

## Mobile device to induce heat-stress on grapevine berries

Kai Müller<sup>1</sup>, Manfred Stoll<sup>1</sup>, Marco Hofmann<sup>1</sup> and Matthias Friedel<sup>1</sup>

<sup>1</sup> Hochschule Geisenheim University, 65366 Geisenheim, Hessen, Germany

\*Corresponding author: [KaiKonstantin.Mueller@hs-gm.de](mailto:KaiKonstantin.Mueller@hs-gm.de)

**Keywords:** fruit surface temperature, heat-stress, sunburn, thermography, *Vitis vinifera*.

### Abstract

Studying heat stress response of grapevine berries in the field often relies on weather conditions during the growing season. We constructed a mobile heating device, able to induce controlled heat stress on grapes in vineyards. The heater consisted of six 150 W infrared lamps mounted in a profile frame. Heating power of the lamps can be regulated individually by a control unit consisting of a single board computer and six temperature sensors to reach a pre-set temperature. Sunburn necrosis symptoms were artificially induced with the aid of the device for *Vitis vinifera* L. cvs. Bacchus and Silvaner. A logistic regression model was derived from temperature data and visual sunburn assessment. The model showed a significant impact of the cultivar on the occurrence of sunburn necrosis symptoms and confirmed a higher susceptibility of Bacchus compared to Silvaner. Silvaner showed a higher chlorophyll and carotenoid concentration in berry skins and a lower skin weight compared to Bacchus, but sunburn susceptibility was not related to these parameters. The device allows the alteration of berry temperatures in a wide range of applications in the field, like phenotyping for heat tolerance. In combination with infrared thermography, the effects of controlled heat stress can be studied with a lower dependency on weather conditions.

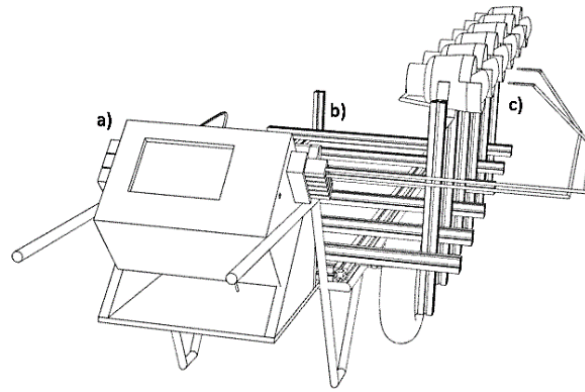
### Introduction

Heat damage on grapevine berries poses challenges for grape growers and breeders in the course of climatic changes with the expected increase in the frequency of heatwaves. Elevated temperatures during ripening lead to lower acidity, higher pH, higher sugar levels and lower anthocyanin content (Venios et al., 2020). Berries exposed to extreme heat and radiation develop symptoms of sunburn necrosis or sunburn browning (Gambetta et al., 2021). Their occurrence has not yet been linked to determined threshold temperatures of the berry surface, in contrast to other fruit such as apple (Schrader et al., 2001).

Several approaches have been conducted to study the effects of heat stress on grapevine berries. While some studies induced heat stress on a whole plant level, e.g. with potted plants in controlled environments (Carbonell-Bejerano et al., 2013; Hulands et al., 2014), others implemented stationary systems in the field using fans to alter berry temperature (Tarara et al., 2000) or increased bunch zone temperature with a construction consisting of polycarbonate sheets (Sadras & Soar, 2009).

Temperature of single berries within one cluster can vary significantly, depending on the environmental conditions and the cluster morphology. Therefore, measuring the temperature of a cluster on single points with e.g., thermocouples may lead to inaccurate measurements. Temperature hotspots or the distribution of berry temperatures within one cluster cannot be shown adequately. With the precise and non-invasive technology of thermal imaging, berry surface temperature can be measured leading to more information of the temperature distribution (Stoll & Jones, 2007).

