Drought effects on root and needle terpenoid content of a coastal and an interior Douglas fir provenance

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Douglas fir (Pseudotsuga menziesii) is a conifer species that stores large amounts of terpenoids, mainly monoterpenoids in resin ducts of various tissues. The effects of drought on stored leaf terpenoid concentrations in trees are scarcely studied and published data are partially controversial, since reduced, unaffected or elevated terpenoid contents due to drought have been reported. Even less is known on the effect of drought on root terpenoids. In the present work, we investigated the effect of reduced water availability on the terpenoid content in roots and needles of Douglas fir seedlings. Two contrasting Douglas fir provenances were studied: an interior provenance (var. glauca) with assumed higher drought resistance, and a coastal provenance (var. menziesii) with assumed lower drought resistance. We tested the hypothesis that both provenances show specific patterns of stored terpenoids and that the patterns will change in response to drought in both, needles and roots. We further expected stronger changes in the less drought tolerant coastal provenance. For this purpose, we performed an experiment under controlled conditions, in which the trees were exposed to moderate and severe drought stress. According to our expectations, the study revealed clear provenance-specific terpenoid patterns in needles. However, such patterns were not detected in the roots. Drought slightly increased the needle terpenoid contents of the coastal but not of the interior provenance. We also observed increased terpenoid abundance mainly in roots of the moderately stressed coastal provenance. Overall, from the observed provenance-specific reactions with increased terpenoid levels in trees of the coastal origin in response to drought, we conclude on functions of terpenoids for abiotic stress tolerance that might be fulfilled by other, constitutively expressed mechanisms in drought-adapted interior provenances.

Keywords: climate change, coastal provenance, Douglas fir, drought stress, interior provenance, terpenoids.

Introduction

Climate change will cause a considerable temperature increase and changes in precipitation patterns together with a higher frequency of extreme weather events such as drought periods, heat waves, heavy rainfall and storms, particularly in the Northern hemisphere (IPCC 2013). Climate models predict that precipitation during summer will decrease considerably in Central and Northern Europe (IPCC 2013). Consistent with these model projections,
hints of enhanced summer warming and increased frequency and strength of summer drought events have been observed in Europe (Schär et al. 2004, Seneviratne et al. 2006). It is thought that the expected environmental changes will strongly affect growth, reproduction, defense and communication processes of plants (Peñuelas and Staudt 2010). Moreover, drought stress is assumed to affect soil nutrient availability of plants, as well as the uptake capacities of plant roots and, consequently, the nutrient status of trees (Kreuzwieser and Gessler 2010).

To withstand drought stress periods, trees respond at the morphological (e.g., by decreased leaf area and altered leaf structure), physiological (e.g., stomatal closure, activation of autotrophic respiration, accumulation of osmolytes and stress resistant proteins) and molecular (e.g., altered gene expression patterns) level (Bréda et al. 2006). Drought stress is well known to cause elevated levels of reactive oxygen species (ROS) (Rennenberg et al. 2006). Plants possess a great arsenal of mechanisms involved in detoxification of these radicals including the antioxidants ascorbate, glutathione, and α-tocopherol (Tausz et al. 2004, Rennenberg et al. 2006), the Halliwell-Asada cycle which is active in different cell compartments (Noctor et al. 2012), as well as the xanthophyll cycle in chloroplasts (Munné-Bosch and Alegre 2000). Further protection against oxidative stress is the biosynthesis of volatile terpenoids, which is thought to quench ROS (Vickers et al. 2009, Possell and Loreto 2013), however, it is not understood if drought stress might be alleviated by volatile terpenoids (Blanch et al. 2009).

Terpenoids are synthesized from isopentenyl diphosphate (IPP) and its allylic isomer, dimethylallyl diphosphate (DMAPP) (McGarvey and Croteau 1995). These compounds are synthesized in plants by two independent pathways, the cytosolic mevalonic acid (MVA) and the plastidic methylethylthiol phosphate (MEP) pathway (Dudareva et al. 2013). Terpenoids can be stored in plants in specialized structures (Gershenzon and Croteau 1991). Conifers, for example, possess resin ducts to accumulate terpenoids (Wu and Hu 1997) and in such species, terpenoids can make up around 1–2% of the leaf dry weight in conifer species (Blanch et al. 2009).

