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RESEARCH PAPER

Elevation of night-time temperature increases terpenoid emissions from *Betula pendula* and *Populus tremula*

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Abstract

Volatile organic compounds (VOCs) are expected to have an important role in plant adaptation to high temperatures. The impacts of increasing night-time temperature on daytime terpenoid emissions and related gene expression in silver birch (*Betula pendula*) and European aspen (*Populus tremula*) clones were studied. The plants were grown under five different night-time temperatures (6, 10, 14, 18, and 22 °C) while daytime temperature was kept at a constant 22 °C. VOC emissions were collected during the daytime and analysed by gas chromatography–mass spectrometry (GC-MS). In birch, emissions per leaf area of the C11 homoterpene 4,8-dimethy1-nona-1,3,7-triene (DMNT) and several sesquiterpenes were consistently increased with increasing night-time temperature. Total sesquiterpene (SQT) emissions showed an increase at higher temperatures. In aspen, emissions of DMNT and β-ocimene increased from 6 °C to 14 °C, while several other monoterpenes and the SQTs (*Z*,*E*)-α-farnesene and (*E*,*E*)-α-farnesene increased up to 18 °C. Total monoterpene and sesquiterpene emission peaked at 18 °C, whereas isoprene emissions decreased at 22 °C. Leaf area increased across the temperature range of 6–22 °C by 32% in birch and by 59% in aspen. Specific leaf area (SLA) was also increased in both species. The genetic regulation of VOC emissions seems to be very complex, as indicated by several inverse relationships between emission profiles and expression of several regulatory genes (*DXR*, *DXS*, and *IPP*). The study indicates that increasing night temperature may strongly affect the quantity and quality of daytime VOC emissions of northern deciduous trees.

Key words: *Betula pendula* (birch), gene expression, isoprene, monoterpene, night-time temperature, *Populus tremula* (aspen), sesquiterpene, volatiles.

Introduction

Most plant species have the ability to synthesize and release volatile organic compounds (VOCs) (Owen and Peñuelas, 2005; Vickers *et al.*, 2009). The precursors of terpenoids are dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) (Schwarz, 1994), which are synthesized by the methylerythritol 4-phosphate (MEP) pathway [i.e. the deoxyxylulose-5-phosphate (DXP) or non-mevalonate pathway] in chloroplasts and by the mevalonate (MVA) pathway in the cytoplasm (Rodríguez-Concepción and Boronat, 2002; Rodríguez-Concepción *et al.*, 2004; Owen and Peñuelas, 2005). These pathways are independent, but interact through metabolic cross-talk (Hemmerlin *et al.*, 2003; Rodríguez-Concepción *et al.*, 2004). Monoterpenes in non-storing plants and isoprene (C_5) in isoprene-producing

plants are volatilized after production and rapidly lost (Niinemets *et al.*, 2004). Semi-volatile sesquiterpenes (SQTs) are easily adsorbed on vegetation surfaces such as leaves and bark, and are later observed by above-canopy flux measurement techniques after re-release from foliage (Helmig *et al.*, 2004). There could also be delayed release of SQTs from surfaces in the morning (Schaub *et al.*, 2010).

The biological function of many volatiles is still poorly understood, although several experiments have indicated clear functions for some of these volatiles within a plant (Holopainen, 2004). The effect of biotic interactions on VOC emissions from plants and long-distance signalling in plant defence has been studied intensively during the last two decades (Gouinguene and Turlings, 2002; Vallat *et al.*, 2005; Gershenzon, 2007; Heil and Ton, 2008). Studies of VOC emission response to abiotic factors have traditionally focused on isoprene (Sharkey *et al.*, 2008), but in recent years increasing research concerning C6, C10, and C15 volatiles has been published (for reviews, see Laothawornkitkul *et al.*, 2009; Vickers *et al.*, 2009; Yuan *et al.*, 2009). The ecophysiological functions of VOCs include plant–plant communication and plant–herbivore interactions (De Moraes *et al.*, 2001; Ibrahim *et al.*, 2005; Blande *et al.*, 2007) as well as attraction of natural enemies to their herbivore prey (Holopainen, 2004; Vuorinen *et al.*, 2004; Mumm *et al.*, 2008). Abiotic factors such as temperature, light, and the availability of carbon for the synthesis of VOCs are known to control the short-term emission of isoprenoids (Grote and Niinemets, 2008; Sharkey *et al.*, 2008). The emission of stored monoterpene (MTs) in ducts, glands, or trichomes is controlled mainly by temperature and/or light, while the emission of isoprene, which is not stored, is also clearly light and temperature dependent (Staudt and Bertin, 1998; Kesselmeier and Staudt, 1999; Dindorf *et al.*, 2006; Loreto *et al.*, 2007). However, recent studies have also shown that SQT emission increases exponentially with temperature (Tarvainen *et al.*, 2005; Helmig *et al.*, 2007). In addition, rising temperature can also affect the quality of volatile emissions from plants (Gouinguene and Turlings, 2002). There is increasing evidence that VOCs, especially isoprene, provide plants with protection against high temperature (Rennenberg and Schnitzler, 2002; Velikova and Loreto, 2005; Peñuelas and Munne-Bosch, 2005; Sharkey *et al.*, 2008; Wiberley *et al.*, 2008). In addition to thermotolerance, isoprene emissions provide tolerance to ozone and other reactive oxygen species (Vickers *et al.*, 2009) and may function as a 'safety valve' to remove unwanted metabolites and dissipate excess energy in high light conditions (Rosenstiel *et al.*, 2004). Besides this, biogenic VOCs play a very important role in atmospheric chemistry as they participate in the formation of ozone in NO_x-polluted atmospheres and quench ozone in unpolluted environments (Lerdau and Slobodkin, 2002).

HMGR (hydroxymethylglutaryl CoA reductase), DXS (1-deoxy-D-xylulose 5-phosphate synthase), and DXR (1-deoxy-D-xylulose 5-phosphate reductoisomerase) are important enzymes in producing the first intermediates specific to cytosolic MVA and plastidial MEP pathways. IPP isomerase mediates the conversion of IPP (produced in the mevalonate pathway) to DMAPP, which is the precursor required for isoprenoids (Hoeffler *et al.*, 2002), while isoprene synthase (IspS) produces isoprene from DMAPP through the plastidial MEP pathway (Schwender *et al.*, 1997). The function of several enzymes and related genes has been documented for both MVA and MEP pathways (McGarvey and Croteau, 1995; Rodriguez-Concepción and Boronat 2002; Sasaki *et al.*, 2007). For example, the expression of genes encoding DXS and DXR that act on the MEP pathway produces DMAPP, which is the substrate for IspS which produces isoprene (Sasaki *et al.*, 2007). Both IspS activity and isoprene emissions were increased by heating in transgenic *Arabidopsis*, providing evidence that one of the physiological functions of isoprene emission could be the protection of the plants from heat stress (Sasaki *et al.*, 2007).

For this study, two predominant species in the northern environment, silver birch (*Betula pendula*) and European aspen (*Populus tremula*), were selected for their different emission profiles. *Betula pendula* is an MT emitter and *P. tremula* is an MT and isoprene emitter (Hakola *et al.*, 1998), while both species emit SQTs (Zhang *et al.*, 1999). So far, very little is known about intraspecific variation in VOC emissions for these species (Vuorinen *et al.*, 2005), although large variation in several stress adaptations (e.g. against oxidative stress) and leaf metabolism has been reported in birches and aspens (Häikiö *et al.*, 2007; Oksanen *et al.*, 2007).

