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Fotossíntese e transcriptoma da variedade Sauvignon Blanc (*Vitis vinífera* L.) em vinhedos de altitude elevada de Santa Catarina

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Orientador: Prof. Drª. Rosete Pescador
Co-orientador: Dr. Alberto Fontanella Brighenti

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O presente trabalho em nível de doutorado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

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Certificamos que esta é a **versão original e final** do trabalho de conclusão que foi julgado adequado para obtenção do título de doutor em Ciências.

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“*Dovunque mi arrampichi io sono seguito da un cane chiamato Ego.”*

(Friedrich Wilhelm Nietzsche)

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RESUMO

De acordo com as previsões das mudanças climáticas, regiões tradicionalmente produtoras de vinho podem ser severamente afetadas em relação ao aumento da temperatura media global. Neste contexto, novas regiões vitícolas de clima frio estão ganhando destaque. No Brasil sobressaem-se as regiões acima de 1000 metros de altitude de Santa Catarina, com um clima vitícola frio, que proporciona condições ótimas para elaboração de vinhos finos. Uma das variedades mais bem adaptadas a estas regiões é a Sauvignon Blanc. Nesta perspectiva, este trabalho tem o objetivo de caracterizar fotossinteticamente a variedade Sauvignon Blanc submetida a diferentes épocas de desfolha, além de investigar as alterações na composição fenólica e no transcriptoma da pele da baga de cachos expostos e não expostos a radiação solar, durante o ciclo 2015/2016 em São Joaquim/SC. O experimento foi realizado em uma área de produção comercial localizada a uma altitude de 1.293m, à latitude 28º15'13" S e longitude 49º57'02" W. As análises e coletas de amostras foram realizadas desde a brotação até a maturação fisiológica dos frutos. Foram realizadas medições do crescimento dos ramos, área foliare medição da fluorescência da clorofila *a*.. Para avaliação de pigmentos foi usado um método não destrutivo através de um clorofilômetro portátil, e realizadas extrações de clorofila *a*, *b* e carotenóides. A quantificação de flavonóis e análise da expressão de genes relacionados à exposição à radiação UV foram realizadas com bagas de plantas desfolhadas nos estádios fenológicos floração total e mudança de cor. Diferentes épocas de desfolha afetaram fortemente o metabolismo da planta, reduzindo a emergência de novas folhas e estimulando a expansão foliar. Na maturação, folhas com área acima de 160 cm^2 apresentaram o maior rendimento fotossintético e compunham aproximadamente 50% do dossel das plantas desfolhadas. Neste estádio fenológico, o alto rendimento fotossintético, acompanhado da ausência de decréscimo no conteúdo de pigmentos fotossintéticos, apontam para uma senescência tardia das folhas de Sauvignon Blanc em regiões altas de Santa Catarina, o que proporciona maior acúmulo de reservas para o próximo ciclo. Com relação à fotossíntese, observou-se uma redução no rendimento fotossintético em todas as plantas durante o crescimento dos frutos, e este decréscimo parece estar intimamente relacionado com as baixas temperaturas nesta fase. As plantas desfolhadas na floração apresentaram uma redução mais acentuada de fotossíntese logo após a desfolha, em relação aos outros tratamentos, no entanto, na maturação, foi observada compensação fotossintética em todos os tratamentos de desfolha. Com relação à composição da baga, a desfolha afetou positivamente o conteúdo de flavonóis na pele da baga, bem como, foi observada a expressão de genes relacionados à elevada radiação UV. O tempo de exposição da baga foi relacionado positivamente com o conteúdo de compostos fenólicos, portanto, considerando este aspecto, desfolhas precoces são indicadas para obter frutos de melhor qualidade para elaboração de vinhos. Uma abordagem molecular envolvendo as vias de sinalização ativadas em folhas submetidas a baixas temperaturas e elevada radiação podem contribuir para a compreensão da manutenção da fotossíntese e do alto vigor da variedade Sauvignon Blanc observados neste estudo.

Palavras-chave: *Vitis vinifera* L, fotossíntese, desfolha, fluorescência da clorofila.

RESUMO EXPANDIDO

Introdução

A vitivinicultura é uma atividade econômica de extrema importância, sendo a uva a terceira fruta mais produzida no mundo. Os maiores produtores mundiais de vinho são a Itália, França, e Espanha. Contudo, em decorrência do processo de globalização, das ameaças das mudanças climáticas à qualidade das uvas e vinhos das regiões tradicionais e de consciência mercadológica de que os produtos vinícolas são uma oportunidade de desenvolvimento regional, quer seja pela exportação, quer pelo desenvolvimento enoturístico, novas regiões vitícolas estão desenvolvendo-se e ganhando visibilidade no mercado internacional. Entre os países que demonstram potencial para produção de uva viníferas, está o Brasil, com destaque para o estado de Santa Catarina, principalmente para elaboração de vinhos finos. Neste estado, novas regiões vitivinícolas em zonas de altitude acima de 1.000 metros como São Joaquim, têm produzido os chamados "vinhos finos de altitude". Estas regiões apresentam terroir completamente diferente em macro e microclimas, que contribue para a produção de vinhos com características distintas e elevada qualidade. Entre as variedades cultivadas, a variedade Sauvignon Blanc *(Vitis vinifera)* destaca-se como a variedade branca melhor adaptada a elevada altitude. O vinho produzido a partir desta cultivar é marcado por sua acidez acentuada e alta complexidade aromática. Neste sentido, a compreensão dos fatores que impactam o terroir é fundamental para compreender as diferentes respostas de uma mesma variedade às restrições ambientais locais e a projeção destas adaptações ao fruto. Com estas informações é possível programar estratégias de manejo do vinhedo, para manipulação da composição química do vinho, a fim de superar as limitações existentes. Muitos estudos têm sido realizados sobre os efeitos dos fatores climáticos, manejo do dossel, tipo de solo e disponibilidade de água na acumulação de compostos fenólicos na baga, e os resultados indicam que quase todos os fatores podem afetar, em diferentes graus, a composição da uva. Entre as tecnicas de manejo, a remoção de folhas basais para aumentar a exposição à luz solar e a temperatura da baga, tem demonstrado promover uma redução significativa do rendimento e a melhor composição da uva de forma quase sistemática. Esta técnica estimula principalmente a eficiência fotossintética do dossel, além de melhorar a eficiência do uso da água e a tolerância a fotoinibição. Além disso, o estágio fenológico no qual é feita a remoção das folhas tem demonstrado ser dominante sobre outros fatores de variabilidade. Outros fatores importantes a serem considerados em regiões altas é a elevada radiação UV e a baixa temperatura media, embora danos causados pela radiação UV natural sejam raros, tem sido demonstrado que a videira apresenta mecanismos efetivos de proteção, tanto nas folhas quanto nos frutos, através da biossíntese de flavonóis, que são interessantes no âmbito enológico. Os principais compostos fotoprotetores estimulados pela exposição UV são a quercetina e o kaempferol. A mudança no perfil dos flavonóides, bem como o aumento desses compostos, tem sido relacionada ao aumento da expressão de vários genes envolvidos em sua biossíntese como forma de adaptação à alta exposição solar. Desta forma, este trabalho avaliou as respostas fotossintéticas das folhas durante todo o ciclo da planta e as modificações em nível de expressão gênica na pele da baga, com ênfase na elevada exposição solar típica de regiões altas.

Objetivos

Avaliar o efeito da época da desfolha no desempenho fotossintético das folhas da variedade Sauvignon Blanc, e no conteúdo de flavonóis e expressão gênica na pele da baga, no ciclo 2015/2016 sob condições climáticas de elevada altitude no município de São Joaquim.

Metodologia

O experimento foi conduzido em um vinhedo comercial de Sauvignon Blanc, localizado em São Joaquim, Estado de Santa Catarina (28º 17 '39 "S e 49º 55' 56" W, entre 1200 e 1400 m.s.l) durante a safra 2015/2016. As videiras (enxertadas em Paulsen 1103) foram plantadas em uma linha norte-sul, com espaçamento de 3,0 m (linha) × 1,5 m (videira), conduzidas em cordão duplo com broto vertical posicionando-se 1,2 m acima do solo. O clima da região é caracterizado como mesotérmico úmido com verões amenos, ou Cfb na classificação de Köppen. O experimento foi realizado com delineamento de blocos casualizados; quatro blocos foram utilizados no total, com cinco plantas por bloco, e medições foram realizadas em dois ramos por planta. Os tratamentos consistiram em cinco tempos diferentes de desfolha, usando a metodologia descrita por Baillod e Baggiollini (1993): (a) controle (C), não tratado, (b) 50 dias após a brotação (DAB), (c) baga em tamanho grão pimenta aos 65 DAB; (d) baga em tamanho grão ervilha aos 78 DAB; (e) mudança de cor da baga aos 121 DAB; (f) 15 dias após a mudança de cor da baga aos 140 DAB. Comprimento da parte aérea, área foliar (LA) e índice SPAD foram medidos a cada duas semanas, desde a brotação até a colheita em dois brotos por planta. O índice SPAD foi realizado usando um medidor de clorofila (Chl) (SPAD-502, Konica Minolta, Tóquio, Japão). Além disso, índice SPAD e a quantificação de Chl a, b e carotenóides (car) foram realizados em folhas de diferentes áreas (S1: 0 - 8 cm^2, S2: 8,1 - 37 cm^2, S3: 37,1 - 88 cm^2, S4: 88,1 - 160 cm^2, S5: 160,1 - 260 cm^2, 260,1 - 385 cm^2) na floração, mudança de cor e maturação. A fluorescência de Chl a (ChlF) foi medida com um fluorômetro (Mini-PAM, Walz, Effeltrich, Alemanha) entre 08:00 e 10:00 e entre 12:00 e 14:00, e foi determinado o rendimento quântico efetivo de PSII (ΦPSII), ΔF / Fm ', e no pré-amanhecer, quando foi determinado o rendimento quântico máximo (Fv / Fm). Análises dos sólidos solúveis totais da baga (° Brix), acidez total e pH foram avaliados em bagas coletadas na mudança de cor e na maturação plena. Os flavonóides foram separados por HPLC usando um instrumento Waters1525 equipado com um detector de díodos (DAD) e uma coluna de fase reversa (RP18 250 mm × 4 mm, 5 M) com uma pré-coluna (Phenomenex, Castel Maggiore, BO, Itália). A quantificação foi realizada a 370 nm com os correspondentes padrões externos (quercetina e kaempferol). Para a extração de RNA da pele da baga, foi usado o kit SpectrumTM Plant Total RNA (Sigma-Aldrich, St. Louis, MO). Actina e ubiquitina foram utilizados como referência. A eficiência de amplificação foi calculada a partir de dados brutos usando o software LingReg PCR. Os resultados foram representados como média ± DP. Os dados obtidos nos tratamentos de desfolha e não desfolhado foram analisados por análise de variância (ANOVA) seguida pelo teste de Tukey ($P \leq 0,05$).

Resultados e Discussão

A temperatura está entre os fatores mais importantes que determinam o crescimento da videira. Neste estudo, o pico de crescimento do ramo entre os estágios fenológicos 5-6 e 7-8 folhas totalmente expandidas coincidiu com o aumento da temperatura média, isso está de acordo com observações anteriores de que uma redução de 2ºC na temperatura média diária pode reduzir drasticamente o comprimento da parte aérea em regiões mais frias, especialmente durante a brotação. As temperaturas entre

15°C e 17°C durante o período de desenvolvimento da inflorescência levaram a uma redução no rendimento fotossintético em todos os tamanhos de folhas. Um aumento da temperatura do ar de 1ºC permitiu que as folhas maduras atingissem valores ótimos de ΦPSII às 08:00 h na fase de floração; no entanto, isso diminuiu ao meio-dia. Isso deve-se ao fato de que, até o final da floração, aproximadamente 60% da área foliar está exposta ao sol, alterando os processos estomáticos e não estomáticos no período de maior radiação solar sob baixas temperaturas e acionando mecanismos de dissipação de energia para evitar a fotoinibição. A redução de 1ºC na temperatura média do ar e o aumento da radiação fotossinteticamente ativa (RFA) no estágio grão pimenta induziram uma acentuada diminuição no ΦPSII, mesmo em folhas maduras, que foram rapidamente recuperadas nas temperaturas mais quentes e maior radiação solar durante as fases fenológicas posteriores. O processo de aclimatação ao frio é cumulativo, mas pode ser revertido ou reiniciado, dependendo das flutuações de temperatura. Provavelmente, isso está relacionado à temperatura durante a formação inicial das folhas, que conferiu maior tolerância à fotoinibição em condições frias. A partir do estágio grão ervilha, as temperaturas mínimas permaneceram acima de 18ºC e os valores de Fv/Fm permaneceram próximos de 0,8, o limiar considerado saudável para uma planta terrestre. Houve exceções: folhas com menos de 88 cm^2 apresentaram Fv/Fm reduzido neste mesmo estágio de desenvolvimento. A baixa capacidade fotoquímica das folhas jovens está de acordo com as menores razões Chl a/b. A diminuição no tamanho da antena PSII resulta em menos energia de excitação no PSII com uma diminuição correspondente na intensidade de fluorescência do PSII. Nos estágios finais de maturação, a temperatura aumentou 1,5 ° C em comparação aos estágios anteriores e as plantas apresentaram valores de ΦPSII muito próximos ao valor ótimo de 0,8. O conteúdo de pigmentos nas folhas S4 e S5 na maturação permaneceu estável e os valores de ΦPSII permaneceram altos, significando que as folhas ainda não haviam entrado em senescência. Isso está provavelmente relacionado a resposta da videira a intensidade e momento da remoção das folhas basais. Está bem documentado que a remoção de folhas aumenta a atividade fotossintética por unidade de área foliar das folhas restantes, ou seja, a planta mantém as folhas restantes o mais fotossinteticamente ativas possível, a fim de compensar a perda geral de área foliar. Além disso, a manutenção da taxa de fotossíntese pós-colheita sugere que a capacidade da planta de acumular reservas durante esse período não foi limitada pela demanda por fotossintatos. Com relação ao amadurecimento tecnológico (TSS, pH e acidez), no presente estudo, não foi observado nenhum efeito após os tratamentos de desfolha. Isso concorda com os dados apresentados por estudos anteriores, cuja desfolha não modificou esses parâmetros em Sauvignon Blanc. Contudo, o aumento da exposição à luz no estágio de plena floração foi eficaz para melhorar o acúmulo total e individual de flavonóides, enquanto a desfolha na mudança de cor da baga não apresentou diferenças em relação às plantas sombreadas. A expressão gênica foi realmente afetada pelos tratamentos de desfolha. Considerando os genes envolvidos nas etapas de biossíntese de flavonóides, a desfolha em plena floração induziu a expressão das diferentes isoformas do VvCHS, que produziram substratos para a biossíntese de flavonóides dois meses depois. Por outro lado, o VvFLS e o VvMYB12 foram imediatamente induzidos após a remoção das folhas e bem correlacionados entre si e com o acúmulo de flavonoides. A hiperacumulação de flavonóides é um mecanismo primário de resposta de defesa das plantas para proteger da radiação UV; portanto, a regulação positiva diferencial das isoformas VvCHS pode atuar como um mecanismo regulador para produzir continuamente substratos para a biossíntese de flavonoides

após alta exposição a UV a longo prazo. Diferentemente, na desfolha durante a mudança de cor, apenas a regulação positiva do VvCHS2 foi detectada e provavelmente uma quantidade menor de precursores de flavonol foi produzida. Os transcritos VvCHS2, VvMYB12 e FLS4 são mais abundantes na desfolha durante a mudança de cor e podem estar relacionados à exposição solar das bagas nesse estágio específico de desenvolvimento, que corresponde às mais altas taxas de radiação UV. Respostas rápidas de FLS4 e MYB foram relatadas em estudos anteriores. É bem documentado o aumento no teor de quercetina e caempferol em resposta ao UV-B na variedade Sauvignon blanc, no entanto, o padrão de regulação gênica encontrado neste estudo parece ser influenciado pela exposição à luz solar desde os estágios iniciais. Além disso, diferentemente dos estudos realizados em uvas Malbec em grandes altitudes, o acúmulo de flavonóides nas uvas Sauvignon Blanc ocorreu sem reduzir o crescimento das bagas, assim como a remoção das folhas tem um efeito mínimo na maturação das bagas. Esta pesquisa é o primeiro passo para elucidar o estado de aclimatação do Sauvignon Blanc sob a combinação específica de condições ambientais em regiões de clima frio da América do Sul. As respostas de defesa à exposição à alta radiação UV e às baixas temperaturas durante a brotação resultam em ajustes na expressão gênica e no desempenho fotossintético desta variedade.

Considerações Finais

As baixas temperaturas reduzem o rendimento fotossintético da variedade Sauvignon Blanc em São Joaquim, principalmente durante a formação e o início do crescimento da baga. Embora o estresse pelo frio tenha afetado os parâmetros de fluorescência da clorofila, não interferiram no conteúdo de pigmentos. A remoção das folhas basais impactou significativamente o crescimento vegetativo. A desfolha a qualquer momento entre a floração e a mudança de cor da baga reduziu o surgimento de novas folhas, o que poderia indicar um dreno de carbono durante o crescimento e a maturação dos frutos. O tamanho do ramo, área foliar e o índice SPAD mostraram-se indicadores fracos das complexas mudanças na atividade foliar; no entanto, a taxa na qual as folhas se expandiram para o tamanho máximo mostrou que as plantas Sauvignon Blanc não apenas foram capazes de se adaptar à desfolha, bem como os valores de ΦPSII mostraram que a fotossíntese era mais ativa em plantas desfolhadas. Com relação a concentração de flavonóides, o tempo de exposição mostrou-se importante, uma vez que a desfolha precoce contribuiu para maior acúmulo de flavonóides. A radiação UV parece ser um fator importante na tipicidade do vinho Sauvignon Blanc em São Joaquim. Este trabalho é pioneiro na investigação de expressão gênica durante o desenvolvimento da videira em regiões de alta altitude de Santa Catarina; portanto, novos estudos devem ser conduzidos para investigar a produção das EROs e outros compostos relacionados à exposição a UV naturalmente elevada em regiões altas, bem como, o efeito desta radiação na fisiologia das folhas.

