Thermostability of Photosynthesis of *Vitis aestivalis* and *V. vinifera*

Sorkel Kadir¹

Department of Horticulture, Forestry, and Recreation Resources, Kansas State University, Manhattan, KS 66506

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ABSTRACT. High temperature adversely affects photosynthetic rates and thylakoid activities in many species, but photo synthesis response to heat stress is not well defined in grapes (Vitis L.). Genotypes within species respond differently to high temperatures, indicating a genetic variability for the trait. The objective of this study was to determine the physiological responses of two grape species to high temperature, at the whole-plant level and at the cellular level. Gas exchange, relative chlorophyll content, and chlorophyll fluorescence of intact leaves and thermostability of extracted thylakoids of the American (V. aestivalis Michx.) 'Cynthiana' and European (V. vinifera L.) 'Semillon', 'Pinot Noir', 'Chardonnay', and 'Cabernet Sauvignon' wine grapes were evaluated. One-year-old vines were placed in controlled environmental chamber held at 20/15, 30/25, or 40/35 °C day/night for 4 weeks. Net CO₂ assimilation (A) rate, stomatal conductance (g_s), transpiration (E) rate, chlorophyll content, and chlorophyll fluorescence of intact leaves were measured at weekly intervals. Chlorophyll fluorescence of thylakoids extracted from V. aestivalis 'Cynthiana' and V. vinifera 'Pinot Noir' subjected to temperatures ranging from 20 to 50 °C was measured. Optimal temperatures for photosynthesis were 20/15 °C for 'Cynthiana' and 'Semillon' and 30/25 °C for the other three V. vinifera cultivars. The A, g., E, chlorophyll content, and chlorophyll fluorescence values at 40/35 °C were lower in 'Cynthiana' than 'Pinot Noir'. In general, reduction of A coincided with decline in g, in 'Cynthiana', whereas no strong relationship between A and g, was observed in V. vinifera cultivars. Variable chlorophyll fluorescence (Fv) and the quantum efficiency of photosystem II (Fv/Fm) of intact leaves for all the cultivars decreased at 40/35 °C, with severe decline in 'Cynthiana' and 'Cabernet Sauvignon,' moderate decline in 'Semillon' and 'Chardonnay', and slight decline in 'Pinot Noir'. A distinct effect of high temperature on Fv and Fv/Fm of 'Cynthiana' was exerted after 2 weeks of exposure. Prolonged-exposure to 40/35 °C led to 78% decrease in Fv/Fm in 'Cynthiana', compared with 8% decrease in 'Pinot Noir'. In general, Fv and Fv/Fm of extracted thylakoids declined as temperature increased, with more decline in 'Cynthiana' than in 'Pinot Noir'. Based on A rates and Fv/Fm ratios, results showed that 'Cynthiana' has lower optimal temperature for photosynthesis (20/15 °C) than 'Pinot Noir' (30/25 °C). Chlorophyll fluorescence responses of intact leaves and extracted thylakoids to high temperatures indicate that 'Pinot Noir' possess higher photosynthetic activity than 'Cynthiana'. Results of this work could be used in selection programs for the development of heat resistant cultivars in the warmest regions.

Productivity of temperate species falls significantly at high temperatures. Photosynthesis is the most sensitive process at high temperatures, especially during vegetative stages (Salvucci and Crafts-Brandner, 2004) and reproductive growth (Moffatt et al., 1990). Photosystem II (PSII) has been reported to be more sensitive than photosystem I (PSI) to heat stress (Berry and Björkman, 1980). The PSII reaction center is especially damaged mostly near the water-oxidizing complex (Wise et al., 2004). Wheat (*Triticum aestivium* L.) PSII has greater sensitivity to heat stress than rice (*Oryza sativa* L.) and millet [*Pennesitum glaucum* (L.) R. Br.] PSII (Al-Khatib and Paulsen, 1999); thus differences within genotypes indicate significant genetic variability for the trait. The adverse effect of high temperature on thylakoid activities has been reported to be more severe than that on the chloroplast envelope or stromal enzyme components (Santarius, 1975).

Inhibition of photosynthesis by high temperature is due to alteration in thylakoid membranes integrity (Gounaris et al.,

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1983; Schrader et al., 2004) and reduction of the activation state of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Kobza and Edward, 1987). Dissociation of the major light-harvesting complex of PSII from the core complex can be attributed to the increased fluidity of the thylakoid membrane (Armond et al., 1980). Damaged thylakoid membranes by high temperature show an increase in the amount of initial chlorophyll fluorescence emission (Kim and Portis, 2005), which indicates damage to the electron transport system of photosynthesis. The ability to resist heat stress differs among plants grown at contrasting thermal environments due to the ability of a plant to accumulate heatshock proteins and increase thermostability of the membranes (Haldimann and Feller, 2005) by altering thylakoid lipid composition (Li et al., 2003; Sharkey, 2005; Tardy and Havaux, 1997). Progressive acclimation to increased temperature significantly improved thermostablity of the thylakoid membranes and photosynthetic electron transport of pea (*Pisum sativum* L.) plants (Haldimann and Feller, 2005).

Heat stress can reduce net CO_2 assimilation (A) rate, stomatal conductance (g_s) and transpiration (E) rates, and increase leaf temperature (Dubey, 1997; Falk et al., 1996). Nevertheless, g_s in blackberry (*Rubus* L. subgenus *Rubus* Watson) was not the limiting factor to A at temperatures up to 35 °C (Stafne et al., 2001). Reduction in photosynthesis has been reported to be related to biochemical factors (Ferrini et al., 1995; Medrano et al., 2003) rather than to g_s (Hancock et al., 1992).

