STOP AND SMELL THE GRAPES: ALTERING CULTIVAR-TYPICAL AROMAS IN 'GEWÜRZTRAMINER' (*VITIS VINIFERA* L.) BERRIES VIA REGULATED DEFICIT IRRIGATION AND CROP LOAD MANAGEMENT

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Abstract

Grape growers use viticultural practices such as deficit irrigation and crop load management via cluster thinning to improve phenolics and aromas in red grapes and wines; however, the impact of these practices on grape terpenes – key aromatics for quality in wines – remains largely unknown. I performed two three-year studies to investigate the effect of deficit irrigation and crop load management strategies on the accumulation of terpenes in Gewürztraminer grapes grown in the Okanagan Valley (BC, Canada). Yield and grape sugars were reduced by deficit irrigation treatments; however, effects were minimal when the deficit was applied late in the season. Applying late deficit allowed to save ~30% of irrigation water compared to standard irrigation. Total free terpenes were not affected by deficit irrigation treatments, but the concentration of key terpenes for the aroma of Gewürztraminer wines, such as geraniol and citronellol, was significantly increased in grapes exposed to water deficit late in the season. This irrigation treatment did not affect the expression of terpene genes, suggesting that the increased concentration of specific terpenes was not regulated at the transcriptional level. Reducing the crop load stimulated sugar accumulation, particularly if this reduction was applied early in the season. Reducing the crop load early during the season also increased the terpene levels before the commercial harvest (20-21 ^{o}Brix), but had no effects at harvest, suggesting a faster accumulation of terpenes during ripening. The peak of expression of several terpene synthases occurred before the commercial harvest. Expression of two terpene synthases was increased in grapes of grapevines subjected to crop load reduction early in the season. My studies indicate that it is possible to modulate terpenes in vineyards by managing irrigation and crop load. However, I observed that the seasons had a stronger effect on terpenes than the treatments investigated.

Lay Summary

The terpenes are key aromatics that affect the quality of several grapes and wines. Two separate studies were conducted with the aim to elucidate the impact of irrigation and the reduction of the amount of crop on a vine – strategies used by grape growers to improve grape and wine quality – on the terpene levels in Gewürztraminer grapes.

Specific terpenes were increased when irrigation was withheld from the onset of ripening until harvest. This deficit irrigation strategy reduces irrigation volumes ~30% and has no impact on yield.

Reducing the crop load per vine via cluster thinning before the beginning of ripening stimulates ripening as measured by faster sugar and terpene accumulation. My results show that leaving a light crop on the vine ripens Gewürztraminer grapes 15 days earlier than in vines with high crop.

Preface

Both grapevine trials were initially devised by Dr. Simone Castellarin.

The deficit irrigation trials were performed at Hidden Terrace Vineyard from 2016 to 2018. Andrew Peller Limited and MRS Management tended all aspects of viticulture apart from those modified for the experiment. MRS Management installed control values into the initial irrigation system for precise irrigation control. MRS Management also provided additional assistance via lending farm equipment, supplies, and personnel when requested. Experiment design and implementation was developed collaborately by Dr. Castellarin and Eugene Kovalenko. Most in-field data collection was performed by Eugene Kovalenko with usual exception during harvest and winter pruning when assistance was provided by Dr. Castellarin and current lab members Yifan Yan, Junfang Wang, and Qiushuo Feng. In 2017, former lab member, Elisabeth Barrows, assisted with all aspects of field work data collection and recording. Dr. Rob Guy provided access to and use of the LI-COR 6400 gas exchange analyzer.

The crop load management trials were performed at Whitetail Vineyard from 2016 to 2018. All aspects of viticulture apart from those modified for the experiment were tended to by Constellation Brands until 2017 afterwards which management switched to Arterra Canada. However, the change in management did not alter the ground crew. Additional assistance via lending farm equipment, supplies, and personnel were provided when requested. Experiment design and implementation was developed collaborately by Dr. Castellarin and Tyler Abbey. Most in-field data collection was performed by Eugene Kovalenko with usual exception during harvest and winter pruning when assistance was provided by Dr. Castellarin and lab members Yifan Yan, Junfang Wang, Tyler Abbey, and Qiushuo Feng. In 2017, former lab member,

Elisabeth Barrows, assisted with all aspects of field work data collection and recording. Dr. Rob Guy provided access to and use of the LI-COR 6400 gas exchange analyzer.

