Acclimation of isoprene emission and photosynthesis to growth temperature in hybrid aspen: resolving structural and physiological controls

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Abstract

Acclimation of foliage to growth temperature involves both structural and physiological modifications, but the relative importance of these two mechanisms of acclimation is poorly known, especially for isoprene emission responses. We grew hybrid aspen (*Populus tremula* x *P. tremuloides*) under control (day/night temperature of 25/20 °C) and high temperature conditions (35/27 °C) to gain insight into the structural and physiological acclimation controls. Growth at high temperature resulted in larger and thinner leaves with smaller and more densely packed chloroplasts and with lower leaf dry mass per area (M_A). High growth temperature also led to lower photosynthetic and respiration rates, isoprene emission rate and leaf pigment content and isoprene substrate dimethylallyl diphosphate pool size per unit area, but to greater stomatal conductance. However, the declining characteristics were similar when expressed per unit dry mass, indicating that the area-based differences were primarily driven by M_A. Acclimation to high temperature further increased heat stability of photosynthesis, and increased activation energies for isoprene emission and isoprene synthase rate constant. This study demonstrates that temperature acclimation of photosynthetic and isoprene emission characteristics per unit leaf area was primarily driven by structural modifications, and we argue that future studies investigating acclimation to growth temperature must consider structural modifications.

Keywords

dimethylallyl diphosphate; isoprene emission; isoprene synthase; structural controls; photosynthesis rate; physiological controls; temperature acclimation

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Introduction

Photosynthesis and respiration are considered the primary biological components regulating the biosphere-atmosphere carbon exchange (Ciais *et al.* 2001; Mahecha *et al.* 2010). However, there is a large and commonly ignored flux of biogenic volatile organic compounds (BVOC) generated by pathways of secondary metabolism that can significantly contribute to the carbon balance of the biosphere (Arneth *et al.* 2008; Guenther *et al.* 2012; Ashworth *et al.* 2013). Among BVOC, a central role is played by isoprene that has the greatest worldwide flux attributable to a single volatile compound (Guenther *et al.* 1993; Li & Sharkey 2013).

Both the primary and the secondary plant metabolism are strongly dependent on temperature, but the temperature sensitivities of different processes differ among plant species, and they often vary with growth conditions (Way & Oren 2010; Centritto *et al.* 2011; Smith & Dukes 2013; Way & Yamori 2014). In particular, acclimation to growth temperature can importantly alter the shape of the temperature responses of respiration and photosynthesis (Berry & Björkman 1980; Hopkins 1998), and isoprene emission (Wiberley *et al.* 2008). Although acclimation of physiological processes to long-term temperature is a well-known phenomenon, the degree of acclimation strongly varies among plant species and for different processes for reasons not yet fully understood (Berry & Björkman 1980; Tjoelker *et al.* 1999; Bunce 2000; Yamasaki *et al.* 2002; Loveys *et al.* 2003; Fares *et al.* 2011). Understanding such acclimation responses and mechanisms of acclimation are important in predicting plant responses to seasonal and year-to-year differences in temperature environments as well as the responses to future climate (Smith & Dukes 2013).

Acclimation of photosynthesis to high temperature is characteristically associated with shifts of the optimum temperature of photosynthesis to higher temperatures (Berry & Björkman 1980; Turnbull *et al.* 2002; Sage & Kubien 2007). Improved heat stability of photosynthesis is typically associated with changes in the stability of thylakoid membranes as the result of increases in the degree of membrane lipid saturation (Berry & Björkman 1980; Björkman *et al.* 1980; Devos *et al.* 1998; Sharkey *et al.* 2001). Given that the temperature responses of Rubisco and photosynthetic electron transport have different temperature sensitivities, temperature acclimation can also result from changes in the partitioning of rate-limiting photosynthetic proteins among the partial determinants of photosynthesis (Hikosaka 1997; Hikosaka *et al.* 1999). In addition, growth temperature driven changes in the overall content of rate-limiting components of photosynthesis machinery can result in changes in maximum photosynthetic rates (Björkman *et al.* 1980; Stitt & Hurry 2002; Yamori *et al.* 2005; Sage & Kubien 2007).

In isoprene-emitting species, heat-stability can be rapidly altered by isoprene emission that is thought to dissolve in photosynthetic membranes and stabilize membrane lipids (Singsaas & Sharkey 2000; Sharkey & Yeh 2001; Velikova *et al.* 2011; Velikova *et al.* 2012) as well as serve as a lipid-soluble antioxidant detoxifying heat stress generated reactive oxygen species and peroxidized membrane lipids (Vickers *et al.* 2009; Velikova *et al.* 2012; Possell & Loreto 2013). Thus, isoprene is considered particularly advantageous in responding to rapid

increases in leaf temperature such as those occurring during lightflecks (Behnke *et al.* 2007; Way *et al.* 2011; Behnke *et al.* 2013).

Isoprene emission capacity can adapt to temperature environment, typically increasing after changes in leaf temperature environment (Sharkey *et al.* 1999; Funk *et al.* 2003; Wiberley *et al.* 2008) as the result of enhanced expression of isoprene synthase gene and genes of the chloroplastic 2-C-methyl-D-erythritol 4-phosphate pathway responsible for isoprenoid synthesis in chloroplasts (Wiberley *et al.* 2008; Wiberley *et al.* 2009; Vickers *et al.* 2010). However, most work on isoprene emission responses to temperature environment stems from transfer experiments where plants developed at a certain temperature have been transferred to the new temperature environment. The effect of plant growth at different temperature environments per see has been investigated only in a few studies (Wiberley *et al.* 2008; Hartikainen *et al.* 2009; Fares *et al.* 2011) with contrasting results showing either enhanced (Wiberley *et al.* 2008), similar (Monson *et al.* 1992; Hartikainen *et al.* 2009; Centritto *et al.* 2011; Fares *et al.* 2011) or reduced emissions (Hartikainen *et al.* 2009) in plants grown under the higher temperature.

Although most work on temperature acclimation has focused on physiological responses, growth at different temperatures can also importantly modify leaf structure, whole plant leaf area and whole plant sink-source relationships (Starck *et al.* 1993; Morcuende *et al.* 1996; Hartikainen *et al.* 2009; Muhl *et al.* 2011; Xu *et al.* 2012). Structural modifications such as changes in chloroplast number, cell size and cell number per unit leaf area are relevant as they can change the amount of rate-limiting enzymes per unit leaf area and result in changes in the process rate per unit leaf area at any given temperature without concomitant changes in physiological potentials of single cells (Niinemets & Sack 2006; Niinemets *et al.* 2009; Poorter *et al.* 2009). It has been demonstrated that growth temperature dependent changes in respiratory (Ow *et al.* 2008) and photosynthetic activities (Yamasaki *et al.* 2002; Yamori *et al.* 2005; Hartikainen *et al.* 2009; Ibrahim *et al.* 2010) were importantly affected by modifications in leaf dry mass per unit area, which connects area and mass based process rates (Poorter *et al.* 2009). However, the role of growth temperature driven structural modifications in acclimation of photosynthesis and isoprene emission has not been routinely studied.

The effects of growth temperature on isoprene emission vs. temperature response relationships also vary among the studies with responses ranging from improved thermal sensitivity (Monson *et al.* 1992) to minor changes or even reduced thermal sensitivity (Fares *et al.* 2011). Given that the temperature response of isoprene emission is driven by both the temperature effects on isoprene synthase activity and its immediate substrate dimethylallyl diphosphate pool size (Rasulov *et al.* 2010), gaining insight into such contrasting responses requires information of growth temperature effects on both partial controls of isoprene emission. As isoprene emission and photosynthetic carbon metabolism are strongly linked (Li & Sharkey 2013), improved heat-resistance of leaves grown at higher temperature can result in maintenance of greater DMADP pools at higher temperature, thereby improving the isoprene emission at higher temperatures.

Here we studied the effects of growth temperature on foliage photosynthetic and structural characteristics and isoprene emission in hybrid aspen (*Populus tremula* x *P. tremuloides*) with the specific aim to separate the structural and physiological controls on growth temperature acclimation. A recently developed in vivo method for estimation of DMADP pool size and determination of isoprene synthase rate constant and in vivo isoprene synthase kinetic characteristics (Rasulov *et al.* 2009a; Rasulov *et al.* 2011) was used to separate the substrate and enzyme level controls on isoprene emission under different conditions. Our results indicate that growth temperature did modify the heat resistance of photosynthesis, and altered the temperature sensitivity of photosynthesis and isoprene emission rate, but the differences in foliage photosynthesis and isoprene emission rates per unit area were primarily driven by structural changes.

Material and methods

Seedlings of hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.) clone H200 (Rasulov *et al.* 2009a; Rasulov *et al.* 2009b; Rasulov *et al.* 2011) were used in the experiments. Dormant seedlings kept in a cold storage were planted in 4 L plastic pots filled with a mixture of peat and sand (1:1) and maintained at room temperature of 22 °C until dormancy was broken in 2-3 days. Thereafter, the seedlings were moved to Percival AR-95 HIL growth chambers (CLF PlantClimatics) with contrasting temperature regimes (day/ night) of 25/20 °C (lower temperature, control) and 35/27 °C (higher temperature). The ambient CO_2 concentration was between 380-420 μmol mol^{-1}, the relative humidity was maintained between 60-65% (corresponding to moderate differences in daytime water vapor pressure deficit of ca. 1.1 kPa for the control and 1.9 kPa for the high temperature treatment) and the quantum flux density of 500 μmol m^{-2} s^{-1} was provided for 14 h light period. The plants were watered daily to field capacity with the Hoagland nutrient solution for optimum water and nutrient supply. All measurements were conducted with fully-expanded 20-24 days old leaves exhibiting peak physiological activity.

