Photosynthetic Activity of Six Hop (*Humulus lupulus* L.) Cultivars under Different Temperature Treatments

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Abstract. The Pacific Northwest grows the majority of hops in the United States; however, the region is experiencing an increase in the number of days with high heat. In addition, there is an increased interest in growing hops in other warmer regions of the United States. To understand how hop plants respond to high temperatures, we measured several physiological traits of six hop cultivars under a range of temperatures from 15 to 45 °C. We found that hop plants achieved maximal carbon assimilation at temperatures of 21 to 39 °C when given sufficient water. At temperatures of 41 °C and higher, all cultivars experienced declines in carbon assimilation. This was likely due to multiple effects on the cell, including damage to photosystem II (PSII), as reflected in declines in F_v/F_m , damage to membrane integrity as reflected in electrolyte leakage at high temperatures, and declines in Rubisco activity likely due to degradation of Rubisco activase, as reflected in declines in $V_{c,max}$. ‘Cascade’, ‘Willamette’, and ‘Southern Brewer’ may be good candidates for growing in warm climates because all experience relatively high rates of carbon assimilation at high temperatures and did not experience significant declines in F_v/F_m or increases in electrolyte leakage. ‘Chinook’ appeared susceptible to extreme heat stress and exhibited evidence of irreparable damage to PSII and membrane integrity at 45 °C.

The majority of hops in the United States are grown in the Yakima Valley of the Pacific Northwest (PNW), a region that is increasingly experiencing extreme high temperatures. Climate models predict an increase in the frequency of heat waves in Washington state

in upcoming decades compared with 1970–99, particularly in the south-central hop-growing regions (Salathé et al., 2010). Regional brewers have expressed increasing interest in local sources of hops in other regions of the country as well, including regions that historically have not been considered ideal for hop production because they lack low temperatures for sufficiently long winter dormancy (Bauerle, 2019; Neve, 1991). As the PNW prepares for increasing heat waves, and as production expands in warmer regions of the country, there is a need to understand the response to heat in common cultivars grown in the United States and to describe differences in heat tolerance among cultivars to identify lines for breeding new cultivars with increased abiotic stress tolerance.

Heat principally limits the photosynthetic activity of plants (Berry and Bjorkman, 1980). Allakhverdiev et al. (2008) identified three components of the photosynthetic system that are sensitive to heat damage: the photosystems themselves, particularly photosystem II (PSII); the ATP-generating electron transport chain; and the carbon assimilation process. Heat affects the photosystems by causing the dissociation of manganese (Mn) molecules

from the oxygen-evolving complex (OEC) in PSII (Enami et al., 1994, 1998; Nash et al., 1985), by disrupting the distribution of absorbed light energy from the light-harvesting complex to the core of PSII (Pastenes and Horton, 1996), and by disrupting the integrity of the D1 protein (Yoshioka et al., 2006). Heat also disrupts membrane fluidity, leading to the breakdown of the thylakoid membrane integrity, which leads to disruptions in the electron transport chain and ATP synthesis (Gounaris et al., 1983; Inaba and Crandall, 1988). Finally, heat disrupts the Rubisco activase protein (Salvucci and Crafts-Brandner, 2004; Sharkey, 2005), leading to inactivation of the carboxylating enzyme and, ultimately, to cessation of carbon assimilation.

We measured several photosynthetic and physiological traits of six hop cultivars in response to a range of temperatures. ‘Cascade’, ‘Centennial’, and ‘Chinook’ are some of the most commonly grown varieties in the PNW and other regions. ‘Willamette’ is a triploid variety that our preliminary work has suggested has higher water use efficiency. ‘Southern Brewer’ is a high alpha variety developed in South Africa for shorter summer days that has been reported to have higher heat tolerance. ‘Pride of Ringwood’ is a high alpha variety developed in Australia that also may have higher heat tolerance. These findings will provide valuable information for growers developing hop yards in the increasingly warm PNW or in other warmer regions of the United States and for breeding programs aiming to develop cultivars more resilient to high temperatures.