The aim of this study was to investigate the influence of night-time warming on the VOC emission rates of birch (*B. pendula*) and aspen (*P. tremula*), originating from areas with low night temperatures. Current global warming has increased surface humidity in the Northern hemisphere (Dai, 2006), which may alleviate the minimum night temperatures. An attempt was also made to identify some of the genetic trends associated with VOC emission rates by comparing expression levels of key genes from the plastidic MEP pathway and the cytoplasmic MVA pathway. In addition to temperature treatment comparisons, differences in emission profiles and related gene expression between the genotypes were studied within these species. It is hypothesized that increasing night-time temperature stimulates the biosynthesis of plant VOCs through activation of the above-mentioned key genes, and this activation is reflected in the daytime emissions of these compounds. Furthermore, it is hypothesized that the ratio of specific VOCs in the emission profiles could be altered and that there is variation among the genotypes in warming responses in both species.

Materials and methods

Plant material

Birch (*B. pendula* Roth) and aspen (*P. tremula* L.) seedlings from clones (Bp8, Bp17, Bp26, and Pt2.2, Pt5.2, Pt6.0, respectively) were used in this study. The clones were randomly selected from naturally regenerated birch and mixed forests from south-eastern Finland, and represent natural genotypic variation within populations. The birch material was micropropagated from three

genotypes growing in a stand in Punkaharju (61°48′N, 29°18′E), used for intensive biodiversity studies. Plant material for aspen was selected from three different locations in central Finland. Since aspen individuals that grow close to each other might well be of the same genotype (Suvanto and Latva-Karjanmaa, 2005), the material was selected from distant locations, but within the same latitude to ensure comparable climatic conditions between the locations.

The experiment started 1.5 months after the planting of micropropagated plantlets in 2006. Plantlets (20–40 cm in height at the beginning of the experiment) were grown at varying night-time temperatures from 13 October to 24 November. One genotype of each species (Bp26 and Pt2.2) was selected to study the daytime volatile emissions. To compare genotypes of both species, all clones were grown at a night-time temperature of 14 °C.

Growth conditions

The plants were planted into plastic pots (Ø 15 cm) containing peat and vermiculite (1:1) and grown in controlled-environment plant growth chambers (Conviron PGW36, Controlled Environments Limited, Winnipeg, Manitoba, Canada) for 6 weeks. Five different night-time temperatures from 6 °C to 22 °C were used with intervals of 4 °C (Fig. 1). The daytime temperature was constant (22 °C) in all treatments between 10:00 am and 4:00 pm. Temperatures were set to decrease in the evening between 4:00 pm and 9:00 pm, stabilizing at each of the night-time temperatures. In the morning, temperatures rose from 5:00 am to 10:00 am back to the daytime temperature. Relative humidity was a constant 60% in all treatments. Light was increased in steps during 2 h in the morning (between 4:00 am and 6:00 am) and decreased similarly between 8:00 pm and 10:00 pm. Light was provided by fluorescent lamps and light bulbs. Daytime photosynthetically active radiation (PAR) was ~300 μmol m^{-2} s^{-1}.

To avoid the consequences of possible differences between the individual chambers, the seedlings were moved weekly from one chamber to another, where the growth conditions were reprogrammed. In addition, the positions of the pots inside the chambers were changed twice a week. Seedlings were watered daily, and fertilizer (Kekkilä, Superex nro5, N:P:K 11:4:25) was added weekly in solutions approximating a total N input of 35 kg ha^{-1} $year^{-1}$.

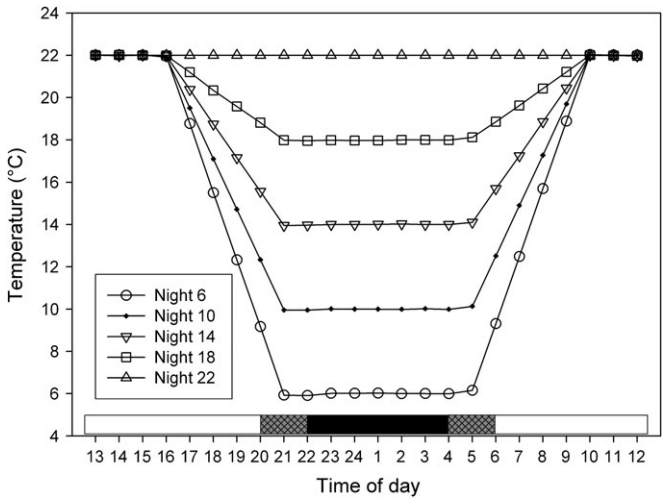

Fig. 1. Temperature profiles in the growth chambers during the experiment with birch (*Betula pendula*) and aspen (*Populus tremula*) between 13 October and 24 November 2006. Light rhythm is shown at the bottom of the figure, black representing darkness, grey mid-light, and white full-light.

Volatile collection and analysis

The tops of the main shoot with young and mature leaves (~26 for birch) and (~13 for aspen) were enclosed inside pre-cleaned (120 °C) Multi-Purpose Cooking Bags (Polyethylene terephthalate, 25×55 cm in size, Look, Terinex Ltd, UK) (Ibrahim *et al.*, 2008). Before inserting the seedling into the bag, one of the top corners of the bag was cut with scissors and an air flow of 750–800 ml min^{-1} (Stewart-Jones and Poppy, 2006; Ibrahim *et al.*, 2008) was passed through a charcoal filter and used to flush out residual contamination for 15–20 min. Thereafter, the test seedling and the Teflon tube were inserted into the bag through its mouth and the bag was tightened around the stem with gardening wire. After enclosure of the foliage, the bags were flushed for a further 15 min (Mäntyla *et al.*, 2008; Ibrahim *et al.*, 2010). The air flow was reduced to 300 ml min^{-1} before the start of sampling. The sampling tube was inserted into the bag through the top corner. The filtered replacement air with ambient CO_2 concentration was scrubbed with MnO_2 to remove ozone and pumped into the bag.

The volatile emissions from the enclosed seedlings were collected into steel sample tubes filled with a 50%:50% combination of Tenax TA and Carbopack B (100 mg of each mesh 60/80, Supelco, Bellofonte, PA, USA) adsorbents. The sampling air flow was 200 ml min^{-1}. The sampling time was 1 h and all samples were collected in a time period between 9:30 am and 4:30 pm with replicates from all the treatments distributed evenly across this period. Compounds were thermally desorbed at 250 °C for 10 min and cryofocused in a cold trap at –30 °C. The compounds were injected onto an HP-5MS capillary column (length 50 m, id 0.2 mm, film thickness 0.33 μm, J&W Scientific USA, Agilent Technologies). The column temperature was held first at 40 °C for 1 min, increasing to 210 °C at a rate of 5 °C min^{-1} and rising to a final temperature of 250 °C at a rate of 20 °C min^{-1}. The carrier gas was helium with constant pressure of 20.7 psi. The samples were analysed by gas chromatography–mass spectrometry (GC-MS; Hewlett Packard GC 6890, MSD 5973). The spectra of external standards for available compounds and the Wiley library (John Wiley & Sons, Ltd, Chichester, UK) were used to identify the adsorbed compounds (Ibrahim *et al.*, 2008; Himanen *et al.*, 2009). Commercially available reference substances were used to quantify the emissions. The concentrations of the MTs α-terpinene, α-terpinolene, and (*E*)-β-ocimene were quantified assuming that the responses were the same as that of the MT α-pinene in the standard (Vuorinen *et al.*, 2007; Himanen *et al.*, 2009). The SQTs β-bourbonene, 3,7-guaiadiene, (*Z*,*E*)-α-farnesene, α-amorphene, and (*E*,*E*)-α-farnesene were quantified using the response of the SQT α-humulene in the standard (Himanen *et al.*, 2009).