Measurements of gas-exchange rates and isoprene emission

An ultra-fast gas-exchange system (system half-time of approximately 0.15 s) was used for the measurements (Laisk *et al.* 2002; Rasulov *et al.* 2010). Briefly, the leaf is enclosed in a clip-on type leaf chamber with a volume of volume 2.4 cm^3 and a cross-sectional area of 8.04 cm^2. The leaf chamber is thermostatted, and the upper leaf surface is glued to the chamber glass window by a starch paste. Direct measurements by thin thermocouples and energy balance calculations indicated that leaf temperature was maintained within 0.5°C of the leaf chamber water jacket. The system has two identical gas lines where different gas concentrations can be prepared and almost instantaneously, in less than 1 s, switched between the sample and the reference line. Such a setup is particularly appropriate for fast measurement of transient physiological responses, avoiding delays in the detection of leaf gas-exchange response signals due to stabilization of gas concentrations. The enclosed leaf area was illuminated with a Schott KL 1500 light source equipped with a heat-reflecting filter (Optical Coating Laboratory, CA, USA). The air was mixed from pure nitrogen, oxygen, and CO_2 using calibrated dynamic mixers, and the air flow rate through the system was kept at 0.5 mmol s^{-1} (Laisk *et al.* 2002). A LI-6251 infrared gas analyzer (LI-Cor, Inc.,

Lincoln, Nebraska, USA) was used to measure the CO_2 concentration, a custom-made microsychrometer was used for the water vapor concentration, and a proton transfer reaction mass spectrometer (PTR-MS, high-sensitivity version; Ionicon Analytik GmbH, Innsbruck, Austria) for the isoprene concentration. The response time of the PTR-MS was approximately 0.1 s and the isoprene detection limit about 10 pmol mol^{-1}. The instrument was calibrated with an isoprene standard mixture of 5.74 µmol mol^{-1} isoprene in N_2 (Hills Scientific, Boulder, Colorado, USA).

The standard conditions used in these experiments were the leaf chamber CO_2 concentration of 360 µmol mol^{-1}, O_2 concentration of 21%, leaf temperature of 30 °C, light intensity (photosynthetic quantum flux density) of 650 µmol m^{-2} s^{-1}, and water vapor pressure deficit between the leaf and the atmosphere (V_D) of 1.7 kPa. The measurements were conducted with attached leaves, and the protocol for all measurements included leaf enclosure, leaf stabilization under the standard conditions until the steady-state rates of CO_2 and H_2O vapor exchange and isoprene emission were established, and then changing either CO_2 (360 vs. 1200 µmol mol^{-1}), O_2 (21 vs. 2%), light (quantum yield measurements) or temperature (temperature response curves) to measure different environmental responses. For the temperature response curves, leaf temperature was changed in steps of 5 °C, and each temperature change was followed by returning to the standard temperature and leaf stabilization as in Rasulov et al. (2010). Chamber air water vapor concentration was adjusted at each temperature to maintain the constant V_D and avoid changes in stomatal conductance due to altered V_D.

The quantum yields for CO_2 exchange (α_{CO2}) and isoprene emission (α_I) were measured over the incident light range of 20 - 75 µmol m^{-2} s^{-1} where the influence of the Kok effect is negligible. In addition, the initial slopes of the net assimilation vs. CO_2 response curves over an ambient CO_2 range of 0-100 µmol mol^{-1} at both O_2 concentrations of 21% and 2% were also measured at the standard light intensity of 650 µmol m^{-2} s^{-1}.

All leaf gas-exchange characteristics were calculated according to von Caemmerer and Farquhar (1981). The maximum carboxylase activity or Rubisco (V_{cmax}) was estimated from the initial slopes of the CO_2 response curve at both O_2 concentrations and the capacity for photosynthetic electron transport (J_{max}) from the high-CO_2 concentration (1200 µmol mol^{-1}) measurements according to Niinemets et al. (1999b).

Measurements of chlorophyll fluorescence

A PAM 101 fluorimeter (Walz GmbH, Effeltrich, Germany) was used to measure chlorophyll fluorescence characteristics simultaneously with CO_2 and water vapor exchange. A Schott KL 1500 light source provided saturated pulses of white light of 14000 µmol m^{-2} s^{-1} for 2 s to measure either the dark-adapted (F_m) or light-adapted (F_m') maximum fluorescence yields, while another Schott KL 1500 with a 720-nm narrow-pass interference filter (Andover Corp., Salem, NH, USA) was used for far-red light to estimate the dark-adapted minimum fluorescence yield (F_0). The fluorimeter operation frequency was 1.6 kHz for the darkened leaves and 100 kHz for the illuminated leaves and during saturated pulses. F_m and F_0 were typically measured 20 min. after darkening. The dark-adapted PSII quantum yield was computed as $(F_m - F_0)/F_m$ and the quantum yield of illuminated leaves, Φ_{PSII}, as

$(F_m' - F)/F_m'$, where F is the steady-state fluorescence yield. The rate of photosynthetic electron transport from chlorophyll fluorescence (J_{ETR}) was further calculated as (Schreiber *et al.* 1994):

$$J_{ETR} = \Phi_{PSII} \xi \beta Q, \quad (1)$$

where Q is the incident quantum flux density, ξ is the leaf absorptance (0.86 for the control and 0.81 for the high temperature grown plants) and β is the fraction of absorbed light used to drive PSII electron transport (taken as 0.5 in this study).

Foliar structural measurements and determination of chlorophylls and carotenoids

Immediately after the gas-exchange measurements, leaf discs of 4 cm^2 were taken between the major leaf veins for estimation of leaf fresh and dry mass. For anatomical measurements, leaf samples were taken with a razor blade between the major veins from the central part of the leaves and cut into ca. 1 x 1 mm pieces for fixation.

The fresh mass of the leaf discs was estimated immediately, and the dry mass after oven-drying at 70 °C for at least 48 h. Additional leaf discs for foliar pigment analyses were frozen at -80 °C until the analysis. The frozen discs were pulverized in liquid N_2 and the pigments were extracted with 80% ice-cold acetone with some $CaCO_3$. The absorptances of the pigment solutions were measured with a dual-beam Shimadzu UV2550PC spectrophotometer at the wavelengths of 663.2, 646.8 and 470 nm, and the pigment contents were calculated according to the equations of Lichtenthaler and Buschmann (2001).

Anatomical measurements

Preparation of foliar samples for light and transmission electron microscopy (TEM) follows the protocol of Copolovici et al. (2011). Briefly, the samples were fixed by infiltrating with buffered (0.2 M phosphate buffer, pH = 7.2) 2% glutaric aldehyde, dehydrated in ethanol series and embedded in Epoxy Embedding Medium (Sigma-Aldrich Chemie, Steinheim, Germany). Semi-thin (1.5–2 μm) cross- and paradermal sections and ultrathin (70–90 nm) cross-sections were cut with a Leica EM UC7 ultramicrotome (Leica Mikrosysteme GmbH, Austria, Vienna) equipped with a 45° diamond knife (Diatome, Hatfield, PA, USA).

The semi-thin sections were stained for 30 s with Toluidine Blue as described in Burns (1978) and Mercer (1963), viewed at 400-1000x magnification with a Zeiss Axioplan 2 microscope and the digital images were taken with an AxioCam HRc (Carl Zeiss Microscopy, Jena, Germany). Five cross-sections per leaf sample were measured in three locations for total leaf thickness, palisade mesophyll and spongy mesophyll thickness, and chloroplasts and cells were counted in these sections. On the same sections, leaf thickness, palisade mesophyll and spongy mesophyll thickness were measured. In addition, chloroplast and cell numbers were also estimated from 10 paradermal sections per sample, 5 cut through the palisade and 5 through the spongy mesophyll. From these measurements, the chloroplast number per leaf area (n_{chl}) was calculated as:

$$n_{\mathrm{chl}} = n_{\mathrm{c,\ p}} n_{\mathrm{chl,\ p,\ p}} n_{\mathrm{chl,\ p,\ c}} + n_{\mathrm{c,\ s}} n_{\mathrm{chl,\ s,\ p}} n_{\mathrm{chl,\ s,\ c}}, \quad (2)$$

where $n_{\mathrm{c,p}}$ is the number of cells in the palisade and $n_{\mathrm{c,s}}$ that in the spongy parenchyma per unit leaf area, $n_{\mathrm{chl,p,p}}$ is the chloroplast number per cell in the palisade parenchyma estimated from the paradermal section and $n_{\mathrm{chl,p,c}}$ that estimated from the cross-section, and $n_{\mathrm{chl,s,p}}$ is the chloroplast number in the spongy parenchyma estimated from the paradermal section and $n_{\mathrm{chl,s,c}}$ that estimated from the cross-section.

Ultrathin sections for TEM were contrasted with uranyl acetate and lead citrate (Copolovici *et al.* 2011 for details) and samples were viewed with a Philips Tecnai 10 TEM (FEI, Eindhoven, Netherlands) with an accelerating voltage of 80 kV. Digital images were taken with Olympus Veleta 2K x 2K sidemounted TEM CCD camera (Olympus Soft Imaging Solutions GmbH, Germany). Magnifications of 7000–10000x were used to measure chloroplast length, thickness and area (ca. 40 chloroplasts were measured in each case). All anatomical characteristics were separately measured for palisade and spongy mesophyll. Digital image analysis was carried out with UTHSCSA Image Tool for Windows 3.00 (UTHSCSA, Texas, USA).

Determination of the dimethylallyl diphosphate (DMADP) pool size (C_{DMADP})

The postillumination method of Rasulov et al. (2009a; 2010) was used to measure the pool size of the immediate precursor pool responsible for the isoprene emission in the steady state. The method is based on the assumption that postillumination isoprene release continues at the expense of the available precursor pool synthesized prior to leaf darkening (Rasulov *et al.* 2009a; Rasulov *et al.* 2010; Li *et al.* 2011). Thus, the size of this pool (C_{DMADP}) was determined by integrating the fast, 200–400 s after darkening, dark decay of isoprene release (Rasulov *et al.* 2009a; Rasulov *et al.* 2010).

In addition to DMADP, the isoprene precursor, isopentenyl diphosphate (IDP) can be converted in the dark to DMADP by IDP isomerase and thus, contribute to the dark release of isoprene (Li *et al.* 2011). Although there is a variation in chloroplastic IDP/DMADP ratios among studies (Ramos-Valdivia *et al.* 1997; Zhou *et al.* 2013) likely indicating differences in the activity of chloroplastic IDP isomerase (Brüggemann & Schnitzler 2002), studies are in agreement that IDP/DMADP ratios are small. Nevertheless, even for relatively high IDP/DMADP ratios of ca. 0.5 (Weise *et al.* 2013), the method of Rasulov (2009a; 2010) has provided DMADP pool size estimates in excellent agreement with destructive chemical estimations (Weise *et al.* 2013).