Materials and Methods

Plant material and experimental conditions.

In Feb. 2019, rhizomes were removed from a single plant from the cultivars Cascade, Centennial, Chinook, Pride of Ringwood, Southern Brewer, and Willamette growing at the USDA-ARS Hop Research fields in Corvallis, OR. The plants were clonally propagated by dividing the rhizomes into six parts and planted in 1-gallon pots with potting soil (Sun Gro Horticulture, Agawam, MA) containing Sphagnum peatmoss, perlite, proprietary nutrient charge, screened pumice, gypsum, dolomite limestone, and proprietary wetting agent; they were allowed to break dormancy in a greenhouse. The plants were maintained there for 4 months and constantly cut back to encourage root growth. Trial 1 plants were moved to Conviron PGR14 growth chambers after 4 months of root growth; trial 2 plants were moved to the growth chambers after 5 months of root growth. Each trial consisted of three plants of each cultivar. Plants were watered regularly and fertilized biweekly with a 24N–8P–16K Miracle-Gro fertilizer (Scotts, Marysville, OH) according to the manufacturer’s instructions.

Once moved to the growth chambers, the plants were grown under a photoperiod of 14 h of daylight at 24 °C and 10 h of night at 12 °C to approximate early growth season conditions in the PNW. Irradiance was maximized at 875 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. We began by measuring physiological traits at 24 °C; temperatures were

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then decreased by 3° every 24 h (21, 18, and 15 °C). The plants were allowed to recover for >24 h at 24 °C; then, temperatures were increased every 24 h (27, 30, 33, 36, 39, 41, and 45 °C).

Gas exchange and fluorescence measurements.

Gas exchange and chlorophyll fluorescence measurements were performed using a LI-6400 Portable Photosynthesis System with a 6400-40 LCF sensor head (LI-COR Biosciences, Lincoln, NE). A single measurement was performed on a mature leaf as well as on a younger leaf at approximately the second or third node below the youngest, fully unfurled leaf because young and mature senescing tissues have different sensitivities to heat stress (Karim et al., 1999; Marias et al., 2017).

Dark-adapted F_0 and F_v/F_m measurements were performed after 10 h of dark adaptation and 3 h of exposure to the experimental temperature. We optimized the measuring intensity according to the manufacturer's instructions (using the LI-6400/LI-6400XT version 6 manual) at 0.4 and used a flash duration of 0.8; all other settings complied with the manufacturer's suggested settings for determining F_v/F_m .

Gas exchange and light-adapted fluorescence measurements were then performed after ≥ 2 h of light adaptation and after >4 h at the experimental temperature. Gas exchange measurements were performed under saturating photosynthetically active radiation (PAR) of $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under atmospheric CO_2 concentrations (400 ppm) controlled by a mixer and flow rates of $400 \mu\text{mol}\cdot\text{s}^{-1}$. The block temperature was set to the experimental temperature. The humidity of the reference air was adjusted to ambient levels to the best of our ability to avoid VPD-caused stomatal closure. Measurements were recorded when carbon assimilation and stomatal conductance (g_s) reached stability, which occurred after ≈ 2 min. Light-adapted fluorescence measurements were performed on the same leaf immediately following gas exchange measurements. We optimized the flash intensity at 8 according to the manufacturer's instructions (using the LI-6400/LI-6400XT version 6 manual); all other LCF settings complied with the manufacturer's suggested settings for determining PSII efficiency (Φ_{PSII}).

Following survey measurements, a rapid A/C_i curve was performed at each temperature on one plant of the cultivars Cascade, Centennial, Chinook, and Southern Brewer during trial 1, and for one plant of the cultivars Cascade, Centennial, Chinook, and Willamette during trial 2. A/C_i curves could only be created for four plants per day due to time constraints; preliminary measurements indicated carbon assimilation began to decrease in hop plants due to natural circadian patterns ≈ 9 h after daybreak. The default settings under the auto-program ACi2 were used, with CO_2 concentrations of 400, 300, 200, 100, 50, 10, 0, 400, 400, 500, 600, 800, 1000, and $1200 \mu\text{mol}\cdot\text{CO}_2$. Measurements were set to record after standard stability settings, with a minimum wait time of 2 min and a maximum wait time of 4 min.

