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NEW SOUTH WALES Wine

What management strategies are most effective in minimising sunburn damage in Chardonnay vineyards around Orange?



FINAL REPORT FOR INCUBATOR PROJECT

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1. Abstract

Sunburn damage manifests as a browning of the berry skin, and depending on the severity of the damage can lead to cracking and shrivelling of the grapes which reduces the visual and aromatic quality of the grapes causing economic losses to grape and wine producers as a result losses in yield and wine quality. In areas where leaf removal is a common practice, better understanding of the best moment to conduct this practice could help mitigate this problem. During the 2018-19 season the effect of leaf removal timing on sunburn incidence and on the accumulation of photoprotective compounds was evaluated on Chardonnay grapes from 2 vineyards located at different altitudes in the Orange region. Leaf removal led to higher final concentrations of all photoprotective compounds when compared to the non-defoliated control. Marked differences were observed in the accumulation patterns between berries defoliated early (end of flowering) and those defoliated later (véraison), with earlier accumulation, and thus better acclimation in those that were exposed early. Ultimately, sunburn damage was higher in grapes defoliated at véraison. There were no significant differences between yield, total soluble sugars, pH, titratable acidity and YAN between treatments within each vineyard.

2. Executive Summary

Leaf removal is a necessary practice in cool climate vineyards to decrease disease pressure. However, this practice can also overexpose berries and lead to sunburn, particularly in the event of heatwaves. Sunburn damage is linked to the accumulation of melanin-like pigments and degradation of chlorophyll. When exposed to sunlight, particularly UV-A and UV-B radiation, grapes also produce flavonol compounds (i.e. quercetin glycosides) and carotenoids which have been shown to be involved in UV screening and act as photoprotective compounds.

This research project evaluated the impact of leaf removal timing (no defoliation, defoliation after the end of flowering and defoliation at véraison) on the severity of sunburn symptoms in Chardonnay grapes from two vineyards located at different altitudes in the Orange region. The accumulation of photoprotective compounds was also assessed. No significant differences were observed in relation to pH, titratable acidity, total soluble solids and YAN between treatments within each vineyard. Results showed a higher incidence of sunburn damage on berries that were defoliated after véraison when compared to those where leaf-removal occurred earlier in the season, after the end of flowering. Higher final concentrations of both flavonoids and carotenoids were observed in berries that had been exposed to light (through both early and late defoliation) than in the non-defoliated controls. Although no significant differences were found in the concentration of most of the evaluated photoprotective compounds at harvest between the early and late defoliated treatments, marked differences were observed in the pattern of accumulation – concentration of these compounds rose rapidly in berries defoliated at flowering and remained high throughout the rest of the season - which appear to have led to better acclimation and a lower degree of sunburn in the berries that were exposed at the end of flowering. 2019 was a particularly harsh season which broke most of the national records for the month of January. When ripening occurred mainly during this month (lower vineyard), higher sunburn damage was observed. An app (RotBot) was also tested to determine whether image analysis could be used as an objective tool to evaluate sunburn damage. Results showed that image analysis is a valuable tool that could be used by producers after further calibration of the algorithm. In areas at risk of sunburn where defoliation is a common practice, the results suggest that performing leaf-removal as soon as the end of flowering would help protect berries from sunburn without affecting yield and sugar accumulation.

3. Aims and Background

Sunburn damage commonly appears as browning of the berry surface when these are directly exposed to sunlight in combination with high temperatures and ultraviolet radiation (Gregan et al., 2012; Hofmann et al., 2006; Rustioni et al., 2015, Rustioni et al., 2014). Under severe conditions it can lead to complete berry shrivelling (Australian Wine Research Institute, 2015). Sunburn also results in the appearance of off-odours and oxidised characters in the wine. Appearance of symptoms is modulated by variety, berry developmental stage, ambient temperature, water availability and viticultural practices (i.e. row orientation, training system and defoliation) (Gregan et al., 2012; Hofmann et al., 2006; Hulands et al., 2014; Rustioni et al., 2015, Rustioni, et al. 2014).

Leaf removal increases air penetration and sunlight exposure to reduce disease pressure (Australian Wine Research Institute, 2015; Gregan et al., 2012; Mosetti et al., 2016). Depending on how and when this practice is conducted, the effects of leaf removal can be variable and can even lead to a higher degree of sunburn if bunches are overexposed. Previous research indicates that if performed before véraison, defoliation leads to an early accumulation of flavonoids and carotenoids which would protect the berry from UV damage and reduce the intensity of sunburn (Hofmann et al., 2006; Joubert et al., 2016; Merzlyak et al., 2002; Price et al., 1995; Solovchenko and Schmitz-Eiberger, 2003) but evidence is lacking. Additionally, assessments of sunburn damage are based exclusively on visual inspection of the berries. This makes comparison of damage difficult between sites and any grading of fruit highly subjective.

The aim of this project was to compare the impact of leaf removal timing (post-flowering vs. véraison) on the appearance and severity of sunburn symptoms in Chardonnay grapes. It also aimed to determine whether performing early leaf removal increases photoprotective compound production and its impact on grape composition, and whether image analysis could be used to evaluate objectively the severity of sunburn damage.

4. Materials and Methods

4.1 Experimental design

Field trials were conducted over the 2018-2019 season in two commercial vineyards; Cumulus (32°58’47.4” S, 148°57’42.8” E, 612 m., CUM) and Balmoral (33°16’03.9” S, 148°59’59.0” E, 884 m., BAL), located at two different elevations (612 m. and 884 m. respectively) in the Orange region, New South Wales, Australia. The trial used Chardonnay grapevines (clone 110V1) planted in 2000 on own roots (Cumulus, CUM) and grafted onto Shiraz in 1995 (Balmoral, BAL). Row orientation was North-South, with 3.0 m between-row and 2.0 m in-row spacing for both sites. The vines were trellised to a double cordon with a vertical shoot-positioning system. Both sites featured red brown earth soil (Northcote, 1979). Vines were drip irrigated in 3 hour intervals as needed.

Treatments were comprised of a control (no leaf removal, T1), an early leaf-removal treatment (EL27, T2, Coombe, 1995) and a late leaf removal treatment (EL35, T3). Early leaf removal was performed on the 6th of December 2018 at both vineyards, and late leaf removal on the 8th of January 2019 in Cumulus and 23rd of January in Balmoral. Consistent with local practice, all leaves and lateral shoot were removed in the fruiting zone (45 cm above the cordon) on the east side of the canopy. The fruiting zone of T2 and T3 was kept exposed for the duration of the trial through continuous leaf-removal. Treatments were laid out in a completely randomised arrangement across three rows, separated by two buffer rows. Each replicate included six vines, with sampling constrained to the four central vines, leaving the two external vines as buffers. All treatments were harvested on the 4th of February 2019 in Cumulus and on the 1st of March 2019 in Balmoral.

4.2 Climatic monitoring

Canopy mesoclimate (temperature and relative humidity) was assessed every 10 minutes with a dual-channel logger (TinyTag TGP-4500; Hastings Data Loggers, NSW, Australia) located in the middle of the canopy. Ultraviolet radiation and photosynthetically active radiation (PAR) were measured hourly using a UV sensor (Apogee, Campbell Scientific Australia Pty Ltd, Queensland, Australia) and a sun quantum sensor (Apogee, Campbell Scientific Australia Pty Ltd). UV and quantum sensor signals were scanned and recorded by two data acquisition systems (AM-25T and CR-1000, Campbell Scientific Australia Pty Ltd) from EL29 until harvest. Sunlight interception in the bunch level was measured using a light sensitive tape (Optoleaf Y-1W, Taisei Fine Chemical Co., Ltd, Tokyo, Japan).

4.3 Measurements and sample and data collection

Vine leaf area was calculated as the sum of the area of all the leaves of each vine. To this effect 10 shoots from three plants from each row were collected, defoliated and the number of leaves counted. The leaf area index (LAI) for the ten shoots was then calculated using a LI-3100C leaf area meter (LICOR, Nebraska, USA).

Treatments were harvested at commercial maturity (~21 °Brix) and yield per plant was estimated by counting and weighing all bunches in each plant. Fifteen bunches per repetition and treatment, chosen aleatory, were reserved for image collection. Sunburn damage was visually evaluated on samples from the Balmoral vineyard by counting all affected berries and grading according to sunburn level (L0-L2). The total number of berries of these bunches were also counted.

