

## Original Article

## Diurnal patterns in Scots pine stem oleoresin pressure in a boreal forest

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## ABSTRACT

Coniferous tree stems contain large amounts of oleoresin under positive pressure in the resin ducts. Studies in North-American pines indicated that the stem oleoresin exudation pressure (OEP) correlates negatively with transpiration rate and soil water content. However, it is not known how the OEP changes affect the emissions of volatile vapours from the trees. We measured the OEP, xylem diameter changes indicating changes in xylem water potential and monoterpene emissions under field conditions in mature Scots pine (*Pinus sylvestris* L.) trees in southern Finland. Contrary to earlier reports, the diurnal OEP changes were positively correlated with temperature and transpiration rate. OEP was lowest at the top part of the stem, where water potentials were also more negative, and often closely linked to ambient temperature and stem monoterpene emissions. However, occasionally OEP was affected by sudden changes in vapour pressure deficit (VPD), indicating the importance of xylem water potential on OEP as well. We conclude that the oleoresin storage pools in tree stems are in a dynamic relationship with ambient temperature and xylem water potential, and that the canopy monoterpene emission rates may therefore be also regulated by whole tree processes and not only by the conditions prevailing in the upper canopy.

*Key-words:* *Pinus sylvestris*; monoterpene emissions; resin storage; temperature; water balance.

## INTRODUCTION

Pines, like most conifers, produce oleoresin in the living epithelial cells lining the resin ducts and store the fluid resin in duct cavities in stems, needles and roots. Traditionally oleoresin in trees has been studied because of its defensive features, because it has an important role in preventing for example, insect-induced damage in coniferous forests. Indeed, the ability of pines to survive, for example, a bark-beetle attack has been explained by the chemical composition of oleoresin, the pressure of oleoresin inside the resin ducts, as well as the amount and flow rate of oleoresin from wounds (Vité 1961, Trapp & Croteau 2001, Strom *et al.* 2002, Perrakis 2008). Certain constituents of oleoresin, such as the monoterpenes

limonene, 3-carene and  $\beta$ -pinene, at the same time attract the pest insects, but also prevent their damage (Smith 1965, Phillips & Croteau 1999, Seybold *et al.* 2006), and the flow of oleoresin may indicate the ability of a tree to defend itself (Lorio 1994).

Oleoresin exudation pressure (OEP) varies approximately from 3 to 12 bars depending on the tree species, tree properties and environmental conditions, and it has been linked to the parameters of water balance in a tree (Schopmeyer *et al.* 1952, Bourdeau & Schopmeyer 1958, Vité 1961, Lorio & Hodges 1968a,c, Helseth & Brown 1970). Transpiration rate, as well as the moisture content of air and soil, has been discovered to influence pine OEP (Vité 1961, Lorio & Hodges 1968b,c, Helseth & Brown 1970, Neher 1993). According to Vité (1961), the deficiency of water or rapid transpiration decreases the turgid pressure inside the epithelial cells lining resin ducts, which in turn decreases the pressure inside the resin ducts and the amount of oleoresin produced. On the other hand, shrinkage in tracheids caused by water deficiency decreases the physical pressure exerted on the oleoresin (Bourdeau & Schopmeyer 1958, Helseth & Brown 1970). Indeed, the diurnal pattern of OEP and stem xylem diameter do often match (Helseth & Brown 1970, Neher 1993), because the changes in the tension of water inside water conducting tracheids affect the diameter of a tree's xylem (Irvine & Grace 1997, Perämäki *et al.* 2001, Mencuccini *et al.* 2013). The changes in xylem diameter are thus proportional to changes in xylem water potential (Irvine & Grace, 1997, Perämäki *et al.* 2001, Mencuccini *et al.* 2013). Thus, OEP and the flow of oleoresin from a wounded stem have been observed to be highest during the night when transpiration rates are at their minimum, and vice versa.

Despite extensive research into the defensive features of pine oleoresin and its connections to stem water potential of a tree, the links between OEP and other functions and physiological processes of a tree have been largely neglected. Oleoresin may actually form an important feedback mechanism between the tree water balance and climate. The OEP in the stem may affect the emissions of climatically important biogenic volatile organic compounds (BVOC) from coniferous trees, because the oleoresin contains large amounts of volatile monoterpenes (e.g. Bäck *et al.* 2012). BVOCs contribute to the growth of atmospheric aerosol particles and the formation of clouds; thus indirectly they contribute to cooling the climate (Janson *et al.* 2001, Kulmala *et al.* 2004a,b, Tunved *et al.* 2006, Kulmala *et al.* 2013). Now, if drought, which is associated with warming climate, influences OEP and thus also BVOC

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emissions, this could be a new important feedback mechanism for ecosystem–atmosphere interactions. Despite substantial research in the last decades (see e.g. Tarvainen *et al.* 2005, Guenther *et al.* 2006, Arneth *et al.* 2008, Harrison *et al.* 2013), the sources and especially the physiological factors affecting incident BVOC emission rates are still insufficiently understood.

The monoterpene emissions in boreal regions originate largely from coniferous trees (Rinne *et al.* 2000), but despite the above findings it is not known what role stem oleoresin dynamics play in emissions, and what regulates the OEP in the stems of boreal tree species. Hence, in this study the OEP of Scots pine (*Pinus sylvestris* L.) was studied in a typical pine forest in southern Finland. We hypothesized that, like in other pine species, also in Scots pine the dynamics of OEP are linked to transpiration driven variation in the xylem water potential and follow xylem diameter on a diurnal scale, being highest at night and lowest in daytime. In addition, we aimed at determining if the variations in OEP can be linked to volatile monoterpene emissions from the stem.

## MATERIALS AND METHODS

The measurements were conducted at the Helsinki University SMEAR II field measurement station (Station for Measuring Forest Ecosystem–Atmosphere Relations) in Hyytiälä, southern Finland (61°51'N, 24°17'E, 181 m above sea level) (Hari & Kulmala 2005). The station is located in an even-aged, approximately 50-year-old Scots pine stand of medium fertility that was established by sowing after prescribed burning in 1962. The site is described in detail in Ilvesniemi *et al.* (2010). The averages of high frequency data for photosynthetically active radiation (PAR), air temperature, precipitation and soil

water content and temperature at the site are presented in Table 1.

