DO HIGH TEMPERATURE EXTREMES IMPACT BERRY TANNIN COMPOSITION?

Authors:Julia GOUOT[1,2*], Jason SMITH[1,3], Bruno HOLZAPFEL[1,4], Celia BARRIL[1,2]

[1]*National Wine and Grape Industry Centre, Wagga Wagga, New South Wales, 2678, Australia*
[2]*School of Agricultural and Wine Sciences, Charles Sturt University, Wagga Wagga, New South Wales, 2678, Australia*
[3] *New South Wales Department of Primary Industries, Orange, New South Wales, 2800, Australia*
[4]*New South Wales Department of Primary Industries, Wagga Wagga, New South Wales, 2678, Australia*

*Corresponding author: *jgouot@csu.edu.au*

Abstract:

Context and purpose of the study - Flavonoids, including flavonols, anthocyanins, and tannins, are important contributors to grape and wine quality, and their biosynthesis is strongly influenced by bunch microclimate. While the synergistic effect of light and temperature has been intensively examined on flavonoids in relation to bunch exposure, studies targeting the sole effect of high temperature have mostly focused on anthocyanins during the ripening period. With tannin biosynthesis starting around flowering, heatwaves occurring earlier in the grape growing season could be critical. Only a few papers report the impact of temperature on tannin synthesis and accumulation; to date, none have examined the effect of high temperature extremes which, in the context of climate change, relates to increases in heatwave intensity.

Material and methods - Three potted-vine experiments were conducted inside a UV-transparent glasshouse during the 2016-17 and 2018-19 seasons. Using fans blowing hot air onto individual bunches without affecting light exposure, several temperature-related parameters were tested on well-irrigated Shiraz vines. In order, these examined high day and/or night temperatures after fruit set (E-L 31, Coombes, 1995), day temperature intensities (Low: LT, High: HT and Very High: VHT) and durations (3 to 39 h) after véraison (E-L 36, ~10 °Brix), and high day temperature at two phenological stages (E-L 31 and/or E-L 36). Berries were sampled at regular intervals, peeled, ground, and skin and seed tannin composition individually analysed by LC-MS/MS after phloroglucinolysis.

Results - During Experiment 1, heat treatments were applied for three days (+8 °C) and/or three nights (+6 °C), with day maximum temperature reaching 44.8 °C and night maximum temperature reaching 32.8 °C. Berry size was immediately affected by day temperature, while skin tannin exhibited small differences with an increase in percentage of galloylation 15 days after the end of the treatment. During Experiment 2, LT, HT and VHT respectively reached a maximum of 37, 45, and 53 °C. VHT considerably impacted on berry physiology and composition, regardless of the treatment duration (12 or 30 h), leading to berry desiccation. Tannins extracted from the dried skin were significantly reduced with some flavan-3-ol subunits proportionally more degraded than others. While the effect on skin was substantial, seed tannins were only slightly affected. Night temperature at E-L 31 (Experiment 1) and day HT at E-L 36 (Experiment 2) affected other primary metabolites but not tannin composition. Experiment 3, conducted during the 2018-19 season, combined parameters for which tannin composition was affected during season 2016-17 to confirm observed trends.

Keywords: Berry composition, Bunch heating, Day, Heat stress, High temperature, Phenological stage, Tannins.

1. Introduction.

DO HIGH TEMPERATURE EXTREMES IMPACT BERRY TANNIN COMPOSITION?

Julia GOUOT[1,2], Jason SMITH[1,3], Bruno HOLZAPFEL[1,4] and Celia BARRIL[1,2]

[1] National Wine and Grape Industry Centre, Wagga Wagga, Australia.
[2] School of Agricultural and Wine Sciences, Charles Sturt University Wagga Wagga, Australia.
[3] New South Wales Department of Primary Industries, Orange, Australia.
[4] New South Wales Department of Primary Industries, Wagga Wagga, Australia.

Introduction

Flavonoids, including flavonols, tannins and anthocyanins, are important contributors to grape and wine quality, and their biosynthesis is strongly influenced by bunch microclimate. While the synergistic effect of light and temperature on flavonoids has been extensively examined in relation to bunch exposure, studies targeting the sole effect of high temperature have mostly focused on anthocyanins during the ripening period [1]. With tannin biosynthesis starting around flowering, heatwaves occurring earlier in the grape growing season could be critical. To date, no studies have examined the effect of high temperature extremes on tannins. In the context of climate change, this would relate to increases in heatwave intensity. Skin and seed tannins differ in size (mean Degree of Polymerisation, mDP), composition (% of galloylation) and subunit concentration: catechin (C), epicatechin (EC), epicatechin gallate (ECG), gallocatechin (GC) and epigallocatechin (EGC) (Fig. 1) [1,2].