Electrolyte leakage and pigment concentrations.

In a third trial, we measured the percent

electrolyte leakage and pigment concentrations of plants that were grown from softwood cuttings in 10-cm square pots and provided ample water from a bottom tray to mitigate the effects of pot size on soil moisture. At temperatures of 33, 36, 39, 41, and 45 °C, six 1-cm discs of tissue from six different leaves were excised, capturing both young and mature leaf tissues. The discs were incubated in 20 mL of de-ionized water in 50-mL tubes at room temperature overnight; the conductivity of the solution was measured after 20 h using a temperature-compensated EC meter (model 1056; Amber Science, Inc., Eugene, OR). The tubes were then steam-sterilized in an autoclave for 15 min at 110 °C to disintegrate the cell materials. After cooling, the total conductivity was measured again, and the electrolyte leakage values were calculated as a percentage of maximum conductivity.

To assess pigment concentrations, two 1-cm discs were excised from a dark green mature leaf and a lighter green younger leaf below the youngest fully unfurled leaf at temperatures of 33, 36, 39, 41, and 45 °C. These discs were placed in a 1.5-mL microcentrifuge tube on ice, kept in a dark box, and soaked in 1 mL of methanol overnight at 4 °C. The methanol was removed and placed in a 10-mL glass test tube and diluted to 5 mL methanol to adjust the concentration to the appropriate range for the spectrophotometer. We read absorbance on a Spectronic 20 (Milton Roy Company, Ivyland, PA) at ≈ 470 , 653, and 666 nm. The concentrations for chlorophyll *a* and *b* and carotenoids were calculated according to Lichtenthaler (1987), with updates for spectrophotometers with a resolution range of 1 to 4 nm (Wellburn, 1994).

Data analysis. All analyses were performed using R version 3.4.3. For photosynthetic data, outliers were identified using boxplots and removed from the dataset based on the assumption that they could represent a calibration error introduced by the LI-6400. To assess the statistical significance of differences, we performed one-way analyses of variance to test a model of differences within traits among cultivars at each temperature using transformed or untransformed data as appropriate; these tests were followed by pairwise Tukey's honestly significant difference tests, and significance was evaluated against a Bonferroni-adjusted alpha value of 0.003 for 15 pairwise comparisons. We used the R package plant ecophys (Duursma, 2015) to fit A/C_i curves and calculate parameters using the default nonlinear regression fit method. Temperatures reported are air temperatures because of known biases in leaf temperature measurements (Still et al., 2019).

Results

For most hop cultivars tested, carbon assimilation (A) was highest between 21 and 39 °C, but some continued to maintain relatively high levels of A even at 41 °C (Fig. 1A). Most differences in A among cultivars were not significant at a Bonferroni-adjusted alpha level at any temperature tested here. 'Cascade' maintained high levels of A between 18 and 39 °C; only the extreme

low (15 °C) and extreme high (45 °C) levels tested during this experiment caused reductions in A in this cultivar. 'Centennial' achieved slightly higher A than 'Cascade' at lower temperatures. 'Willamette' achieved the highest level of A at the high temperatures of 39 to 41 °C. Carbon assimilation in 'Chinook' was significantly lower at 41 °C than in 'Willamette' ($P < 0.001$). Most cultivars had low rates of g_s at 15 to 18 °C despite adequate soil moisture; at 15 °C, g_s decreased to levels that introduce stomatal limitations to A (Medrano et al., 2002). Rates of g_s reached the maximal level at 27 °C and then declined with increasing temperatures, although 'Cascade' had significantly higher g_s rates at 36 °C [$F_{(5,29)} = 6.65$; $P < 0.001$] and warmer compared with the other cultivars tested (Fig. 1B). Leaf transpiration (E) was positively correlated with temperature and tended to be higher in 'Cascade' at higher temperatures; E in 'Cascade' was significantly higher than it was in 'Pride of Ringwood' at 36 to 39 °C ($P < 0.003$) (Fig. 1C). Intercellular CO_2 concentrations (C_i) remained steady between ≈ 250 and $300 \mu\text{mol}/\text{air}$ at most temperatures, but they were highly variable within cultivars at 15 °C. 'Cascade' and 'Chinook' had higher C_i at the extreme high temperature of 45 °C [$F_{(5,27)} = 3.90$; $P = 0.008$] (Fig. 1D).