Terpenoids are thought to play important roles in abiotic (heat, oxidative stress) and biotic stress defense (Loreto and Schnitzler 2010, Loreto et al. 2014). In conifers, they are involved in the defense against herbivores and pathogens (Martin et al. 2003, Gershenzon and Dudareva 2007, Loreto et al. 2014). Even though several functions of terpenoids in stress alleviation are well understood (Vickers et al. 2009), the effect of different forms of stress on the abundance of terpenoids in plants is less investigated. Some previous work studied the effects of drought on leaf and wood terpenoid concentrations in conifers. The results were, however, very inconsistent, ranging from drought-induced increases in monoterpenoid levels in xylem oleoresin and stems of conifers (Pinus taeda: Hodges and Lorio 1975, Picea abies, Pinus sylvestris: Turtola et al. 2003) to decreased levels of some monoterpenoids even in the same species (P. taeda: Gilmore 1977). Similarly, drought increased the monoterpenoid concentrations in needles of conifers and deciduous plants (P. abies: Kainulainen et al. 1992, Pinus halepensis, Quercus ilex, Quercus cocifera: Llusia and Peñuelas 1998, Rosmarinus officinalis, P. halepensis: Llusia et al. 2006, Salvia officinalis: Nowak et al. 2012), but did not affect their concentrations in other species (Cistus albidus: Llusia and Peñuelas 1998). In addition, it has been demonstrated that different provenances of the same conifer species can strongly vary in their terpenoid content (Von Rudloff 1972, Manninen et al. 2002) and it is not clear if different provenances adapted to specific site conditions react in the same manner to a given environmental stress. These ambiguous results on the effects of drought stress on terpenoid contents of aboveground tree parts are contrasted by a complete lack of knowledge on drought-induced changes in root terpenoids, although root terpenoids may be equally affected by abiotic stress.

In the present study, we therefore examined the effects of drought stress on the needle and root terpenoid levels in young Douglas fir trees. For this purpose, we performed an experiment under controlled environmental conditions in which two Douglas fir provenances from contrasting origins, namely from a coastal and an interior habitat, were exposed to limited soil water availability and were then assessed during the development of moderate and severe drought stress. We tested the hypotheses (i) that severe drought stress causes increased levels of terpenoids in leaves and roots of Douglas fir, and (ii) that provenance-specific reactions will appear with the more drought-sensitive coastal provenance exhibiting stronger drought responses than the more drought-adapted interior provenance.

**Materials and methods**

**Plant material**

One-year-old Douglas fir saplings were studied in the present experiment. Interior Douglas fir trees of provenance ‘Fehr Lake’ (INT, seedlot: FDI 39,481; Southern interior British Columbia, Canada, zone TOA, Thompson Okanagan Valley, arid, hot, sub-zone IDF xh 3, seeds collected near Fehr Lake) were provided from BC Timber Sales (Vernon, BC, Canada), while the coastal Douglas fir provenance ‘Snoqualmie’ (COA, seedlot: pme 07 (797) 412-10; USA, Washington, North Cascades, west side zone Snoqualmie) was supplied by the Forestry Commission, Wykeham Nursery (Sawdon, UK). The climate conditions of the natural origins of the two Douglas fir provenances are described in detail by Du et al. (2016). Briefly, the annual precipitation sum during summer was much lower for INT (333 mm) than for COA (2134 mm). Mean annual temperatures were 5.8 and 7.94 °C for INT and COA, respectively. The seedlings were transplanted in 3 l pots containing a commercial substrate (Container substrate 1 medium + GreenFibre basic, pH 5.3,
Klasmann-Deilmann GmbH, Geeste, Germany) and NPK fertilizer (N170 + P200 + K230 + Mg100 + S150 mg L⁻¹) and then transferred into a walk-in environmental chamber (VB 8018, Vötsch Industrietechnik GmbH, Balingen-Frommern, Germany) at the Institute for Landscape Biogeochemistry, Leibniz Centre for Agricultural Landscape Research in Müncheberg, Germany. Throughout the experiment, the environmental chamber was illuminated (photosynthetically active radiation: 500 μmol m⁻² s⁻¹) for 16 h with metal halide lamps (Powerstar HQI-BT 400 W/D PRO Daylight, Osram GmbH, Munich, Germany) every day and the temperature was kept at 21 °C during day and night. Seedlings were watered regularly with a 1:1 mixture of tap water and distilled water to maintain a target soil water tension of ~2.5 pF. The trees were acclimated to these conditions for 3 months prior to the experiment. Subsequently, on 20 July, 10 seedlings of each provenance were exposed to drought conditions by water deprivation and 10 seedlings were normally watered as controls.