¹Assistant Professor and corresponding author; E-mail address: skadir@ksu.

Damage to the photosynthetic apparatus results in reduction in CO₂-fixation, ultimately, reduction in the rate of growth. Most of the photosynthate of grape is used for respiration, which is required for growth and organ maintenance (Blanke, 1990). High temperature affects the partitioning and translocation of photosynthates to plant parts other than the clusters (Sepulveda et al., 1986), thus affecting fruit set and berry compositions. Fruit set is inhibited by temperatures above 35 °C due to reduction in ovule or pollen viability and pollen tube growth, which contribute to smaller berry size and yield reduction (Ewart and Kliewer, 1977; Song and Ko, 1999). Extended periods of high temperature delays fruit maturation, reduces total titratable acidity (Kliewer, 1971), increases pH (Kliewer, 1968), and reduces berry color development (Kliewer, 1970).

Grapes are generally grown in the warmest regions in the world, where maximum air temperature can reach more than 40 °C (Williams et al., 1994), but the optimum temperature for photosynthesis has been established for some grapevines to be between 25 and 35 °C (Mullins et al., 1992). The wide variation in temperature range is due to species, cultivars, environmental conditions, or seasonal temperature variation (Ferrini et al., 1995; Shiraishi et al., 1996; Song and Ko, 1999). Temperatures above 35 °C generally reduce photosynthesis for both the American and *V. vinifera* species (Gamon and Pearcy, 1990), but the *V. vinifera* cultivars can survive temperatures up to 40 °C for a short period of time (Mullins et al., 1992).

For the last 15 years, grape has become one of the most productive and important specialty crops throughout the midwestern United States. The American and French-American hybrid wine grapes are widely grown in the midwestern United States due to their cold hardiness. 'Cynthiana' is one of the most widely planted *V. aestivalis* red wine cultivars, due to its cold hardiness and production of rich, dark-red wines with spicy, raspberry-scented aromas.

Little information exists on the mechanism by which V. vinifera cultivars survive high temperatures compared to the V. aestivalis 'Cynthiana' wine grape; therefore, the work presented in this paper was to assess thermostability of photosynthesis of Vitis germplasms over moderate to high temperatures. The objectives of this study were to determine optimum temperatures for photosynthesis, measure responses of grape vines to high temperature, and rank the relative heat tolerance of cultivars. On a cellular level and based on results of chlorophyll fluorescence of intact leaf that showed 'Cynthiana' was the most sensitive to high temperature while 'Pinot Noir' was the least sensitive, thylakoid membrane activity of both was compared under high temperatures to establish thermostability of the two cultivars. Results of this work could be used in selection programs for the development of winegrape cultivars resistant to high temperature for the warmest zones.

Material and Methods

PLANT MATERIALS AND TREATMENTS. Self-rooted *V. aestivalis* red winegrape 'Cynthiana' was compared with four *V. vinifera* winegrape cultivars grafted onto 3309 rootstocks: 'Semillon' clone 5 (white); 'Pinot Noir' clone 667 (red); 'Chardonnay' clone 4 (white); and 'Cabernet Sauvignon' clone 169 (red). Dormant 1-year-old vines (Double A Vineyards, Fredonia, N.Y.) were planted in polyethylene pots (16.25 × 16.25 × 12.5 cm) containing a mixture of 1 sand: 1 peatmoss: 1 perlite (by volume). Each pot had six drainage holes at the bottom to facilitate water drainage.

Vines were placed in the greenhouse at $22/17 \pm 3$ °C day/night (D/N) temperatures, $50\% \pm 10\%$ relative humidity (RH), and 16/8 h photoperiods. Supplemental light intensity on a horizontal plane above the canopy was $200~\mu \text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density (*PPFD*) [400–700 nm, measured with LI-188B Integrating Quantum/Radiometer/Photometer and LI-190sB sensor (LI-COR, Lincoln, Nebr.)] using Hydrofarm grow lights with 400-W, high-pressure sodium S-51 type-lamps (Hydrofarm Products, Petaluma, Calif.). Vines were irrigated to full pot capacity (Olson et al., 2000) and fertilized weekly with a commercial fertilizer containing $300~\mu \text{g}\cdot\text{L}^{-1}$ nitrogen, $250~\mu \text{g}\cdot\text{L}^{-1}$ phosphorus, and $220~\mu \text{g}\cdot\text{L}^{-1}$ potassium (Miracle-Gro; Scotts Miracle-Gro Products, Port Washington, N.Y.).

After 5 weeks, vines were pruned to a single shoot per vine, and gas exchange, relative chlorophyll content, and chlorophyll fluorescence of the most recent fully expanded leaf were measured. Measurements before applying temperature treatments were considered as the control. Vines per cultivar were divided into three groups, each of four vines, placed in one of the three growth chambers (CMP 3244; Conviron, Asheville, N.C.) for 4 weeks. Growth chambers were set at $20/15 \pm 1$ °C, $30/25 \pm 1$ °C, and $40/35 \pm ^{\circ}C$ 1 D/N temperatures, 16/8 h photoperiods, and 550 μ mol·m⁻²·s⁻¹ *PPFD*. Humidity was not regulated, but was monitored to be in the range from $40\% \pm 10\%$ during the light period to $70\% \pm 10\%$ RH during the dark period. Vines at 20/15, 30/25, and 40/35 °C were watered as needed to full pot capacity. Besides daily watering, plants at 40/35 °C were misted every 30 min to prevent secondary injury due to desiccation. Gas exchange, relative chlorophyll content, and chlorophyll fluorescence of intact leaves were measured at weekly intervals, for 4 weeks at the temperature treatments, between 0900 and 1100 HR. Leaves from 5-week-old self-rooted 'Cynthiana' and 'Pinot Noir' clone 667 grafted onto 3309 rootstock grown in the greenhouse under similar conditions described earlier, were collected and thermostability of extracted thylakoids was measured.