Palavras-chave: *Vitis vinifera* L, fotossíntese, desfolha, fluorescência da clorofila.

ABSTRACT

According to predictions on climate change, traditional wine-producing regions may be severely affected by increasing temperatures. In this context, new wine-growing cool-climate regions are gaining prominence. In Brazil, the high regions of Santa Catarina, the cold wine-growing climate provides excellent conditions for high-quality wines elaboration. One of the varieties better adapted to this region is Sauvignon Blanc. In this perspective, this study is aimed to investigate the effect of defoliation times on photosynthetic yield of cv. ' Sauvignon Blanc', in addition, to investigate the changes in phenolic composition and berry skin transcriptome of exposed and not exposed cluster to solar radiation during the cycle 2015/2016 in São Joaquim / SC. The experiment was carried out in an area of commercial production located at 1293m a.s.l at latitude 28º15'13 "S and longitude 49º57'02" W. Samples were analyzed from budding to maturity. Measurements of shoot growth, leaf area, and chlorophyll a fluorescence were performed. A non- destructive method was used to evaluate pigments using a portable chlorophyllmeter, in addition, chlorophyll a, b and carotenoids quantification were performed. Flavonols content and gene expression related to UV-exposure were carried out with berries of defoliated plants at full bloom and veraison. Defoliation times strongly affected the plant metabolism, reducing the emergence of new leaves and increasing leaf expansion. At maturity, leaves with an area above 160 cm2 showed the highest photosynthetic yield and composed approximately 50% of defoliated plants canopy. In this phenological stage, the high photosynthetic yield and the maintenance of photosynthetic pigments, evidence a late senescence of Sauvignon Blanc leaves in high regions of Santa Catarina, which increases the reserves accumulation for next cycle. Regarding photosynthesis, a reduction in photosynthetic yield was observed in all plants during berry growth, and this decrease seems to be closely related to the low temperatures at this stage. Full bloom defoliated plants showed a more pronounced reduction of photosynthesis following defoliation, in relation to the other defoliation times, however, at maturity, photosynthetic compensation was observed in all defoliation times. Regarding berry composition, the defoliation positively affected the flavonols content and gene expression related to high UV-fluence was observed. The time of berry exposure was positively related to phenolic compounds content, therefore, considering this aspect, early defoliation is indicated to obtain better grape berry quality. A molecular approach involving the signaling pathways activated in leaves under low temperatures and high radiation can contribute to the understanding of photosynthesis maintenance and high vigor of the Sauvignon Blanc plants observed in this study.

Keywords: *Vitis vinifera* L, photosynthesis, defoliation, chlorophyll fluorescence.

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LISTA DE ABREVIATURAS E SIGLAS

ChlF – Chl *a* fluorescence

DAB – Days after bud burst

DMSO - Dimethylsulfoxide

GDD – Growing Degree Day

LA – Leaf area

Tmax – Maximum temperature

Tmin – Minimum temperature

SUMÁRIO

1 INTRODUÇÃO E JUSTIFICATIVA

A vitivinicultura é uma atividade econômica de extrema importância, sendo a uva a terceira fruta mais produzida no mundo, com mais de 73 milhões de toneladas por ano em 7,5 milhões de hectares de área plantada (OIV,2018). Os maiores produtores mundiais de vinho são a Itália, França, e Espanha. Contudo, em decorrência do processo de globalização, das ameaças das mudanças climáticas à qualidade das uvas e vinhos das regiões tradicionais e de consciência mercadológica de que os produtos vinícolas são uma oportunidade de desenvolvimento regional, quer seja pela exportação, quer pelo desenvolvimento enoturístico, novas regiões vitícolas estão desenvolvendo-se e ganhando visibilidade no mercado internacional (JONES, 2006; STOCK et al., 2007).

Previsões de mudanças climáticas estimam um aumento de duas vezes nos níveis de CO_2 atmosférico nas áreas vitícolas europeias nos próximos 50 anos (IPPC, 2007; EEA, 2013; SCHULTZ, 2000), o que resultaria em reduções na disponibilidade de água no solo variando de 20% na Europa Central a 70% na Península Ibérica e Ilhas Baleares. No entanto, a produção europeia de uvas já apresenta declínio devido às condições climáticas, ao mesmo tempo em que o consumo mundial de vinhos é crescente, e em 2014 apresentou um aumento de 8% em comparação com o período 2000-2012, atingindo 243 milhões de hectolitros. Sendo assim, o aumento no consumo e a redução da produção de vinho nos países tradicionais estão promovendo o crescimento de novas áreas produtoras, fora da Europa, que tem como principal característica a presença de condições climáticas favoráveis à cultura da videira (OIV, 2014).

Entre os países que demonstram potencial para produção de uva viníferas, está o Brasil, que apresenta clima temperado e subtropical nas regiões vitivinícolas produtoras de vinhos finos com uma colheita por ano. Em 2014, a produção nacional de vinhos atingiu 237,15 milhões de litros, embora apenas 40,9 milhões (em torno de 17%) tenham sido produzidos de uvas *V. vinifera* (MELLO, 2015). Provavelmente por este motivo, 80,9% dos vinhos consumidos no país ainda sejam importados (UVIBRA, 2014).

Entre os estados brasileiros produtores de uva, Santa Catarina é o terceiro com maior produção, principalmente para elaboração de vinhos finos, tendo 4,23 mil hectares plantados, e uma produção de 52 mil toneladas no ano de 2014 (IBGE, 2014). Neste estado, novas regiões vitivinícolas em zonas de altitude acima de 1.000 metros como São Joaquim, em que a altitude chega a 1.400m, tem produzido os chamados “vinhos finos de altitude” (VIANNA et al., 2016). Estas regiões apresentam *terroir* completamente diferentes em macro e microclimas, e seus vinhos já apresentam características distintas e elevada qualidade (BURIN et al., 2011; GRIS et al., 2011; MALINOVSKI et al., 2012). Segundo o Sistema de Classificação Climática Multicritério Geovitícola (Sistema CCM Geovitícola) (TONIETTO e CARBONNEAU, 2004), São Joaquim, apresenta um clima vitícola "frio, de noites frias e úmido" (IH_{-2} IF_{+1} IS_{-2}). Essa condição, aliada à baixa latitude, proporciona deslocamento do ciclo produtivo da videira, expondo as plantas no período de maturação a temperaturas noturnas amenas, o que permite a maturação fenólica mais completa (índice de maturação elevado, maior acidez total, valores de ºBrix > 20, sementes escuras no ponto de colheita, caracterizando taninos “macios”) e a elaboração de vinhos de alta qualidade (BONIN e BRIGHENTI, 2006; BORGHEZAN et al. 2011; VIEIRA et al., 2011; MALINOVSKI et al., 2012; BRIGHENTI et al., 2014). É uma região recente na indústria do vinho (menos de 20 anos) e uma das principais características que diferem a viticultura de altitude das demais regiões produtoras do Estado é que ela não esta associada com a colonização italiana ou com qualquer tipo de viticultura tradicional. É uma atividade realizada por empresários, voltada para a produção de vinhos finos, com o uso de alta tecnologia e práticas de manejo modernas (BRIGHENTI et al., 2016; CARVALHO-JUNIOR e MOSSINI, 2011). Dadas às condições privilegiadas da região e a alta qualidade dos vinhos produzidos, o setor vitivinícola catarinense ainda é incipiente, embora gere em torno de dois mil empregos diretos, e a produção de um milhão de garrafas de vinho por ano, nas 35 vinícolas de altitude do estado (ALVES, 2015).

Nesta perspectiva, a inter-relação entre os vitivinicultores, empresas, universidades, centros de pesquisa, e outros, tem um papel crucial na competitividade da atividade, principalmente na busca de ferramentas de diferenciação valorizadas no

mercado (BENKO, 2001). A Denominação de Origem (DO) é um exemplo de estratégia de mercado interessante no âmbito do agronegócio, e se aplica a produtos que possuem atributos qualitativos indissociáveis das características próprias de uma região ou microrregião bem delimitada, sejam elas relativas ao clima, ao solo, à história ou à mão-de-obra (ALCOFORADO, 2002). Contudo, o processo de diferenciação através da DO exige a comprovação científica das características qualitativas diferenciais relacionadas ao *terroir.*

Verificar a influência de fatores isolados sobre a resposta de uma espécie perene é complexo, e a avaliação da exata influência do *terroir* sobre a qualidade da uva e do vinho torna-se um estudo multidisciplinar. O *terroir* possui como elementos primários características como tipo de solo, clima (radiação, temperatura e precipitação) e topografia (URHAUSEN et al., 2011; BRESCIA et al., 2002). A compreensão dos fatores que impactam o *terroir* é fundamental para compreender as diferentes respostas de uma mesma variedade às restrições ambientais locais e a projeção destas adaptações ao fruto (LI et al., 2011). Com estas informações é possível programar estratégias de manejo do vinhedo, para manipulação da composição química do vinho, a fim de superar as limitações existentes (HANNAH et al., 2013).

Neste sentido, estudos têm sido realizados sobre os efeitos dos fatores climáticos, manejo do dossel, tipo de solo e disponibilidade de água na acumulação de compostos fenólicos na baga, e os resultados indicam que quase todos os fatores podem afetar, em diferentes graus, a composição da uva (ŠUKLJE et al., 2014; KENNEDY et al., 2002; PRADO et al., 2007).

Em São Joaquim, um dos principais fatores relacionados ao *terroir* é a elevada altitude, que é especialmente importante e deve ser considerado como potencial limitante da produtividade da planta e qualidade da uva, assim como a baixa temperatura durante a brotação (BRIGHENTI et al, 2014). A fotossíntese é um processo altamente resiliente, e nesse sentido as videiras demonstram ser tolerantes a baixas temperaturas, embora seu crescimento seja fortemente afetado (HENDRICKSON et al., 2003, 2004).

O vigor da planta está relacionado com a relação fonte/dreno, portanto, direta e indiretamente as baixas temperaturas podem afetar o ganho de carbono. Além disso, é preciso considerar que as técnicas comuns de manejo do vinhedo, como remoção das folhas basais para aumentar a exposição à luz solar e a temperatura da baga e o manejo de desponte, que evita a competição dos ápices vegetativos pelos fotoassimilados, enquanto promove o deslocamento dos mesmos para os tecidos reprodutivos e formação de metabólitos secundários, afetam o equilíbrio fonte/dreno da planta (CHAVES et al., 2007; SMART; ROBINSON, 2008; SHELLIE; GLENN, 2008).

Vários artigos foram publicados sobre os efeitos da remoção de folhas no período de pré-floração, seja ela manual ou mecânica, na qualidade da uva (PONI et al., 2006, 2008; INTRIERI et al. 2008; DIAGO et al. 2009). Os resultados mostraram que a redução significativa do rendimento e a melhor composição da uva foram obtidos de forma quase sistemática. Esta resposta mostra que o controle fisiológico imposto através da remoção de folhas na pré-floração é dominante sobre outros fatores de variabilidade, ou seja, o estágio fenológico que a desfolha é realizada também deve ser considerado. Em estudos relacionados à recuperação fotossintética pós-desfolha, tanto nos ramos, quando no dossel, as videiras desfolhadas recuperaram a capacidade fotossintética, atingindo níveis semelhantes às videiras não desfolhadas até o inicio da mudança de cor das bagas (PONI et al., 2008). Em um estudo sobre a remoção de folhas na pré-floração, Palliotti et al. (2011) constataram que a eliminação de ~80% da área foliar, aumentou a eficiência fotossintética do dossel, especialmente durante o período de mudança de cor das bagas, e induziu um desempenho melhor na eficiência do uso da água e na tolerância a fotoinibição. A videira também ajusta a distribuição de proteínas e clorofila para a densidade da copa, de modo que as folhas mais expostas à luz têm maior capacidade fotossintética por unidade de área foliar (PETRIE et al., 2000). De fato, menos de 20% das folhas representam frequentemente mais de 80% da assimilação total de carbono de uma videira. Portanto, a área de folhas expostas de um vinhedo é mais importante que sua área foliar total, porque quanto mais energia solar for interceptada, maior será a produção de biomassa e o potencial de produção. Além disso, a taxa de fotossíntese

é influenciada pela idade e posição da folha (PONI et al. 1994, MEDRANO et al., 2015) e o excesso de vigor, por exemplo, pode causar declínio da taxa de fotossíntese por unidade foliar, bem como, a redução da área foliar pode causar o oposto (PETRIE et al., 2000). Portanto, medidas de folhas individuais podem não representar o rendimento fotossintético da planta inteira e esta extrapolação de valores pode ser uma limitação importante para a aplicabilidade dos resultados da pesquisa.

Outro fator importante a ser considerado em regiões altas é a elevada radiação UV, principalmente durante a maturação das bagas. Na América do Sul, o potencial climático das regiões de alta altitude, como Mendoza na Argentina, tem sido estudado principalmente em relação à exposição de videiras a altas radiações UV. Nessa região, tem sido demonstrado que a videira apresenta mecanismos efetivos de proteção, tanto nas folhas quanto nos frutos. No entanto, a resposta dos frutos, através da biossíntese de flavonóis, que são compostos fenólicos absorventes de UV e possuem propriedades antioxidantes, são interessantes no âmbito enológico (BERLI et al., 2008, 2011, 2013, 2015). Os danos causados pela radiação UV natural, em condições de campo, mesmo em elevadas altitudes são raros, e sua interferência na qualidade da uva é altamente dependente do estágio de desenvolvimento das bagas (JOUBERT et al., 2016). Os principais compostos fotoprotetores estimulados pela exposição UV são a quercetina e o kaempferol (BERLI et al., 2010, 2011). Entretanto, em vinhedos de altitude, ocasionalmente, pode ocorrer aumento das Proteínas PR que causam escurecimento nos vinhos brancos (COLAS et al., 2012; LIU et al., 2014).

Na região de São Joaquim, muitos trabalhos foram desenvolvidos com o objetivo de avaliar a adaptação das variedades e caracterizá-las em relação à fenologia, produção e qualidade da uva. Considerando estes estudos, e compreendendo a complexidade de estudar plantas perenes em campo, o presente trabalho optou por realizar uma abordagem fotossintética da resposta da variedade Sauvignon Blanc aos diferentes tempos de desfolha, bem como o estudo das respostas da baga em nível molecular em relação a exposição à maior radiação solar na região de São Joaquim.

Este trabalho está estruturado em dois capítulos que consistem em dois artigos, sendo o primeiro relacionado às respostas fotossintéticas das folhas, com ênfase nas

baixas temperaturas durante todo o ciclo da planta e o segundo relacionado às modificações em nível de expressão gênica na pele da baga, com ênfase na elevada exposição solar típica de regiões altas.

1.1 OBJETIVOS

1.1.1 Objetivo Geral

Avaliar o efeito da época da desfolha no desempenho fotossintético das folhas da variedade Sauvignon Blanc, e no conteúdo de flavonóis e expressão gênica na pele da baga, no ciclo 2015/2016 sob condições climáticas de elevada altitude no município de São Joaquim.

2 INFLUENCE OF DEFOLIATION TIME ON VEGETATIVE GROWHT AND PHOTOSYNTHESIS OF SAUVIGNON BLANC (*Vitis vinifera* L.) UNDER HIGH ALTITUDE CONDITIONS

2.1 ABSTRACT

Low temperature is the major factor limiting growth of grapevines. In a field experiment in high altitude regions with annual mean temperature between 12°C and 18ºC, defoliation times were investigated during the 2015/2016 cycle, with the aim to evaluate the impact of this practice on vegetative growth and photosynthetic yield on cv. 'Sauvignon Blanc'. Leaves were categorized by size, and Chlorophyll *a* fluorescence was used to investigate photosynthetic parameters. Early defoliation, at full bloom, resulted in a decrease of the quantum yield of photosystem II (ΦPSII) in all leaf sizes. Defoliated plants between pepper-corn size and veraison showed faster ΦPSII recovery. All defoliation times reduced the emergence of new leaves and increased leaf expansion. At maturation, all defoliation treatments showed ΦPSII significantly higher than non-defoliated plants. Despite the low temperatures, vegetative growth was not affected, and no chronic photoinhibition was observed. Our study provides direct evidence of the defoliation times on growth and photosynthetic dynamics.

Keywords: chlorophyll fluorescence; *Vitis vinifera*; high altitude; low temperature; growth analysis.

2.2 INTRODUCTION

Wine production has only been established within Santa Catarina State, Brazil, within the last two decades (Brighenti *et al.* 2013), but their products have already shown to be of high quality (Falcão *et al*. 2007, Gris *et al.* 2010, Burin *et al.* 2011, Malinovski *et al.* 2012, Borghezan *et al.* 2014). According to Brighenti *et al.* (2013), Sauvignon Blanc grapes (*Vitis vinifera*) stand out as the white variety best adapted to the high-altitude vineyards; the wine made from this cultivar is marked for its high

quality, marked acidity, and high aromatic complexity. Similar to the Marlborough region of New Zealand, low night temperatures and few hot days allow fruit and wine to retain acidity (Montero *et al.* 2016).

