PRELIMINARY INVESTIGATIONS ON SUNBURN IN CHARDONNAY GRAPEVINE VARIETY

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ABSTRACT

The aim of this investigation was to determine if a temperature response curve can be used to describe sunburn in grape berries. Trials were carried out at the Viticulture and Enology Department (University of California, Davis) on cv Chardonnay (clone 29) grown under both field and greenhouse conditions.

Greenhouse plants were two years old, grown in 5 L pots, and watered daily with a modified Hoagland's nutrient solution. The vines were pruned to two shoots with one or two clusters per shoot, and the shoots were vertically trained to approximately 1.5 m. Field-grown vines were clone 29 grafted onto 101-14 rootstock, planted at 2.5 x 3.7 m spacing, cane pruned, and VSP trained. Rows were north-south oriented.

In order to increase the temperature of the berry surface, solar radiation was concentrated using a normal reading lens with different magnifications degrees. Temperature was measured with a copper-constantan thermocouples attached to the berry surface. Experiments were performed just before harvest. Sunburn was caused by using different ranges of temperatures held constant for 2 or 5 minutes in the case of greenhouse plants and 5, 10, and 15 minutes in the case of field-grown plants. The effects of treatments were rated on visual basis by a panel of 3 people at one day intervals for three or four consecutive days after the treatments. On the last day, treated berries were harvested and analyzed for cell viability and membrane integrity using the fluorescein diacetate (FDA) technique.

In greenhouse grown vines, a temperature of 38-40 °C for 5 minutes was sufficient to cause visual symptoms of sunburn two days after the treatments, even if no cells were

permanently damaged. In field-grown vines, 5 minutes at 40-43 °C caused 12.4% cell mortality and permanent surface deformation.

In conclusion, exposure of berries to a surface temperature of 40-43 °C appears to be effective in causing sunburn in greenhouse and field-grown plants. The radiation regime experienced by the cluster during the growing season may be important to determine the critical level of temperature causing sunburn.

Keywords: Solar radiation, berry temperature, cell mortality, sunburn necrosis, FDA.

INTRODUCTION

Sunburn is a physiological injury that significantly affects fruit quality. In apple, three different types of sunburn damage have been characterized: sunburn necrosis, sunburn browning and photo-oxidative sunburn (Felicetti and Schrader, 2009). Sunburn necrosis is heat-induced and results from thermal death of exocarp and mesocarp cells. In apple, sunburn necrosis was induced when the fruit skin temperature reached 52 ± 1°C for 10 minutes (Schrader at al., 2003). Sunburn browning is a sublethal skin injury caused by a concomitant exposure of the fruit to high solar radiation and high temperature (on apple fruit, 46-49 °C for 1 hour). A discoloration of the epidermal tissue with different damage depth occurs at the beginning sunburn browning; later the tissues become soft and, in the more serious injury, the mesocarp cells die. Grapevines can be cultivated in areas where high light is frequently associated with high air temperatures, often above 40 °C, but little is known about the incidence of sunburn. Greer et al. (2006) have suggested that 5-15% of grapes were sunburned as a consequence of high light and high summer temperatures in some Australian areas. Greater incidence of sunburn was also cited by Spayd et al. (2002) on south facing fruit of east-west oriented rows, and by Tarara et al. (2005) on west facing fruit of north-south oriented rows.

Meteorological conditions, variety susceptibility, cultural practices, and the physiological condition of the plants may have a role on sunburn inception and evolution.

In this study we attempt to determine the temperature response of sunburn necrosis in *Vitis vinifera* L. cultivar Chardonnay.

MATERIAL AND METHODS

The trials were carried out at the Viticulture and Enology Department (University of California, Davis) on cv Chardonnay (clone 29) grown in the field and in the greenhouse.

Plant in greenhouse were two years old, and were grown in 5 L pots filled with a mixture of GrowCoir^{TM} (Greenfire Co., Ltd., Sacramento, CA, USA) clay pellets, and perlite (4:1:1 by volume). Air temperature and relative humidity were of 30 ± 3 °C and 40 ± 10% during the day and 20 ± 3 °C; 70 ± 10%; natural light reached a daily maximum PAR of 1,200 μmol photons m^{-2} s^{-1}. Vines were pruned to two shoots with one or two clusters per shoot, and the shoots were vertically trained to approximately 1.5 m. Vines were daily fully watered with a modified Hoagland's nutrient solution (in mM: NO^{3-}, 6.85; NH^{4+}, 0.43; PO_4^{3-}, 0.84; K^+, 3.171; Ca^{2+}, 2.25; Mg^{2+}, 0.99; SO_4^{2-}, 0.50; and in lM: Fe^{2+}, 28.65; Mn^{2+}, 4.91; BO_3^{3-},24.05; Zn^{2+}, 1.83; MoO_4^{2-}, 0.17; Cu^{2+}, 2.52) with EC1.00 dS m^{-1} at pH 5.75.