Berry samples (n = 170 berries) were collected every two weeks starting at EL29 until commercial harvest (EL38, ~21 °Brix) only from the exposed face of bunches on the eastern side of the canopy. Fifty berries (transported on ice) were used to determine basic chemical parameters which included fresh weight per berry, total soluble solids (TSS), pH and total acidity (TA) and ammonium and α-amino acid concentrations. Total soluble solids were measured using a digital refractometer (PR-101, Atago, Tokyo, Japan) and total acidity and pH were measured using an automatic titrator (Metrohm Fully Automated 59 Place Titrando System, Metrohm AG, Herisau, Switzerland) to an end point of pH 8.2. The ammonium and α-amino acid concentrations of the juice were determined using a commercially available enzymatic assay kit, designed for an Arena discrete analyser (Thermofisher, Scoresby, Australia). The yeast assimilable N (YAN) concentration was subsequently calculated from the ammonium and free amino N (FAN) (Iland et al., 2004).

The remainder sample were immediately flash frozen in the field using liquid nitrogen and were used for carotenoid, total polyphenol and flavonoid analysis. Samples for carotenoid and total polyphenol analysis were deseeded and ground under liquid nitrogen (A11 basic analytical mill, IKA, Selangor, Malaysia). One gram of frozen grape berry powder were used for carotenoid determination. Extraction and analysis was performed as outlined by Wehrens et al. (2013) and (Young et al., 2016). Total polyphenol content was determined according to Downey and Rochfort (2008) using 1 g of previously ground sample. Absorbance was determined at 280 and 354 nm using a benchtop spectrophotometer (Helios (α) UV-Vis spectrophotometer, Thermo Electron, Osterode, Germany). Flavonoid content was determined using the skin of twenty berries, previously freeze-fried and ground, according to Gouot et al. (2019). Analysis was carried out on an Agilent LC system consisting of a binary pump G1312B, a HiP autosampler G1367E (maintained at 8 °C), a diode array detector 6412B and a triple quadrupole tandem spectrometer (QQQ).

Vine leaf area was calculated as the sum of the area of all the leaves of each vine. To this effect 10 shoots from three plants from each row were collected, defoliated and the number of leaves counted.

Leaf area for the ten shoots was then calculated using a LI-3100C leaf area meter (LICOR, Nebraska, USA).

4.4 Statistical analysis

Data was analysed using Matlab and R. One-way and two-way ANOVA and Tukey's HSD test was used to identify significant differences between means ($P < 0.05$). Images were then processed using Rotbot to evaluate percentage of damage (Hill et al., 2014).

5. Results and Discussion

5.1 Climatic monitoring

2019 was an exceptionally hot year in all of Australia, including the Orange region, and summer 2019 was named the warmest on record ("Heat wave events headline a record warm summer for Australia," 2019). Figure 1 shows temperatures recorded in the canopy level (every 10 min.) using a Tinytag datalogger. The Cumulus and Balmoral vineyards experienced a maximum temperature of 46.3 and 41.3 °C respectively on January 16th. Given how temperature exacerbates sunburn symptoms, this is of particular relevance to the final results obtained. In all, Cumulus experienced 40 days of the growing season over 35 °C and 17 days over 40 °C, while the Balmoral vineyard saw temperatures rise above 35 °C during 23 days and over 40 °C, during 2 days. Other important climatic parameters such as total radiation, average daily maximum solar radiation and rainfall are listed in Table 1. The 2018-19 vintage was a particular dry one, which only saw an average of 69 mm of rainfall at both sites.

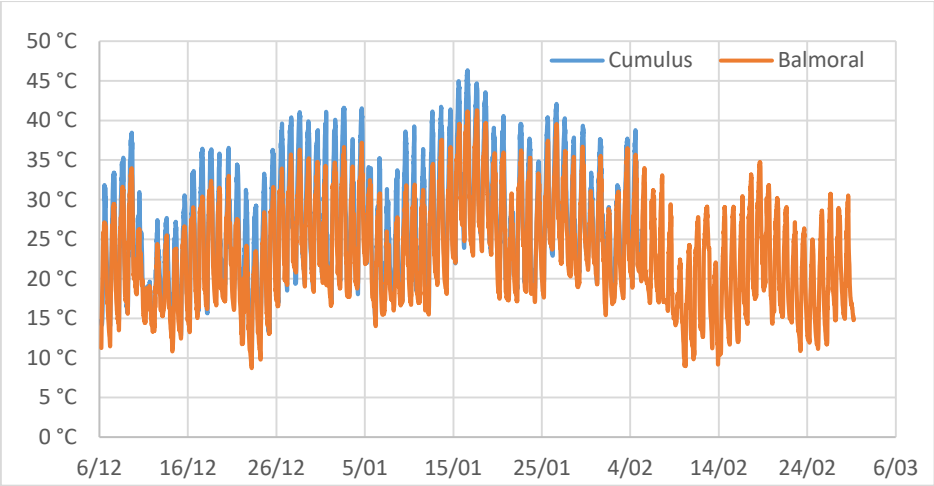


Figure 1. Canopy temperature measures between December 2018 and harvest (February 2019 (CUM) and March 2019 BAL) at two vineyard sites.

UV radiation (250-400 nm) was monitored on a daily basis in both vineyards between the end of flowering and harvest. Contrary to what was expected, daily UV average values were higher at the Cumulus vineyard that is located at a lower altitude than Balmoral. Differences over the entire growing season were not considered to be significant. Although both sites had drip irrigation installed, stem water potential measurements revealed that plants at both sites were in a moderate to severe water stress deficit at the beginning of the season which progressed into a more severe stress by harvest. However there were no significant differences in the level of stress between plants in a same vineyard despite leaf removal treatments. It must be noted that high levels of water stress exacerbate the incidence of sunburn (Australian Wine Research Institute, 2015).

Table **1.** Monthly total radiation, average daily maximum solar radiation, average temperature and rainfall from December 2018 until January 2019

Month	Total Radiation (mol/m^2)	Average daily max. solar radiation (µmol/m^2.s)	Avg. temp (°C)	Rainfall (mm)
BALMORAL				
Dec	1241	2039	21.9	91
Jan	1590	2028	26.0	68
Feb	1378	1943	20.9	48
CUMULUS				
Dec	1296	2034	24.1	61
Jan	1369	1880	28.2	69
Feb	226	1804	25.6	75

5.2 Visual evaluation of sunburn symptoms

Fifteen bunches from all repetitions of T1-T3 were collected at Balmoral during harvest and inspected visually for sunburn symptoms. Sunburn damage causes the appearance of melanin-like pigments and manifests as browning of the berry skin (Rustioni et al., 2014). The browning intensity of BAL samples was classified on a scale ranging from 0 to 2 (Fig. 2), and all berries in each category were counted (Table 2). These results were later compared to those obtained by using the computer app "RotBot" (Hill et al., 2014). RotBot analyses the pixel distribution of a given image for a particular hue value. Different hue values were assayed in order to improve classification.

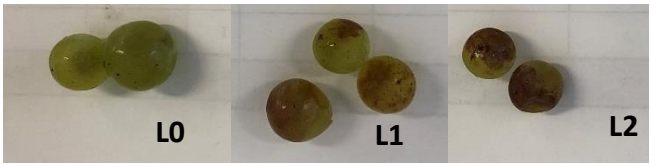


Figure 2. Berries catalogued according to sunburn damage intensity (L0-L2)

Appendix 2 provides images of representative bunches in each treatment and vineyard. As can be observed from the images (Fig. S1 and S2), samples that were not defoliated (Fig. S1A) experienced in general the least amount of sunburn, as long as these possessed good leaf covering. All exposed bunches from CUM, including non-defoliated control samples suffered varying degrees of sunburn (Fig. S1A and B).