Experimental design and sample collection

Details of the drought experiment are provided by Du et al. (2016) and are therefore given here only briefly. Plants were watered daily to keep a target volumetric soil water content of ~50%. To start the treatment, the water supply of drought stressed seedlings was lowered stepwise (to ~20% of the water supplied to the controls) to induce moderate drought; this period lasted for 21 days. Thereafter (10 August), water supply was completely stopped, to cause severe drought (reaching 6–15% soil water content); this period also lasted for 21 days (until 31 August). Soil moisture contents were determined by EC200 sensors (EC5, Decagon Devices, Inc., Pullman, WA, USA). Needle and root material was harvested from four to five plants of each provenance and stress level: seedlings exposed to water stress (i) for 29 days (i.e., 21 days of reduced water supply plus 8 days without any irrigation, 19 August) (mild stress, MS); or (ii) for 41–42 days (i.e., 21 days of reduced water supply plus 20–21 days without irrigation, 31 August) (severe stress, SS). At each time point, four to five normally watered seedlings of each provenance were also harvested as controls. Needles excised from twigs and fine roots (diameter less than 2 mm) were immediately shock-frozen in liquid nitrogen (N₂), homogenized with mortar and pestle in liquid N₂, and stored at ~80 °C until terpenoid analysis. As a physiological measure of the drought effect, pre-dawn twig water potential (Ψtwig) was assessed using the method of Scholander et al. (1965) as modified by Rennenberg et al. (1996).

Gas exchange measurements

As a sensitive measure of tree drought stress, gas exchange measurements were performed 1–2 days before harvesting plant material. For this purpose, twigs containing current-year needles of Douglas fir provenances were carefully inserted into the 3 cm² leaf chamber of a portable gas exchange measuring unit (LI-6400, LI-COR Bioscience Inc., Lincoln, NE, USA). The chamber was illuminated with 1000 μmol m⁻² s⁻¹ photosynthetic photon flux density and flushed with a flow rate of 650 μmol s⁻¹ air, which was adjusted to contain 400 ppm CO₂, 45% relative humidity and a leaf temperature of 25 °C. Twigs were adapted to these conditions until net CO₂ assimilation rates reached an equilibrium, which took about 15 min. Subsequently, rates of net CO₂ assimilation (A), stomatal conductance of water vapor (gH₂O) and C₄/C₃ ratios (i.e., leaf internal CO₂ concentrations over cuvette CO₂ concentrations) were logged every 2 min over a period of 10 min. Gas exchange parameters were calculated automatically by the measuring device by applying the equations of von Caemmerer and Farquhar (1981). Subsequently, parameters were corrected for leaf area in the cuvette. Values were averaged for each twig; five biological replicates were analyzed per provenance, treatment and time point.

Terpenoid extraction by stir bar sorptive extraction

Aliquots of 25 mg frozen leaf or root powder were added to 500 μl methanol. The samples were mixed on a thermostirrer (Eppendorf AG, 22,331 Hamburg, Germany) at 30 °C, 1400 rpm for 20 min. After centrifugation at room temperature and 14,000 rpm for 5 min, the supernatant was diluted 1:50 (leaves) or 1:12.5 (fine roots) with appropriate amounts of distilled water and 2-carene was added as an internal standard. Terpenoid enrichment was performed by stir bar sorptive extraction. For this purpose, stir bars coated with 1 mm of the adsorbent polydimethylsiloxane (PDMS) (Twister®, Gerstel, Mülheim, Germany) were added to the diluted supernatants. Samples were shaken on a thermostirrer at 30 °C and 1400 rpm for 60 min to allow equilibration of terpenoid adsorption on stir bars. Finally, the stir bars were removed from the solution, briefly dried with lint-free paper tissue and placed into a thermodesorption tube (Gerstel).