For both projects, frozen grape sample preparation was conducted in the Castellarin Lab at UBC Vancouver collaboratively by the following current and former lab members in no order: Marie Nosten, Ricco Tindjau, Sicheng Li, Louise Thoor, Morgane Clairenbeaud, Eugene Kovalenko, and Tyler Abbey. Gas chromatography mass spectroscopy data acquisition was performed collaboratively by the following current and former lab members in no order: Ricco Tindjau, Morgane Clairenbeaud, Eugene Kovalenko, and Tyler Abbey. Key guidance was provided by Lina Madilao who maintains the mass spectroscopy facility. RNA extraction, cDNA synthesis, and real-time qPCR were largely performed by Ricco Tindjau with assistance from Changzheng Song. Bartosz Kozak, Changzheng Song, Junfang Wang, and Ricco Tindjau acquired/designed, tested, and verified the primers utilized. Preliminary tests of gene expression were performed by Eugene Kovalenko. Data compilation and analysis were performed by Eugene Kovalenko.

Preliminary and unpublished results were presented at several conferences from 2017 to 2019. Abstracts were published and posters presented for the 68th and 70th National Conference of the American Society of Enology and Viticulture: **Kovalenko Y**., Abbey T., Nosten M., Kozak B., and Castellarin S.D. (2017) Crop Load Management to Improve Ripening and Aromatic Contents in White Grapes in the Okanagan Valley. ASEV 68th National Conference Technical Abstracts: Posters. p81., **Kovalenko Y**., Abbey T., Nosten M., Kozak B., and Castellarin S.D. (2017) Irrigation Management for Improving Ripening and Aromatic Contents in White Grapes in the Okanagan Valley. ASEV 68th National Conference Technical Abstracts: Posters. p81., **Kovalenko Y**., Tindjau R., and Castellarin S.D. (2019) Impact of Crop Load

Management on Terpene Concentration of Gewürztraminer Grapes in the Okanagan Valley. ASEV 70th National Conference Technical Abstracts: Oral Presentations. p65., and the 18th to 19th Enology and Viticulture Conference and Tradeshow put on by British Columbia Wine and Grape Council: **Kovalenko Y**., Nosten M., and Castellarin S.D. (2017) Deficit Irrigation Strategies for Improving the Armoatic Contents and Ripening in Gewürztraminer Grapes. BCWGC 18th Enology and Viticulture Conference Abstracts: Posters., **Kovalenko Y**., Tindjau R., and Castellarin S.D. (2019) Impact of Crop Load Management on Terpene Concentration of Gewürztraminer Grapes in the Okanagan Valley. BCWGC 19th Enology and Viticulture Conference Abstracts: Posters., and **Kovalenko Y**., Tindjau R., and Castellarin S.D. (2019) Impact of Deficit Irrigation on Terpene Concentration of Gewürztraminer Grapes in the Okanagan Valley. BCWGC 19th Enology and Viticulture Conference Abstracts: Posters. Posters and presentations were presented by Eugene Kovalenko.

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List of Abbreviations

[Total]	Total Concentration
a.s.l.	Altitude Above Sea Level
ADH	Alcohol Dehydrogenase
ANOVA	Analysis Of Variance
AP47	Actin Protein, Clathrin-Associated Protein
BC	British Columbia
bHLH	Basic Helix-Loop-Helix
CAD	Canadian Dollar
CAR	Carboxen
cDNA	Complementary DNA
CLM	Crop Load Management
cm	Centimetre
CN	Control
CoA	Coenzyme-A
Ct	Threshold Cycle
CYP	Cytochrome P450
DAA	Days After Anthesis
DI	Deficit Irrigation
DMAPP	Dimethylallyl Pyrophosphate
DOX	1-Deoxy-D-Xylulose 5-Phosphate
DVB	Divinylbenzene
DXR	1-Deoxy-D-Xylulose 5-Phosphate Reductase
DXS	1-Deoxy-D-Xylulose 5-Phosphate Synthase
ED	Early Deficit\
EU	Experimental Units
FPP	Farnesyl Pyrophosphate
FPPS	Farnesyl Pyrophosphate Synthase
FVT	Free Volatile Terpenoids
FW	Fresh Weight
g	Gram
GC	Gas Chromatography
GDD	Growing Degree Days
GER	Geranial Reductase
GL	Gigalitre
GPP	Geranyl Pyrophosphate
GPPS	Geranyl Pyrophosphate Synthase
GT	Glucoside Transferase
h	Hour
ha	Hectare
HC	High Crop
HDR	4-Hydroxy-3-Methylbut-2-Enyldiphosphate Reductase
HMGR	3-Hydroxy-3-Methylglutaryl Reductase