Table 2. Classification of berries at harvest according to sunburn damage, Balmoral vineyard

Treatment	%L1	%L2	Sunburnt berries (L1 and L2, %)	RotBot (%)
T1	13	2	15	24
T2	26	8	34	48
T3	18	26	44	55

Visual inspection of BAL bunches (Table 2) revealed that T3 berries had a higher proportion of berries (26%) with darker pigmentation (L2), and overall higher proportion of sunburnt berries (44%) than T1 and T2. These results were then compared to those obtained using RotBot, and a maximum correlation value (R^2) of 0.47 was obtained. A better correlation (R^2=0.56) when only L2 was considered. Given that RotBot was initially programmed to detect botrytis affected bunches, and that no change was made to the algorithm, these results were considered encouraging. These moderate correlation values were mainly due to misclassifications of healthy grapes.

Comparison of all images from both vineyards and RotBot results, showed that a higher percentage of berries burned to a higher intensity when leaf removal was performed at véraison (55% BAL and 73% CUM, Fig. S1C and S2C) rather than earlier, at the end of flowering (48% BAL and 70% CUM, Fig. S1B and S2B). Sunburn incidence was higher in the Cumulus vineyard than in Balmoral, which was to be expected considering overall temperatures at both vineyards during the ripening period (Fig. 1). Berries react differently to high temperatures according to maturity level, and are more susceptible to sunburn once they start softening (Hulands et al., 2014). Véraison at CUM occurred on the 7th of January whilst it occurred on the 23rd of January in BAL. As a consequence, berries in CUM softened when temperatures were at their highest, which led to higher overall sunburn incidence in CUM defoliated samples than BAL, as well as a higher degree of desiccation in T3 samples (Fig. S1). As noted by Hulands et al. (2014), there is a high correlation between the level of sunburn damage and light intensity, temperature and maturity level. These authors observed, that berries at mid-stage of development, when treated with a light intensity of 660 µmol m^{-2} s^{-1} exhibited more sunburn damage at 38 °C (50% damage) than at 30 °C (8% damage). Ripe berries incurred an even higher degree of damage, when temperatures reached 38 °C, these researchers reported a 90% sunburn damage.

5.3 Leaf area index (LAI) and yield

LAI was calculated for both vineyards and all treatments. Leaf area of non-defoliated treatments was 11.1 and 8.0 m^2 for BAL and CUM respectively (Table 3). This was calculated to be roughly one third higher that of the defoliated treatments (T2 and T3) which had average LAIs of 8.05 (BAL) and 5.7 cm^2 (CUM).

As experienced by others previously (Kliewer and Dokoozlian, 2005), leaf removal, regardless of when it was conducted, did not significantly affect bunch weight or yield (Table 3) at harvest, indicating that the remaining leaf area (1.4-1.8 m^2/kg) was sufficient to ripen the fruit to the desired 21-22 °Brix. Only the number of bunches per plant was affected by the treatments at the Balmoral vineyard (Table 3) where early defoliation of the canopy zone appears to have improved fruit setting by 17%. There were no significant differences between both vineyards in regards to yield at harvest, despite the lower bunch weight of the fruit at CUM. This is due to a higher bunch load in this vineyard. Bunch weight was significantly lower (-29%) than that of BAL, partly due to the higher temperatures and higher cropping rates.

Table 3. Mean values of bunch number, bunch weight, yield and leaf area index (LAI) per plant and LAI per kg of fruit for both sites at harvest

	No. of bunches/vine	**Bunch weight (g)**	**Yield (kg)**	**LAI (m^2)**	**LAI/kg fruit (m^2/kg)**
BALMORAL					
T1	36 ± 3a*	130 ± 16	4.49 ± 0.78	11.1 ± 3.3	2.45 ± 0.43b
T2	40 ± 2ab	120 ± 160	4.84 ± 0.96	8.6 ± 2.6	1.77 ± 0.37ab
T3	42 ± 1b	130 ± 32	5.25 ± 1.30	7.5 ± 2.0	1.42 ± 0.16a
CUMULUS					
T1	50 ± 12	90 ± 20	4.55 ± 1.15	8.0 ± 0.6	1.81 ± 0.33
T2	42 ± 6	90 ± 20	3.85 ± 1.60	5.6 ± 1.3	1.72 ± 1.05
T3	51 ± 6	90 ± 20	4.45 ± 1.16	5.8 ± 1.8	1.43 ± 0.68

*Means within each column followed by different letters are statistically different at p≤ 0.05, within a same vineyard. Values within a column not followed by any letters are not significantly different.

5.4 Basic chemical parameters

Samples were harvested at commercial maturity (~21-22 °Brix, Table 4) on February 7th at Cumulus and March 1st at Balmoral. Despite the removal of leaves, there were no significant differences in berry weight, pH, TA, TSS and YAN between treatments within a same vineyard. As observed in previous studies (Young et al., 2016), leaf removal does not appear to affect individual sugars and acids. However, significant differences were observed between both vineyards for TSS, TA and YAN. The fruit at BAL was harvested at a slighter higher final TSS (~22.4 °Brix) than at CUM (~21.4 °Brix). As a result of the higher temperatures (Sweetman et al., 2014) experienced at CUM (Fig. 1), TA at harvest was significantly lower than that of BAL samples (Table 4).

Table 4. Mean values of berry weight, pH, titratable acidity (TA), total soluble solids (TSS) and yeast assimilable nitrogen (YAN) for all treatments at harvest from the Balmoral and Cumulus vineyards.

	50 berries weight (g)			pH			TA (g/L)			TSS (°Brix)			YAN (mg/L)		
BALMORAL															
T1	69.4	±	4.9	3.45	±	0.06	6.34	±	0.86	22.4	±	0.4	217	±	19
T2	69.5	±	4.0	3.39	±	0.03	6.56	±	0.36	22.7	±	0.1	170	±	34
T3	66.3	±	0.5	3.44	±	0.04	6.47	±	0.49	22.1	±	0.4	227	±	21
CUMULUS															
T1	52.7	±	8.0	3.54	±	0.06	5.21	±	0.41	21.4	±	1.0	240	±	18
T2	54.4	±	7.3	3.31	±	0.62	4.77	±	0.59	21.8	±	0.7	249	±	52
T3	53.8	±	4.6	3.69	±	0.11	4.71	±	0.27	21.1	±	0.3	241	±	25

5.5 Total polyphenol and flavonoids analysis by spectrophotometry

Total polyphenols and flavonoids were measured by analysing the absorbance of extracted deseeded berry samples at 280 and 354 nm. This method was chosen since spectrophotometers are now common in many wineries. Different protocols were assayed, and although the extraction methodology outlined by Downey and Rochfort (2008) gave the best results, other extraction methods used for colour analysis, such as the one described by Iland et al. (2004) which are more commonly used in wineries, gave comparable results.

According to the 2-way ANOVA analysis, there were no significant differences between the final contents of both total polyphenols and flavonoids at harvest for each treatment between vineyards. However, significant differences were detected between the final concentration of total polyphenols and flavonoids at harvest in the control (T1) and the two defoliated treatments (T2 and T3, Fig. 3). Exposure to light, and in particular, UV radiation, has been demonstrated to increase total polyphenol and flavonoid content in berries in previous studies (Gregan et al., 2012; Keller and Torres-Martinez, 2004; Lafontaine et al., 2005; Liu et al., 2015; Price et al., 1995; Song et al., 2015). As such, exposed

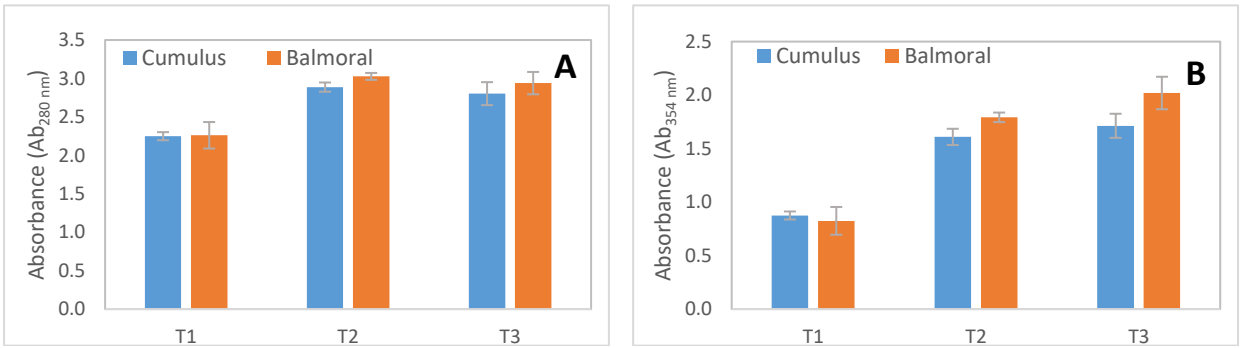


Figure 3. Total polyphenol (DO280, A) and flavonoid (DO 354,) content at harvest for all three treatments, non-defoliated control (T1), early defoliation (T2) and late defoliation. Error bars indicate standard error

berries (T2 and T3) contained up to 24% more total polyphenols and 57% more total flavonoids than control berries (T1) at harvest.

