SATI	CFPA	SAAPPA/SASPA	DFTS	Winetech
tarryn@satgi.co.za Tel: 021 863-0366	inmaak@mweb.co.za Tel: 021 872-1501	theresa@hortgro.co.za Tel: 021 882-8470	dappies@dtd.co.za Tel: 021 870 2900	andraga@winetech.co.za Tel: 021 276 0499
				x

FINAL REPORT

(2018)

PROGRAMME AND PROJECT LEADER INFORMATION

	Research Organisation Programme leader	Research Team Manager	Project leaders
Title, initials, surname	Prof MA Vivier	Prof MA Vivier	Prof MA Vivier/Dr PR Young
Present position	Professor	Professor	Professor/Researcher
Address	Institute for Wine Biotechnology, Department of Viticulture and Oenology, SU	Institute for Wine Biotechnology, Department of Viticulture and Oenology, SU	Institute for Wine Biotechnology, Department of Viticulture and Oenology, SU
Tel. / Cell no.	021 808 3773	021 808 3773	021 808 3773
Fax	021 808 3771	021 808 3771	021 808 3771
E-mail	mav@sun.ac.za	mav@sun.ac.za	mav@sun.ac.za/pryoung@sun.ac.
	Co-worker	Co-worker	
Title, initials, surname	Dr JP Moore		
Present position	Senior Researcher		
Address	Institute for Wine Biotechnology, Department of Viticulture and Oenology, SU		
Tel./Cell no.			
Fax	021 808 3771		
E-mail	moorejp@sun.ac.za		

1. PROJECT INFORMATION

Research Organisation Project number	IWBT Vivier 2015 – 1
Project title	DST Title: An integrated approach to study the impacts of climate change on berry composition Winetech title: Interpreting the grapevine-environment interaction
Short title	Grapevine-Environment Interaction

Fruit kind(s)	Grapes		
Start date (mm/yyyy)	01/2015	**End date** (mm/yyyy)	12/2017

Key words	Genotype-environment-interactions; metabolomics; transcriptomics, environmental profiling; row-orientation; berry cell wall profiling

Approved by Research Organisation Programme leader (tick box) | x |

2. EXECUTIVE SUMMARY

Objectives & Rationale
This study used state-of-the-art technologies to study and interpret the impacts of changing environmental factors on grapevine biology, organ development and berry quality in vineyards to ultimately understand how grapevine functions in interaction with its complex and changing environment. Established molecular, analytical and statistical techniques were applied to characterised vineyards in Elgin (cv Sauvignon Blanc) and Robertson (cv Shiraz) to link berry compositional changes and plant physiological responses to developmental and/or environmental cues.

Methods
Grape berries were sampled at three phenological stages that represent the green, véraison, and ripe stages of berry development. In the Elgin vineyard (Sauvignon Blanc), representative grape berries were sampled in the morning (09h00, AM) from UV-exposed and UV-shaded grapes in a high light and a low light bunch microclimate. In the Robertson vineyard (Shiraz) representative grape berries were sampled from the four row-orientations (N/S, NE/SW, E/W and NW/SE) from both sides of the canopy, separately, in the morning (09h00/AM) and the afternoon (16h00/PM). Berry samples were flash-frozen in the vineyard, homogenised and stored at -80ºC. Targeted chemical analysis, as well as cell wall analysis were performed to profile and quantify the levels of key quality-associated metabolites, including major sugars and organic acids, amino acids, phenolics, volatiles and carotenoids and chlorophylls. Climatic variables (including temperature, light, relative humidity) were measured to correlate metabolite changes to abiotic cues in the microclimate.

Results & Discussion
In the Elgin vineyard (Sauvignon Blanc), photosynthetically active berries in the early/green developmental stages, used oxygenated carotenoids (xanthophylls) to respond to light (including UVB) exposure. The response to UVB differed between the high- and low light microclimates. Ripe berries, however, responded to changes in light quality and quantity, by forming compounds that have direct antioxidant and/or "sun-screening" abilities (e.g. volatiles and polyphenols). Interestingly, the different microclimates did not change the cell wall composition of the ripe berries, indicating that the berries utilised metabolites (and not structural/morphological changes) to both sense and acclimate to their microclimates. Light quality (PAR and UVB) and quantity (high-light/low-light) differentially impacted individual metabolites that are linked to quality-imp act factors in the grapes and wines (notably carotenoids, phenolics, and monoterpenes).

In the Robertson vineyard (Shiraz) developmental stage, row orientation, canopy facet (morning-exposed versus afternoon-exposed) and time of day (AM versus PM) all impacted berry composition, but it was possible to identify metabolites that was regulated very similarly irrespective of their microclimates, whereas others, such as some secondary metabolites and amino acids were highly responsive to changes in the microclimates, not only over the season, but also during the day/night cycles.

Conclusions
Grapevine berries are remarkably responsive to their immediate environment and can react to adjust the berry composition to cope with the stresses perceived in the microclimate. These acclimation responses through metabolites can range from rapid (daily fluctuations of the carotenoids) versus long-term seasonal responses (amino acids and volatiles). The results also highlighed that both red (Shiraz) and white (Sauvignon Blanc) cultivars in a cool climate (Elgin) and warm climate (Robertson) were remarkably conserved in their responses to light and temperature and the data underlines the significant plasticity of grapevine and the potential to manipulate the berry composition by long term (row orientations) and seasonal (canopy manipulations) decisions. The approach and the results obtained provide insight into the plant mechanisms that allow a solid theoretical base to evaluate, plan and predict the outcomes of microclimatic impacts on berry metabolism.

3. PROBLEM IDENTIFICATION AND OBJECTIVES

Problem: Grapevine is considered the most economically important fruit crop worldwide. However, it has been proposed that one of the greatest challenges facing the grape and wine industries during this century, will be maintaining high quality sustainable grape production in a changing environment. This challenge will require a deeper understanding of grape biology, and the impacts of various factors in the vineyard environment.

The objectives in the proposed project is to study the influence of light (both quantity and quality) and temperature on berry metabolism and cell wall structure throughout the berry development in characterised (model) vineyards. The study will make use of state-of-the-art technologies to study and interpret the impacts of changing environmental factors on grapevine biology, organ development, and berry quality in vineyards, following a grapevine "field-omics" approach. Ultimately, the proposed work will provide a deep understanding of how a grapevine functions in interaction with its complex and changing environment.

4. DETAILED REPORT

PERFORMANCE CHART (January 2015-December 2017))

AIMS/OBJECTIVES	MILESTONES/ACTIVITIES AND ACHIEVEMENTS	TARGET DATE	COMPLETION DATE
AIM 1: CONSTRUCTING A FIELD-OMICS EXPERIMENTAL WORKFLOW			
OBJECTIVE 1.1 Establish an experimental framework for the full project in terms of treatments, sampling, analysis and data-integration	**ACTIVITY 1: Project planning session to develop detailed project plan based on existing/preliminary data.** Two model vineyards were used: Elgin Sauvignon Blanc (UV-B attenuation) and Robertson Shiraz (Row orientation)	June 2015	June 2015
AIM 2: MODULATING THE MICROCLIMATE OF DEVELOPING AND RIPENING GRAPEVINE BERRIES IN MODEL VINEYARDS AND PROFILING OF THE TRANSCRIPTOMIC AND METABOLOMIC RESPONSES			
OBJECTIVE 2.1 Comprehensive analyses of berry samples from the model vineyards	**ACTIVITY 1: Gene expression analyses of berry samples** Transcriptome analysis of the Sauvignon Blanc samples were completed, but not for the Shiraz experiment, due to the fact that we were not allowed by the ARC, as coordinators of the vineyard, to collect samples for that purpose.	June 2015 –Dec 2016	Dec 2016 for SB; halted for Shiraz
	ACTIVITY 2: Metabolite profiling of berry samples using HPLC, UPLC and GC-MS analyses All samples analysed, yielding metabolite profiling data that had been evaluated with statistical and multivariate data analyses methods.	June 2015 –Dec 2017	Dec 2017

	ACTIVITY 3: Data analysis of the individual datasets as well as integrative, multivariate statistics to combine the different datasets. Data was successfully analysed and several publications and theses have been prepared. More papers are still being prepared and the datasets provide an important resource that could be used for further analysis and outputs.	Continuous	Dec 2017
OBJECTIVE 3.1: Profiling the construction of the wine grape cell wall during ripening	ACTIVITY 1: Classification of ripening grapes with cell wall tools and spectroscopic technologies in characterized technologies in characterised vineyards (Sauvignon Blanc and Shiraz) The cell wall composition of the berries within the Elgin Sauvignon Blanc (UVB attenuation) project were unaltered by leaf removal (exposure) and UVB attenuation. This highlights that the plants did not require physical/morphological changes in response to the treatment but rather modulated stress-responsive metabolites in the berry skin (carotenoids, flavanols, etc). Cell wall analysis of the Shiraz berry skins and pulp provided a cell wall model for Shiraz berries at different ripenening stages.	October 2014- April 2016 (amended for December 2017	Completed Dec 2017
	ACTIVITY 2: Development of a bioinformatical workflow to analyse cell wall gene expression *in silico* and from our own data. The cell wall gene families have been sub-grouped in three main groups- the gene families within these sub-groups are currently being studied to provide appropriate gene lists to explore the gene expression patterns with an *in silico* approach. No outputs have been prepared from this activity and will still lead to more outputs and insight.	December 2014 – December 2017)	Completed Dec 2017
	ACTIVITY 3: Integration of datasets using statistical methods Statistical and multivariate methods were successfully implemented in the data analysis.	December 2014 – December 2017	Completed Dec 2017
AIM 4: DEVELOPING A MORE INTEGRATED UNDERSTANDING OF GRAPE BERRY RIPENING			
OBJECTIVE 4.1 Finding links between the datasets	**ACTIVITY 1: Data analysis and integration** An iterative process that was implemented utilising existing data sets and expanded as additional datasets were completed.	Continuous	Completed March 2018

b) WORKPLAN (MATERIALS AND METHODS)

VINEYARD SITE SELECTIONS

Two distinct vineyard sites formed part of this study:

1. A Sauvignon blanc model vineyard in the Elgin area was selected to study the effect of light quality and quantity, with a particular focus on UVB impacts on berry growth and metabolism. This study was an expansion of a previous research project that contributed to establishing and characterising the model vineyard in Elgin. The Elgin region and the specific vineyard site were classified according to conventional viticultural climatic indices based on macroclimatic weather station data (i.e. regional) as well as mesoclimatic data (local vineyard). Climatic classification indices characterized the Elgin region as a temperate region with moderate to cool nights. At more than 250 m above sea level, Elgin is a high-altitude wine grape-growing region in South Africa. The elevation combined with the proximity to the Atlantic Ocean (and consequent exposure to the cooling sea breeze) makes it the fourth coolest wine-grape-growing region in South Africa. This site was chosen as a typical moderate climatic site for the production of a commercially desirable style of cv Sauvignon Blanc wine.

The vines were orientated in a north-west (NW), south-east (SE) row direction and trained on a vertical shoot positioned (VSP) trellis system. Spur pruning to two buds was employed during winter and diligent canopy management occurred throughout the growing season. No water constraints were noted due to the high moisture content of the deep shale soils, as was confirmed by stem water potential measurements. The experimental plot included three rows from which 16 panels were identified. Two controls and two treatments were applied randomly over the 16 panels with each control/treatment being repeated four times. Each panel consisted of four consecutive vines and represented a biological repeat (Figure 1A shows a diagram of the plot layout).

Light and UVB conditions were manipulated by an early partial leaf removal in the bunch zone and UVB-excluding/attenuating acrylic sheets, respectively. Four distinct microclimates were generated that differed in light quality and quantity. The four microclimates created included: (1) a “shaded” treatment (low light = LL) characterized by no alteration to the canopy in the bunch zone and its exposure to sunlight (i.e. no leaf removal; no UVB-attenuation); (2) the “exposed” treatment (high light = HL) and involved the early removal of leaves and laterals in the bunch zone (leaf removal; no UVB attenuation). The UVB-attenuation involved the implementation of UVB-excluding sheets that block >99% of UVB light, in the bunch zones of (3) shaded and (4) exposed treated vines, to create LL-UVB and HL-UVB, respectively (Figure 1B).