Terpenoid analysis

Terpenoids were analyzed on a gas chromatograph (model 7890A, Agilent, Waldbronn, Germany) equipped with a thermodesorption/cold injection system (TDU-CIS) (Gerstel) and connected to a mass-selective detector (5975C, Agilent). Thermodesorption tubes containing the terpenoids loaded stir bars were heated up to 240 °C to release the terpenoids adsorbed. A helium gas stream channeled the terpenoids into the CIS where they were cryofocused at −100 °C. Subsequently, the CIS was heated to 240 °C in order to release the volatilized terpenoids onto the separation column (DB-624, Agilent) at a helium flow of 1 ml min⁻¹. The oven temperature program began at 40 °C which was kept for 5 min; temperature then increased at a rate of 6 °C min⁻¹ until 100 °C was reached, when the temperature ramp sped up to 16 °C min⁻¹ until the column reached the final temperature of 230 °C. Transfer line and MS source temperatures were adjusted to 280 and 230 °C, respectively. Electron impact ionization energy was 70 eV and the MSD was operated in full scan mode from 40 to 300 m/z.
Raw data files were processed with the freely available AMDIS (automated mass spectral deconvolution and identification system, version 2.69) software supplied by NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA). For compound identification, mass spectra were searched against the NIST database and by the use of external monoterpenoid standards. Peak alignment was done with SpectConnect (Styczynski et al. 2007). Terpenoids were quantified with calibration curves of representative terpenoids (i.e., α-pinene for monoterpenes, linalool for oxygenated monoterpenoids, citronellyl acetate for acetylated monoterpenoids and α-caryophyllene for sesquiterpenoids). Terpenoid concentrations in needles and roots were calculated based on dry weight.

Statistical analysis

Student’s t-test on the software package SigmaPlot 11.0 (Systat Software GmbH, Erkrath, Germany) was applied for statistical analysis. To ensure that data matched normal distribution in order to perform t-tests, data were transformed to denary logarithms if required. For data still failing normality test after normalization, the Mann–Whitney Rank Sum Test was employed to determine the significance of differences. Partial least-square discriminant analysis (PLS-DA) was performed using the freely available web-based software package MetaboAnalyst 3.0 (Xia et al. 2009, 2012, 2015, http://www.metaboanalyst.ca). Before running PLS-DA, raw data of terpenoid concentrations in roots and needles were subjected to a natural logarithm transformation (‘generalized logarithm transformation’) within MetaboAnalyst 3.0.

Results

Drought effects on leaf physiology are independent of provenance

Determination of $\Psi_{\text{twig}}$ indicated that the initial moderate reduction in water supply did not considerably affect plant water status (Figure 1) although soil water tension was significantly enhanced from $\sim$2.5 pF (well-watered controls) to 4.2 pF during that time (data not shown). We consider the seedlings in this state as moderately stressed (MS). After prolonged exclusion of water supply soil water tension further increased ($\sim$7.5 pF) and $\Psi_{\text{twig}}$ significantly decreased causing clear differences between stressed trees and controls; we refer the trees in this state as severely stressed (SS). It is noteworthy that despite the assumed higher drought stress resistance of INT, provenance-specific differences in $\Psi_{\text{twig}}$ were not observed (Figure 1).

Compared with twig water potential, leaf gas exchange reacted more sensitively to reduced water availability, but in a more provenance-specific way (Figure 2). COA showed significantly reduced stomatal conductance ($g_{\text{H2O}}$) under moderate drought (Figure 2F). The same trend but to a lesser extent occurred in INT (Figure 2E). Under full water deprivation both provenances showed significantly reduced stomatal conductance. Net CO$_2$ assimilation rates (Figure 2A and B) of both provenances were significantly reduced under both drought regimes compared with the normally watered control trees. Interestingly, drought seemed to cause different $C_i/C_a$ ratios in the two provenances. Whereas this ratio was unaffected by water stress in INT, it slightly decreased in COA seedlings (Figure 2C and D).

Figure 1. Effects of moderate (MS) and severe (SS) drought stress on twig water potential of the two Douglas fir provenances Fehr Lake (INT, interior) and Snoqualmie (COA, coastal). Asterisks over bars indicate significant difference at $P < 0.05$ between treatments as calculated by Student’s t-test.

Figure 2. Mean (± SE) of net CO$_2$ assimilation rate (A, B), $C_i/C_a$ ratio (C, D) and stomatal conductance (E, F) in the needles of interior (INT) and coastal (COA) Douglas fir seedlings under moderate (MS) and severe (SS) drought stress. Asterisks over bars indicate significant difference at $P < 0.05$ between treatments as calculated by Student’s t-test.