Examination of the accumulation patterns during the ripening season revealed that although there were no significant differences between the final concentrations of polyphenols and flavonoids in T2 and T3 measured by UV-vis spectrometry, there were indeed differences in the synthesis rates throughout the ripening season. Figure 4A and B show the concentration of flavonoids at each point of sampling for both vineyards. As can be observed in both figures, early removal of leaves, at the end of flowering stimulated the production of these compounds in T2 berries since early on in the season. By véraison, early exposure of berries to sunlight appears to have caused a two-fold accumulation of flavonoids in T2 samples, when compared to T1 and T3. Once leaves were removed in T3, the concentration of these compounds increased rapidly to the same levels (or slightly higher) than T2.

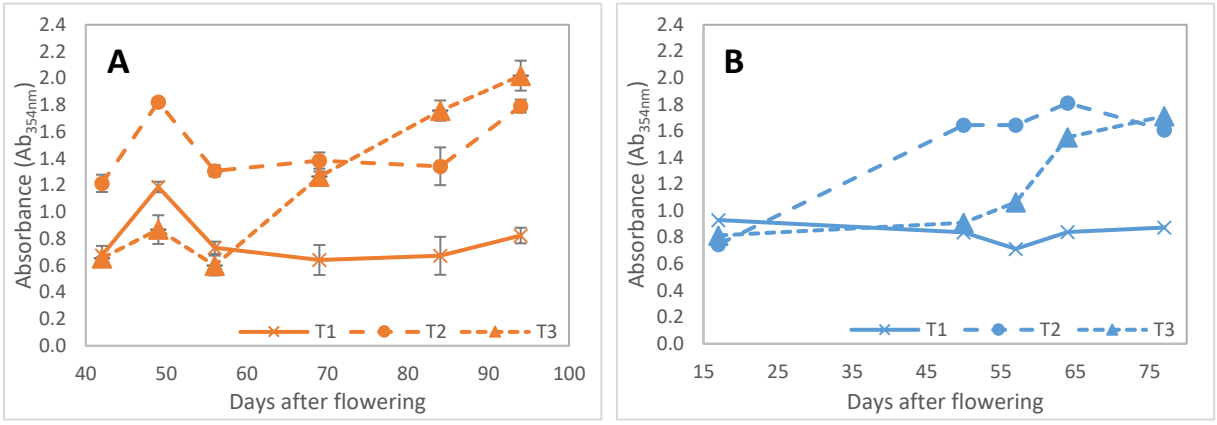


Figure 4. Total flavonols measured at DO354 nm in all treatments (T1-T3) for both vineyards during the ripening season. (A) Balmoral, (B) Cumulus.

5.6 LCMS

Flavonoids are well known photoprotective compounds and have been extensively researched in red berries. Flavonoids have been shown to act both as UVB screens and as antioxidants, helping berries acclimate to higher radiation situations (Joubert et al., 2016). UVB radiation regulates the phenylpropanoid pathway and the synthesis of flavonoids in grape epidermis (Carbonell-Bejerano et al., 2014). LCMS analysis confirmed all of the trends observed in the results obtained by UV-Vis spectrophotometry, and provided further insight into the response of flavonoids to leaf removal timing. As observed in the previous section, the main effect of leaf removal timing was observed in regards to synthesis kinetics rather than final concentrations (Appendix 3, Fig. S3A-H). Earlier defoliation caused an early rise in the concentration of flavonoids so that by véraison, T2 berries contained 2-2.5 times the amount of all flavonoids compared to the T1 or T3 berries. However, differences were observed between the behaviour of quercetin-3-glucoside (Q3G) and the other flavonoids analysed. Whilst the concentration of Q3G in T3 quickly increased after leaf removal, up to levels similar to those observed in T2 (Fig. S3A-B), this trend was not observed in the other three flavonoids. Despite leaf removal, the concentration of isorhamnetin-3-glucoside (I3G), myricetin-3-glucoside (M3G) and laricitrin-3-glucoside (L3G) remained low until the end of the season (Fig. S3C-H). I3G, M3G, and L3G are only synthesised early on during ripening. By the time defoliation occurred in T3, these berries no longer possessed the capacity to synthesise these photoprotective compounds.

5.7 Carotenoids

Carotenoids and chlorophylls are photosynthetic pigments that perform important light harvesting functions in plants. Like the flavonoids, carotenoids and chlorophylls also serve as photoprotectants in high light situations (Lashbrooke et al., 2010). With the exception of zeaxanthin, most of these

pigments are only produced during the early stages of berry development, and thereafter their concentration decreases, reaching their lowest levels at harvest (Joubert et al., 2016; Lashbrooke et al., 2010). Grapes lose their ability to synthesise most carotenoids due to the disappearance of chloroplasts (Baumes et al., 2002). This trend was observed for all measured compounds (except zeaxanthin, Fig. S4A and B), with maximum values being attained at véraison.

Examination of the concentration of the carotenoids and chlorophylls throughout the ripening period revealed that early leaf removal caused the concentration of all measured carotenoids to increase significantly in T2 samples. Late leaf removal, which occurred at véraison did not have this same effect. The only carotenoid that increased in T3 following defoliation was zeaxanthin, confirming that all photoprotective compounds were more responsive to light in the early stages of berry development and that acclimation to light stress occurs early on, as observed by others previously (Joubert et al., 2016). Under high light conditions, violaxanthin is converted to zeaxanthin in order to dissipate the excess absorbed energy as heat and to scavenge reactive oxygen species (Lashbrooke et al., 2010). Higher amounts of this compound were detected in T2 (2.98 mg/g FW, CUM and 3.58 mg/g FW, BAL) and T3 (3.12 mg/g FW, CUM; 3.16 mg/g FW, BAL) than in T1 in both vineyards (2.02 mg/g FW, CUM; 0.915 mg/g FW, BAL; Fig. 5A and 5B). As observed in the case of the flavonoids, the concentration of this compound was higher in T2 than in T1 and T3 at the beginning of the season, following early leaf removal. The concentration of zeaxanthin in T3 increased dramatically after leaf removal at véraison (Fig S4A and B).

One of the main roles of chlorophyll is to minimize photoxidative damage under excess radiation conditions. Rustioni et al. (2014) demonstrated that a quadratic relationship exists between the appearance of brown, melanin-like pigments related to sunburn and the disappearance of chlorophyll. Two-way ANOVA analysis revealed that at harvest, the main source of differences was vineyard location rather than the treatments themselves for all compounds except chlorophyll a and b, and zeaxanthin. At harvest, the exposed (T2 and T3) berries from CUM contained significantly lower concentrations of chlorophyll a and b than those from BAL, and also exhibited the highest levels of sunburn (Fig. 5 C-D).

6. Conclusion and Recommendations

Leaf removal is a common practice in cool viticultural areas which increases exposure and airflow in the bunch zone to reduce disease pressure. However, it can also overexpose berries and lead to sunburn, and consequent economic loss. The main aim of this study was to determine the best timing to remove leaves in cool viticultural areas in order to minimise sunburn damage. Results showed that when performed at the end of flowering, sunburn incidence was lower than when this practice was performed later in the season (at véraison). Earlier defoliation promoted the synthesis of photoprotective compounds such as the flavonoids, carotenoids and chlorophylls, during the early stages of berry development. The earlier accumulation of these compounds appears to have protected the berries from greater damage when they were at their most vulnerable, once they started to soften. By véraison, berries loose the capacity to synthesise most of these compounds, and when leaf removal is performed at the stage, the berry lacks the capacity to acclimate to the new high light conditions by producing more of these photoprotective compounds. This was of particular importance during a vintage like 2019, where plants were very stressed by a combination of extremely high temperatures and low availability of water. Although other alternatives could be envisioned to reduce the incidence of sunburn, such as the use of sunblocks or shade cloths, these increase production costs, and are only envisioned for the highest quality grapes. Leaf removal is a practice that is commonly applied in cool viticultural areas such Orange, and simply choosing the right moment to do it can minimise the economic losses experienced by these producers due to a problem such as sunburn without any additional cost.

