Isoprenoid emission variation of Norway spruce across a European latitudinal transect

van Meeningen, Ylva; Wang, Min; Karlsson, Tomas; Seifert, Ana; Schurgers, Guy; Rinnan, Riikka; Holst, Thomas

Published in:
Atmospheric Environment

DOI:
10.1016/j.atmosenv.2017.09.045

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Isoprenoid emission variation of Norway spruce across a European latitudinal transect

Ylva van Meeningen a, *, Min Wang a, Tomas Karlsson a, Ana Seifert a, Guy Schurgers b, Riikka Rinnan c, Thomas Holst a, c

a Lund University, Department of Physical Geography and Ecosystem Science, Silvegatan 12, 223 62 Lund, Sweden
b University of Copenhagen, Department of Geosciences and Natural Resource Management, Øster Voldgade 10, 1350 Copenhagen K, Denmark
c University of Copenhagen, Terrestrial Ecology Section, Department of Biology, Universitetsparken 15, DK-2100 Copenhagen Ø, Denmark

HIGHLIGHTS

- Isoprenoid emission from Norway spruce were measured at seven sites in Europe.
- There were little differences in standardized emission rates across Europe.
- The emission profile differed between sites, but was less distinct for cloned trees.
- Emission patterns were potentially influenced by tree height, season and year.

ABSTRACT

Norway spruce (Picea abies) is one of the dominant tree species in the European boreal zone with the capacity to grow over large areas within Europe. It is an important emitter of biogenic volatile organic compounds (BVOCs), which can act as precursors of photochemical smog and ozone and contribute to the formation and growth of secondary organic aerosols (SOA) in the atmosphere.

Isoprenoid emissions were measured from Norway spruce trees at seven different sites, distributed from Ljubljana in Slovenia to Piikkiö in Finland. Four of the sites were part of a network of genetically identical spruce trees and contained two separate provenances. The remaining three sites were part of other networks which have been used to conduct studies in the European boreal zone.

There were minimal differences in the standardized emission rates between sites and across latitudes. The emission profile differed between provenances and sites, but there were not any distinct patterns which could be connected to a change in latitude. By using genetically identical trees and comparing the emission rates between sites and with genetically different trees, it was observed that the emission patterns were mostly influenced by genetics. But in order to confirm this possible stability of the relative emission profile based on genetics, more studies need to be performed.

The effects of branch height, season and variation between years on observed emission pattern variations were also investigated. There were indications of potential influences of all three factors. However, due to different experimental setups between measurement campaigns, it is difficult to draw any robust conclusions.

© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Biogenic volatile organic compounds (BVOCs) are emitted in large variations from different types of plants, where some of the most prominent compound groups are isoprene (ISO, consisting of one C5 unit), monoterpenes (MTs, consisting of two C5 units) and sesquiterpenes (SQTs, consisting of three C5 units) (Kesselmeier and Staudt, 1999; Holopainen, 2011). The primary functions for plants to release BVOCs are to attract pollinators, defend against herbivores and pathogens, serve as signals in plant-plant communication and to give protection against abiotic stresses such as high temperature, high irradiation or oxidative stress (Dudareva and Pichersky, 2008;...
Laathaworkitkul et al., 2009; Vickers et al., 2009; Holopainen and Gershenzon, 2010; Loreto and Schnitzler, 2010; Penuelas and Staudt, 2010; Maffei, 2010). But once released into the atmosphere, BVOCs can influence the atmospheric chemistry and Earth’s radiative balance (Di Carlo et al., 2004; VanReken et al., 2006; Beerling et al., 2007; Paasonen et al., 2013). BVOCs can act as precursors of photochemical smog and ozone, and also contribute to its destruction depending on the levels of NOx in the atmosphere (VanReken et al., 2006; VanReken et al., 2006; Spracklen et al., 2008; Paasonen et al., 2013; Ehn et al., 2014). Both SOA and modifications of cloud properties have the potential to affect incoming solar radiation and thus influence global warming (VanReken et al., 2006; Laathaworkitkul et al., 2009; Paasonen et al., 2013).

Forests are significant sources of BVOCs, in particular of isoprene and MTs (Kesselmeier and Staudt, 1999; Holopainen, 2011; Laffineur et al., 2011). One of the largest vegetation zones in the world is the boreal zone, consisting of approximately 33% of the total worldwide forest cover across Eurasia and Northern America (FAO, 2001). Boreal forests emit less BVOCs in comparison to temperate or tropical regions on a global scale, but are important contributors to regional emission budgets (Guenther et al., 1995, 2012; Acosta Navarro et al., 2014; Sindelarova et al., 2014). The emission budgets are influenced by the high reactivity rates of BVOCs, which depend on the structure of the BVOCs released and the chemical composition of the air at the local scale where BVOCs are emitted (Atkinson and Arey, 2003). The emission from the boreal zone is often characterized by MTs, but there can be considerable isoprene and SQT emissions as well (Tarvainen et al., 2007; Rinne et al., 2009). The boreal zone yields substantial amounts of SOA in the atmosphere, which plays an important role in the global radiation balance (Claeys et al., 2004; Tunved et al., 2006; Bonn et al., 2009).

Norway spruce (Picea abies) is one of the dominant tree species of the European boreal zone, but it is also widely distributed throughout other parts of Europe (Janson et al., 1999; Grabmer et al., 2006; Filella et al., 2007; Skjøth et al., 2008; Kivimäenpää et al., 2013). Norway spruce is a low or moderate monoterpene emitter and a low isoprene emitter (Janson et al., 1999; Kesselmeier and Staudt, 1999; Simpson et al., 1999; Grabmer et al., 2006). It has the capacity to emit BVOCs from storage structures in resin ducts, needles and the bark, but there can also be a significant contribution from de novo emissions (Ghirardo et al., 2010).