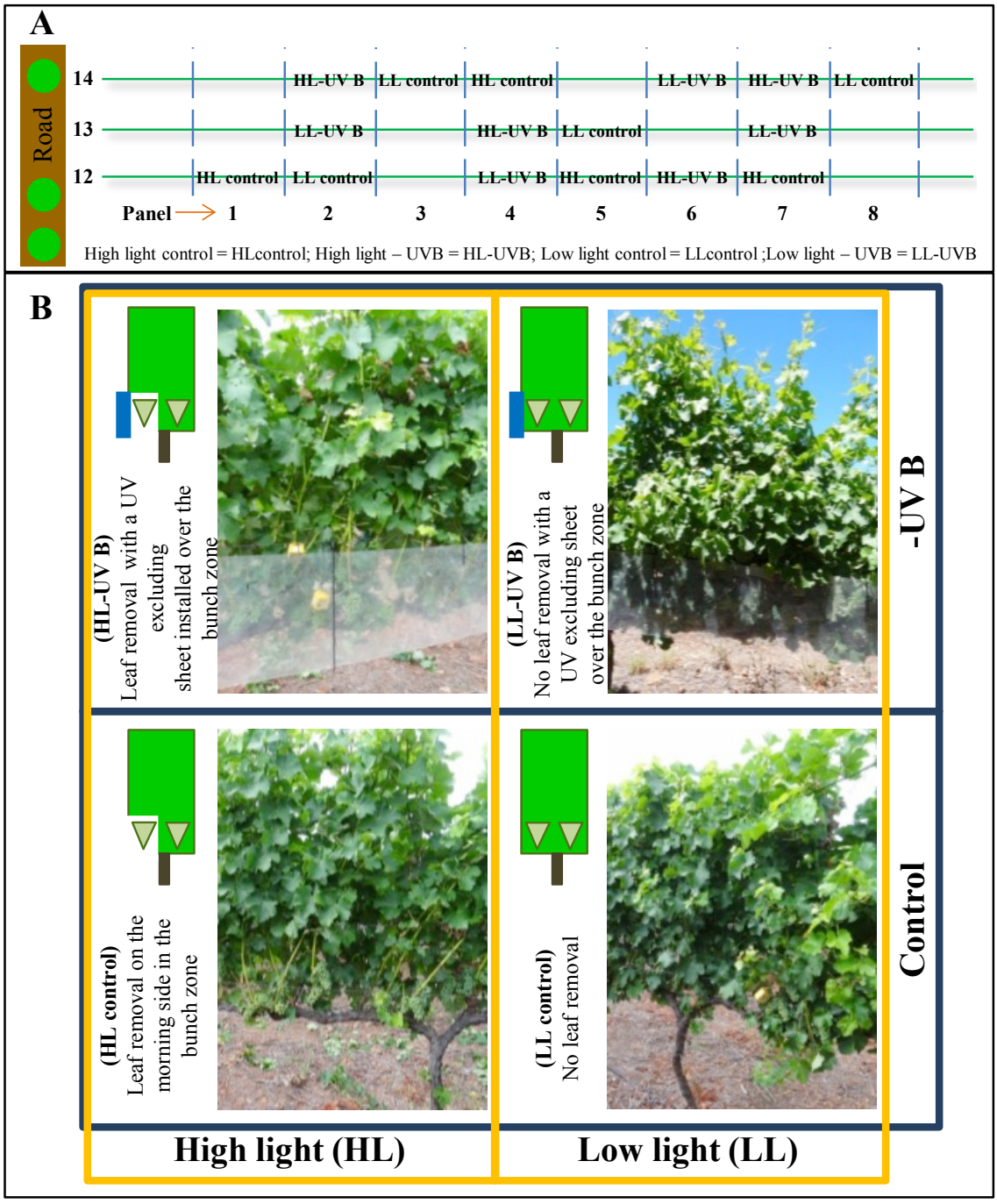


Figure 1. The Elgin Sauvignon Blanc experimental layout of the treatments within the plot (A) and the four light environments created by leaf removal and UVB attenuation (B). (Figure from Joubert et al., 2016).

2. A Shiraz model vineyard in the Robertson area was selected to study the effect of row-orientation on berry metabolism and quality. The vineyard establishment and experimental layout are described (Winetech project number "IWBT 2015-01", "100557", and "WW 12-20" as well as a Hunter et al: https://doi.org/10.1016/j.agrformet.2016.06.013). Briefly, *Vitis vinifera* L. cv Shiraz (clone SH9C grafted onto 101-14 Mgt) was established in 2003 at the Agricultural Research Council's Infruitec-Nietvoorbij experimental farm in Robertson (within the Breede River Valley grape growing region of South Africa: 33°49′35S/19°52′53E). The terrain is characterised as flat with predominantly clay-loam soil type. The vines were planted in four row orientations: North/South (N/S), North-East/South-West (NE/SW), East/West (E/W) and North-West/South-East (NW/SE) with a 1.8 m vine- and 2.7 m row spacing. There were five replicates for each of the four row orientations resulting in 20 blocks (refer to Figure 2). Vines were trained on four-wire VSP trellising system and pruned to two buds per spur. Due to the typical low rainfall for the Robertson region, vines received weekly supplementary drip irrigation.

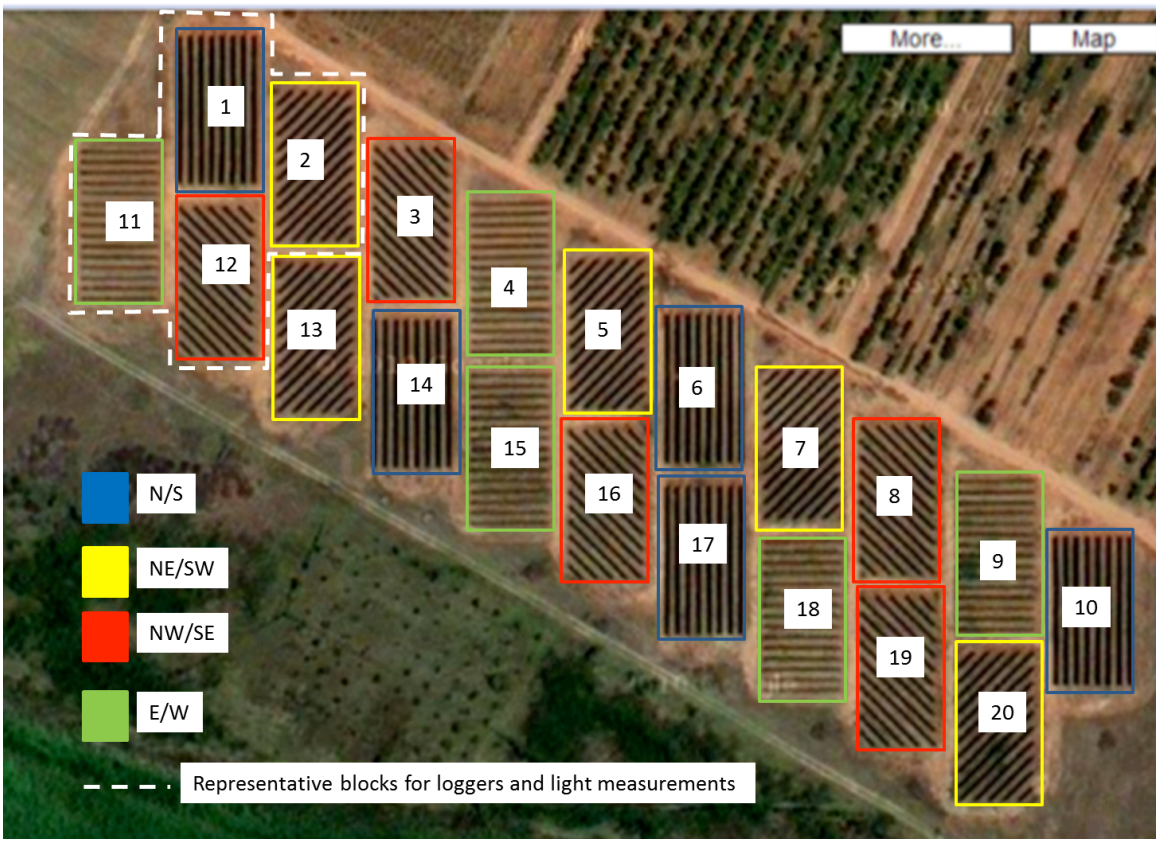


Figure 2. The Robertson Shiraz row-orientation plot layout showing the respective row orientations and five biological repeats for each. The vineyard was planted and managed by Dr Kobus Hunter of ARC Nietvoorbij.

SAMPLING

For both vineyard sites, grape berries were sampled at three distinct phenological stages that represented the (1) green (~EL-31), (2) véraison (~EL35) and (3) ripe (~EL38) stages of berry development. Each berry sample consisted of at least 48 representative berries with at least four biological replicates sampled for each treatment. Berries harvested at each phenological stage were immediately flash frozen in N_2 (l) in the vineyard, homogenised, and stored at -80ºC until required. For the Elgin vineyard, representative berries were sampled at 09h00 from the morning-exposed (i.e. NE-facing facet). In the Robertson vineyard (Shiraz), representative berries were sampled from the four row-orientations (N/S, NE/SW, E/W and NW/SE) from both sides of the canopy (separately) in the morning (09h00) and the afternoon (16h00), for each sampling date.

MESO- AND MICROCLIMATIC MEASUREMENTS

The mesoclimatic measurements were obtained from adjacent weather stations. The canopy and bunch microclimate temperature were measured throughout the growth season (with 15 min intervals) using

dual channel temperature data loggers (TinyTag Plus 2, TGP-4520, Gemini Data Loggers (UK) Ltd., Chichester, United Kingdom) with two thermistor flying lead probes. These probes were positioned within representative bunches from each side of the panel for each of the four row orientations.
Spot measurements for photosynthetic active radiation (PAR) in the bunch zone were collected before each sampling with a ceptometer (Accupar model LP-80 Decagon Devices). Ambient PAR (full sunlight) was measured before and after each canopy PAR measurement. PAR values were expressed relative to the ambient light measurement on the sampling day (i.e. as a percentage relative to ambient sunlight at the time of sampling).
Additional solar radiation sensors (Vantage Pro2TM solar radiation sensors Davis Instruments, California, USA) were installed In the Elgin vineyard both inside and outside of the canopy. The outer unit measured the ambient solar radiation while the internal sensors measured the solar radiation that penetrated the canopy and reached the bunch zone. A solar sensor was placed in the bunch zone of each of the four light environments to determine the degree of light penetration in each case. UV radiation was measured using sensors (Apogee SU-100 UV sensors. Apogee Instruments Inc., Utah, USA) which were positioned similarly to the solar radiation sensors; one externally to measure ambient UV and one placed in the bunch zone of each created light environment. The solar and UV sensors were attached to two loggers (DataTaker DT82E data logger, Thermo Fisher Scientific Australia Pty Ltd, Victoria, Australia) which recorded measurements throughout berry development (Figure 3).

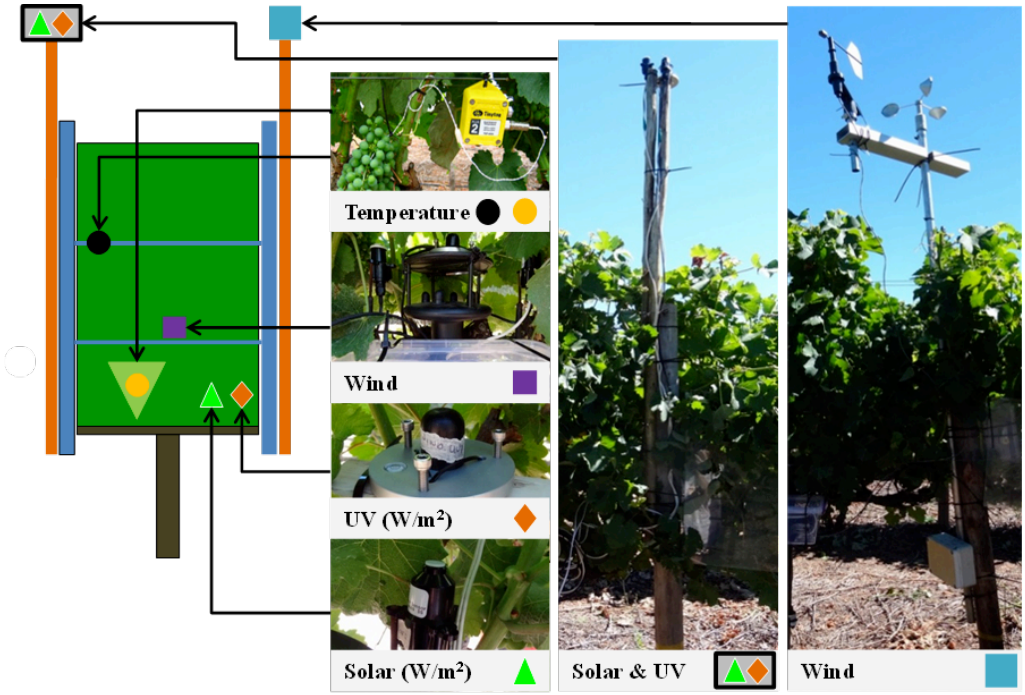


Figure 3. Schematic showing the relative positions of the climatic loggers in the Elgin Sauvignon Blanc trial. (Figure from Joubert et al., 2016).

EXTRACTION AND ANALYSIS OF BERRY METABOLITES

Figure 4 summarises the metabolites and their classes targeted for analysis in this project.

Analysis of major sugars and organic acids

Major sugars (glucose and fructose) and organic acids (malate, tartrate and succinate) were extracted from frozen, homogenised whole berries (100±5 mg) and analysed using HPLC as described in Eyeghe-Bickong et al. (2012).

Analysis of photosynthetic pigments

Photosynthetic pigments (carotenoids and chlorophylls) were extracted from frozen, homogenised whole berries (250±5 mg) and analysed using UPLC as described in Young et al. (2016).

Analysis of volatile aroma compounds

Volatile organic compounds (flavour and aroma compounds) were extracted from frozen, homogenised whole berries (500±5 mg) and analysed using HS-SPME-GC-MS as described in Joubert et al. (2016).

Analysis of amino acids
Amino acids were extracted from homogenised whole berries (200±5 mg) and analysed using HPLC as described in Du Plessis et al. (2017).

Analysis of phenolics
Phenolics were extracted from homogenised whole berries (200±5 mg) and analysed using UPLC as described in Du Plessis et al. (2017).