Vines in the field were Chardonnay (clone 29) grafted onto 101-14 rootstock placed at 2.5 x 3.7 m spacing, cane pruned and VSP trained. Rows were north-south oriented. Standard canopy management of the area were applied during the growing season. In particular, leaves were removed from the cluster zone of the canopy during the last week of July.

Both in the field and the greenhouse, reading lenses were used to heat berries in situ and to induce sunburn necrosis. In particular, solar radiation was directed by mean of commercial reading lenses with different magnification degrees (1.00, 1.50, 2.00, 2.50, 3.00, 3.50) and the light spot was oriented on the berry surface where the copper constantan thermocouple was attached. Berry surface temperature was recorded by CR21X datalogger(Campbel Scientific) at 60 seconds time interval.

The measurements were done just before harvest on 5 berries replications for each time and temperature combination. In greenhouse plants the temperature intervals were: 36-38 °C, 38-40 °C, 40-42 °C, 42-44 °C, and 44-46 °C. Temperature was kept constant within the each interval for 2 and 5 minutes by changing the distance between lens and berry surface (exposure time). In the field, the temperature were: 40-43 °C, 43-45 °C and 45-48 °C, and two additional exposure times were utilized, i.e.: 10 and 15 minutes. Berries were chosen from the middle part of the cluster.

Sunburn damage was established by a panel of 3 people using a scorecard and a visual scale (0 = no symptoms; 1 = the berry shows a bright gold-ralphyellow color, without any deformation of the berry; 2 = the berry shows a mustard-dark goldenrod and a light deformation; 3 = the berry shows a dark orange-yellow ochre color, and a light deformation; 4 = the berry shows a dark orange-yellow ochre color and a strong deformation; 5 = the berry shows a dark orange-yellow ochre color and a extremely severe deformation). Photos of the berry were also taken. Observations started just after treatment and were made every 24 hours for four consecutive days in the greenhouse and five consecutive days in the field. The last day the treated berries were harvested, cut by the pedicel and immediately transported to the laboratory and analyzed for the cell viability and membrane integrity by means of the fluorescine diacetate (FDA) technique (Krasnow et al. 2008). The staining solution was made by adding 2µL of a 4.8 mM stock FDA solution (in acetone) to 1 mL of sucrose solution balanced to approximately the same osmolarity as the berry and, within 10 min of being made, about 250 µL of this staining solution was placed over the entire cut surface and held there by surface tension for at least 20 min to allow FDA uptake prior to visualization (Krasnow et al. 2008).

Berries with sunburn damage were sectioned longitudinal to the treated area and observed with a MZ12 Leica stereomicroscope with illumination a MZ12 Leica HBO 100 mercury lamp fitted with a Leica GFP Plus Fluor filter (450–490 nm) magnitude 2.5 X. The photos were taken with a Leica DC 300F camera linked to the microscope. Stained (living) and unstained (dead) area were easily hand traced and measured by Image-J software (NIH) and the mortality index was calculated as the sunburned to non-sunburned area ratio.

Data of the damage score was analyzed using randomized block design. Data of cell mortality were transformed using the equation: $\arcsin(\sqrt{\text{mortality index}})$ and analyzed by a two-way analysis of variance (ANOVA) to determine if significant differences existed among treatments. Student-Newman-Keuls method was used to determine which means were statistically different ($p < 0.05$).

RESULTS AND DISCUSSION

Vines acclimated under the greenhouse conditions showed the first symptoms of sunburn necrosis after two minutes of exposure at temperature of 42-44 °C. Symptoms were found after two days with a damage score of 1.4 on a scale of 5, after three days (Figure 1a). The highest temperatures (44-46 °C) had a significantly increased level of damage to 2.8. After five minutes of exposure, symptoms of sunburn necrosis were found after two days at 38-40 °C temperature, and by the last day of the observations the damage score was 2.8 (Figure 1b). The highest levels of damage were found three days after five minutes of exposure at temperature of 42-44 °C and 44-46 °C. At these temperatures, symptoms of sunburn necrosis were found one day after the exposure (Figure 1).