Leaf- and canopy scale emission models can be used to investigate the effect of BVOC emissions on atmospheric chemistry and aerosol formation. As BVOCs have different reactivity with OH radicals and ozone due to their different molecular structures (Atkinson and Arey, 2003), it is of importance to consider the composition of the emissions from different plant species (Niinemets et al., 2010). At a spatial-, biome- or global-scale, the emissions are typically determined by dividing the plants into plant functional types (PFT) for which an average emission potential has been determined. However, the emission capacities for different plant species within a PFT can vary greatly and the parameterization chosen for a PFT might vary for different models (Guenther et al., 2006; Arneth et al., 2010; Niinemets et al., 2010; Monson et al., 2012). The vegetation has a capacity to adapt their emission patterns over environmental gradients, which leads to large uncertainties between emissions and geographical location (Niinemets et al., 2010; Bourtsoukidis et al., 2012; Harrison et al., 2013) that are difficult to include in models. The observed geographic variability may be related to adaptation to local growing conditions, hybridization or to existing genetic variation (Staduit et al., 2004; Bäck et al., 2012; Steinbrecher et al., 2013), but may also be affected by microclimatic conditions, adaptations at different canopy heights and by seasonal development (Keenan et al., 2009; Niinemets et al., 2010; Grote et al., 2013).

The importance and impact of genetic diversity and growing condition adaptations on the BVOC emission patterns has been highlighted by several studies. Nerg et al. (1994) studied Scots pine (Pinus sylvestris) seedlings grown at different latitudes in Estonia and Finland, and reported latitudinal effects on terpene concentration in pine shoots. Bäck et al. (2012) measured BVOC emission from Scots pine trees and found that there existed different chemotypes attributed to genetic variation within the same stand. Persson et al. (2016) measured genetically identical trees of English oak (Quercus robur), European beech (Fagus sylvatica) and Norway spruce and found that the relative compound contribution remained fairly stable between individuals of the same species. These studies highlight an existing uncertainty regarding the importance of genetic variation for observed BVOC emissions relative to the effect of local environmental conditions across latitudes (Kesselmeier and Staudt, 1999; Bäck et al., 2012; Persson et al., 2016).

The aims for this study are (I) to investigate the potential variability for the emission rates of Norway spruce across a latitudinal transect in Europe and (II) to examine how the observed emission patterns are influenced by genetic diversity and height within the canopy, but also time of season and variation between years will be considered.

2. Methods

2.1. Site descriptions

Measurements were performed at seven sites along a latitudinal transect across Europe, stretching from Ljubljana in Slovenia to Piiikkiö in Finland (Fig. 1). The sites were chosen as they represent both the boreal ecosystem and cover a wide range where spruce has the capacity to grow. It is a collaboration of four separate
projects with different measurement campaigns, where one to five campaigns were performed per site. Each campaign lasted between one and three weeks. The details of location, tree height, tree age, sampling date, number of performed campaigns, measurement year, annual average temperature (°C), annual total precipitation (mm), average Photosynthetically Active Radiation (PAR) and average temperature (°C) during campaigns can be found in Table 1.

Four of the sites, namely Ljubljana (Slovenia), Grafrath (Germany), Taastrup (Denmark) and Piikkiö (Finland), are part of the International Phenological Garden (IPG) network in Europe which have been used to perform long-term phenological observations on naturally occurring plant species (Chmielewski et al., 2013). The advantage of the network is that all plants are clones, which means the genetic variation between plants is negligible. Ljubljana, Grafrath and Piikkiö were established between 1962 and 1965, whilst Taastrup was begun in 1971 (www.agrar.hu-berlin.de). Within the IPG network, there are two provenances of Norway spruce which are divided into early spruce (SE), with an early budburst, and late spruce (SL) which experiences budburst approximately one week later.

The remaining sites did not contain genetically identical trees, but are part of other networks used to perform studies in the European boreal zone. Hyltemossa research station is located in southern Sweden, whilst Norunda research station is located about 30 km north of Uppsala in Sweden. Both sites are run by the International Phenological Garden (IPG) network in Europe which many), Taastrup (Denmark) and Piikkiö (Finland), are part of other networks used to perform studies in the European boreal zone. Hyltemossa research station is located in southern Sweden, whilst Norunda research station is located about 30 km north of Uppsala in Sweden. Both sites are run by the Integrated Carbon Observation System in Sweden (ICOS). Hyltemossa is dominated by Norway spruce with a small fraction of Downy birch, whilst Norunda is part of a boreal forest dominated by 80–123-years old Norway spruce and Scots pine (Lagergren et al., 2005). The site in Skogaryd is located in south-western Sweden, approximately 50 km from the west coast and covers an area of roughly 30 km². The site is part of Swedish Infrastructure for Ecosystem Science (SITES) (Shendryk et al., 2014) and mainly contains coniferous trees, dominated by Norway spruce and Scots Pine (Wallin et al., 2015) (Fig. 1).