Estimation of the rate constant, the maximum rate (V_{max}) and Michaelis-Menten constant (K_{m}) for isoprene synthase

The DMADP pool size (C_{DMADP}) decreases after darkening due to DMADP conversion into isoprene according to a first-order reaction kinetics under strongly substrate-limited conditions (Loreto *et al.* 2004; Rasulov *et al.* 2009a; Rasulov *et al.* 2009b). Given further that the isoprene synthase activation state does not vary during the light-dark transient (as confirmed by the rapid rise of isoprene emission upon illumination of the darkened leaves),

isoprene emission rate at any given time t after darkening, $I(t)$, is driven by the C_{DMADP} pool size that has remained at this time, $C_{DMADP}(t)$ (the integral of isoprene emission rate from a given time t until cessation of isoprene emission). Thus, paired values of $I(t)$ vs. $C_{DMADP}(t)$ were derived for the entire dark decay and the linear parts of this relationships were used to estimate the rate constant of isoprene synthase (k, s^{-1}) as in our previous studies (Rasulov *et al.* 2010; Sun *et al.* 2012b; Rasulov *et al.* 2014).

In vivo V_{max} and K_m for isoprene synthase were estimated from the Hanes-Wolff plots ($C_{DMADP}(t)/I(t)$ vs. $C_{DMADP}(t)$) using the full dark decay of isoprene emission. The values of both characteristics were expressed per unit leaf area and dry mass.

Data analyses

Considering that fixation of 6 molecules of CO_2 is needed for the formation of one molecule of isoprene (e.g., Niinemets *et al.* 1999c), the fraction of carbon lost due to isoprene emission was estimated as $6I/A$, where I is the isoprene emission rate and A the net assimilation rate.

The temperature responses of net assimilation rate, photosynthetic electron transport rate, and isoprene emission characteristics (trait p_i) were fitted by an Arrhenius-type temperature response function with an optimum:

$$p_i = \frac{e^{c_i - \Delta H_{a,\,i}/RT}}{1 + e^{(\Delta S_i T - \Delta H_{d,\,i})/RT}}, \quad (3)$$

where c_i is the scaling constant for the trait i, $H_{a,i}$ (J mol^{-1}) is the activation energy, $H_{d,i}$ (J mol^{-1}) is the deactivation energy, S_i (J mol^{-1} K^{-1}) is the entropy term, T (K) is the leaf temperature, and R (8.314 J mol^{-1} K^{-1}) is the gas constant. The temperature optimum for the trait i, $T_{opt,i}$, (Niinemets *et al.* 1999a) was further calculated as:

$$T_{opt,\,i} = \frac{\Delta H_{d,\,i}}{\Delta S_i + R \ln\left(\frac{\Delta H_{d,\,i}}{\Delta H_{a,\,i}} - 1\right)}. \quad (4)$$

Dark respiration and stomatal conductance vs. temperature response curves that did not exhibit an optimum were fitted by exponential relationships in the form:

$$p_i = e^{c_i - \Delta H_{a,\,i}/RT}. \quad (5)$$

Note that the traditional estimate of temperature responses of chemical and biological processes, Q_{10}, the reaction rate at temperature $T + 10$ relative to the rate at temperature T is given as.

$$Q_{10, i} = e^{\frac{\Delta H_{a, i}[1/T - 1/(T+10)]}{R}}. \quad (6)$$

The Q_{10} concept assumes that the rate of the exponential increase (slope) of the process rate with increasing temperature is independent of temperature. Eq. 5 does not include such an assumption and is thus more flexible than the traditional Q_{10} analysis. Nevertheless, for rapid comparisons with other reported data, we also calculated the Q_{10} values. Treatment means for all traits ($n = 4$-32) were compared by analyses of variance (ANOVA) using Statistica 6.0 (StatSoft, Inc., Tulsa, OK, USA) and the growth temperature effects were considered significant at $P < 0.05$.

Results

Growth temperature effects on foliage structural characteristics

Increases in growth temperature were associated with about 1.2-fold larger and about 1.4-fold thinner leaves (Fig. 1, Table 1) with 1.6-fold lower leaf dry mass per unit area (M_A, Table 1). Decreases in leaf thickness primarily resulted from decreases in the thickness of palisade (1.6-fold) and to a smaller degree in spongy mesophyll thickness (1.3-fold, Fig. 1a, b, Table 1). The ratio of palisade to spongy mesophyll thickness also decreased from an average ($\pm$ SE) of 1.60 ± 0.08 to 1.22 ± 0.06 with increasing growth temperature ($P < 0.001$ for the treatment effect). However, leaf density, the ratio of M_A to leaf thickness was similar among the treatments (0.34 g cm^{-3} for the control and 0.30 g cm^{-3} for plants grown at the higher temperature).

Decreased leaf thickness was also associated with reduced number of chloroplasts per unit leaf area, and with reduced chloroplast size (Table 1). In addition, leaf pigment contents per unit leaf area also decreased. Changes in these traits were relatively smaller than in leaf thickness and M_A, and the number of chloroplasts per leaf dry mass was 1.2-fold greater in the high-temperature-grown plants (Table 1). Ultrastructural measurements also indicated that leaf chloroplasts of the high-temperature-grown plants had barely visible starch grains, while large starch grains were visible in the chloroplasts in the control treatment (Table 1, Fig. 1c, d).

Effects of growth temperature on foliage photosynthetic and respiratory activities

In analyzing the effects of growth temperature on foliage photosynthetic characteristics, we first focus on the effects at the standard measurement temperature of 30 °C, and then consider modifications in the temperature responses. At the standard temperature, acclimation to the higher growth temperature resulted in reductions in net assimilation rate per unit leaf area at the ambient CO_2 concentration (Fig. 2a), and this reduction was associated with decreased maximum carboxylase activity of Rubisco (V_{cmax}, Table 2), decreased photosynthetic electron transport rate estimated from chlorophyll fluorescence at ambient CO_2 concentration (J_{ETR}, Fig. 2c) and from the CO_2-saturated net assimilation rate (J_{max}, Table 2) per unit area. Analogously to the net assimilation rate, dark respiration rate per unit area (Fig. 2e, Table 2) and the quantum yield of photosynthesis for the incident light

(Table 2) were lower in the warmer treatment. The latter difference reflected lower chlorophyll content per leaf area (Table 1) and leaf absorptance (data not shown) in plants grown at the higher temperature. Conversely, stomatal conductance and intercellular CO_2 concentration were greater in plants grown at the higher temperature (Fig. 3, Table 2).

The growth temperature dependent changes in assimilation rates and foliage biochemical capacities were strongly driven by temperature effects on leaf structure. In fact, dark respiration rate, J_{max} and net assimilation rate per dry mass were not significantly different among the treatments (Table 2, Fig. 2), while the CO_2-limited photosynthetic electron transport rate per dry mass (electron transport rate when photosynthesis is Rubisco-limited, Fig. 2d) was greater at higher growth temperature. However, V_{cmax} per dry mass was still greater for the control treatment (Table 2), indicating that the similarity of net assimilation rate per dry mass among the treatments reflected a greater intercellular CO_2 concentrations due to higher stomatal conductance (Fig. 3).

Increased growth temperature shifted the optimum temperature of net assimilation to higher temperatures (Fig. 2a, b), and an analogous response was observed for the CO_2-limited photosynthetic electron transport rate (Fig. 2c, d). In addition, the activation energies, H_A (Eq. 3) were smaller in plants grown at the lower temperature ($P < 0.05$ for both comparisons). This, together with greater optimum temperatures resulted in greater mass-based net assimilation and electron transport rates at higher temperature (Fig. 2b, d). On the other hand, growth temperature did not affect the shapes of the dark respiration (Fig. 2e, f) and stomatal conductance (Fig. 3) vs. temperature responses ($P > 0.3$ for the treatment effects on H_A for both traits).

Influence of growth temperature on isoprene emission characteristics at the standard measurement temperature of 30 °C

As with the photosynthesis data, in this section, we first analyze the differences in growth treatments at the standard measurement temperature of 30 °C, and then focus on differences in the shape on temperature responses. On a leaf area basis, both the isoprene emission rate (I) and the pool size of the substrate for the isoprene synthase enzyme, dimethylallyl diphosphate (DMADP, C_{DMADP}), were greater in the control than in the high temperature treatment (Fig. 4a, c, Table 2). However, on a leaf dry mass basis, I and C_{DMADP} values were similar among the growth temperature treatments (Fig. 4b,d, Table 2). Furthermore, the rate constant for isoprene synthase (k) was not significantly different between the growth temperature treatments (Fig. 5, Table 2). The percentage of photosynthetic carbon used for isoprene emission was also not significantly different among the treatments at this measurement temperature (Fig. 6). Similarly to the quantum yield for photosynthesis, the quantum yield for isoprene emission for the incident light was greater in the control treatment (Table 2).

The apparent kinetic characteristics of isoprene synthase, the maximum activity at substrate-saturated conditions, V_{max} per unit area (Fig. 7a) and the Michaelis-Menten constant for the area-based C_{DMADP} (Fig. 7c) were both greater in the control treatment. However, similarly to I and C_{DMADP} (Fig. 4b, d), V_{max} per unit dry mass (Fig. 7b) and K_m for dry-mass based C_{DMADP} (Fig. 7d) were not different among the growth temperatures.

Modification of the temperature responses of isoprene emission characteristics by growth temperature

Growth temperature had minor effects on the optimum temperatures (T_{opt}) of isoprene emission characteristics (Figs. 4-5, 7) with only a slightly greater T_{opt} for apparent maximum isoprene synthase activity (V_{max}) in the control leaves (Fig. 7a). On the other hand, for the measurement temperature range of 25-35 °C, the average activation energy (H_A) for I was 77.9 ± 1.7 kJ mol^{-1} for the control treatment and 108 ± 5 kJ mol^{-1} for the high-temperature-grown plants ($P < 0.01$ for the differences among the treatments). These activation energies correspond to a Q_{10} of 2.77 ± 0.05 for the control and 4.14 ± 0.29 for the high-temperature treatment. The values of H_A for C_{DMAPD} for the same temperature range were 63 ± 7 kJ mol^{-1} ($Q_{10} = 2.31 \pm 0.22$) for the control and 76 ± 14 kJ mol^{-1} ($Q_{10} = 2.9 \pm 0.6$) for the high temperature treatment ($P > 0.4$ for the difference among the treatments). Thus, the effects of measurement temperature on and growth temperature sensitivity of I and k were larger than those for C_{DMADP}.

The differences among treatments in activation energies for k and I were ultimately associated with greater k and mass-based I values at higher temperatures (Fig. 4-5). As the result of much lower temperature sensitivity of photosynthesis than isoprene emission, the percentage of photosynthetic carbon lost due to isoprene emission increased with increasing temperature, and this increase was greater for plants grown under the lower temperature (Fig. 6).