In the field no symptoms of sunburn were observed when berries were exposed for two minutes at the three lowest ranges of temperature (40-43, 43-45 and 45-48 °C). Sunburn necrosis symptoms were evident two days after 5 minutes of exposure at 40-43 °C and 43-45 °C. Treated berries reached the maximum level of damage after 4 days from the treatment (Figure 2a, b). The same evolution of the damage was observed also after 10 and 15 minutes of exposure at 40-43, 43- and 45-48 °C (data not shown), while at 45-48 °C for 5 minutes, light symptoms of sunburn necrosis were found just after one day from treatment (Figure 2a). Sections of grape berries stained with FDA showed clear bright fluorescent outlines of living mesocarp cells, while no stained area showed dead cells; some of these were killed as an effect of the treatments (Figure 3). Five minutes of exposure at 40-43 °C caused a cell mortality of 12.4%. At the same temperature, the increase of exposure time to 10 and 15 minutes caused an increase of cell mortality (13.0% and 15.9%, respectively) but the differences were significant only for the 15 minute treatment (Figure 4). An average sunburned area of 19.5% was observed at 43-45 °C with no differences among the times of exposure. Exposure at 45-48 °C caused a cell mortality of about 18% with statistical differences between the 3 exposure time treatments (Figure 4). Within the same value of exposure time significant differences were observed in cell mortality for the treatments 40-43 °C and 43-45 °C (Figure 4).

Under greenhouse conditions, sunburn necrosis started to occur when the berry surface temperature was maintained in a range of 42-44 °C for at least 2 minutes or at 38-40 °C for 5 minutes. In the field, severe symptoms of sunburn necrosis were also induced after 5 minutes of exposure at a temperature ranging from 40-43 °C. Our experiment however, does not clarify the role of light in the induction of the sunburn injury. Following the terminology of Schrader et al. (2001), sunburn necrosis was induced, in the field on cultivated apple trees, when the fruit skin temperature reached 52 ± 1°C for 10 minutes. The authors concluded that at this temperature the presence of light was not necessary to induce severe necrosis to apple fruit. Nagakawa et al (1960), working on Muscat of Alexandria grape, induced symptoms of sunburn necrosis simply by heating the berries at 35 °C for 3.5 hours, or at about 40 °C for 1 hour. The same symptoms, described by Nagakawa and co-authors (1960), were found in our experiment but the sunburn induction period was only of 5 minutes presumably for the concomitant presence of very high light. Rabinowitch et al. (1974) reported that typical sunscald injury was induced in mature-green tomato fruit under controlled conditions. Their induction periods were: 18 hours at 45.1°C, or 28 hours at 40.8°C. At these temperatures, light in the visible spectrum was found to be essential, as 13% of the fruit escaped injury in the dark.

CONCLUSIONS

Our data should provide insight into the response of grape berries to temperature and sunburn damage evolution. The exposure of berries at temperature of 36-38 °C appears to be effective to cause sunburn in greenhouse grown plants, while in the field this threshold was 4-6 °C higher.

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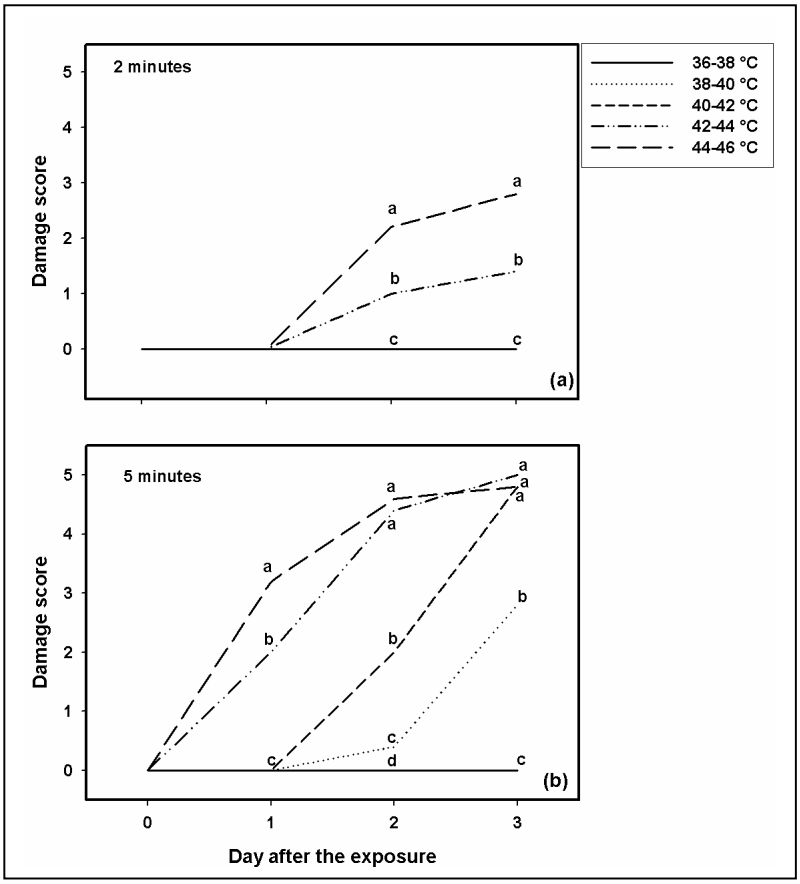