Our goal was to establish a method to induce a reproducible heat stress on grapevine berries with less dependency on weather conditions by constructing a mobile device. By using infrared radiation as the source of heat, the modification of photosynthetic active radiation or the creation of artificial air convection is avoided.



**Figure 1.** Schematic drawing of the moveable heating device. a) Control Unit with touchscreen b) Modular profile system mounted on a long barrow c) Heating unit consisting of spots, lamps and temperature sensor.

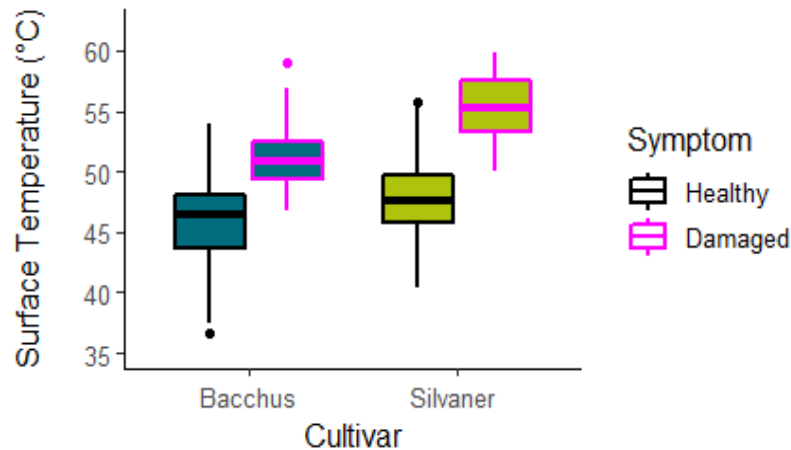
## Materials and Methods

A modular profile system (b), mounted on a long barrow supports six heating units (c) and allows for their positioning with three translational and two rotational degrees of freedom. Each heating unit consists of a 150 W infrared radiant heater mounted on spots and a PT1000 temperature sensor. The sensor provides the actual temperature (AT) and is placed in a central position within the cluster that is being heated. The AT is altered through the Control Unit (a) using a Proportional Integral Control algorithm to regulate the power supply to each heater individually by comparing AT with a desired temperature setpoint (TS). All temperature data and additional information are displayed on a touchscreen in real time and can be stored on the single-board computer running the processes of the Control Unit.

On the 7<sup>th</sup> of September 2021, clusters of the cultivars Bacchus and Silvaner (16.5–17.5 °Brix) were heated with TS set to 50 °C for 30 minutes (n=6). The berry surface temperature (BST) was measured by thermal imaging (InfRec R500EX, Fa. Nippon Avionics, JAP) immediately after heating was stopped and analyzed for single berries with the associated software. Sunburned berries were visually determined by digital images. Berry samples of heated, but not damaged and damaged berries from the same cluster were collected and immediately frozen in liquid nitrogen. Further on, berries were weighed on a fine scale, measured in height and length with a caliper, peeled under CO<sub>2</sub>-atmosphere and ground to a fine powder before freeze-drying. Of the freeze-dried powder, 35 µg of the berry skin were extracted in 100% acetone and used for UV-VIS spectroscopy. The derived contents of Chlorophyll (Chl) *a*, Chl *b*, total Chl and total Carotenoids were calculated as proposed by Lichtenthaler & Buschmann (2001) and converted to a pigment concentration in µg/mm<sup>2</sup>. For the conversion, the data derived from berry dimension measurements was used in the assumption of an ellipsoid berry shape. Statistical Analysis was performed using R statistical software. For determining statistical differences of threshold temperatures between the cultivars, a binary logistic regression ( $p < .001$ ) was carried out. A Principal Component Analysis (PCA) was performed to cluster cultivars to visualize the differences between cultivars and the relation of berry physical and chemical parameters with the occurrence of sunburn necrosis phenomena.