The diurnal pattern of OEP was measured in several periods over two consecutive summers, from 30 July to 3 August in 2012 and from 6 May to 30 August in 2013. In 2013, we already started the measurements in April, but the first time OEP values that exceeded our detection limit were observed was in early May. The system for measuring OEP was a modification of that by Vité (1961) and consisted of a brass tube that was fastened into a hole drilled into a tree stem, and a pressure gauge (Swagelok 316SS and WIKA 111.16.40.16) attached to one end of the brass tube (Fig. 1). Before measurements, the accuracy of all the pressure gauges was tested with compressed air. Inside the brass tube and pressure gauge, there was glycerine fluid to transmit the changes of OEP and to prevent oleoresin from crystallizing inside the pressure gauge. The brass tube was approximately 10 cm long and 3.17 mm in outer diameter, whereas the drilled hole was 3 mm in diameter and approximately 4 cm deep.

Before drilling the 4-cm-deep hole and fastening the brass tube, approximately 1 × 1 cm of bark surface was removed, and a cone-shaped starting hole was drilled. Then, the longer hole was drilled slightly tangentially, so that it punctured as many resin ducts as possible. After drilling, the brass tube was pushed into the stem to approximately 3.5 cm deep. The junction point of the brass tube was sealed with silicone in order to attach the brass tube better to the tree and to prevent oleoresin flowing past the brass tube. The best seal was, however, spillover resin crystallizing around the brass tube. Finally, a pressure gauge was attached to the free end of the brass tube. The success of the installation was verified by some pressure being observable after a half an hour and data collection started approximately 24 h after the installation (Vité 1961,

**Table 1.** The climatic characteristics during the measurements, and the long-term (1981–2010) climate averages at the site (SMEAR II Hyytiälä). The phenological phases during the intensive measurement periods are given in the bottom row

Measurement period		2012			2013		
		30 Jul–3 Aug	10–15 May	27–31 May	23–28 Jun	15–17 Jul	26–30 Aug
Average temperature °C	This study <sup>a</sup>	16.3	11.9	17.2	19.4	12.9	13.7
	1981–2010 <sup>b</sup>	13.8	8.9	8.9	13.3	16	13.8
Daily min / max temperature °C	This study <sup>a</sup>	12.3 / 20.7	6.1 / 17.4	12.1 / 22.7	14.7 / 24.5	8.5 / 16.7	8.3 / 19.5
	1981–2010 <sup>b</sup>	9.5 / 19.1	2.8 / 14.9	2.8 / 14.9	7.6 / 18.9	10.8 / 21.6	9.5 / 19.1
Average soil temperature °C	This study <sup>a</sup>	14.6	6.8	10.3	13.9	12.2	12.3
	1997–2013 <sup>a</sup>	13.4	4.7	7.0	10.7	12.9	12.0
Soil water content m <sup>3</sup> /m <sup>3</sup>	This study <sup>a</sup>	0.23	0.27	0.21	0.18	0.13	0.12
	1997–2013 <sup>a</sup>	0.20	0.32	0.28	0.24	0.21	0.19
Phenological phase <sup>c</sup>		Growth of needles and stem diameter ended, root growth continues	Growth of buds and stem diameter just begun	Elongation of shoots and stem diameter continue, needle growth beginning	Shoot elongation ended, growth of needles and stem diameter continue	Growth of needles and stem diameter continue	Growth of needles and stem diameter ended, root growth continues

<sup>a</sup><http://www.atm.helsinki.fi/smartsMEAR/>, <sup>b</sup>Pirinen *et al.* (2012) and <sup>c</sup>Aalto *et al.* (2014).



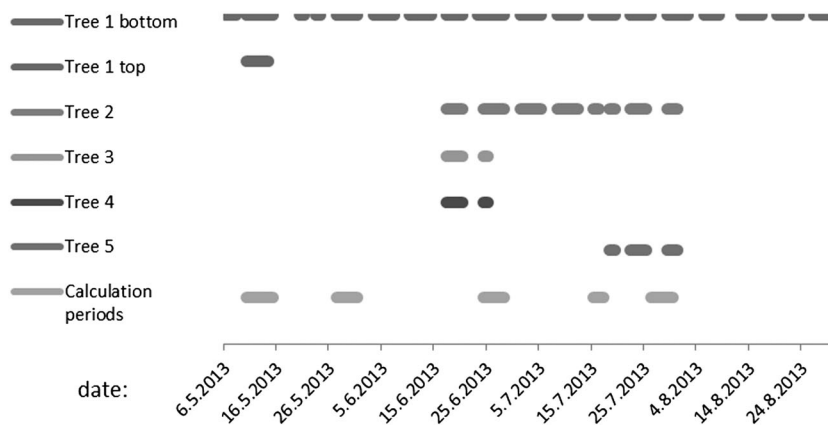
**Figure 1.** Components of the oleoresin exudation pressure (OEP) measuring system with Swagelok gauge (top) and the brass tube and WIKA pressure gauge attached to a pine tree at SMEAR II (bottom).

Perrakis 2008). The OEP was recorded at 30 min intervals using the time lapse feature of a digital camera installed facing the pressure gauge. The pressure gauge photos were transformed into data manually by reading the pressure from the photos, the resolution being 0.1 bar.

We had six pressure gauges which were installed on five trees (Trees 1 to 5) over the two summers (Fig. 2). In 2012, the measurements were taken only from Tree 1; this was the main measurement tree throughout 2012 and 2013. In

addition during the measurement periods of 2013, one gauge was continuously attached at breast height to Tree 1, this was photographed, and its data was used for the calculations. The manually read and thus less frequent data from other trees (Trees 2 to 5) were used to support the analysis and to examine any differences between the trees. Simultaneous successful measurements from three trees were performed for a period of 13 d (18 to 31 July 2013), whereas at other times one to two trees were being measured simultaneously (Fig. 2). In addition, the OEPs at two different heights of Tree 1 were measured simultaneously from 8 to 15 May 2013 (Fig. 2). The exact locations of the pressure gauges and brass tubes had to be changed on average every three weeks because of resin crystallization inside the brass tubes and the holes, indicated by the gradual dampening of diurnal variations and gradual lowering of pressure values. These about three weeks of constant measurements by one gauge are referred to as measurement periods below. However, the installations were always located close to each other so that the results could be compared, because according to Perrakis (2008), measurements on the different sides of a tree may differ. The breast-height diameter of Tree 1 was 29 cm, and the range of other tree diameters was from 14 to 26 cm.

