹⁷ *	9.1x10 ⁻¹² *
limonene	136.2	0.0886	2850	253*	4.49	1.5x10 ⁻¹⁰ ***	4.4x10 ⁻¹⁶ ***	
myrcene	136.2	0.0421	6300	265*	4.34	1.9x10 ⁻¹⁰ ***	4.4x10 ⁻¹⁶ ***	
1,8-cineole	154.2	19.1	13.27	253*	2.61	2.3x10 ⁻¹¹ ***		
bornyl acetate	196.3	0.118 **	44.3 ***	30.4***	3.86 **	7.7x10 ⁻¹² ***		
longicyclene	204.4	0.966 ***	2422 ***	11.5***	5.60 ***	9.4x10 ⁻¹² ***		
iso-longifolene	204.4	0.375 ***	25939 ***	6.4***	6.12 ***	9.6x10 ⁻¹¹ ***	1.1x10 ⁻¹⁷ ***	
β-caryophyllene	204.4	0.245 ***	69914 ***	4.2***	6.30 ***	2.0x10 ⁻¹⁰	1.2x10 ⁻¹⁴	1.9x10 ⁻¹¹
aromadendrene	204.4	0.345 ***	29688 ***	5.3***	6.13 ***	6.2x10 ⁻¹¹ ***	1.2x10 ⁻¹⁷ ***	
α-humulene	204.4	0.0683 ***	165160 ***	2.0***	6.95 ***	2.9x10 ⁻¹⁰	1.2x10 ⁻¹⁴	3.5x10 ⁻¹¹

18

19 Water sol., H, VP, log[KOW]: *)Niinemets and Reichstein (2002) **)Niinemets and Reichstein (2003)
 20 ***)ChemSpider. Reaction rate constants: *)Rinne et al., (2007) ***)ChemSpider.

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3 **Appendix B: BVOCs in shoot emissions and surface waxes**

		a-pinene	myrcene	carene	limonene	b-pinene	camphene	p-cymene	monoterpenes total	a-humulene	aromadendrene	longicyclene	iso-longifolene	b-carophyllene	unknown1	unknown2	unknown3	unknown4	unknown5	unknown6	sesquiterpenes total	1,8-cineol	bornylacetate	others total	TOTAL
Emissions, µg/m ³ /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	1.2	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
	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
	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
	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
	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
	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
	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
	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
	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
	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
	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
	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
	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
	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
	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
	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

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		a-pinene	myrcene	carene	limonene	b-pinene	camphene	p-cymene	monoterpenes total	a-humulene	aromadendrene	longicyclene	iso-longifolene	b-carophyllene	sesquiterpenes total	1,8-cineol	bornylacetate	others total	TOTAL
Emissions, % of total	Tree 1	35.9	35.7	13.6	0.0	8.4	4.0	0.0	97.7	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.0	2.3	100.0
	Tree 2	44.9	17.8	12.3	2.7	7.2	11.4	0.2	96.4	0.8	0.0	0.8	0.0	0.0	1.7	1.6	0.3	1.9	100.0
	Tree 3	57.5	15.8	17.4	0.1	4.5	3.0	0.0	98.3	1.2	0.0	0.0	0.0	0.0	1.2	0.5	0.0	0.5	100.0
	Tree 4	44.4	13.4	17.5	11.8	7.3	2.7	0.8	97.9	0.5	0.5	0.0	0.0	0.0	0.9	1.0	0.2	1.2	100.0
	Min	35.9	13.4	12.3	0.0	4.5	2.7	0.0	96.4	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.5	100.0
	Max	57.5	35.7	17.5	11.8	8.4	11.4	0.8	98.3	1.2	0.5	0.8	0.0	0.0	1.7	2.3	0.3	2.3	100.0
	Mean	45.7	20.7	15.2	3.7	6.8	5.3	0.2	97.6	0.6	0.1	0.2	0.0	0.0	1.0	1.4	0.1	1.5	100.0
	SD	8.9	10.2	2.6	5.6	1.7	4.1	0.4	0.8	0.5	0.2	0.4	0.0	0.0	0.7	0.8	0.1	0.8	0.0
In waxes, % of total	Tree 1 s 1	55.7	0.8	26.3	2.0	0.0	2.6	0.0	87.4	0.9	0.0	0.0	0.2	8.1	9.2	0.2	3.1	3.3	100.0
	Tree 1 s 2	51.6	0.5	18.5	5.6	2.7	1.3	1.2	81.3	1.5	0.0	0.0	5.0	10.5	17.1	0.4	1.3	1.7	100.0
	Tree 1 s 3	46.1	5.8	22.6	14.2	0.0	0.0	0.3	89.0	0.8	0.0	0.0	0.8	6.9	8.5	0.0	2.6	2.6	100.0
	Tree 2 s 1	47.7	3.7	15.8	8.2	0.0	1.5	0.2	77.1	2.1	0.0	0.0	3.1	15.8	20.9	0.4	1.5	1.9	100.0
	Tree 2 s 2	33.9	3.3	18.4	10.5	6.8	2.5	0.3	75.7	2.7	0.0	0.0	0.7	14.4	17.8	0.6	5.8	6.4	100.0
	Tree 2 s 3	10.0	17.4	21.7	40.0	3.9	0.0	0.0	93.0	0.7	0.0	0.0	0.0	5.2	5.9	0.9	0.2	1.1	100.0
	Tree 3 s 1	40.7	5.8	19.2	16.3	2.3	1.8	0.3	86.3	1.2	0.0	0.0	0.9	9.6	11.7	0.5	1.5	2.0	100.0
	Tree 3 s 2	38.4	5.8	13.0	19.5	4.9	3.5	0.9	86.1	1.1	0.0	0.0	2.2	7.2	10.6	0.6	2.7	3.4	100.0
	Tree 3 s 3	44.8	3.3	19.4	9.2	1.8	1.6	0.2	80.3	1.6	0.0	0.0	3.7	12.2	17.5	0.4	1.8	2.2	100.0
	Tree 4 s 1	57.3	1.0	11.1	2.0	2.7	2.5	0.4	77.0	1.2	0.0	0.0	9.0	9.5	19.6	0.4	2.9	3.4	100.0
	Tree 4 s 2	22.2	10.9	16.4	38.1	4.2	1.1	0.3	93.2	0.5	0.0	0.0	0.8	4.0	5.3	0.9	0.6	1.5	100.0
	Tree 4 s 3	28.3	7.4	23.7	20.6	3.9	1.0	0.0	84.9	1.6	0.0	0.0	0.6	11.8	14.0	0.6	0.5	1.1	100.0
	Min	10.0	0.5	11.1	2.0	0.0	0.0	0.0	75.7	0.5	0.0	0.0	0.0	4.0	5.3	0.0	0.2	1.1	100.0
	Max	57.3	17.4	26.3	40.0	6.8	3.5	1.2	93.2	2.7	0.0	0.0	9.0	15.8	20.9	0.9	5.8	6.4	100.0
	Mean	39.7	5.5	18.8	15.5	2.8	1.6	0.3	84.3	1.3	0.0	0.0	2.2	9.6	13.2	0.5	2.0	2.5	100.0
	SD	14.1	4.8	4.4	12.6	2.1	1.0	0.4	6.0	0.6	0.0	0.0	2.6	3.6	5.4	0.3	1.5	1.5	0.0