### Table 1

<table>
<thead>
<tr>
<th>Study site and coordinates</th>
<th>Tree heights (m)</th>
<th>Tree age (yr)</th>
<th>No of trees and samples</th>
<th>Measurement months and no. of campaigns</th>
<th>Study years</th>
<th>Temperature (°C)</th>
<th>Precipitation (mm)</th>
<th>PAR in chambers (µmol m⁻² s⁻¹)</th>
<th>Temperatures in chambers (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ljubljana 46°04’N, 14°30’E</td>
<td>17</td>
<td>52</td>
<td>3 (17)</td>
<td>May (1)</td>
<td>2014</td>
<td>10.9</td>
<td>1362</td>
<td>1000</td>
<td>17.8</td>
</tr>
<tr>
<td>Ljubljana 46°04’N, 14°30’E</td>
<td>17</td>
<td>53</td>
<td>3 (21)</td>
<td>Oct (1)</td>
<td>2015</td>
<td>10.9</td>
<td>1362</td>
<td>1000</td>
<td>12.5</td>
</tr>
<tr>
<td>Ljubljana 46°04’N, 14°30’E</td>
<td>17</td>
<td>54</td>
<td>3 (51)</td>
<td>Apr–May (3)</td>
<td>2016</td>
<td>10.9</td>
<td>1362</td>
<td>1000</td>
<td>18.5</td>
</tr>
<tr>
<td>Grafrath 48°18’N, 11°17’E</td>
<td>2.5, 20</td>
<td>5, 51</td>
<td>6 (50)</td>
<td>Jun (1)</td>
<td>2014</td>
<td>8.5</td>
<td>877</td>
<td>1000</td>
<td>25.7</td>
</tr>
<tr>
<td>Grafrath 48°18’N, 11°17’E</td>
<td>3, 20</td>
<td>7, 53</td>
<td>4 (22)</td>
<td>Jun (1)</td>
<td>2016</td>
<td>8.5</td>
<td>877</td>
<td>1000</td>
<td>22.8</td>
</tr>
<tr>
<td>Taastrup 55°40’N, 12°18’E</td>
<td>15</td>
<td>42</td>
<td>4 (76)</td>
<td>Jul–Aug (1)</td>
<td>2013</td>
<td>7.5</td>
<td>583</td>
<td>1000</td>
<td>20.2</td>
</tr>
<tr>
<td>Taastrup 55°40’N, 12°18’E</td>
<td>15</td>
<td>43–45</td>
<td>4 (70)</td>
<td>Jul (3)</td>
<td>2014</td>
<td>7.5</td>
<td>583</td>
<td>1000</td>
<td>20.9</td>
</tr>
<tr>
<td>Hyltemossa 56°06’N, 13°25’E</td>
<td>14–18</td>
<td>30</td>
<td>4 (27)</td>
<td>Jul (1)</td>
<td>2016</td>
<td>8.0</td>
<td>800</td>
<td>1000</td>
<td>20.8</td>
</tr>
<tr>
<td>Skogaryd 58°23’N, 12°09’E</td>
<td>25</td>
<td>53</td>
<td>1 (40)</td>
<td>Oct (1)</td>
<td>2015</td>
<td>6.2</td>
<td>709</td>
<td>134</td>
<td>8.9</td>
</tr>
<tr>
<td>Norunda 60°05’N, 17°29’</td>
<td>25</td>
<td>119</td>
<td>1 (90)</td>
<td>Jun (1)</td>
<td>2014</td>
<td>5.4</td>
<td>520</td>
<td>250</td>
<td>17.8</td>
</tr>
<tr>
<td>Norunda 60°05’N, 17°29’</td>
<td>25</td>
<td>119</td>
<td>1 (73)</td>
<td>Jul (1)</td>
<td>2014</td>
<td>5.4</td>
<td>520</td>
<td>289</td>
<td>29.0</td>
</tr>
<tr>
<td>Piikkiö 60°23’N, 22°30’E</td>
<td>12</td>
<td>49</td>
<td>4 (19)</td>
<td>Jul–Aug (1)</td>
<td>2015</td>
<td>5.9</td>
<td>698</td>
<td>1000</td>
<td>24.2</td>
</tr>
</tbody>
</table>

---

a = http://meteo.aro.gov.si.
b = http://www.wetter-by.de.
c = Jensen et al., 1997.
d = http://www.icos-sweden.se.
f = Aubinet et al., 2010.
g = http://en.ilmatieteenlaitos.fi.
with PAR (Li-Cor Li-190, USA). In Skogaryd, two chambers were mounted on a scaffold at two heights (2.5 m and 3.5 m). In Norunda, one chamber was installed 20.0 m above the ground, whilst another chamber measured the emission rates at a lower level (3.0 m in June and 11.0 m in July).

In all campaigns, a hydrocarbon trap (Alltech, Associates Inc., USA) containing MnO2-coated copper nets was fixed between the ambient air inlet and the chamber inlet to absorb all the VOCs and ozone from the incoming air stream. Sample air passed through stainless steel cartridges (Markes International Limited, Llantrisant, UK) packed with adsorbents Tenax TA (a porous organic polymer) and Carbograph 1TD (graphitized carbon black). The air samples were extracted by using flow-controlled pocket pumps (Pocket Pump, SKC Ltd., Dorset, UK). The sampling flow rate was 200 ml min⁻¹ and the collected volume for each sample was between 5 and 6 L. Blank samples were also collected in order to acknowledge possible background contamination in the ambient air. After BVOC measurements were performed, the needles inside the chambers were harvested, dried until the biomass weight was constant and weighed to get the dry weight (g(dw)). All measurements were performed during daytime (8:00–17:00).

A total of 556 cartridges were collected and analyzed. All cartridges were sealed with Teflon-coated septa. Samples and stored at 3°C until analysis. For all sites except Norunda, the BVOCs samples were analyzed by gas chromatograph–mass spectrometer following thermal desorption at 250°C at the University of Copenhagen (for details, see van Meeningen et al., 2016). For standardization, pure standard solutions of isoprene, α-pinene, camphene, δ-phellandrene, limonene, eucalyptol, γ-terpinene, linalool, aromadendrene and α-humulene in methanol (Fluka, Buchs, Switzerland) were injected into adsorbent cartridges in a stream of helium. When quantifying BVOCs without a standard solution, α-pinene was used for MTs and α-humulene was used for SQTs. The chromatograms from the analysis were identified from the mass spectra in the NIST library and analyzed with the MSD ChemStation Data Analysis software (G1701CA C00.00 21 Dec 1999; Agilent Technologies, Santa Clara, CA, USA). For the samples collected in Norunda, the analysis of sample tubes was done by the Finnish Meteorological Institute (Atmospheric Composition Unit, Helsinki, Finland). The samples were desorbed at 300°C, and the standard solutions were camphene, 3-carene, 1,8-cineole, limonene, linalool, myrcene, α-pinene, β-pinene, terpinolene, longicyclene, isolongifolene, β-caryophyllene, aromadendrene, α-humulene, 2-methyl-3-buten-2-ol (MBO), p-cymene and bornylacetate. Isoprene was calibrated using a gaseous standard.

2.3. Normalization of BVOC emission rates

The BVOC emission rate (E) from all of the samples was defined by the mass of compound per dry biomass weight and time (Hakola et al., 2003):

\[
E = (C_2 - C_1) \times F \times m^{-1}
\]  

(1)

where E is the emission rate (in μg g⁻¹ h⁻¹), C₂ is the concentration of compound in the sample (μg l⁻¹), C₁ is the VOC concentration in the inlet air (μg l⁻¹), here considered to be zero, as the incoming air was filtered free of VOCs, F is the air flow rate into the chamber (l min⁻¹), and m is the dry weight of the needle biomass (g). In order to make comparisons between emission spectra and amounts from different sites, all emission rates were normalized to standard light and temperature conditions (PAR, 1000 μmol m⁻² s⁻¹ and T, 303 K). The algorithm for light dependent compounds presented by Guenther et al. (1993) was used for isoprene and is expressed as:

\[
I = I_S C_T C_L
\]  