Unfortunately, the tendency of Sauvignon Blanc fruits to develop in a compact cluster provides an excellent environment for bunch rot to thrive under the frequent rainfall that occurs in São Joaquim during the season when berries are undergoing maturation (Wurz *et al*, 2017). Frequently, high rainfall and emergence of disease determine the harvest date independently of full fruit maturation (Mosetti *et al.* 2016). Many recent studies have investigated the effect of basal leaf removal on disease rates in Sauvignon Blanc (Mosetti *et al.* 2016*,* Wurz *et al.* 2017), as well as fruit biochemical composition (Liu *et al*. 2014, Parker *et al.* 2015, Beslic *et al.* 2016, Young *et al.* 2016, Borghezan *et al.* 2017), wine chemical composition (Kozina *et al.* 2008*,* Martin *et al.* 2016), and carbohydrate reserve (Parker *et al.* 2014, Greven *et al.* 2016).

The climatic conditions when leaves are removed result in changes in aromatic compound composition (Sivilotti *et al.* 2017). The authors of this study speculated that leaf removal may have had an effect on the availability of photosynthates and other key metabolites the affect fruit ripening. The effect of defolation and time of defoliation on the distribution of leaf area (LA) and photosynthesis within the remaining leaves is largely unstudied, especially in cool climates. Low temperature at budbreak is typical of high altitude regions and could be a limiting factor on plant productivity and grape quality (Brighenti *et al*. 2014).

Grapevine photosynthesis has been shown to be cold tolerant; however, low temperatures reduced grapevine shoot growth, as well as temperature-dependent carbon grain (Hendrickson *et al.* 2003,2004). Leaf age and position affect photosynthesis rates (Poni *et al.* 1994, Medrano *et al.* 2015), and an increase in the source:sink ratio can cause photosynthesis rates to decline (Petrie *et al.* 2000). However, measurements of individual leaves could not represent the photosynthetic yield of the whole-plant and this extrapolation of values can be an important limitation to the applicability of the research results. In this context, the objective of this study is to investigate the effect of the different defoliation times on shoot length, LA, leaf

growth, and photosynthetic yield of the Sauvignon Blanc variety under low temperatures.

2.3 MATERIALS AND METHODS

The experiment was conducted in a commercial Sauvignon Blanc vineyard located in São Joaquim, Santa Catarina State, (28º 17' 39" S e 49º 55' 56" W, between 1200 and 1400 m a.s.l) during the 2015/2016 vintage. The vines (grafted on Paulsen 1103) were planted in 2004 in a north-to-south row orientation, with a 3.0 m (row) × 1.5 m (vine) spacing, trained on a double cordon with vertical shoot positioning 1.2 m above the ground, and covered with anti-hail protection screen. The soils of the vineyard location fall into the classes Humic Cambisol, Litholic Neosol and Haplic Nitosol, developed from riodacito and basalt rock. The climate of the region is characterized as moist mesothermic with mild summers, or Cfb in the classification of Köppen (EMBRAPA, 2004). The experiment was performed with a randomized block design; four blocks were used in total with five plants per block, and measurements were taken on two branches per plant. Treatments consisted of five different defoliation times, using the methodology described by Baillod and Baggiollini (1993): (a) control (C), untreated, (b) full bloom defoliation at 50 days after budding (DAB), (c) at time in which grape fruits are pepper-corn size stage at 65 DAB, (d) berry pea size stage at 78 DAB, (e) veraison defoliation at 121 DAB, (f) 15 days after veraison at 140 DAB. Climatic data (mean hourly air temperature, rainfall and photosynthetically-active radiation (PAR) were recorded from September to February by a weather station of the EPAGRI (Company of Agricultural Research and Rural Extension of Santa Catarina). Growing Degree Day (GDD) units were calculated using the daily mean of Tmax and Tmin with a minimum threshold of 10ºC and no maximum threshold applied.

2.3.1 Growth parameters and Photosynthetic pigments

The following grapevine growing parameters were measured every two weeks from budding to harvest in all plants: shoot length, leaf area (LA), and SPAD index in two shoots per plant. SPAD index was performed using a chlorophyll (Chl) meter (SPAD-502, Konica Minolta, Tokyo, Japan). The LA was estimated through the

mathematical model obtained by Borghezan *et al.* (2010); LA per plant (m²) was estimated by multiplying the average LA per shoot by the number of shoots per plant. In addition, SPAD index and quantification of Chl *a*, *b* and carotenoids (car) were performed on leaves of different areas (S1: 0 – 8 cm^2, S2: 8.1 - 37 cm^2, S3: 37.1 - 88 cm^2, S4: 88.1 - 160 cm^2, S5: 160.1 - 260 cm^2, 260.1 – 385 cm^2) at full bloom, veraison, and maturity stage. Chl and SPAD index were measured at the same point on each leaf.

For each area range, thirty 100 mg fresh tissue samples were incubated with 7.0 mL dimethylsulfoxide (DMSO) for two hours at a 65°C water bath in dark conditions without maceration. After filtering, the volume was adjusted to 10 mL and the assessment was done by spectrophotometry (A) measuring the optical density at 649 and 665 nm. Wellburn (1994) equations were used:

Chl *a* = [12.19 * (A665) - 3.45 * (A649)](1)

Chl *b* = [21,99 * (A649) - 5.32 * (A665)](2)

Total Chl = sum of Chl *a* and Chl *b*

2.3.2 Chl *a* fluorescence

Chl *a* fluorescence (ChlF) was measured with a pulse-amplitude-modulated photosynthesis yield analyzer (Mini-PAM*,* Walz, Effeltrich, Germany). During the light period, between 08:00 h and 10:00 h and between 12:00 h and 14:00 h, effective quantum yield of PSII (ΦPSII), $\Delta F/F_m'$, was determined, where $\Delta F = F_m' - F_t$, F_m' is the maximum fluorescence yield of illuminated leaves and Ft is the instantaneous fluorescence of illuminated leaves measured briefly before application of a saturation pulse, according to Genty *et al.* (1989). At pre-dawn, maximal quantum yield (F_v/F_m), was determined, where $F_v = F_m - F_0$, F_m maximum fluorescence in leaves acclimated to dark and F_0 is the basal fluorescence of fully oxidized reaction.

2.3.3 Statistical analysis

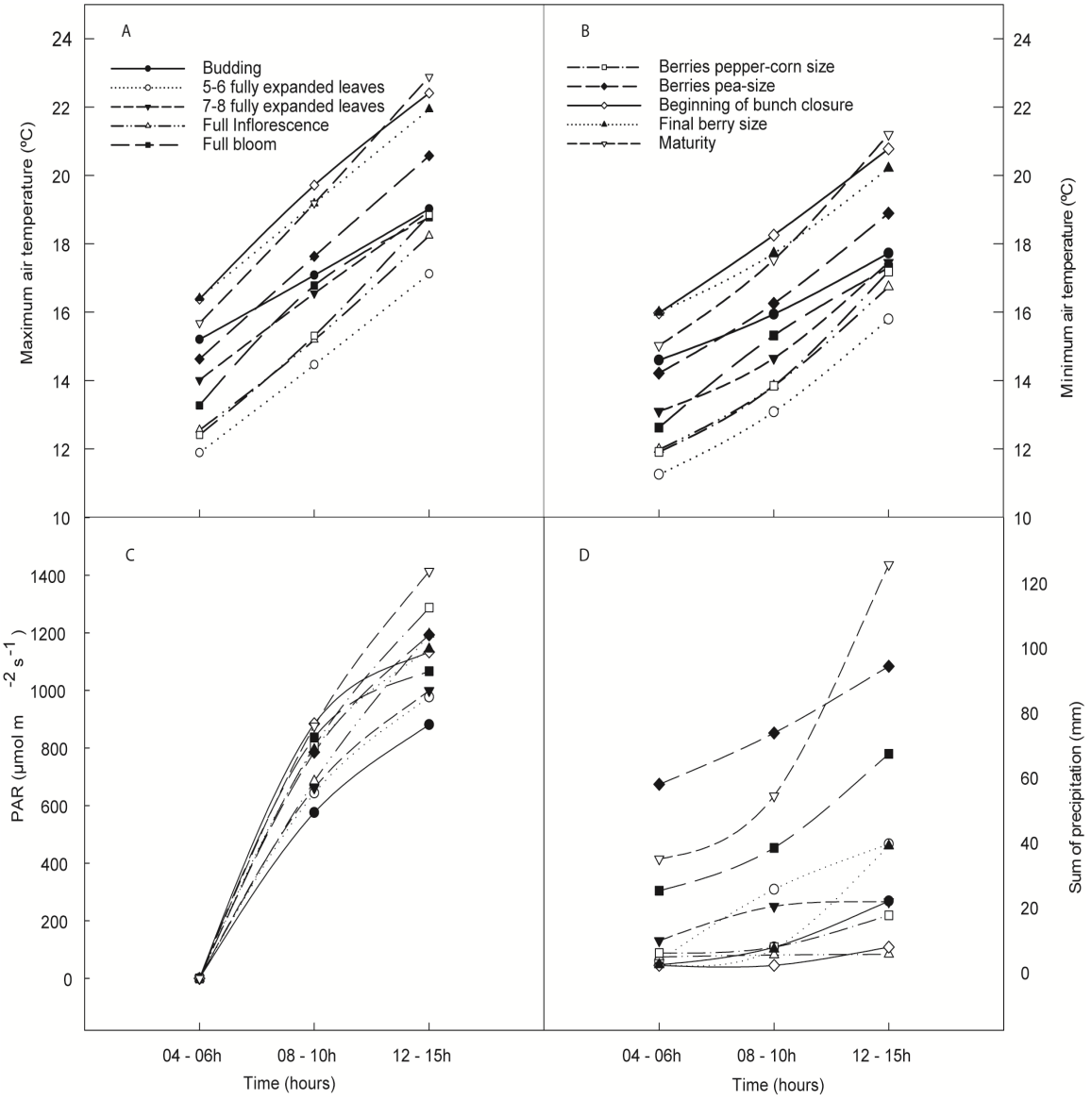
Results were represented as mean ± SD. Data obtained from the defoliated and non-defoliated treatments were analysed by analysis of variance (ANOVA) followed by Tukey's test with acut-off at $P \leq 0.05$ using R (R Core Team, 2011).

2.4 RESULTS

2.4.1 Weather conditions

The season 2015/2016 was characterized by lower temperatures and high rate of rainfall as showed in Fig. 1. The highest air temperatures occurred between the final berry size stage and berry maturation (Fig. 1*A*). The minimum temperatures recorded during the cycle were far lower during the expansion of the first 6 leaves. The nighttime temperatures remained low throughout the cycle with an average of 14ºC (Fig. 1*B*). GDD from budding to harvest was 977. Total rainfall over the same period was 973 mm. Photosynthetically-active radiation (PAR) was highest during fruit maturation, coinciding with the highest precipitation rate (Fig. 1*C,D*).

Figure 1 – (A) Maximum air temperature (ºC), (B) minimum air temperature (ºC), (C) PAR, photosynthetically active radiation (µmol $m^{-2}s^{-1}$), and sum of precipitation (mm) at predawn (04:00 - 06:00h), morning (08:00 - 10:00 h) and midday period (12:00 - 15:00 h) from budding to maturation (2015/2016 cycle) of cv. Sauvignon Blanc in São Joaquim.



2.4.2 Shoot growth measurements

Peaks of shoot growth were observed between stages 5-6 fully expanded leaves and inflorescence development (59%) and from this stage to full bloom (45%) (Table 1). The highest percentage of shoot growth coincides with a 2ºC temperature increase in relation to the previous stage. In the same period, the LA increased 70%, mainly due to the production of new leaves.

Table 1 – Mean (± SD) for shoot length (cm), LA (m^2 $plant^{-1}$), SPAD values Sauvignon Blanc (*Vitis vinifera* L.).

Shoot length (cm)
Defoliation time

Phenological stage	Control	Full bloom	Berry pepper-corn size	Berry pea size	Veraison	15 days after Veraison
5 - 6 fully expanded leaves	15.6 ± 2.3					
7 - 8 fully expanded leaves	23.8 ± 2.1					
Developed inflorescence	57.7 ± 6.7					
Full bloom	105.2 ± 6.5					
Berry pepper-corn size	133.9 ± 18.2	141.5 ± 21.0				
Berry pea size	168.6 ± 21.8	168.4 ± 23.3	186 ± 8			
Beginning of bunch closure	219.2 ± 23.3	225.3 ± 20.2	202.2 ± 24.6			
Final berry size	273.5 ± 26.2	275 ± 25.6	247.2 ± 34	258.5 ± 24.5		
Veraison	112.6 ± 20	125.5 ± 53.6	121 ± 34	129.6 ± 12.6	149 ± 31.3	
Maturity	112.6 ± 20	125.5 ± 53.6	121 ± 34	121 ± 3.5	137.6 ± 25.3	131.1 ± 10.6
Leaf area (m^2 $plant^{-1}$)						
5 - 6 fully expanded leaves	0.58 ± 0.1					
7 - 8 fully expanded leaves	1.06 ± 0.2					
Developed inflorescence	3.64 ± 0.5					
Full bloom	7.22 ± 0.9					
Berry pepper-corn size	10.91 ± 1.4	10.53 ± 0.9				
Berry pea size	15.04 ± 1.2	12.86 ± 0.5	13.8 ± 0.5			
Beginning of bunch closure	18.33 ± 0.9	16.96 ± 1.5	14.7 ± 1.3			
Final berry size	17.52 ± 2.5	16.83 ± 2.8	15.9 ± 1.5	17.1 ± 3.7		
Veraison	12.06 ± 2.5	7.74 ± 1.2	10.3 ± 0.02	11.1± 1.9	8.21 ± 1.9	
Maturity	11.13 ± 2.7	8.05 ± 1	10.4 ± 0.1	11.4 ± 0.9	8 ± 1.2	9.55 ± 1.3
SPAD index						
5 - 6 fully expanded leaves	20.2 ± 0.6					
7 - 8 fully expanded leaves	22.5 ± 0.9					
Developed inflorescence	25.5 ± 1.2					
Full bloom	28.8 ± 1.2					
Berry pepper-corn size	28.9 ± 1.3	29.3 ± 0.9				
Berry pea size	31.7 ± 1.4	31.8 ± 1.6	29.2 ± 1.9			
Beginning of bunch closure	32.1 ± 1.5	33 ± 1.2	31.4 ± 1.3			
Final berry size	33.1 ± 1.4	34 ± 1.4	32.1 ± 1.3	34.9 ± 2.2		
Veraison	34.8 ± 2.3	35.1 ± 2.7	39.4 ± 3.3	37.2 ± 2.3	29.8 ± 2.4	
Maturity	36.1 ± 2.3	38.2 ± 1.8	40.5 ± 3.6	39.6 ± 0.4	30.4 ± 2.4	33.7 ± 3.5

Subsequent to flowering, a regular shoot growth of 20% per stage was observed until shoots were trimmed after fruits reached their full size. LA, as expected, increased with leaf expansion. The timing of leaf removal had no significant impact on

shoot length, LA, or Chl content (SPAD) overall when all leaves of a branch were measured and their values averaged. However, there was a wide range of values, and categorizing leaves by their size tended to give more consistent responses.

2.4.3 Photosynthetic pigments

As expected, leaf size strongly influenced its pigment content (Table 2). Younger leaves, S1 and S2, showed Chl *a* content five-fold lower than fully expanded leaves at all measured phenological stages.

Table 2 – Mean (± SD) for SPAD, Chl *a*, Chl *b*, the ratio of Chl (*a*/*b*) and car. Different letters within the same row denotes significant differences ($p < 0.05$) between phenological stages. (**continue**)

		SPAD	
LA	**Flowering**	**Veraison**	**Maturity**
S1	15.3 ± 1.4^{a}	16.7 ± 2.7^{a}	11.6 ± 2.6^{b}
S2	16.7 ± 1.3^{a}	17.3 ± 2.4^{a}	11.0 ± 1.6^{b}
S3	22.0 ± 2.8^{b}	25.7 ± 3.2^{ab}	30.0 ± 9.8^{a}
S4	25.4 ± 3.3^{c}	31.1 ± 2.5^{b}	37.1 ± 2.8^{a}
S5	31.5 ± 3.7^{c}	34.6 ± 4.0^{b}	41.6 ± 2.7^{a}
S6	37.4 ± 3.2^{a}	38.2 ± 3.8^{a}	38.8 ± 2.9^{a}
		Chl *a* (g kg^{-1} FM)	
S1	0.30 ± 0.1^{b}	0.49 ± 0.2^{a}	0.35 ± 0.1^{b}
S2	0.50 ± 0.1^{ab}	0.59 ± 0.2^{a}	0.42 ± 0.1^{b}
S3	0.86 ± 0.2^{c}	1.20 ± 0.2^{b}	1.62 ± 0.7^{a}
S4	1.09 ± 0.2^{b}	1.24 ± 0.2^{b}	1.84 ± 0.3^{a}
S5	1.48 ± 0.2^{b}	1.45 ± 0.3^{b}	2.07 ± 0.2^{a}
S6	1.81 ± 0.3^{a}	1.87 ± 0.4^{a}	1.73 ± 0.3^{a}
		Chl *b* (g kg^{-1} FM)	
S1	0.13 ± 0.04^{b}	0.14 ± 0.07^{b}	0.19 ± 0.04^{a}
S2	0.11 ± 0.03^{b}	0.14 ± 0.05^{ab}	0.17 ± 0.03^{a}
S3	0.16 ± 0.05^{c}	0.27 ± 0.08^{b}	0.42 ± 0.17^{a}
S4	0.19 ± 0.05^{c}	0.31 ± 0.06^{b}	0.45 ± 0.12^{a}
S5	0.33 ± 0.06^{b}	0.43 ± 0.25^{ab}	0.52 ± 0.05^{a}
S6	0.40 ± 0.10^{b}	0.52 ± 0.12^{a}	0.42 ± 0.09^{ab}

Table 2 – Mean (± SD) for SPAD, Chl *a*, Chl *b*, the ratio of Chl (*a*/*b*) and car. Different letters within the same row denotes significant differences ($p < 0.05$) between phenological stages. (**end**)

		SPAD	
LA	**Flowering**	**Veraison**	**Maturity**
		Chl a/b ratio	

S1	2.31	3.50	1.84
S2	4.55	4.21	2.47
S3	5.38	4.44	3.86
S4	5.74	4.00	4.09
S5	4.48	3.37	3.98
S6	4.53	3.60	4.12
		Car (g kg^{-1} FM)	
S1	0.26 ± 0.08^{a}	0.18 ± 0.05^{b}	0.31 ± 0.05^{a}
S2	0.26 ± 0.03^{a}	0.22 ± 0.06^{b}	0.28 ± 0.04^{a}
S3	0.33 ± 0.05^{b}	0.37 ± 0.06^{b}	0.58 ± 0.22^{a}
S4	0.40 ± 0.05^{b}	0.38 ± 0.09^{b}	0.60 ± 0.11^{a}
S5	0.47 ± 0.08^{b}	0.42 ± 0.11^{b}	0.71 ± 0.09^{a}
S6	0.60 ± 0.12^{a}	0.62 ± 0.17^{a}	2.60 0.13^{a}

The greatest change in Chl *a* content was found in S3 leaves at the veraison and berry maturation stages with a two-fold and four-fold increase in relation to S2 leaves. Chl *b* content of all sizes of leaves was higher at maturity. The highest increases in car content among the different sizes were observed at veraison. The fully expanded leaves, S6, showed SPAD, Chl *a* and car content high in all measured phenological stages. At maturity, S4 leaves showed the highest values for all parameters. Chl *a/b* ratio increased with leaf development until S4 size and then declined. Likewise, the highest Chl *a/b* ratios were observed at flowering stage and then declined until maturity stage.