Traits that reflect the health of the light-harvesting system (PSII efficiency or Φ_{PSII} and ETR) for the different cultivars displayed trends similar to those observed in A . However, these traits reached peak rates at temperatures between 27 and 31 °C (Fig. 1E and G), which was slightly warmer than the temperatures at which we observed peak rates of A . 'Willamette' had slightly higher Φ_{PSII} and ETR at 41 °C [$F_{(5,28)} = 3.65$; $P = 0.01$]. Nonphotochemical quenching (NPQ), as a measure of stress to the photosynthetic apparatus, was highly variable at 15 °C; then, it remained steady between 24 and 39 °C and increased sharply at 41 and 45 °C (Fig. 1H). F_0 is the initial dark-adapted chlorophyll fluorescence level with Q_A fully oxidized and "open." This measure decreased from 15 to 27 °C; then, it continued to increase from 33 to 41 °C. In 'Chinook', it increased significantly at 45 °C (Fig. 2A). The measure of photoinhibition F_v/F_m remained at nonstress levels (≈ 0.8) for temperatures 15 to 33 °C. At 36 °C, 'Chinook' and 'Southern Brewer' began to show early signs of damage. However, 'Chinook' was the only cultivar to experience significantly lower F_v/F_m ratios at 41 °C [$F_{(5,30)} = 8.96$; $P < 0.001$] and 45 °C [$F_{(5,30)} = 4.09$; $P = 0.006$] (Fig. 2B).

The maximum carboxylating efficiency of Rubisco ($V_{c,\text{max}}$) was positively correlated with temperature until 39 °C ($R^2 = 0.83$; $P < 0.001$); at temperatures warmer than 39 °C, $V_{c,\text{max}}$ decreased in all cultivars (Fig. 3). $V_{c,\text{max}}$ was similar among cultivars at temperatures of 15 to 30 °C. Although more replication is necessary, the $V_{c,\text{max}}$ appeared to increase more overall in 'Southern Brewer' compared with the other cultivars at temperatures >30 °C. 'Chinook' displayed the lowest $V_{c,\text{max}}$ value at 45 °C.

The percentage of electrolytes leaked from cell tissue was relatively steady for all

Fig. 1. Photosynthetic traits measured at 1000 $\mu\text{mol photons/m}^2/\text{s}$ for each cultivar. Error bars represent the SEM value for six plants. (A) Carbon assimilation (A). (B) Stomatal conductance (g_s). (C) Leaf transpiration (E). (D) Interacellular carbon concentration (C_i). (E) Photosystem II efficiency (Φ_{PSII}). (F) Apparent quantum yield (Φ_{CO_2}). (G) Electron transfer rate (ETR). (H) Nonphotochemical quenching (NPQ).

the cultivars at temperatures between 33 and 39 °C, but they increased sharply in ‘Pride of Ringwood’, ‘Chinook’, and ‘Centennial’ at 45 °C [$F_{(5,18)} = 19.61$; $P < 0.001$]. However, ‘Cascade’, ‘Southern Brewer’, and ‘Willamette’ displayed only slight increases at 45 °C (Fig. 4). It should be noted that all cultivars showed visible damage to leaf tissue in the form of leaf curling and cell death at leaf edges at this temperature.