(2)

where I is the emission rate at a given leaf temperature and flux of PAR. I_S is the standardized emission rate at standard light and temperature conditions. C_T and C_L are the temperature and light correction factors, calculated by the following equations:

\[
C_T = \frac{2C_1 PAR}{\sqrt{1 + 2^{2PAR^2}}}
\]  

(3)

and

\[
C_L = \exp\left(\frac{C_1(T - T_0)(314 - T_0)}{RT_0^2}\right)
\]  

(4)

where R (8.314 J K⁻¹ mol⁻¹) is the ideal gas constant and α (0.0027), C₁ (1.066), C₇₁ (95 000 J mol⁻¹), C₇₂ (230 000 J mol⁻¹) and T₉ (314 K) are empirical coefficients (Guenther et al., 1993). For monoterpenes and sesquiterpenes, the algorithm for temperature dependent compounds by Guenther et al. (1993) was used and is described as:

\[
M = M_S \exp(β(T - T_0))
\]  

where M is the monoterpene or sesquiterpene emission rate at a given leaf temperature, whilst β (0.09 K⁻¹) is an empirical coefficient.

2.4. Statistical analysis

The significant difference between sites, provenances and years was analyzed by a Kruskal-Wallis test on all of the different groups. If the test resulted in significant differences, a Mann-Whitney U test followed by Bonferroni correction was performed to determine significant groups. Differences in the emission rates between different heights were tested using Mann-Whitney U-tests.

Partial least squares regression (PLS) was performed to investigate possible connections between factors such as ambient and past average temperatures, height, latitude, PAR and season (X-variables) and the emitted BVOCs (Y-variables). Season was defined as the number of active growing days where the average daily temperature had exceeded 5°C for five consecutive days. Analysis was performed using SIMCA (Umetrics, version 13.0.3.0, Umeå, Sweden).

3. Results

3.1. Latitudinal BVOC emission profiles and influential environmental factors

The total average normalized emission rate of BVOC was 2.74 ± 2.96 μg (g dw)⁻¹ h⁻¹ (mean ± standard deviation), of which MT (1.59 ± 1.91 μg (g dw)⁻¹ h⁻¹) was the most commonly emitted BVOC group followed by isoprene (0.94 ± 1.84 μg (g dw)⁻¹ h⁻¹) and SQTs (0.30 ± 0.88 μg (g dw)⁻¹ h⁻¹). The average release of isoprene, MTs and SQTs between sites are in line with previous studies on Norway spruce (Table 2). The sites Taastrup, Skogaryd and Norunda had different emission rates in comparison to remaining study sites. Taastrup had a higher total MT emission, due to high emission contribution from needle branch measurements done in 2013. Skogaryd had a total emission which was a fifth of the average total emission rate from all measured sites. But it should be noted that the site was only visited in October, whilst the other sites had a
The majority of their measurements taken between May and August. Norunda had the highest emission of isoprene, mostly originating from the lower parts within the canopy (Table 2).

A total of 23 terpene compounds were detected from the measurement sites, which were isoprene, 17 MTs and five SQTs. The most emitted compounds from a majority of the sites were α- and β-pinene and limonene. Other compounds with high emission rates were camphene and 3-carene. Early spruce had in general a higher emission of α- and β-pinene whilst late spruce emitted more isoprene and limonene. For the sites Hyltemossa and Skogaryd, about a third of the emissions came from pinene, whilst Norunda had high emission rates of isoprene. Average isoprene emissions for Grafrath was 0.64 μg (g dw)^{-1} h^{-1} and for Norunda 2.58 μg (g dw)^{-1} h^{-1}, which was significantly higher in comparison to the major study sites (P < 0.05). MT emissions from Norunda were 1.02 μg (g dw)^{-1} h^{-1}, which was significantly less in comparison to Grafrath (1.60 μg (g dw)^{-1} h^{-1}). Taaststrup had an average MT emission of 2.63 μg (g dw)^{-1} h^{-1}, which was higher compared to Ljubljana (1.17 μg (g dw)^{-1} h^{-1}) and Norunda. Average SQT emissions were significantly lower for Grafrath (0.10 μg (g dw)^{-1} h^{-1}) and Norunda (0.38 μg (g dw)^{-1} h^{-1}) in comparison to the other sites. In regards to separate compounds, Taaststrup had high emission rates of pinene (0.91 μg (g dw)^{-1} h^{-1}) and low emission rates of camphene (0.14 μg (g dw)^{-1} h^{-1}), whilst Norunda differed in 3-carene emission due to low emission rates (<0.01 μg (g dw)^{-1} h^{-1}). Limonene emission varied most between sites (Fig. 2).

There were little changes in the emission patterns of specific compounds with changes in latitude. Within the IPG network, the contribution of limonene in relation to the total BVOC emission increased slightly with latitude; from 12% in Ljubljana to 23% in Piikkiö. The relative emission of pinene on the other hand ranged between 16 and 23% between samples and was fairly similar between the IPG sites. For the sites Hyltemossa, Skogaryd and Norunda, the relative percentage of limonene was lower, with 16% of the total emission at Hyltemossa and 7–8% for Skogaryd and Norunda. For total BVOC emission, α-pinene and β-pinene had a relative contribution of 30–32% for Hyltemossa and Skogaryd and 10% for Norunda. In the case of Norunda, isoprene contributed with 64% to the total emission. But if only MTs were considered, the relative compound contribution of pinene would be in range of both Hyltemossa and Skogaryd. Fig. 3 shows the average emission profile of individual MTs for each of the sites.

As the emission rates and emission profiles varied between the study sites, a PLS was used to investigate if there was any specific climatic factor which had a higher tendency to affect the observed emission patterns. It should be noted that the aim of this PLS-analysis was to investigate the connection between the compound emissions (isoprene, MTs and SQTs) and the climatic conditions experienced on site, and not to build a good prediction model for each of the studied compounds. The PLS-analysis revealed that the samples were separated according to latitude and measurement height on the y-axis and temperature on the x-axis (Fig. 4a). Height and latitude were strongly correlated. In Fig. 4a, data from higher latitudes and canopy heights are positioned towards the bottom whilst lower latitudes and canopy heights are positioned at the upper part of the figure. On the x-axis of this figure, data points with higher temperatures are positioned to the right whilst lower temperatures are positioned to the left. The number of active growing days (season) was not as strong driver as temperature on the x-axis, but is part in separating sites with measurements taken in October (Ljubljana and Skogaryd in 2015) from the sites where measurements were done in the middle of the summer (Fig. 4a). The PLS-analysis also revealed that the individual compounds are poorly explained by the model (Fig. 4b); the included climatic factors explained 7.8% of the total emission variation (Fig. 4c).