IEC	Ion Extracted Chromatogram
IPP	Isopentenyl Pyrophosphate
kg	Kilogram
km	Kilometre
LA	Leaf Area
LC-E	Light Cropping, Early Thinning
LC-L	Light Cropping, Late Thinning
LD	Late Deficit
Lmv	Leaf Main Vein Length
LOD	Limit Of Detection
LOQ	Limit Of Quantitation
m	Metre
MC-E	Medium Cropping, Early Thinning
MC-L	Medium Cropping, Late Thinning
MEP	2-*C*-Methyl-D-Erythritol 4-Phosphate
mg	Milligram
Mha	Megahectare
ML	Megalitre
mL	Millilitre
MLWP	Midday Leaf Water Potential
MPa	Megapascel
MS	Mass Spectroscopy
MTBE	Methyl Tert-Butyl Ether
MVA	Mevalonic Acid Pat
MYB	Myeloblastosis Transcription Factor
n.s.	Not Significant
NADP/NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Center For Biotechnology Information
NIST	National Institute Of Standards And Technology
PAR	Photosynthetically Active Radiation
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PD	Prolonged Deficit
PDMS	Polydimethylsiloxane
PQA	Point Quadrat Analysis
PVT	Potential Volatile Terpenoids
qPCR	Quantitative PCR
R	Pearson Correlation Coefficient
R2	Coefficient Of Determination
s	Second
TA	Titratable Acidity
TPS	Terpene Synthase
TSS	Total Soluble Solids

Tukey's HSD	Tukey's Honestly Significant Difference
ug	Microgram
uL	Microlitre
USD	United States Dollar
UV	Ultra-Violet
VOC	Volatile Organic Compounds
VOCs	Volatile Organic Compounds
WD	Water Deficit
ΨLeaf	Potential, Leaf - Used Here as Equivalent to MLWP

Acknowledgements

I would like to begin by acknowledging that the land on which this research was operated from is the unceded territory of the Coast Salish Peoples, including the territories of the xwməθkwəy̓əm (Musqueam), Skwxwú7mesh (Squamish), Stó:lō and Səl̓ílwətaʔ/Selilwitulh (Tsleil- Waututh) Nations. Furthermore, I would like to acknowledge that the land upon which the vineyard for Chapter 3 was grown has been leased from the Osoyoos Indian Band since 1998, situated in the larger Sylix/Okanagan Nation.

I have witnessed unwavering support and trust in my decisions and progress from friends, family, and colleagues. Without the dutiful and consistent technical and laboratory support from Lina Madilao, Ricco Tindjau, and Marie Nosten, neither project would have progressed to their current status. Additionally, I would like to acknowledge the field- and lab-support from Steve McDonald, Mark Antonello, and Karen Gillis on the deficit irrigation project and from Scott Carlson, Ruiping Cheng, Dave Carson, Troy Osborne, and Michael Watson on the crop load management study.

I also wish to acknowledge all funding providers for supporting these projects through grants and scholarships: Mitacs in conjunction with British Columbia Wine and Grape Council, Investment Agriculture Foundation of British Columbia, the Natural Sciences and Engineering Research Council of Canada, the American Society of Enology and Viticulture, the University of British Columbia, and the Land and Food Systems Faculty.

Dedication

This thesis is dedicated to my grandparents who have become an evergreen inspiration to my academic career.

Chapter 1: Introduction

1.1 Impetus of Studying Grapevines and Terpenes

Grapevines (*Vitis vinifera* L.) are an economically important crop that produce a variety of products from table grapes to preserves, to wines. For grapevine cultivation, a yearly average of 7 million hectares (or 0.5 % of total global agricultural crop area) was seen committed to bearing grapes from 2006 to 2016 (Anderson et al, 2016). From this 7.6 Mha, approximately 70 million tonnes (billion kg) of grapes per annum were produced, half of which were used for wine, producing 25 billion L of wine (Anderson et al, 2016). In 2015, approximately $30 billion USD was spent globally on wine amounting to 14 billion L of wine import/export volume between nations (Anderson et al, 2016). Vineyards in Canada averaged 0.16% of the world's total grapevine bearing area in 2016 (Anderson et al., 2016). As of 2016, 720 wineries were established in Canada with 369 in B.C. mostly distributed in the Okanagan Valley among 8 sub-regions: Kelowna/Lake Country, Peachland/Summerland, Naramata Bench, Skaha Bench, Okanagan Falls, Oliver, Golden Mile Bench, and Osoyoos (British Columbia Wine Institute, 2019). The other wine grape producing regions in B.C. include Vancouver Island, Gulf Islands, Fraser Valley, Kootenays, Lillooet, Shuswap, and Thompson Valley (British Columbia Wine Institute, 2019). In terms of vineyards, of Canada's approximate 12.150 ha, approximately 4,000 ha are in B.C. of which 84.1% are in the Okanagan Valley (Reid Hurst Nagy INC., 2014). Overall, the wine and grape industry contributes an approximate $9 billion CAD annually to the Canadian economy, that is $1 million every hour, and employs over 37,000 Canadians (Frank, Rimerman + Co. LLP, 2017).