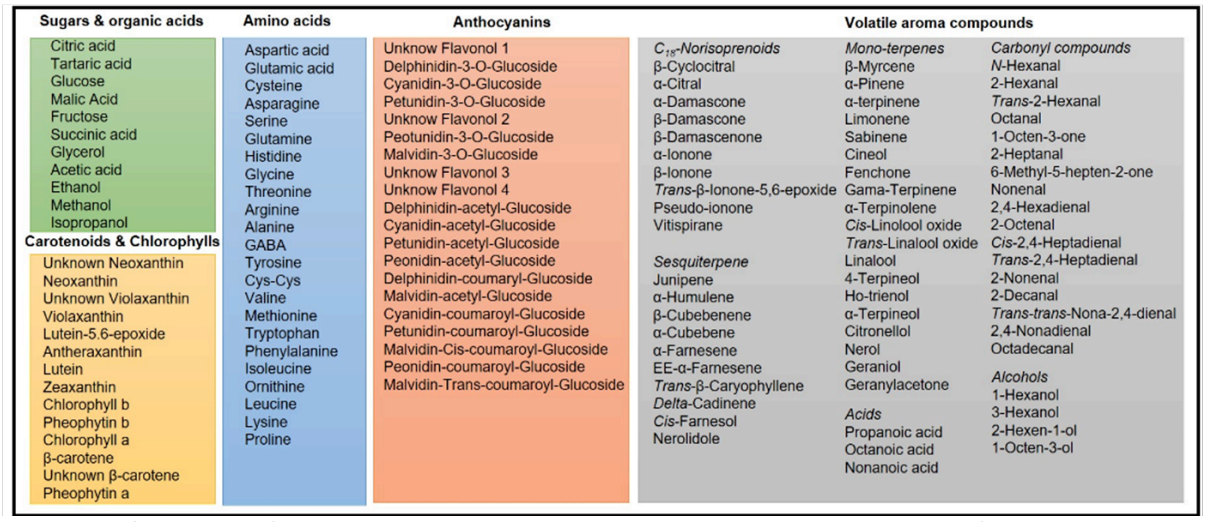


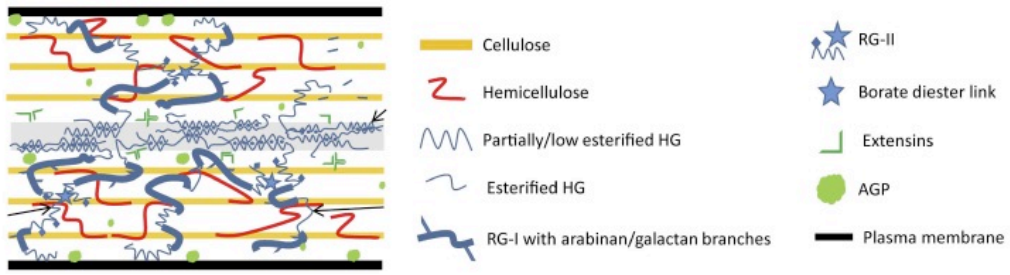
Figure 4. Summary of metabolites classes and individual metabolites targeted for chemical analysis (from Eyéghé-Bickong et al., 2017).

DATA ANALYSIS
Statistica (version 13) and Microsoft Excel (version was used for standard statistical analysis and testing. Multivariate data analysis was performed using SIMCA (Sartorius, version 14.1).

CELL WALL METHODS
Extraction of alcohol insoluble fraction (AIR) from berry tissues
The alcohol insoluble fraction (AIR) used for cell wall compositional analysis was isolated from homogenised tissue berry skin and pulp tissue using the method described in Ortega-Regules et al. (2008) using the modifications outlined in Zietsman et al. (2017).

CoMPP and monosaccharide composition analysis of berry cell wall material
Comprehensive Microarray Polymer Profiling (CoMPP) analysis of the AIR fraction was conducted in Denmark (Institute of Molecular Biology, University of Copenhagen, Denmark). The tissue was sequentially extracted to obtain the pectin and hemicellulose rich fractions using CDTA (diamino-cyclo-hexane-tetra-acetic acid) and then NaOH respectively. The arrays printed with these fractions were then probed with a panel of monoclonal antibodies (mAbs) and carbohydrate binding modules (CBMs) (Moller et al., 2007; Moore et al., 2014). The target specific epitopes related to cell wall polymers. This method was described in Moller et al. (2007) and has been validated for grapevine leaf (Moore et al., 2014) and berry tissue (Moore et al., 2014). A list of the probes used in the analysis is provided in Table 1, with an indication of how these probes correspond to the different cell wall polymers. A mean spot signal was calculated and each of the arrays was normalised to the highest signal in the dataset. A cut-off value of 5 was instated whereby any values calculated to be lower than 5 were said to be zero. The monosaccharide composition of the AIR fractions was also determined using GC-MS as described in Zietsman et al. (2015) and expressed as pM/mg AIR. Two representative samples per tissue type per treatment were selected for this analysis.

Table 1. A list of the probes used in the CoMPP analysis. A representation of the cell wall is presented and the probes and their targets are shown in relation to the cell wall structural model. Different colour components correlate with the coloured text in the table to give an indication of the cell wall components targeted by the different antibodies (Adapted from Zietsman et al., 2017).



Monoclonal antibody	Reference
HG (homogalacturonan) partially/de-esterified (mAb JIM5)	(Verhertbruggen et al., 2009)
HG partially esterified (mAb JIM7)	(Verhertbruggen et al., 2009)
HG partially/de-esterified (mAb LM18)	(Verhertbruggen et al., 2009)
HG partially/de-esterified (mAb LM19)	(Verhertbruggen et al., 2009)
HG partially esterified (mAb LM20)	(Verhertbruggen et al., 2009)
HG Ca^{2+} dimers (mAb 2F4)	(Liners et al., 1989)
RG-I (rhamnogalacturonan-I), 6 unbranched disaccharide (mAb INRA-RU1)	(Ralet et al., 2010)
RG-I, 2 unbranched disaccharide (mAb INRA-RU2)	(Ralet et al., 2010)
α-1,4-D-galactan (mAb LM5)	(Jones et al., 1997)
α-1,5-L-arabinan (mAb LM6)	(Willats et al., 1998)
Linearized α-1,5-L-arabinan (mAb LM13)	(Moller et al., 2008)
β-1,4-D-(galacto)(gluco)mannan (mAb LM21)	(Marcus et al., 2010)
β-1,4-D-(gluco)mannan (mAb LM22)	(Marcus et al., 2010)
β-1,3-D-glucan (mAb BS-400-2)	(Moller et al., 2008)
Xyloglucan (XXXG motif) (mAb LM15)	(Marcus et al., 2010)
Xyloglucan (mAb LM25)	(Pedersen et al., 2012)
β-1,4-D-Xylan (mAb LM10)	(McCartney et al., 2005)
β-1,4-D-Xylan d/arabinoxylan (mAb LM11)	(McCartney et al., 2005)
Cellulose (crystalline) (mAb CBM3a)	(Blake et al., 2006)
Extensin (mAb LM1)	(Neumetzler et al., 2012)
Extensin (mAb JIM11)	(Smallwood et al., 1994)
Extensin (mAb JIM20)	(Smallwood et al., 1994)
AGP (arabinogalactan proteins) (mAb JIM8)	(Pennell et al., 1991)
AGP (mAb JIM13)	(Yates et al., 1996)
AGP (mAb LM14)	(Moller et al., 2008)
AGP,b-linked GlcA (mAb LM2)	(Yates et al., 1996)

c) RESULTS AND DISCUSSION

The results and discussions for the two vineyard trial sites will be presented and discussed separately, for clarity.

THE ELGIN SAUVIGNON BLANC TRIAL: THE IMPACT OF UVB ON GRAPE BERRY METABOLITES IN A HIGH AND LOW LIGHT MICROCLIMATE

The findings of this section are expanded on in peer-reviewed scientific publications (Joubert et al., 2016; Du Plessis et al., 2017) as well as the associated PhD theses (Du Plessis, 2017 and Honeth, 2018) that are available for additional context. Here we highlight only a selection of prominent results that will be contextualised and discussed.

Characterization of the microclimates in the canopy and bunch zones confirmed that high light and low light microclimates were successfully established and that the UV attenuation was successful, obtaining four distinct microclimates as planned

The data obtained from the loggers confirmed that solar radiation (PAR) and UVB exposure were statistically significantly different in the respective microclimates generated for this study (Figure 5). These measurements were crucial for quantifying the microclimatic variables and the subsequent interpretation of the metabolite responses.

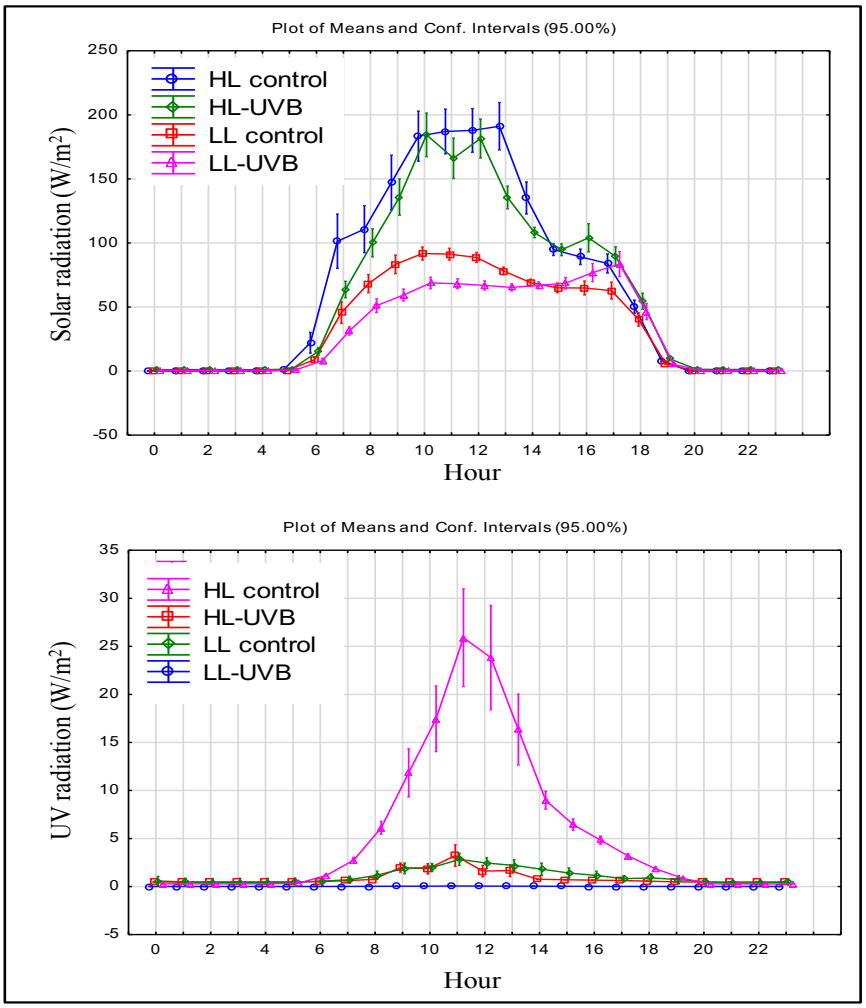
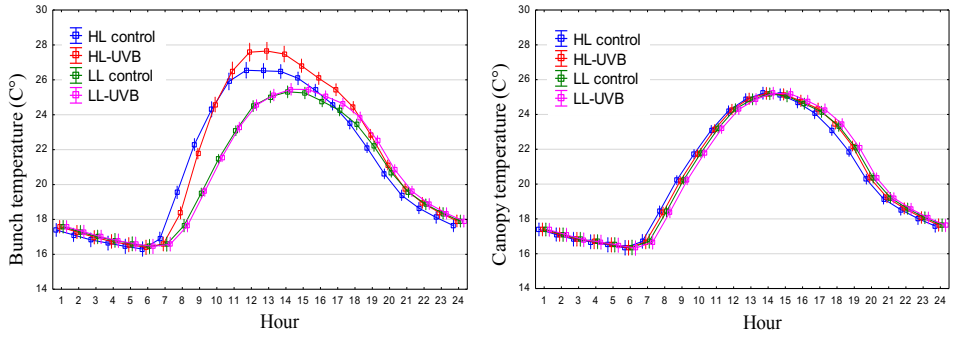


Figure 5. The mean hourly seasonal (i.e. from berry set to harvest) solar radiation (PAR) and UV radiation data (mean ±95% confidence interval) for each light environment. The first hour corresponds to 00h00-01h00. **The data confirms that four distinct microclimates, modulated in light (PAR) exposure and UVB exposure was obtained in the model vineyard.** (Figure from Joubert et al., 2016).

The specifications of the acrylic sheets used in the trial are reported to block out 99% of UV light. This was verified by measuring the UV radiation both behind and in front of the sheets. The UV-excluding sheets would block UVB (280-315 nm) since it excluded wavelengths between 280 and 350 nm. When evaluating the HL and LL environments separately, ANOVA plots furthermore showed that the HLcontrol (high light with UV exposure) and HL-UVB (high light without UV exposure) treatment (and similarly the LLcontrol and LL-UVB treatment) had similar solar radiation exposure levels, respectively, confirming that the UV-excluding sheets did not change the solar radiation (PAR) in addition to the impacts of the leaf removal treatment that successfully established the HLcontrol and LLcontrol microclimates.

The leaf removal and increased exposure lead to slight differences in the bunch temperature between the HL and LL microclimates, but the UV-excluding sheets did not lead to additional differences in temperature within the HL (i.e. HLcontrol versus HL-UVB) or LL microclimates (Figure 6). The canopy temperatures were similar between all four the experimental scenarios.



Parameters		High light + UV	High light - UV B	Low light + UV	Low light - UV B
Bunch temperature (C°)	Mean±SD	21.1±5.3	21.4±5.6	20.4±4.6	20.5±4.7
	Minimum	7.4	7.8	8	8
	Maximum	37.1	39.6	33.7	33.8
Canopy temperature (C°)	Mean±SD	20.3±4.6	20.4±4.7	20.4±4.6	20.4±4.7
	Minimum	7.4	7.4	7.4	7.3
	Maximum	33.9	34.1	34	34.1

Figure 6. The mean bunch and canopy minimum, maximum and mean ± SD) temperatures for all light environments and the corresponding kinetics showing the mean hourly bunch and canopy temperatures mean ± 95% confidence interval). The first hour corresponded to 00h00-01h00. (Figure from Joubert et al., 2016).

Developmental and treatment impacts on berry metabolites

When analysing the berry metabolites, the developmental stage (i.e. green/véraison/ripe) had the strongest effect on the measured chlorophyll, carotenoid and xanthophyll pool sizes, and the latter two pools were also significantly affected by both the exposure of the berries, as well as UVB attenuation. These results prompted a more in-depth analysis in a subsequent season on the photosynthetically-related pigments, as well as volatile compounds in reaction to UVB attenuation.

OPLS-DA plots using developmental stage (Figure 7A) or light exposure (Figure 7B) as Y- variables, and the corresponding coefficient plots of compounds that contributed most to the models, highlighted metabolites that responded to the two factors. Separation in the samples was observed according to developmental stage with both primary and secondary metabolites contributing, in varying degrees, to the observed separation. Similarly, variation in light exposure also resulted in a clear separation between samples, confirming the influence of a high light and low light environment on berry metabolism (Figure 7B). The metabolites mainly responsible for the separation, the xanthophylls, were similar to those previously reported by Young et al. (2016) in response to light exposure.