One period from 2012 and five periods from 2013 measurements were used in the detailed data analysis. These periods chosen are referred to as calculation periods below. The calculation periods were chosen from the data of Tree 1 on the basis of measurement installations, and on the time frame the measurements remained functional so that the calculation periods covered all the measurement periods and the different phases of the growing season. The data for the first 24 h and approximately three days before the end of each measurement period were omitted from the analysis. This was done because the measurements could only be started 24 h after the installation, as the OEP normally soared for a few hours just after installing the gauge, and it took some time for the setup to reach equilibrium (Vité 1961, Perrakis 2008). During the measuring period, the diurnal pattern of OEP remained similar in all the data, although the actual OEP values continuously decreased. This is presumably because of the partial crystallization of resin in the drilled hole, because it gradually prevents the movement



**Figure 2.** The periods and location (in Tree 1) of measurements, and the calculation periods in 2013.



of oleoresin and thus diminishes the magnitude of observed changes in OEP. Eventually also the diurnal variation reached the detection limit, and then the measurements were stopped. Consequently, because of the small diurnal OEP variation, the last days of a measurement period could not be used for analysis. The calculation periods chosen for detailed analysis were 10–15 May, 27–31 May, 23–28 June, 15–17 July and 26–30 August, 2013.

The xylem diameter of Tree 1 was measured with linear displacement transducers (Solartron Inc., Model AX/5-0/5, Bognor Regis). The sensors were used as point dendrometers attached at breast height with custom made metal frames. The measurement frequency was  $1 \text{ min}^{-1}$ . See Perämäki *et al.* (2001) for a detailed description of the xylem diameter change setup. Tree gas exchange measurements from the top branches were used to estimate the transpiration rate (see e.g. Kolari *et al.* 2009). The xylem tension was calculated from trees in the same stand using the same value for xylem elastic modulus as Perämäki *et al.* (2001), i.e. 1 GPa.

Stem monoterpene emissions from Tree 1 were measured in 2013. The measurements were conducted with a dynamic flow-through fluorinated ethylene propylene (FEP) foil enclosure  $396 \text{ cm}^2$  in area surrounding the stem as in Vanhatalo *et al.* (2015) and attached at a height of about  $12 \text{ m}^{-1}$  where stem was about 9 cm thick. The sample flow was  $11 \text{ min}^{-1}$ , and samples of this flow were directed to an online proton transfer reaction-quadrupole mass spectrometer (PTR-Q-MS, Ionicon Analytik GmbH, Innsbruck, Austria). The sample flow was compensated by ambient supply air flow. In order to obtain the emission rate, also the monoterpene concentration in the supply air was measured before each sampling. In order to avoid high humidity and temperature as well as condensation and accumulation of volatiles in the enclosure air and surfaces, in between the samplings, the enclosure was flushed with ambient supply air at a lower flow rate of about  $0.51 \text{ min}^{-1}$ . Mixing of enclosure air was achieved with a rather high, continuous air flow rate through the enclosure. The air temperature inside the enclosure was followed with a thermocouple. The emission measurements were done at three hour intervals (three consequent measurements at a time), yielding 24 measurements per day. Here we report emissions of  $m/z$  137 ( $\text{amu}+1$ ), corresponding to the sum of monoterpenes. PTR-Q-MS was calibrated with standard gases every second week. The method for calculation of emissions as well as calibration is described in Taipale *et al.* (2008) and Kolari *et al.* (2012). In 2012, while OEP measurements were being taken, the monoterpene emission measurements were not running because of technical problems.

The visible downward trends of OEP in 2012 and 23–28 June, 2013, were corrected by fitting a function to the measured OEP and using the anomaly from the trend in the analysis. We used simple linear functions, because the differences between different fittings (linear, exponential and logarithmic) were negligible in the cases of the periods of data in question, and linear model was the simplest to employ. The trend was most likely produced by the gradual crystallization of oleoresin inside the brass tubes which inhibits its movement and thus its ability to create pressure inside the pressure gauge. Because

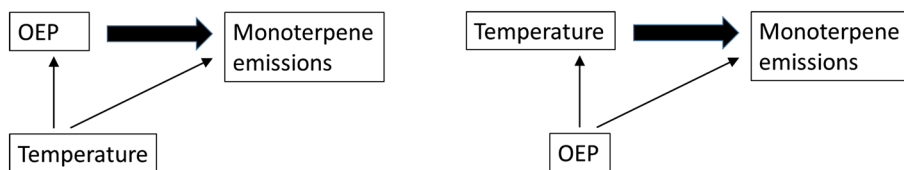
this is not a physiological phenomenon related to the phenomena studied here, it would have biased the analyses if it had been left uncorrected.

In order to clarify the xylem water potential effect, we also calculated temperature corrected OEP. This was done by simply modelling OEP as a function of temperature, and subtracting the OEP values obtained from this model from the measured OEP values. As to the type of function used for the correction (exponential, linear, logarithmic or polynomial), the best fit for the period of data in question was selected. Both the measured and temperature corrected OEP were analysed against environmental and physiological variables, such as temperature, xylem diameter and BVOC emissions from the stem. For each six calculation periods, Pearson correlations between OEP, temperature, xylem diameter and monoterpene emissions were calculated, as well as the averages of correlation coefficients over all the periods. As well for each calculation period, linear regression models explaining OEP firstly by using temperature and then by using temperature and water balance variables (the diameter of xylem, vapour pressure deficit (VPD) and transpiration) were constructed, and their averages of  $R^2$  and root-mean-square error (RMSE) were summarized over all the periods. The relationships between OEP and temperature, as well as OEP and the xylem diameter, and OEP and monoterpene emissions were analysed by fitting different functions for each period separately. We tested two function shapes: linear and exponential. They correspond to the different processes that can partly explain the OEP, for example thermal expansion and the effect of vapour pressure.