Figure 1. Sunburn damage score, in greenhouse grown Chardonnay vines, after 2 or 5 minutes of exposure at different temperatures. Data were collected at maturation on five single berries and observed by a panel of three people (n = 15) per each range of temperature. Different lowercase letters indicate significant differences ($p < 0.05$) within temperature range. Non significant differences were not reported.

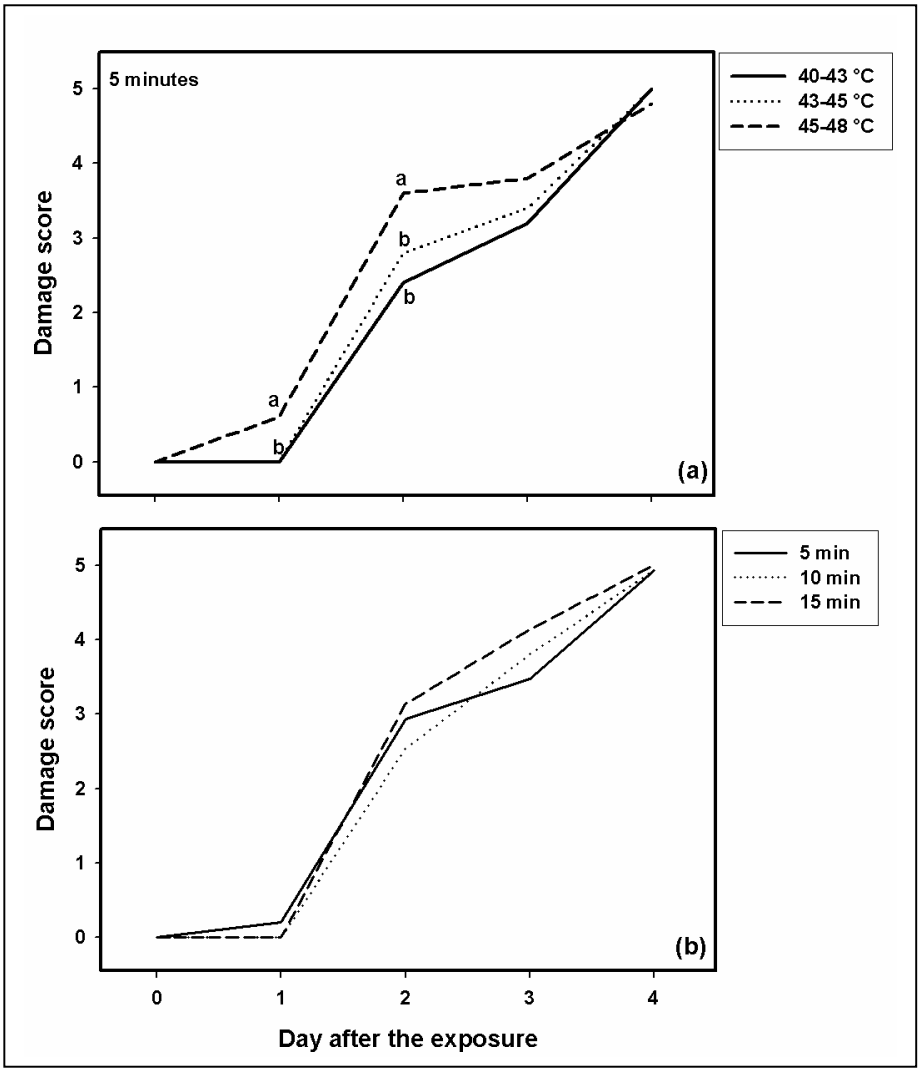

Figure 2. (a) Sunburn damage score at different temperatures. Data were collected at maturation in field-grown Chardonnay vines on five single berries (n = 15) per each range of temperature and time of exposure. In b) each data point represents the average of five single berries by three ranges of temperature and three observers (n = 45). Different lowercase letters indicate significant differences ($p < 0.05$) between temperature. Non significant differences were not reported.