Analogously, the temperature sensitivity was greater for the isoprene synthase apparent V_{max} than for the apparent K_m (Fig. 7). The Q_{10} values for V_{max} were 2.65 ± 0.08 for the control and 3.51 ± 0.15 for the high temperature treatment ($P < 0.005$ for difference among treatments), while the corresponding Q_{10} values for K_m were 2.16 ± 0.26 for the control and 2.18 ± 0.46 for the high temperature treatment ($P > 0.9$ for the treatment effect).

Discussion

Modification of leaf structural characteristics and pigment content by growth temperature

Our study demonstrates that acclimation to high growth temperature led to profound changes in anatomy, morphology and chemistry of hybrid aspen leaves (Fig. 1, Table 1). As an integral change, high-temperature-grown leaves were thinner with lower leaf dry mass per unit area (M_A). In addition, high growth temperature led to less densely packed mesophyll, with fewer mesophyll cells and fewer chloroplasts per unit leaf area (Fig. 1, Table 1). Despite lower number of chloroplasts per unit leaf area, the chloroplasts were smaller, and chloroplast number per unit leaf dry mass was greater. These results are in a broad agreement in past studies demonstrating significant modifications in leaf anatomy and chloroplast characteristics (Jin *et al.* 2011), and decreased leaf thickness (Körner *et al.* 1989; Hartikainen *et al.* 2009; Muhl *et al.* 2011) in plants grown in warmer temperatures.

On the other hand, leaf expansion growth was enhanced, resulting in larger leaves (Table 1) and overall greater whole canopy leaf area and mass (data not shown). Such an enhancement of leaf expansion growth is a characteristic response to high growth temperatures in well-watered plants (Wardlaw & Bagnall 1981; Hewitt *et al.* 1985; Granier *et al.* 2000) and might

reflect both enhanced light interception and enhanced sink activity, i.e. enhanced consumption of photoassimilates, at high temperatures. In fact, chloroplasts in high-temperature-grown leaves were essentially depleted of starch (Table 1, Fig. 1c, d) in agreement with observations in other studies (Buttrose & Hale 1971; Gandin *et al.* 2011). In addition to a reduction of starch content due to enhanced sugar export from chloroplasts, there is also evidence that high temperatures might inhibit gluconeogenesis and increase sucrose synthesis (Ito *et al.* 2009; Yamakawa & Hakata 2010). Soluble sugars importantly enhance the thermotolerance of photosynthetic apparatus (Hüve *et al.* 2006; Hüve *et al.* 2012; Sun *et al.* 2013), and thus, a possible elevation of soluble sugar content can be an important mechanism improving the heat resistance of photosynthesis.

Structural control of temperature acclimation of photosynthetic and respiratory characteristics

Our results demonstrate strong reductions in area-based foliage pigment content, biochemical potentials, and net assimilation and dark respiration rates in high temperature grown plants (Tables 1-2, Fig. 2). These results are analogous with previous studies demonstrating enhanced photosynthetic rates and greater photosynthetic electron transport and Rubisco carboxylase activity (Huner 1985; Mawson & Cummins 1989; Yamasaki *et al.* 2002; Yamori *et al.* 2005) and number and size of mitochondria and respiratory activity (Jin *et al.* 2011) per unit leaf area in plants grown at lower temperatures. However, as our study further demonstrates, the mass-based photosynthetic characteristics differed much less among the treatments, indicating that the photosynthetic potentials of single leaf cells were not strongly affected by changes in growth temperature (Table 2, Fig. 2). An increase of M_A with increasing growth light availability is well-known (Niinemets & Sack 2006; Niinemets 2007; Niinemets & Anten 2009; Poorter *et al.* 2009), and this increase has been associated with increases in protein content and photosynthetic rate per area (Niinemets & Sack 2006; Niinemets 2007). Our results further underscore the important role of structural controls in temperature acclimation of photosynthesis.

In addition to the rates of photosynthesis, the quantum yield of photosynthesis for an incident light also decreased with increasing irradiance (Table 2) and this reduction was associated with reduced chlorophyll content per area (Table 1) and reduced leaf absorptance as has also been reported in several other studies (Hikosaka *et al.* 1999; Yamori *et al.* 2005). As chlorophyll content per dry mass did not vary with growth temperature (Table 1), modifications in leaf structure were also responsible for the reduction of quantum yields for the incident light in the high-temperature-grown plants (Table 2). Nevertheless, changes in chlorophyll content typically play a much smaller role in whole plant light interception than alterations in total leaf area (Niinemets 2007).

Differently from most of the photosynthetic characteristics, stomatal conductance was strongly enhanced upon growth at high temperatures, leading to enhanced intercellular CO_2 concentration (Fig. 3). In fact, as the maximum carboxylase activity of Rubisco per dry mass was somewhat decreased, enhanced intercellular CO_2 concentration was responsible for similar mass-based net assimilation rates in different temperature treatments. Enhanced stomatal conductance at higher growth temperature can be a structural response associated

with greater stomatal size and/or density as reported in some studies (Ghosh *et al.* 1996; Pandey *et al.* 2007) although not always (Crawford *et al.* 2012). Maintenance of high stomatal conductance at higher temperatures clearly plays an important role in convective cooling of leaves (Crawford *et al.* 2012), and also importantly compensates for the reduced biochemical capacity of photosynthetic machinery by increasing the internal CO_2 concentration.

The maximum electron transport rate per dry mass estimated from the net assimilation rates at high CO_2 concentration did not differ among temperature treatments. However, in calculation of the capacity for photosynthetic electron transport rate at high CO_2 concentration, we did not consider the possible limitation of photosynthesis by triose phosphates that can lead to underestimation of the true electron transport capacity (Sharkey 1985). Given the overall strong coordination of Rubisco activity and electron transport capacity, the difference in growth temperature effects on Rubisco carboxylase and electron transport capacities might reflect a stronger feedback inhibition of photosynthesis at higher CO_2 in plants grown at lower temperature (e.g., Pammenter *et al.* 1993 for a discussion). Such a possible feedback limitation of maximum electron transport rate is also supported by reduced use of assimilates in plant growth as indicated by accumulation of starch in lower temperatures.

Effects of growth temperature on temperature responses of photosynthesis and respiration rates

In addition to structural modifications, growth at the higher temperature resulted in significantly improved heat resistance of net assimilation rates as evident in greater activation energies and optimum temperatures (T_{opt}) of net assimilation rates and CO_2-limited electron transport rates (Fig. 2a-d). Such an improved heat resistance is in line with past observations as discussed in the Introduction and is typically associated with increased lipid saturation in thylakoid membranes (Pearcy 1978; Raison 1986; Kunst *et al.* 1989; Mawson & Cummins 1989; Shanklin & Cahoon 1998), and can also reflect increased leaf sugar contents as discussed above. In addition, increases in T_{opt} of photosynthesis can partly reflect increased stomatal conductance that reduces the rate of photorespiration at any given temperature and thereby shifts the optimum temperature to higher temperatures (Leegood 1995; Medlyn *et al.* 2002).

Past studies have indicated that the activation energy for the dark respiration often decreases upon growth at higher temperatures (Atkin & Tjoelker 2003; Atkin *et al.* 2005). However, there was no evidence of changes in the shape of dark respiration vs. temperature response curve in our study (Fig. 2e, f) suggesting either that the mitochondrial capacity for respiration was unaffected by high growth temperature or that changes in the capacity were obscured by alterations in respiratory substrate availability. The latter possibility is plausible given the altered sink/source relations in plants grown at different temperatures.

Isoprene emission characteristics in relation to growth temperature

As with the area-based photosynthetic characteristics, isoprene emission rate, DMADP pool size (Table 2, Fig. 4) and apparent maximum rate and K_m for isoprene synthase at 30 °C

(Table 2, Fig. 7) were greater in low-temperature-grown plants. However, none of these characteristics was affected by growth temperature when expressed on a leaf dry mass basis (Fig. 4, 7 and Table 2), indicating that differences in area-based isoprene emission characteristics were primarily driven by leaf structural modifications. Furthermore, the rate constant of isoprene synthase (Fig. 5) and the fraction of carbon lost due to isoprene emission (Fig. 6) also did not depend on growth temperature at the measurement temperature of 30 °C, further indicating that isoprene emission scaled to DMADP pool size and net assimilation rate similarly in both temperature treatments. Contrasting growth temperature effects on the area-based isoprene emission rates have been observed across the studies (see Introduction), and we argue that these contrasting effects primarily result from study-to-study differences in leaf morphological response to growth temperature. We also emphasize that the effects of longer-term temperature on isoprene emission characteristics can be different for transfer experiments where leaves developed at a given temperature are transferred to another temperature. In these experiments, any acclimation to changed conditions is primarily biochemical and physiological (Geron *et al.* 2000; Mayrhofer *et al.* 2005; Wiberley *et al.* 2008; Sun *et al.* 2012a).

Temperature response of isoprene emission characteristics

As demonstrated previously (Rasulov *et al.* 2010; Li *et al.* 2011), the optimum temperature for isoprene emission (Fig. 4a, b) is co-determined by the temperature effects on the DMADP pool size (Fig. 4c, d) and on the isoprene synthase rate constant (Fig. 5). Thus, the key issue is how the share of different controls is affected by acclimation to different growth temperatures. Our data do demonstrate that the DMADP pool size starts to decrease earlier with increasing temperature (Fig. 4c, d) than the isoprene synthase rate constant or V_{max} of isoprene synthase (Fig. 7a, b). Furthermore, our data demonstrate that K_m of isoprene synthase increases with increasing temperature, indicating that progressively higher DMADP pools are needed to reach a given value of isoprene emission rate (Fig. 7c, d). Such a temperature-dependent increase of K_m is characteristic for many enzymes and indicates weaker associations in the enzyme-substrate complex at higher temperature (Hajdu *et al.* 2009).