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INT and COA show provenance-specific terpenoid patterns in needles but not in roots

Total terpenoid contents differed strongly between roots and needles, amounting to around 23–28 mg g⁻¹ dry weight (DW) in needles of INT and 17–25 mg g⁻¹ DW of COA (Figure 3A and B), but only to ~2–5 mg g⁻¹ DW in the roots of both provenances (Figure 3C and D). Individual terpenoids in needles indicated a clear provenance-specific pattern (Figure 4). In INT, camphene was the most abundant terpenoid (42%), followed by α-pinene (21%), β-pinene (9%), (-)-bornyl acetate (8%) and tricyclene (7%) (Figure 4 and see Table S1 available as Supplementary Data at Tree Physiology Online). This was in contrast to needles of COA, where β-pinene (55%) dominated followed by α-pinene (16%), β-citronellol (5%) as well as 3-carene and camphene (each 4%).

Drought only slightly affected total terpenoid contents in needles (Figure 3A and B). Needles of both provenances showed slightly increased levels of many terpenoids including six sesquiterpenoids under moderate and severe drought stress. Most of these effects were, however, not statistically significant (see Table S1 available as Supplementary Data at Tree Physiology Online).

In roots of both provenances a very different pattern of terpenoids was observed compared with the needles (Figure 5 and see Table S2 available as Supplementary Data at Tree Physiology Online).
Drought effects on root and needle terpenoid content

Whereas we identified 33 different terpenoids in the needles, only 14 monoterpenoids and the sesquiterpenes α-longipinene and (+)-longifolene were observed in roots of Douglas fir (see Tables S1 and S2 available as Supplementary Data at Tree Physiology Online). Roots of both provenances did not differ in their terpenoid composition, both containing high amounts of 3-carene (most abundant), α-pinene (second most abundant), sabinene, β-pinene, β-phellandrene and β-myrcene. Moderate drought significantly increased total terpenoid contents in roots of COA, an effect not seen under severe drought (Figure 3D). This increase was mainly due to significantly increased contents of most monoterpenoids except α-pinene, β-pinene, α-terpinolene and camphene (see Table S2 available as Supplementary Data at Tree Physiology Online). However, there was no effect of severe drought on total terpenoid content in COA, and, consistently, also no effect on the content of any root terpenoid—although there seemed to be a trend to higher contents also under these conditions. In COA there was an increase in the terpenoid levels of the controls indicating a developmental effect during the course of the experiment (see Table S2 available as Supplementary Data at Tree Physiology Online). In contrast to COA, root terpenoid contents of INT did not show clear changes due to drought stress. Only the contents of the low abundance β-cymene and p-cymene increased under severe drought as compared with non-stressed control plants.

We performed PLS-DA to test for common patterns of the different leaf and root samples of the Douglas fir provenances investigated (Figure 6). The analysis clearly demonstrated different clustering of root and leaf samples. However, whereas root samples of the two provenances did not form their own clusters, needle samples could be assigned to either INT or COA. The respective loading plots indicate the influence of each compound for the spatial orientation of individual samples in the two-dimensional matrix. It became obvious that β-cymene (‘B’), numbers indicate the compounds in the loading plot; compounds are listed in Tables S1 and S2 available as Supplementary Data at Tree Physiology Online), p-cymene (‘1’1), and α-terpinolene (‘10’), p-cymenol (‘12’) as well as the sesquiterpenes α-longipinene (‘14’) and (+)-longifolene (‘15’) were main drivers for the separation of roots from needles (Figure 6). In contrast, the monoterpenes α-terpinolene (‘18’), β-pinene (‘4’), camphene (‘2’), and Z-(β)-ocimene (‘20’), the oxygenated monoterpenes (-)-bornyl acetate (‘34’), and the sesquiterpenoids α-cubebene (‘36’) and nerolidol (‘41’) were the most important compounds causing the provenance-specific clustering of the needle samples.

Discussion

The present work aimed at elucidating the effects of drought stress on the terpenoid content and composition in needles and roots of two Douglas fir provenances originating from contrasting habitats. For this purpose, Douglas fir seedlings were exposed to successively increasing drought intensities. In a first phase, water supply was reduced leading to significantly increased soil water tension in the soil substrate of both provenances (Figure 1; Du et al. 2016).