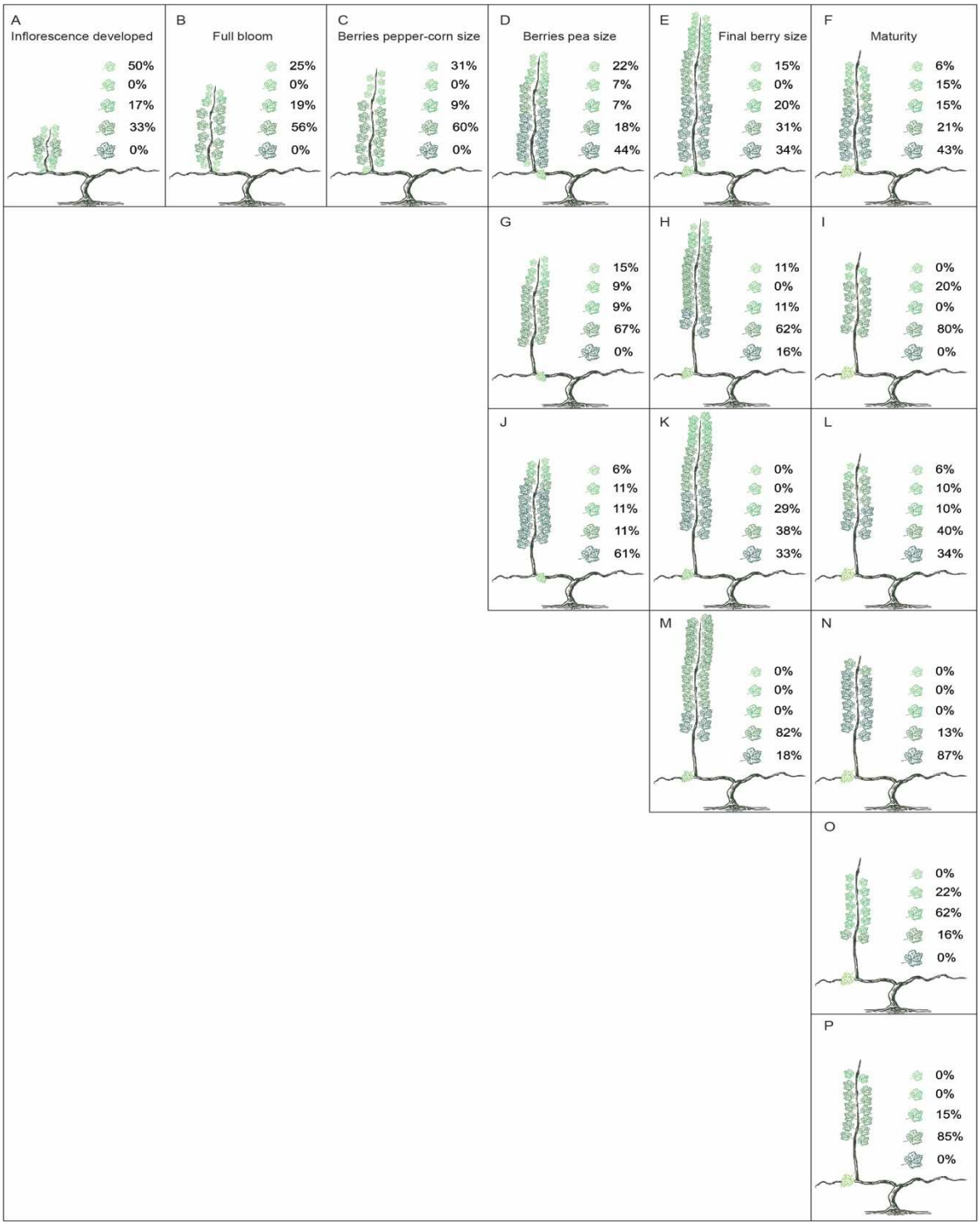
2.4.4 Chlorophyll fluorescence

Leaves, categorized into sizes after previous data showed that it was more indicative of plant behavior than pooled data from branches, were then tested for effective photochemical quantum yield of PSII (ΦPSII) and maximum photochemical efficiency of PSII (F_v/F_m). To allow ChlF measurements, leaves of size S1 (0-8 cm^2) and S2 (8.1-37 cm^2) were grouped, and from now called S1. ChlF of control plants was measured from the inflorescence development to maturity (Fig. 2), while ChlF of the treated plants was measured according to the defoliation time. We assumed that leaves in control and treated plants were the same until defoliation.

The inflorescence development stage was strongly marked by the emergence of new leaves; at this stage, 50% of plant leaves had area smaller than 37 cm^2 (Fig. 2*A*). Although, there was a significant difference in the photosynthetic pigments content, ChlF measurements at 08:00 h showed no significant differences in ΦPSII between the different leaf sizes(Fig. 3*A*). After 16 days at the full-bloom stage, a considerable leaf expansion was observed, with 56% of measured leaves growing into the S4 category (Fig. 2*B*). Interestingly, there were no significant differences in ΦPSII of S1 and S4 leaves (Fig. *3B*), although S4 leaves had shown the highest Chl *a/b* ratio of all leaf sizes during flowering. A decline in S3 and S4 leaf ΦPSII was observed at noon; however, S1 leaf ΦPSII increased.

At the berry peppercorn phenological stage, there was in increase in both new leaf emergence and leaf expansion (Fig. 2*C*). At this stage, ΦPSII of S4 leaves was significantly higher than in S1 leaves (Fig. 3*C*). At pea-size stage, the ChlF of the defoliated plants at full bloom and at the phenological stage of berries peppercorn size were also evaluated. At this stage, a reduction in emergence of new leaves in non-defoliated plants was observed (Fig. 2*D*), which was composed of 44% of fully expanded leaves (S5). Further, the ΦPSII of S4 and S5 leaves were significantly higher than the smaller leaves. At 08:00 h, only S3 leaves showed a reduction in ΦPSII (Fig. 3*D*).

Figure 2 - Representation of non-defoliated and defoliated plants of Sauvignon Blanc in São Joaquim through the following phenological stages: inflorescence development, full bloom, berry pepper-corn size, berry pea size, berry final size, and maturity.



(*A,B,C,D,E,F*) Non-defoliated plants; (*G,H,I*) Defoliated plants at full bloom; (*J,K,*L) Defoliated plants at berry pepper-corn size; (*M,N*) Defoliated plants at berry pea size; (*O*) Defoliated plants at veraison; (*P*) Defoliated plants 15 days after veraison.

At noon, the smaller leaves showed reduced values of ΦPSII. In this stage, defoliated plants at full bloom (30 DAD) did not show completely expanded leaves (Fig. 2*G*), and fewer new leaves emerged compared to control plants. The F_v/F_m of S3 and

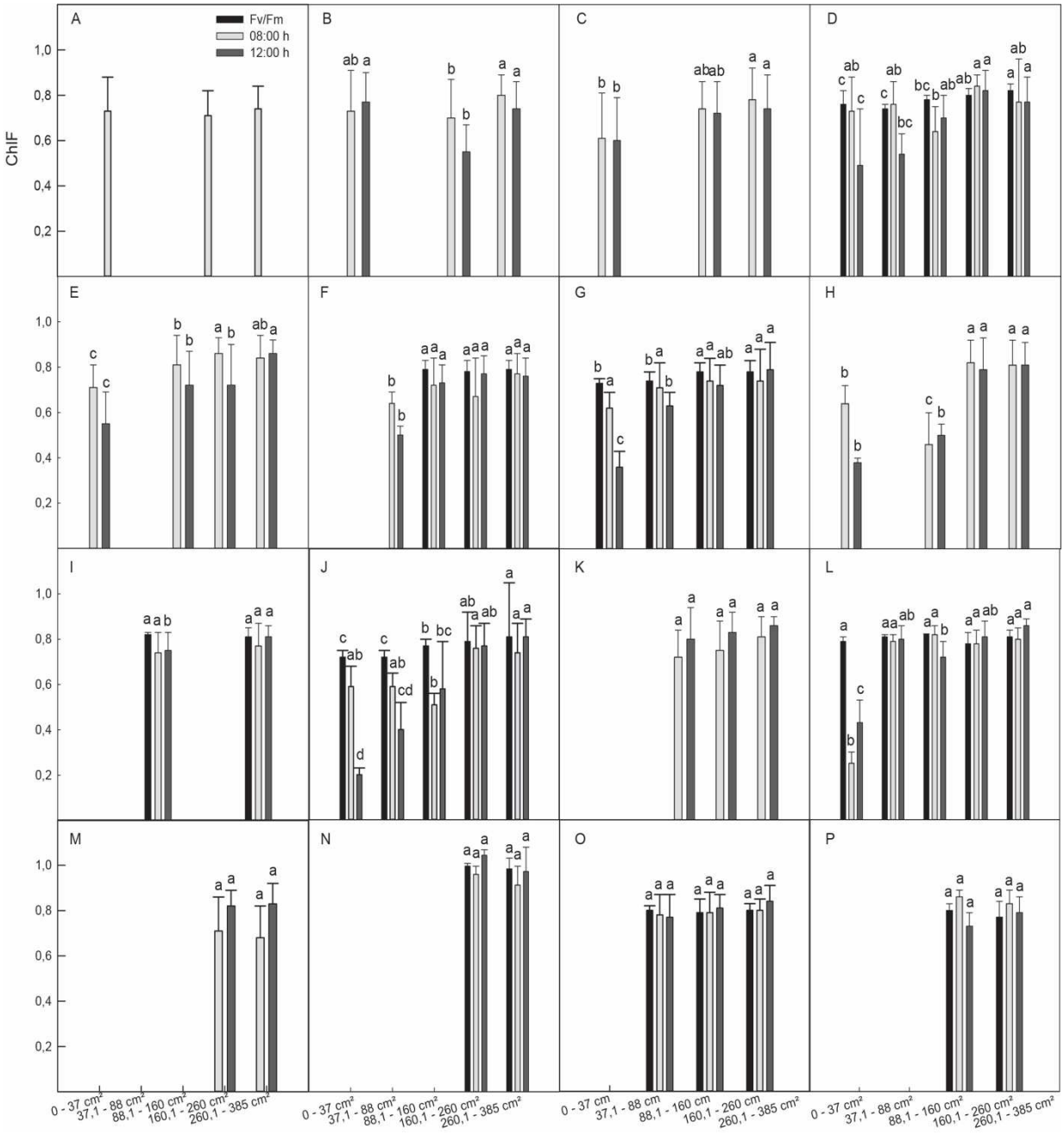
S4 leaves of plants defoliated during blooming were significantly higher than smaller leaves. (Fig. 3*G*). At 08:00 h there were no significant differences between leaf sizes, although at noon S3 and S4 leaves had shown a higher ΦPSII. It is important to note that, after defoliation, plants were exposed to an average 0.7ºC reduction in air temperature, with night temperatures reaching 11.9 ºC, and a precipitation reduction of 2.5-fold in relation to the previous stage. Plants defoliated at berry peppercorn size (15 DAD) (Fig. 2*J*) showed 61% of fully expanded leaves. Emergence of new leaves was even more reduced in relation to the control plants and plants defoliated at full bloom stage. ΦPSII and F_v/F_m was significantly higher in the larger leaves, and at noon compared to 08:00 h (Fig. 3*J*).

In the case of plants defoliated at the peppercorn berry-sized stage, plants were exposed to an average increase of 2 °C in air temperature and a precipitation increase of 3.7-fold compared to the previous stage. At the final berry-size, plants defoliated at pea-size were also evaluated. At this stage, no F_v/F_m measurements were performed. In control plants, though there was a balanced division between the leaf sizes (Fig. 2*E*), ΦPSII was significantly higher in fully expanded leaves (Fig. 3*E*).

In plants defoliated at full bloom (58 DAD), there was a reduction of new leaf emergence; however, existing leaves were found to expand with a total of 16% reaching full expansion from 0% in the previous stage (Fig. 2*H*). At this stage, ΦPSII was significantly higher in S4 and S5 leaves than in smaller ones (Fig. 3*H*). In plants defoliated at berry peppercorn size (43 DAD) (Fig. 2*K*) and at pea size (30 DAD) (Fig. 2*M*), no new leaf emergence was observed, as well as no significant differences in ΦPSII and F_v/F_m between leaf sizes (Fig. 3*K,M*). Plants defoliated at the pea-size stage were exposed to an increase of 2°C in air temperature, and a precipitation decrease of 3.2-fold regarding the previous stage.

Shoot trimming was performed between the pea-sized stage and veraison. Finally, at maturity, plants defoliated at veraison and 15 days after veraison were also evaluated. At berry maturation, foliar expansion was observed in the control plants after shoots were trimmed (Fig. 2*F*).

Figure 3 – ΦPSII at 08:00 and 12:00 and Fv/Fm in leaves of control and defoliated treatments.



(*A,B,C,D,E,*F) Non-defoliated plants at inflorescence development, full bloom, berry pepper-corn size, berry pea size, final berry size, and maturity, respectively; (*G,H,I*) Defoliated plants at full bloom during berry pea size, final berry size and maturity, respectively; (*J,K,L*) Defoliated plants at berry pepper-corn sizeduring berry pea size, final berry size and maturity, respectively; (*M,N*) Defoliated plants at berry pea size during final berry size and maturity, respectively;(*O*) Defoliated plants at veraison during maturity; (*P*) Defoliated plants 15 days after veraison during maturity.

ΦPSII of S3, S4, and S5 leaves were significantly higher than the smaller leaves, which agrees with the Chl *a* content of this leaves at this stage (Fig. 3*F*). At this stage, there was a reduction of S5 leaves in plants defoliated at full bloom (92 DAD) after the removal of the apical portion (Fig. 2*I*). No significant differences were

observed in F_v/F_m and ΦPSII at 08:00 h; however, ΦPSII was significantly higher in S4 leaves at noon (Fig. 3*I*). In plants defoliated at berry peppercorn size (78 DAB), new leaves were formed after shoot trimming (Fig. 2*L*).

No significant differences were observed in F_v/F_m between leaf sizes. At 08:00 h only S1 leaves showed lower ΦPSII. At noon, S1 and S3 leaves showed significantly lower ΦPSII (Fig. 3*L*). In plants defoliated at pea size (64 DAD), no new leaves emergence was observed, however, a marked increase in leaf expansion was observed (Fig. 2*N*). Regarding the plants defoliated at veraison (26 DAD), it should be considered that these plants were submitted to shoot trimming before basal leaf removal. Although it was observed in these plants, a leaf composition with lesser areas than plants of others defoliation times, which implied in smaller LA per plant (Fig. 2*O*), ΦPSII is high (Fig. 3*O*). In plants defoliated 15 days after veraison (12 DAD), 100% of the remaining leaves are S3 and S4 (Fig. 2*P*), and no significant differences in ΦPSII and F_v/F_m between leaf sizes are observed (Fig. 3*P*).

2.5 DISCUSSION

Temperature is among the strongest factors determining grapevine growth. A 2 ºC decrease in the average daily temperature can severely reduce shoot length in the colder areas, especially during bud-break in early spring (Hendrickson et al. 2004, Brighenti et al. 2014). The temperature increases between the phenological stages 5 - 6 and 7 - 8 fully expanded leaves seems to supports these observations because peak shoot growth coincided with increasing temperature. This is in agreement with previous observations that fully-developed, photosynthetically-active leaves are required for sustained shoot growth (Hendrickson et al. 2004). Even before new leaves begin to export photosynthates, the plant depends on stored carbon and other reserves, and these are removed more slowly by shoots growing in low temperatures than shoots growing at higher ones, thus limiting leaf growth (Keller and Tarara. 2010).