Despite the similarity of mass-based isoprene emission characteristics at 30 °C (Table 2), and similarly high optimum temperatures for isoprene emission between 40-41 °C (Fig. 4a, b), a number of important differences were observed among the growth temperature treatments in responses of isoprene emission characteristics to higher temperatures (Figs. 4-7). In low-temperature-grown plants, the activation energies were almost identical to those observed in hybrid aspen grown in similar conditions (Rasulov *et al.* 2010). However, in the current study, the activation energy for isoprene emission was greater in leaves grown at greater temperature, and this was associated with greater mass-based emission rates at higher temperatures (Fig. 4b). These effects resulted from a greater activation energy for isoprene synthase rate constant (Fig. 5), while the activation energy for DMADP pool size did not differ among the treatments, suggesting that the rate of substrate production was not affected by growth temperature. This seems surprising given the improved heat tolerance of photosynthetic apparatus in high-temperature-grown plants (Fig. 2). In fact, plants grown at the lower temperature used a greater fraction of photosynthetic carbon for isoprene emission

at higher temperatures (Fig. 6). It is plausible that in high-temperature-grown plants with a greater growth rate and reduced sink limitation of photosynthesis, alternative pathways for DMADP consumption for higher molecular mass isoprenoids (Rasulov *et al.* 2014 for a discussion) were also more strongly activated such that the availability for DMADP for isoprene synthesis was ultimately similar for both treatments.

As isoprene synthase activity is affected by pH and cellular ionic medium characteristics (Sasaki *et al.* 2005; Köksal *et al.* 2010) possible growth temperature effects on subcellular environment might also play a role in the observed patterns. Thus, differences in the isoprene synthase rate constant (Fig. 5) and V_{max} (Fig. 7a, b) might reflect changes in chloroplastic conditions in plants grown at different temperatures. Such changes are likely given that heat stress gradually leads to leakiness of chloroplastic membranes and reduced proton gradient (Schrader *et al.* 2004; Zhang & Sharkey 2009) bringing pH in actively photosynthesizing chloroplasts (ca. 7.8-8) closer to the cytosolic values (6-6.5). Given that the pH optimum for isoprene is ca. 8, such a reduction of pH would importantly affect the rate of isoprene synthesis, especially in low-temperature-grown plants with lower heat resistance of membranes.

Apart from subcellular effects, *Populus* has multiple isoprene synthase genes (Vickers *et al.* 2010), and expression of a more heat-resistance isoprene synthase might provide a complementary explanation for improved temperature kinetics. However, so far there is no evidence of differences in heat resistance of different isoprene synthase isoforms as well as on the regulation of expression of different isoforms by growth temperature. Finally, smaller chloroplasts (Table 1) with almost missing starch grains might also mean reduced diffusive limitations between the sites of DMADP synthesis and isoprene synthase. Overall, these results are consistent with improved heat stability of isoprene synthesis in high temperature acclimated leaves, but do not support the suggestion of a greater requirement of isoprene synthesis to cope with sustained high temperatures.

Conclusions

Our study demonstrates that increases in growth temperature resulted in major changes in leaf anatomy and morphology and that these structural modifications mainly drove the treatment effects on foliage photosynthetic and isoprene emission characteristics expressed per unit leaf area. While structural acclimation to growth light regime is well-recognized (Niinemets & Anten 2009; Poorter *et al.* 2009), temperature acclimation studies do not routinely address growth temperature effects on leaf structure. As our study demonstrates, consideration of structural effects clearly provides insight into the mechanisms of temperature-driven photosynthetic acclimation. In fact, structural changes might explain the study-to-study differences in growth temperature effects on foliage area-based physiological characteristics that have been difficult to explain so far.

Higher growth temperature also enhanced the heat stability of photosynthetic characteristics, but had surprisingly minor effects on the heat stability of isoprene emission traits. As noted in the Introduction, there are important study-to-study differences in the effects of growth temperature on the shape of the temperature response of isoprene emission (Monson *et al.*

1992; Fares *et al.* 2011). We argue that such contrasting effects most likely result from differences in how growth temperature affects the isoprene precursor DMADP pool size. In our study where a higher growth temperature was associated with enhanced sink activity, growth temperature effects on DMADP pool size were minor. However, much stronger pathway upregulation may occur when primary metabolism is curbed by limited sink activity (Harding *et al.* 1990; Starck *et al.* 1993). Although our study demonstrated that growth temperature might also affect the isoprene synthase characteristics, we argue that the overall pathway flux can be potentially more strongly affected by DMADP pool size and isoprene synthase expression (Rasulov *et al.* 2009b; Rasulov *et al.* 2010; Li *et al.* 2011; Li & Sharkey 2013). We conclude that analyses of temperature acclimation of photosynthesis and isoprene emission should consider both structural and physiological adjustments and that more experimental studies are needed to gain insight into the effects of sink strength on expression of isoprenoid synthesis pathway.

Acknowledgements

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Summary statement

High growth temperature reduced area-based photosynthetic and isoprene emission characteristics in hybrid aspen, and these effects were primarily driven by structural modifications. Acclimation to high temperature also enhanced stomatal conductance, improved heat resistance of photosynthetic apparatus and increased the activation energy of isoprene emission resulting in a greater fraction of photosynthetic carbon emitted as isoprene at higher temperatures. These results emphasize the important roles of both leaf structural and physiological changes in high-temperature acclimation of photosynthesis and isoprene emission.
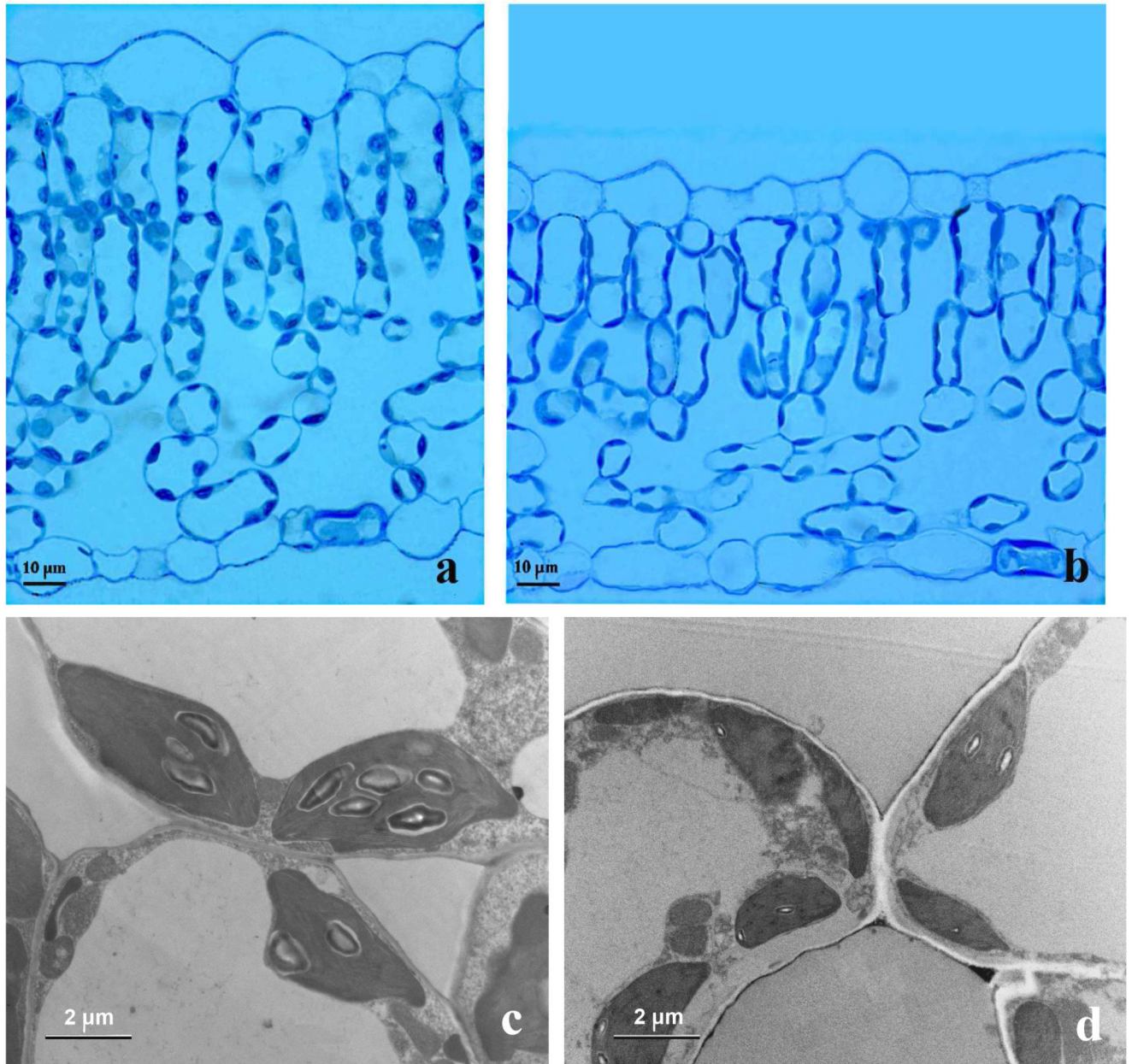
Fig. 1.
Representative cross-sections of hybrid aspen (*Populus tremula* X *P. tremuloides*, clone H200) leaves (a, b) and chloroplasts (c, d) developed under control (day/night temperatures of 25/20 °C) and high temperature (35/27 °C) conditions.
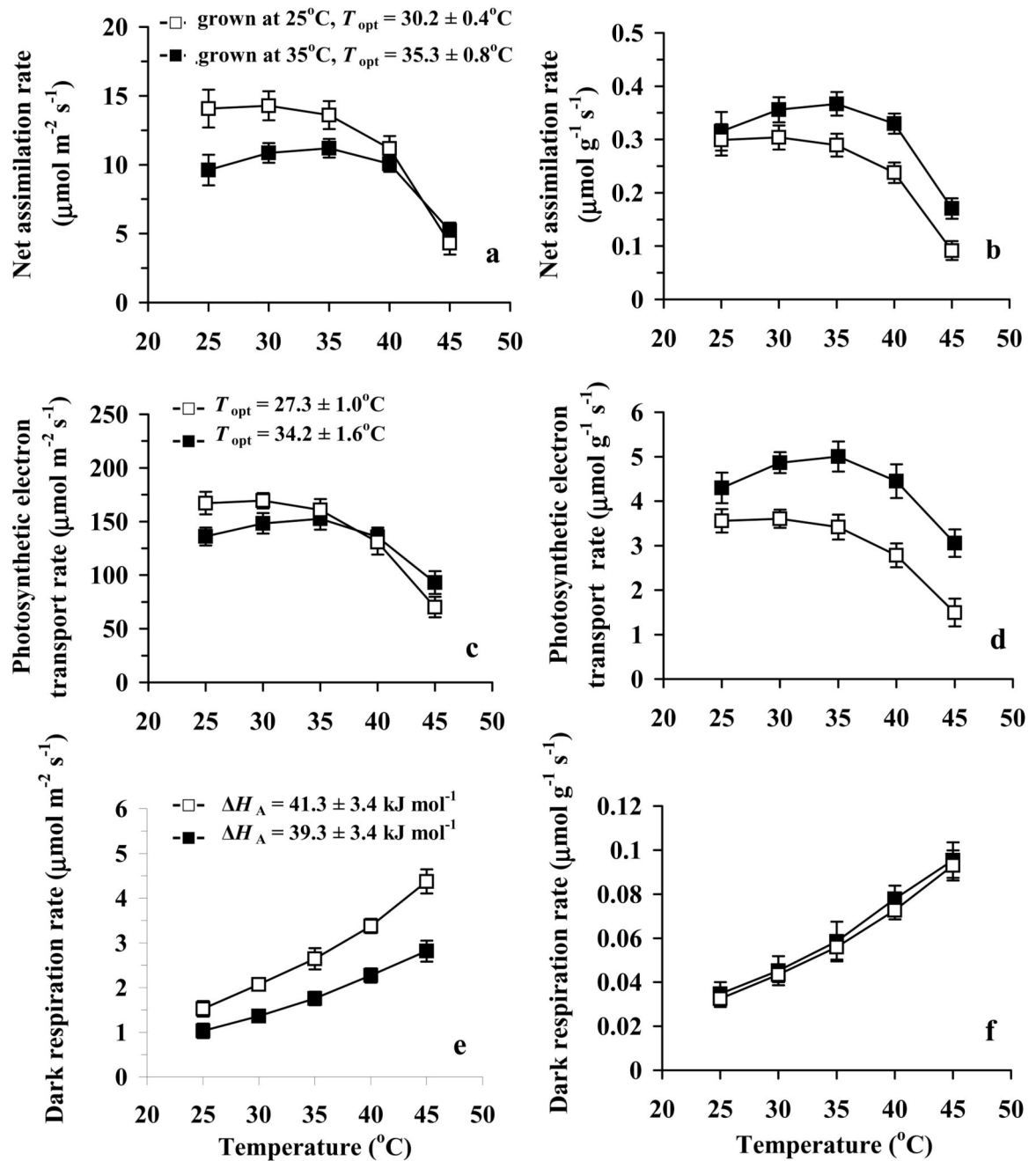
Fig. 2.
Temperature dependencies of light-saturated net assimilation rate (a, b), photosynthetic electron transport rate estimated from chlorophyll fluorescence (Eq. 1, c, d) and dark respiration rate (e, f) per unit leaf area (a, c, e) and per unit dry mass (b, d, f) in hybrid aspen grown at day/night temperatures of 25/20 °C (control) and 35/27 °C (high temperature treatment). Data were fitted by Eq. 3 (a-d) and Eq. 5 (e, f), and the values of the estimated optimum temperature (T_{opt}, Eq. 4) are indicated for (a) and (c) and the values of the activation energy H_a (Eq. 5) for (e). The optimum temperatures in (a) and (c) are