Gas exchange and relative chlorophyll content. Gas exchange was measured by using LI-6400 open system portable photosynthesis measurement (LI-COR). Leaf net CO₂ assimilation (A) rate and stomatal conductance (g_s) were determined for 4 weeks. Due to equipment failure, transpiration (E) rates were determined for weeks three and four only. An intact leaf was placed in the leaf chamber (6.0 cm²) and exposed to 2000 $\mu \text{mol·m-2·s-1}$ PPFD and CO₂ concentration of 400 $\mu \text{L·L-1}$. Data were recorded after $\approx \! 30$ to 45 s, when CO₂ and stomatal conductance stabilized.

Leaf chlorophyll meter (SPAD-501; Minolta Corp., Osaka, Japan) was used to measure indirect index of chlorophyll content under temperature treatments. Three SPAD measurements (38 mm² total leaf area) were taken from locations between the veins. Data were averaged for each leaf of the replicated grapevines to represent one observation.

INTACT LEAF CHLOROPHYLL FLUORESCENCE. Leaf chlorophyll fluorescence emission was determined using a pulse-modulated fluorescence monitoring system (FMS-1; Hansatech Instruments Ltd., Norfolk, England) operated in the Fv/Fm mode. The fluorometer probe was placed 7 mm away from the leaf surface and measurements were made at steady state of 2000 μ mol·m⁻²·s⁻¹ and saturating state of 5000 μ mol·m⁻²·s⁻¹ for 0.7 s when all of the PSII reaction centers were reduced. Initial fluorescence (Fo) when plastoquinone electron acceptor pool (Qa) is fully oxidized, maximal fluorescence (Fm) when Qa is transiently fully reduced, variable fluorescence (Fv = Fm – Fo), and maximum quantum

efficiency of PSII (Fv/Fm) were recorded. Three readings from the center of the leaf of each vine were averaged to represent one observation.

THYLAKOID EXTRACTION AND TREATMENTS. Chloroplast thylakoids were extracted as described previously (Al-Khatib and Paulsen, 1990). Leaves were homogenized with a Polytron (Brinkmann Instruments, Westbury, N.Y.) for 1 min in ice-cold medium of 330 mm sorbitol, 40 mm N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (Hepes-NaOH) (pH 7.0), 30 mm KCl, and 3 mm MgCl₂, then all subsequent operations were carried out at 0 °C. The homogenate was centrifuged at 750 g_n for 1 min, and the supernatant was centrifuged at 2500 g_n for another 2 min. The pellet from the second centrifugation was suspended in 10 mm NaCl, centrifuged at 2500 g_n for 10 min, and washed twice before being taken up in the original extraction medium. Chlorophyll concentration was 30 mg·mL⁻¹, preparation for temperature treatments were made within 30 min. Temperature treatments were imposed on 0.2 mL chloroplast thylakoid samples in thin glass tubes placed in several controlled-temperature baths (Haake Buchler Instruments, Saddle Brook, N.J.) set at 20, 30, 40, 45, or 50 °C for 2 min, with gentle agitation. After 2 min, samples were taken out of each bath and kept in ice prior to measurement. The above procedure was carried out under room temperature (22–23 °C) and lighting of 15 μ mol·m⁻²·s⁻¹ *PPFD*.

THYLAKOID FLUORESCENCE MEASUREMENT. Chlorophyll fluorescence was conducted according to the method of Al-Khatib and Paulsen (1990). Fluorescence was measured by adding 0.2 mL of thylakoid-extract, containing 2 mg·L⁻¹ chlorophyll and 3 mL of PSII media to the Hansatech DW1 oxygen electrode unit (King's Lynn, England). The PSII media consisted of 50 mm HEPES-NaOH (pH 7.6), 15 mm 3-(3,4-dichlorophenyl)-1,1-dimethylurea, 10 mm KCl, and 5 mm MgCl₂. The plant efficiency analyzer [PEA (Hansatech Instruments Ltd., Norfolk, England)] sensor head was placed over the unit, and thylakoids were dark-adapted by covering the unit with a black fabric for 2 min before measurement. The oxygen electrode unit was fitted with two Hansatech FDP photodiode detectors protected by 685- and 740-nm center bandpass interference filters with bandwidths of 12-nm (Ealing Electro-Optics, South Natick, Mass.) to detect fluorescence. Excitation radiation from a quartz halogen lamp (Oriel Corp., Stamford, Conn.) equipped with Ilex shutter (Melles Griot, Rochester, N.Y.), Schott BG-39 shortpass filter (586-nm cutoff), and neutral density filters (Schott Glass Technologies, Duryea, Pa.) provided 100 μmol·m⁻²·s⁻¹ irradiance. Temperature of the unit was maintained at 22 °C by a Haake A80 refrigerated circulating bath (Haake-Buchler, Saddle Brook, N.J.). Fluorescence photodiode signals were processed by an EXP-16 multiplexer and DAS-16 converter (MetraByte Corp., Taunton, Mass.) interface-computer system.