However, these results remain to be confirmed during different climatic conditions. In white grapes, defoliation is a practice aimed primarily at reducing disease pressure. During a year such as 2019, disease pressure was very low but the chances of sunburn extremely high. In such a case, defoliation should not be practiced. It would be valuable to repeat this study under less extreme conditions. Furthermore, although there is extensive research on the effect of flavonoids on the sensory attributes of red wine, very little is known about what impact an increase in these compounds would have on white wine quality. Further studies are required to determine if these compounds compromise consumer acceptance of the final wine.

This study also demonstrated that it is possible to assess sunburn damage through image analysis. The development of a better performing tool would require further calibration of a predictive model, and could provide both winemakers and grape producers with a much needed objective tool to help them grade their grapes and negotiate their value.

7. Extension

- An article (Appendix 5) detailing the project background and aims, in addition to relevant literature information, was published in the Wine and Viticulture Journal as an introduction to the experiment (Australian and New Zealand Grapegrower and Winemaker, No.661, p28-30, Feb 2019).
- Details of the project were covered by various print, radio and television news media outlets in February 2019 and the results of the project are scheduled to be covered by WIN News in July 2019.
- Results from this study were presented to the grapegrowers and winemakers during the AWRI roadshow in the Hunter Valley and Orange in May 2019. They will also be presented at the NSW innovation forum in July 2019 and to the industry partner at the NSW DPI Spring Vine Health Field day workshops in September 2019.
- A poster outlining the results of this study will be presented in July's Australian Wine Industry Technical conference and trade exhibition.

8. Researcher benefit and feedback

Participation in Wine Australia's Incubator initiative has allowed me the opportunity interact closely with grapegrowers to address a more practical concern. It has been an enriching learning opportunity, and has provided me with the chance to be project leader, strengthening my research and project management skills. Reflecting on the whole initiative, I would recommend that project start dates be aligned with the grapevine developmental cycle. This would allow researchers to better utilise the full 12-month period and so address the specific industry concern properly.

Appendix 1

References

Australian Wine Research Institute, 2015. Ask the AWRI: Effects of hot and dry conditions. Australian & New Zealand Grapegrower and Winemaker. 612, 38.

Baumes, R., Wirth, J., Bureau, S., Gunata, Y., Razungles, A., 2002. Biogeneration of C13-norisoprenoid compounds: experiments supportive for an apo-carotenoid pathway in grapevines. Anal. Chim. Acta, 458, 3–14.

Carbonell-Bejerano, P., Rodríguez, V., Royo, C., Hernáiz, S., Moro-González, L.C., Torres-Viñals, M., Martínez-Zapater, J.M., 2014. Circadian oscillatory transcriptional programs in grapevine ripening fruits. BMC Plant Biol., 14, 78.

Downey, M.O., Rochfort, S., 2008. Simultaneous separation by reversed-phase high-performance liquid chromatography and mass spectral identification of anthocyanins and flavonols in Shiraz grape skin. J. Chromatogr. A, 1201, 43–47.

Gouot, J.C., Smith, J., Holzapfel, B., Barril, C., 2019. Single and cumulative effects of whole-vine heat events on Shiraz berry composition. OENO One, 53.

Gregan, S.M., Wargent, J.J., Liu, L., Shinkle, J., Hofmann R., Winefield, C., Trought, M., Jordan, B., 2012. Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. Aust. J. Grape Wine Res., 18, 227–238.

Heat wave events headline a record warm summer for Australia, 2019. Winetitles. URL https://winetitles.com.au/heat-wave-events-headline-a-record-warm-summer-for-australia/ (accessed 3.7.19).

Hill, G.N., Evans, K.J., Beresford, R.M., Dambergs, R.G., 2014. Comparison of methods for the quantification of botrytis bunch rot in white wine grapes. Aust. J. Grape Wine Res. 20, 432–441.

Hofmann, R.W., Jordan, B.R., Winefield, C.S., Stilwell, S.A., Shinkle, J.R., Wargent, J.J., 2006. UV radiation in New Zealand: implications for grape quality. National Institute of Water & Atmospheric Research.

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. Eur. J. Hortic. Sci. 79, 1–7.

Iland, P., Bruer, N., Edwards, G., Weeks, S., Wilkes, E., 2004. Chemical Analysis of Grapes and Wine: Techniques and Concepts. Patrick Iland Wine Promotions, Campbelltown, Australia.

Joubert, C., Young, P.R., Eyéghé-Bickong, H.A., Vivier, M.A., 2016. Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to uv exposure differentially in high and low light (shade) conditions. Front. Plant Sci. 7.

Keller, M., Torres-Martinez, N., 2004. Does UV radiation affect winegrape composition? Acta Hortic. 640, 313–319.

Kliewer, W.M., Dokoozlian, N.K., 2005. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. Am. J. Enol. Viticult., 56, 170-181.

Lafontaine, M., Schultz, H.R., Lopes, C., Balo, B., Varadi, G., 2005. Leaf and fruit responses of ‘Riesling’ grapevines to UV-radiation in the field. Acta Hortic. 689, 125–132.

Lashbrooke, J.G., Young, P.R., Strever, A.E., Stander, C., Vivier, M.A., 2010. The development of a method for the extraction of carotenoids and chlorophylls from grapevine leaves and berries for HPLC profiling. Aust. J. Grape Wine Res. 16, 349–360.

Liu, L., Gregan, S.M., Winefield, C., Jordan, B., 2015. From UVR8 to flavonol synthase: UV-B-induced gene expression in Sauvignon blanc grape berry. Plant Cell Environ. 38, 905–919.

Merzlyak, M.N., Solovchenko, A.E., Chivkunova, O.B., 2002. Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. Plant Physiol. Biochem., 40, 679–684.

Mosetti, D., Herrera, J.C., Sabbatini, P., Green, A., Alberti, G., Peterlunger, E., Lisjak, K., Castellarin, S.D., 2016. Impact of leaf removal after berry set on fruit composition and bunch rot in "Sauvignon blanc." VITIS,55, 57–34.

Price, S.F., Breen, P.J., Valladao, M., Watson, B.T., 1995. Cluster sun exposure and quercetin in Pinot noir grapes and wine. Am. J. Enol. Vitic. 46, 187–194.

Rustioni, L., Milani, C., Parisi, S., Failla, O., 2015. Chlorophyll role in berry sunburn symptoms studied in different grape (*Vitis vinifera* L.) cultivars. Sci. Hortic. 185, 145–150.

Rustioni, L., Rocchi, L., Guffanti, E., Cola, G., Failla, O., 2014. Characterization of grape (*Vitis vinifera* L.) berry sunburn symptoms by reflectance. J. Agric. Food Chem. 62, 3043–3046.

Solovchenko, A., Schmitz-Eiberger, M., 2003. Significance of skin flavonoids for UV-B-protection in apple fruits. J. Exp. Bot. 54, 1977–1984.

Song, J., Smart, R., Wang, H., Dambergs, B., Sparrow, A., Qian, M.C., 2015. Effect of grape bunch sunlight exposure and UV radiation on phenolics and volatile composition of *Vitis vinifera* L. cv. Pinot noir wine. Food Chem. 173, 424–431.

Sweetman, C., Sadras, V.O., Hancock, R.D., Soole, K.L., Ford, C.M., 2014. Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. J. Exp. Bot. 65, 5975–5988.

Wehrens, R., Carvalho, E., Masuero, D., Juan, A. de, Martens, S., 2013. High-throughput carotenoid profiling using multivariate curve resolution. Anal. Bioanal. Chem. 405, 5075–5086.

Young, P.R., Eyeghe-Bickong, H.A., du Plessis, K., Alexandersson, E., Jacobson, D.A., Coetzee, Z., Deloire, A., Vivier, M.A., 2016. Grapevine plasticity in response to an altered microclimate: Sauvignon blanc modulates specific metabolites in response to increased berry exposure. Plant Physiol. 170, 1235–1254.