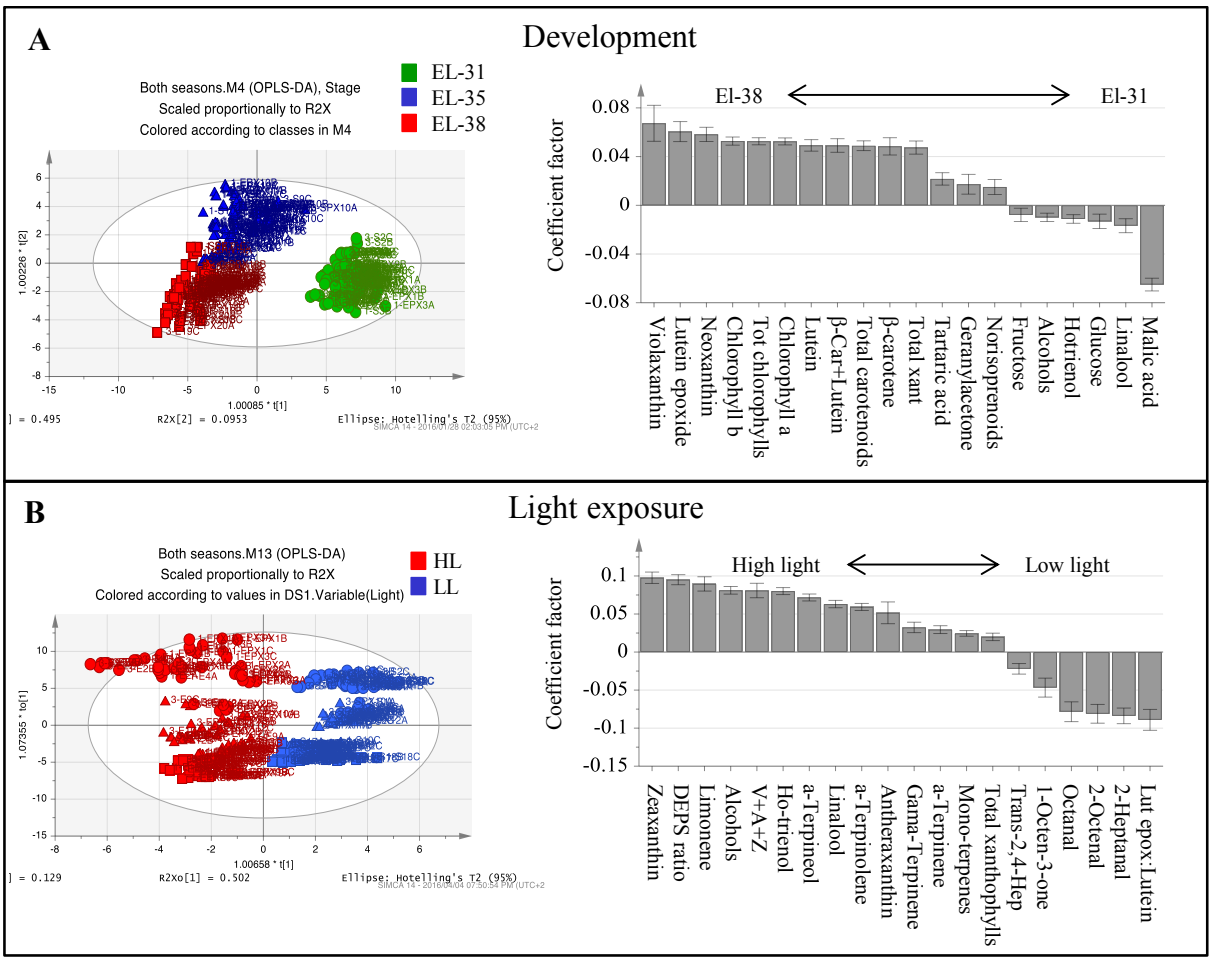


Figure 7. Multivariate data analysis as an overview and to identify and visualise metabolites responsive to the various factors. OPLS-DA models were generated for all metabolic data over both experimental seasons for developmental stage (A) and light exposure (B). Each OPLS-DA is accompanied by a co-efficient plot of metabolites that contributed most to the respective models. These were chosen according to the individual variable importance plots (VIP) and included the top compounds with a VIP ≥0.5. Shapes of the sample icons denote the respective developmental stages: EL-31 (●), EL-35 (▲) and EL-38 (■).(Figure from Joubert et al., 2016).

To better elucidate the subtle effects of UVB attenuation, OPLS-DA plots were created for the early and late stages of development separately. It was clear that different metabolites contributed to the separation in the green (Figure 8A) versus ripe berries (Figure 8B). The corresponding coefficient plots of compounds that contributed most to the models, highlighted specific xanthophylls and volatile aroma compounds that responded to UVB radiation/attenuation.

To simplify and visualise the data according to the main focus of the study (“What is the impact of UVB on berry metabolites and how is it different from exposure?”); compounds that responded to the variation in light exposure and/or UVB-attenuation were used to create Venn diagrams per developmental stage (Figure 9). Fisher LSD Post Hoc tests were used to identify statistically significant changes. Interestingly, in the pre-ripening stages, all compounds that responded to exposure, also responded to UVB attenuation. These compounds therefore differed in amplitude, and not in presence or absence. In the ripening stage, however, compounds were identified that responded exclusively to UVB attenuation.

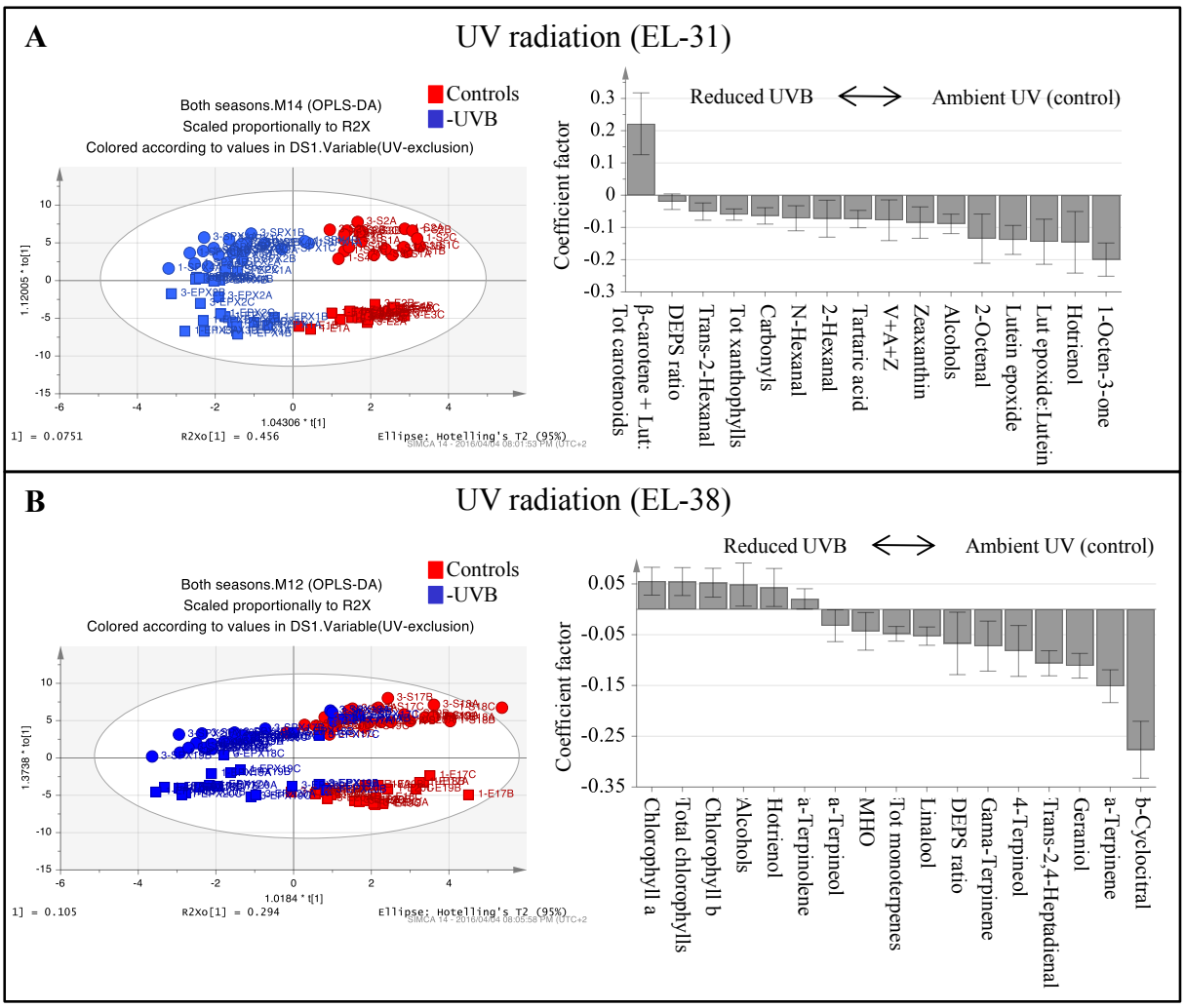


Figure 8. Multivariate data analysis as an overview and to identify and visualise metabolites responsive to the various factors. OPLS-DA models generated for all metabolic data over both experimental seasons for the early (A) and late (B) developmental stages, separately. The attenuation of UVB was used as the y-factor in both models. Each OPLS-DA is accompanied by a co-efficient plot of compounds which contributed most to the respective models. These were chosen according to the individual variable importance plots (VIP) and included the top compounds with a VIP ≥0.5. Shapes of the sample icons denote the respective exposure: High Light (■) and Low Light (●).(Figure from Joubert et al., 2016).

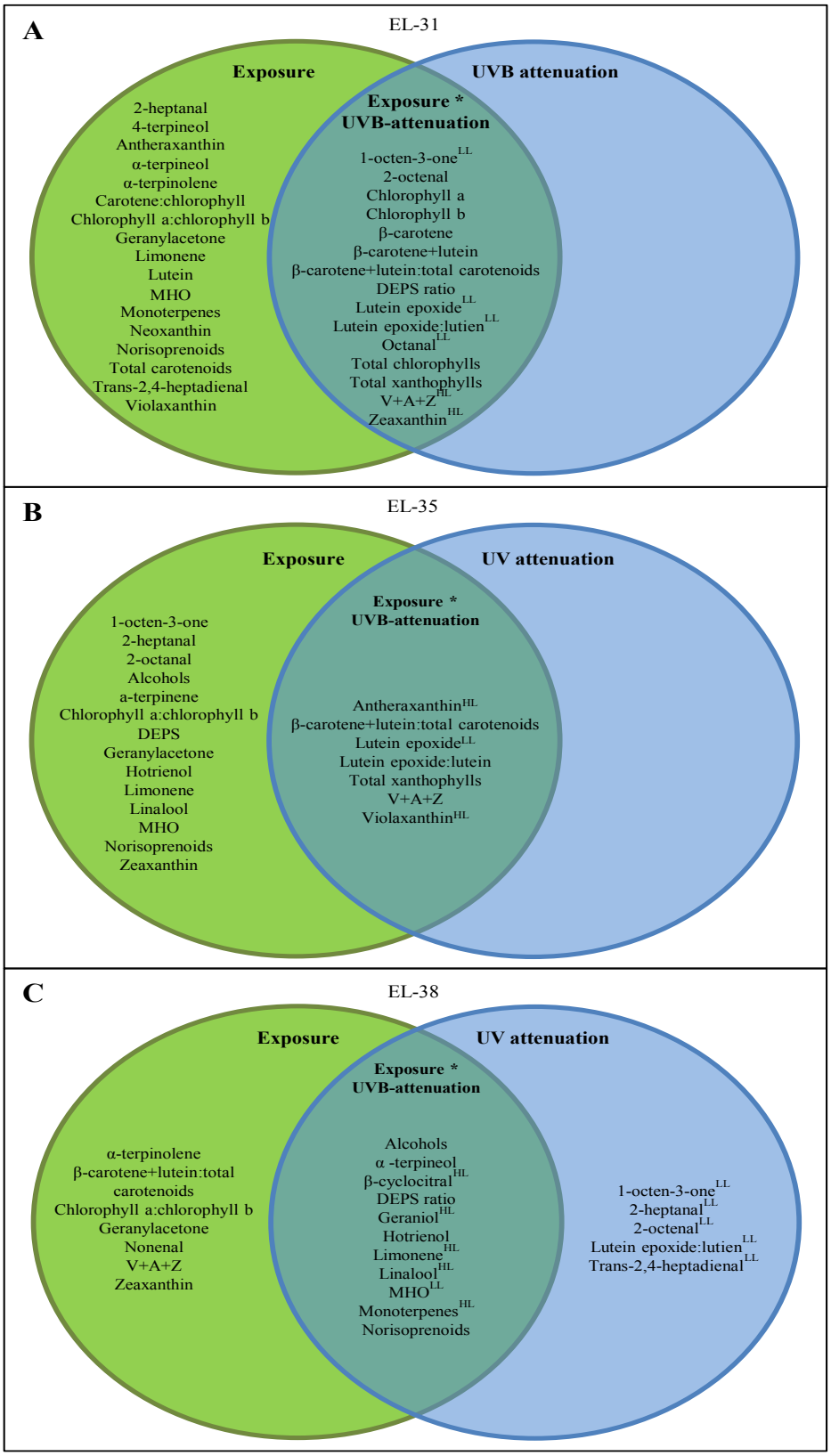


Figure 9. Composite view of all metabolites statistically significantly affected by light exposure (exposed versus control) and UVB (UVB or UVB-exclusion) per developmental stage. A Venn diagram was used to show the metabolites that responded to light exposure (green circle), UVB attenuation (blue circle) and both (intercept) in the early (A), véraison (B), and late (C) developmental stages. Compounds were selected for representation based on significance in a repeated measures ANOVA and Fisher LSD Post Hoc tests (adjusted p-value, q-value ≤0.05). All metabolites presented have a q-value ≤0.05. Metabolites with a log2-fold-change of ≥0.5 are indicated by a "HL" for the high light- or "LL" for the low light microclimate. (Figure from Joubert et al., 2016).