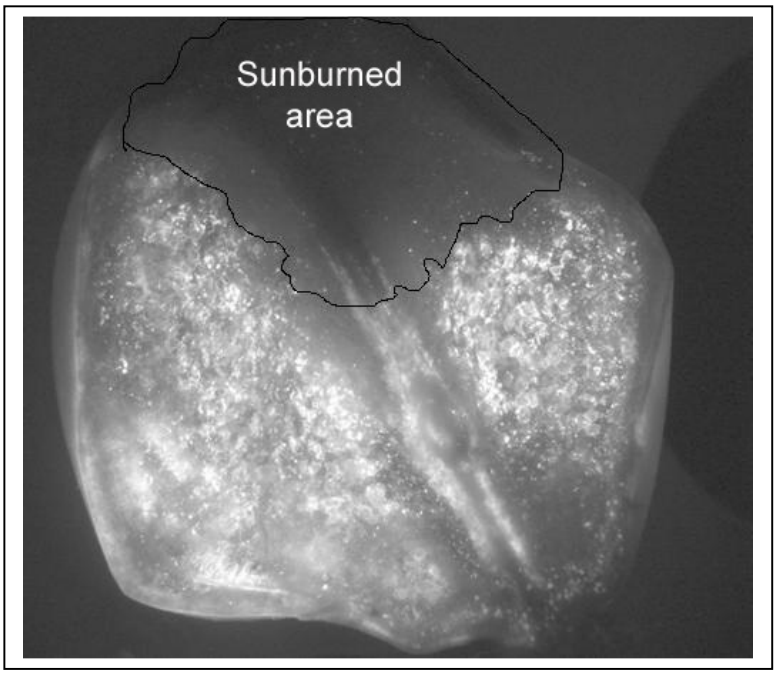

Figure 3. FDA fluorescence images of longitudinally sectioned Chardonnay (clone 29) berry, picked at maturation after four days after 5 minutes of exposure at 43-45°C. Sunburned area, traced by hand, is also showed.

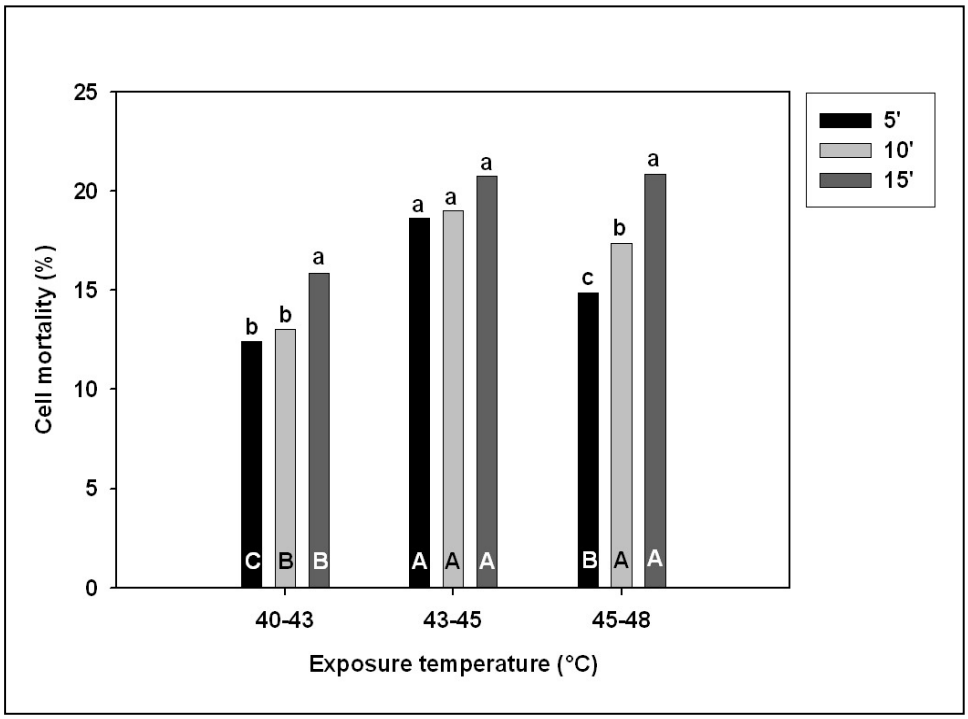

Figure 4. Field experiment. Percentage of sunburned area or cell mortality with respect to the total area of the berry. Different capitol letters indicate significant differences ($p < 0.05$) between temperature, different lowercase letters indicate significant differences ($p < 0.05$) between time of exposure.