3.2. The influence of genetics on observed BVOC emission patterns

The sites within the IPG network were used to study how the BVOC emissions from Norway spruce might have adapted to local growing conditions without considering genetic variation. Grafrath was significantly different (P < 0.05) from the other sites in regards to its isoprene emissions. The average emission from Grafrath (0.64 μg (g dw)^{-1} h^{-1}) was two to four times higher in comparison to all remaining sites and provenances, except for late spruce in Taastrup (0.50 μg (g dw)^{-1} h^{-1}). The isoprene emission rate from early spruce at Taastrup (0.22 μg (g dw)^{-1} h^{-1}) was low and significantly different from Ljubljana (0.32 μg (g dw)^{-1} h^{-1}) and...
Grafrath. Total MT emission from early spruce in Grafrath (1.88 mg g\(^{-1}\)C\(^01\) h\(^{-1}\)) and late spruce in Taastrup (2.95 mg g\(^{-1}\)C\(^01\) h\(^{-1}\)) were significantly different from Ljubljana (1.17 mg g\(^{-1}\)C\(^01\) h\(^{-1}\)) and late spruce in Piikkiö (0.70 mg g\(^{-1}\)C\(^01\) h\(^{-1}\)) due to higher average MT rates (Fig. 5). There were no significant differences in SQT emissions. In regards to separate compounds, late spruce in Taastrup had higher emission rates of \(\alpha\)-pinene (1.44 mg g\(^{-1}\)C\(^01\) h\(^{-1}\)) and lower emission rates of limonene (0.82 mg g\(^{-1}\)C\(^01\) h\(^{-1}\)) in comparison to many of the other sites (Fig. 5). There were no significant emission differences between sites for camphene and 3-carene.

The importance of genetics and latitudinal adaptation was further investigated by comparing the emission pattern variation between genetically identical trees and genetically different trees over similar latitudinal ranges. The sites Taastrup and Piikkiö were chosen for comparing genetically identical sites and the sites Hyltemossa and Norunda for the genetically different sites. These four sites were chosen as the latitudinal differences between sites are relatively similar, with approximately one latitude degree difference between the two sites compared. For Norunda, the emission rate from 20 m up in the canopy was used for the comparison.

For early and late spruce in Taastrup (n = 25) and Piikkiö (n = 19), there were significant differences (P < 0.05) in the emission of isoprene, 3-carene, limonene, other compounds, total MT emission and the total BVOC emission. However, if only late spruce was considered (n = 19 for Taastrup and n = 10 for Piikkiö), then there were no statistical differences (P > 0.05). This comparison could not be done for early spruce as there was only one tree

---

**Fig. 2.** Statistically significant differences between measurement sites Ljubljana (L), Grafrath (G), Taastrup (T), Piikkiö (P), Hyltemossa (H) and Norunda (N). Statistics were done on the summarized emission of isoprene (a), monoterpenes (b), sesquiterpenes (c), pinenes (d), limonene (e), camphene (f) and 3-carene (g) across measurement sites. A grey box indicates a statistical difference (P < 0.05) between the two sites for a particular BVOC group or compound. All measurements performed in April and October were excluded from the dataset, which means that the site Skogaryd was not included in the statistical analysis. Statistical analysis was performed by doing a Kruskal-Wallis test and when it was statistically significant it was followed by Mann-Whitney U test with Bonferroni correction.

**Fig. 3.** Individual MT compound contribution to the total emission of MTs (mean ± SE) from the measurement sites Ljubljana (Slovenia), Grafrath (Germany), Taastrup (Denmark), Piikkiö (Finland), Hyltemossa (Sweden), Skogaryd (Sweden) and Norunda (Sweden). Other compounds include tricyclene, \(\alpha\)-thujene, sabiine, \(\alpha\)-phellandrene, \(\alpha\)-terpinene, \(\beta\)-phellandrene, eucalyptol, ocimene, \(\gamma\)-terpinene, terpinolene and linalool.
available in Taastrup and too few measurements from Piikkiö. For the sites Hyltemossa (n = 27) and Norunda (n = 72), there were significant differences (P < 0.05) in the emissions of isoprene, 3-carene, other compounds and total emission of MTs. When the genetically identical trees from Taastrup and Piikkiö were compared with the emissions from Hyltemossa and Norunda, all compounds except for the total BVOC emission were significantly different, with an average emission rate of 2.76 ± 1.88 and 1.73 ± 1.55 µg (dw)^{-1} h^{-1} for Taastrup and Piikkiö and 1.76 ± 1.36 and 1.75 ± 1.59 µg (dw)^{-1} h^{-1} for the Hyltemossa and Norunda.

Fig. 4. Partial least squares regression (PLS) on the biogenic volatile organic compound emissions (circle in the loading plot (b) = isoprene; squares = monoterpenes (M); triangles = sesquiterpenes (S)) from the different latitudinal sites, height within the canopy and measurement years. The scores of the factors (a) are shown together with the loading variables (b). The variance explained of the independent data (X) and dependent data (Y) are shown within the parenthesis. The independent variables shown in the loading plot (b) were ambient temperature (°C), average temperature over the past two and four days (°C), daily ambient Photosynthetically Active Radiation (PAR), height within the canopy (m), latitude and season. Season was defined as the number of active growing days where the average daily temperature had exceeded 5 °C for five consecutive days. Dependent variables were the compounds isoprene (ISO), tricyclene (M1), α-thujene (M2), α-pinene (M3), camphene (M4), sabinene (M5), β-pinene (M6), myrcene (M7), α-phellandrene (M8), 3-carene (M9), α-terpinene (M10), limonene (M11), β-phellandrene (M12), eucalyptol (M13), ocimene (M14), γ-terpinene (M15), terpinolene (M16), linalool (M17), caryophyllene (S1), β-farnesene (S2), α-farnesene (S3), α-humulene (S4) and cis-α-bisabolene (S5).
3.3. The impact of height