Appendix 2

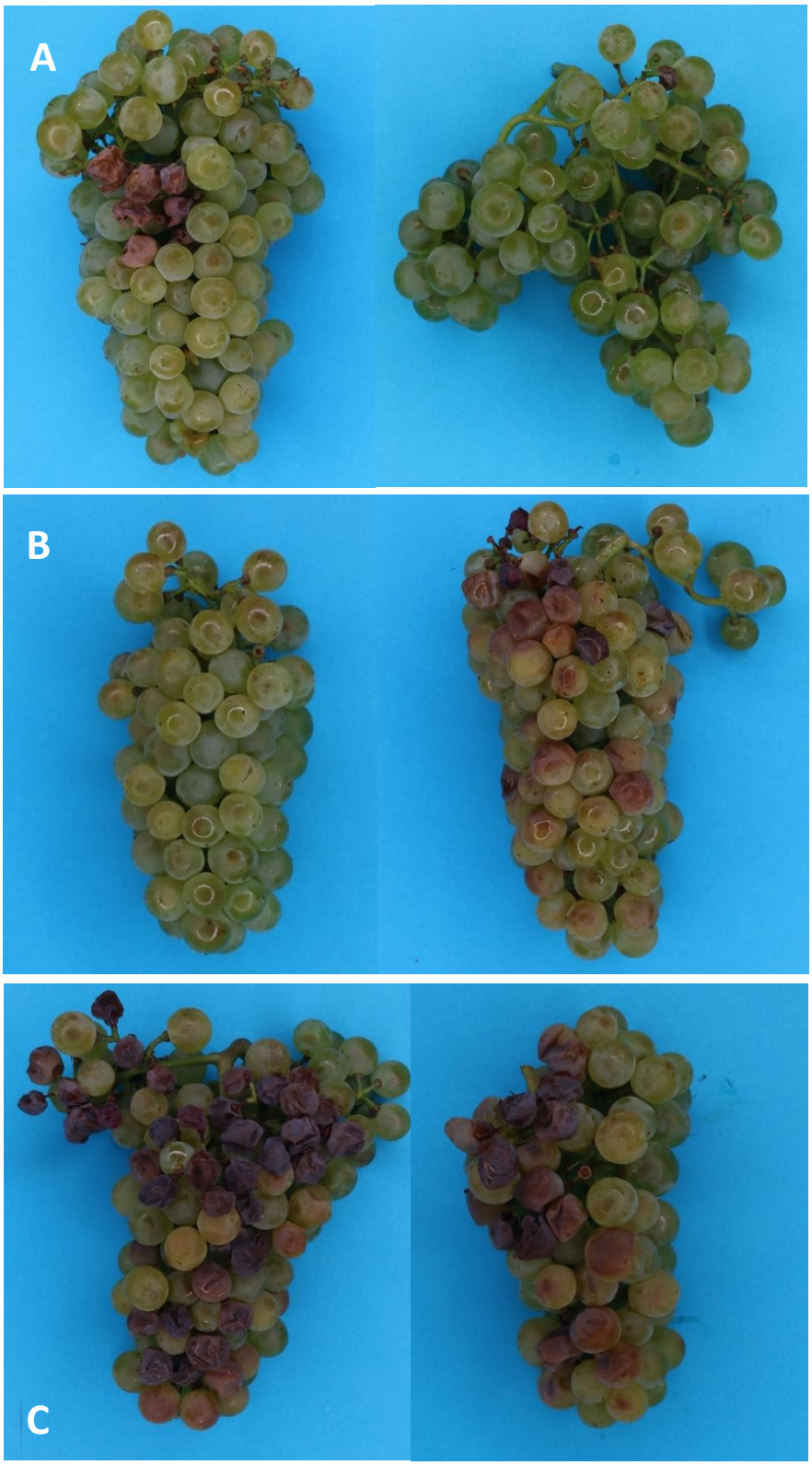


Figure S1 – Images of bunches of Chardonnay berries at harvest from Cumulus vineyard subjected to different defoliation timings. (A) T1, non-defoliated, (B) T2, early defoliation and (C) T3, late defoliation

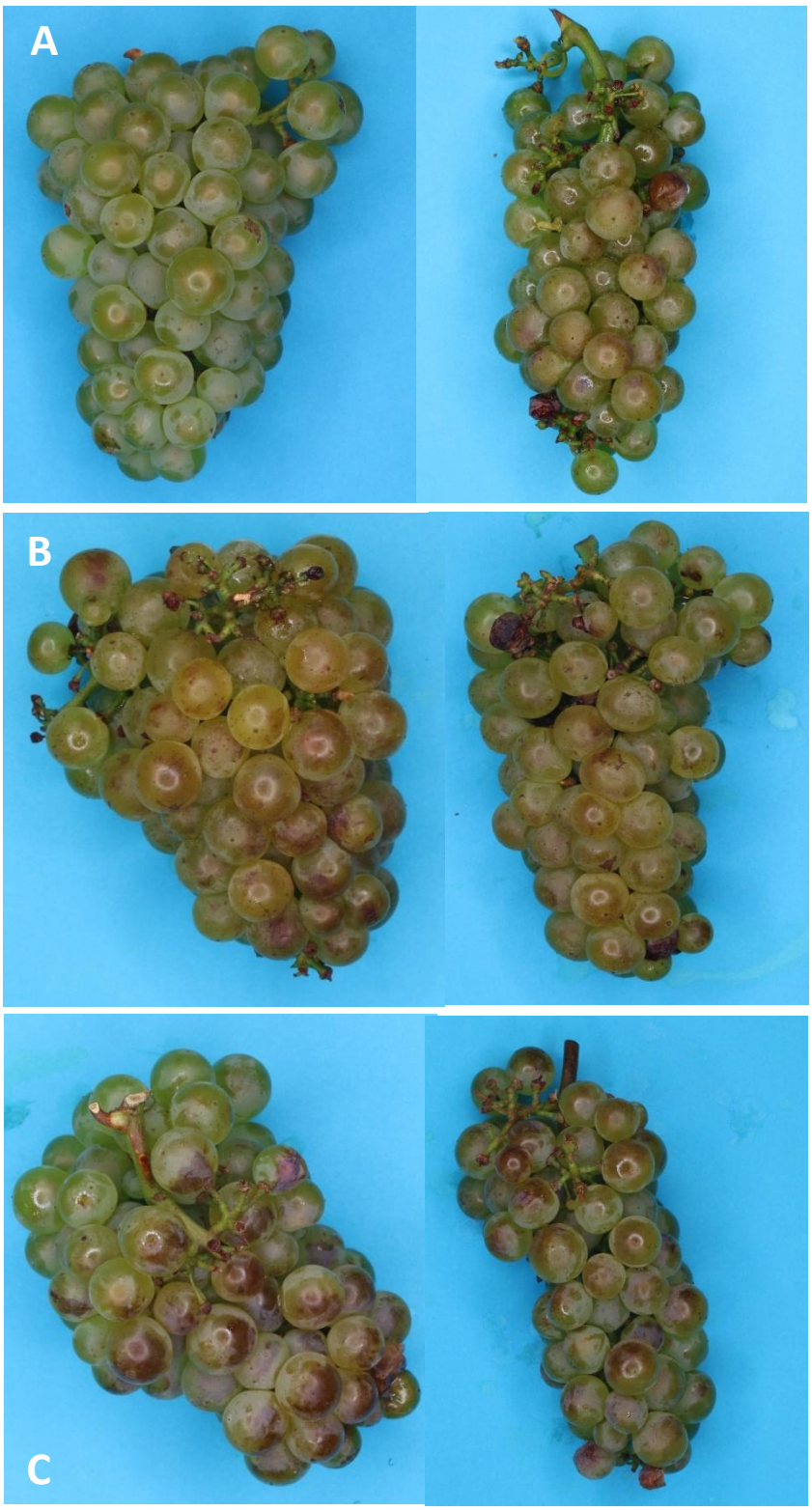


Figure S2 – Images of bunches of Chardonnay berries at harvest from Balmoral vineyard subjected to different defoliation timings. (A) T1, non-defoliated, (B) T2, early defoliation and (C) T3, late defoliation