Specific xanthophylls responded to UVB attenuation in predominantly the green photosynthetically active berry stages

During the early stages of development, the xanthophylls zeaxanthin and lutein epoxide were identified as being the most responsive to UVB attenuation. Interestingly, the responses to UVB attenuation differed between the HL and LL environments. The attenuation of UVB in the HL environment resulted in a statistically significant decrease in zeaxanthin (Figure 10). This in turn resulted in a smaller xanthophyll pool size (violaxanthin, antheraxanthin, zeaxanthin) and a consequent lowered de-epoxidation state (DEPS ratio) in those samples (Figure 10). Although this was particularly obvious at the green berry stage, the lower xanthophyll pool, and consequent lower DEPS ratio, was consistently seen throughout berry development in the HL-UVB microclimate, but decreasing with developmental stage progression. Furthermore, the attenuation of UVB in the LL environment also resulted in a decreased V+A+Z pool and a lowered DEPS ratio in the green stage (Figure 10), although the effect was less pronounced compared to HL.

A significant difference in the levels of lutein epoxide between the LLcontrol and LL-UVB contrasts was also confirmed, clearly showing that UVB exposure in LL conditions is involved in the metabolism of lutein epoxide. Since lutein levels did not change, the Lx:L ratio was consequently significantly affected in the green developmental stage and to a lesser degree at the harvest stage (Figure 10).

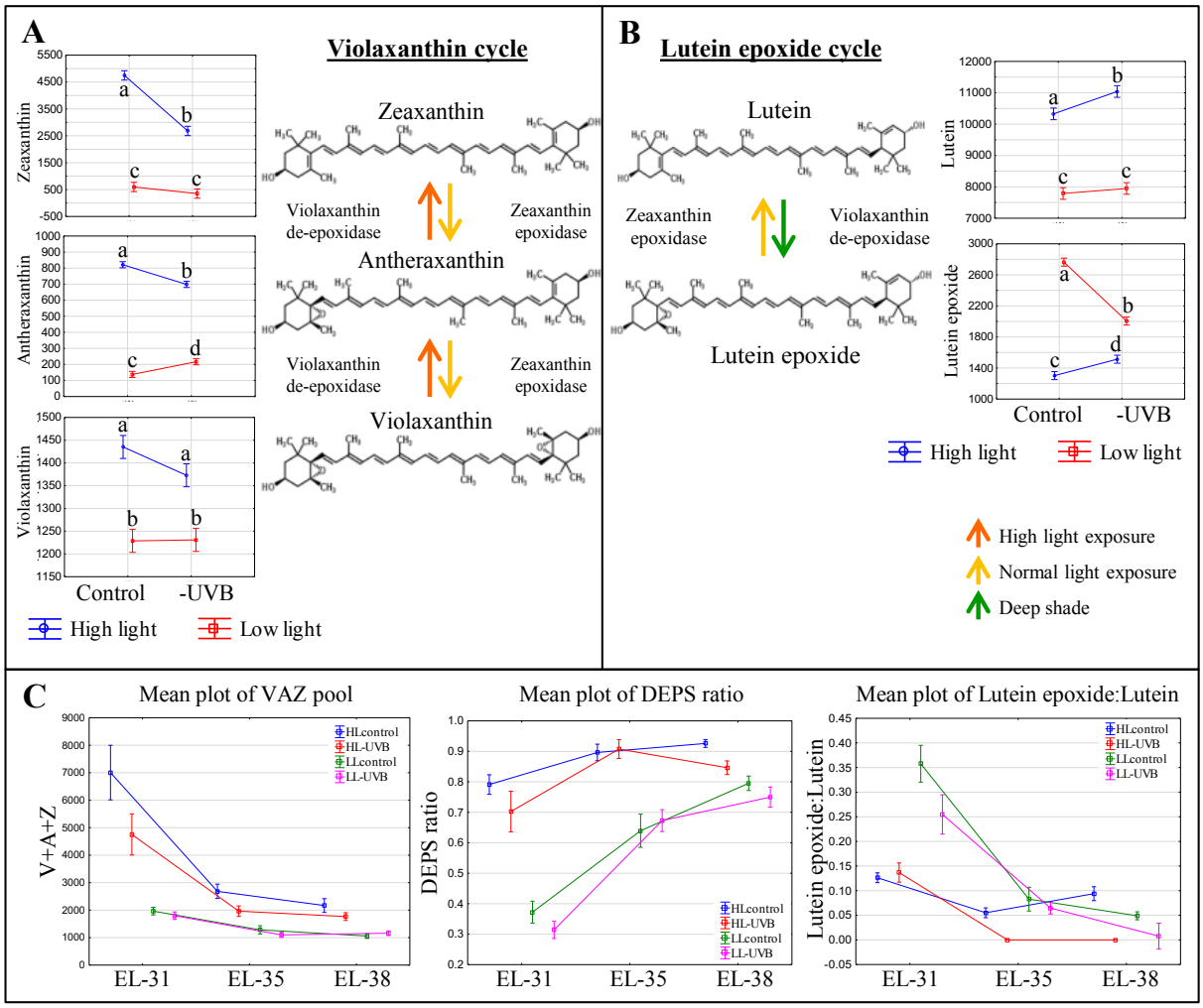


Figure 10. Contextualisation of the data into grapevine metabolic pathways of physiological relevance during light stress. The violaxanthin (A) and lutein epoxide (B) cycles with the ANOVA graphs for their associated xanthophylls in the green developmental stage (EL-31). Different letters indicate significant difference ($p \leq 0.05$). The mean plots of the associated xanthophyll pool (V + A + Z), DEPS ratio and lutein epoxide:lutein ratio for both high- and low light environments over all developmental stages (C). (Figure from Joubert et al., 2016).

In the ripe berry stages, specific volatiles responded to UVB attenuation
UVB attenuation was shown to affect specific volatile compounds in the ripe developmental stage (EL-38). These included monoterpenes, carotenoid-derived norisoprenoids and certain C6 compounds. In the HL environment, certain monoterpenes and norisoprenoids were decreased by UVB attenuation, leading to larger monoterpene and norisoprenoid pools in the HL control samples (Figure 11) and confirming that UVB exposure stimulates volatile organic compounds (VOCs) in exposed berries. Under LL conditions, however, both the monoterpene and norisporenoids pools were decreased relative to the HL microclimate and UVB attenuation resulted in no further statistically significant differences between the LLcontrol and LL-UVB microclimates.

Interestingly, under LL conditions, different VOC profiles as well as contents of individual volatile compounds were observed when comparing the LLcontrol with the UVB attenuated microclimate (LL-UVB) in ripe berry samples. Certain straight chain aldehydes and ketones (e.g. 1-octen-3-one, 2-heptanal and trans-2,4-heptadienal), decreased with UVB attenuation. Conversely, a significantly higher concentration of C6 compounds, including trans-2-hexenal and N-hexanal were observed when UVB was attenuated in the LL environment. This is the opposite of the scenario in HL, where the HLcontrol had more total C6 compounds than the HL-UVB (Figure 11).

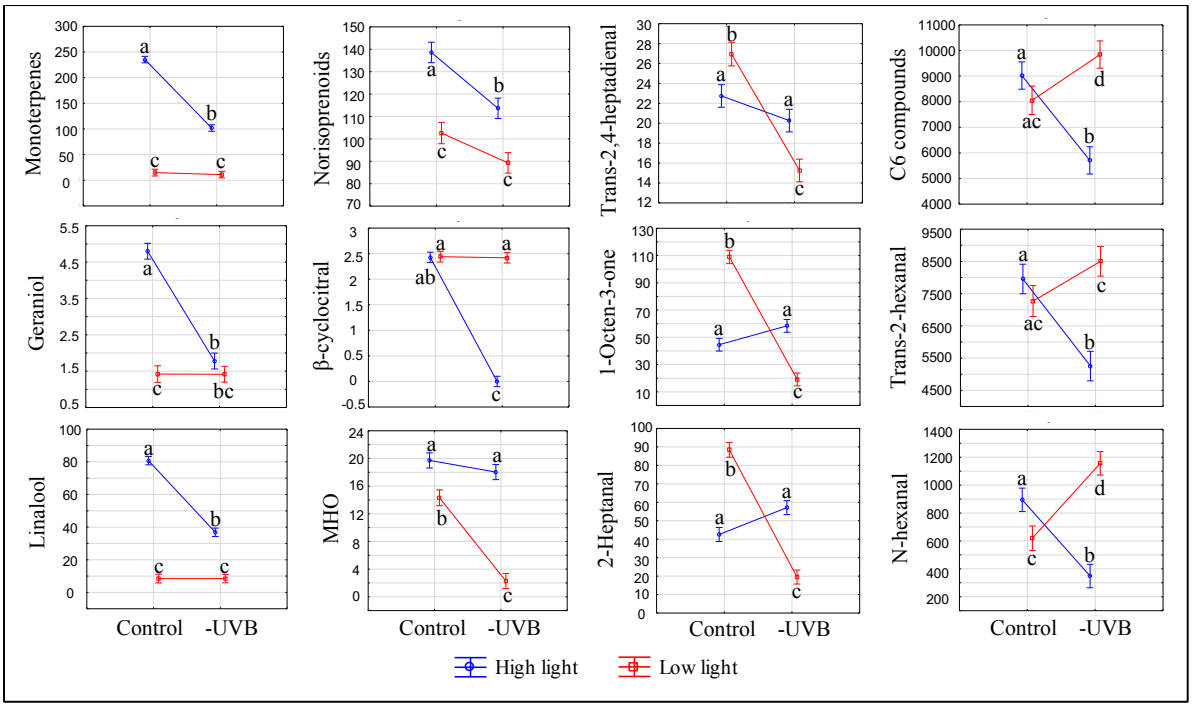


Figure 11. **Impact of light quality and quantity on volatiles in the berries at the ripe (harvest) date.** Analysis of variance (ANOVA) plots for selected volatiles, including monoterpenes, norisoprenoids and C_6-compounds measured at the late developmental stage (EL-38). The results for both high light and low light environments are represented. Different letters indicate significant difference ($p \leq 0.05$).

Furthermore, to control for well-known metabolite responses to UV, samples were also analysed for polyphenols. As expected, total polyphenolics, and specifically the flavonol quercetin-glucoside, was significantly reduced with UVB attenuation in the HL microclimate, most notably in the early developmental stages (Figure 12), although this pattern followed through to harvest. No statistical significances were seen in the LL microclimate (LLcontrol versus LL-UVB) in either the early- or late developmental stages.

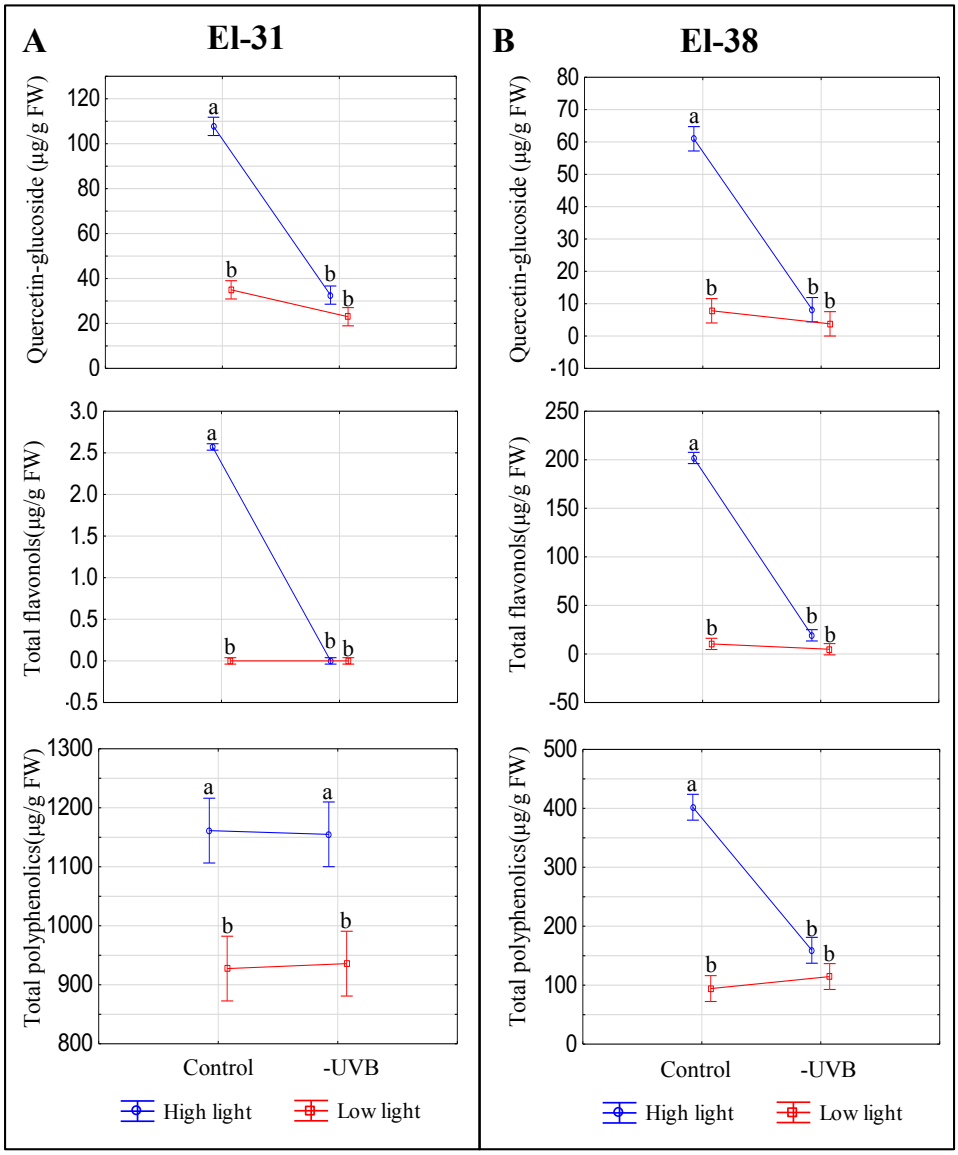


Figure 12. Impact of light quality and quantity on phenolics in the berries in the early (green-) and ripe (harvest) developmental stage. The ANOVA plots for quercetin-glucoside, total flavonols and total polyphenolics measured at the early (EL-31) (A) and late developmental stage (EL-38) (B). The results for both high light and low light environments are represented. Quercetin-glucoside is expressed in µg/g fresh weight. The pooled compounds are expressed relative to quercetin-glucoside (µg/g fresh weight). Different letters indicate significant differences ($p \leq 0.05$).