In the present study, temperatures between 15 °C and 17 °C during the inflorescence development led to a reduction in photosynthetic yield in all leaf sizes. An air temperature increase of 1ºC allowed mature leaves to reach optimum ΦPSII

values at 08:00 h at the flowering stage; however, this decreased at noon. Until the end of flowering, approximately 60% of the LA is exposed to the sun, altering stomatal and non-stomatal processes in the period of higher solar radiation under low temperatures, and triggering energy dissipation mechanisms to avoid photoinhibition (Hendrickson et al. 2003, Sawicki et al. 2012). Thus, in addition to a reduction in stomatal conductance, a reduced rate of RuBP regeneration may be occurring simultaneously. The reduction of 1ºC in the mean air temperature and an increase of PAR at the berry peppercorn size stage induced a marked decrease in ΦPSII, even in mature leaves, which was quickly recovered in the warmer temperatures and greater solar radiation during later phenological stages.

The process of acclimatization to the cold is cumulative but can be either reversed or restarted, depending on the fluctuations in temperature (Keller 2012). This is likely related to temperature during early leaf formation, which conferred higher tolerance to photoinhibition under cold conditions (Boese and Huner 1992). Keller and Tarara (2010) observed that plant growth was primarily affected by temperatures during leaf formation, only in spring, also, lower bud-break temperatures slow all growth processes, although leaf-area expansion appeared to be less responsive to temperature than either shoot elongation rate or leaf appearance rate. In the present study, low temperatures reduced the photosynthetic yield of Sauvignon Blanc in São Joaquim, principally during the formation and beginning of the growth of the berry.

The Fv/Fm fluorescence parameter is linearly correlated with the concentration of functional PSII reaction centers (Lee et al. 1999). From the pea-size stage and onward, minimum temperatures remained above 18ºC. Subsequently, Fv/Fm values remained close to 0.8, the threshold considered healthy for a terrestrial plant (Maxwell and Johnson 2000). There were exceptions: leaves with less than 88 cm² showed reduced Fv/Fm at pea-size stage even more pronounced in defoliation plants. Developmental stage is an important factor that determines the responses of young and old grapevine leaves under stressful conditions. In a study comparing 10-day-old young leaves (6 cm^2) and 30-day-old mature leaves (80 cm²), Pinto et al. (2011) observed high expression of Early light-inducible proteins (ELIPs) in the younger leaves, however, the ELIPs expression was highly temperature-dependent, occurring

only above 13 °C and reaching an optimum at around 30 ◦C. ELIPs play an important role in the Chl accumulation, protecting them from degradation during assembly of PSII reaction centers.

The decrease in the PSII antenna size results in less excitation energy in PSII with a corresponding decrease in PSII fluorescence intensity. The low photochemical capacity of young leaves is in accordance with the lower Chl a/b ratios. At the final berry-sized and maturity stages, the temperature increased 1.5 °C compared to previous stages and plants showed ΦPSII values very close to the optimum value of 0.8. The ideal temperature for photosynthesis tends to increase by approximately 1°C for each increase of 2 to 3°C during growth temperature; however, this ideal temperature decreases with increasing altitude due to decreasing overall temperature (Schultz 2000).

Low temperatures during early spring followed by intense PAR can cause degradation of the thylakoid structure and distortion in light-dependent photosynthetic reactions (Suzuki et al. 2011). Grapevine photosynthesis may have a highly-efficient photoprotective mechanism because a significant and sustained reduction in Fv/Fm in field-grown grape leaves under low temperatures has not been reported (Hendrickson et al. 2003). Although the cold stress had affected ChlF parameters in the present study, Chl content did not decrease. Similarly, pigment content in S4 and S5 leaves at maturity remained stable and ΦPSII values remained high, signifying that leaves had not yet entered senescence. This is accord with Bertamini and Nedunchezhian (2003), who observed that senescent leaves presented low proportions of Chl a/b due to the high degradation of Chl between maturity and senescence.

Moreover, the response of the vine depends largely on the level and timing of manipulation (Kliewer and Dokoozlian 2005); defoliation times had a strong impact on leaf growth dynamics, as well as photosynthetic yield. It has been well documented that leaf removal increases the photosynthetic activity per unit LA of remaining leaves (Petrie et al. 2000, 2003, Parker et al. 2014). Parker et al. (2014) suggested that, during maturity, the demand for photosynthesis increases, and has the potential to maintain high even in mature leaves. In addition, the authors suggested that the

decline of photosynthesis attributed to leaf aging may in part caused by increasing LA in non-defoliated plants.

LA reduction through basal leaf removal could have the opposite effect; the plant may maintain their remaining leaves as photosynthetically-active as possible in order to compensate for the overall loss in LA . The maintenance of photosynthesis rate post-harvest suggests that the plant's ability to accumulate reserves during this period was not limited by the demand for photosynthates Removing the basal leaves significantly impacted grapevine growth. Defoliation at any time between flowering to after veraison reduced the emergence of new leaves, which could indicate a carbon sink during fruit growth and maturation. Branch size, LA, and Chl (SPAD) were shown to be poor indicators of the complex changes in foliar activity; however, the rate at which leaves expanded to their maximum size showed that Sauvignon Blanc plants were not only capable of adapting to defoliation, but the ΦPSII values comparing defoliated to non-defoliated plants showed that photosynthesis was more active in defoliated plants.

Our study highlighted several further areas of study for grapes grown at high-altitudes and low-temperatures, namely the underlying changes in carbon assimilation, movement, and accumulation; and the effect of UV radiation on leaf physiology.

3 GENE EXPRESSION AND FLAVONOL COMPOSITION RESPONSE TO DEFOLIATION TIME ON CV. 'SAUVIGNON BLANC' BLANC (*Vitis vinifera* L.) UNDER HIGH ALTITUDE CONDITIONS

3.1 ABSTRACT

Leaf removal around bunches is a common practice to increase berries sunlight exposure. However, exposure to high solar radiation in high altitude vineyards strongly affect the berry phenolic composition. Until now no transcriptomic data have been reported related the mechanisms that underlie the effects of defoliation in high altitude regions of Santa Catarina. The aim of this study was to analyze the effects of defoliation at full bloom (FBD) and at veraison (VD), on flavonol composition and gene expression, focusing on effects of UV-exposure. We conducted analyses of the main berry compositional parameters, berry skin flavonoids and berry skin transcriptome on FBD and VD berries sampled during berry growth, veraison, and maturity. Flavonol concentration was much lower in shaded plants than in defoliated plants in all measured phenological stages. The longer exposure time increased the flavonols content, although there were no significant differences between defoliation times at maturity. Gene expression related to low and high UV-fluence was observed in control and defoliated plants. Our results provide insight into the metabolic processes affected in berries by the defoliation practice and the impact the final berry ripening traits.

Keywords: Grapevine, Defoliation, Flavonols, Altitude, Gene expression.

3.2 INTRODUCTION

Climate change over the next 20 years could lead to a shift in the current wine-growing zoning. Occurrence of droughts and increasing air temperatures directly affect grapevines physiology in the traditional producing regions, and in this context, new wine-growing regions are developing and taking importance in the international market, mainly the cool climate regions, which are likely to benefit from that gradual warming trend (Hannah et al., 2013; Shaw, 2016; Drappier et al.,2017; Wolkovich et al., 2017; Bornmann et al., 2017). That is the case of Santa Catarina state in Brazil, where a high-altitude wine-growing region has been highlighted. High levels of solar radiation and low temperatures positively influence fruit quality (Brighenti et al., 2013; 2017).

'Sauvignon Blanc' stands out as the white variety of grapevine that has better adapted to the altitude vineyards in Santa Catarina state (Brighenti et al. 2013). Specific terroir features of this region allow developing better-quality white wines with

high intensity, complexity, and aromatic compounds related to fruity (apple, pear, banana) and floral aroma descriptors (Marcon Filho 2015). In South America, the climatic potential of high altitude regions has been studied mainly in relation to the exposure of grapevine to high UV-radiation. Solar radiation reaching the Earth's surface includes infrared radiation (wavelengths higher than 700 nm), photosynthetically active radiation (PAR, 400–700 nm) and UV-component, that is made up of 95% UV-A (315–400 nm) and 5% UV-B (280–315 nm).

UV levels may vary depending on changes in altitude, latitude, season and time of the day, nevertheless, the seasonal variation in UV-A levels is significantly smaller than that of UV-B (Seckmeyer, et al., 2008; McKenzie et al. 2003, 2007; Dag Brune et al., 2001; Caldwell et al., 1997). Also, although predominant, UV-A is less efficient than UV-B in mediating biological responses, because relatively small amounts of UV-B induce diverse morphological, physiological and biochemical responses in higher plants (Frohnmeyer and Staiger 2003; Kakani et al. 2003).

In high altitude regions of South America, it has been demonstrated that red grape cultivars present effective mechanisms of UV-B protection, through the biosynthesis of flavonoids and in particular of flavonols, which are phenolic compounds absorbing UV-B and possessing antioxidant properties (Berli et al. 2008, 2010, 2011, 2013, 2015). Recently, many studies have focused on understanding UV-B specific and non-specific signaling pathways in response to low and high fluence rates, since changes in reactive oxygen species (ROS) and antioxidant metabolism, as well as flavonoid accumulation, occurs under both conditions (Hideg et al., 2013; Zhang et al., 2017; Liu et al., 2014), i.e., are increased under solar exposure, however, depending on the developmental stage of the berries (Joubert et al., 2016).

The two flavonols quercetin and kaempferol are photoprotective compounds with high antioxidant capacity that respond to high UV-B in altitude vineyards, even in leaves and berries of grapevine (Berli et al., 2010, 2011) and a different qualitative flavonol profile is observed under high UV-B fluence (Gregan et al., 2012; Liu et al., 2014). The change in the flavonol profile, as well as the increase of these compounds, have been related to the increased expression of a number genes involved in their biosynthesis as a way of adapting to high sun exposure (Liu et al., 2014; Joubert et al.,

2016). In plants, UV radiation is perceived by the specific photoreceptor UVR8, which initiates signaling through transcription factors involved in the flavonoid biosynthesis pathway (Brown et al., 2016).

Following UV-radiation perception, the monomerization of UVR8 occurs and its monomeric form interacts with the protein COP1, leading finally to the transcriptional activation of HY5 (Cloix et al., 2012), which can promote the flavonoids biosynthesis by binding to MYB transcription factors or directly to flavonoid biosynthetic genes (Stracke et al., 2010). Furthermore, it is well established that UV-B stimulate the expression of various genes induced by wound and defense signaling pathway, as PR proteins (Jenkins and Brown, 2007).

Leaf removal is a commonly used canopy management tool to increase sun exposure and improve berry composition (Young et al., 2016). In high regions of Santa Catarina, it has been demonstrated that leaf removal near flowering stage provides a reduction in berry acidity and increase of pH, together with a reduction in the severity of Botrytis bunch rot (Wurz et al., 2016). On the other hand, in altitude vineyards, the increase in sun exposure linked to defoliation can increase the accumulation of pathogenesis-related (PR) proteins which can cause ‘hazing’ in the wine, due to early UV-radiation exposure of the berries (Colas et al. 2012; Liu et al., 2014, Tian et al., 2015).

Until now, no studies concerning the effects of the increasing sun exposure on flavonols accumulation in altitude vineyards under naturally high UV fluence are reported. In this research we compared the impact of two timing of defoliation (at flowering and at veraison) on Sauvignon Blanc berry composition cultivated in an high altitude vineyard located in Santa Catarina (Brazil), focusing on flavonol accumulation and on the associated changes in the expression of genes involved in their biosynthesis and regulation. Moreover, the molecular strategy for UV perception is examined at molecular level to better explain the relation between increasing UV and berry composition in a UV-rich natural environment.

3.3 MATERIALS AND METHODS

3.3.1 Plant material

The experiment was conducted in a commercial *Vitis vinifera* L. cv. Sauvignon Blanc vineyard located in São Joaquim, Santa Catarina State, (28º 17' 39" S e 49º 55' 56" W, between 1,200 and 1,400 m a.s.l) in the 2015/2016 vintage. The vines (grafted on Paulsen 1103) were planted in 2004, in a north to south row orientation, with a 3.0 m (row) × 1.5 m (vine) spacing and trained on a double cordon with vertical shoot positioning 1.2 m above the ground and covered with anti-hail protection screen. The soils of the vineyard location fall into the classes Humic Cambisol, Litholic Neosol and Haplic Nitosol, developed from riodacito and basalt rock. The climate is of the moist mesothermic type with mild summers, Cfb in the classification of Köppen (EMBRAPA, 2004). The experiment had randomized block design, with four blocks, and five plants per plot. Treatments consisted of two defoliation times, using the methodology described by Baillod and Baggiollini (1993): (a) control (C), untreated; (b) full bloom defoliation (FBD), manual removal of the six basal leaves at full-bloom (JD 322 in 2015), (c) veraison defoliation (VD), manual removal of the six basal leaves at veraison (JD 27 in 2016).

3.3.2 Climatic parameters and sample collection

Weather data (mean hourly air temperature, rainfall, photosynthetically active radiation (PAR) and UVAB radiation were recorded from September to February by a weather station of the EPAGRI (Company of Agricultural Research and Rural Extension of Santa Catarina). Berry samples were collected from each vine in trial at the following time: JD 350 (28 days after FBD), JD 13 (58 days after FBD), JD 47 (92 days after FBD, 26 days after VD). Berries were sampled randomly from both sides of the vines and were used to perform biochemical and molecular analyses.

3.3.3 Analyses of berry total soluble solids (°Brix), total acidity and pH

On JD 27 and JD 47 samples of twenty berries each were collected to perform total soluble solids (°Brix), total acidity and pH analyses. The berries were weighed and after crushing, the must obtained was immediately filtered through a strainer and a drop used for °Brix analysis with a temperature-compensating CR50 refractometer

(Maselli Misure Spa, PR, Italy). Then, 5 ml of the same must was diluted in seven volumes of double distilled water and titrated with 1N, 0.5N or 0.25N NaOH (Sigma-Aldrich, St. Louis, MO, USA) with a Crison Compact Tritator (Crison, Barcelona, Spain) to estimate pH and titratable acidity (expressed as g/L of tartaric acid equivalents).

3.3.4 Flavonols extraction and separation by HPLC

Flavonols extraction was performed on 20 berries sampled for each vine. Berry skins were soaked in 100 mL methanol for 24 h, then the extracts were subjected to acid hydrolysis of flavonols glucosides to analyze the concentration of each flavonol aglicone (Mattivi et al., 2006). Flavonols were separated by HPLC using a Waters1525 instrument equipped with a diode array detector (DAD) and a reversed phase column (RP18 250 mm × 4 mm, 5 M) with a pre-column (Phenomenex, Castel Maggiore, BO, Italy). Flavonols were quantified at 370 nm with the corresponding external standards (quercetin and kaempferol) purchased from Extrasynthese (Genay, France).

3.3.5 RNA extraction and RT-PCR analyses

Total RNA was isolated from ~350 mg of the ground berry skin using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich, St. Louis, MO). RNA quality and quantity were determined using a Nanodrop 2000 instrument (Thermo Scientific, Wilmington, DE). cDNA was synthesized from total RNA as described by Pastore et al. (2011). The gene-specific primers responsible of flavonol biosynthesis and regulation (VvFLS4, VvFLS5 and MYB12) and those for genes involved in UV-B radiation signalling pathway (UVR8, HY5, CHS1 and VvCHI4a) were retrieved from Liu et al., 2014. Actin (Pastore et al. 2011) and ubiquitin1 (Bogs et al., 2005) genes were used as references. Primers and cDNA were mixed with the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, United States) and the reaction was carried out on an ABI PRISM StepOne Sequence Detection System (Applied Biosystems, Foster City, CA, United States) using the cycling conditions reported in Pastore et al. (2017). Non-specific PCR products were identified by the dissociation curves. Amplification efficiency was calculated from raw data using LingReg PCR software (Ramakers et al., 2003). The mean normalized expression (MNE)-value was

calculated for each sample referred to the actin and ubiquitin1geometric average expression according to the Simon equation (Simon, 2003).

3.3.6 Statistical analysis

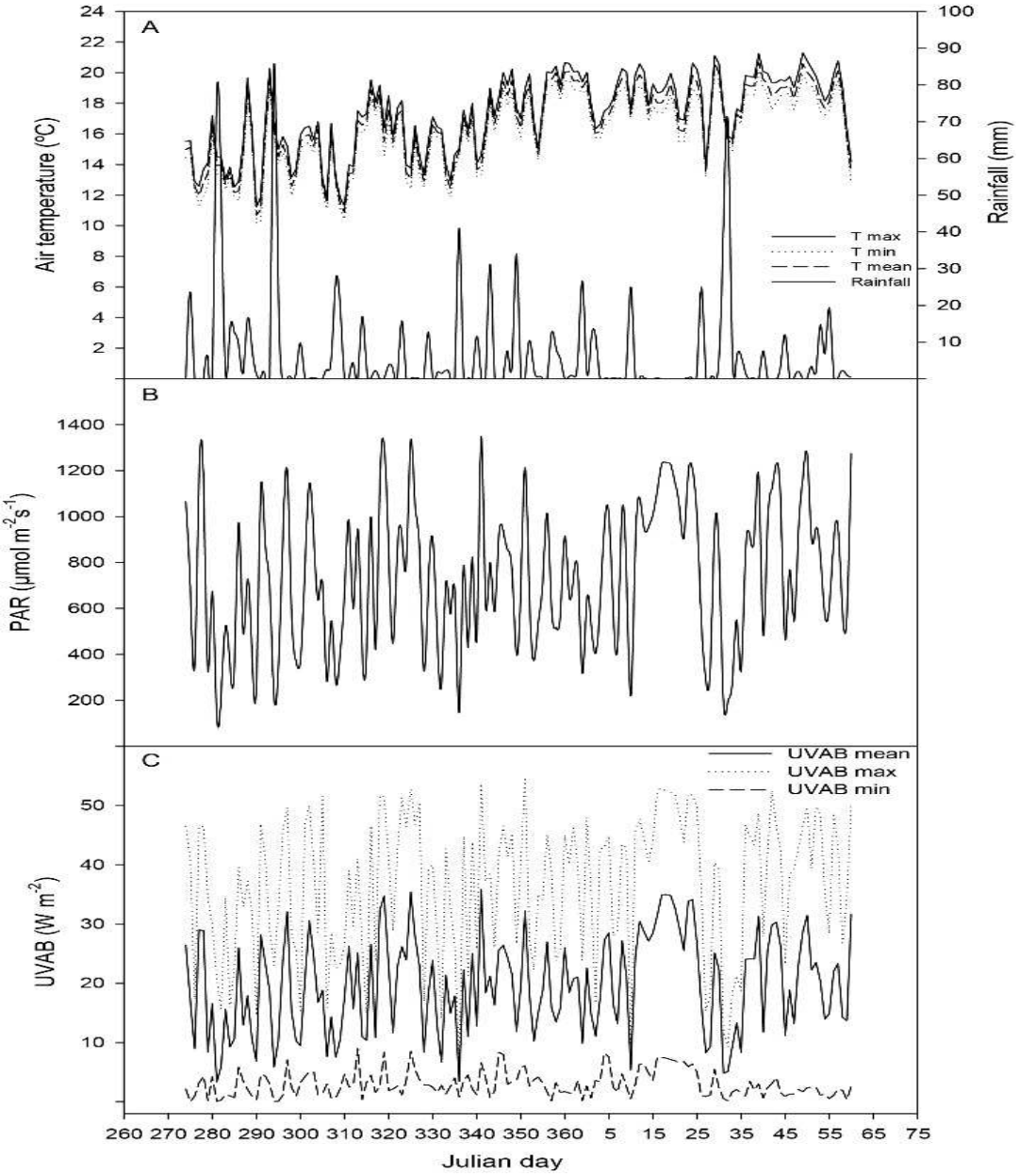
Data represent the means ± standard deviation of three replicates. Data obtained from the defoliation and non-defoliated treatments were analysed by analysis of variance (ANOVA) followed by Tukey's test with acut-off at $P \leq 0.05$.