Measurement of physiological parameters

After each volatile collection, gas exchange was measured with a Li-Cor 6400 photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA). Photosynthesis was measured from one mature leaf of each seedling used for VOC collections. The temperature in the cuvette was 22 °C and a light-emitting diode (LED) light source was used to provide light at the level of PAR 400 μmol m^{-2} s^{-1}. CO_2 concentration was 360 ppm and air flow through the cuvette was a constant 500 μmol s^{-1}. After measurements, the leaves used for VOC collection were scanned and their areas were analysed (Adobe Photoshop Elements 3.0, Adobe Systems Inc., San Jose, CA, USA). Leaves were oven dried (70 °C for 2 d) for determination of dry weights. Four seedlings from each genotype and treatment were used to obtain the other growth data. Leaf areas of the whole seedlings were estimated based on area analyses of every second leaf of the seedling, taking into account a mass relationship of analysed and non-analysed areas. Specific leaf area (SLA; leaf area per unit dry weight) was calculated for the whole seedling.

Quantitative real-time PCR

For gene expression analysis, samples were taken the day after VOC measurements from three Pt2.2 and three Bp26 seedlings growing under each of the five temperature treatments. Genotypic comparison was made of three genotypes grown at a night-time temperature of 14 °C. The fifth and eighth leaves from the top of the seedling were collected, frozen in liquid nitrogen, and stored in a deep freezer (–70 °C) until RNA extractions. The mRNA levels were analysed using quantitative real-time PCR (qRT-PCR). Total RNA was isolated from the leaf samples using the cetyltrimethylammonium bromide (CTAB) method (Chang *et al.*, 1993). The DNase I-treated total RNA was reverse transcribed using an oligo(dT_{18}) primer and DyNAmo 2 step SYBR Green qPCR kit (Finnzymes). QRT-PCRs were carried out using a Dynamo HS SYBR Green kit (Finnzymes) in a 20 µl reaction volume with 0.5 µM gene-specific primers and 2 µl of diluted cDNA, deriving from 5–15 ng of total RNA, as a template.

The quantitative PCR primers (Table 5) were designed from *P. tremula* sequences obtained in this study by amplifying with primers designed from an alignment of *Populus* expressed sequence tags (ESTs) that had homology to *P. alba*×*P. tremula ispS* (AJ294819), *P. trichocarpa DXS* (EU693019), *Arabidopsis* isopentenyl-diphosphate delta-isomerase (*IPP1*; At5g16440), and *Arabidopsis* 3-hydroxy-3-methylglutaryl COA reductase (*HMG1*, At1g76490). DXR primers were designed from *P. tremula* ESTs that have a high homology to *P. trichocarpa DXR* (EU693020) as well as to *Arabidopsis DXR* (At5g62790). The quantitative PCR primers for *B. pendula DXS*, *DXR*, and *HMGR* were designed using a silver birch EST library (Aalto and Palva, 2006). The Q-PCR amplicons were sequenced.

α-Tubulin was chosen as a reference gene in aspen and in birch (GI: 6723477, GenBank). The amplicon lengths and the primer sequences are given in Table 5. The reactions were performed in triplicate in an iCycler iQ Real-time PCR (Bio-Rad). The PCR conditions were: 95 °C for 15 min, followed by 35 cycles of 95 °C for 15 s, 57 °C for 20 s, and 72 °C for 20 s. After final annealing (72 °C, 5 min) and redenaturation (95 °C, 1 min), a melt curve analysis was done by decreasing the temperature from 95 °C to 60 °C at 0.5 °C intervals. The fold change in gene expression was calculated using the comparative Ct method ($2^{-\Delta\Delta Ct}$) (Livak and Schmittgen, 2001).

Table 1. Volatile organic compounds (ng m^{-2} h^{-1}) emitted from birch (*Betula pendula*, clone 26) under different night-time temperatures
Values are means ±SE (n = 9). Means followed by the same letters are not significantly different ($P \leqslant 0.05$).