For wine and other grape-derived products, chemical composition of the grape berry strongly affects the product quality. Wine aroma and flavour are heavily influenced by the

volatile composition in the grape including compounds such as norisoprenoids, alcohols, carbonyls, phenylpropenoids, methoxypyrazines, thiols, and terpenoids (Robinson et al, 2014). Volatile organic compounds (VOCs) may be present in grapes as their free molecular form or covalently bound to other molecules (Robinson et al, 2014). Bound VOCs are typically non-volatile thus do not affect aroma/flavour until chemically or enzymatically released during fermentation or aging (Robinson et al, 2014). Other factors influencing wine aroma and flavour are microbiologically-derived secondary metabolites, acid- and enzyme-catalysis of berry constituents, oxidation, and oak-derived compounds (Robinson et al, 2014).

In Gewürztraminer, an aromatic white-wine cultivar and the third most grown white grape cultivar in the Okanagan by tonnage (11.4% of total white grape tonnage) (Mount Kabau Wine Services, 2015), terpenoids are key odorants that contribute to the overall flavour of the wine (Guth, 1997a; Guth, 1997b; Ong and Acree, 1999; Martin et al., 2012).The particular terpenoids of interest are monoterpenes such as geraniol/nerol, cis-rose oxide, linalool, and citronellol (Guth 1997). Terpenoids are subject to modulation by the plant in response to biotic or abiotic stress (Robinson et al., 2014). Not only are terpenoids crucial to the quality of Gewürztraminer wines, but they are also the single largest family of all known plant metabolites encompassing 40,000 structures and have uses in traditional and modern practices such as pharmaceuticals, fragrances, food supplements, and pesticides (Bohlmann and Keeling, 2008).

1.2 Terpene Biosynthesis

1.2.1 Terpenoid Precursors

Plant terpenoids are synthesized from two isomeric C_5 precursors, dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) (Schwab and Wüst, 2015). DMAPP and IPP are formed cytosolically in the mevalonic acid (MVA) pathway and plastidically in the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway (Rohmer, 1999; Lange et al, 2000) from glycolysis products: pyruvate and glyceraldehyde-3-phosphate for MEP and two acetyl-CoA for MVA (Figure 1). Three enzymes are known to control metabolic flux in the MEP pathway (DXS: 1-deoxy-D-xylulose-5-phosphate synthase, DXR: 1-deoxyxylulose-5-phosphate reductoisomerase, and HDR: 1-hydroxy-2-methyl-butenyl 4-diphosphate reductase), while only one enzyme largely determines flux for the MVA pathway (HMGR: 3-hydroxy-3-methylglutaryl coenzyme A reductase) (Vranova et al., 2013). Studies of Gewürztraminer berries demonstrated an increase in transcript abundances for the first and last step enzymes DXS3 and HDR that parallel accumulation of monoterpenoids starting ~60 days after blooming (Martin et al., 2012). Similar findings were reported with berries from Moscato Bianco, Italia x Big Perlon (*V. vinifera x V. vinifera*), and Moscato Bianco x Wr63 (*V.vinifera x V.riparia)* (Battilana et al., 2009; Battilana et al., 2011). This is paralleled in other plant systems such as *DXS* over-expression lines in *Arabidopsis* (Wright et al., 2014). HDR and HMGR are the final steps in the MEP and MVA pathways, respectively; both produce IPP but only HDR generates DMAPP. However, isomerases exist that interconvert DMAPP and IPP (Schwab and Wüst, 2015). The C_{10} compound geranyl diphosphate (GPP) is generated from DMAPP and IPP by GPP synthase (GPPS) (Schwab and Wüst, 2015).