significantly different among the treatments ($P < 0.005$ for (a) and $P < 0.02$ for (c)). The measurements in a-d were conducted at an ambient CO_2 concentration of 360 μmol mol^{-1} and at an incident quantum flux density of 650 μmol m^{-2} s^{-1}. Data are means ± SE ($n = 4$).
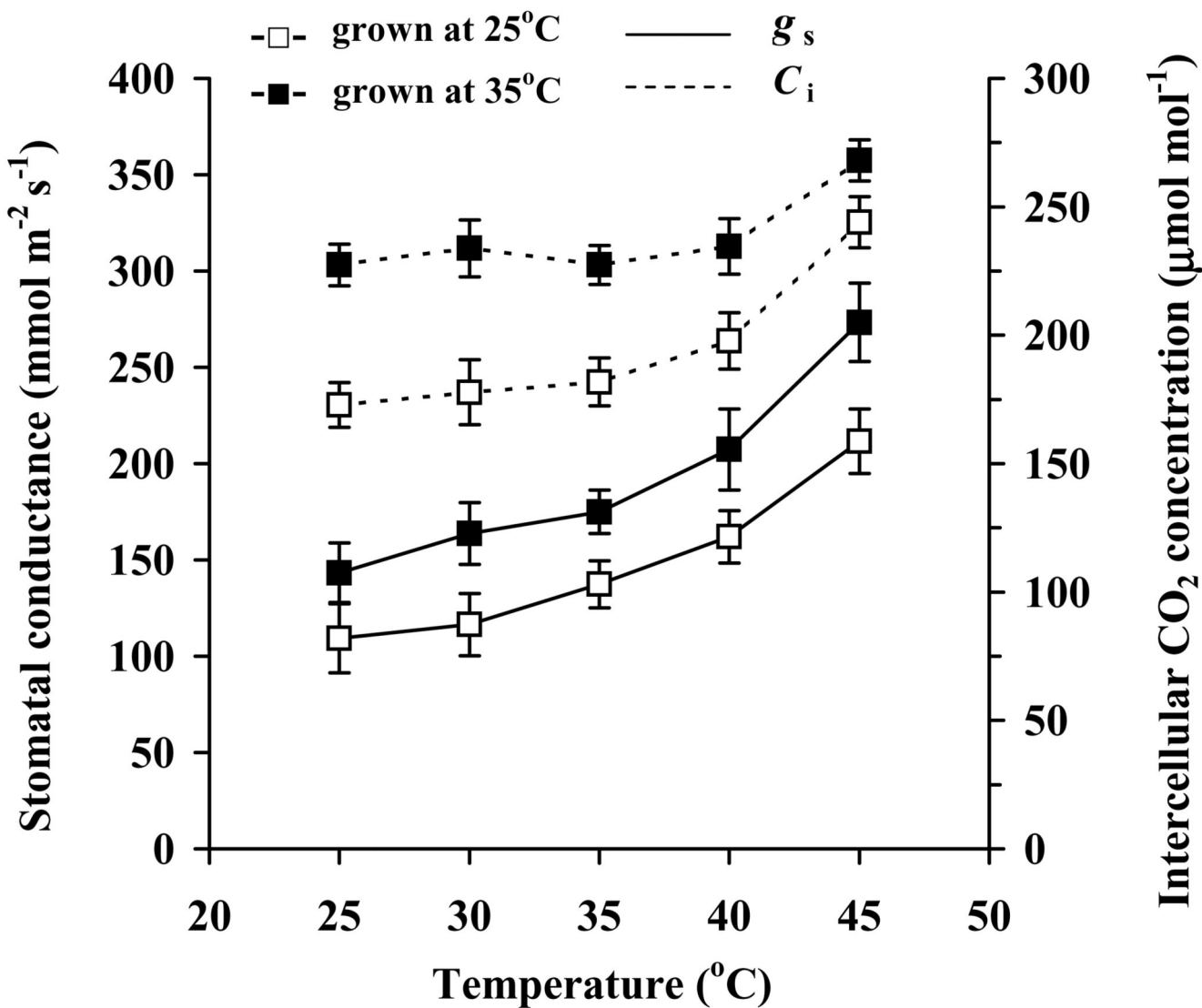
Fig. 3.
Temperature responses of stomatal conductance to water vapor and intercellular CO_2 concentration in hybrid aspen grown at the contrasting temperature regimes, control (day/night temperature 25/20 °C) and high temperature (35/27 °C). Measurement conditions, data presentation and replicates as in Fig. 2.
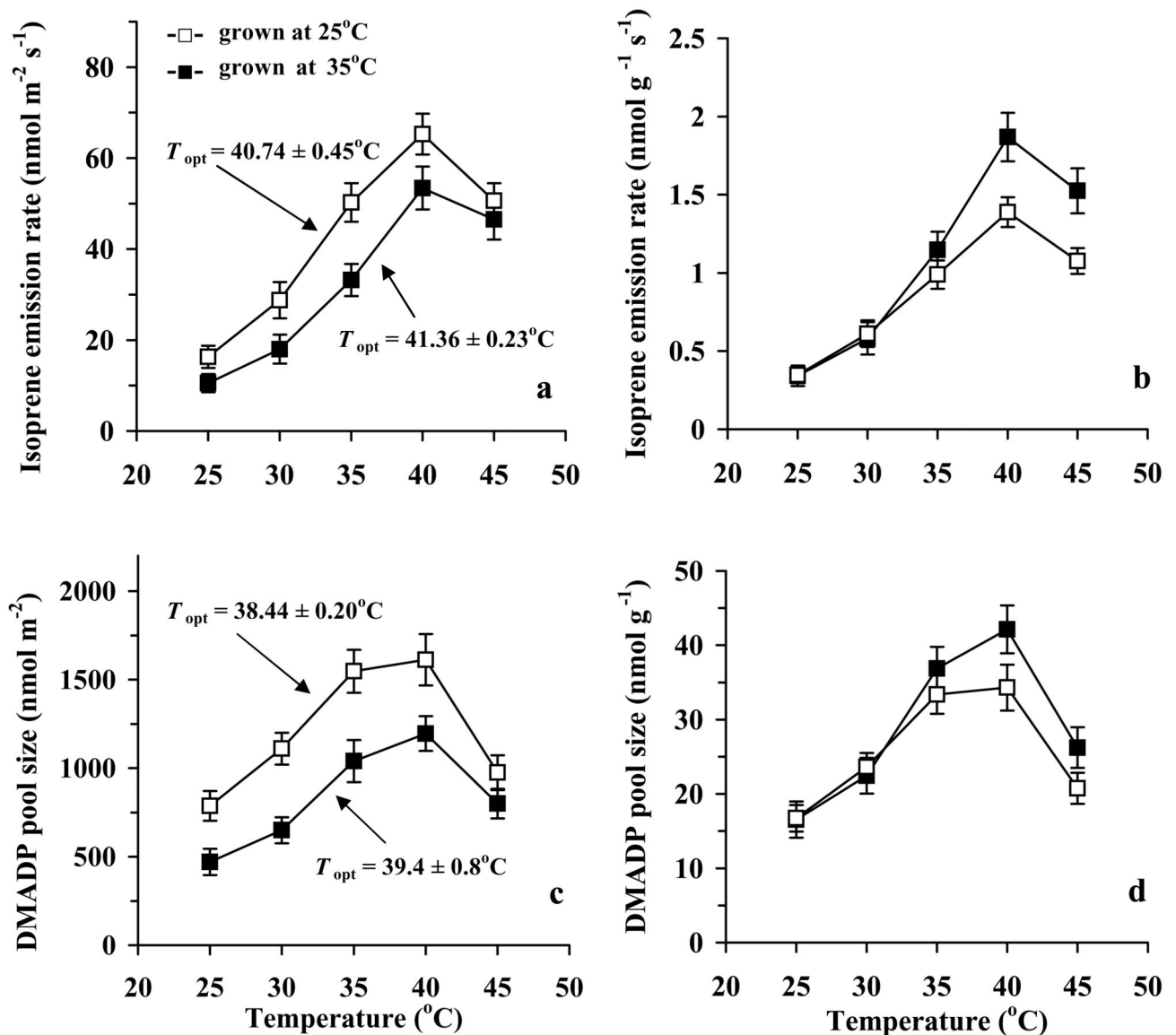
Fig. 4.
Temperature dependencies of isoprene emission rate (a, b) and the isoprene synthase substrate dimethylallyl diphosphate (DMADP) pool size (c, d) per unit leaf area (a, c) and per unit leaf dry mass (b, d) in hybrid aspen in dependence on growth temperature conditions, control (day/night temperature 25/20 °C) vs. high temperature (35/27 °C). Measurement conditions and data presentation as in Fig. 2. The optimum temperatures for isoprene emission and DMADP pool size were not significantly different among the treatments ($P > 0.1$ for both).