At Taastrup (2013) and Norunda (2014), samples were taken at different heights within the canopy in order to investigate possible emission pattern variations. Focus has been on the emissions of isoprene, MT, SQT and dominant MT compounds (α-pinenes, β-pinene, camphene, 3-carene and limonene) from different canopy heights (Table 3). For early spruce in Taastrup, there was a significant difference (P < 0.05) for limonene emission, between canopy level of 2.0 m (2.47 μg (g(dw))⁻¹ h⁻¹) in comparison to 12.5 m (0.07 μg (g(dw))⁻¹ h⁻¹) in comparison to 12.5 m (0.02 μg (g(dw))⁻¹ h⁻¹). The remaining compounds showed no statistical difference (P > 0.05) between canopy heights. For late spruce in Taastrup, camphene emission was significantly lower at 2.0 m (0.01 μg (g(dw))⁻¹ h⁻¹) than at 12.5 m (0.1 μg (g(dw))⁻¹ h⁻¹), whilst limonene emission was lower at 2.0 m (0.57 μg (g(dw))⁻¹ h⁻¹) than at 12.5 m (2.32 μg (g(dw))⁻¹ h⁻¹) and at 5.0 m (0.69 μg (g(dw))⁻¹ h⁻¹) than at 12.5 m. For Norunda, all BVOC emissions between 3.0 m and 20.0 m height within the canopy were significantly different. The emissions of isoprene (3.79 μg (g(dw))⁻¹ h⁻¹) and MT (1.51 μg (g(dw))⁻¹ h⁻¹) at 3.0 m height were higher in comparison to the emissions at 20.0 m above the ground (isoprene 0.98 μg (g(dw))⁻¹ h⁻¹ and MT 0.60 μg (g(dw))⁻¹ h⁻¹). The total MT emission was not significantly different for canopy levels of 11.0 m and 20.0 m, but there was a significant difference for the compounds β-pinene (0.1 μg (g(dw))⁻¹ h⁻¹) for 11.0 m and <0.01 μg (g(dw))⁻¹ h⁻¹ for 20.0 m) and 3-carene. It should be noted though that no 3-carene emission was on 11.0 m due to a high 3-carene concentration in the blank samples (Table 3).

3.4. Indications of annual and seasonal emission pattern fluctuation

Within the IPCC network, Ljubljana and Grafrath were visited in 2014 and 2016 at approximately the same time within the growing season. Comparisons between years were done on the main BVOC groups and the emission rates were not significantly different (P > 0.05) between measurement years. Taastrup was visited between June and August in 2013–2016. Isoprene emission was significantly different for both 2013 (0.19 μg (g(dw))⁻¹ h⁻¹) and 2015 (1.12 μg (g(dw))⁻¹ h⁻¹) in comparison to remaining measurement years (P < 0.05). In 2013, the emission of isoprene was low in comparison to the remaining measurement years, whilst 2015 had a higher emission rate. MT emission was significantly different in 2013 (4.17 μg (g(dw))⁻¹ h⁻¹) and between 2014 (1.57 μg (g(dw))⁻¹ h⁻¹) and 2015 (0.69 μg (g(dw))⁻¹ h⁻¹) (P < 0.05). Both provenances of spruce had MT emission rate three to four times as high in 2013 as the average emission rate in the other years. There were also differences in MT emission rates between 2014 and 2015, where the average emissions in 2014 were higher in comparison to the emission rates in 2015 (Fig. 6).

In regards to seasonal development, most of the BVOC measurements were taken between May and August, but for Ljubljana (2015) and Skogaryd, measurements were performed in October. The average standardized emission rates in October were approximately a third to a sixth of the total average standardized emission rates measured between May and July (Table 1; Fig. 6). For Ljubljana, where the emission profile in October and May were compared, early spruce had a higher contribution to the total emission of camphene (43.1%) and limonene (23.72%) in October, whilst in May there was a higher contribution of isoprene (17.46%) and SQTs (23.6%). Late spruce had a higher emission of isoprene (43.06%) and SQT (36.02%) in October and a higher contribution to the total emission of pinenes (20.48%) and other compounds (24.83%, which for Ljubljana were tricyclene, terpinenes, eucalyptol and linalool) in May. Both provenances showed a decrease in the total emission contribution of other compounds, but without a decrease in the number of emitted compounds (data not shown).

4. Discussion

4.1. Separating latitudinal adaptation from genetic diversity

Norway spruce and Scots pine are important BVOC emission contributors in European boreal ecosystems (Rinne et al., 2009). In
comparison to Scots pine, less emission studies have been undertaken on Norway spruce despite it being one of the most common coniferous tree species growing in Europe (Rinne et al., 2009; Yassaa et al., 2012). This study presents the average standardized emission rates of Norway spruce within the European boreal zone, but also over a larger latitudinal range across Europe. The emission rates across the sites were in range of other earlier studies (Janson, 1993; Christensen et al., 2000; Janson and de Serves, 2001; Hakola et al., 2003; Grabmer et al., 2006; Yassaa et al., 2012; Bourtsoukidis et al., 2014; Hakola et al., 2017) and there was minimal emission rate difference between the southernmost site and the northernmost site. Taastrup, Skogaryd and Norunda were sites which differed most from the other sites in regards to their emission patterns. Even though these sites are located in the northern range of this study, the observed emission rate differences are not necessarily due to their geographical location. The total MT emission rate at Taastrup in 2013 was higher and significantly different from the other sites. The measurement years between 2014 and 2016 were in range of the other visited sites. BVOC emission at Skogaryd was only measured in October and had lower emission rates in comparison to sites measured during the growing season. Norunda had the highest average isoprene emission, with high emission rates at 3.0 m and 11.0 m in the canopy. This is most likely due to a strong emission response during high irradiation events in different parts of the canopy.

Apart from limonene, the emission rates of other compounds in the southernmost sites Ljubljana and Grafrath were not significantly different from each other. The spruce tree in Norunda differed most from the trees in the remaining sites, both in regards to BVOC emission groups and for separate compounds. One reason could be that the tree in Norunda was standing in a much denser forest in comparison to the other sites, leading to a possible difference in shade acclimation.