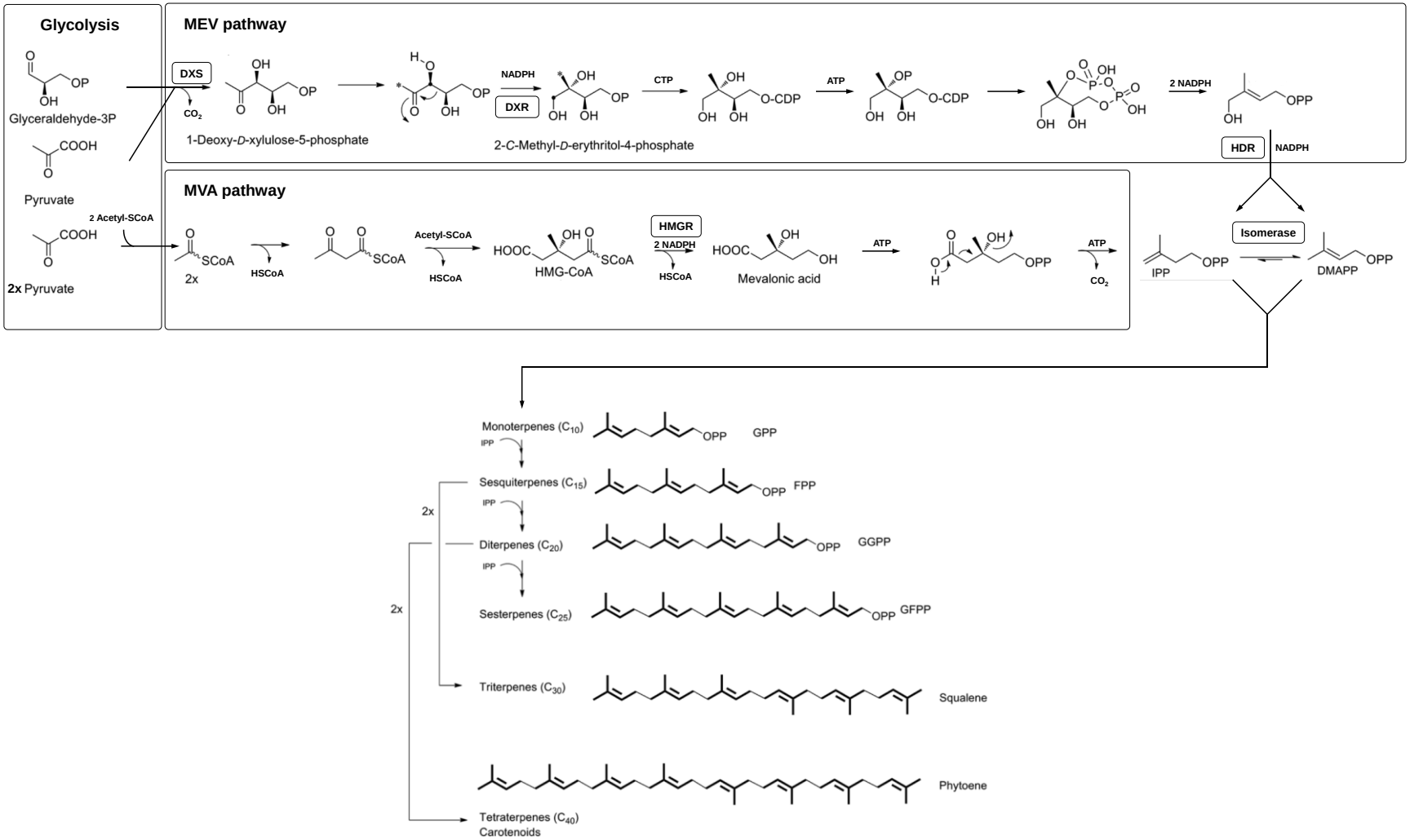

Figure 1: Overview of the MEP and MVA biosynthetic pathways to terpenoid skeletons via IPP/DMAPP from glycolysis in higher plants. Enzymes that control metabolic flux are highlighted in boxes. This figure was adapted from Schwab and Wüst (2015). DXS: 1-deoxy-D-xylulose-5-phosphate synthase. DXR: 1-deoxyxylulose-5-phosphate reductoisomerase. HDR: 1-hydroxy-2-methyl-butenyl 4-diphosphate reductase. HMGR: 3-hydroxy-3-methylglutaryl coenzyme A reductase. IPP: isopentenyl diphosphate. HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A. DMAPP: dimethylallyl diphosphate. IPP: isopentenyl diphosphate. DMAPP: dimethylallyl diphosphate. GPP: geranyl diphosphate. FPP: farnesyl diphosphate. GGPP: geranylgeranyl diphosphate. GFPP: geranylfarnesyl diphosphate