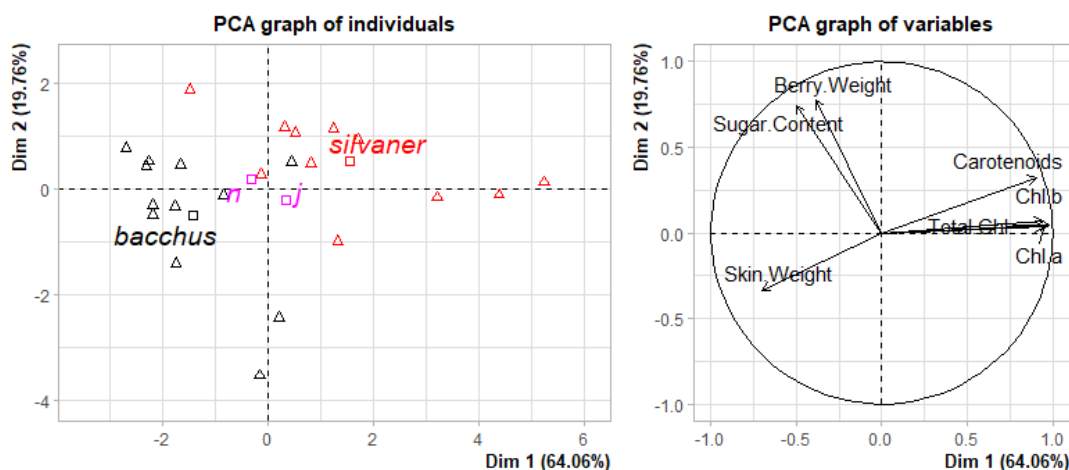
## Results and Discussion

A binary logistic regression was performed showing that the predictors Surface Temperature and Cultivar were significant predictors for the occurrence of sunburn on grapevine berries (Chi-Square = 248.52, df = 2,  $p < .001$ ). Surface Temperature and Cultivar showed significance at the level of 0.1% (*Surface Temperature*, Wald=83.30,  $p < .001$ ; *Cultivar* Wald=34.60,  $p < .001$ ). The odds ratio (OR) for Surface Temperature was 2.153 (97.5% CI 1.850 – 2.576) and 0.069 (97.5% CI 0.027 – 0.160) for Bacchus and Silvaner respectively. The odds of berries showing sunburn symptoms is 2.15 times higher with Surface Temperature increasing by 1°C and likewise 14.49 (1/OR) times higher for Bacchus compared to Silvaner (Figure 2). By predicting a 50% chance for a berry to develop sunburn symptoms within 30 min of heating (LD<sub>50</sub>) from the model, it was estimated that Bacchus approached LD<sub>50</sub> at 49.6 °C compared to Silvaner at 53.1 °C (data not shown).



**Figure 2.** Boxplots of Berry Surface Temperatures (BST) of two cultivars Bacchus and Silvaner, heated with the aid of the mobile device. Berries were divided in symptomatic and non-symptomatic for sunburn necrosis. A logistic regression indicated significance for the predictors Cultivar on the induction of sunburn symptoms (Wald = 34.60,  $p < .001$ )

The data obtained by pigment analysis and berry parameters is summarized by PCA in Figure 3, where the first two principal components (PCs) explain 83.8 % of the total variability. The first PC was strongly associated with the factor cultivar, while the second PC was rather associated with the presence of sunburn symptoms. Individuals of cv. Silvaner showed higher values in variables Chl *a*, Chl *b*, Total Chl and Carotenoids and lower values in sugar content (° Oechsle) and berry skin weight compared to cv. Bacchus. Bacchus had a thicker berry skin (higher skin weight at equal berry weight), which is in accordance with Alleweldt et al., (1981) who reported a relatively thick epidermis and hypodermis for Bacchus compared to other cultivars. Hence, skin thickness seems to be unrelated to sunburn susceptibility and is not a suitable parameter for heat tolerance phenotyping. Sunburnt berries within the individual cultivars had higher concentrations of sugars and pigments and a lower berry weight, rendering it unlikely that the pigment concentration itself may be related to heat tolerance. Within-cultivar heat tolerance rather seemed to be related to the phenological stage, with heat tolerance increasing during the ripening phase. This is in line with the literature (reviewed by Gambetta et al., 2021).