EXPERIMENTAL DESIGNS AND DATA ANALYSIS. The experiments were randomized complete-block designs with factorial arrangement of cultivar \times temperature \times time for the intact leaf study and cultivar \times temperature for the thylakoid heat treatment study. Treatments were replicated four times for the intact leaf study and six times for the thylakoid heat treatment study. The replications in the thylakoid heat study were independent extractions. Experiments were repeated; data are averaged across both runs since there was no interaction between treatments and runs. Temperature treatment response was determined by conventional analyses of variance (ANOVA) or general linear model (GLM) as appropriate. Least significant differences (LSD) among means were tested at P=0.05, and precision was measured by coef-

ficient of variation (cv) percentage. Standard errors of treatment means were calculated. Data are presented as percentages of measurements taken before temperature treatments. A correlation coefficient between photosynthetic parameters was established. Data were tested for homogeneity of variance and normality of distribution (Ramsey and Schafer, 1997). Three-way interactions of cultivar × temperature × time were found for all intact leaf parameters, except a two-way interaction for cultivar × temperature was statistically significant for the transpiration rate. There was significant two-way interaction for chlorophyll fluorescence parameters for the thylakoid heat treatment study.

Results

GAS EXCHANGE. All cultivars subjected to 1 week of 20/15 °C had more than 95% of the net CO_2 assimilation (A) rates of the control (Fig. 1). After 4 weeks of exposure to 20/15 °C,

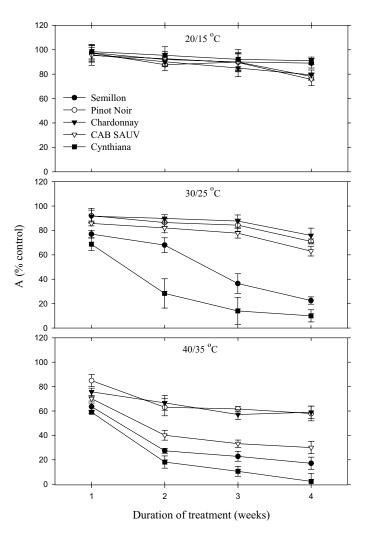


Fig. 1. Net CO₂ assimilation rate (A) of attached leaves of *Vitis vinifera* 'Semillon', 'Pinot Noir', 'Chardonnay', 'Cabernet Sauvignon' (CAB SAUV), and *V. aestivalis* 'Cynthiana' after temperature treatments. Attached leaves were on 35-d-old plants grown at 20/15, 30/25, or 40/35 °C day/night (D/N) and 16/8 h photoperiod for 4 weeks. Photosynthetic rates were measured at CO₂ concentration of 400 μL·L-¹, at weekly intervals. Vertical lines through data points represent the se; values smaller than symbols are not shown. Control values (μmol·m-²-s-¹) were: 'Semillon' (16.1), 'Pinot Noir' (15.1), 'Chardonnay' (15.9), 'Cabernet Sauvignon' (15), and 'Cynthiana' (12.9). The control treatment was measured at 22/17 ± 3 °C D/N and 16/8 h photoperiod.

temperature had no significant effect on A rates in 'Cynthiana' or 'Semillon', compared to week one, whereas A decreased in 'Pinot Noir', 'Chardonnay', and 'Cabernet Sauvignon'. 'Cynthiana' and 'Semillon' had the highest A rates (91% and 89%, respectively), compared with an average of 78% for the other cultivars.

After 1 week at 30/25 °C, A was reduced for 'Semillon' and 'Cynthiana', whereas A rates were not altered in the other cultivars at this duration of treatment. Photosynthetic activity in 'Cynthiana' and 'Semillon' declined as time of exposure to 30/25 °C increased to 4 weeks. At a given time, reduction in A rate was more pronounced in 'Cynthiana' than in 'Semillon'. 'Chardonnay' (75%) and 'Pinot Noir' (72%) had the highest A rates after 4 weeks of exposure to 30/25 °C. Leaf photosynthesis of all the cultivars decreased at 40/35 °C. In general and throughout the experiment, 'Cynthiana' had the lowest A rates, whereas 'Pinot Noir' and 'Chardonnay' had the highest rates. Photosynthesis of 'Cynthiana' at 40/35 °C was reduced to 19% after 2 weeks of exposure, compared with 63% and 66% in 'Pinot Noir' and 'Chardonnay', respectively. 'Cynthiana' leaves were nearly killed showing dry leaf surface with very few green spots after 4 weeks of exposure to high temperature, whereas A rate was reduced to 58% and 59% for 'Pinot Noir' and 'Chardonnay', respectively.

Stomatal conductance (g_s) differed distinctly among the cultivars (Fig. 2). Throughout the experiment, g_s at 20/15 °C was the highest in 'Cynthiana', although 'Semillon' had similar rate at week one. After more than 3 weeks of exposure to 20/15 °C, 'Pinot Noir' (37%) and 'Chardonnay' (32%) had the lowest g_s, whereas 'Semillon' and 'Cabernet Sauvignon' were intermediate. Stomatal conductance of 'Cynthiana', 'Chardonnay', and 'Cabernet Sauvignon' at 30/25 °C was significantly reduced after 1 week of exposure. At the end of the experiment, g_s values were higher in 'Pinot Noir' (63%) and 'Semillon' (67%) than in the other cultivars. Vines of 'Pinot Noir' and 'Cynthiana' grown at 40/35 °C for 4 weeks had the lowest g_s with 26% and 16% of the control, respectively, whereas 'Cabernet Sauvignon' had the highest value (92%).