3.4 RESULTS

3.4.1 Climatic Data

The 2015/2016 season was characterized by high rate of rainfall correlated with lower solar radiation due to the high frequency of cloudy days. Vegetative and reproductive Sauvignon Blanc cycle occurred under mild median temperature (16.7 ºC) and thermal amplitude around 9 ºC. The maximum air temperature varied between 11 ºC and 21 ºC, and the highest air temperatures of the season occurred between veraison and full maturation, in February. Total rainfall from budburst to harvest was 973 mm. Peaks of precipitation were observed at the beginning of budburst, inflorescence development and veraison (Fig. 4A). Photosynthetically active radiation (PAR) and ultraviolet radiation (UV) reach a maximum (1349,4 µmol $m^{-2}s^{-1}$ and 53,6 Wm^{-2} respectively) between full bloom and pea size phenological stages, and then PAR remained above 900 µmol $m^{-2}s^{-1}$ and UV around 30 Wm^{-2} until veraison beginning. From the veraison a marked decrease of the PAR and UV (around 600 µmol $m^{-2}s^{-1}$ and 28 Wm^{-2}) radiations was observed (Fig. 4B, C).

Figure 4 – Characterization of the mesoclimate: Seasonal trends (1 October, 2015 - 29 February, 2016) of (A) diurnal air mean, maximum and minimum temperature and mean daily rainfall (B) daily mean of photosynthetically active radiation (PAR) and (C) daily mean, maximum and minimum ultra violet radiation (UV).



3.4.2 Analyses of berry ripening

To investigate the effect of the time of defoliation under specific high altitude climatic conditions, samples taken on JD 27 (corresponding to veraison phase) and JD 47 (full-maturation) were analysed for berry TSS, total acidity and pH. On JD 27, there was a slight decrease in pH and TSS in the FBD treatment (10 weeks after leaf removal), as well as an increase in acidity (Table 3) in comparison to C. On the same day, which correspondend to five days after leaf removal at veraison, VD treatment

showed a very similar behavior to C. On JD 47 (full maturation), the berry maturation was similar in all treatments.

Table 3 – Influence of full-bloom (FBD) and veraison defoliation (VD) on agronomic parameters and ripening parameters of Sauvignon Blanc berries compared to non-defoliated controls (C) on JD 27 and 47.

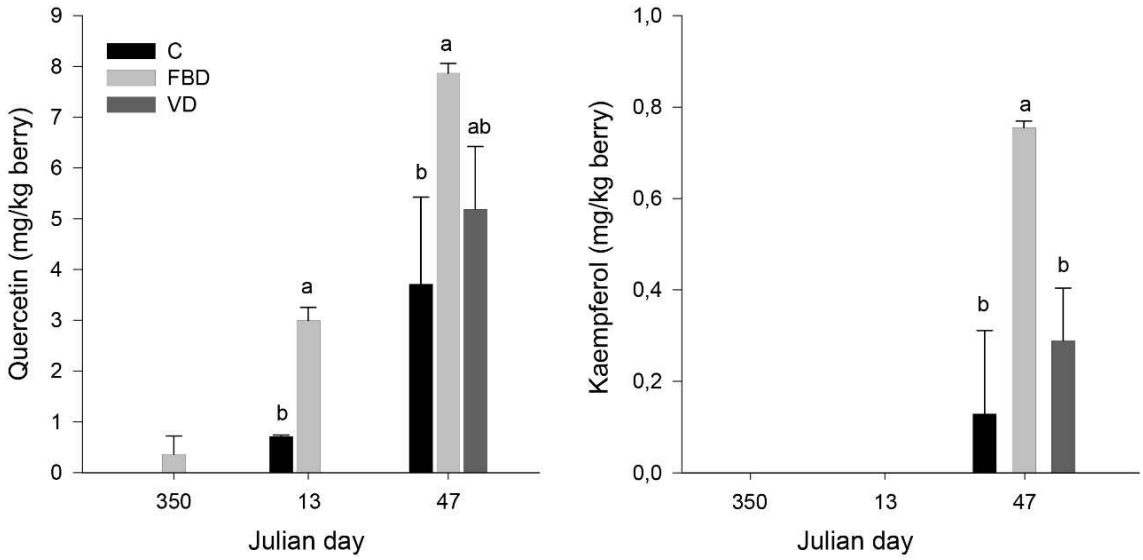
	JD 27			JD 47			
	C	**FBD**	**VD**	**C**	**FBD**	**VD**	**Treat.**
Berry mass (g)	1.3 ± 0.1	1.4 ± 0.3	1.5 ± 0.1	1.9 ± 0.3	2 ± 0.4	2.1 ± 0.2	ns
TSS (◦Brix)	14.2± 1.6	10 ± 5.4	12.3 ± 1.6	17.2 ±1.9	17.8± 1.6	17.1 ± 0.8	ns
TA ($g.L^{-1}$)	18.8± 3.4	23.7 ± 9.2	18.1 ± 1.6	9.2 ± 3.7	9.2 ± 2	9.3 ± 0.4	ns
Must pH	3.1 ± 0.7	2.9 ± 0.2	3.1 ± 0.1	3.5 ± 0.2	3.6 ± 0.2	3.5 ± 0.1	ns

ns, no significant differences between the treatments

3.4.3 Analyses of flavonol concentration and composition via HPLC

To verify the flavonol responses following the defoliation treatments, samples collected on JD 350 (30 days after FBD), JD 13, and JD 47 were analyzed for the flavonols quercetin, myricetin and kaempferol. As previously reported for Sauvignon Blanc (Mattivi et al., 2006) myricetin was not detectable nor in C, neither in FBD and VD: Total concentration of flavonols, as sum of quercetin and kaempferol, reached its maximum at harvest, ranging from 3.8 mg/kg berry in C to around 9 mg/kg berry in FBD. VD did not differentiate from C and FBD in terms of total flavonol concentrations. Quercetin was the most abundant flavonol analyzed in all samples, regardless the treatment and the ripening phase, anda statistically significant difference was observed in quercetin between control and FBD in all sampling dates, included the first, in which it seems that in C the biosynthesis of quercetin was not yet started (Fig. 5*A*).

Figure 5 – Concentration of quercetin and kaempferol (mg/Kg berry) on JD 350, 13 and 47 in Sauvignon Blanc subjected to defoliation at full bloom (FBD) and veraison (VD) and in control vines (C) in 2015/2016 cycle.

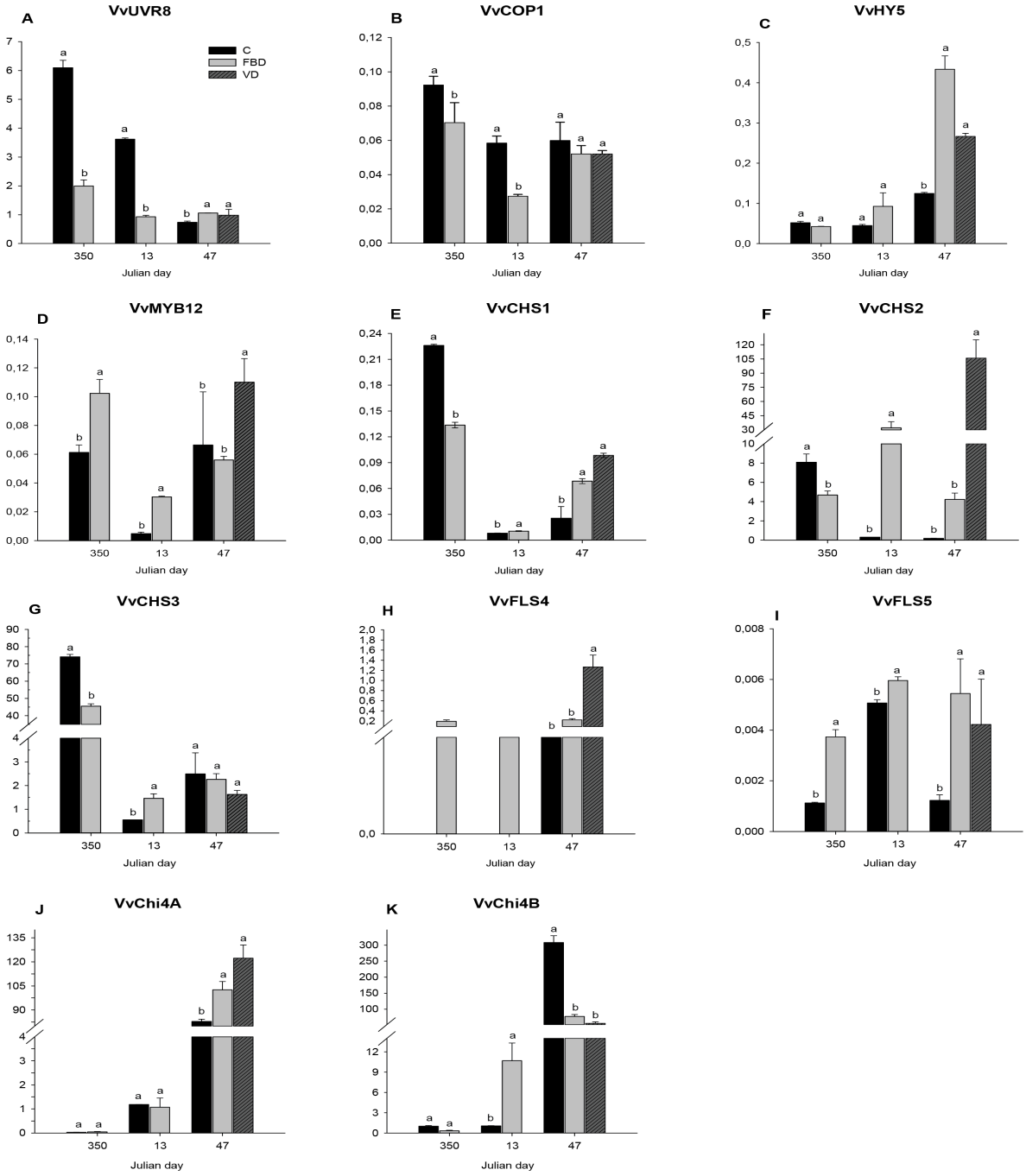


Differently from what observed for quercetin, kaempferol was detected in all treatments only on JD 47 reaching a peak in FBD treatment, with a concentration twofold higher than VD and C.

3.4.4 Gene expression on genes involved in flavonol biosynthesis, UV regulation and perception in the berry.

In order to assess the effect of the defoliation treatments on flavonol biosynthesis, we analyze the expression of the genes involved in the regulation and biosynthesis of flavonol compounds in C, FBD and VD berry skin. Interestingly, two of the three VvCHS gene isoforms (VvCHS1 and VvCHS3), shared in C a quite similar trend of transcription in the different developmental stages, with a maximum detected on JD 350, followed by a strong decrease on JD 13 and a further increase in the third sampling date. On the other hand, after the peak of expression in the first day, CHS2 shows in C only very low levels of expression during the rest of ripening (Fig. 6F).

Figure 6 – The effects of leaf removal on transcript abundance of (A) VvCHS1, (B) VvCHS2, (C) VvCHS3, (D) VvFLS4, (E) VvFLS5, (F) VvMYB12, (G) VvUVR8, (H) VvCOP1, (I) VvHY5, (J) VvChi4a, (K) VvChi4b on JD 350, 13 and 47 in Sauvignon Blanc subjected to defoliation at full bloom (FBD) and veraison (VD) and in control vines (C) in 2015/2016 cycle.



Considering the defoliation treatments, all CHS isoforms showed before a decrease on JD 350 and then an increase in expression on JD 13 in FBD in comparison to C. At JD 47, when also VD treatment was taken into account, no differences were detected between C, FBD and VD in the expression of VvCHS3, while VvCHS1 was induced in both defoliation treatments in comparison to C and VvCHS2 only in VD (Fig. 6E,F).

The key genes of the biosynthetic pathway of flavonols are flavonol synhtases (FLS) among which we investigated FLS4 and FLS5, which has been shown the most related to light responses (Fujita et al., 2006; Liu et al., 2014; Loyola et al., 2016). VvFLS4 expression was strongly related to sun exposure, mainly on JD 350 and JD 13, when a peak of expression was detected in FBD, while it was completely suppressed in C (Fig.6H). On JD 47, VD treatment had a significantly higher expression of VvFLS4 than FBD treatment and C. VvFLS5 also showed a high light-induced response being always more induced in both defoliation treatments, although its expression was also observed in C at all stages (Fig.6I).

In grapevine, among the MYB transcription factors, only VvMYB12 showed a significant response in relation to UV-B-induced flavonols biosynthesis (Liu et al., 2014). In the present study, VvMYB12 showed a significant response to sun exposure, with significantly higher transcription on JD 350 in the FBD treatment and on JD 47 in the VD treatment (Fig.6D).

In plants, UV radiation is perceived by the specific photoreceptor UVR8, which initiates signaling through transcription factors involved in the flavonoid biosynthesis pathway (Brown et al., 2005). Following UV-radiation perception, the monomerization of UVR8 occurs and its monomeric form interacts with COP1, leading to the transcriptional activation of HY5 (Cloix et al., 2012), which can promote the flavonoids biosynthesis by binding to MYB transcription factors and also binding directly to flavonoid biosynthetic genes (Stracke et al., 2010).

In the present study, UVR8 and COP1 gene expression was observed in all berry developmental stages, but no significant UV specific response was observed, except for UVR8 at JD 47, where an induction of expression was detected in both defoliation treatments in comparison to C. On the other hand, HY5 was present at all berry development stages but its expression was significantly higher at on JD in the VD treatment (Fig.6C).

To investigate genes involved in the high-UV pathway, we tested PR genes (VvChi4A, 4B) reported by Liu et al., 2015 to be involved in UV via high fluence in controlled environment experiments with Sauvignon Blanc. The highest levels of VvChi4A/4B expression were observed post-veraison, however, with completely

opposite patterns (Fig.6J,K). The expression of VvChi4A showed to be influenced by sun exposure, with significantly higher levels in defoliation treatments. In contrast, VvChi4B showed to be influenced by sun exposure on JD 350, but with significantly higher levels in C on JD 47.

3.5 DISCUSSION

Several studies have shown that higher UV-radiation in high altitude vineyards of South America directly affect the phenolic compounds and antioxidant capacity of grape berry (Berli et al. 2008, 2011, 2014, 2015; Gil et al., 2013; Alonso et al., 2016). However, the majority of the recent studies aiming to investigate the effect of UV-radiation on the grapevine composition were conducted in mid altitudes regions (up to 400 meters), (Joubert et al., 2016; Del-Castillo-Alonso et al., 2016; Young et al., 2016; Šuklje et al., 2014; Carbonell-Bejerano et al. 2014) or in controlled environment (Loyola et al., 2016; Liu et al. 2014; Martínez-Luscher et al., 2014). Moreover, most of the researches conducted in the highlands of South America have been carried out under high temperatures and water deficit conditions.

In addition to high radiation, the altitude vineyards located in Santa Catarina (Brazil) are quite different from the environments taken until now into account, being characterized by a high frequency of rainy days during ripening, which may negatively affect berry ripening (Gardin et al., 2012; Brighenti et al., 2017), and by mild temperatures, that instead can enhance the accumulation of flavonoids, since these compounds are favored by low (< 30 °C) than by high temperatures (Pastore et al., 2017). To our knowledge, this is the first study conducted under naturally high UV-radiation rates in cool climate grapevine production regions of South America aiming to unravel the effects of such particular environmental conditions on flavonol accumulation and on the molecular mechanisms that underlie their biosynthesis and regulation and the perception of UV radiation in Sauvignon Blanc. In the present research, no effect following both defoliation treatments was observed on berry weight and on technological ripening (TSS, pH, and acidity). This agrees with data presented by Sivilotti et al. (2017) and Mosetti et al. (2016), which defoliation did not modify these parameters on Sauvignon Blanc,

This study highlights the effect of the timing of defoliation in affecting the biosynthesis of flavonols under high altitude environments. Differently from what observed under low altitude on white and red grapevine cultivars (Friedel et al., 2016; Pastore et al., 2013), increase in light exposure at full bloom stage was effective in enhancing total and individual flavonols accumulation, while the effect following defoliation at veraison showed no differences from shaded plants.