Figure 13 proposes an overview model of the respective responses and highlights the importance of the developmental stage (early or late) as well as the microclimate (HL or LL) on the metabolites that are differentially produced and proposed to play a role in the acclimation responses. The data presented supports the hypothesis that plants in shade are less acclimated and consequently more susceptible (on e.g. a clear day) than the exposed (HL) more acclimated counterparts (typically displaying higher flavonols, higher photo-protective xanthophylls, and/or antioxidant volatiles, depending on the developmental stage). In the absence of UVB, less acclimation has potentially occurred in the LL-UVB and the plants will be more susceptible (to e.g. sunflecks) than the more acclimated HL-UVB counterparts. Here we show that these general plant responses are active in grapevine berries with developmental stages displaying distinctive responses.

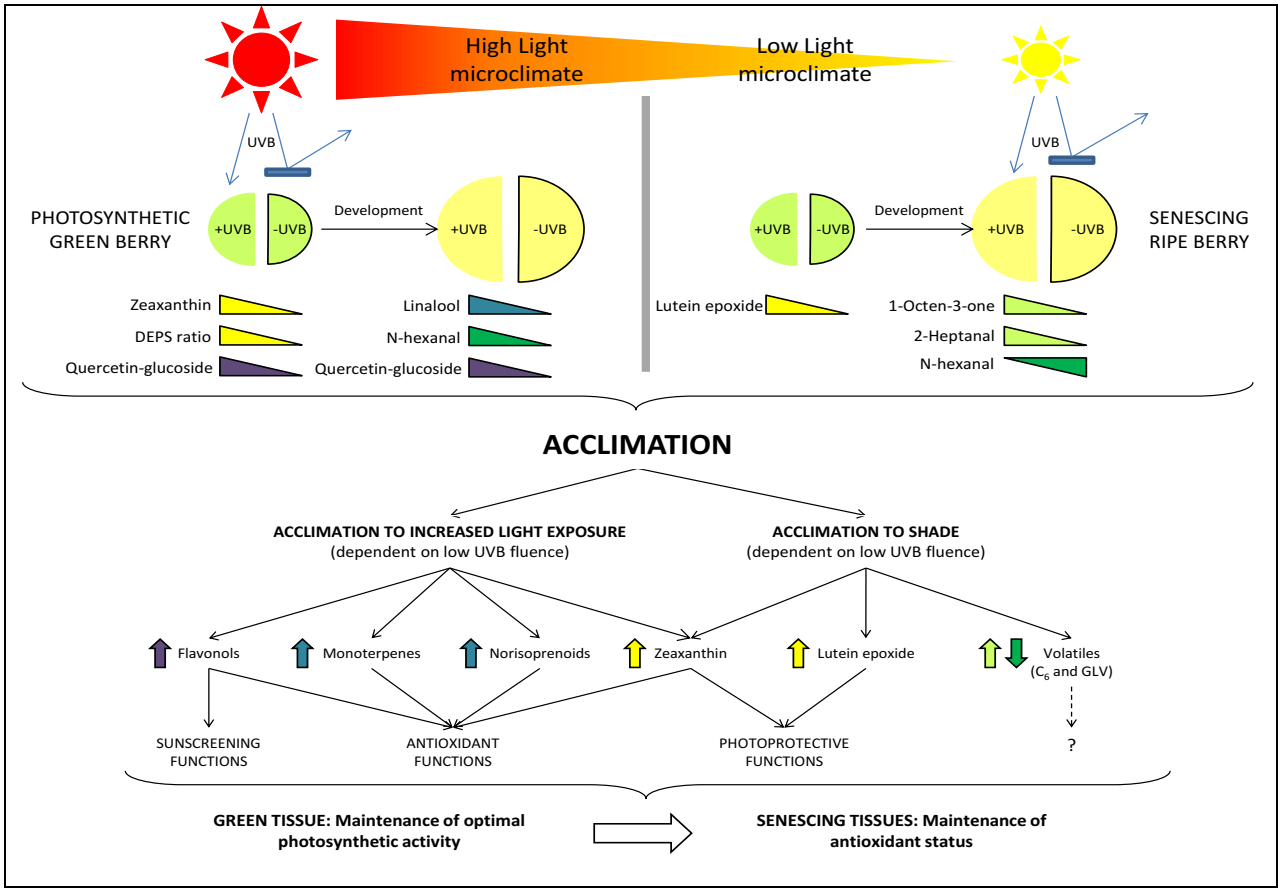


Figure 13. A comprehensive model summarizing the results of the study. In each light environment (HL and LL) both early and late developmental stages are represented as well as the attenuation of UVB. The coloured triangles indicate those compounds which reacted to UVB attenuation in each case, indicating the presence of an acclimation response in the berries. Each of the compound groups perform a specific function in the berry tissue and contribute to the acclimation of the berry via various physiological processes. These processes differ depending on the tissue type and are therefore associated with the developmental stage of the berry.

Cell wall analysis of ripe berry skin and pulp tissues from the Elgin Sauvignon Blanc

Profiling data of the skin and pulp cell wall samples showed high levels of homogalacturonans (HG) and rhamnogalacturonan with its associated side chains extracted from the CDTA fraction with some extensins and AGPs. These levels were generally higher in the pulp tissue as opposed to the skin tissue, with a few exceptions seen in specific epitopes. Additionally, strong signals for the epitopes linked to glucans, xyloglucans, cellulose, extensins and AGPS were seen in the hemicellulose rich fraction (NaOH) as well as epitopes linked to rhamnogalacturonan with its associated side chains, particularly in the pulp tissue. No statistically significant differences were however observed between the grapes from the four microclimates. To support the CoMPP profiling analysis, the AIR from the samples was also subjected to monosaccharides analysis which confirmed that the samples did not differ significantly between the controls and UVB attenuation samples in either the HL or LL microclimate. The altered microclimates therefore affected berry metabolism without causing morphological changes to the grape cell walls.

THE ROBERTSON SHIRAZ TRIAL: THE IMPACT OF ROW-ORIENTATION ON GRAPE BERRY METABOLITES

Row orientation created distinct canopy microclimates that differed in the timing and extent of exposure with regard to temperature and/or sunlight:

A vineyard's global position largely dictates its macroclimate. Various physical characteristics of the vineyard, including topography (aspect and slope), altitude and soil, will further influence the vineyard mesoclimate. The vineyard row orientation dictates the timing and intensity of light exposure in the

microclimate. The impact of four row orientations (N/S, NE/SW, E/W and NW/SE) on the microclimate were characterised per canopy side (facet) in the morning (09h00/AM) and afternoon (16h00/PM) and the consequent impact on berry composition. Figure 14 indicates the respective position of the sun at the sampling times in context of the row orientation trial. Photosynthetic Active Radiation (PAR) measurements for the different row orientations at the two sampling times are shown in Figure 15, clearly indicating which of the row orientations lead to the most diverse light conditions in total levels as well as shifts during the daily cycles (N/S), versus the E/W orientation that exhibited lower levels and little change during comparing the morning and afternoon sampling.

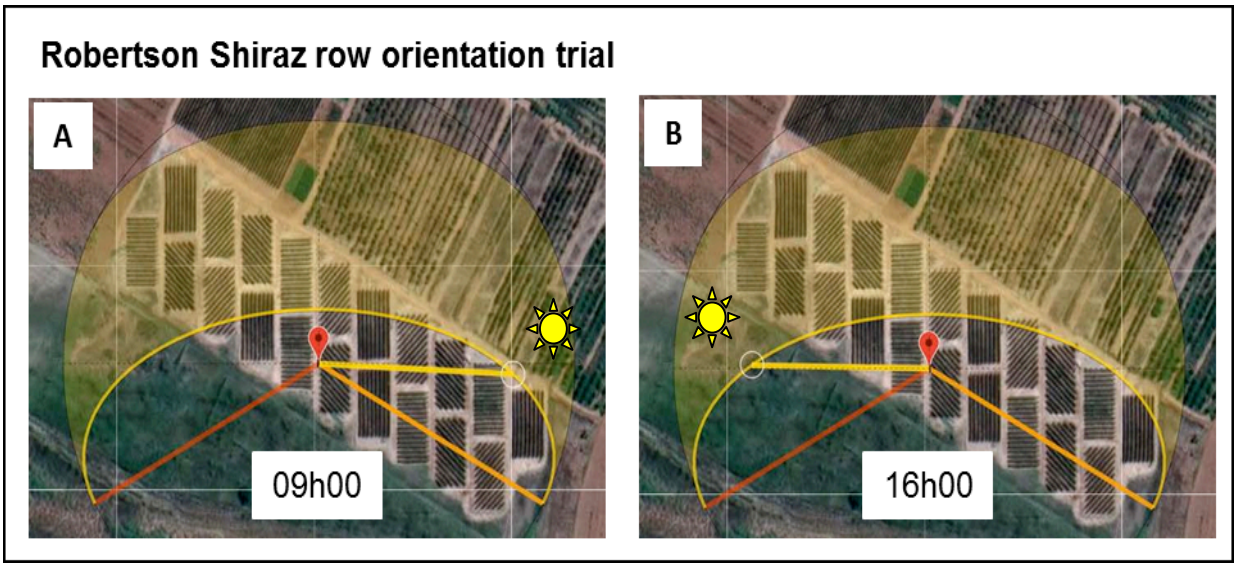


Figure 14. **The Robertson Shiraz row-orientation trial showing the respective position of the sun at the sampling times.** The position of the sun at (A) the AM (09h00), and (B), the PM (16h00) sampling times. Light orange line, indicates the position of the sun at sunrise; yellow line, position of the sun at sampling times and dark orange line, position of the sun at sunset.

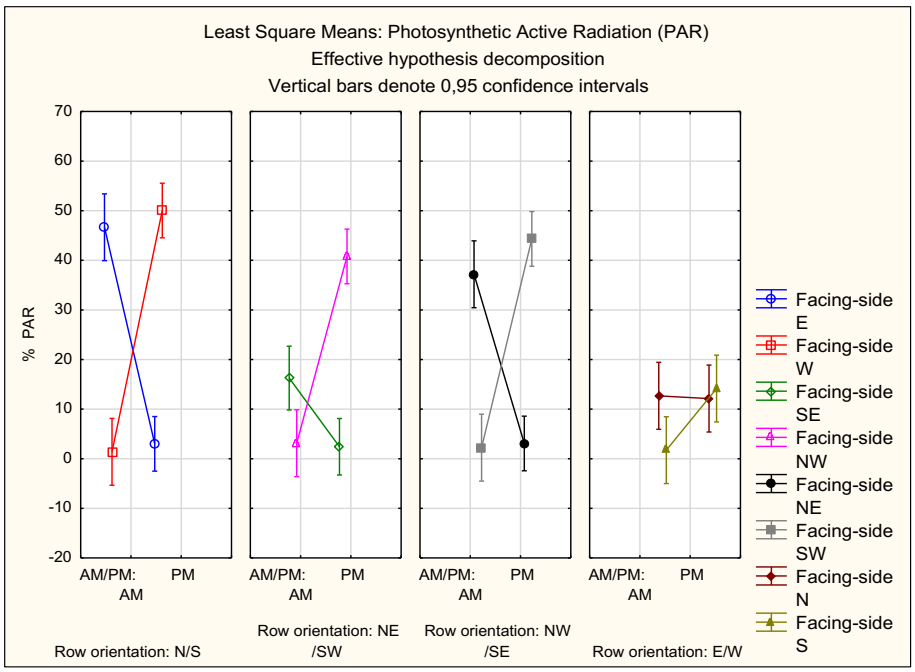


Figure 15. **Photosynthetic Active Radiation (PAR) measurements were taken before and after each sampling time and expressed as a percentage relative to the ambient (full) sunlight.** The two facets of each row orientation are shown in the AM (09h00) and PM (16h00).

Microclimatic measurements were also used to characterise the temperature and light in the bunch zones of the four row orientations in the two facets of the canopy. Figure 16 indicates the daily average temperatures in the four row orientations in the two facets of the canopy, whereas Figure 17 shows the impact row orientation had on the cumulative bunch temperature in the two facets of the canopy. It was clear that the morning exposed microclimates were on average cooler than the afternoon exposed microclimates, but that the morning exposed microclimates experienced quick changes in temperature, whereas the afternoon exposed microclimates experienced more gradual increases during the day.

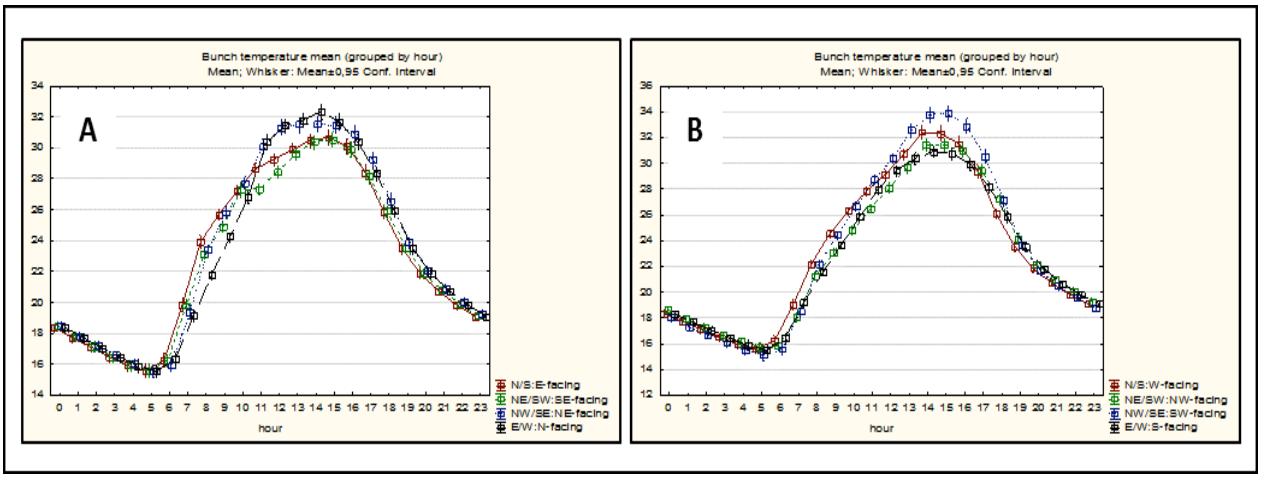


Figure 16. **Temperature loggers in the bunch zones were used to determine the daily average bunch temperatures in the respective facets**: (A) "morning"-exposed facet, (B) "afternoon"-exposed facet.