Time of exposure had no effect on E rates; nevertheless there was two-way interaction for cultivar × temperature. Data presented in Table 1 are average of 2 weeks. 'Cynthiana' at 20/15 °C had the highest E rate of 2.1 mol·m-2·s-1, whereas 'Pinot Noir' had the lowest rate of 0.56 mol·m-2·s-1. At 30/25 °C, 'Semillon' (2.4 mol·m-2·s-1) and 'Pinot Noir' (1.85 mol·m-2·s-1) had the highest rates compared to 'Chardonnay' (0.64 mol·m-2·s-1); 'Cynthiana' and 'Cabernet Sauvignon' were intermediate. 'Cynthiana' leaves at 40/35 °C transpired the least (0.55 mol·m-2·s-1), whereas 'Cabernet Sauvignon' leaves transpired the most (3.96 mol·m-2·s-1). In general, *V. vinifera* cultivars transpired the most at high temperature, with an average rate of 2.63 mol·m-2·s-1, compared with 'Cynthiana'.

CHLOROPHYLL CONTENT. After 2 weeks of exposure to 20/15 °C, relative chlorophyll content (SPAD units) was not altered in any of the cultivars (Fig. 3). More than 3 weeks of exposure, however, chlorophyll content increased in 'Semillon' to 133% of the control, but decreased to the same level of week one in 'Cabernet Sauvignon' (109%). No significant changes were observed in the other cultivars.

After 1 week of exposure to 30/25 °C, chlorophyll content of 'Cynthiana' was the highest (137%), although a significant decline to 75% was observed after 4 weeks of exposure. Among the *V. vinifera* cultivars, 'Semillon' had the highest chlorophyll content of 130% after 4 weeks of exposure to 30/25 °C, whereas no change was observed in the other cultivars. There were significant

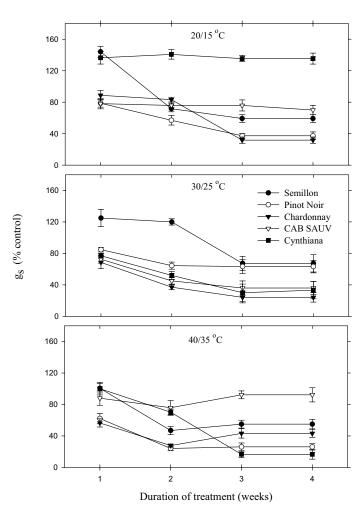


Fig. 2. Stomatal conductance (g_s) of attached leaves of *Vitis vinifera* 'Semillon', 'Pinot Noir', 'Chardonnay', 'Cabernet Sauvignon' (CAB SAUV), and *Vitis aestivalis* 'Cynthiana' after temperature treatments. Attached leaves were on 35-d-old plants grown at 20/15, 30/25, or 40/35 °C day/night (D/N) and 16/8 h photoperiod for 4 weeks. Stomatal conductance was measured at CO₂ concentration of 400 μL·L⁻¹, at weekly intervals. Vertical lines through data points represent the SE; values smaller than symbols are not shown. Control values (mmol·m-²-s-¹) are: 'Semillon' (0.144), 'Pinot Noir' (0.198), 'Chardonnay' (0.206), 'Cabernet Sauvignon' (0.088), and 'Cynthiana' (0.209). The control treatment was measured at 22/17 ± 3 °C D/N and 16/8 h photoperiod.

Table 1. Intact leaf transpiration rate (E) of *Vitis vinifera* 'Semillon', 'Pinot Noir', 'Chardonnay', 'Cabernet Sauvignon', and *V. aestivalis* 'Cynthiana' in response to 20/15 °C, 30/25 °C, or 40/35 °C under controlled environmental conditions.²

			Cabernet	
Semillon	Pinot Noir	Chardonnay	Sauvignon	Cynthiana
Leaf transpiration rate [H ₂ O (mol·m ⁻² ·s ⁻¹)]				
		20/15 °C		
1.15	0.56	1.02	1.58	2.1
		30/25 °C		
2.40	1.85	0.64	1.17	1.04
		40/35 °C		
2.95	1.66	2.01	3.96	0.55
$\mathrm{LSD}_{0.05}$		0.6		

Average of 2 weeks. Equipment failure prevented recording data from weeks 0, 1, and 2.