Gene expression was really affected by defoliation treatments. Considering the genes involved in the biosynthetic steps of flavonoid and flavonols biosynthesis, it is interesting to note that FBD enhanced the expression of the different VvCHS isoforms, which produce substrates for the biosynthesis of flavonols two months later (JD 13) the defoliation treatment. On the other hand VvFLS and VvMYB12 were immediately induced after the removal of the leaves and well correlated to each other and with the flavonol accumulation too. Hyper-accumulation of flavonoids is a primary defense response mechanism of plants to protect from UV irradiation (Li et al., 1993), therefore, the differential up-regulation of the VvCHS isoforms can act as a regulatory mechanism to continuously produce substrates for flavonol biosynthesis following long-term high UV-exposure (Zhang et al., 2017). Different is the situation observed in VD, where only the up-regulation of VvCHS2 was detected and probably a lower amount of flavonol precursors were produced.

VvCHS2 was up-regulated under stressful situations, as reported by Soubeyrand et al., (2014) in vines without nitrogen fertilization as compared to high supplementation and by Castellarin et al., (2007) in water-stressed vines. VvCHS2, VvMYB12, and FLS4 transcripts are more abundant in VD treatment and may be related to the sun exposure of the berries at this specific stage of development, that matches the highest UV-radiation rates. VvCHS2 may be directly regulated by a VvMYB transcription factor in response to light treatment (Harris et al., 2013), and rapid responses of FLS4 and MYB was reported previously (Loyola et al., 2016; Czemmel et al., 2009; Matus et al., 2009). The identity of FLS5 in relation to the accumulation of flavonols is unclear, the most highly ranked case is FLS4. Nevertheless, FLS5 has also been related to flavonol synthesis (Kobayashi et al., 2010; Fujita et al., 2006; Villano et al., 2017; Liu et al., 2014). In fact, increased quercetin and kaempferol content in

response to UV-B has been reported in Sauvignon grapevine (Martin et al., 2016; Gregan et al., 2012; Liu, Gregan, Winefield, & Jordan, 2015) nevertheless, the gene regulation pattern found in this study seems to be influenced by the sunlight exposure from early stages.

The effect of high solar radiation and mild temperatures on flavonols profile well documented for the accumulation of flavonoids (Pastore et al., 2017; Del-Castillo-Alonso et al., 2016; Cohen et al., 2008, 2012; Azuma et al., 2012). Furthermore, unlike the studies carried out by Berli et al., 2011 in Malbec grapes at high altitudes, flavonols accumulation in Sauvignon Blanc grapes took place without reducing berry growth, as well the leaf removal has a minimal effect on berry maturation.

In this last measured stage, genes related to PR proteins (VvChi4A and VvChi4B) showed opposite transcription patterns, but present at all developmental stages. Constitutive expression of VvChi4 appears to be induced at high levels in grapes during ripening (Shukla and Jalil, 2014). However, as reported by Liu et al., 2014, in the present study, VvChi4 seems to be enhanced by high-UV exposure.

The current research is the first step to elucidating the state of acclimatization os Sauvignon Blanc under the specific combination of environmental conditions in cool climate regions of South America. Defense responses to exposure to high UV-radiation result in adjustments in gene expression, which can be ROS-mediated signaling (Noctor et al., 2006). Quercetin and kaempferol are very competent ROS-scavengers, although quercetin is most efficient (Berli et al., 2010). UV-induced increase in the quercetin:kaempferol ratio seems to be closely related to ROS scavenging activity (Agati e Tattini, 2010; Fini et al., 2011; Hideg et al., 2013). Although it has been reported that the biosynthesis of flavonols in Sauvignon Blanc is increased through low fluence UVB response pathway (Tian et al., 2015), we observed a similar behavior under UV-radiation of high altitude.

3.6 CONCLUSION

Leaf removal had a determining impact on flavonols concentration and phenolic profile under high altitude regions. Exposure time has been shown important, since, early defoliation contributed to higher flavonol accumulation. Gene expression

related to both high and low UV-fluence was observed. UV radiation seems to be an important factor in the Sauvignon Blanc wine's typicality in São Joaquim. This work is a pioneer in gene expression investigation during berry development in high altitude regions of Santa Catarina, therefore, further studies should be conducted to investigate the ROS production and other compounds related to high UV-fluence.

REFERENCES

ALCOFORADO, I. G. A construção de mercados certificados - uma abordagem neo institucionalista. In: **CONGRESSO BRASILEIRO DE ECONOMIA E SOCIOLOGIA RURAL**, 2002, Passo Fundo, RS. Anais. Passo Fundo: SOBER, 2002.

Alonso, R., Berli, F.J., Fontana, A., Piccoli P., Bottini, R. 2016. Malbec grape (Vitis vinifera L.) responses to the environment: Berry phenolics as influenced by solar UV-B, water deficit and sprayed abscisic acid. Plant Physiology and Biochemistry 109, 84–0.

ALVES, F. **Vinícolas de altitude investem para fomentar o enoturismo em Santa Catarina.** Disponível em: <http://ndonline.com.br/florianopolis/noticias/245828-vinicolas-de-altitude-investem-para-fomentar-o-enoturismo-em-santa-catarina.html.> Acesso em: 02 de agosto de 2015.

Azuma, A., Yakushiji, H., Koshita, Y., Kobayashi, S. 2012. Flavonoid biosynthesis-related genes in grape 481 skin are differentially regulated by temperature and light conditions. Planta 236, 1067– 080.

Baillod, M., Baggiollini, M. 1993. Les stades repères de la vigne. Revue Suisse de Viticulture, Arboriculture, Horticulture. 25, 7–9.

BENKO, G. A recomposição dos espaços. **Revista Internacional de Desenvolvimento Local**, v.1, n. 2, p.7-12, mar. 2001.

Berli, F., D'Angelo, J., Cavagnaro, B., Bottini, R., Wuilloud, R., Silva, M.F. 2008. Phenolic composition in grape (Vitis vinifera L. Cv. Malbec) ripened with different solar UV-B radiation levels by capillary zone electrophoresis. Journal of Agricultural and Food Chemistry. 56, 2892–2898.

Berli, F.J., Moreno, D., Piccoli, P., Hespanhol-Viana, L., Silva, M.F., Bressan-Smith, R., Cavagnaro, J.B., Bottini, R. 2010. Abscisic acid is involved in the response of grape (Vitis vinifera L.) cv. Malbec leaf tissues to ultraviolet-B radiation by enhancing ultraviolet-absorbing compounds, antioxidant enzymes and membrane sterols. Plant Cell and Environment 33, 1–10.

Berli, F.J., Fanzone, M., Piccoli, P., Bottini, R., 2011. Solar UV-B and ABA are involved in phenol metabolism of Vitis vinifera L. increasing biosynthesis of berry skin polyphenols. Journal of Agricultural and Food Chemistry 59, 4874–4883.

Berli, F.J., Alonso, R., Bressan-Smith, R. & Bottini, R., 2013. UV-B impairs growth and gas exchange in grapevines grown in high altitude. Physiol. Plant. 149, 127–140.

Berli, F.J., Alonso, R., Beltrano, J., Bottini, R. 2015. High-altitude solar UV-B and abscisic acid sprays increase grape berry antioxidant capacity. Am J Enol Vitic. 66(1), 65–72.

Bertamini, M., Nedunchezhian, N.: Photosynthetic functioning of individual grapevine leaves (*Vitis vinifera* L. cv. Pinot noir) during ontogeny in the field. Vitis. **42**:13-17, 2003.

Bešlić, Z., Todić, S., Marković, N. *et al.* Influence of early basal leaf removal on yeald components and must quality on cv. Sauvignon Blanc. Annals of the University of Craiova - Agriculture, Montanology. **XLVI**, 2016.

Boese, S.R., Huner, P.A.: Developmental History Affects the Susceptibility of Spinach Leaves to in Vivo Low Temperature Photoinhibition. Plant Physiology. **99**:1141-1145,1992.

Bogs, J., Downey, M.O., Harvey, J.S., Ashton, A.R., Tanner, G.J., Robinson, S.P., 2005. Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. Plant Physiol. 139, 652–663.

BONIN, V., BRIGHENTI, E. Aspectos climáticos e produção de vinhos finos na serra catarinense. In: **ENCONTRO NACIONAL SOBRE FRUTICULTURA DE CLIMA TEMPERADO**, Fraiburgo. Anais. Epagri, 368 p., 2006.

Borghezan, M., Gavioli, O., Pit, F.A. *et al*. Modelos matemáticos para a estimativa da área foliar de variedades de videira à campo (*Vitis vinifera* L.). Cienc. Tec. Vitivinic. **25**: 1-7, 2010.

BORGHEZAN, M.; GAVIOLI, O.; PIT, F.A.; SILVA, A.L. da. Comportamento vegetativo e produtivo da videira e composição da uva em São Joaquim, Santa Catarina. **Pesquisa Agropecuária Brasileira**, v.46, p.398-405, 2011.

Borghezan, M., Villar, L., da Silva, T.C. *et al*. Phenology and Vegetative Growth in a New Production Region of Grapevines: Case Study in São Joaquim, Santa Catarina, Southern Brazil. Open Journal of Ecology. **4**:321-335, 2014.

Borghezan, M., Pit, F.A., Gavioli, O. *et al*. Canopy management on ripening of 'Sauvignon Blanc' grapevine grown in São Joaquim, Brazil. Acta Hortic. **1188**. DOI 10.17660/ActaHortic.2017.1188.11, 2017.

BRESCIA, M. A., CALDAROLA, V., DEGIGLIO, A., BENEDETTI, D., FANIZZI, F. P., SACCO, A. Characterization of the geographical origin of Italian wines based on traditional and nuclear magnetic resonance spectrometric determinations. **Anal. Chim. Acta**, 458, 177- 186, 2002.

Brighenti, A. F., Brighenti, E., Bonin, V., Rufato, L. 2013. Caracterização fenológica e exigência térmica de diferentes variedades de uvas viníferas em São Joaquim, Santa Catarina – Brasil. Ciência Rural. 43, 1162–1167.

Brighenti, A.F., Silva, A.L., Brighenti, E. *et al*. Desempenho vitícola de variedades autóctones italianas em condição de elevada altitude no Sul do Brasil. Pesquisa Agropecu. Bras. **49**: 465-474 , 2014.

BRIGHENTI, A.F., BRIGHENTI, E., PASA, M. Vitivinicultura de altitude: realidade e perspectivas, 2016.

Brown, B.A., Cloix, C., Jiang, G.H., Kaiserli, E., Herzyk, P., Kliebenstein, D.J., Jenkins G.I. 2005. A UV-B-specific signaling component orchestrates plant UV protection Proc. Natl Acad. Sci. USA. 102, 18225–18230.

Burin, V. M., da Silva, A. L., Malinovski, L. I. *et al*. Characterization and multivariate classification of grapes and wines of two Cabernet Sauvignon clones. Pesquisa Agropecuária Brasileira. **46**:474-481, 2011.

Carbonell-Bejerano, P., Diago, M.P., Martínez-Abaigar, J., Martínez-Zapater, J.M.,Tardáguila, J., Núñez-Olivera,E.2014. Solar ultraviolet radiation is necessary to enhance grapevine fruit ripening transcriptional and phenolic responses. BMC Plant Biol. 14,183.

CARVALHO JUNIOR, L.C.C., MOSSINI, M.A. Cadeia produtiva de uvas e vinhos de Santa Catarina: uma análise das transformaço es entre os seus segmentos. Revista Textos de Economia 14 (1), 2011.

Castellarin, S.D., Matthews, M.A., Gaspero, G.D., Gambetta, G.A., 2007. Water deficitsaccelerate ripening and induce changes in gene expression regulating flavonoidbiosynthesis in grape berries. Planta 227, 101–112.

CHAVES, M.M., SANTOS, T.P., SOUZA, C.R., ORTUNO, M.F., RODRIGUES, M.L., LOPES, C.M., MAROCO, J.P., PEREIRA, J.S. Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. **Annals of applied biology,** v. 150, n. 2, p. 237-252, 2007.

Cloix, C., Kaiserli, E., Heilmann, M., Baxter, K.J., Brown, B.A., O'Hara, A., Smith, B.O., Christie, J.M., Jenkins, G.I. 2012. C-terminal region of the UV-B photoreceptor UVR8 initiates signaling through interaction with the COP1 protein. Proc Natl Acad Sci USA. 109, 16366–70.

Cohen, S.D., Tarara, J.M., Kennedy, J.A. 2008. Assessing the impact of temperature on grape phenolic metabolism. Analytica Chimica Acta. 621, 57–67.

Cohen, S. D., Tarara, J. M., Gambetta, G. A., Matthews, M. A., Kennedy, J. A. 2012. Impact of diurnal temperature variation on grape berry development, proanthocyanidin accumulation, and the expression of flavonoid pathway genes. Journal of experimental botany. 63, 2655–2665.

Colas, S., Afoufa-Bastien, D., Jacquens, L., Clément, C., Baillieul, F., Mazeyrat-Gourbeyre, F., Monti-Dedieu L. 2012. Expression and In Situ localization of two major PR proteins of grapevine berries during development and after UV-C exposition. PLoS ONE. 7, e43681.

Czemmel, S., Stracke, R., Weisshaar, B., Cordon, N., Harris, N.N., Walker, A.R., Robinson, S.P., Bogs, J. 2009. The grapevine R2R3-MYB transcription factor

VvMYBF1 regulates Xavonol synthesis in developing grape berries. Plant Physiol. 151, 1513–1530.

Del-Castillo-Alonso, M-A., Diago, M.P., Tomás-Las-Heras, R., Monforte, L., Soriano, G., Martínez-Abaigaa, J., Núñez-Olivera, E. 2016. Effects of ambient solar UV radiation on grapevine leaf physiology and berry phenolic composition along one entire season under Mediterranean field conditions. Plant Physiology and Biochemistry. 109, 374–386.

DIAGO, M. P., TODA, M.T., PONI, S., TARDAGUILA, J. Early leaf removal for optimizing yield components, grape and wine composition in Tempranillo (*Vitis vinifera* L.). In: **16th International GiESCO Symposium**, 2009.

Drappier, J., Thibon, C., Rabot, A., Geny-Denis, L. 2017. Relationship between wine composition and temperature: Impact on Bordeaux wine typicity in the context of global warming-Review. Crit Rev Food Sci Nutr. 24, 1–17.

EEA - EUROPEAN ENVIRONMENT AGENCY Annual report 2013 and EMAS environmental statement 2013. Disponível em: < https://www.eea.europa.eu//publications/eea-annual-report-2013>. Acesso em fevereiro 2018.

EMBRAPA – Empresa Brasileira de Pesquisa Agropecuária. Solos do Estado de Santa Catarina. Rio de Janeiro: Embrapa Solos. Boletim de Pesquisa e Desenvolvimento. 721, 2004.

Falcão, L. D., de Revel, G., Perello, M. C. *et al*. A survey of seasonal temperatures and vineyard altitude influences on 2-methoxy-3-isobutylpyrazine, C13-norisprenoids, and the sensory profile of Brazilian Cabernet Sauvignon wines. Journal of Agricultural and Food Chemistry. **55**:3605-3612, 2007.

FAO - Food and Agriculture Organization of the United Nations. Disponível em: <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor>. Acesso em fevereiro, 2018.

Fini, A. Brunetti, C., Ferdinando, M.D., Ferrini, F., Tattini, M. 2011. Stress-induced flavonoid biosynthesis and the antioxidant machinery of plants. Plant Signal. Behav. 6, 709–711.

Fujita, A., Goto-Yamamoto, N., Aramaki, I., Hashizume, K. 2006. Organspecific transcription of putative flavonol synthase genes of grapevine and effects of plant hormones and shading on flavonol biosynthesis in grape berry skins. Biosci Biotechnol Biochem. 70, 632–638.

Genty, B., Briantais, J. M., Baker, N. R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochimica et Biophysica Acta. **990**:87-92, 1989.

Gil, M., Esteruelas, M., González, E., Kontoudakis, N., Jiménez, J., Fort, F., Canals, J.M., Hermosín-Gutiérrez, I., Zamora, F., 2013. Effect of two different treatments for reducing grape yield in Vitis vinifera cv Syrah on wine composition and quality: berry thinning versus cluster thinning. J. Agric. Food Chem. 61, 4968–4978.

Godfrey, D., Able, A.J., Dry, I.B. 2007. Induction of a Grapevine Germin-Like Protein (VvGLP3) Gene Is Closely Linked to the Site of Erysiphe necator Infection: A Possible Role in Defense?. Mol Plant Microbe Interact. 20(9), 1112–25.

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

Greven, M. M., Neal, S. M., Tustin, D. S. *et al*. Effect of Postharvest Defoliation on Carbon and Nitrogen Resources of High-Yielding Sauvignon Blanc Grapevines. Am J Enol Vitic. **67**:315-326, 2016.

Gris, E. F., Burin, V; M., Brighenti, E. *et al*. 2010. Phenology and ripening of *Vitis vinifera* L. grape varieties in São Joaquim, southern Brazil: a new South American wine growing region. Ciencia e Investigación Agraria. **37**:61-75, 2010.

GRIS, E.F., MATTIVI, F., FERREIRA, E.A., VRHOVSEK, U., FILHO, D.W., PEDROSA, R.C., BORDIGNON-LUIZ, M.T. Stilbenes and tyrosol as target compounds in the assessment of antioxidant and hypolipidemic activity of Vitis vinifera red wines from Southern Brazil**. J Agric Food Chem** 59:7954–7961, 2011.