Appendix 3

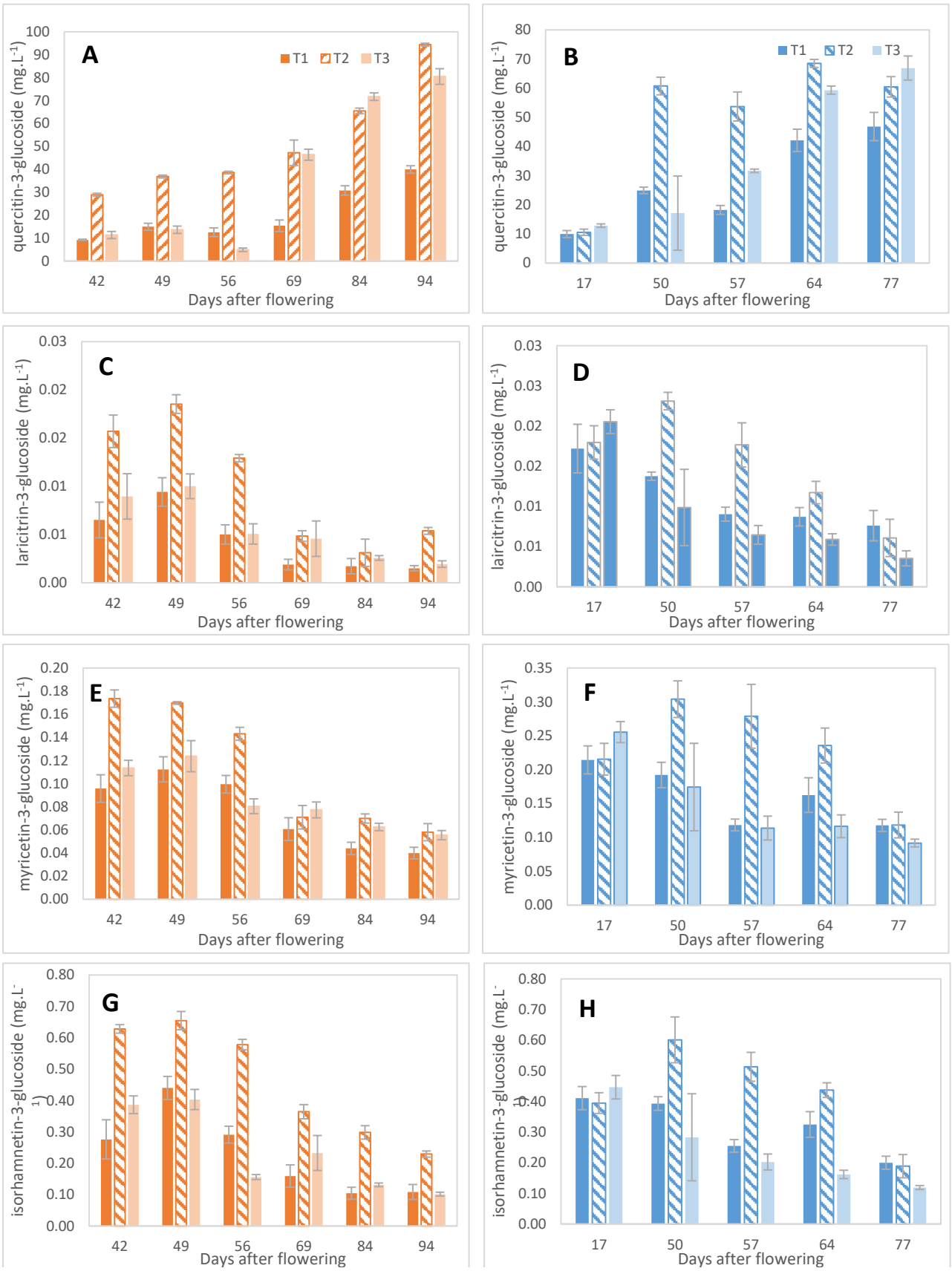


Figure S3. Evolution of quercetin-3-glucoside (A and B), laricitrin-3-glucoside (C and D) myricetin-3-glucoside (E and F) and isorhamnetin-3-glucoside (G and H) concentrations in treatments T1-T3 during ripening. (A, C, E, G) Balmoral and (B, D, F and H) Cumulus.

Appendix 4

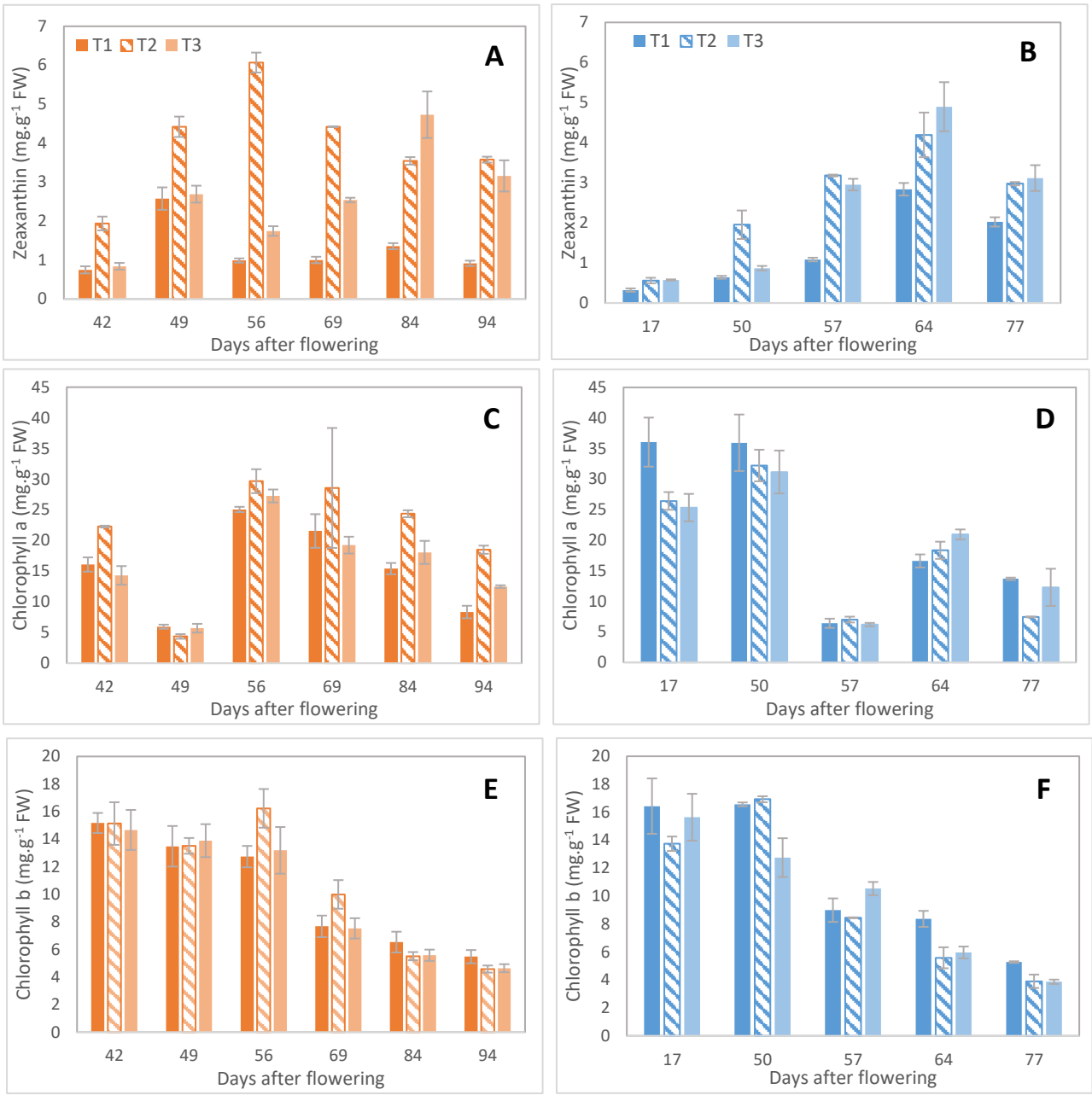


Figure S4. Evolution of zeaxanthin (A and B), chlorophyll a (C and D) and chlorophyll b (E and F) concentrations in treatments T1-T3 during ripening. (A, C, E) Balmoral and (B, D, F) Cumulus.