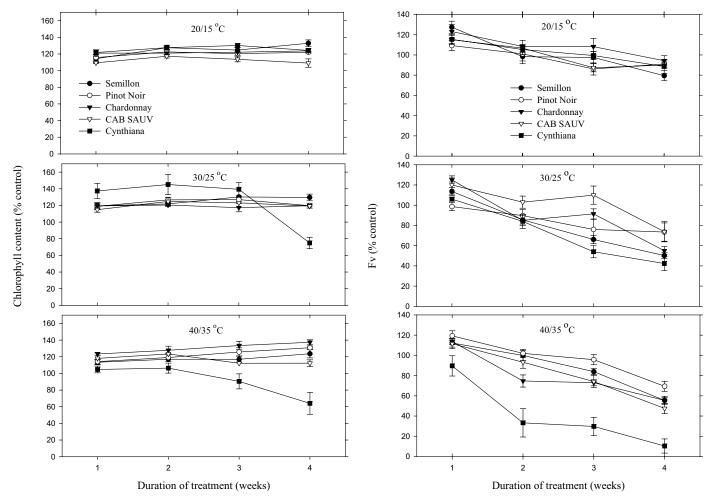


Fig. 3. Relative chlorophyll content of attached leaves of *Vitis vinifera* 'Semillon', 'Pinot Noir', 'Chardonnay', 'Cabernet Sauvignon' (CAB SAUV), and *V. aestivalis* 'Cynthiana' after temperature treatments. Attached leaves were on 35-d-old plants grown at 20/15, 30/25, or 40/35 °C day/night (D/N) and 16/8 h photoperiod for 4 weeks. Chlorophyll content was measured with the leaf chlorophyll meter, at weekly intervals. Vertical lines through data points represent the se; values smaller than symbols are not shown. Control values (SPAD units) are: 'Semillon' (30.46), 'Pinot Noir' (33.11), 'Chardonnay' (31.15), 'Cabernet Sauvignon' (32.63), and 'Cynthiana' (30). The control treatment was measured at 22/17 ± 3 °C D/N and 16/8 h photoperiod.

Fig. 4. Chlorophyll variable fluorescence (Fv) of attached leaves of *Vitis vinifera* 'Semillon', 'Pinot Noir', 'Chardonnay', 'Cabernet Sauvignon' (CAB SAUV), and *V. aestivalis* 'Cynthiana' after temperature treatments. Attached leaves were on 35-d-old plants grown at 20/15, 30/25, or 40/35 °C day/night (D/N) and 16/8 h photoperiod for 4 weeks. Chlorophyll fluorescence was measured as described in the Materials and Methods, at weekly intervals. Vertical lines through data points represent the SE; values smaller than symbols are not shown. Control values (relative units) are: 'Semillon' (1233), 'Pinot Noir' (1291), 'Chardonnay' (1277), 'Cabernet Sauvignon' (1226), and 'Cynthiana' (1302). The control treatment was measured at 22/17 ± 3 °C D/N and 16/8 h photoperiod.

nificant differences between the cultivars grown at 40/35 °C. At 1 week of exposure, 'Chardonnay' had 123% chlorophyll content, which was the highest level, whereas 'Cynthiana' had the least (104%). In contrast to *V. vinifera* cultivars, reduction in chlorophyll content was more pronounced in 'Cynthiana' after more than 2 weeks of exposure. After 4 weeks exposure to 40/35 °C, 'Chardonnay', 'Pinot Noir', and 'Semillon' had 137%, 131%, and 124%, respectively, chlorophyll content, compared with 64% for 'Cynthiana'.

LEAF CHLOROPHYLL FLUORESCENCE. Variable fluorescence of 'Semillon' declined by 23% after 2 weeks of exposure to 20/15 °C, compared with week one. No changes were recorded in the other cultivars (Fig. 4). The Fv of 'Chardonnay', 'Cynthiana', and 'Semillon' grown at 30/25 °C for 2 weeks decreased by 32%, 25%, and 21%, respectively, whereas no change was observed in 'Pinot Noir' or 'Cabernet Sauvignon'. Four weeks of 30/25 °C significantly reduced Fv of all the cultivars, although the reduction was more severe in 'Cynthiana' (60%), 'Chardonnay'

(57%), and 'Semillon' (56%), compared with 'Pinot Noir' (25%) or 'Cabernet Sauvignon' (38%). Variable fluorescence decreased rapidly in all the cultivars after 2 weeks of 40/35 °C. Except for 'Chardonnay', Fv decline was less evident in *V. vinifera* cultivars than in 'Cynthiana'. Reduction of Fv after 2 weeks at 40/35 °C was 63% in 'Cynthiana', relative to an average decline of 19% in the *V. vinifera* cultivars. Four weeks of exposure to 40/35 °C, Fv in 'Cynthiana' was reduced to 10% of the control, approximately an 89% reduction. 'Pinot Noir' had the highest Fv value (69%) after 4 weeks of exposure to 40/35 °C, whereas 'Cynthiana' had the lowest value.

Differential responses of Fv/Fm of all the cultivars to temperature treatments are shown in Fig. 5. After 2 weeks at the lowest temperature (20/15 °C), Fv/Fm ratio in 'Semillon' was decreased by 11%, compared with that of week one, whereas no temperature response was evident in the other cultivars. As the time of exposure extended to 4 weeks, Fv/Fm of 'Semillon' was reduced by 16%, whereas no significant changes were observed

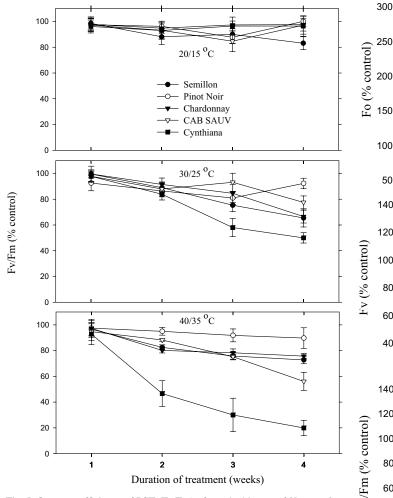


Fig. 5. Quantum efficiency of PSII (Fv/Fm) of attached leaves of *Vitis vinifera* 'Semillon', 'Pinot Noir', 'Chardonnay', 'Cabernet Sauvignon' (CAB SAUV), and *V. aestivalis* 'Cynthiana' after temperature treatments. Attached leaves were on 35-d-old plants grown at 20/15, 30/25, or 40/35 °C day/night (D/N) and 16/8 h photoperiod for 4 weeks. Fv/Fm was measured as described in the Materials and Methods, at weekly intervals. Vertical lines through data points represent the SE; values smaller than symbols are not shown. Control values (relative units) are: 'Semillon' (0.793), 'Pinot Noir' (0.797), 'Chardonnay' (0.801), 'Cabernet Sauvignon' (0.797), and 'Cynthiana' (0.811). The control treatment was measured at 22/17 ± 3 °C D/N and 16/8 h photoperiod.