Figure 5. Contents of the 10 most abundant terpenoids (and other monoterpenoids and sesquiterpenoids) in roots of two Douglas fir provenances. The interior (INT) (A, C) and coastal (COA) (B, D) provenances were exposed to moderate (MS) (A, B) or severe (SS) (C, D) drought stress and individual terpenoid contents were quantified by gas chromatography–mass spectrometry based on dry weight. Data are means ± SE of at least five biological replicates each. Statistically significant differences at P < 0.05 between treatments were calculated by Student’s t-test and are indicated by asterisks.
However, only the assumed less drought-tolerant coastal provenance COA showed lowered pre-dawn \( \Psi_{\text{twig}} \) (Figure 1); this observation might be due to a relatively more conservative water use of the INT provenance adapted to a more arid climate, which prevented stronger reductions in leaf water potential. In a subsequent phase, water supply was completely stopped causing further enhanced soil water tension, which significantly affected pre-dawn \( \Psi_{\text{twig}} \) of both provenances. These pre-dawn \( \Psi_{\text{twig}} \) suggest severe drought stress of the Douglas fir seedlings (Andrews et al. 2012, Woodruff 2014), an assumption which is supported by strongly impaired nitrogen metabolism and accumulation of osmolytes in the needles of the seedlings (Du et al. 2016).

Besides seedling \( \Psi_{\text{twig}} \), we determined needle gas exchange as a sensitive indicator of plant water stress. In accordance with many other studies (Chaves 1991), drought considerably affected the seedlings’ gas exchange (Figure 2). The range of gas exchange rates (A: 6.4–9.4 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), \( g_{\text{H}_{2}O} \): 77–191 mmol m\(^{-2}\) s\(^{-1}\)) of non-stressed trees was very similar to that determined in other studies with Douglas fir (Lerdau et al. 1995, Constable et al. 1999, Lewis et al. 2001, Manter 2002, Poulson et al. 2002, Snow et al. 2003, Warren et al. 2003, Junker and Ensminger 2016). As expected, drought impaired plant gas exchange (net \( \text{CO}_2 \) assimilation by \( \sim 80\% \), transpiration and stomatal conductance by \( \sim 70\% \)). The assumed less drought-tolerant coastal provenance COA seemed to react earlier to the stress than the interior provenance. However, under severe drought the provenance-specific differences disappeared. This reaction to drought is in good agreement with results of Poulson et al. (2002) and Warren et al. (2003) showing a very similar 80% drop in net \( \text{CO}_2 \) assimilation rates in drought-stressed Douglas fir trees. It is assumed that decreased photosynthetic activity may be caused by stomatal limitation, but also by physiological adjustments and/or indirect effects of oxidative stress (Cornic and Fresneau 2002, Flexas et al. 2004a, 2004b, Chaves et al. 2009). Interestingly, the \( C_i/C_a \) ratios revealed provenance-specific differences. The drought-caused lower \( C_i/C_a \) ratio in COA (compared with controls of COA) indicates the expected stomatal limitation of net \( \text{CO}_2 \) assimilation rates (Cornic and Massacci 1996, Flexas and Medrano 2002). The unaffected \( C_i/C_a \) ratio of drought-stressed INT seedlings (compared with INT controls), however, rather suggests non-stomatal limitation of net \( \text{CO}_2 \) assimilation, because at identical leaf internal \( \text{CO}_2 \) concentrations (\( C_i \) values), photosynthesis is reduced (Brodribb 1996, Cornic and Massacci 1996). This difference between the two provenances is surprising and more studies focusing on seedling net \( \text{CO}_2 \) assimilation under drought are required for a better mechanistic understanding and to elucidate if the different patterns are connected to plant drought tolerance. There are several possible reasons for non-stomatal limitation of photosynthesis including increased mesophyll resistance for \( \text{CO}_2 \) or impaired ATP synthesis under drought (Cornic and Massacci 1996, Tezara et al. 1999, Cornic 2000, Flexas and Medrano 2002, Flexas et al. 2004b). The results obtained by Du et al. (2016), who analyzed the nitrogen metabolism of the same seedlings of the present experiment, provide hints that drought stress impaired abundance and/or activity of Rubisco, contributing to non-stomatal limitation of photosynthesis.