Changes to pigment ratios can indicate damage to the light-harvesting complexes. There were no significant changes in the ratio of chlorophyll *a* to chlorophyll *b* at the temperatures tested (Fig. 5A), and the concentration of carotenoids within cultivars also remained relatively steady over the temperatures tested here (Fig. 5B). Only ‘Willamette’ and ‘Pride of Ringwood’ had significantly higher antioxidant carotenoid

concentrations at 41 °C [$F_{(5,42)} = 11.94$; $P < 0.001$], and these concentrations decreased at 45 °C.

Discussion

The major hop-growing regions of the United States are experiencing an increase in the number of days with extreme high temperatures. At the same time, there is increased

Fig. 2. Dark-adapted chlorophyll fluorescence measurements for each cultivar. Error bars represent the SEM value for six plants. (A) F_0 . (B) F_v/F_m .

Fig. 3. The maximum carboxylating efficiency ($V_{c,max}$) of Rubisco for each cultivar. Error bars, when present, represent the SEM.

be better suited for growth in warmer regions of the United States, and to identify cultivars for use in breeding programs to increase resilience to abiotic stress.

Carbon assimilation and Φ_{CO_2} reached maximum rates between 21 and 39 °C, whereas Φ_{PSII} and ETR in these hop cultivars all reached maximum rates at slightly warmer temperatures between 27 and 39 °C (Fig. 1). This range is slightly warmer than the range considered optimum for most C₃ photosynthetic plants at 20 to 35 °C (Sage et al., 2008); however, the shape of the temperature response curve was typical of most C₃ plants.

Indicators of stress on the photosynthetic system, NPQ (Fig. 1H), and F_v/F_m (Fig. 2B) also indicated that this range of temperatures from 24 to 39 °C was optimum for growth, although there were some early indications of photoinhibition in 'Chinook' and 'Southern Brewer' at 39 °C (Fig. 2B). F_0 increases sharply under heat treatments, and this is attributed to the irreversible dissociation of the light-harvesting complex, the reversible inactivation of PSII, and the reduction of QA by plastoquinone from the PSII reaction center (Yamane et al., 1997, 1998, 2000); in these experiments, F_0 increased sharply in 'Chinook' at 45 °C.

Broadly speaking, this range of 21 to 39 °C appeared to be the range of optimal growth for the hop cultivars tested in this study, and the range of 27 to 39 °C produced maximal values for both Φ_{PSII} and Φ_{CO_2} . There was little statistically significant variation among cultivars; however, 'Pride of Ringwood' appeared to not reach maximum levels of A until 30 °C and 'Willamette' remained near maximal levels at 41 °C when other cultivars appeared to suffer reductions in A due to heat (Fig. 1A).

interest among growers in expanding production to different parts of the country. A more pressing issue for growth in other regions of the United States will be an appropriate photoperiod for cone production (Bauerle, 2019; Neve, 1991); however, high temperatures during the growing season are also a valid concern. Most plants appear to have an optimum temperature for photosynthesis and can acclimate to different temperature ranges (Berry and Bjorkman, 1980). However, some

plants are better adapted to higher temperatures than others and are better able to acclimate to higher photosynthesis and higher productivity. We tested six hop cultivars to observe differences in their photosynthetic responses to increasing high temperatures. Our goals were as follows: 1) to identify the range of temperatures at which commonly grown hop cultivars photosynthesize, 2) to describe differences among cultivars in their tolerance to heat to identify cultivars that might

Fig. 4. Percent electrolyte leakage at different temperatures. Error bars represent the SEM value for four plants.

'Pride of Ringwood' generally did not thrive under these experimental conditions, and plants remained small throughout the experiment. A different photoperiod may produce different results in 'Pride of Ringwood', but the photoperiod tested was chosen to represent early-season conditions in the PNW when rapid bine growth occurs.