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9



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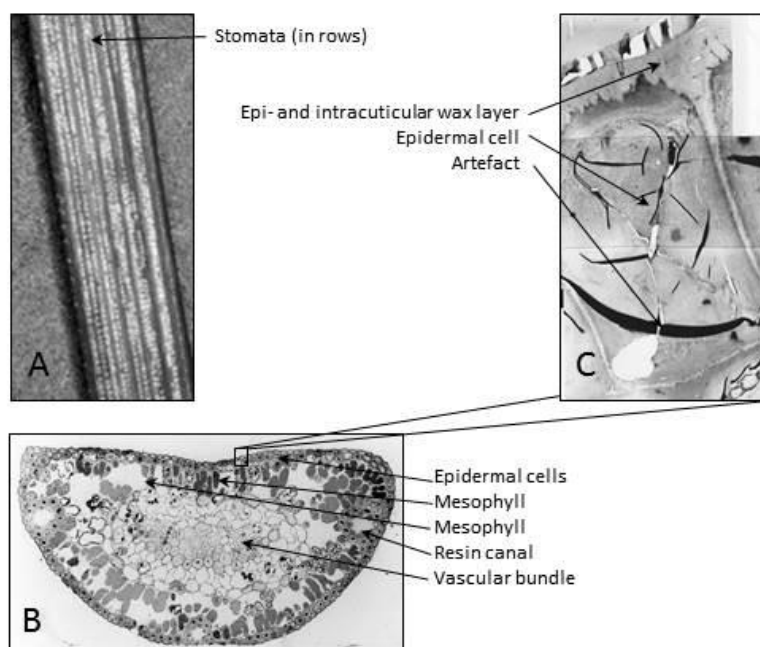
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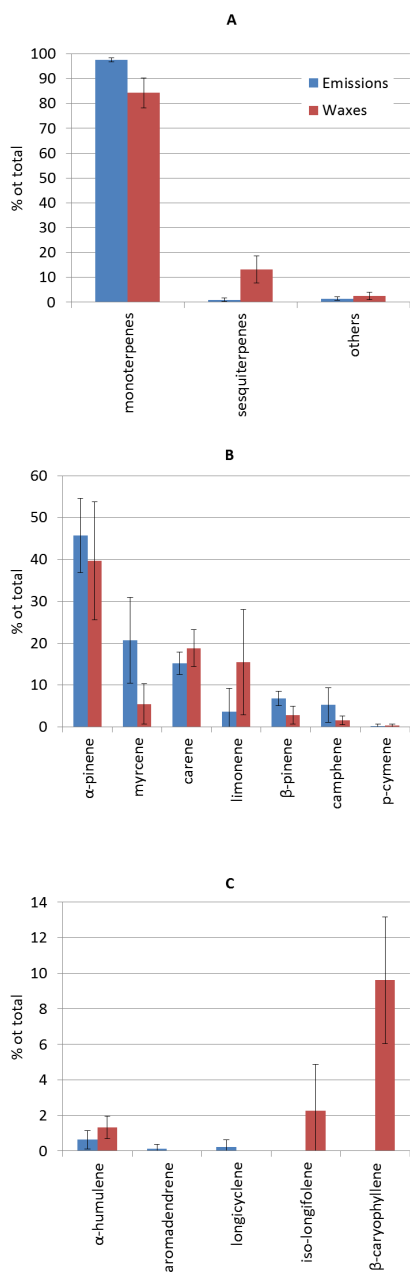
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2 Fig. 1. Pine needle structure. A. The abaxial side of the needle with rows of stomata covered
3 with epicuticular waxes. B. Cross-section of a needle. C. An epidermal cell with epicuticular
4 layer.
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2 Fig. 2. Relative amounts of terpenes in the pine shoot emissions and needle surface waxes,
3 average % of total, with standard deviation. A: relative abundancies of each compound group,
4 B: monoterpenes, C: sesquiterpenes. The unknown sesquiterpenes found in the waxes are not
5 included.