in the other cultivars. As time of exposure to 30/25 °C increased, Fv/Fm ratios decreased in 'Cynthiana', 'Semillon', and 'Chardonnay'. There was 13% reduction in 'Pinot Noir' after 3 weeks of exposure; nevertheless, plants recovered thereafter and reached the same Fv/Fm ratio (93%) as that of week one. After 4 weeks of exposure to 30/25 °C, 'Cynthiana' Fv/Fm was reduced the most to 50%, relative to the *V. vinifera* cultivars.

Two weeks at 40/35 °C, Fv/Fm ratios decreased in all the cultivars, except in 'Pinot Noir'. A sharp decline in 'Cynthiana' was observed by 2 weeks of exposure to 40/35 °C, which was reduced to 46% while other cultivars were above 80%. After 4 weeks of exposure to 40/35 °C, Fv/Fm ratio of 'Cynthiana' was reduced to 20% of the control, compared with 90% in 'Pinot Noir', 76% in 'Chardonnay', 73% in 'Semillon', and 57% in 'Cabernet Sauvignon'.

EXTRACTED THYLAKOIDS FLUORESCENCE. Initial chlorophyll fluorescence (Fo) of extracted thylakoids from 'Cynthiana' and 'Pinot Noir' was not affected by 20 °C (Fig. 6A). Significant dif-

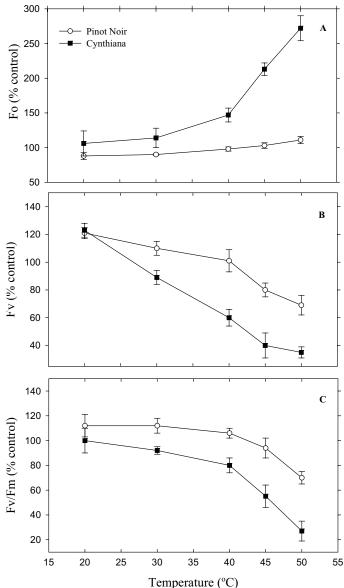


Fig. 6. Initial chlorophyll fluorescence (Fo) (**A**), variable fluorescence (Fv) (**B**), and quantum efficiency of PSII (Fv/Fm) (**C**) of extracted thylakoids of *Vitis vinifera* 'Pinot Noir' and *V. aestivalis* 'Cynthiana' after temperature treatments of 20, 30, 40, 45, or 50 °C for 2 min. Thylakoids were extracted from leaves on 35-d-old plants grown at 22/17 ± 3 °C day/night (D/N) and 16/8 h photoperiod for 4 weeks. Thylakoid extraction, treatments, and fluorescence measurements are as described in the Materials and Methods. Vertical lines through data points represent the se; values smaller than symbols are not shown. Control values (relative units) for Fo, Fv, Fv/Fm, respectively, are: 'Pinot Noir' (49, 14, 0.2367) and 'Cynthiana' (53, 12, 0.1943). The control treatment was measured at room temperature (22–23 °C).

ferences between the two cultivars were evident as temperature increased by 10 °C. The Fo increased in 'Cynthiana', compared with that of 'Pinot Noir', by 21%, 33%, 52%, and 59% at 30, 40, 45, and 50 °C, respectively. From 30 to 40 °C, Fo of 'Pinot Noir' thylakoids increased by 8%, whereas 22% increase was recorded for 'Cynthiana'. The Fv (Fig. 6B) of the thylakoids was reduced more in 'Cynthiana' than in 'Pinot Noir.' Most of the decline in 'Cynthiana' was due to a decline in Fm (data not shown) and increase in Fo. Reduction in Fv was reflected in a steady decline in Fv/Fm (Fig. 6C).

Discussion

Shiraishi et al. (1996) reported that optimal temperature for A is higher for *V. vinifera* cultivars than that of *V. aestivalis* cultivars, which was attributed to selection conducted for the cultivars under different temperature conditions in the cultivated areas (Chaves et al., 1987). In our study, optimal temperature for photosynthesis in 'Cynthiana' was 20/15 °C, whereas 30/25 °C was optimum for 'Pinot Noir' and most of the other *V. vinifera* cultivars. At 40/35 °C, 'Cynthiana', followed by 'Semillon', were the most sensitive cultivars; 'Pinot Noir' was the most resistant; and the other *V. vinifera* cultivars were intermediate.

Net CO_2 assimilation rate (Fig. 1), g_{s} (Fig. 2), chlorophyll content (Fig. 3), E (Table 1) rates, and intact leaf Fv/Fm (Fig. 5) were significantly lower in 'Cynthiana' at high temperature, relative to 'Pinot Noir' and most of the *V. vinifera* cultivars. 'Cynthiana' showed a linear decline in A rate as temperature increased to 40/35 °C. These findings support previous work that A declines in temperate fruit crops as temperature increases from 20 to 30 °C (Hancock et al., 1992).