Appendix 5

Climate Change

What is the best time to remove leaves to minimise sunburn?

Joanna Gambetta, Bruno Holzapfel and **Leigh Schmidtke** from the National Wine and Grape Industry Centre are evaluating three different timings of defoliation in Chardonnay vines grown around Orange in New South Wales.



Heavily sunburnt winegrape berries.

Background

Sunburn is caused by exposure to a combination of sunlight, high temperatures and ultraviolet radiation[1-4]. Just as in humans, sunburn in grapes generally appears as a browning of the skin and is due to the accumulation of polymerised melanin-like pigments in both white and red varieties. In red grapes, it also leads to a loss of red coloration. In extreme situations, sunburn can lead to complete berry shrivelling and consequent loss of yield[5]. Finally, sunburnt fruit leads to an overall loss of wine quality, higher oxidation, increased bitterness and the appearance of off-odours. A number of variables such as grape variety, berry developmental stage, ambient temperature, UV level, water availability and viticultural practices (i.e. row orientation, training system and defoliation)[1,5] affect the intensity of the symptoms.

Leaf removal, or partial defoliation of the canopy, is a common technique in (cool climate) vineyards to reduce the incidence of disease (namely fungal pathogens), improve quality and to increase colour stability of wines from red cultivars. Leaf removal in the bunch zone increases air penetration and sunlight to allow grapes to dry off quicker after a rain event and, depending on grape variety, can help to decrease the perception of green characters and increase that of tropical fruit[6]. However, depending on how and when this practice is conducted, the effects of leaf removal can be variable and can even lead to a higher degree of sunburn if bunches are overexposed.

Research indicates that berries actually possess the capacity to acclimatise to higher sunlight situations by producing photoprotective compounds such as flavonoids and carotenoids[7-9]. As such, leaf removal before véraison may induce higher levels of these photoprotective compounds, thus protecting berries from UV damage and reducing the intensity of sunburn[7-12], but further research is required to confirm this.

The past few vintages have seen some of the hottest summers on record in cool grapegrowing regions like Orange where the amount of sunburnt berries has been on the rise. Producers in the area were concerned as not only does sunburn result in lower yields, but it also impacts fruit quality. In severe cases A-grade fruit can be downgraded to C-grade, which reduces the price of the fruit by approximately 50% (in the order of $1200/t). Therefore the combination of reduced quantity and quality of grapes from a vineyard can lead to sizeable economic losses, becoming a significant issue for grape and wine producers.

This project was shaped in collaboration with grapegrowers from the Orange region. After trying unsuccessfully to grapple with this problem, they contacted Bruno Holzapfel and myself [Joanna Gambetta] to determine a better strategy for leaf removal that does not subject the grapes to excessive exposure. As they explained, their frustration derived from not knowing when it would be best to remove leaves. So far they had attempted different strategies with mixed results. Not removing leaves was not an option due to sanitary concerns. Although alternative solutions, such as the use of sunblock and shade cloths, exist these only add to the cost of vineyard management, whilst leaf removal is a practice already conducted. The question was, when would be the best time to remove leaves? Options were: to wait until as late as possible in the growing season to protect the berries as much as possible from sun and wind exposure; or remove leaves early in the season to allow berries to acclimatise to sunlight and heat when the temperatures are still relatively mild, and allow them to synthesise as many photoprotective compounds as possible?

The trials

Thanks to Wine Australia's incubator scheme, we were given the opportunity to conduct defoliation trials in season 2018-19. Working with Chardonnay grapes in two vineyards in Orange (Cumulus and See Saw), located at two different altitudes (roughly 600 and 900m above sea level), we are testing the effect of UV intensity as well as timing of leaf removal. Producers in the area have also reported that although cooler than the lower vineyards, they were experiencing



Setting up the defolition trial.



A section of the defoliation treatment.

higher levels of sunburn in the vineyards located at higher altitudes.

The trial comprises two phases, an early and a late leaf removal. Early leaf removal was conducted early in December, between the end of flowering and fruitset. Late leaf removal was done at véraison, which occurred in early January in the lower site, and towards the end of January in the higher site. All leaves were removed in the bunch zone (up to 45cm above the cordon) only from the western side of the canopy. All treatments were conducted in triplicate and alternated in a completely randomised design across nine rows. Each replicate consisted of 18 vines, with the two outer vines and rows of each plot being used as buffers between treatments. In order to monitor the different parameters that influence sunburn, we installed sensors to measure canopy temperature and relative humidity, photosynthetically active radiation, UV, and bunch temperature. We will be collecting samples regularly to monitor the concentration of flavonoids and carotenoids. Visual results will be obtained by harvest and we are aiming to finalise results by the end of June 2019.

How burnt is too burnt?

Adding to the problem is the subjectivity inherent in grading fruit and evaluating the severity of sunburn. A final aim of this project is to create a visual record of sunburn, allocate a score to each level and to correlate it with more objective parameters such as total polyphenol content.

In all, we are aiming to create guidelines that Chardonnay producers can follow depending on the vintage forecast, and a visual record that indicates quantity and quality impacts from sunburn. This information can be used to help grade fruit and contribute to the enhancement of sunburn management in following seasons.

References

[1]Rustioni, L.; Rocchi, L.; Guffanti, E.; Cola, G. and Failla, O. (2014) Characterization of grape (*Vitis vinifera* L.) berry sunburn symptoms by reflectance. J. Agric. Food Chem. 62:3043-3046.

[2]Rustioni, L.; Milani, C.; Parisi, S. and Failla, O. (2015) Chlorophyll role in berry sunburn symptoms studied in different grape (*Vitis vinifera* L.) cultivars. Sci. Hort. 185:145-150.

[3]Hofmann, R.W.; Jordan, B.R.; Winefield, C.S.; Stilwell, S.A.; Shinkle, J.R. and Wargent, J.J. (2006) UV radiation in New Zealand: implications for grape quality. National Institute of Water & Atmospheric Research.

[4]Gregan, S.M.; Wargent, J.J.; Liu, L.; Shinkle, J.; Hofmann, R.; Winefield, C. and Jordan, B. (2012) Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of Sauvignon Blanc grapes. Aust. J. Grape Wine Res. 18:227-238.

[5]The Australian Wine Research Institute (2015) Ask the AWRI: Effects of hot and dry conditions. Australian & New Zealand Grapegrower & Winemaker 612:38.

[6]Mosetti, D.; Herrera, J.C.; Sabbatini, P.; Green, A.; Alberti, G.; Peterlungi, E.; Lisjak, K. and Castellarin, S.D. (2016) Impact of leaf removal after berry set on fruit composition and bunch rot in 'Sauvignon Blanc'. Vitis 55:57-34.

[7]Joubert, C.; Young, P.; Eyeghe-Bickong, H. and Vivier, M. (2016) Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (shade) conditions. Front. Recent Dev. Plant Sci. 7:786.

[8]Liu, L.; Gregan, S.; Winefield, C. and Jordan, B. (2015) From UVR8 to flavonol synthase: UV-B-induced gene expression in Sauvignon Blanc grape berry. Plant, Cell & Environment 38:905-919.

[9]Young, P.; Eyeghe-Bickong, H.; du Plessis, K.; Alexandersson, E.; Jacobson, D.; Coetzee, Z.; Deloire, A. and Vivier, M. (2016) Grapevine plasticity in response to an altered microclimate: Sauvignon blanc modulates specific metabolites in response to increased berry exposure. Plant Physiol. 170:1235-1254.

[10]Hofmann, R.W. (2014) UV effects in plants-case studies from New Zealand with a northern hemisphere twist. Paper presented at the 2014 NIWA UV Workshop, Auckland.

[11]Merzlyak, M.N.; Solovchenko, A.E. and Chivkunova, O.V. (2002) Patterns of pigment changes in apple fruits during adaptation to high sunlight and sunscald development. Plant Physiol. Biochem. 4:679-684.

[12]Solovchenko, A.E. amd Schmitz-Eiberger, M. (2003) Significance of skin flavonoids for UV-B protection in apple fruits. J.Exp. Bot. 54:1977-1984.