'Cascade' had high levels of A over a range of temperatures 24 to 39 °C (Fig. 1A); therefore, it appears to be a strong candidate for growing in the increasingly warm PNW, for growing in other warm areas of the country, and for breeding programs for increased abiotic stress tolerance. 'Willamette' had higher A, Φ_{PSII} , Φ_{CO_2} , and ETR than 'Cascade' at 41 °C (Fig. 1). In regions where temperatures are likely to reach >40 °C, 'Willamette' may also have strong growth potential if the photoperiod is appropriate for cone production. 'Chinook' appears to be a poor candidate for warmer climates because it had some of the lowest A rates at temperatures of 39 to 45 °C (Fig. 1A) and exhibited signs of irreparable damage to the photosystems and cell membranes at the extreme high temperature of 45 °C, as seen by the rapid increase in F_o (Fig. 2A) and electrolyte leakage (Fig. 3) and the rapid decrease in F_v/F_m (Fig. 2B).

The positive correlation of E and temperature (Fig. 1C) suggested that high A was, in part, a result of the cooling effect of evapotranspiration; therefore, a reliable water source to supply that evapotranspiration at high temperatures is necessary. This study did not examine the often-concomitant stresses of heat and drought. However, almost all hop production fields in the PNW are irrigated, and drought is more easily alleviated than high temperatures. g_s reached peak rates at 27 °C; then, it decreased in most cultivars except 'Cascade', which remained high throughout

the experiment. The observed decrease in g_s at temperatures >27 °C was not low enough to introduce stomatal limitations to A (Medrano et al., 2002). Therefore, decreases in A were not due to stomatal closure resulting from vapor pressure differences (VPD) between the reference airflow and humidity. Relative humidity was poorly controlled in the confines of the growth chambers and tended to increase with increased temperatures, which is not typical of field conditions. Decreases in the relative humidity and VPD caused stomatal closure during mid-day heat and have been shown to be a significant limiting factor for A in the field (Pons and Welschen, 2003). These factors would likely introduce stomatal limitations to A in field-grown hops during heat waves that would be in addition to the limitations to A produced by the heat alone.

The traits we measured also allowed us to track the health status of the three major sites of cellular damage as a result of high heat exposure. We were able to compare the integrity of PSII by measuring F_o and F_v/F_m , the integrity of the membranes with electrolyte leakage, and the efficiency of carbon assimilation using $V_{c,max}$.

The OEC of PSII is generally considered a component of the photosynthetic system most sensitive to heat stress (Allakhverdiev et al., 2008; Berry and Bjorkman, 1980). High temperature stress is known to cause the loss of Mn from the OEC (Enami et al., 1994, 1998; Nash et al., 1985). Several extrinsic proteins and cofactors have been associated with stabilizing the Mn cluster, and it is the loss of these proteins and cofactors under heat stress that leads to the loss of Mn, subsequent inactivation of PSII, and degradation of the D1 protein (Yoshioka et al., 2006). Destabilization of PSII results in sharp

increases in F_o and significant decreases in F_v/F_m . Under these experimental conditions, PSII in hops appeared relatively robust in temperatures of 15 to 41 °C. There was early evidence of a decrease in F_v/F_m in 'Southern Brewer' and 'Chinook' at 36 °C, and all cultivars began to experience some decreases in F_v/F_m at 45 °C. However, these experimental conditions only produced a sharp rise in F_o and significant decreases in F_v/F_m , suggesting severe and irreparable damage to PSII in one cultivar at 45 °C. Damage to PSII due to heat stress produces reactive oxygen species in the plant, leading to increases in lipophilic antioxidants such as carotenoids (Das and Roychoudhury, 2014; Suzuki and Mittler, 2006), which have been shown to stabilize the thylakoid membrane by decreasing membrane fluidity (Havaux, 1993). Under temperatures 33 to 45 °C, an increase in carotenoids was only seen in 'Pride of Ringwood' and 'Willamette' at 41 °C. The levels of antioxidant carotenoids remained relatively steady among samples of the same cultivar but were lowest in 'Chinook' samples (Fig. 5). The lack of increases in carotenoids within cultivars could be because carotenoid concentrations were already high at 33 °C when samples were first obtained. Increases in carotenoid concentrations in 'Willamette' may partially explain this cultivar's resilience to heat stress; however, similar increases in the less resilient 'Pride of Ringwood' suggest that carotenoids are not the primary factor influencing heat resilience.