In general, reduction in A of the *V. vinifera* cultivars at 40/35 °C was more closely related to changes in Fv/Fm rather than g_s . 'Pinot Noir' had the highest A rate (Fig. 1) and Fv/Fm ratio and the lowest g_s and E rates, compared with the other *V. vinifera* cultivars, after 4 weeks of exposure to 40/35 °C. Nevertheless, reduction of A in 'Cynthiana' was related to reduction in both g_s and Fv/Fm ratio. Net CO_2 assimilation rates were strongly related to Fv/Fm, with an average correlation coefficient (r) of 0.92 (data not shown) across the cultivars, whereas A was less related to g_s with an average correlation coefficient of 0.61 (data not shown). Photosynthetic rate of 'Cynthiana' was related to g_s with correlation coefficient of 0.94, whereas average correlation for A and g_s for *V. vinifera* cultivars was 0.50.

Results of studies on g_s rates and their relationship to A during heat stress have been inconsistent. Some studies reported that g_s rates increase with increasing temperature (Rogers et al., 1981), whereas others showed a decline (Candolfi-Vasconcelos and Koblet, 1991; Chaves et al., 1987; Ferrini et al., 1995). Decline of g_s has been attributed to internal CO_2 accumulation (C_i), water stress, and hormonal changes that control stomatal activities (Gomez-Del-Campo et al., 2004). Reduction in A rates under drought stress, with no effect on g_s , has been attributed to nonstomatal factors such as carbohydrate accumulation, reduction in ribulose-1,5-bisphosphate regeneration, or photoinhibition (Medrano et al., 2003). In this study, drought stress was not a factor and measurement of hormonal changes during heat stress was not conducted; thus, photoinhibition, or a decrease in quantum efficiency of PSII, might be one of the main factors for A reduction.

A positive relationship between g_s and E rates is observed in this study. The lowest g_s values of 'Pinot Noir' and 'Cynthiana' after 4 weeks of exposure to high temperature coincided with the lowest E rates, which agrees with earlier report that g_s is positively related to E rate (Stafne et al., 2001). Chlorophyll content (Fig. 3) of 'Cynthiana' declined as temperature increased to 40/35 °C, whereas no changes were recorded in most of the *V. vinifera* cultivars. Leaves of 'Pinot Noir' and 'Chardonnay' had the highest A rates and chlorophyll content at 40/35 °C, whereas 'Cynthiana' leaves had the lowest values. These findings indicate that a positive relationship between A and chlorophyll content existed, which agrees with earlier reports that an increase in A is related to an increase in chlorophyll content at 35 °C (Ferrini et al., 1995). Higher chlorophyll content of 'Pinot Noir' relative

to 'Cynthiana' indicates that the latter was not utilizing the light harvesting pigments as efficiently as 'Pinot Noir' (Candolfi-Vasconcelos and Koblet, 1991).

Environmental stresses increase Fo and decrease Fv/Fm, which indicates dissociation of the light harvesting pigments from the PSII core, resulting in reduction in photochemical efficiency of PSII (Kitao et al., 2000; Wise et al., 2004). Thermostability of extracted thylakoid behaved similarly to whole-plant responses to high temperature. After 2 min exposure to temperatures higher than 20 °C, extracted thylakoids from 'Cynthiana' showed an increase in Fo (Fig. 6A) and decrease in Fv (Fig. 6B) and Fv/Fm (Fig. 6C). Reduction in Fv was more severe in 'Cynthiana' than in 'Pinot Noir'. Most of the decline of Fv in 'Cynthiana' was due to a decline in Fm (data not shown) and an increase in Fo. The lowest Fv/Fm values indicate that electron transfer from PSII in 'Cynthiana' subjected to temperature higher than 20 °C is less efficient than that of 'Pinot Noir' (Kitao et al., 2000; Sharkey, 2000). A sharp decline in leaf Fv/Fm at 40/35 °C was a good indicator that irreversible damage occurred to PSII of 'Cynthiana'. The high resistance of intact leaves of 'Pinot Noir' to 40/35 °C could be attributed to thermostability of the thylakoid membranes subjected to temperatures ranging between 20 to 50 °C. Results of this study agree with an earlier report that thermostability of resistant thylakoids is essential for the stability of photosynthesis (Al-Khatib and Paulsen, 1999; DeEll and Toivonen, 2000). Responses of chlorophyll fluorescence of intact leaves and extracted thylakoids to high temperatures indicate that 'Pinot Noir' possesses higher potential photosynthetic activity at high temperatures than 'Cynthiana'.

This study was conducted to determine how two *Vitis* species responded to three temperatures under controlled environmental conditions. Differences among grapevine cultivars in response to heat stress have been identified. 'Pinot Noir' was the most resistant cultivar, having minimal decline in A, chlorophyll content, and less decline in Fv/Fm, compared with 'Cynthiana', which proved to be the cultivar most sensitive to high temperature. Optimal temperatures for photosynthesis were 20/15 °C and 30/25 °C for 'Cynthiana' and most of the *V. vinifera* cultivars, respectively. Photosynthesis in 'Cynthiana' was affected both by stomatal activities and by decline in the Fv/Fm ratio, whereas A was less related to g_e, but more to Fv/Fm ratio in *V. vinifera* cultivars.

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