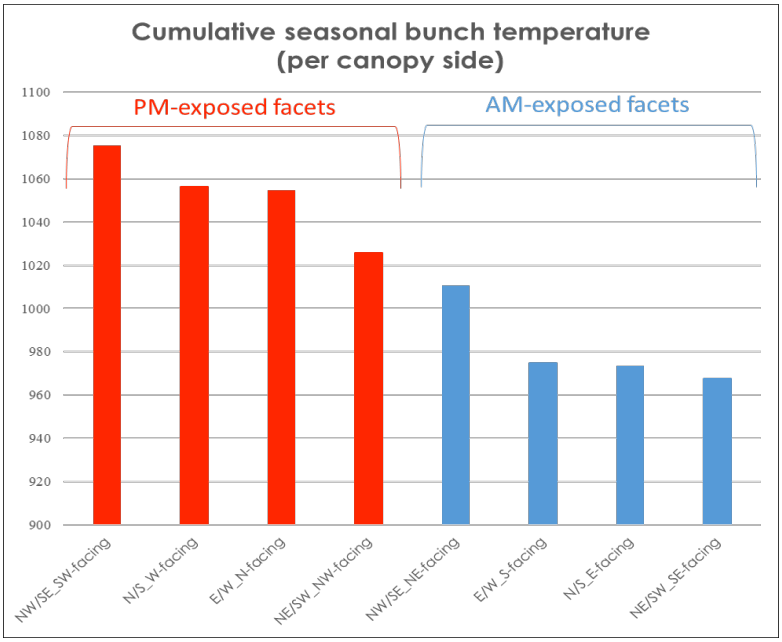


Figure 17. **Seasonal cumulative bunch temperature accumulation in the two facets of each of the four row orientations.** Red, afternoon-exposed facet; Blue, morning-exposed facet.

These results confirmed that the row orientations established very diverse microclimates and that not only the seasonal microclimatic differences are important, but also changes that occur on a daily cycle in both sides of the canopies of the different row-orientations.

These microclimatic differences caused modulations in metabolite composition of specific metabolites or metabolite classes (dependent on developmental stage):

The berry samples from the three developmental stages that were analysed for the primary and secondary metabolites can be used to evaluate the impacts of the different row orientations. Cell wall profiling also provided a cell wall model for the of Shiraz berries from the vineyard, showing the differences between the skin and pulp cell walls (Figure 18).

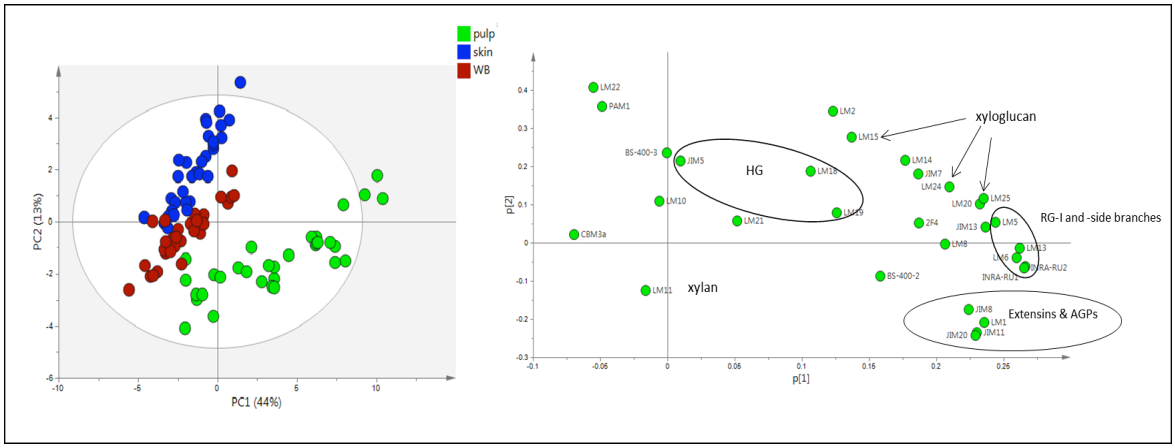


Figure 18. Pectin-rich fraction extracted from the isolated cell walls of Shiraz grapes at different ripeness levels. The PCA shows separation due to tissue type (skin, pulp and whole berry ["WB"].

The data generated from the experimental setup is complex (multifactorial) and can therefore be interrogated from a number of perspectives. For example, the data can be used to identify the metabolites primarily responsive to:

1. Developmental stage (Green, véraison, ripe);
2. Row orientation (N/S, NE/SW, E/W and NW/SE);
3. Canopy side (or facet, e.g. "morning"-exposed versus "afternoon"-exposed); or
4. Time of day (AM versus PM).

The first approach was to use all the data and perform hierarchical clustering to provide an overview of the data. It provided proof that the biological repeats sampled grouped together, providing confidence in the experimental design of the experiment and showing that the green, veraison and ripe samples formed three big groupings as expected. The same approach was also applied to visualise the metabolic impacts of each row orientation. In Figure 19, the clustering of the N/S row-orientation is shown.

Looking deeper into the metabolites it was clear that some metabolites, particularly primary metabolites and some pigments were remarkably well regulated and followed predictable patterns and were largely unaffected by any of the factors investigated (for an example, refer to Figure 20).

On the other hand, the data also showed that the berries adapted to their microclimates by using a number of very responsive secondary metabolites to deal with differences in timing, duration and degree of exposure to temperature and/or light, as shown in Figure 21 for a number of these types of responsive metabolites.

The AM/PM sampling regime also pointed to metabolic plasticity in daily acclimation cycles in the berries, depending on their microclimates. This aspect provides novel insight that metabolites, many of them quality impact factors such as flavonoids and aroma-compounds are labile, not only over a season and in response to microclimatic features, but could also change (increasing and decreasing) during a typical day/night cycle. The data showed that the berries responded to their different microclimates by implementing slight adjustments to their metabolism on a continuous basis.

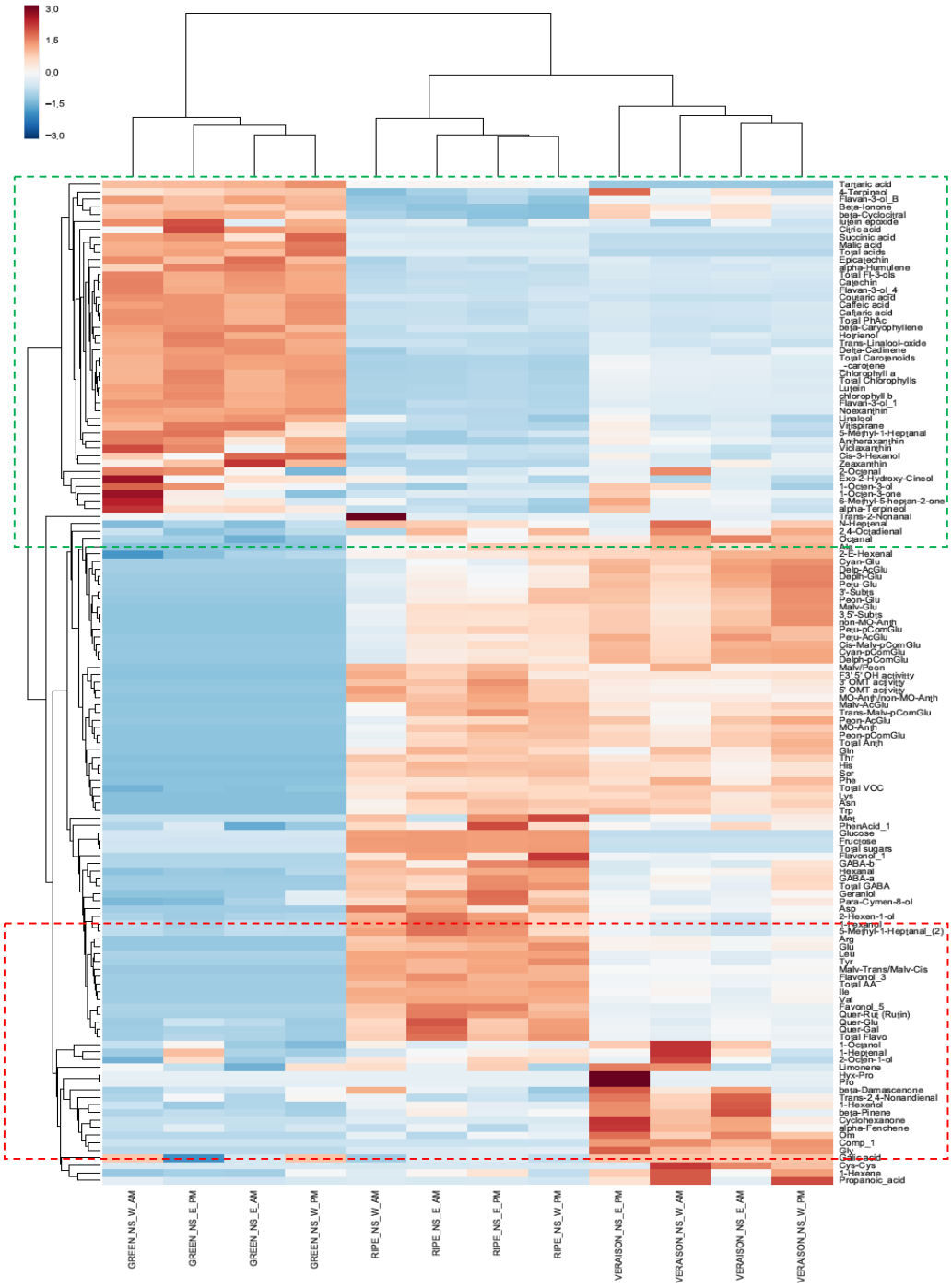


Figure 19. Hierarchical cluster analysis (HCA) zooming in to the N/S row orientation (only) and includes all developmental stages, canopy facets, and sampling times. To illustrate (as in Figure 18 above), the dashed red box shows the metabolites with a similar pattern to glucose and fructose (i.e. metabolites that increase with berry development). The dashed green box groups the metabolites with a similar pattern to malic acid and chlorophylls (i.e. metabolites that decrease during berry development). **Hierarchical cluster analysis (HCA) provides an accessible, data-rich overview of the complexity of the metabolite data generated in the study.**

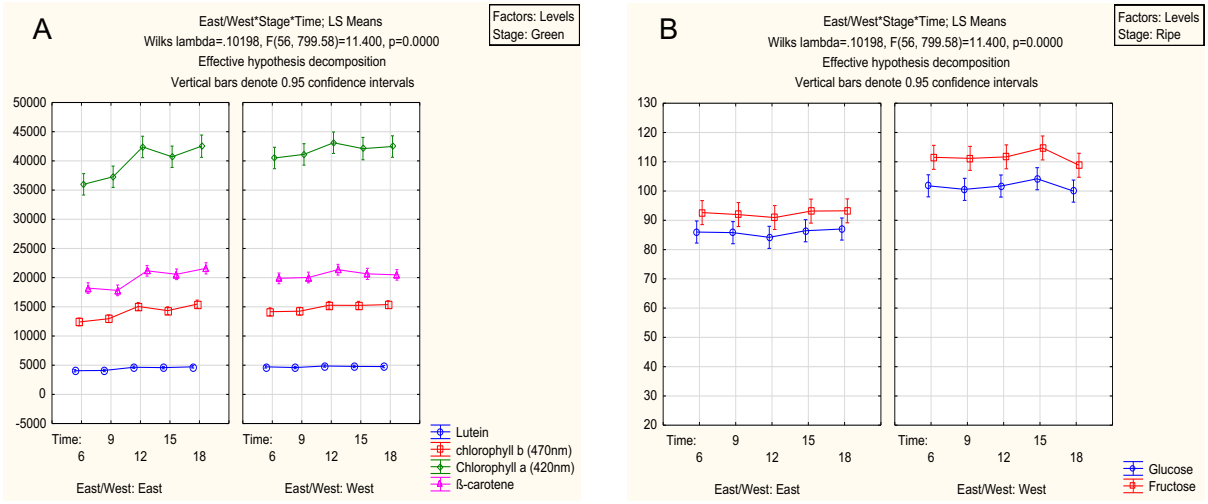


Figure 20. Some berry metabolites and metabolite classes were remarkably constant, irrespective of their environment. Photosynthetic pigments in the green stage (A) and major sugars in the ripe stage(B) were unaffected in the N/S row orientation by time of day, and minimally affected by the canopy side, with the PM-exposed W-facing side having higher sugars.