Compounds	Night-time temperatures (°C)				
	6	10	14	18	22
Monoterpenes					
α-Thujene	6.5±4.3 a	4.4±4.4 a	2.9±2.9 a	0.0±0.0 a	0.0±0.0 a
α-Pinene	321.9±17.8 b	979.6±451.0 c	341.5±30.5 b	210.0±27.0 a	343.8±33.2 b
Sabinene	6344.7±2839.5 a	1592.7±812.3 a	9210.0±46267.8 a	4675.6±2390.8 a	6072.6±3856.1 a
β-Pinene	290.1±27.6 b	558.6±72.8 c	290.3±20.9 b	186.1±30.0 a	293.3±28.8 b
β-Myrcene	186.1±12.1 a	345.5±53.0 b	220.4±30.8 a	170.0±32.6 a	233.8±23.0 a,b
Limonene	537.7±32.1 a,d	949.0±122.2 c	565.4±62.5 a,b	386.0±55.8 a	636.8±71.0 b,d
1,8-Cineole	311.1±25.1 b	490.5±37.2 c	289.4±19.0 a,b	186.3±27.3 d	337.5±35.0 b
γ-Terpinene	157.8±75.2 a	375.4±195.4 a	207.1± 105.1 a	123.4±61.3 a	225.6±94.0 a
α-Terpinene	0.0±0.0 a	0.0±0.0 a	24.4±24.4 a	34.1±25.1 a	0.0±0.0 a
α-Terpinolene	251.1±16.0 a,c,d	364.5±60.7 b	248.0±18.1 c	177.7±19.7 a	322.3±24.3 b,d
Linalool	728.6±273.3 a	688.1±163.2 a	1081.0±352.2 a	751.7±168.8 a	1803.2±572.5 a
β-Ocimene	255.4±13.5 a	938.8±63.2 c	655.1±81.6 b	3072.0±531.5 d	852.6±57.2 c
Alloocimene	3.1±3.1 a	7.5±7.5 a	0.0±0.0 a	30.8±17.7 a	18.2±18.2 a
Total monoterpenes	9394.2±2748.2 a	21629.3±8727.5 a	13135.4±4628.7 a	10003.4±2625.8 a	11139.6±3771.5 a
Homoterpenes					
DMNT	3.5±3.5 a	27.3±13.6 a,b	45.0±14.2 b,c	80.6±28.1 c	337.3±109.0 d
Sesquiterpenes					
α-Copaene	676.7±308.8 a	1321.7±630.3 a	1282.1±517.6 a,b	5152.3±3477.4 a,b	5327.4 ±.2338.9 b
α-Humulene	204.7±97.4 a	366.2±186.0 a	322.2±129.0 a	1265.8±790.0 a,b	2270.8±996.0 b
δ-Cadinene	265.2±124.3 a	564.8±268.1 a,b	514.7±224.1 a,b	2598.7±1547.0 b,c	5817.5±2527.0 c
β-Bourbonene	26.1±17.7 a	275.0±255.6 a	391.0±178.3 b	2284.2±1524.1 b,c	4286.0±1821.4 c
(*E*)-Caryophyllene	442.8±271.1 a	1002.0±518.1 a,b	649.7±307.7 a,b	2344.2±1333.2 b,c	3561.8±1379.0 c
3,7-Guaiadiene	1432.8±792.7 a	1934.5±1154.8 a	1002.5±516.5 a	3168.8±2026.7 a	1801.1±667.5 a
(*Z*,*E*)-α-Farnesene	122.2±88.2 a	440.0±291.5 a	373.3±224.4 a	918.3±569.7 a	1110.4±647.4 a
α-Amorphene	9.7±6.6 a	354.3±240.4 a	27.5±14.3 a	641.1±442.2 a,b	1597.6±709.1 b
(*E*,*E*)-α-Farnesene	5334.5±2090.0 a	8221.0±3563.0 a	8859.0±4156.3 a	27631.1±13018.7 a,b	40682.0±14324.6 b
γ-Cadinene	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	477.1±315.5 b	880.0±378.0 b
Total sesquiterpenes	8514.6 ±3086.1 a	14479.0±5994.3 a	13422.0±5021.4 a,b	46482.2±21410.4 b,c	67334.3±21118.3 c
Non-terpenes					
(*Z*)-3-Hexenyl acetate	1630.0±392.5 a	5079.2 ±3199.4 a,b	3102.5±374.1 b	4194.2±1103.3 a,b,c	12884.0±4680.1 c
(*Z*)-3-Hexenyl isovalerate	810.0±266.0 a	1686.3±1200.8 a	1349.3±535.6 a	1961.1±752.3 a	3409.2±1263.6 a
(*Z*)-3-Hexenyl butyrate	216.2± 64.4 a	518.4±343.1 a	231.6±73.5 a	280.5±100.7 a	565.3±220.1 a
(*Z*)-3-Hexenyl tiglate	55.1±53.0 a	273.0±270.3 a	214.3±103.7 a	134.1±91.5 a	320.4±193.0 a
Total non-terpenes	2711.1±714.0 a	7556.8±4990.0 a	4897.7±875.6 a	6569.9±1724.7 a,b	17179.0±5456.6 b

Statistical analysis

Statistical analyses were performed using the statistical package SPSS for Windows (14.0 and 15.0). Analysis of variance (ANOVA) followed by Tukey's multiple comparisons was used for the analysis of normally distributed volatile compounds. Compounds which were not normally distributed were analysed with the non-parametric Kruskal–Wallis test followed by Mann–Whitney test. Leaf growth and net photosynthesis data were also analysed by ANOVA/Tukey, and linear regressions for leaf area and SLA were calculated with Microsoft Office Excel 2007.

Results

Volatile emission

In birch, the emission of the homoterpene DMNT increased significantly with increasing night temperature, while the monoterpene β-ocimene peaked at 18 °C. The emission of SQTs β-bourbonene, (*E*,*E*)-α-farnesene, and γ-cadinene increased significantly (Table 1) with elevated night-time temperature, while the emission of α-copaene, α-humulene, δ-cadinene, (*E*)-caryophyllene, and α-amorphene increased clearly at night temperatures of 18–22 °C compared with lower temperatures (Table 1). Among the non-terpenes (NTs), emissions of the green leaf volatile (GLV) (*Z*)-3-hexenyl acetate was significantly higher at 22 °C than at lower (6–14 °C) night temperatures (Table 1). Emissions of the MTs α-pinene, β-pinene, β-myrcene, limonene, 1,8-cineole, and α-terpinolene differed between the treatments, but did not show any correlation with night temperature (Table 1). Total SQTs were significantly increased at higher night temperatures (Table 1). Results from the three birch clones indicated that there were no significant differences between the clones in terms of individual compounds; however, clone Bp8 emitted higher total MTs, while Bp17 emitted higher total NTs than the other two clones (Table 2).

Table 2. Volatile organic compounds (ng m^{-2} h^{-1}) emitted from three different birch (*Betula pendula*) clones at 14 °C night-time temperature

Values are means ±SE (n=3). Means followed by the same letters are not significantly different ($P \leqslant 0.05$).

Compounds	Birch clones		
	8	17	26
Monoterpenes			
α-Thujene	0.0±0.0 a	76.4±76.4 a	0.0±0.00 a
α-Pinene	873.4±351.7 a	820.7±557.1 a	361.3±4.854 a
Sabinene	1277.2±379.0 a	3828.2±2991.5 a	710.3±302.5 a
β-Pinene	980.6±323.1 a	410.3±188.6 a	286.4±83.3 a
β-Myrcene	407.6±227.6 a	226.0±50.4 a	860.0±656.1 a
Limonene	1210.8±427.6 a	352.0±214.6 a	553.0±26.8 a
1,8-Cineole	634.7±291.6 a	191.2±17.4 a	251.0±87.6 a
δ3-Carene	0.0±0.0 a	265.3±265.3 a	0.0±0.0 a
γ-Terpinene	188.1±135.0 a	137.7±120.3 a	22.2±1.5 a
α-Terpinolene	202.8±103.8 a	221.5±111.8 a	303.1±15.3 a
α-Terpinene	0.0±0.0 a	94.2±94.2 a	28.7±28.7 a
Linalool	7193.1±4703.2 a	604.8±275.9 a	1545.0±822.5 a
β-Ocimene	1299.1±392.4 a	318.1±120.0 a	855.2±226.0 a
Alloocimene	12.4±12.4 a	0.0±0.0 a	0.0±0.0 a
Total monoterpenes	14356.2±6558.3 a	7470.1±2653.0 a	5776.± 153.0 a
Homoterpenes			
DMNT	59. 0±38.0 a	30.4±30.4 a	32.54±19.0 a
Sesquiterpenes			
α-Copaene	512.6±364.7 a	14213.5±13552.5 a	289.0±46.0 a
α-Humulene	234.8±169.8 a	3210.2±3029.5 a	95.4±14.0 a
δ-Cadinene	230.1±230.1 a	6506.4±6233.5 a	104.2±13.4 a
β-Bourbonene	593.3±249.6 a	72.8±72.8 a	5611.1±5611.1 a
(*E*)-Caryophyllene	681.5±355.6 a	386.0±115.2 a	5225.4±3425.7 a
3,7-Guaiadiene	1468.4±1168.5 a	165.3±165.3 a	2178.6±2178.6 a
(*Z*,*E*)-α-Farnesene	132.5±132.5 a	0.0±0.0 a	531.0±457.2 a
α-Amorphene	117.0±85.2 a	0.0±0.0 a	2298.7±2298.7a
(*E*,*E*)-α-Farnesene	21136.1±18807.6 a	140.6±76.3 a	49216.6±32215.6 a
γ-Cadinene	72.7±72.7 a	0.0±0.0 a	1425.4±1425.4 a
Total sesquiterpenes	26371.3±21362.7 a	25960.4±22028.5 a	71753.5±51523.2 a
Non-terpenes			
(*Z*)-3-Hexenyl acetate	2798.5±953.7 a	4586.5±1826.6 a	2821.4±991.4 a
(*Z*)-3-Hexenyl isovalerate	563.0±549.5 a	2485.2±2275.0 a	19.5±9.8 a
(*Z*)-3-Hexenyl butyrate	142.7±87.8 a	1135.4±890.0 a	67.6±40.0 a
Total non-terpenes	3504.1± 1021.8 a	8207.0±4742.2 a	2908.5±1032.0 a