Subsequently, terpene synthase (TPS) enzymes utilize GPP to generate several monoterpene scaffolds via a coupled isomerization-cyclization reaction (Schwab and Wüst, 2015). GPPS and certain TPS activity have been shown to localize to plastids (Feron et al., 1990; Soler et al., 1992; Hardie et al., 1996; Tholl et al., 2004). Thus, monoterpenoids are primarily formed plastidically via the MEP (May et al., 2013). The MVA pathway largely contributes to the sesquiterpene (C_{15}) metabolic pool via a cystolically localized farnesyl diphosphate synthase (FPPS) and TPS a-subfamily enzymes (Schwab and Wüst, 2015). The plastidic-MEP and cytosolic-MVA pathways are metabolically isolated due to their cellular localization, however some evidence for "cross-talk" has been demonstrated at the level of IPP (Kreuz and Kleinig, 1981; Lutke-Brunkhaus and Kleinig, 1987; Feron et al., 1990) and potentially GPP (Bick and Lange, 2003; Flugge and Gao, 2005). Regardless, monoterpene production largely depends on the carbon flux through the MEP pathway, since metabolite flux for MEP often exceeds that of the MVA pathway (Dudareva et al., 2005; Wu et al., 2006).

1.2.2 Monoterpenoid Synthesis

Monoterpenoid molecular skeletons are synthesized by various terpene synthases (TPSs) from plastidically synthesized GPP (Chen et al., 2011; Tholl, 2015) via a coupled isomerization-cyclization through diphosphate hydrolysis generating acyclic, monocyclic, and bicyclic isomers depending on the TPS (Martin et al., 2010). There exist 69 putative intact *TPS* genes grouped into 5 subfamilies (Martin et al., 2010) in grapevines. Presently, only subfamilies TPS-b and TPS-g have members known to synthesize monoterpenes (Martin et al., 2010).

The functions of the TPS-b subfamily have been characterized as mainly multiproduct synthases, for example, the major products of TPS44 and TPS45 are (+)-α-pinene, (+)-α-limonene, (+)-α-camphene, and (+)-α-phellandrene, myrcene, terpinolene, respectively (Martin

et al., 2010). More than half of the TPS-b genes can generate an isomer of ocimene and TPS47 in particular has been characterized as a (*E*)-α-ocimene synthase and a (*E,E)*-β-farnesene (sesquiterpene) synthase (Martin et al., 2010). TPS31 and TPS44 have been observed to produce an oxygenated monoterpene, linalool, and TPS31 solely generates the (3R) isomer (Martin et al., 2010). As with other plants, TPS-b genes in grapes fall into two clades, clade I mainly generate cyclic monoterpenes and clade II mainly generate acyclic monoterpenes (Martin et al., 2010). This subfunctionalization is mirrored in other genera such as *Lotus japonica* and *Malus x domestica*, suggesting speciation proceeded this functional split (Martin et al., 2010). For grapevines, this subfamily accounts for many cyclic and acyclic monoterpenes and a few monoterpenols.

TPS-g genes exclusively catalyze the synthesis of acyclic terpenols and can be catergorized by three types of enzymes based on their substrate ranges (Martin et al., 2010). TPS54 and TPS56 represent one type and they accept GPP and FPP as substrates to generate (3S)-linalool and (*E*)-nerolidol (Martin et al., 2010). TPS57 represents the second group that also has an additional function to accept and catalyze geranylgeranyl diphosphate into (*E,E)*-gernayl linalool (Martin et al., 2010). Geraniol is the only product from the activity by the third type of TPS-g enzyme represented by three functionalized genes, i.e. GwGer, CSGer, and PNGer (TPS54 isoforms) (Martin et al., 2010). TPS-g genes have been suggested to be mainly responsible for linalool and geraniol synthesis *in vivo* throughout development (Martin et al., 2010).

1.2.3 Monoterpenoid Functionalization

Further functionalization can occur on monoterpenoids after TPS activity such as hydroxylation, reduction, oxidation, dehydration, acylation and others, which helps produce the