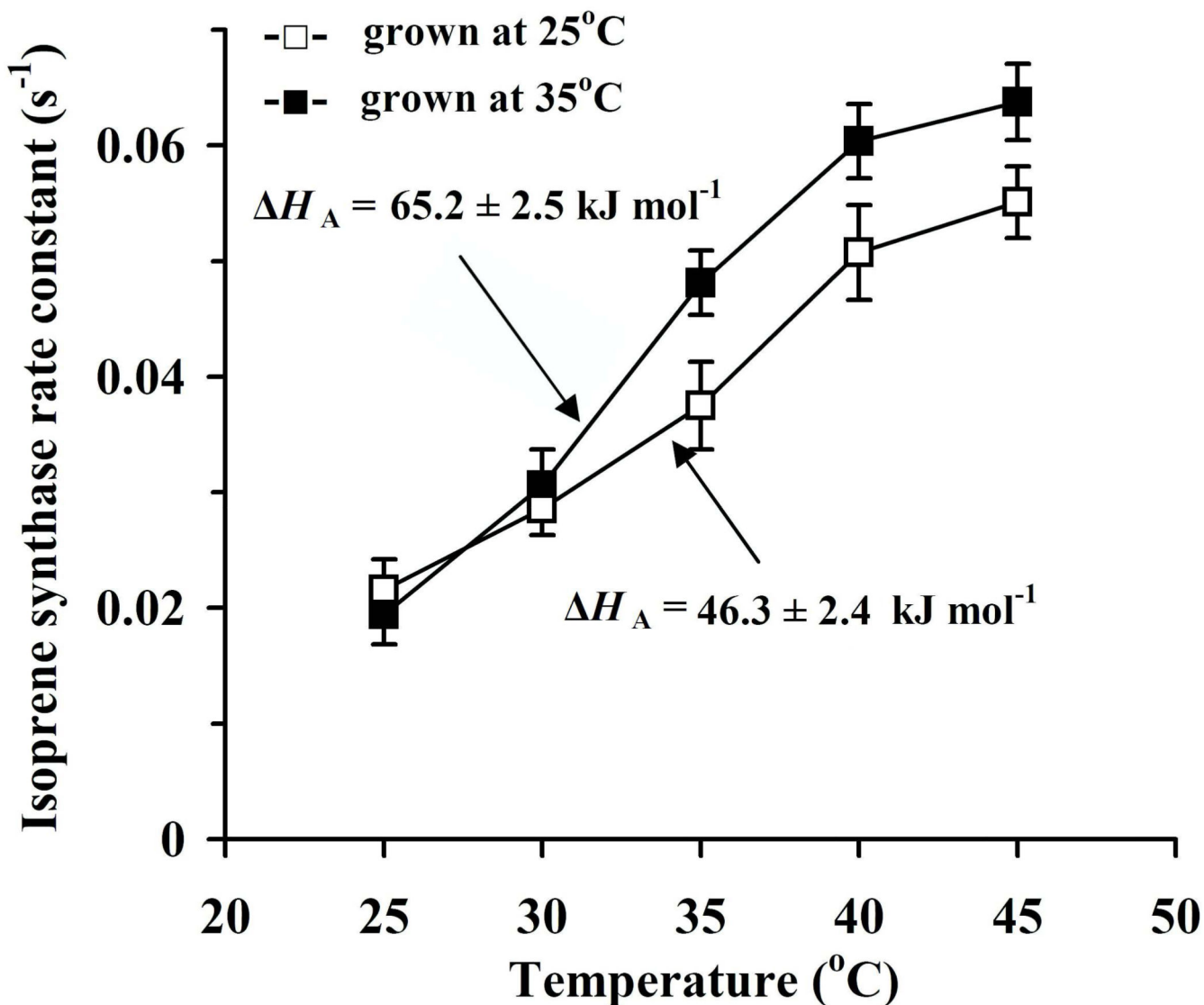
Fig. 5.
Temperature dependence of the rate constant of isoprene synthase (k) in hybrid aspen grown under control (day/night temperature 25/20 °C) and high temperature (35/27 °C) conditions. The first order reaction rate constant was estimated as the slope of the linear part of dimethylallyl diphosphate pool size vs. isoprene emission rate relationship through the dark decay of isoprene emission and corresponds to the environmental conditions (ambient CO_2 concentration of 360 μmol mol^{-1}, O_2 concentration of 21% and incident quantum flux density of 650 μmol m^{-2} s^{-1}) prior to leaf darkening. The number of replicates and data presentation as in Fig. 2. The activation energies for k (H_a, kJ mol^{-1}, Eq. 3) are also demonstrated ($P < 0.001$ for the difference among the treatments).
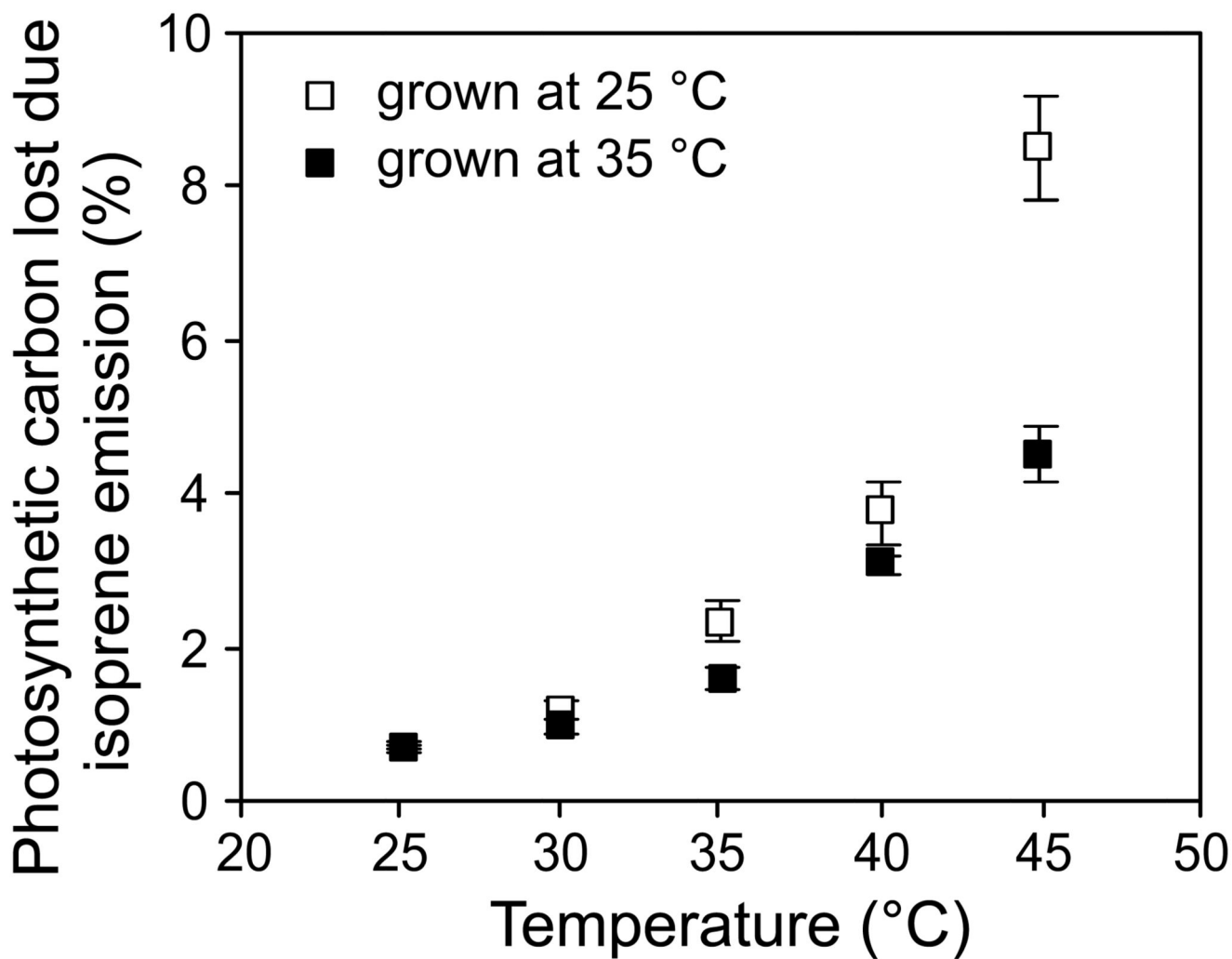
Fig. 6.
Temperature-dependent changes in the percentage of photosynthetic carbon lost due to isoprene emission in hybrid aspen leaves under control (day/night temperature 25/20 °C) and high temperature (35/27 °C) treatments.
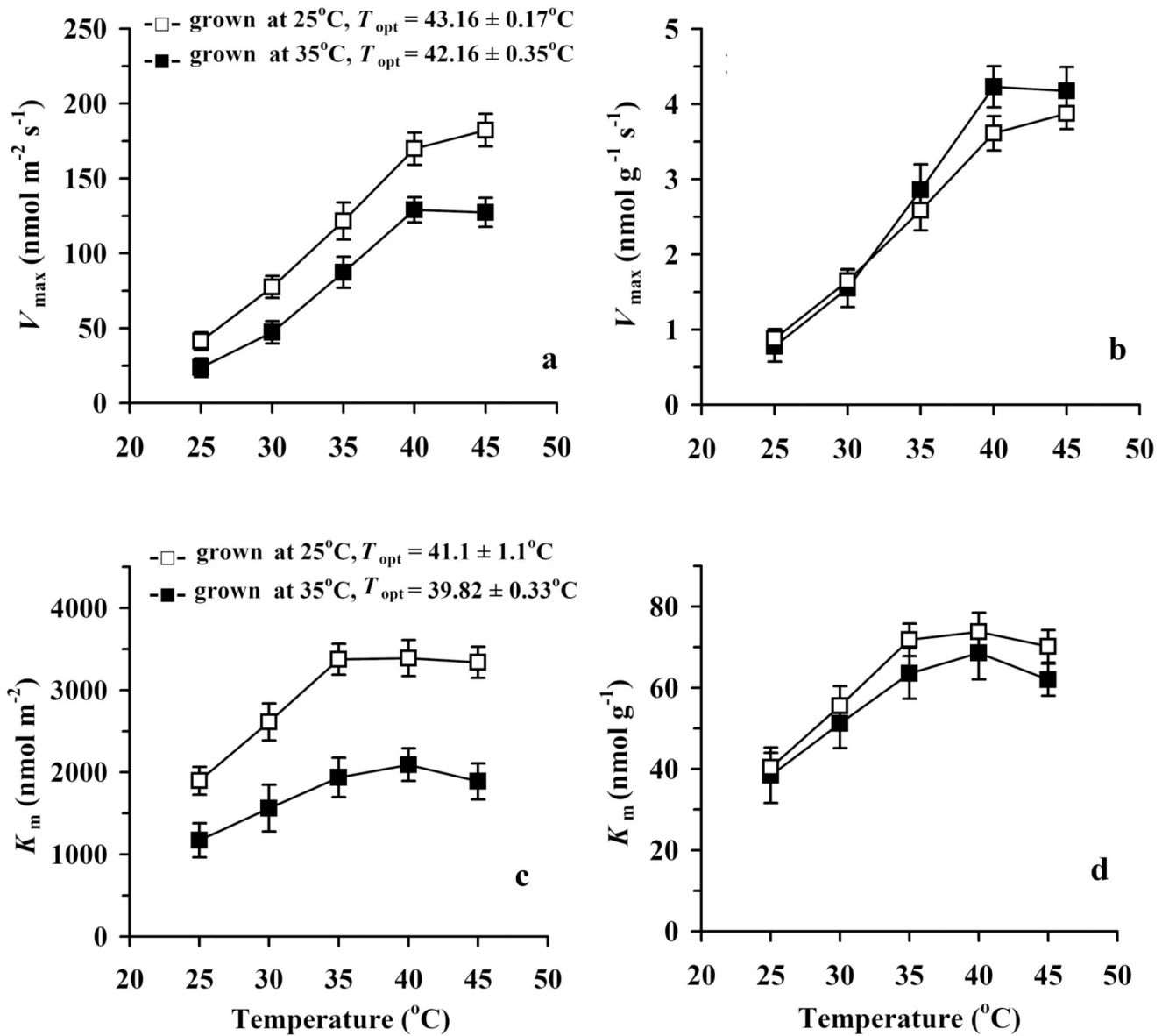
Fig. 7.