In aspen, the isoprene emission did not differ between the night temperature treatments (Table 3). Emissions of the homoterpene DMNT and the MT β-ocimene showed constant increases from 6 °C to 14 °C, while the MT β-pinene and the MT alcohol linalool increased from 6 °C to 18 °C. The SQT (*Z*,*E*)-α-farnesene was emitted at a significantly higher rate at 18 °C while the SQT (*E*,*E*)-α-farnesene showed consistent increases from 6 °C to 18 °C. Among the NTs, emissions of the GLVs (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenyl isovalerate, and (*Z*)-3-hexenyl butyrate were increased consistently with night temperatures of 6 °C to 14 °C (Table 3). The total SQTs were increased consistently across the range of 6–18 °C, while NTs were increased across the range of 6–14 °C (Table 3).

Comparisons between the three clones of aspen indicated that clone Pt5.2 emitted significantly higher amounts of the MTs α-pinene, β-myrcene, and 1,8-cineole than clones Pt2.2 and Pt6.0, although clone Pt2.2 emitted more total MTs than Pt5.2 and Pt6.0 (Table 4). The MT β-ocimene and the homoterpene DMNT were emitted at significantly higher rates by clone Pt2.2 than the other two clones, while the highest emission rate of the SQT (*E*)-caryophyllene was from clone Pt6.0, differing significantly from clone Pt5.2 (Table 4). The highest isoprene emission was from clone Pt5.2 (Table 4).

Growth and photosynthesis

Daytime average photosynthesis related to VOC emissions was practically unaffected by night-time temperature, as it was not possible to detect any significant differences between the treatments (Fig. 2a). There was also no significant change in total leaf biomass in either species (Fig. 2b), although total leaf area increased due to warmer night-time temperatures in both species (Fig. 2c). In aspen, the increase in total leaf area was 53% from 6 °C to 22 °C (linear regression, r^2=0.99). In birch, there was no change in total leaf area up to 14 °C, but after that a similar linear increase (linear regression, r^2=0. 97) resulted in a 32% increase from 6 °C to 22 °C. In both species SLA also

Table 3. Volatile organic compounds (ng m^{-2} h^{-1}) emitted from aspen (*Populus tremula*, clone 2.2) under different night-time temperatures

Values are means ±SE (n = 9). Means followed by the same letters are not significantly different ($P \leqslant 0.05$).

Compounds	Night-time temperatures (°C)				
	6	10	14	18	22
Isoprene[a]	48.5±7.0 a	53.6±9.0 a	45.0±7.4 a	52.5± 8.0 a	35.3±4.7 a
Monoterpenes					
α-Pinene	77.5±14.4 a	217.3±128.0 a	186.0±74.4 a	97.6±16.5 a	83.4±6.6 a
Sabinene	2941.0±1566.4 a	1864.3±1206.5 a	4015.2±1922.4 a	5922.0±3374.3 a	2867.6±1686.4 a
β-Pinene	48.6±10.0 a	49.7±13.4 a	86.2±12.8 b	108.4±21.0 b	90.0±12.1 b
β-Myrcene	16.5±7.6 a	16.2±7.0 a,c	45.5±9.2 b	50.7±15.3 b,c	43.2±9.1 b
Limonene	74.3± 11.2 a	87.8±15.0 a	113.2±10.4 a	132.5±22.5 a	112.3±10.1 a
1,8-Cineole	16.4±10.1 a	9.7±7.2 a	25.8±6.0 a	35.0±9.1 a	22.4±7.6 a
γ-Terpinene	80.5±54.5 a	29.4±29.4 a	63.7±44.6 a	104.7±63.1 a	61.6±40.7 a
α-Terpinolene	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	19.3±14.7 a	0.0±0.0 a
α-Terpinene	0.0±0.0 a	0.0±0.0 a	16.±16.0 a	0.0±0.0 a	0.0±0.0 a
Linalool	28.2±14.3 a	126.1±40.8 a,b	425.3±113.6 b,c	441.3±161.0 b	346.3±102.5 c
β-Ocimene	89.0±29.8 a	277.2±60.5 b	402.4±71.0 b,c	399.1±57.5 b	615.4±73.7 c
Total monoterpenes	3372.±1642.7 a	2677.6±1283.0 a	5379.2±1852.3 a	7310.4±3442.3 a	4242.2±1722.0 a
Homoterpenes					
DMNT	57.0±31.6 a	303.5±128.0 b	1343.1±469.0.c	1086.0±547.0 c	1103.6±447.2 c
Sesquiterpenes					
α-Copaene	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	10.4±10.4 a
α-Humulene	719.2±335.0 a	355.1±184.1 a	716.3±313.7 a	731.6±331.0 a	859.2±478.4 a
δ-Cadinene	0.0±0.0 a	6.6±6.6 a	0.0±0.0 a	8.1±8.1 a	9.7±9.7 a
(*E*)-Caryophyllene	2641.6±1202.2 a	1541.5±780.7 a	2581.5±1120.0 a	2752.8±1233.0 a	3305.2±1771.3 a
(*Z*,*E*)-α-Farnesene	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	1235.3±799.2 b	0.0±0.0 a
(*E*,*E*)-α-Farnesene	502.1±285.8 a	3796.6±1790.1 b	4905.0±2224.1 b	28340.0±17803.3 c	5857.1±3489.8 b
Total sesquiterpenes	3863.0±1342.6 a	5699.8±2515.0 a	8202.7±3024.6 a	33067.7±19249.4 a	10041.6±5135.8 a
Non-terpenes					
(*Z*)-3-Hexenyl acetate	196.2± 31.7 a	356.8±64.0 b	3404.8±818.1 c	2828.4±1176.4 c	2597.4±1010.4 c
(*Z*)-3-Hexenyl isovalerate	0.0±0.0 a	65.3±45.5 a,b	653.2±351.8 b,c	501.3±228.0 c	351.0±244.1 b,c
(*Z*)-3-Hexenyl butyrate	0.0±0.0 a	32.1±25.1 a,b	314.0±140.4 c	258.1±141.3 c	202.5±160.2 b,c
Methyl salicylate	20.1±12.3 a	299.0±143.2 a	251.2±131.0 a	327.3±208.4 a	182.3±109.5 a
Total non-terpenes	216.3±33.0 a	753.2±116.1 b	4623.1±1205.0 c	3915.0±1403.1 c	3333.3±1299.0 c

[a] Isoprene concentrations are presented in μg m^{-2} h^{-1}.

Table 4. Volatile organic compounds emitted (ng m^{-2} h^{-1}) from three different aspen (*Populus tremula*) clones at 14 °C of night-time temperature

Values are means ±SE (n=3). Means followed by the same letters are not significantly different ($P \leq 0.05$).