over 40,000 observed terpenoid structures (Croteau et al., 1991; Croteau et al., 2000; Dudareva et al., 2004; Pichersky et al., 2006; Luan et al., 2006).These reactions alter the final wine profile by producing other powerful odorants (e.g., rose-oxide) and compounds for glycosylation which are released during or after vinification (Schwab and Wüst, 2015). Monoterpenoids with a free hydroxyl group are precursors for glycosidic conjugation by glycosyltransferases (GTs) (Schwab and Wüst, 2015). This includes original TPS products and ones generated from post-TPS activity. Glycosylated terpenoids are non-volatile compounds that are released during vinification by *V.vinifera* endogenous and exogenous glycosidases (Hjelmeland and Ebeler, 2015). Bound terpenes are found in abundance in grape berry mesocarp and exocarp of the aromatic cultivars Scheurebe and Muscat varieties (Luan et al., 2004; Luan et al., 2006), Gewürztraminer (Martin et al., 2012), and in Viognier (Wang et al., 2019). Recent efforts have been successful in cloning and characterizing heterologous grape UDP-monoterpenol GTs from berries, leaves, flowers, and roots (Bönisch et al., 2014a; Bönisch et al., 2014b). GT14a and three GT15 isoforms have been identified as linalool, geraniol, citronellol, and nerol GTs (Schwab and Wüst, 2015). Expression of GTs peaks after veraison, signalling their importance in ripening, specifically in monoterpenol glycoside accumulation (Wen et al., 2015). Glycoside-conjugated monoterpenols are stored in the vacuole as water-soluble compounds that are odorless and non-toxic (Hjelmeland and Ebeler, 2015). In fact, high concentrations of monoterpenols induce apoptosis in plants cells, thus glycosylation may be required for cell survival. Aromatic varietals such as Gewürztraminer and Riesling can store up to 90 % of their total volatile terpenoid fraction as glycosidically bound (Reynolds and Wardle; 1989; Reynolds et al., 1996; Ghaste et al., 2015).

1.2.4 Regulation of Monoterpenoid Biosynthesis

Little is known about the regulation of monoterpene biosynthesis in grapevine. However, a recent study by Savoi et al. (2016) suggested the presence of a few transcription factors that correlate significantly with the monoterpenes linalool, α-terpinol, and nerol, including MYB24 (*VIT_14s0066g01090*), C2H2 Zinc Finger (*VIT_07s 0005g02190*), and Constans-like (*VIT_19s0014g 05120*). Furthermore, in Savoi et al. (2016), promoters of the top 100 genes correlating with linalool, α-terpinol, and nerol were significantly enriched for MYB recognition and drought-responsive motifs. Moreover, *MYB24*, was strongly upregulated under solar UV radiation in Tempranillo skins (Carbonell-Bejerano et al., 2014), and its upregulation mirrored the upregulation of two linalool synthases, two 1,8-cineole synthases, one geraniol 10-hydroxylase, as well as one flavonol synthase and two flavonol glycosyltransferases. Also, *MYB24* is homologous to *Arabidopsis* MYBs that are involved in regulating terpenoid biosynthesis (Reeves et al., 2012). Interestingly, MYBF1 is a characterized transcription factor that regulates flavonol biosynthesis in response to UV irradiation (Matus et al., 2008) and was upregulated in Tempranillo skins exposed to solar UV (Carbonell-Bejerano et al., 2014). *MYB24* as well as three bHLH transcripts show similar upregulation to *MYBF1*'s transcripts suggesting that *MYB24* might have a role in UV- and general abiotic-stress response (Carbonell-Bejerano et al., 2014).

1.2.5 Monoterpene Accumulation in the Grape Berry

Monoterpenes appear to accumulate in the exocarp (skin) and mesocarp (pulp) (Park et al., 1991) and are thought to be largely synthesized *de novo* in plastids in berry tissue (Bravdo et al., 1990; Hardie et al., 1996). Total monoterpenes typically constitute < 10 ppm of berry mass (Girard et al., 2002; Martin et al., 2010; Ghaste et al., 2015). For Gewürztraminer, the terpenes

citronellol, linalool, geraniol, nerol, and rose oxide are associated with characteristic-varietal flavour (Guth, 1997a, b). These terpenes accumulate in free and glycoside-bound fractions and demonstrably accumulate in correlation with the MEV-pathway and TPS gene expression (Martin et al., 2010).

1.2.6 Impact of Viticultural Strategies on Berry Ripening and Monoterpene Accumulation

In order to achieve desired varietal aroma / berry composition, vineyard management utilizes many strategies to increase ripening and flavour such as deficit irrigation or shoot/cluster thinning (Robinson et al., 2014). The effects of irrigation management on grape composition are dependent on cultivar (Mirás-Avalos et al., 2017). For example, berry weight of Albariño berries remains unaffected by water deficit compared to Sauvignon Blanc or Riesling; however, this may depend on the severity of water stress (Mirás-Avalos et al., 2017). In general, ripening and berry quality are positively associated with moderate water stress applied via deficit irrigation (Robinson et al., 2014; Mirás-Avalos et al., 2017). Crop load management practices such as crop thinning directly affect the sink-source relationship, positively affecting berry ripening and quality (Robinson et al., 2014). It is common practice for vineyard managers to apply either deficit irrigation or crop thinning on red varieties; however, these practices are usually applied from veraison to harvest (Alonso et al., 2016; Goldammer, 2018). Currently, there is a lack of studies comparing deficit irrigation treatments applied at various developmental stages, especially in white wine varieties. Of the studies concerning deficit irrigation on white grapes, many lack well-irrigated controls, are carried out in hot climates, or examine only a subset of chemical, biological, and physiological factors (Bouzas-Cid et al., 2018 a, b; Vilanova et al.,