We applied elaboration techniques (Babbie 2007) to evaluate the mutual roles of OEP and temperature as determinants of the stem monoterpene emissions. Elaboration is a logical tool that helps to clarify the aims of the analysis and supports interpretation of its results. It is widely used in social sciences to clarify messy dependencies, but applies as well to analysis of, e.g. ecological data. Firstly, we tested whether the effect of OEP on monoterpene emissions could actually be explained by temperature, i.e. exogenously (Fig. 3, left). Secondly, we examined whether OEP mediates the effect of temperature on monoterpene emissions (Fig. 3, right). For this, three linear regression models, separately for each of the time periods, were constructed. In the two starting models, OEP and temperature individually served as the only explanatory variable whereas in the third model they were both present. By comparing the starting model coefficients of OEP and of temperature to those based on the third model the mutual roles of these variables as determinants of monoterpene emissions can be uncovered. For earlier ecological applications of elaboration, see e.g. Penttilä *et al.* 2006 (with two explanatory variables) and Ekholm *et al.* 2015 (with several explanatory variables).

## RESULTS

Remarkably, a similar systematic diurnal dynamic variation of OEP in the stems of all the studied trees was apparent in the results of both the summers of 2012 and 2013: the OEP was



**Figure 3.** A schematic model of the elaboration analysis. On the left, the OEP is the focal variable, and the interference of temperature on its effect on monoterpene emissions is studied. On the right, the temperature is the focal variable, and whether its effect on monoterpene emissions is partly mediated through OEP is studied.

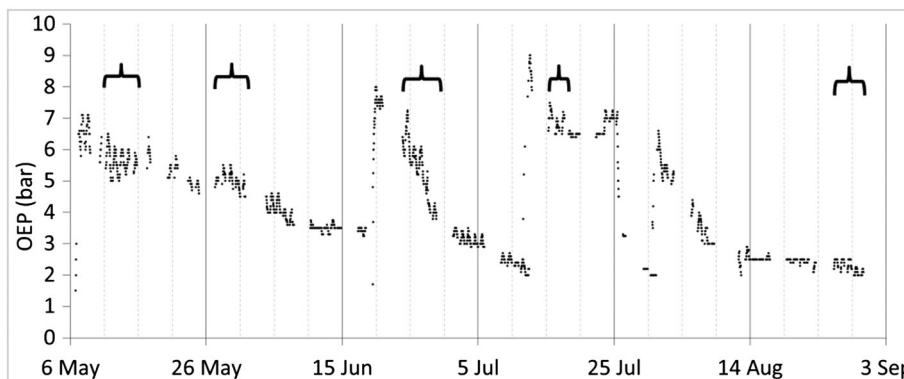
always highest after noon, from 1300 to 1500 h, and lowest before sunrise, from 0300 to 0600 h (Fig. 4). This pattern was similar during all the measuring periods regardless of the pressure gauge used for the measurements, measuring height or tree. In 2012, the mean OEP for Tree 1 was 6.5 bars. In 2013, the mean OEP for Tree 1 was approximately 5 bars and 3 bars for the other trees (Trees 2 to 5), the between-tree variation in mean OEP being 1.6 bars. The pressure at the top of Tree 1's stem was lower than at the bottom (Fig. 6) the average difference being 1.4 bars. From the installation of gauge towards the end of the measurement period both the absolute value and the diurnal amplitude of OEP diminished, but the diurnal pattern with respect to the timing of daily OEP minima and maxima remained unchanged. The daily range of OEP in all trees was approximately 0.5–1 bar (1.5 bar in 2012), and the range from the start to the end of one measurement period, the maximal observed change in OEP, was approximately from 7 to 2 bars.

The ambient temperature measured under the canopy from the height of 4.2 m correlated strongly positively with OEP during all the calculation periods (Table 2) and OEP quickly reflected the changes in temperature. For example, on the afternoon of 25 June the sudden drop in temperature was apparent in the OEP, too (Fig. 7). Temperature alone explained the diurnal pattern of measured OEP well, the average coefficient of determination ( $R^2$ ) of a linear regression model explaining OEP by temperature being 0.77 over the 2013 periods. The relation between temperature and OEP was exponential during the 2013 calculation periods (Fig. 5) except for the last one, 26–30 August, when it was linear. However, the differences between the coefficients of explanation of linear and exponential fittings were small

(number of observations, depending on the calculation period, from 113 to 243), so a definitive relation was not found.

However, during the warmest days of each measurement period, the OEP was not always at its highest value, and there were certain times when the changes in OEP could not be directly explained by the changes in temperature. These times seemed to be often connected to sudden changes in VPD that were not clearly linked to temperature. For example, in the afternoon of 26 June, OEP started to decrease following the descending VPD value, although the ambient temperature stayed high for slightly longer (Fig. 7). This is remarkable, because supposedly there should be a lag of ten to some tens of minutes in the response of OEP to ambient temperature, as the temperature of the wood changes slowly and indirect effects cause delays, too. Although VPD itself did not correlate consistently with OEP (data not shown), it slightly increased the explanatory power of the linear regression model explaining OEP. A linear regression model including temperature, VPD, xylem diameter and transpiration explained OEP better ( $R^2 = 0.88$ , RMSE = 0.14) than a model with only temperature ( $R^2 = 0.77$ , RMSE = 0.11) in 2013. During four out of five calculation periods, VPD was as a statistically significant variable in the regression models.