**Douglas fir shows provenance-specific terpenoid composition in needles but not in roots**

With \( \sim 24 \text{ mg g}^{-1} \text{ DW} \) the total terpenoid contents in needles of both Douglas fir provenances were comparable to data reported in earlier work with this species ranging between 1.5 and 43 \( \text{ mg g}^{-1} \text{ DW} \) (Figure 3; Lerdau et al. 1995, Constable et al. 1999, Litvak et al. 2002, Pureswaran et al. 2004) and 1–11 \( \text{ mg g}^{-1} \) fresh weight (i.e., \( \sim 2–25 \text{ mg g}^{-1} \text{ DW} \); Gambriel and Cates 1995, Zou and Cates 1995). The terpenoid composition clearly differed between the interior and the coastal provenance (Figure 6). Such pattern is well known from the pioneer work of Von Rudloff (1972) studying the leaf oils of many coastal and interior (also called ‘Rocky Mountain’) varieties of

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**Figure 6. Score plot (upper panel) and loading plot (lower panel) of a partial least-square discriminant analysis (PLS-DA) of the terpenoid contents in needles and roots of the Douglas fir provenances Fehr Lake (INT) and Snoqualmie (COA).** The loading plot indicates the terpenoids that are responsible for the sample distribution in the score plot. Terpenoids belonging to the numbers indicated in the loading plot are given in Tables S1 and S2 available as Supplementary Data at *Tree Physiology* Online.
Douglas fir. The provenances investigated in the present study fit well into the chemotype scheme proposed by Von Rudloff (1972, 1975), with INT containing high levels of camphene, (-)-bornyl acetate and tricyclene and a β-pinene to α-pinene ratio <1, which is typical for interior provenances, whereas COA showed low amounts of these terpenoids but a β-pinene-to-α-pinene ratio >2 as expected for a coastal provenance (Figures 4 and 5; Von Rudloff 1972, 1975). The reasons for provenance-specific patterns are not fully understood, but evolutionary adaptation to the environmental conditions at the natural sites might be assumed (Loreto et al. 2009). The typical terpenoid composition of the two varieties (coastal vs interior) might be considered an adaptation to different environmental conditions with a more humid climate and smaller maximal differences between winter and summer temperatures in coastal areas than inland. Loreto et al. (2009) proposed such adaptation when comparing terpenoid emissions of different chemotypes of cork oak trees (limonene emitter in provenances from humid Portuguese areas, β-pinene emitters in dry central Italy provenances). However, past genetic isolation of populations (Loreto et al. 2009) can be another reason for such chemotype formation. In Douglas fir, the observed differences in local adaptation of the two Douglas fir varieties are likely a result of post-glacial migration from two distinct and geographically separated refugial areas (Gugger and Sugita 2010). Provenance-specific terpenoid compositions comparable to Douglas fir have also been reported in wood and leaves of different provenances of Scots pine (Manninen et al. 2002), and for terpenoid emissions from Scots pine (Kivimäenpää et al. 2012), Mediterranean cork oak (Loreto et al. 2009) and boreal Silver birch (Maja et al. 2015).

In our study with Douglas fir provenances, drought did not significantly affect total terpenoid contents in needles, although the contents in COA tended to increase (Figure 3). Particularly under moderate drought we observed a trend towards increased levels of some terpenoids including oxygenated monoterpenes and sesquiterpenes (significant increase for (-)-terpinen-4-ol and β-elemene contents) in COA. In contrast, INT seemed to accumulate the monoterpene β-pinene in needles (see Table S1 available as Supplementary Data at Tree Physiology Online). The effect of drought on terpenoid contents and composition is still controversially discussed; under moderate drought, monoterpenoid content and emission rather remain unaffected or are slightly stimulated. Severe drought, however, seems to impair terpenoid biosynthesis due to a lack of substrate availability as a consequence of inhibited photosynthesis (Peñuelas et al. 2009, Šimpraga et al. 2011, Kleine and Müller 2014, Nogües et al. 2015a, 2015b). It should be mentioned that we studied seedlings with assumed low carbon pools available for terpenoid biosynthesis; the situation for adult trees might be somewhat different due to larger carbon pools and they might react even less sensitively to the stress. Similar to our results, earlier work with other conifers showed increased needle terpenoid contents in response to drought stress (P. abies: Kaunilainen et al. 1992, Pinus ponderosa: Johnson et al. 1997, P. halepensis: Llusíà and Peñuelas 1998, Llusíà et al. 2006). This drought-induced increase in the content of individual terpenoids in the coastal provenance can be due to drought effects on specific terpene synthases, and might reflect an acclimation to the stress. Terpenoid biosynthesis might alleviate drought stress by quenching reducing power, thereby preventing oxidative stress and photoinhibition (Nowak et al. 2012, Selmar and Kleinwächter 2013). As we see moderately enhanced terpenoid production only in needles of drought-stressed COA, we conclude that this assumed less drought-tolerant provenance forms the terpenoids in order to protect the photosynthetic apparatus from oxidative stress, whereas INT is generally better protected because of higher terpenoid contents already under control conditions (Figure 3) or possesses alternative mechanisms reducing oxidative stress. Increased terpenoid contents under water shortage were also explained by reduced growth and allocation of a larger portion of assimilated carbon into terpenoid biosynthesis. It was hypothesized that the resulting terpenoid accumulation could have ecological functions for defense and storage (Peñuelas and Estiarte 1998, Llusíà and Peñuelas 1998, Peñuelas and Llusíà 1999, Blanch et al. 2009).