Both local climate and genetic diversity have been shown to impact observed emission pattern fluctuations (Komenda and Koppmann, 2002; Semiz et al., 2007; Niinemets et al., 2010; Bäck et al., 2012; Steinbrecher et al., 2013), but it can be difficult to separate latitudinal adaptations and genetics apart. By using the IPG network, it was possible to further investigate the impact of possible adaptations to local growing conditions from two provenances of spruce without considering genetic diversity. The two provenances of Norway spruce were slightly different in regards to their emission profiles. Whilst early spruce had a higher relative emission of α- and β-pinene, late spruce emitted more isoprene and limonene. Apart from limonene emission, which increased slightly with increasing latitude, there were no distinct emission pattern changes within the same provenance and between IPG sites. This relative compound similarity across latitudes would suggest that the genetic material of the tree determines the emission profile of the compounds and that adaptation to local growing conditions do not happen instantly, but may have developed over generations.

The importance of genetic diversity was further investigated by comparing the BVOC emissions of genetically identical trees from Taastrup and Piikkiö with genetically different trees in Hyltemossa and Norunda. When all sites were compared, there was a statistical difference for all compounds and sites except for the total amount

### Table 3

The p-values of Mann-Whitney U test for the standardized emission rate (in µg (g dw)^{-1} h^{-1}) of isoprene (ISO), monoterpenes (MT) and sesquiterpenes (SQT) and dominant MT compounds from different canopy heights in Taastrup (2.0 m, 5.5 m and 12.5 m) and Norunda (3.0 m, 11.0 m, 20.0 m). SE stands for early spruce and SL for late spruce. The hyphen marks compounds where there was a lack of data on one of the measurement heights. Statistics were not performed between height levels of 3.0 m and 11.0 m at the Norunda site due to a difference in measurement months between mentioned height levels.

<table>
<thead>
<tr>
<th>Terpene</th>
<th>Taastrup (SE)</th>
<th>Taastrup (SL)</th>
<th>Norunda</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0 m–5.5 m</td>
<td>2.0 m–12.5 m</td>
<td>5.5 m–12.5 m</td>
</tr>
<tr>
<td>ISO</td>
<td>0.66</td>
<td>0.35</td>
<td>0.21</td>
</tr>
<tr>
<td>MT</td>
<td>0.13</td>
<td>0.24</td>
<td>0.98</td>
</tr>
<tr>
<td>SQT</td>
<td>0.24</td>
<td>0.07</td>
<td>0.19</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>0.10</td>
<td>0.19</td>
<td>0.93</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>0.40</td>
<td>0.35</td>
<td>0.94</td>
</tr>
<tr>
<td>Camphene</td>
<td>0.07</td>
<td>&lt;0.01</td>
<td>0.28</td>
</tr>
<tr>
<td>3-Carene</td>
<td>0.66</td>
<td>0.61</td>
<td>0.90</td>
</tr>
<tr>
<td>Limonene</td>
<td>0.03</td>
<td>0.19</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Fig. 6. The average standardized emission rates (in µg (g dw)^{-1} h^{-1}) of isoprene (ISO), monoterpenes (MT) and sesquiterpenes (SQT) from Ljubljana (Lju), Grafrath (Gra) and Taastrup (Taa) for provenances and years. SE stands for early spruce and SL for late spruce, where early spruce has a budburst pattern approximately one week earlier than late spruce. The error bars show the standard deviation for each BVOC group.
of BVOC emissions. But when the same comparison was performed on genetically identical trees, there were significant differences for all compounds and sites. These results highlight that the sites show less of a difference in emission patterns with a change in latitude, most likely due to acclimation to local weather and growing conditions. The non-significant difference between sites and genetically identical trees further suggest that adaptation to local environment do not happen instantly, but may take place over a longer time. In order to confirm this possible similarity of the relative emission profile, similar emission comparisons with more sites and over a longer latitudinal range are suggested.

The potential similarity in the total emission rates across a latitudinal gradient and relative emission profile might be of use in atmospheric emission models. In general for many emission models, the emission potentials are calculated for isoprene, the sum of all MTs and the sum of all SQTs as they represent a significant emission proportion from trees (Arndt et al., 2010; Niinemets et al., 2010). The results presented here provide average standardized emission rates of these three compound groups from spruce over a vast latitudinal range. However, for models which focus on atmospheric chemistry, it is important to also consider the emission profile, as the reactivity of compounds varies by several orders of magnitude due to their molecular structure (Atkinson and Arey, 2003; Niinemets et al., 2010). As the composition might vary due to short-term environmental impacts and genetic diversity between trees, it is difficult to give specific emission ranges of different compounds. However, the observed similarity of emission rates between IPG network sites, which also have had the possibility to acclimate to the local growing conditions, show that the composition seen at different sites can be considered to remain fairly stable with the progression of time.

4.2. Observed BVOC emission patterns in relation to height, season and year

Apart from the studied effects of latitudinal adaptation and genetic diversity on observed emission pattern fluctuations, the effect of measurement height within the canopy, change with season and yearly differences have also been investigated.

In Taastrup and Norunda, emissions were measured at three levels within the canopy. Whilst Taastrup only showed emission pattern differences between levels for a few compounds, Norunda showed statistically significant differences both for compound groups and separate compounds for all measured canopy heights. A possible explanation for the dissimilarity in emission response could be the amount of shade adaptation at different heights and sites. Persson et al. (2016) explained that the insignificant emission difference between canopy heights in Taastrup could be caused by the wide spacing between trees in the phenological garden. This results in relatively high levels of irradiation at lower canopy levels, which minimizes the difference in light adaptation between needle branches from the upper and lower parts of the canopy. In Norunda, the trees were planted with less space in between trees and therefore have quite distinct differences in available light at different canopy levels. Furthermore, the shape and the color of the needles in Norunda were different between canopy heights, with thicker, greener and a higher percentage of newly produced shoots at the upper canopy in comparison to lower levels.

The emission pattern change in regards to time of season has shown to have a substantial effect on observed emission rates (Janson, 1993; Hakola et al., 2003, 2012, 2017; Taryainen et al., 2005). Seasonality has also shown to influence not only the emission amounts, but also changing the compounds’ emission profile (Janson, 1993; Hakola et al., 2003). The majority of the measurements in this study were performed between May and August, which is in the middle of the growing season. But in Ljubljanas and Skogaryd in 2015, measurements were performed in October which is outside the main growing season. In comparison to both the emission differences between campaigns within Ljubljanas and between the remaining sites, the emission rates in October were less than one third of the emissions reported during the middle of the growing season. The trees in Ljubljanas also showed a change in emission profile for both provenances of spruce and season. Early spruce changed from high emissions of isoprene and SQT in May to emitting a higher relative contribution of camphene and limonene in October. Late spruce had high relative compound contribution of pinenes and other compounds in May and emitted more isoprene and SQT in October. However, even though the emission profile changed with the progression of the season, the number of compounds emitted did not change considerably. Skogaryd has only been measured in October and as it is unknown what the emission capacities are during summer season, which makes comparisons of emission profile with other sites unrealistic. However, the differences in emission patterns with remaining sites points to a potential influence of season which would be recommended to study further.