The diurnal pattern of the measured OEP differed considerably from the diurnal pattern of the xylem diameter, and the correlation between measured OEP and xylem diameter was on average negative in 2013 (Table 2, Figs. 5, 6 and 7). However, the correlation between temperature corrected OEP and the xylem diameter was on average positive (Table 2). Correlations between both measured and temperature corrected OEP and xylem diameter varied between

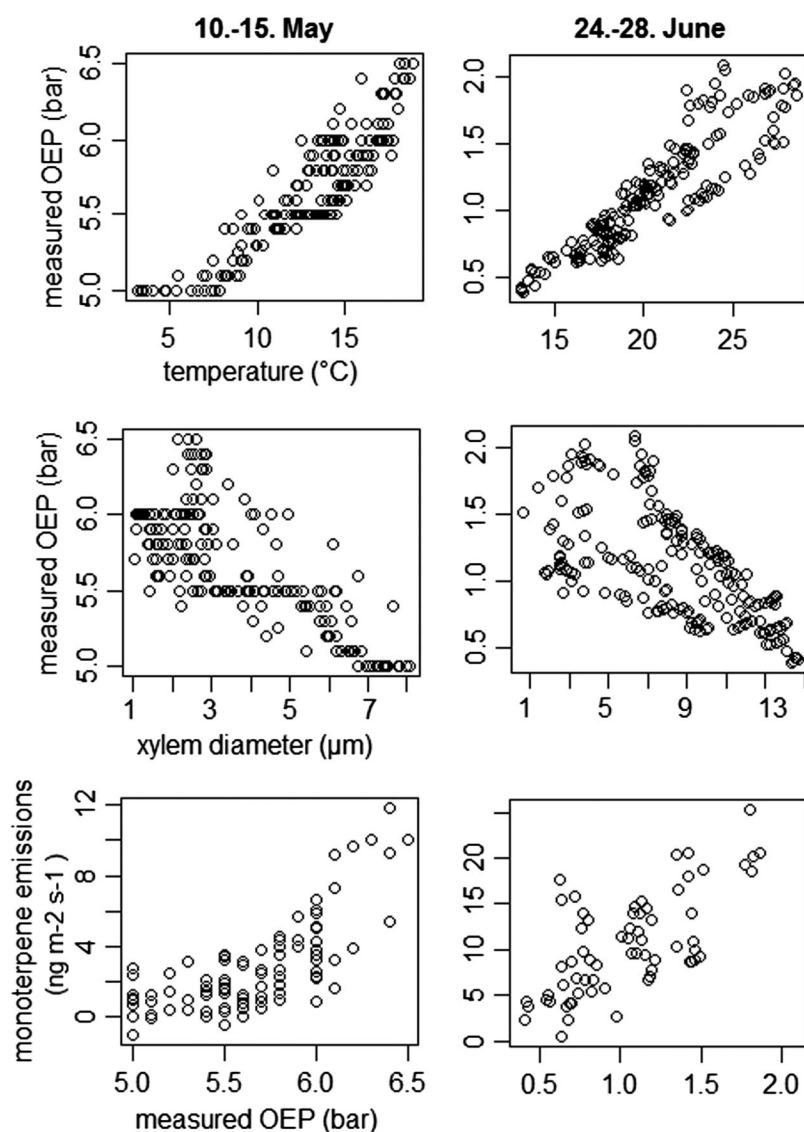


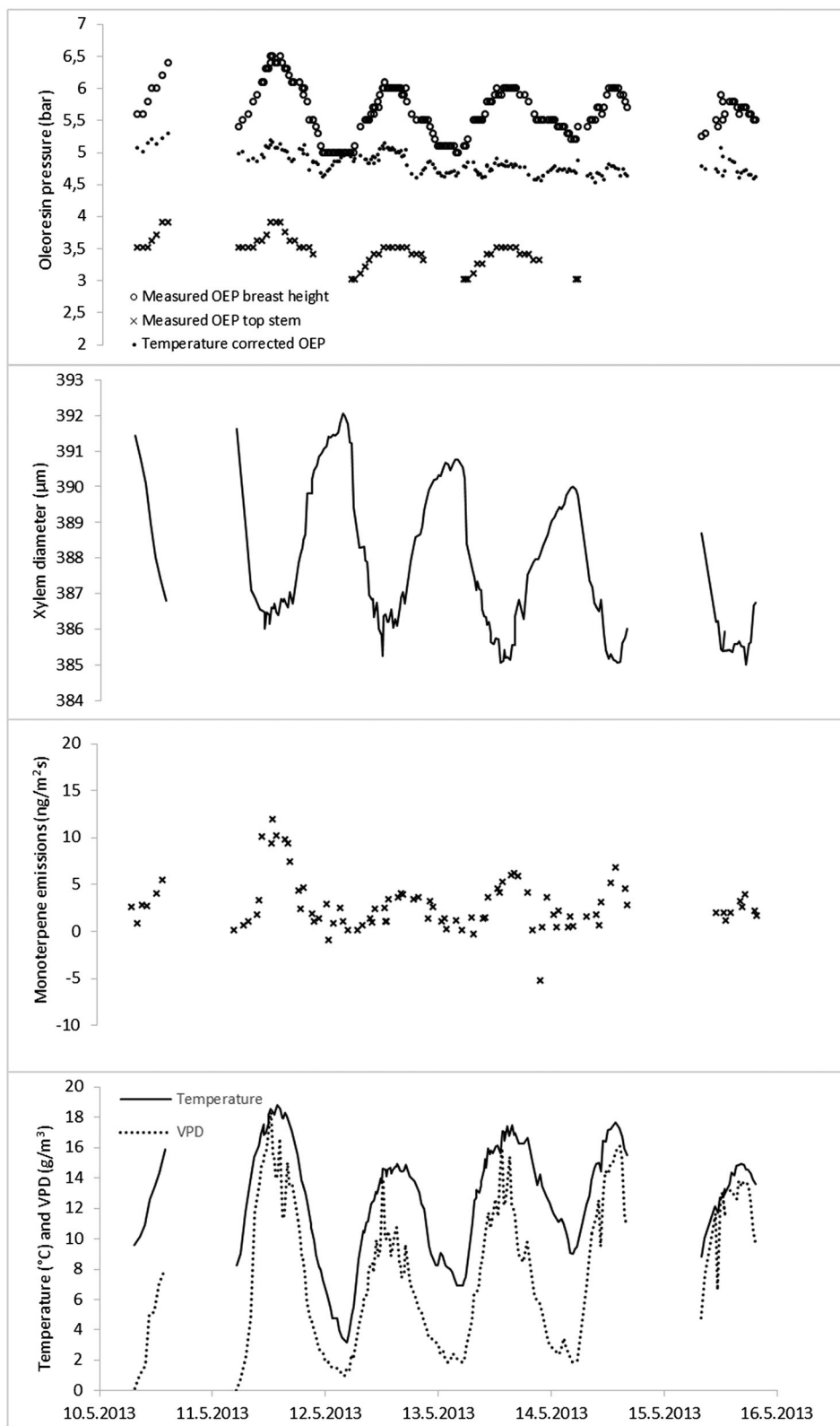
**Figure 4.** The measured dynamics of OEP in the bottom of Tree 1 over the summer of 2013 at SMEAR II station. The calculation periods are marked with braces.