Hannah, L., Roehrdanz, P.R., Ikegami, M., Shepard, A.V., Shaw, M.R., Tabor, G., Zhi, L., Marquet, P., Hijmans, R.J. 2013. Climate change, wine, and conservation. Proc Natl Acad Sci USA. 110, 6907–6912.

Harris, N.N., Luczo, J.M., Robinson, S.P., Walker, A.R. 2013. Transcriptional regulation of the three grapevine chalcone synthase genes and their role in flavonoid synthesis in Shiraz. Aust J Grape Wine Res. 19, 221–229.

Hendrickson L., Ball, M. C., Osmond, C. B. *et al*. Assessment of photoprotection mechanisms of grapevine at low temperature. Functional Plant Biology. **30**: 631-642, 2003.

Hendrickson L, Ball, M. C., Wood, J. T. *et al*. Low temperature effects on photosynthesis and growth of grapevine. Plant Cell Environ. **27**:795–809, 2004.

Henry-Kirk, R.A., Plunkett, B., Hall, M., McGhie, T., Allan, A.C., Wargent, J.J., Espley, R.V. 2018. Solar UV light regulates flavonoid metabolism in apple (Malus x domestica). Plant Cell Environ. 41(3), 675–688.

Hideg, E., Jansen, M.A.K., Strid, A., 2013. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? Trends Plant Sci. 18, 107–115.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Levantamento Sistemático da Produção Agrícola: pesquisa mensal de previsão e acompanhamento das safras agrícolas.** Disponível em: < http://seriesestatisticas.ibge. gov.br/>. Acesso em: fevereiro de 2018.

INTRIERI, C., FILIPPETTI, I., ALLEGRO, G., CENTINARI, M., PONI, S. Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (*Vitis vinifera* L.). **Aust. J. Grape Wine Res.**, v. 14, p. 25-32, 2008.

IPCC - INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE. Climate Change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: **Cambridge University Press**, 996, 2007.

Jacobs, A. K., Dry, I. B., and Robinson, S. P. 1999. Induction of different pathogenesis-related cDNAs in grapevine infected with powdery mildew and treated with ethephon. Plant Pathol. 48, 325–336.

JONES, G. Climate and terroir: impacts of climate variability and change on wine. In: MACQUEEN, R. W., MEINERT, L. D. (Eds.). Fine wine and terroir: the geosciences perspective. St. John‟s, Newfoundland: Geoscience Canada Reprint Series Number 9, **Geological Association of Canada**. 247 p., 2006.

Joubert, C., Young, P. R., Eyéghé-Bickong, H. A., and 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, 786.

Keller, M., Tarara, J. M. Warm spring temperatures induce persistent season-long changes in shoot development in grapevines. Ann. Bot. **106**:131-141, 2010.

Keller, M., Mills, L. J., Harbertson, J. F. Rootstock effects on deficit-irrigated winegrapes in a dry climate: Vigor, yield formation, and fruit ripening. Am. J. Enol. Vitic. **63**: 29-39, 2012.

Kliewer, W.M., Dokoozlian, N. K. Leaf area/crop weight ratios of grapevines: influence on fruit composition and wine quality. American Journal of Enology and Viticulture. **56**: 170-181, 2005.
KENNEDY, J. A., MATTHEWS, M. A., WATERHOUSE, A. L. Effect of maturity and

vine water status on grape skin and wine flavonoids. **American Journal of Enology and Viticulture**, v. 53, p.268–274, 2002.

Kobayashi, H., Suzuki, Y., Ajimura, K., Konno, T., Suzuki, S. 2010. Characterization of phenolic compounds biosynthesized in pink-colored skin of Japanese indigenous Vitis vinifera cv. Koshu grape. Plant Biotechnology Reports. 5, 79–88.

Kozina, B. Influence of basal leaf removal on the chemical composition of Sauvignon Blanc and Riesling wines. Journal of Food, Agriculture & Environment. **6**:28-33, 2008.

Lee, H-Y., Chow, W. S., Hong, Y-N. Photoinactivation of photosystem II in leaves of Capsicum annuum. Physiol Plant.**105**: 377-384, 1999.

Li, J., Ou-Lee, T.M., Raba, R., Amundson, R.G., Last, R.L. 1993. Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. The Plant Cell. 5, 171–179.

LI, Z., HE, Y., NA, W., SONG, L., ZHANG, W., NORM, C., CAO, W., WILFRED, H. T., LIU, H., WANG, S., DU, J., XIN, H., CHANG, LI. Climate and glacier change in southwestern China during the past several decades. **Environ. Res. Lett**, **6** 045404, 2011.

Liu, L., Gregan, S., Winefield, C., Jordan, B. 2014. From UVR8 to flavonol synthase: UV-B-induced gene expression in Sauvignon Blanc grape berry. Plant Cell Environ. 38, 905–919.

Loyola, R., Herrera, D., Mas, A., Wong, D.C.J., Höll, J., Cavallini, E., Amato, A., Azuma, A., Ziegler, T., Aquea, F., Castellarin, S.D., Bogs, J., Tornielli, G.B., Peña-Neira, A., Czemmel, S., Alcalde, J.A., Matus, J.T., Arce-Johnson, P. 2016. The photomorphogenic factors UV-B RECEPTOR 1, ELONGATED HYPOCOTYL 5, and HY5 HOMOLOGUE are part of the UV-B signalling pathway in grapevine and mediate flavonol accumulation in response to the environment. Journal of Experimental Botany. 67, 5429–5445.

Malinovski, L. I., Welter, L. J., Brighenti, A. F. *et al*. Highlands of Santa Catarina/Brazil: A region with high potential for wine production. Acta Hortic. **931**: 433–439, 2012.

Marcon Filho, J. L. Hipólito, J. S., De Macedo, T. A. *et al*. Raleio de cachos sobre o potencial enológico da uva 'Cabernet Franc' em duas safras. Ciencia Rural, Santa Maria. **45**: 2150-2156, 2015.

Martin, D., Grose, C., Fedrizzi, B., Stuart, L., Albright, A., McLachlan, A. 2016. Grape cluster microclimate influences the aroma composition of Sauvignon Blanc wine. 210, 640–647.

Martínez-Lüscher, J., Sánchez-Díaz, M., Delrot, S., Aguirreolea, J., Pascual, I., Gomès, E. 2014. Ultraviolet-B Radiation and Water Deficit Interact to Alter Flavonol

and Anthocyanin Profiles in Grapevine Berries through Transcriptomic Regulation. Plant and Cell Physiology. 55, 1925–1936.

Marx, W., Haunschild, R., Bornmann, L. 2017. Climate change and viticulture—a quantitative analysis of a highly dynamic research field. VITIS Journal of Grapevine Research. 56, 35–43.

Maxwell, K., Johnson, G.N. Chlorophyll fluorescence−a practical guide. Journal of Experimental Botany. **51**: 659-680, 2000.

Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., Velasco, R. 2006. Metabolite profiling of grape: flavonols and anthocyanins. J Agric Food Chem.54, 7692–7702.

Matus, J.T., Loyola, R., Veja, A., Peña-Neira, A., Bordeu, E., Arce-Johnson, P., Alcalde, J.A. 2009. Post-veraison sunlight exposure induces MYB-mediated transcriptional regulation of anthocyanin and flavonol synthesis in berry skins of Vitis vinifera. J Exp Bot. 60, 853–867.

Medrano, H., Tomas, M., Martorell, S. *et al*. From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target. Crop J. **3**: 220-228, 2015.

MELLO, L.M.R.de. Panorama da vitivinicultura brasileira em 2014. **Campo & Negócios**, 12 abril 2015. Disponível em < http://www.revistacampoenegocios.com.br>. Acesso em 15 ago. 2015.

Montero, R., Mundy, D., Albright, A. *et al.* Effects of Grapevine Leafroll associated Virus 3 (GLRaV-3) and duration of infection on fruit composition and wine chemical profile of *Vitis vinifera* L. cv. Sauvignon Blanc. Food Chemistry. **197**: 1177-1183, 2016.

Myburgh, P.A. Water status, vegetative growth and yield responses of *Vitis vinifera* L. cvs. Sauvignon Blanc and Chenin Blanc to timing of irrigation during berry ripening in the Coastal Region of South Africa. S. Afr. J. Enol. Vitic. **26**: 59–67, 2005.

Noctor, G. 2006. Metabolic signalling in defence and stress: the central roles of soluble redox couples. Plant Cell Environ. 29, 409–425.

OIV - International Organisation of Vine and Wine. Disponível em: <http://www.oiv.int/oiv/cms/index>, acesso em julho de 2015.

OIV - International Organisation of Vine and Wine - Statistical Report on World Vitiviniculture 2018. Disponível em: < http://www.oiv.int/public/medias/6371/oiv-statistical-report-on-world-vitiviniculture-2018.pdf>, acesso em dezembro de 2018.

PALLIOTTI, A., GATTI, M., PONI, S. Early leaf removal to improve vineyard efficiency: gas exchange, source-to-sink balance, and reserve storage responses.Am. J. Enol. Vitic. 62: 219–228, 2011.

Parker, A.K., Hofmann, R.W., van Leeuwen, C. et al. Leaf area to fruit weight ratio determines the time of veraison in Sauvignon Blanc and Pinot Noir grapevines. Australian Journal of Grape and Wine Research. **20**:422-731, 2014.

Parker, A.K., Hofmann, R.W., van Leeuwen, C. et al. Manipulating the leaf area to fruit weight ratio alters the synchrony of soluble solids accumulation and titratable acidity of grapevines: implications for modelling fruit development Australian Journal of Grape and Wine Research. **21**:266-276, 2015.

Pastore, C., Zenoni, S., Tornielli, G. B., Allegro, G., Dal Santo, S., Valentini, G., Intrieri, C., Pezzotti, M., Filippetti, I. 2011. Increasing the source/sink ratio in Vitis vinifera (cv Sangiovese) induces extensive transcriptome reprogramming and modifies berry ripening. BMC Genomics, Nova York.12, 631.

Pastore, C., Allegro, G., Valentini, G., Muzzi, E., Filippetti, I. 2017. Anthocyanin and flavonol composition response to veraison leafremoval on Cabernet Sauvignon, Nero d'Avola, Raboso Piave andSangiovese Vitis vinifera L. cultivars. 218, 147-155.

Petrie, P.R., Trought, M.C.T. Howell, G.S. Influence of leaf ageing, leaf area and crop load on photosynthesis, stomatal conductance and senescence of grapevine (*Vitis vinifera* L. cv. Pinot Noir) leaves. Vitis **39**: 31–36, 2000.

Petrie, P.R., Trought, M.C.T., Howell, G.S. *et al*.The effect of leaf removal and canopy height on whole-vine gas exchange and fruit development of *Vitis vinifera* L. Sauvignon Blanc. Functional Plant Biology. **30**:711–717, 2003.

Pinto, F., Berti, M., Olivares, D. *et al*. Leaf development, temperature and light stress control of the expression of early light-inducible proteins (ELIPs) in *Vitis vinifera* L. Environmental and Experimental Botany.**72**:278-283, 2011.

Poni, S., Intrieri, C., Silvestroni, O. Interactions of leaf age, fruiting, and exogenous cytokinins in Sangiovese grapevines under non-irrigated conditions. I. Gas exchange.Am. J. Enol.Vitic. **45**:71-78, 1994.

PONI, S., CASALINI, L., BERNIZZONI, F., CIVARDI, S AND C. INTIERI. Effects of early defoliation on shoot photosyntesis, yield components, and grape composition. **Am. J. Enol. Vitic.**, v. 57, p. 397-407, 2006

PONI, S., BERNIZZONI, F., CIVARDI, S., LIBELLI, N. Effects of prebloom leaf removal on growth of berry tissue and must composition in two red Vitis vinifera L. cultivars. **Aust. J. Grape Wine Res.**, v.15, p.185-193, 2008.

PRADO, R. D. A., YUSTE-ROJAS, M., SORT, X., ANDRÉS-LACUEVA, C., TORRES, M., & LAMUELARAVENTÓS, R. M. Effect of soil type on wines produced from Vitis vinifera L. Cv. Grenache in commercial vineyards. **Journal of Agricultural and Food Chemistry**, v. 55, p. 779–786, 2007.

Ramakers, C., Ruijter, J. M., Deprez, R. H. L., and Moorman, A. F. M. 2003. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci. Lett. 339, 62–66.

Sawicki, M., Jeanson, E., Celiz, V. *et al*. Adaptation of grapevine flowers to cold involves different mechanisms depending on stress intensity. PLoS ONE: **7**(10) e46976, 2012.

Schultz, H. Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. Australian Journal of Grape and Wine Research. **6**:2-12, 2000.

Shaw, T.B. 2016. Climate change and the evolution of the Ontario cool climate wine regions in Canada. Journal of Wine Research. 28:1, 13–45.

SHELLIE, K.; GLENN, D.M. Wine Grape Response to Foliar Particle Film under Differing Levels of Preveraison Water Stress. **HortScience,** v. 43, n. 5, p. 1392-1397, 2008.

Shukla, M., Jalil S.U. 2014 Endochitinase: engineered resistance against fungal plant pathogens. 7(3) 137–142.

Simon, P. 2003. Q-Gene: processing quantitative real-time RT–PCR data. Bioinformatics 19, 1439–1440.

SMART, R., ROBINSON, M. **Sunlight into wine: a hand book for winegrape canopy management**. Winetitles, Adelaide, SA, 88 p, 2008.

Soubeyrand, E., Basteau, C., Hilbert, G., van Leeuwen, C., Delrot, S. Gomès, E. 2014. Nitrogen supply affects anthocyanin biosynthetic and regulatory genes in grapevine cv. CabernetSauvignonberries. Phytochemistry 103, 38–49.

STOCK, M., BADECK, F., GERSTENGARBE, HOPPMANN, D., KARTSCHALL, T.; ÖSTERLE, H., WERNER, P. C., WODISNSKI, M. Perspektiven der Klimaänderung bis 2050 für den Weinbau in Deutschland. In: PIK. R**eport. Potsdam: Institut fuer Klimafolgenforschung**. 132p., 2007.

Stracke, R., Favory, J.J., Gruber, H., Bartelniewoehner, L., Bartels, S., Binkert, M., Funk, M., Weisshaar, B., Ulm, R. 2010. Plant Cell Environ. 33, 88–103.

Šuklje, K., Antalick, G., Coetzee, Z., Schmidtke, L.M., Baša Česnik, H., Brandt, J.,du Toit, W.J., Lisjak, K., Deloire, A. 2014. Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of Vitis vinifera L. cv. Sauvignon Blanc wine. Aust J Grape Wine Res. 20: 223–233

Suzuki, K., Ohmori, Y., Ratel, E. High root temperature blocks both linear and cyclic electron transport in the dark during chilling of the leaves of rice seedlings. Plant Cell Physiology. **52**:1697–1707, 2011.

Tian, B., Harrison, R., Jaspers, M., Morton, J. 2015. Influence of ultraviolet exclusion and of powdery mildew infection on Sauvignon Blanc grape composition and on extraction of pathogenesis-related proteins into juice. 21, 417–424.

TONIETTO, J., CARBONNEAU, A. A multicriteria climatic classification system for grape-growing regions worldwide. **Agricultural and Forest Meteorology**, v.124, p.81-97, 2004.

URHAUSEN, S.; BRIENEN, S.; KAPALA, A.; SIMMER, C. Climatic conditions and their impact on viticulture in the Upper Moselle region. **Climatic Change**, v.109, p.349–37, 2011.

UVIBRA - UNIÃO BRASILEIRA DE VITIVINICULTURA. **Informações de mercado.** Disponível em: <http://www.uvibra.com.br>. Acesso em julho de 2015.

VIANNA, L.F., MASSIGNAN, A.M., PANDOLFO, C., DORTZBACH, D., VIEIRA, V.F. Agronomic and edaphoclimatic characterization high altitude vineyards. **Revista de Ciências Agroveterinárias,** Lages, v.15, n.3, p.215-226, 2016

VIEIRA, H.J., BACK, A.J., SILVA, A.L., PEREIRA, E.S. Comparação da disponibilidade de radiação solar global e fotoperíodo entre as regiões vinícolas de Campo Belo do Sul-sc, Brasil e Pech Rouge, França. **Rev. Bras. Frutic.** *33* (*4*), 1055–1065, 2011.

Villano, C., Rinaldi, A., Lanzillo, C., Moio, L., Tamburrino, A.J., Carputo, D., Frusciante, L., Aversano, R. 2017. Polyphenol content and differential expression of flavonoid biosynthetic pathway genes in berries of Aglianico. Acta Hortic. 1188, 141-148.

Wellburn, A. R. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. **144**: 307–313, 1994.

Wolkovich, E.M., García de Cortázar-Atauri, I., Morales-Castilla, I., Nicholas, K.A., Lacombe, T. 2017. From Pinot to Xinomavro in the world's future wine-growing regions. Nature Climate Change. 8(1), 29–37.

Wurz, D.A., de Bem, B.P., Allebrandt, R., Marcon Filho, J.L., Brighenti, A.F., Outemane, M., Rufato, L., Kretzschmar, A.A. 2017. Timing of leaf removal modifies chemical and phenolic composition of Sauvignon Blanc wine. Bio Web of Conferences. doi: 10.1051/bioconf/20170902027.

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 Physiology. 170 (3), 1235-1254.

Zhang, X., Abrahan, C., Colquhoun, T.A., Liu, C-J. 2017. A Proteolytic Regulator Controlling Chalcone Synthase Stability and Flavonoid Biosynthesis in Arabidopsis. The Plant Cell. tpc.00855.2016; DOI: 10.1105/tpc.16.00855