Compounds	Aspen clones		
	2.2	5.2	6
Isoprene[a]	31.1±10.1 a	39.1±13.8 a	16.3± 8.3 a
Monoterpenes			
α-Pinene	103.7±16.3 a	378.0±40.4 b	67.3±10.0 a
Sabinene	2928.8±2483.4 a	1548.6±616.1 a	250.0±43.4 a
β-Pinene	165.1±62.6 a	341.3±121.4 a	72.0±15.0 a
β-Myrcene	56.0±10.6 a	160.5±10.5 b	15.2±7.6 c
Limonene	5790.3±5681.0 a	340.0±80.3 a	72.±7.0 a
1,8-Cineole	13.3±13.3 a	179.2±14.3 b	0.0±0.0 a
γ-Terpinene	91.1±91.1 a	677.8±654.2 a	0.0±0.0 a
Linalool	1296.4±782.6 a	385.6±200.1 a	136.0±40.7 a
β-Ocimene	760.4±182.8 b	86.1±43.6 a	190.0±85.3 a
Total monoterpenes	11204.9±7788.6 a	4097.0±546.2 a	802.2±95.0 a
Homoterpenes			
DMNT	2080.6±1505.7 b	85.2±85.2 a	46.4±8.8 a
Sesquiterpenes			
α-Copaene	0. 0±0.0 a	5.5±5.5 a	5.5±5.5 a
α-Humulene	68.1±15.8 a	167.6±150.3 a	534.2±467.1 a
δ-Cadinene	0.0±0.0 a	55.2±55.2 a	0.0±0.0 a
(*E*)-Caryophyllene	281.5±90.6 a,b	350.6±28.2 a	295.0±151.1 b
(*E*,*E*)-α-Farnesene	365.8±277.0 a	19213.6±15825.8 a	4793.8±2154.7 a
Total sesquiterpenes	715.5±352.2 a	20034.7±15626.1a	6262.4±2019.0 a
Non-terpenes			
(*Z*)-3-Hexenyl acetate	5425.2±3316.6 a	1469.0±847.2 a	4516.8±1867.6 a
(*Z*)-3-Hexenyl isovalerate	734.6±698.2 a	5691.7±5418.3 a	231.6±112.2 a
(*Z*)-3-Hexenyl butyrate	49.6±27.0 a	579.6±364.0a	116.3±59.2 a
(*Z*)-3-Hexenyl tiglate	0.0±0.0 a	1207.8±1174.8 a	20.3±14.6 a
Total non-terpenes	6209.5±3235.3 a	8948.2±7682.1 a	4885.0±2037.4 a

[a] Isoprene concentrations are presented in μg m^{-2} h^{-1}.

increased with temperature (Fig. 2d). Although a minor effect in SLA was observed between 6 °C and 10 °C and the strongest impact was observed between 10 °C and 14 °C, the increase in general was quite linear, despite the differences between the genotypes (data not shown). Increase in SLA: aspen, 3.5 (cm^2g^{-1})/1 °C, r^2=0.956; birch, 3.5 (cm^2g^{-1})/1 °C, r^2=0.916. SLA was greater in birches than aspens.

Gene expression

In birch, the highest expression of *DXR*, *DXS*, and *IPP* was found at 10 °C, while down-regulation of these genes occurred at higher night-time temperatures (Fig. 3). No major differences in these gene activities were found between the genotypes (Fig. 3). In aspen, expression of *DXR* and *IspS* was down-regulated with increasing night-time temperature, whereas *DXS* and *HMGR* were not clearly affected by temperature treatments (Fig. 4). Genotypic comparisons showed that *IPP* and *IspS* expression was highest in clone Pt5.2 (correlating with the highest isoprene emissions of this clone) and that the lowest expression for all genes was found in clone Pt2.2 (Fig. 4).

Discussion

The results from the present study indicate that elevated night-time temperature enhances daytime emissions of volatiles, especially SQTs and the homoterpene DMNT in birch. Interestingly, DMNT, which is considered to be primarily induced by biotic stresses (Dicke, 2009) including feeding by spider mites (Pinto *et al.*, 2007), mining fly larvae (Wei *et al.*, 2006), and moth larvae (Vuorinen *et al.*, 2007; Mäntylä *et al.*, 2008) and which has ecological significance, for example in attraction of predatory mites (Pinto *et al.*, 2007) and birds (Mäntylä *et al.*, 2008), showed a strong night-temperature dependence in this study. An earlier study by Gouinguene and Turlings (2002) on maize showed that emissions of DMNT and several SQTs from plants stressed by simulated herbivore damage were independent of growth and sampling temperatures ranging from 17 °C to 37 °C. However, exposure to C_6 GLVs, particularly to (*Z*)-3-hexenyl acetate, can trigger DMNT emissions from

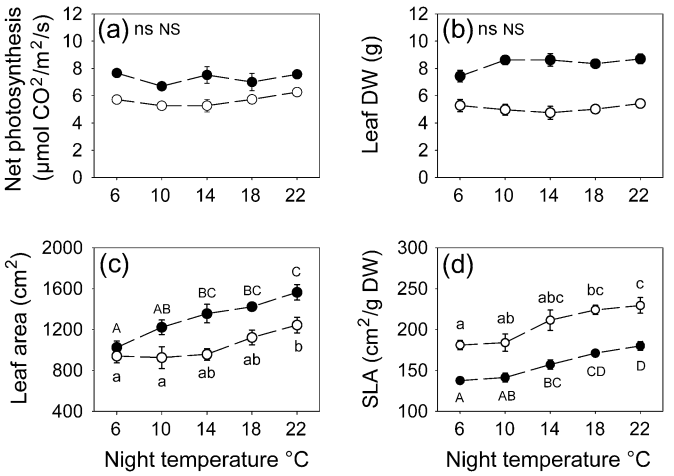

Fig. 2. Daytime average net photosynthesis and leaf growth of *B. pendula* (open circles) and *P. tremula* (filled circles) grown at different night temperatures. (a) Photosynthesis (means ±SE, n=3) was measured after VOC collections from clones 26 and 2.2. Whole seedling (b) leaf biomass, (c) leaf area, and (d) specific leaf area are means ±SE (n=9–12), combining the data of all three studied clones. Significant differences ($P \leqslant 0.05$) between the temperature treatments are indicated by different letters; *B. pendula* (lower case) and *P. tremula* (upper case).