Heat stress causes structural changes in the thylakoid membranes, which are reflected in ion leakage (Inaba and Crandall, 1988; Wahid and Shabbir, 2005; Yang et al., 1996). Evidence of breakdown of the thylakoid membranes indicated by a rapid increase in electrolyte leakage was seen in 'Chinook', 'Centennial', and 'Pride of Ringwood' (Fig. 4). 'Cascade', 'Southern Brewer', and 'Willamette' appear to maintain membrane integrity even at 45 °C; 'Willamette' appears to possibly recover membrane integrity and experiences a reduction in electrolyte leakage at 45 °C compared with 41 °C, which should be investigated further. In *Chlamydomonas*, saturation of membrane lipids acts to stabilize the membranes and PSII under heat stress (Sato et al., 1996). As a triploid cultivar, Willamette may have higher expression of proteins that adjust the lipid composition to increase membrane resiliency; however, from these data, it is not possible to determine the exact mechanism of increased resilience or whether the increased resilience is common among triploids.

The carboxylating enzyme Rubisco exhibits affinities for both CO₂ and O₂, despite the waste of resources involved in fixing O₂ (i.e., photorespiration). Under increasing temperatures, the solubility of CO₂ decreases, thus decreasing CO₂ movement through the aqueous portions of the plant cell, thereby decreasing the availability of CO₂ at the sites of carboxylation. At the same time, the affinity of Rubisco for CO₂ decreases at high temperatures, causing an increase in O₂ fixation and photorespiration (Slattery and Ort, 2019). In addition, the activation state of Rubisco

Fig. 5. The ratio of chlorophyll *a* to chlorophyll *b* at different temperatures (A) and the concentration of total carotenoids at different temperatures (B). Error bars represent the SEM value for four plants.

decrease above a species-specific optimum temperature (Sage et al., 2008). In hops, this transition to decreased activation of Rubisco appears to occur at temperatures >39 °C, at which $V_{c,max}$ appears to decrease. Although the replication was low, the $V_{c,max}$ in 'Southern Brewer' remained high at 41 °C. Rubisco itself is known to be stable at temperatures up to 50 °C (Crafts-Brandner and Salvucci, 2000); therefore, the decline in Rubisco activity at high temperatures seen in all plants is attributed to declines in Rubisco activase activity (Hikosaka et al., 2006; Salvucci and Crafts-Brandner, 2004; Sharkey, 2005; Yamori et al., 2014), which can no longer efficiently remove metabolites from the catalytic sites of Rubisco above certain temperatures and eventually denatures (Feller et al., 1998; Salvucci et al., 2001). Sage et al. (2008) showed that Rubisco activase activity was limiting at temperatures >30 °C in the boreal species black spruce (*Picea mariana*), and that adaptation to high-temperature environments across the range of red maple (*Acer rubrum*) is due to an increased ratio of Rubisco activase to Rubisco (Weston et al., 2007). Differences in $V_{c,max}$ among cultivars at high temperatures may represent different ratios of Rubisco activase to Rubisco or different tolerances of Rubisco activase to heat stress that should be investigated further.

Conclusions

Hops achieved maximal carbon assimilation at temperatures of 21 to 39 °C when given sufficient water. 'Cascade' and 'Willamette' experienced relatively high rates of A at high temperatures, whereas 'Southern Brewer'

appeared to have higher carboxylating efficiency at high temperatures. 'Cascade', 'Willamette', and 'Southern Brewer' did not experience sharp increase in F_o , significant decrease in F_v/F_m , or increases in electrolyte leakage. These cultivars may be good candidates for growth in warm climates. 'Cascade' and 'Southern Brewer' may be good candidates for use as breeding lines to improve abiotic stress tolerance. 'Chinook' appears particularly susceptible to extreme heat stress, exhibiting evidence of irreparable damage to PSII and membrane integrity at the highest temperatures tested in this study.

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