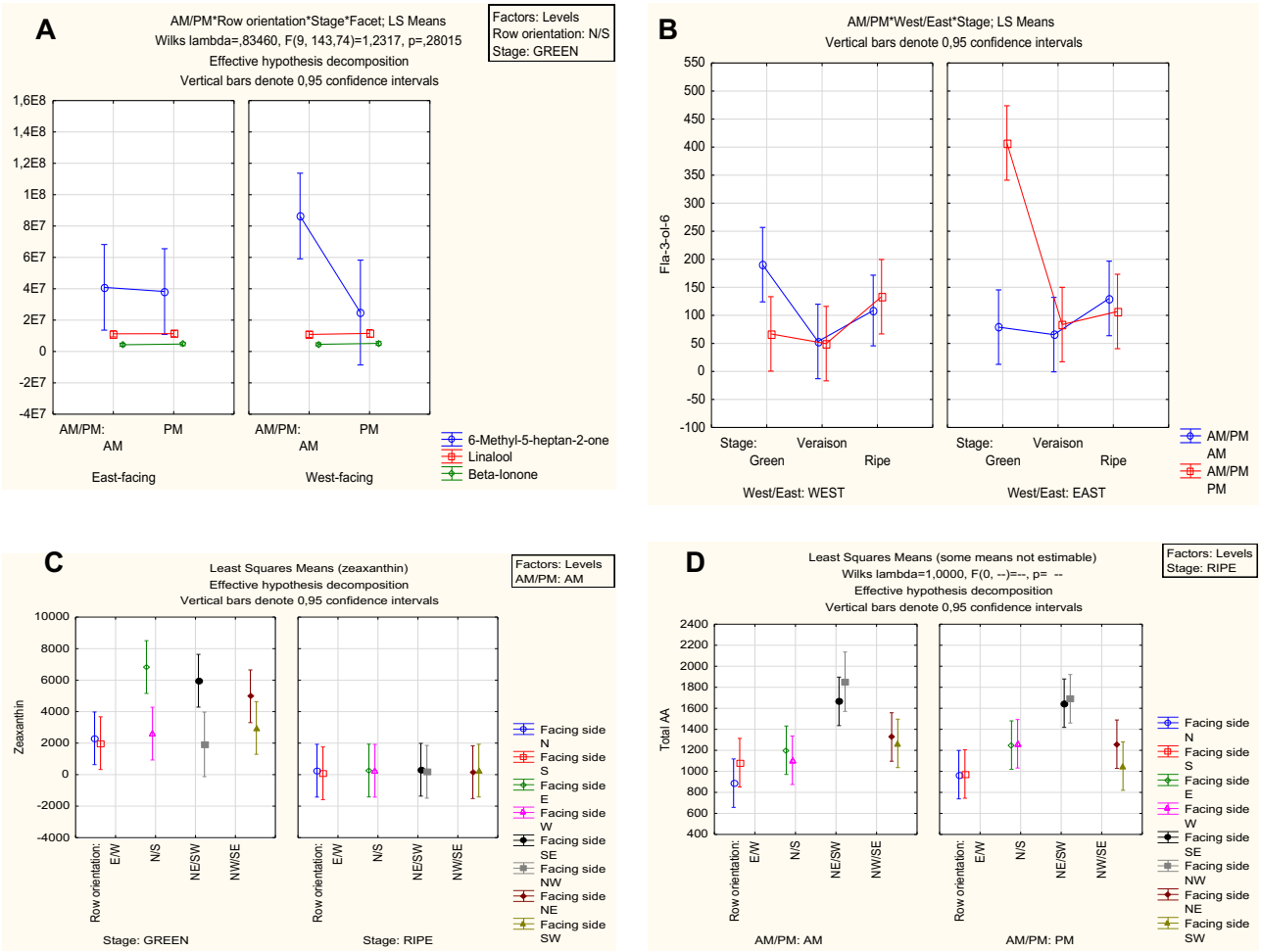


Figure 21. Specific berry metabolites and metabolite classes were remarkably responsive to their immediate environments and were dependent on numerous factors (developmental stage, time of day, or facet). (A) the response of a specific volatile norisoprenoid, b-ionone; (B) flavanols; (C) zeaxanthin; and (D) total amino acids, to time of day and/or facet.

d) CONCLUSIONS

The Elgin Sauvignon Blanc and Robertson Shiraz studies have highlighted the remarkable ability of grapevine cultivars to respond to their microclimates. These responses were shown to be dynamic and specific metabolites responded to changes in light (PAR and UVB) and/or temperature in the microclimates. These responses can range from rapid (daily cycle, e.g. zeaxanthin) to long-term (seasonal, e.g. phenolics) responses.

The Sauvignon Blanc model vineyard proved that sunlight is a complex signal for plants and both the quality (PAR and UVB) and quantity (high light versus low light) can impact grape berry composition. These responses develop over the duration of the season and what occurs in the early stages of the berry development have a lasting impact on the berries in the ripe stage as well. It was possible to identify the metabolites that were responsive to canopy manipulation (leaf removal and UVB-exclusion), and the process of acclimation (to an altered microclimate) was considered the outcome for the berries exposed to the different microclimates. Cell wall analyses showed that the canopy manipulations (leaf removal and UVB-exclusion) did not cause long-term morphological changes, but rather short-term acclimation utilising metabolite changes.

The Robertson row orientation experiments in-stead represented long-term adaptation in berry composition. The vineyard layout (established by the ARC) provided a unique opportunity to dissect the impact of row orientation on berry composition. The experimental setup made it possible to identify metabolites that were differentially affected by row orientation (N/S, NE/SW, NW/SE and E/W) as well as the metabolites that were differentially affected by the canopy side (e.g. morning-exposed versus afternoon-exposed) and the time of day (09h00 versus 16h00). This demonstrated the dynamic responses of metabolite in response to their immediate microclimates. The xanthophylls (e.g. zeaxanthin and antheraxanthin) showed rapid responses to the light and temperature on a daily basis. Amino acids were less responsive to the canopy side and/or time of day, but were very responsive to the row orientation.

Collectively, these studies highlight the remarkable adaptability of grapevine to its microclimate. Berry composition of quality-associated metabolites are responsive and demonstrates the impact that viticultural actions (long-term row orientation or short-term canopy manipulations) can have on berry composition.

5. ACCUMULATED OUTPUTS

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

TECHNOLOGY DEVELOPED:

Analytical methods for targeted metabolite analysis have been developed (previously) and have been implemented for the red (Shiraz) and white (Sauvignon Blanc) grape berry matrix (from different developmental stages):

- Sugars and organic acids: HPLC (RID and DAD) [2015].
- Amino acids: HPLC (fluorescence) [2017].
- Phenolics (i.e flavanols and anthocyanins): HPLC (DAD) and migrated to a faster and more cost efficient UPLC (DAD and MS verification) system; 2017.
- Photosynthetic pigments (carotenoids and chlorophylls): UPLC (DAD) [2015].
- Volatile flavour and aroma compounds: GC-MS; [2016].
- Rapid infra-red (MIR/NIR) spectroscopic analyses are being evaluated (analytical methods listed in points 1-5 provided the reference datasets for calibrations) [2015].
- Grapevine cell wall profiling methods. [2015].

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

The outputs have already formed part of several industry-targeted presentations, for example two presentations have been made at study groups, the Sauvignon Blanc Forum, as well as at symposia of the South African Society for Viticulture and Oenology. Some aspects were already highlighted in Wineland publications, but there is still more scope to produce more of these outputs.

Given the extensive number of scientific publications and theses that were developed as part of this project, a technology transfer plan should be established in partnership with Winetech and VinPro to "package" the information appropriately and to bring awareness of the outcomes. Based on the results, our group has gained recognition for the approach of field-omics and in particular, the insights gained from the study in terms of the impacts of light quality and quantity on berry metabolism of Sauvignon Blanc. Despite the large number of outputs already generated, there are still quite a few unfinished manuscripts in draft that will lead to more popular papers as well.

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

Student Name and Surname	Student Nationality	Degree (e.g. MSc Agric, MComm)	Level of studies in final year of project	Graduation date	Total cost to industry throughout the project
Honours students					
Masters Students					
Davirai Musingarawbi	Zimbabwe	MSc	MSc	2015	R50 000 (approximate project costs)
PhD students					
Anscha Zietsman	South Africa	PhD	PhD	2015	R100 000 (approximate project costs)
Kari du Plessis	South Africa	PhD	PhD	2017	R250 000 (bursary and approximate project costs)
Yu Gao	China	PhD	PhD	2016	R150 000 (bursary and approximate project costs)
Chandre Joubert	South Africa	PhD	PhD	2018	R150 000 (bursary and approximate project costs)
Postdocs					
Dr Hans Eyeghe- Bickong	Gabon	na	na	na	R150 000 (approximate project costs)
Dr Jay Belli Kullan	India	na	na	na	R150 000 (contribution to fellowship and approximate project costs)
Support Personnel (not a requirement for HORTGRO Science)					

PERSONS PARTICIPATING IN THE PROJECT (Excluding students)

Initials & Surname	Highest Qualification	Degree/ Diploma registered for	Race (1)	Gender (2)	Institution & Department	Position (3)	Cost to Project R
MA Vivier	PhD	NA	W	F	IWBT/DVO, SUN	PL	0
PR Young	PhD	NA	W	M	IWBT/DVO, SUN	PL	200 000 p/a
JP Moore	PhD	NA	W	M	IWBT, SUN	Co	0
J Trygg	PhD	NA	W	M	Umea University	Coll	0

(1)Race B = African, Coloured or Indian
W = White

(2)Gender F = Female
M = Male

(3)Position Co = Co-worker (other researcher at your institution)
Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
PF = Post-doctoral fellow
PL = Project leader
RA = Research assistant
TA = Technical assistant/ technician

d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

Peer-reviewed publications:

1. Musingarabwi, Davirai M., Nieuwoudt, Hélène H., Young, Philip R., Eyéghè-Bickong, Hans A. & Vivier, Melané A. (2016) A rapid qualitative and quantitative evaluation of grape berries at various stages of development using Fourier-transform infrared spectroscopy and multivariate data analysis. *Food Chemistry*, 190, Pages 253-262.

2. Yu Gao, Jonatan U. Fangel, William G.T. Willats, Melané A Vivier and John P. Moore. 2016. Effect of intra-vineyard ripeness variation on the efficiency of commercial enzymes on berry cell wall deconstruction under winemaking conditions. *Journal of Agricultural and Food Chemistry*

3. Joubert Chandré, Young Philip R., Eyéghé-Bickong Hans A. & Vivier Melané 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. *Frontiers in Plant Science*. 7, 786. DOI=10.3389/fpls.2016.00786.

4. Du Plessis, K., Young, P. R., Eyéghé-Bickong, H. A., & Vivier, M. A. (2017). The transcriptional responses and metabolic consequences of acclimation to elevated light exposure in grapevine berries. *Frontiers in Plant Science*, 8, 1261.

5. A-F Adam-Blondon, M Alaux, C Pommier, D Cantu, Z-M Cheng, GR Cramer, C Davies, S Delrot, L Deluc, G Di Gaspero, J Grimplet, A Fennell, JP Londo, P Kersey, F Mattivi, S Naithani, P Neveu, M Nikolski, M Pezzotti, BI Reisch, R Töpfer, MA Vivier, D Ware and H

Quesneville. 2016. Towards an open grapevine information system. Horticulture Research (2016) 3, 16056 (1-8).

Popular publications:

1. Eyéghé-Bickong, Hans A., Young, Philip R. & Vivier, Melané A. (2017) Analytical methods to measure grape metabolites – a review. *Oenology research, Winetech Technical.*

2. Zietsman, Anscha Vivier, Melané & Moore, John (2016) Investigating grapevine cell walls *Viticulture research, Winetech Technical.*

3. Young, Philip & Vivier, Melané (2017) Sauvignon Blanc – role of phenotypic plasticity in cultivar typicity. *Viticulture research, Winetech Technical*

e) PRESENTATIONS/PAPERS DELIVERED

1. Joubert, C., Young, P.R., Vivier, M.A. Studying the impact of UV-light on grapevine photosynthetic pigments, sugars and organic acids (*Vitis vinifera* L. cv Sauvignon blanc). MACROWINE 2014. Macromolecules and secondary metabolites of grapevine and wine, Stellenbosch, South Africa (7-10 September 2014).

2. Joubert, C., Young, P.R., Eyeghe-Bickong, H., Vivier, M.A. How do field-grown grapevine berries acclimate to UV exposure in high and low light conditions? Tenth International Symposium on Grapevine Physiology and Biotechnology 2016. Verona, Italy (13-18 June 2016).

3. M.A. Vivier, K. Du Plessis, C. Joubert, H. Eyeghe-Bickong, A. Berry and P.R. Young. 2017. Aroma profiling of Sauvignon blanc: Linking grapes to wine. 40th Society for Viticulture and Oenology Conference (August 2017, Franschhoek). (Invited keynote).

4. Vivier, M. (2016) Field-grown Sauvignon Blanc berries react to increased exposure by controlling antioxidant homeostasis and displaying UV acclimation responses that are influenced by the level of ambient light. Presentation at the Macrowine conference at Changins (Nyon), Switzerland on 27 – 30th of June 2016.

5. MA Vivier, K. Du Plessis, C. Joubert, H. Eyeghe-Bickong, A. Berry, P.R. Young. Many Compounds Linked to “Quality” in Wine Originate from Stresses Experienced by Grape Berries. 10th In Vino Analytica Scientia, Salamanca, Spain (17-20 July 2017). (Invited keynote).

6. Young, P.R., Berry, A., Eyeghe-Bickong HA, Vivier, M.A. Remarkable dynamic nature of macromolecular content in grapevine berries in reaction to microclimatic factors: evidence from a Shiraz row-orientation model vineyard. MACROWINE 2018. Zaragoza, Spain (28-31 May 2018).

6. BUDGET
TOTAL COST SUMMARY OF THE PROJECT

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech/DST	THRIP	OTHER	TOTAL
2015					1 200 000			1 200 000
2016					1 300 000			1 300 000
2017					1 300 000			1 300 000

EVALUATION BY INDUSTRY

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Project number	
Project name	
Name of Sub-Committee*	

Comments on project

Committee's recommendation (Review panel in the case of PHI)

- Accepted. ☐
- Accepted provisionally if the sub-committee's comments are also addressed. Resubmit this final report by_____ ☐
- Unacceptable. Must resubmit final report. ☐

Chairperson_____ Date_____

*SUB-COMMITTEES

Winetech
Viticulture: Cultivation; Soil Science; Plant Biotechnology; Plant Protection; Plant Improvement;
Oenology: Vinification Technology; Bottling, Packaging and Distribution; Environmental Impact; Brandy and Distilling; Microbiology

Deciduous Fruit
Technical Advisory Committees: Post-Harvest; Crop Production; Crop Protection; Technology Transfer
Peer Work Groups: Post-Harvest; Horticulture; Soil Science; Breeding and Evaluation; Pathology; Entomology