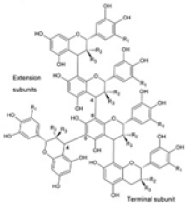
Materials & Methods

Three experiments were conducted inside a UV-transparent glasshouse during the 2016-17 and 2018-19 seasons (Fig. 2). Using fans blowing hot air onto individual bunches without affecting light exposure [3], the effect of temperature on a range of berry growth and composition parameters was tested on well-irrigated potted Shiraz vines:

Parameters	Tissue	
	Skin	Seed
Average mDP	20-30	10
Average galloylation	5%	10 - 20%
Extension subunits*	EC, EGC, ECG, C	EC, ECG, C
Terminal subunits*	C, EC, GC, ECG	C, EC, ECG

*Subunits in order of abundance

Figure 1 Skin and seed tannin compositions and tannin structure

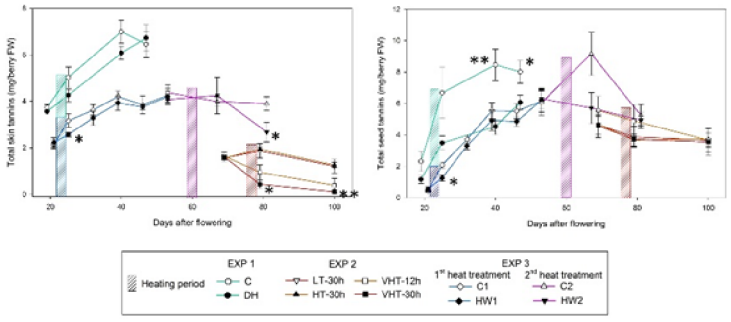


- EXP 1: high day and/or night temperatures 20 days after flowering (E-L 31)
- EXP 2: day temperature intensity (Low: LT, High: HT, Very High: VHT) and duration (3 to 39 h) 15 days after the onset of véraison (E-L 36, ~10 °Brix)
- EXP 3: high day temperature at two phenological stages (E-L 31 and/or E-L 35/36)

In all experiments, berry samples were peeled, ground, and freeze-dried skin and seeds were analysed by LC-MS/MS to determine detailed tannin composition (after phloroglucinolysis) [4].

Figure 2. Graphical summary of the three experiments

Results



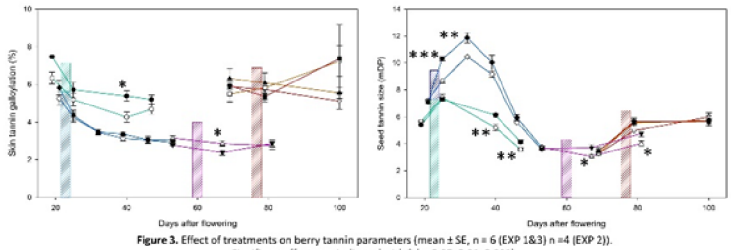


Figure 3. Effect of treatments on berry tannin parameters (mean ± SE, n = 6 (EXP 1&3) n =4 (EXP 2)). Significant effects are indicated with * (p<0.05, 0.01, 0.001).

During EXP 1, heat treatments were applied for three days (+8 °C) and/or three nights (+6 °C), with day maximum temperature reaching 44.8°C and night maximum 32.8 °C. Berry size was immediately decreased under high day temperature and, while skin tannins only exhibited small differences 2 weeks after treatment application, seed tannins were considerably reduced (Fig. 3). During EXP 2, LT, HT and VHT respectively reached a maximum of 34.6, 46.0, and 52.9 °C. VHT considerably impacted on berry physiology and composition, regardless of the treatment duration (12 or 30 h). This led to berry desiccation, and tannins extracted from the dried skin were significantly reduced. While the effect on skin was substantial, seed tannins were only slightly affected. Night temperature at E-L 31 (EXP 1) and HT compared to LT at E-L 36 (EXP 2) did not significantly impact on skin and seed tannin composition. During EXP 3, the 1st heat treatment, with day maximum temperature reaching 40 °C, slightly reduced skin and seed tannins while the 2nd heat treatment, with day maximum temperature reaching 45 °C, led to berry desiccation and a subsequent decrease in skin tannins but no long-term effect on seeds.

When content was affected by high temperature, changes in composition were also sometimes observed. For e.g., in the skin, during EXP 1, the percentage of galloylation (proportion of ECG subunits) was slightly increased around F+40. Contrasted results were observed after véraison with decrease in galloylation (EXP 3). When significantly impacted, size (mDP) of seed tannins was increased in all cases (Fig. 3).

Conclusion

For some treatments, tannin accumulation for well irrigated Shiraz grapevines was only affected for a short period of time following 3 days of high temperature exposure. In most cases, tannin contents were lower in heated berries, but most differences were no longer evident by harvest unless the berries were visually damaged and completely desiccated. In this case, skin tannins were dramatically reduced but seed tannins were preserved. Differences in berry tannin concentration and composition were most likely due to a combination of berry development disruption (berry development slowed with less skin surface and smaller and greener seeds) as well as a potential deregulation of some genes involved in tannin biosynthesis. To confirm the last hypothesis, further work is needed as most genes involved in galloylation and polymerisation are still unknown.

Acknowledgements: This work was supported by the National Wine and Grape Industry Centre and funded by Charles Sturt University. Travel was funded by Wine Australia, National Wine and Grape Industry Centre and Charles Sturt University. The authors acknowledge David Foster, Khoi Nguyen, Jack Zyhalak, Nikolaos Kontoudakis, Michael Loughlin, Vincent West & Lucas Filannino for technical assistance.

References:
[1] Gouot *et al.* (2018) Journal of Experimental Botany, 70, 397-423
[2] Rousserie *et al.* (2019) Journal of Agricultural and Food Chemistry, 67, 1325-1343
[3] Tarara, J. *et al.* (2000) American Journal of Enology and Viticulture, *51*, 182-188
[4] Pinasseau *et al.* (2016) Molecules, 21(10), 1409.