Temperature responses of the apparent maximum capacity, V_{max}, (a, b) and Michaelis-Menten constant K_m (c, d) of isoprene synthase in hybrid aspen grown under control (day/night temperature 25/20 °C) and high temperature (35/27 °C) treatments. Data fitting and presentation as in Fig. 2. The optimum temperatures for V_{max} were significantly different among the treatments ($P < 0.05$), while the optimum temperatures for K_m were not ($P > 0.3$).

Table 1

Leaf structural characteristics and pigment contents (mean ± SE) in hybrid aspen (*P. tremula* x *P. tremuloides*, clone H200) grown at contrasting temperatures

Characteristic	Growth temperature (day/night)	
	25/20 ºC	**35/27 ºC**
Leaf area (cm^2)	67.5 ± 2.4a	82.3 ± 2.7b
Leaf dry mass per unit area (g m^{-2})	47.6 ± 1.7a	30.5 ± 1.1b
Leaf thickness (μm)	133.9 ± 3.3a	93.5 ± 2.8b
Palisade mesophyll thickness (μm)	71.5 ± 3.1a	40.9 ± 2.6b
Spongy mesophyll thickness (μm)	44.3 ± 1.0a	34.4 ± 0.8b
Chloroplast number per cm^2 leaf area (palisade)	$(38.8 ± 2.2).10^6$a	$(29.3 ± 1.9).10^6$a
Chloroplast number per cm^2 leaf area (spongy)	$(7.5 ± 0.5).10^6$a	$(7.1 ± 0.3).10^6$a
Total chloroplast number per cm^2 leaf area (palisade+spongy)	$(46.3 ± 3.7).10^6$a	$(36.4 ± 2.3).10^6$a
Chloroplast length (μm)	4.41 ± 0.17a	4.14 ± 0.18a
Chloroplast width (μm)	1.99 ± 0.09a	1.52 ± 0.11b
Chloroplast area (μm^2)	7.23 ± 0.49a	4.99 ± 0.51b
Starch grain area per chloroplast (μm^2)	1.05 ± 0.11a	0.047 ± 0.11b
Chlorophyll content per area (mg m^{-2})	413 ± 16a	302 ± 12b
Chlorophyll content per dry mass (mg g^{-1})	9.11 ± 0.27a	9.20 ± 0.29a
Chl a/b ratio	2.68 ± 0.11b	2.38 ± 0.08b
Carotenoid content (mg m^{-2})	88.4 ± 2.9a	58.9 ± 2.2b
Carotenoid content per dry mass (mg g^{-1})	1.80 ± 0.05a	1.90 ± 0.05a

Means with the same letter are not statistically different ($P > 0.05$ according to ANOVA, $n = 6$, for leaf structural measurements and pigment contents and $n = 30-42$ for leaf anatomical measurements)

Table 2

Effects of growth temperature on average (± SE) foliage photosynthetic and isoprene emission characteristics in hybrid aspen (*P. tremula* x *P. tremuloides*, clone H200)

Trait	Growth temperature (day/night)	
	25/20 °C	**35/27 °C**
Photosynthetic characteristics		
A_n per area (μmol m^{-2} s^{-1})	14.73 ± 0.40a	11.18 ± 0.35b
A_n per dry mass (μmol g^{-1} s^{-1})	0.310 ± 0.009a	0.346 ± 0.018a
g_s (mmol m^{-2} s^{-1})	122 ± 7a	164 ± 8b
C_i (μmol mol^{-1})	182.3 ± 4.6a	247 ± 11b
R_d per area (μmol m^{-2} s^{-1})	2.07 ± 0.06b	1.36 ± 0.07b
R_d per dry mass (μmol g^{-1} s^{-1})	0.0436 ± 0.0016a	0.0421 ± 0.0023a
α_{CO2} (mol mol^{-1})	0.0441 ± 0.0026a	0.0338 ± 0.0025b
V_{cmax} per area (μmol m^{-2} s^{-1})	62.1 ± 1.6a	32.7 ± 1.4b
V_{cmax} per dry mass (μmol g^{-1} s^{-1})	1.309 ± 0.038a	1.010 ± 0.043b
J_{max} per area (μmol m^{-2} s^{-1})	120 ± 6a	80 ± 6b
J_{max} per dry mass (μmol g^{-1} s^{-1})	2.04 ± 0.06a	2.19 ± 0.15a
Isoprene emission characteristics		
I per area (nmol m^{-2} s^{-1})	28.8 ± 2.8a	17.8 ± 1.0b
I per dry mass (nmol g^{-1} s^{-1})	0.60 ± 0.05a	0.547 ± 0.027a
C_{DMADP} per area (nmol m^{-2} s^{-1})	1110 ± 90a	650 ± 60b
C_{DMADP} per dry mass (nmol g^{-1} s^{-1})	23.5 ± 2.3a	20.0 ± 1.8a
k (s^{-1})	0.0286 ± 0.0021a	0.0306 ± 0.0023a
V_{max} per area (nmol m^{-2} s^{-1})	79.5 ± 2.9a	48.8 ± 3.0b
V_{max} per dry mass (nmol g^{-1} s^{-1})	1.673 ± 0.037a	1.51 ± 0.11a
K_m per area (nmol m^{-2})	2640 ± 180a	1542 ± 30b
K_m per dry mass (nmol g^{-1})	55.6 ± 4.9a	47.7 ± 1.7a
α_I (mmol mol^{-1})	0.0743 ± 0.0010a	0.0700 ± 0.0010b

The photosynthetic characteristics are: A_n - net assimilation rate; g_s - stomatal conductance to water vapor; C_i - intercellular CO_2 concentration; R_d - dark respiration rate; α_{CO2} - quantum yield of photosynthesis for the incident light; V_{cmax} - apparent (C_i-based) maximum carboxylase activity of Rubisco; J_{max} - apparent (C_i-based) capacity for photosynthetic electron transport. The isoprene emission characteristics are: I - isoprene emission rate; C_{DMADP} - dimethylallyl diphosphate (DMADP) pool size; k - rate constant of isoprene synthase; V_{max} - apparent maximum activity of isoprene synthase; K_m - apparent Michaelis-Menten constant for isoprene synthase; α_I - quantum yield of isoprene emission for the incident light.

All measurements were conducted at 30 °C, and saturating light intensity (Q) of 650 μmol m^{-2} s^{-1} was used for all measurements except for the quantum yields. The quantum yields were calculated as the linear slopes of the process rate vs. incident Q over the Q range of 20-75 μmol m^{-2} s^{-1} and at the ambient [CO_2] of 360 μmol mol^{-1} and oxygen concentration of 21%. The value of V_{cmax} is reported as an average of V_{cmax} estimates determined from measurements at 21% and 2% O_2. Use of measurements at different O_2 concentrations renders the apparent V_{cmax} estimates less sensitive to Rubisco kinetic characteristics employed in the calculations.

Each value represents the mean ± SE of four measurements in different plants, and means with different lowercase letters are statistically different at $P < 0.05$ according to ANOVA.