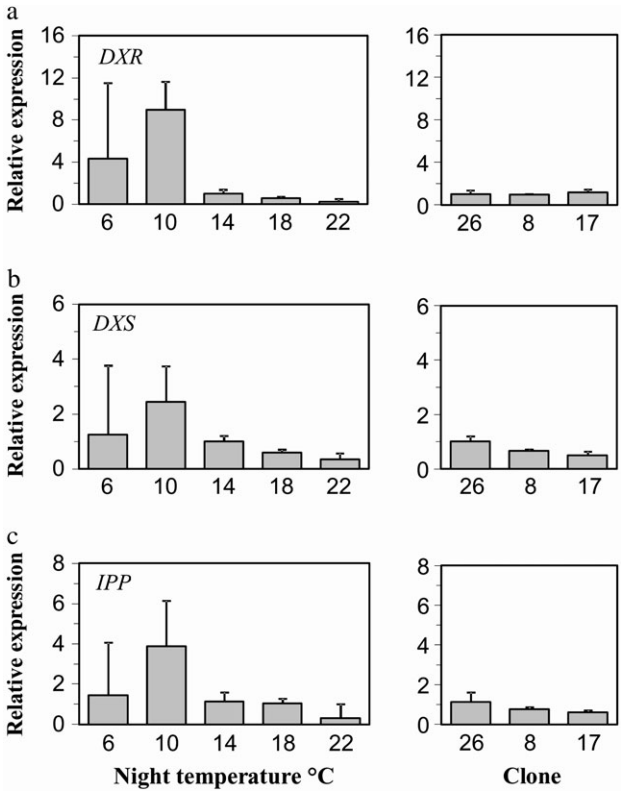

Fig. 3. Expression of (a) *DXR*, (b) *DXS*, and (c) *IPP* in *Betula pendula* clone 26 at night-time temperatures of 6, 10, 14, 18, and 22 °C (left) and at 14 °C in clones 26, 8, and 17 (genotypic comparison, right). The data indicate the relative mRNA expression compared with the expression of clone 26 at 14 °C (value 1); means of three replicates. Error bars=SD.

uninfested maize plants (Yan and Wang, 2006) and prime DMNT emissions from *Populus deltoides*×*nigra* (Frost *et al.*, 2008). These results suggest that increasing night-time temperature may represent a stress factor for northern trees.

In the present study, several SQTs emitted by birch showed a steady increase in emission rate with increasing night-time temperature. In line with these results, *B. pendula* was reported to be a high SQT emitter, with emissions equalling or even exceeding MT emissions (Vuorinen *et al.*, 2005, 2007). Exponential dependencies of both MT and SQT emissions on sampling temperature have previously been found for intact pine trees in branch enclosure experiments (Helmig *et al.*, 2006, 2007). It was reported that SQT emission rates of loblolly pine (*Pinus taeda* L) had a stronger temperature dependency than MT emission rates (Helmig *et al.*, 2007). Helmig *et al.* (2007) were also able to show that a 10 °C increase in night-time temperature increased the night-time emission rate to a level close to the daytime emission rate (at the same temperature), indicating that SQT emissions are strongly modulated by temperature conditions regardless of light. In their study, SQTs were the dominating compounds at temperatures >30 °C, while MTs were more abundant at lower temperatures, indicating that emissions of SQTs are triggered by high temperatures, probably as a direct stress response. Hartikainen *et al.* (2009) did not detect SQT emissions from *P. tremula* saplings, when grown in normal boreal field conditions with monthly mean temperatures below +20 °C. Based on this information, it is suggested that in some tree species such as birch, SQT emissions are temperature dependent and that this may have a positive effect on the thermotolerance of the plants. Accordingly, maximum VOC emissions have also been measured during the warmest summer season in coniferous trees, as reported by Pressley *et al.* (2004) and Kim *et al.* (2005) in the case of MTs, and Tarvainen *et al.* (2005) and Hakola *et al.* (2006) in the case of SQTs. Night-time sampling of VOC emissions and tissue pools was not conducted in the present study and consequently it cannot be estimated how much of the increasing SQT emissions was due to increased biosynthesis or delayed release from plant surfaces (Helmig *et al.*, 2004; Schaub *et al.*, 2010) at higher temperatures.

In the present study, the isoprene emission measured during the daytime was lowest in plants grown at the highest night temperature. This is consistent with the recent findings of Hartikainen *et al.*, (2009) who found a decrease of isoprene emission at high temperatures in field-growing aspen clone Pt2.2. Although, isoprene responses to night-time temperatures have not been previously reported, the present findings suggest that the temperature dependency of isoprene cannot be generalized to all plants. However, the results are inconsistent with several previous studies reporting that isoprene emissions at daytime temperatures are strictly temperature dependent in addition to light dependent (Singsaas *et al.*, 1999; Hanson and Sharkey 2001; Sharkey *et al.*, 2008). Isoprene emission is also known to be induced by several other abiotic factors, such as increased

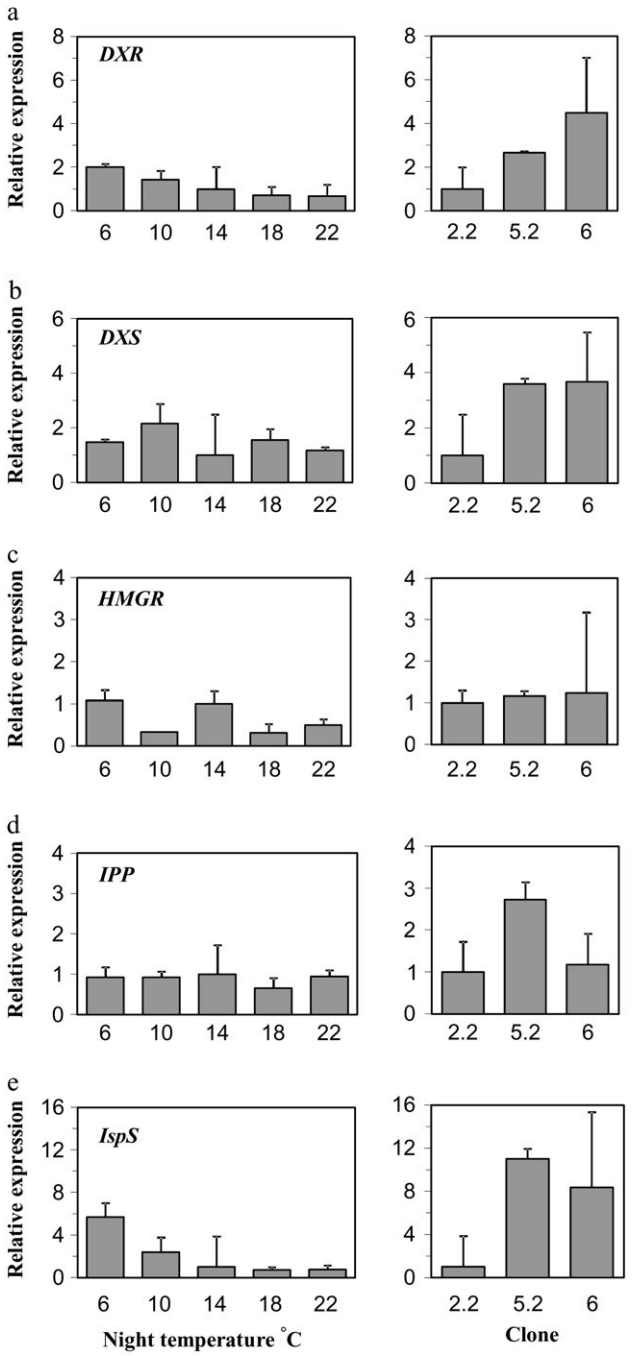

Fig. 4. Expression of (a) *DXR*, (b) *DXS*, (c) *HMGR*, (d) *IPP*, and (e) *IspS* in *Populus tremula* clone 2.2 at night-time temperatures of 6, 10, 14, 18, and 22 °C (left) and at 14 °C in clones 2.2, 5.2, and 6 (genotypic comparison, right). The data indicate the relative mRNA expression compared with the expression of clone 2.2 at 14 °C (value 1); means of three replicates. Error bars=SD.

tropospheric ozone concentrations, as demonstrated in *Phragmites australis* and *Quercus pubescens* by Velikova *et al.* (2005), and by UV-B enhancement, as reported in *Q. gambelii* by Harley *et al.* (1996) and peatland plants by Tiiva *et al.* (2007). Isoprene might play a role in thermoprotection of leaves and especially photosynthetic apparatus (Sharkey *et al.*, 2008, and references therein). Velikova *et al.* (2006) found that exogenous isoprene fumigation reduced the negative effect of high temperatures (38 °C) on photosynthetic activity in *Platanus orientalis* leaves. In the present experiment, photosynthesis was practically unaffected by increased night-time temperature, but the temperatures used were below normal heat stress temperatures. Therefore, the protective role of isoprene cannot be proved in this case.