Apart from a seasonal change in BVOC emission patterns, there might also be differences between measurement years (Hakola et al., 2017). Hakola et al. (2012) compared the emissions from a boreal forest in Hyttiälä between seasons and years. They found that apart from summer, when the emission differences were rather small, the emission profile could differ between measurement years. A similarity between summer season emissions was also indicated in this study for Ljubljanas and Grafrath, which showed no significant difference between measurement years. But as only two summer seasons were compared, the emission similarities during the summer period in general cannot be quantified. For Taastrup, measurements were conducted for four years in a row at approximately the same time in July. For the total emission rates, the emissions were on average higher in 2013 compared to the other years. For the summer season of 2013, Persson et al. (2016) reported a distinct period without rainfall which might have caused drought stress to the spruce trees. Even though the volumetric water content of the soil was not measured, other indications such as dry needles shedding from lower branches whilst being handled suggested that the trees were in some state of water shortage. In a study by Lappalainen et al. (2009) in a coniferous forest, MT concentrations started to decline soon after a summer drought. This drop in emissions might be connected to senescence or needle shedding. The emission rates also differed between measurement years. In 2013, the emission of isoprene was significantly lower in comparison to other measurement years, whilst MTs were higher. 2015 was also a year which differed from remaining measurement years, with high isoprene emissions and lower MT emissions. In relation to other measurement years, 2015 was almost 2 °C colder than average for July (data not shown) and this may have had an influence on the observed emission patterns. Even though the reasons for variable emission patterns only can be speculated in this case, there are indications that past weather events might have had an influence on the emission pattern variations between years.

4.3. Uncertainties related to BVOC measurements from chosen sites and the latitudinal gradient

Several sites which are part of different measurement campaigns were studied in order to get a better representation of the possible growing range of Norway spruce. The inclusion of different measurement campaigns made it possible to investigate different influential aspects of the emission variation, such as genetic
diversity, latitudinal adaptations, potential emission differences between summer and autumn, height within the canopy and between measurement years. But as the different measurement campaigns were not originally set to cover all of the above mentioned aspects, the amount of data collected is not sufficient to quantify their separate importance on observed BVOC emissions. Furthermore, the inclusion of different chamber measurement techniques between studies adds further uncertainty to emission variations between sites. The needle chambers used together with LI-6400 has a disadvantage that the plant needs to be handled for each sample, which might increase the risk of mechanical stress induced emissions. The disadvantage of the transparent branch chambers is that the climatic conditions inside the chamber are more difficult to control, which might have indirectly provided with more stress to the branch inside the chamber due to different climatic conditions.

Apart from the influence of genetic diversity, adaptations to different growing conditions, height, season and measurement year on observed BVOC emissions, the emissions have also been influenced by other factors which have not been thoroughly studied in this setup. In the performed PLS analysis, only 7.8% of the emissions could be explained by temperature, PAR, height, latitude and time of season. Factors which were not included in the analysis but which have shown visible effects on the emission rates are drought, heat, herbivore attack and mechanical damage of the needle twigs or branches. Therefore, more studies investigating how the emission patterns in situ are influenced by these short-term effects would be needed.

In order to compare the emission rates between sites, all measurements were normalized according to the algorithms presented by Guenther et al. (1993). For the normalization of monoterpens and sesquiterpenes, a $\beta$-value of 0.09 was used. But it has been shown by Duhl et al. (2008) that the $\beta$-value could be different between SQTs and MTs when the standardized emissions are calculated. Previous results have indicated that $\beta$ for SQTs could be in the range 0.05–0.29 (Duhl et al. 2008). However, since many factors need to be taken into account when choosing an appropriate $\beta$-value, such as plant species, season and specific compounds, it is hard to decide which value should be used. For the studies reported in Table 2, the $\beta$-value for MTs is between 0.01 and 0.19 and for SQTs 0.02–0.17. As the main purpose of this study was to compare the emission pattern variability for spruce influenced by latitude, genetics and height, the same value for both MTs and SQTs was chosen to simplify the analysis.

5. Conclusions

Norway spruce is an important coniferous source of BVOCs which can be found over a large range in Europe. This study measured the emission rates of genetically identical and genetically different Norway spruce trees at seven European sites. The results showed that the standardized emission rates from different sites within Europe did not vary considerably with a change in latitude. The emission profile differed between provenances and sites, but the emission profile difference was less distinct for genetically identical trees. The study suggests that the spruce isoprenoid emission is potentially more determined by genetic diversity than by adaptation to local growth conditions. This possible stability in isoprenoid emissions could be used to improve the parameterization in different emission models.

It was also indicated that the observed emission rates were influenced by canopy height, time of season and measurement years. But due to different experimental setups between measurement campaigns, it is not possible to quantify the effect of the separate factors. More comprehensive measurements involving the effect of canopy height, season and measurement years would be needed in order to better understand the emission pattern fluctuations.

Acknowledgements

The authors would like to acknowledge Michelle Schollert Reneeerkens and Goshia Sylvester at the University of Copenhagen and Hannele Hakola and Heidi Helén at the Finnish Meteorological Institute for performing the BVOC sample analysis. We thank David Allbrand and Sven-Olof Andersson for their contribution with the field work in Skogary and Anders Båth and Irene Lehner for their help with the field work in Norunda. We would also like to thank Per-Erik Isberg at the department of Statistics in Lund for his advice in statistics. All campaigns were funded by FORMAS (2012–727), VR (2011–3190) and BECC.

References


van Meeningen, Y., Schurgers, G., Rinman, R., Holst, T., 2016. BVC emissions from English oak (Quercus robur) and European beech (Fagus sylvatica) along a latitudinal gradient. Biogeosciences 13, 6067–6080.


Svensson, M., Öquist, M.G., 1999. Inven-
989–998.

Web references