As mentioned above, the present study revealed that two Douglas fir provenances with strongly differing terpenoid composition in the needles did not differ in their terpenoid composition in the roots—a finding similar to that described for two chemotypes of the perennial aromatic plant Tanacetum vulgare (Kleine and Müller 2014) (Figure 6). In both Douglas fir provenances of the present study, main terpenoids in roots were 3-carene, α-pinene, sabinene and β-pinene—together with other terpenoids forming a composition clearly different from the pattern seen in the needles of the same trees (Figure 5 and see Table S2 available as Supplementary Data at Tree Physiology Online). Similar total terpenoid contents (~1.1 and 1.4 mg g\(^{-1}\) DW) were observed in roots of two coastal Douglas fir varieties with the same main compounds but in a slightly different order (i.e., α-pinene > 3-carene > β-pinene > sabinene) (Huber et al. 2005). The different composition and the ~10-times lower total terpenoid contents in roots of Douglas fir (~1.7 – 2.7 mg g\(^{-1}\) DW) compared with the needles of the same trees (~24 mg g\(^{-1}\) DW) suggest that root terpenoids fulfill other functions for the plant than the terpenoids abundant in aboveground plant organs. Earlier studies showed that root terpenoids—particularly sesquiterpenoids—are important for interspecies communication between the root and ectomycorrhizal fungi during establishment of the symbiosis (Ditengou et al. 2015). Other roles of root terpenoids have been demonstrated such as interaction with herbivores, fungi and bacteria (Trowbridge and Stoy 2013, Lavender and Hermann 2014, Kleine and Müller 2014). The work of Huber et al. (2005) also indicates that root abundant terpenoids might play a role in the response to biotic

**Drought effects on root and needle terpenoid content**

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stress, because simulating herbivore attack of Douglas fir roots by methyl jasmonate treatment caused enhanced abundance of numerous terpenoids including monoterpenoids and sesquiterpenoids. Because abiotic stress like drought weakens the plant, thereby enhancing the probability of herbivore attack, the observed biosynthesis of terpenoids might be required to anticipate biotic stress (Kleine and Müller 2014).

Whereas the total terpenoid contents of roots of INT was unaffected by drought in the present study, moderate drought— and only to a minor extent severe drought—caused increased total terpenoid contents in roots of COA (Figure 3). This might be a provenance-specific reaction to cope with the abiotic stress applied. The weaker effect of severe drought on terpenoid contents might be caused by strongly reduced gas exchange and, therefore, limitation of assimilates needed for the biosynthesis of terpenoids. There is only a handful of data available on the effects of drought on root terpenoids of other species and the results are partly contradictory. Whereas roots of Solanum lycopersicum showed decreased levels of linalool and β-caryophyllene under drought (Asensio et al. 2012), other studies observed rather increased terpenoid contents (Kleine and Müller 2014).

Taken together, needle terpenoids of two Douglas fir provenances derived from contrasting habitats strongly differed in composition and content. The occurrence of such chemotypes might be a consequence of an adaptation to the environmental conditions at the sites of origin. In contrast, the very similar terpenoid compositions in roots of the two provenances suggests that there was no selective pressure on root terpenoid composition during adaptation of the provenances to the conditions at their sites of origin. The observed increased terpenoid contents in roots and needles of drought-stressed seedlings of the coastal provenance might be a result of altered carbon allocation under conditions of water shortage resulting in reduced seedling growth and allocation of larger fractions of assimilated carbon to terpenoid biosynthesis. Ecological reasons could be connected to defense against biotic or abiotic stresses. Future studies should particularly elucidate the role of root-borne terpenoids for biotic interactions, such as establishment of ectomycorrhizal symbioses (Ditengou et al. 2015) or defense against pathogen attack (Huber et al. 2005).

Supplementary Data

Supplementary Data for this article are available at Tree Physiology Online.

Conflict of interest

None declared.

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References