The larger total leaf areas of the seedlings with increasing night temperature could mean a consistent increase of all measured emissions. At the forest stand level this kind of change would suggest a considerable increase of daytime reactive VOC emissions. However, calculations of total VOC emissions from whole seedlings cannot be made from these data, because emission profiles probably change both quantitatively (Blande *et al.*, 2007; Frost *et al.*, 2007; Brilli *et al.*, 2009) and qualitatively along the seedling profile from top to base, as is the case with MTs (Brilli *et al.*, 2009) and isoprene in poplars (Sharkey *et al.*, 2008; Brilli *et al.*, 2009). With unaffected net photosynthesis, increasing VOC emissions would indicate changes in carbon balance through enhanced carbon release back to the atmosphere. However, this hypothesis needs to be studied more extensively before further conclusions are drawn.

Increased SLA with night-time temperature is in accordance with the meta-analysis of Poorter *et al.* (2009) reporting that leaf mass per area is lower in plants grown at higher temperatures. Recent results of Hartikainen *et al.* (2009) also demonstrated significantly thinner leaves in the same aspen clones growing in field conditions with diurnally elevated temperature. In that study, thinner leaves had a thinner epidermis, palisade, and spongy mesophyll. A thinner leaf could possibly enable a more rapid diffusion and release of newly synthesized SQTs. Some evidence for this comes from considering the reverse of an example by Vuorinen *et al.* (2004): thicker leaves of elevated CO_2-grown cabbage plants emitted a marginally reduced amount of the SQT (*E*,*E*)-α-farnesene and the homoterpene DMNT, when VOCs were sampled in ambient air (Vuorinen *et al.*, 2004). However, thinner leaves may need more thermal protection.

The present gene expression studies indicated that VOC emissions are not directly regulated by the transcription level, but that the regulation is very complex. In birch, maximum expression of *DXR*, *DXS*, and *IPP* at a night-time temperature of 10 °C coincided with the maximum emissions of total MTs at the same temperature, suggesting that these genes are temperature controlled. However, increasing emission of DMNT with increasing night-time temperature was accompanied by down-regulation of *DXR*, *DXS*, and *IPP*, operating in the MEP pathway. Similarly, in aspen, *DXR* was slightly suppressed when MTs were increasing at warmer night-time temperatures while *IpsS* showed a clearer down-regulation with warming. The high complexity of regulation of volatile emissions was previously demonstrated by Nogués *et al.* (2006) who reported that pool sizes of the immediate precursors (DMAPP and geranyldiphosphate) are inversely related to the emission fluxes of isoprene and MTs through a strong negative control. In addition, Rasulov *et al.* (2009) reported that

Table 5. Primer sequences and qRT-PCR amplicon lengths

Gene	Forward primer	Reverse primer	Amplicon length (bp)
P. tremula			
tub	GATCCTCGTCACGGAAAGTACA	CAGCAACACTGGTAGAGTTGGA	241
DXR	GCCTGATATGCGCTTGCCTAT	CAGCACTGAGGACTCCTGTCA	201
DXS	AGTTGCAGATGCCAGGTTCT	GCTGGTGGTCCATGGTCAAT	216
IPP	GAATGCTGCACAGAGGAAGCT	GCTCCTTCAACTCATCCTGGTT	230
IPS	CTGGAGGCTGAGGTGAGAAGA	GAGCAGTAGCATGAAGGCTAGT	193
HMG	GCTGGTGCTCTAGGTGGATT	CGGATTGCGATGCAAGTTGA	217
B. pendula			
tub	CGGTTCGATGGAGCCTTGAA	CATCAAGCAGCAAGCCATGT	228
DXR	CTCGTCTGTTCTGGCACAGTT	GTCCAGCAGCATATGCGAGAT	194
DXS	CGATGAGGCAGAGCTCTTTCA	CAGCTCTGAACTGCTGTTCCA	207
IPP	CTGACGATGTGCCAGTTGATCA	TGTCCACCACTAGTCTGAACCA	255

isoprene emissions were more dependent on DMAPP pool size than IpsS activity. Mayrhofer *et al.* (2005) have also reported that the regulation of isoprene biosynthesis in Grey poplar (*Populus*×*canescens*) occurs at transcriptional, post-translational, and metabolic levels, and is highly variable with respect to diurnal and seasonal changes in light and temperature. Anyhow, several investigations support that *DXS* and *DXR* play an important role in the control of plant isoprenoid biosynthesis. The rate-limiting step is still unknown, although molecular engineering revealed that overexpression of *DXS* (Estévez *et al.*, 2001) or of *DXR* (Mahmoud and Croteau, 2001) resulted in an enhanced accumulation of isoprenoid end-products.

In the present experiments, *IspS* proved to be more responsive than *IPP* to increasing temperature, showing the highest expression at the lowest night-time temperatures. In contrast to this study, a strong increase in enzymatic activity as a result of the transcriptional up-regulation of the *IspS* gene was reported to be induced by heat (40 °C) in *P. alba* leaves (Sasaki *et al.*, 2005) and (60 °C) in transgenic *Arabidopsis* (Sasaki *et al.*, 2007). Previous studies with Grey poplar reported that the expression rates of *DXR* (*PcDXR*) and *IspS* (*PcISPS*) were correlated with each other (Mayrhofer *et al.*, 2005), which was also evident in the present study. A specific role for *HMGR* in temperature-dependent VOC production could not be demonstrated.

The changes in transcription levels of these selected genes could not explain the differences in VOC emission between the aspen and birch clones. The birch clones were rather similar in their gene expression patterns, although large differences were found in many volatile compounds. Even though aspen showed clearer differences among the clones in terms of *DXR*, *DXS*, *IPP*, and *IspS* expression, these changes were poorly correlated with related VOC emission patterns. Only the highest isoprene emission in clone Pt5.2 matched the highest expression of *IPP* and *IspS* in genotypic comparison.

The experiment demonstrated that increasing night-time temperatures strongly increase daytime emissions of the homoterpene DMNT, several SQTs, and total SQTs (at higher temperatures) in birch and the emission of several MTs up to 18 °C in aspen, while isoprene emissions were not correlated with temperature increase. Although expression of *DXR*, *DXS*, *IPP*, and *IspS* was temperature dependent, further research is needed to understand the complex nature of regulation, which seems to involve inverse relationships with VOC emissions.

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