**Table 2.** Pearson correlation coefficients between measured and temperature corrected oleoresin exudation pressure (OEP), ambient temperature, xylem diameter and monoterpene emission rates in 2012 and 2013.

Measurement period	2012		2013				Average
	30 Jul–3 Aug	10–15 May	27–31 May	23–28 Jun	15–18 Jul	26–30 Aug	
Measured OEPx							
Temperature	0.80**	0.90**	0.77**	0.90**	0.91**	0.90**	0.88
Diameter of xylem	−0.77**	−0.76**	−0.33**	−0.63**	−0.39**	−0.49**	−0.52
Temperature corrected OEPx							
Diameter of xylem	0.005	−0.09	0.46**	0.11	0.43**	0.20*	0.22
Monoterpene emission ratex							
Measured OEP	n.a.	0.72**	0.47**	0.68**	0.72**	0.84**	0.69
Temperature-corrected OEP	n.a.	0.34**	−0.20	0.16	−0.18	0.30*	0.08
Temperature	n.a.	0.67**	0.71**	0.67**	0.69**	0.77**	0.70

\*\*significant at the 0.01 level, \*significant at the 0.05 level, n.a. = not available

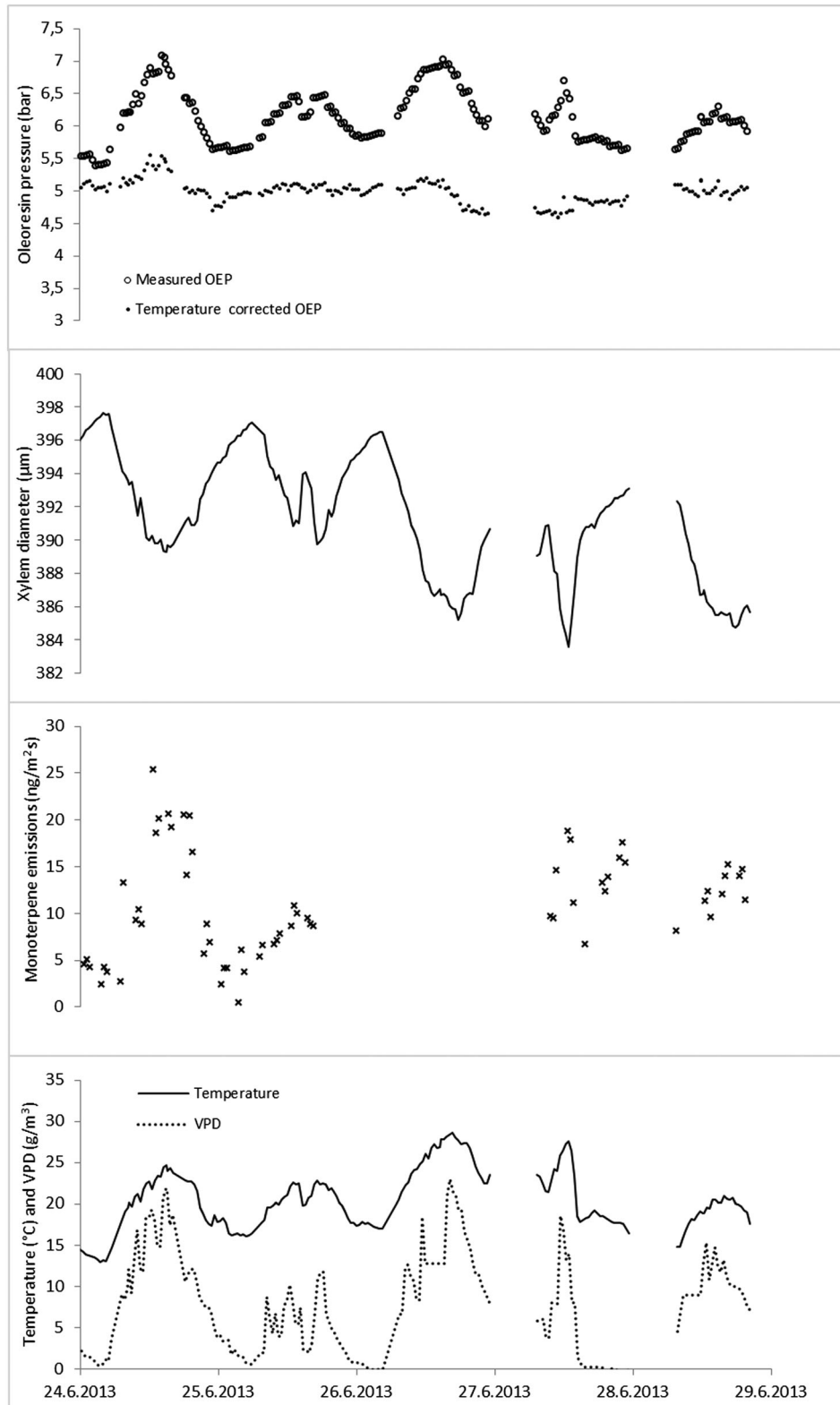
**Figure 5.** The relations between measured OEP, temperature, xylem diameter and monoterpene emissions at SMEAR II station, 10–15 May (left) and 24–28 June (right), 2013.