**Figure 3.** Left: PCA with individuals factor map showing the individuals colored after their category for the variable cultivar. Right: PCA graph of variables showing the loadings of the seven observed parameters.

## Conclusion

The reproducible manipulation of berry temperature in the field poses major challenges. The mobile device presented in this study allows the alteration of berry temperatures in a wide range of applications in the field. With the possibility of setting a target temperature in combination with infrared thermography, the effects of controlled heat stress can be studied in more detail. The influence of the cultivar on the susceptibility to sunburn

necrosis was demonstrated to be significant by comparing berry surface temperatures of healthy and damaged berries when a reproducible heat stress was applied. A PCA indicated that berry skin thickness and pigment concentration in the skins seemed to be unrelated to the heat-tolerance of berries, but susceptibility seemed to increase with sugar accumulation. The presented device may aid as a tool for phenotyping for heat tolerance when reproducible conditions are required.

## References

- Alleweldt, G., Engel, M., & Gebbing, H. (1981). Histologische Untersuchungen an Weinbeeren. *Vitis*, Vol. 20
- Carbonell-Bejerano, P., Santa María, E., Torres-Pérez, R., Royo, C., Lijavetzky, D., Bravo, G., Aguirreolea, J., Sánchez-Díaz, M., Antolín, M. C., & Martínez-Zapater, J. M. (2013). Thermotolerance Responses in Ripening Berries of *Vitis vinifera* L. cv Muscat Hamburg. *Plant and Cell Physiology*, 54(7), 1200–1216. <https://doi.org/10.1093/pcp/pct071>
- Gambetta, J. M., Holzzapfel, B. P., Stoll, M., & Friedel, M. (2021). Sunburn in Grapes: A Review. *Frontiers in Plant Science*, 11, 604691. <https://doi.org/10.3389/fpls.2020.604691>
- Hulands, S., Greer, D. H., & Harper, J. D. I. (2014). The Interactive Effects of Temperature and Light Intensity on *Vitis vinifera* cv. ‘Semillon’ Grapevines. II. Berry Ripening and Susceptibility to Sunburn at Harvest. *Europ.J.Hort.Sci*, 79(1), 1–7.
- Lichtenthaler, H. K., & Buschmann, K. (2001). Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy. *Current Protocols in Food Analytical Chemistry*
- Sadras, V. O., & Soar, C. J. (2009). Shiraz vines maintain yield in response to a 2–4°C increase in maximum temperature using an open-top heating system at key phenostages. *European Journal of Agronomy*, 31(4), 250–258. <https://doi.org/10.1016/j.eja.2009.09.004>
- Schrader, L. E., Zhang, J., & Duplaga, W. K. (2001). Two Types of Sunburn in Apple Caused by High Fruit Surface (Peel) Temperature. *Plant Health Progress*, 2(1), 3. <https://doi.org/10.1094/PHP-2001-1004-01-RS>
- Stoll, M., & Jones, H. G. (2007). Thermal imaging as a viable tool for monitoring plant stress. *OENO One*, 41(2), 77. <https://doi.org/10.20870/oeno-one.2007.41.2.851>
- Tarara, J. M., Ferguson, J. C., & Spayd, S. E. (2000). A Chamber-Free Method of Heating and Cooling Grape Clusters in the Vineyard. *American Journal of Enology and Viticulture*, 51(2), 182.
- Venios, X., Korkas, E., Nisiotou, A., & Banilas, G. (2020). Grapevine Responses to Heat Stress and Global Warming. *Plants*, 9(12), 1754. <https://doi.org/10.3390/plants9121754>