**Figure 6.** OEP at the living canopy (~10 m height) and at breast height, xylem diameter at breast height, stem monoterpene emissions at 12 m, temperature and vapour pressure deficit (VPD) at 4.2 m in SMEAR II, 10–15 May 2013.

the periods as did the shape of relation between the two variables. For four calculation periods in 2013, the relation between OEP and xylem diameter was linear (Fig. 5) and

for one period (27–31 May) the relation could have been either linear or exponential, as the coefficients of explanation ( $R^2$ ) of the different fittings were similar. However,



**Figure 7.** OEP at breast height, xylem diameter at breast height, stem monoterpene emissions at 12 m, temperature and VPD at 4.2 m in SMEAR II, 24–28 June 2013.

because of the very small differences between the fittings for all the periods, the definitive relation between OEP and the diameter of the xylem was not concluded.

Studying the OEPs of the top and the lower stems revealed that the difference between their OEPs correlates strongly with the diameter of the xylem ( $-0.79$ ) and VPD ( $0.77$ ).



Furthermore, we calculated the median value of xylem diameter over the calculation period and used it to divide the OEP differences between the top and the lower stem into two groups. The first group consisted of OEP difference values that corresponded temporally to the values of the xylem diameter over the median, and the second group of those that corresponded to the xylem diameter values under the median. The averages of these two groups differed significantly (over the median xylem diameter OEP was 2.21 and under 2.46 bars). However, as the diurnal variations in the xylem tension calculated from the changes in diameter of the xylem were low, only in the range of 1 bar, the effects of the extremes were not observed.

Although stem monoterpene emission rates varied considerably from hour to hour, the overall pattern appeared to follow both the ambient temperature and the measured OEP (Table 2, Figs. 5 and 6). The shape of the relation between measured OEP and monoterpene emissions in 2013 was linear for three periods (27–31 May, 23–28 June and 15–18 July) (Fig. 5), and exponential for two periods (10–15 May, 26–30 August). However, the differences between different fittings were small (number of observations from 44 to 118). There was not any consistent functional form between temperature corrected OEP and stem monoterpene emissions.

Because temperature appeared to be such an important feature in both the variations of stem monoterpene emission rates and the OEP, the relationship between these three variables was studied using the elaboration approach [explanatory framework based regression analysis (EFRA)]. During all the periods, adding temperature to the model decreased the coefficient of OEP, indicating that temperature is at least partly responsible for the effect of OEP on monoterpene emissions (Table 3). However, as the reduction in OEP coefficient was small during all but one period, 27–31 May, we conclude that only a small part of the effect of OEP on monoterpene

emission rates could be explained by temperature, and most of the effect did not depend on temperature. Conversely, adding OEP to the models considerably reduced temperature's coefficient; thus a large part of the temperature effect on monoterpene emission rates is probably mediated by OEP (Table 3). However, the temperature coefficient remained always non-zero; therefore part of the temperature effect is direct. Because monoterpene emission rates vary substantially during different phases of the growing period (Vanhatalo *et al.* 2015, A. Vanhatalo, personal communication), the temperature and OEP coefficients vary considerably between the different periods (Table 3).

In addition to EFRA analysis, simple linear regression models indicated that both OEP and temperature affect the monoterpene emission rates. The coefficients of determination ( $R^2$ ) for the linear regression models explaining monoterpene emissions with temperature or with OEP were on average very close to each other: 0.481 (temperature) and 0.476 (OEP). With both OEP and temperature in the model, the coefficient of determination was on average 0.54. In addition VPD, PAR and transpiration were introduced into the EFRA models (not shown), but their effects on the coefficients of temperature and OEP were negligible.

## DISCUSSION

As expected, our study showed that the Scots pine stem OEP has a clear and consistent daily pattern that was similar between different trees, measurement locations and pressure gauges throughout summer. However, our observations of the daily pattern in OEP were contrary to the pattern observed earlier: OEP reached the diurnal maxima at the time when the xylem diameter, and thus also water potential, reached its minima and temperature its maxima, i.e. during the early afternoon. The smallest OEPs were measured before sunrise. For example, according to Schopmeyer *et al.* (1952), Bourdeau & Schopmeyer (1958), Vité (1961), Lorio & Hodges (1968a,b) and Helseth & Brown (1970), OEP is high in the night, usually just before sunset, whereas during the warmest time of day it is at its lowest.

One reason for the differences between ours and the former studies could be the climate of the study sites. The other studies have been conducted in clearly warmer climates with presumably higher daily water potential variations, although the weather on our site during the summer of 2013 was slightly warmer than average for the local conditions (Table 1). During all but the fourth measurement period in July, the average monthly temperatures, as well as the minimum and maximum temperatures, were higher than average. In addition, the precipitation during summer 2013 was lower than average on our site. However, the diurnal xylem water potential variation at the stem base, as interpreted from the diameter variation at the stem base, was quite low, only less than 1 bar (Perämäki *et al.* 2001). We seldom experience such low soil water potential levels and droughts affecting vegetation at our measurement site as are found in other more southern sites (Ilvesniemi *et al.* 2010).

**Table 3.** The coefficients of elaboration analysis in 2013. Variable columns present the variables that were used in a linear regression model at any time; the coefficient columns present the coefficients of each variable in each model they were used

Measurement period	Variables of the model	Coefficients of variables	
		OEP	Temperature
10–15 May	OEP	5.01	
	Temperature		0.46
27–31 May	OEP and temperature	4.92	0.01
	OEP	19.18	
	Temperature		2.70
23–28 Jun	OEP and temperature	−6.96	3.18
	OEP	9.78	
	Temperature		1.12
15–18 Jul	OEP and temperature	5.46	0.56
	OEP	3.86	
	Temperature		0.41
26–30 Aug	OEP and temperature	2.80	0.13
	OEP	24.51	
	Temperature		1.19
	OEP and temperature	21.36	0.19

Instead of varying with the water balance of the tree, as suggested in the earlier studies (Vité 1961, Lorio & Hodges 1968b,c, Helseth & Brown 1970, Neher 1993), the OEP covaried best with ambient temperature, and in the linear regression models it was also clearly the best explanatory variable for OEP. This relationship was unaffected by the phase of the growing season or the monthly mean temperature. There are several potential explanations for the strong correlation. Temperature may impact the OEP directly by for example, thermal expansion of woody tissue, changes in viscosity or temperature dependency of the vapour pressure of oleoresin components. Oleoresin components, especially monoterpenes, are more fluid in warm temperatures; this affects the resin flow but should not influence the pressure reading directly as there is no resin flow in the measuring system. However, temperature increases the partial pressure of volatile compounds that could be reflected in pressure changes. If oleoresin is fluid enough only above a certain temperature in the stem, leaving air filled spaces in the measured system before that, temperature effect may explain also the fact that OEP was not detected before May. The above effects of temperature should have a time lag relative to air temperature because of slower temperature changes inside wood. That was not, however, tested here, as the time scales of the measurements are very different, and such an analysis would not have been very fruitful. The OEP measurements were performed every 30 min, the monoterpene emission measurements hourly, but the sapflow and diameter measurements were done at much shorter time intervals. Thus, in order to understand the temporal relationships better, more detailed measurements with shorter time intervals are needed.

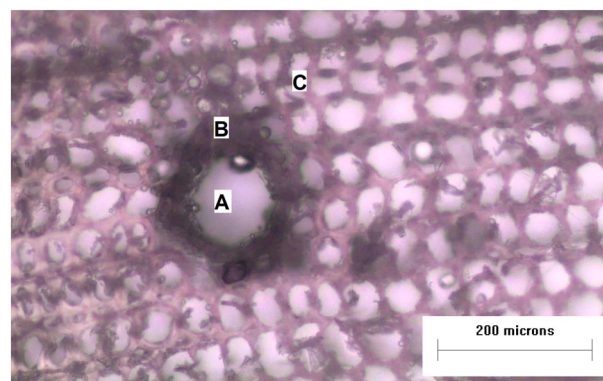
In addition to its physico-chemical effects, temperature may affect OEP through physiological processes in stems. The production of oleoresin in the epithelial cells may be activated at higher temperatures thus increasing the pressure inside the resin ducts. Temperature may also affect OEP indirectly, through higher photosynthetic production, allocation and storage in stems (e.g. Chan *et al.* 2015). This affects both the carbohydrate supply for stem processes (Sauter & van Cleve 1994) and the balance between sugars and starch inside the epithelial cells of resin ducts, as increasing temperature favours sugars over starch (Bhullar & Jenner, 1986). This could increase the osmotic pressure of the living epithelial cells in resin ducts, enlarge the cells as they take up water and cause a reduction in the volume of the resin ducts, and consequently increase the OEP.

Despite the strong correlation, the daily changes in OEP are not completely similar to the changes in temperature, and especially when VPD changes suddenly and independently of temperature, OEP seems to differ from the temperature pattern. Moreover, the linear regression models including water balance variables explain OEP better than models based only on temperature. The measured OEP correlated negatively with the xylem diameter and positively with VPD, whereas the temperature corrected OEP correlated positively with the xylem diameter. Thus, it can be hypothesized that the hydrostatic tension of the stem has an effect on OEP, but this is usually masked by the effect of temperature. In addition,

our results showed that the average OEP was clearly lower the higher the measurement point was in the stem, where the water potential is also more negative. The difference between the top and bottom stem OEPs was larger when VPD was higher and xylem diameter smaller, which also implies that changes in stem water tension affect the OEP. During periods of high water deficiency, hydrostatic tension changes more at the stem top, and this explains the growing difference in OEP between stem top and bottom. The average weather of the period was not observed to have an effect on the relationship between OEP and xylem diameter.

Our results clearly show that the effect of temperature overrides the effects of water status on OEP in Scots pine in a boreal environment under normal summer conditions. The relative importance of different drivers on the OEP, and especially the impacts of temperature and water status may differ between tree species and their growth conditions. We suggest that the processes that regulate the osmotic status of the epithelial cells lining the resin ducts and the changes in the volume of resin duct cavities play a central role in this balance (Fig. 8). On one hand, rising temperature may increase OEP by making the living epithelial cells lining the resin ducts swell and reducing the volume of the resin ducts. On the other hand, rising temperature increases VPD and transpiration which in turn decreases the turgor pressure of epithelial cells, enlarging the volume of the resin ducts and decreasing the OEP. The question is therefore, which processes affect epithelial cells most, and in boreal conditions they appear to be the temperature correlated ones. It might be possible that in boreal Scots pine the effect of water balance on OEP is revealed only by certain extreme conditions, for example during droughts. During summer 2013, no periods of drought were observed, so this effect could not have been reliably detected. However, it cannot be excluded that variables such as the previous reserves of oleoresin and the production of oleoresin building enzymes can have an influence on the relations of OEP.

As for the relationship between OEP, monoterpene emissions and temperature, it is evident that temperature affects monoterpene emissions directly for example by inducing diffusion flux and the evaporation of monoterpenes



**Figure 8.** Light micrograph of a vertical resin duct in Scots pine xylem, from the height of 10.4 m. A: resin duct B: epithelial cells C: tracheids.

from pools (e.g. Niinemets & Reichstein 2003). The effect of temperature induced evaporation of monoterpenes can be estimated by the temperature dependency of the vapour pressures of the chemical compounds in monoterpenes. However, according to the EFRA analysis, also OEP has an important and individual effect on the monoterpene emission rates, which suggests that the influence of temperature is probably partly mediated through OEP. Most likely the mechanism by which temperature affects the relationship between OEP and monoterpene emissions is a mixture of these factors. It can also include some lag that was not observed here because of the frequencies of the OEP and monoterpene emission measurements. Up to the present, the main driving factors in monoterpene emissions from tree canopies have been linked to variations in the light and temperature that the leaves and needles experience, but our results indicate that also the whole tree physiology may be involved in the process, especially as the oleoresin storage pools in stems and roots of resin-storing species are huge. For example, the bark monoterpene concentration in Scots pine is three times higher than that in the needles (Ghirardo *et al.* 2010). A more thorough analysis of the relationship between OEP and emission rates in different seasons is needed to potentially shed additional light on why the monoterpene emission rates in a pine canopy vary so much over time (Hakola *et al.* 2006).

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