2019 a, b). Additionally, many studies on cluster thinning in red and white varieties exist; however, the effects on terpene accumulation remain largely unknown.

1.3 Research Objectives

The following studies aim to characterize the chemical, biological, and physiological response of field-grown Gewürztraminer to moderate water stress via deficit irrigation and crop load adjustment via cluster thinning both applied at various developmental stages. Terpenes are of interest as they are associated with cultivar-specific flavor and aroma in Gewürztraminer (Guth, 1997) and occur in relative abundance in this variety (Reynold and Wardle, 1989).

I hypothesize that application of moderate water stress via deficit irrigation will induce the synthesis of terpenes with early and prolonged application of water deficit being more effective than later application. I also hypothesize that reducing the crop load via cluster thinning will stimulate ripening and induce the synthesis of terpenes with early thinning application having a more positive effect than late application.

Chapter 2: Modulation of Grape Aromas via Deficit Irrigation

2.1 Research Introduction

Grapevines are generally adapted to growing in moderate water deficit conditions as evident by their broad and deep root systems, efficient stomatal control, and ability to adjust osmotic potential (Rodrigues et al., 1993; Patakas and Noitsakis, 1999; Lovisolo et al., 2002). The application of water deficit (WD) via regulated deficit irrigation (DI) in grapes is a viticultural practice used to achieve high-quality fruit in red wine varieties (Matthews and Anderson, 1988, 1989; McCarthy et al., 1997, 2000; Castellarin et al., 2007a; Chaves et al., 2007). The quality of red wines is associated with the concentration of phenolics and aromatics; higher quality generally corresponds to higher concentrations. In the vineyard, techniques that reduce the berry weight, such as deficit irrigation, generally increase the skin:pulp ratio and increase the concentration of phenolics and aromatics – mostly synthesised in the skin – in juices and wines (Matthews and Anderson, 1988, 1989; McCarthy et al., 1997, 2000; Castellarin et al., 2007; Chaves et al., 2007; Austin and Wilcox, 2011, Ghan et al., 2015). Recent studies have indicated that the increase in the metabolite concentration in grapes exposed to WD is not only due to changes in the skin:pulp ratio but also to an induction of the biosynthesis of terpenes, anthocyanins, flavanols, phenylpropanoids, and amino acids (Ojeda et al., 2002; Wample and Smithyman, 2002; Peyrot des Gachons et al., 2005; Castellarin et al., 2007a; Acevedo-Opazo et al., 2010; Chaves et al., 2010; Martínez-Lüscher et al., 2015; Ghan et al., 2015, Castellarin et al., 2016; Savoi et al., 2016; Balint and Reynolds, 2017; Wang et al. 2019).

The timing of DI application is crucial and affects the physiology and metabolism of the berry, as well as yield (Mirás-Avalos et al., 2017). DI applied early during the season, i.e., from fruit set to veraison, results in a larger reduction in berry size and yield than DI applied late

during the season, i.e., from veraison to harvest. This is possibly because cell division in berries occurs early during the season (Considine and Knox, 1981; Coombe and McCarthy, 2000). Thus, the application of DI early in the season impacts both berry cell number and berry expansion, while the application of DI late in the season has only an effect on cell expansion (Hardie and Considine, 1976; Considine and Knox, 1981; Matthews and Anderson, 1989; Coombe and McCarthy, 2000). The application of DI before veraison increases anthocyanin and tannin concentration (but reduces the tannin amount per berry) in the berry and increases anthocyanin and tannin in the derived wines (Matthews et al., 1990; McCarthy et al., 1997; McCarthy et al., 2000; Castellarin et al. 2007; Casassa et al., 2015). Generally, pre-veraison DI also leads to earlier onset of ripening (Castellarin et al., 2007a; Herrera and Castellarin, 2016). Applied after veraison, DI has variable effects on TSS, acidity, and berry weight, but generally increases anthocyanin concentration (Ojeda et al., 2002; Castellarin et al., 2007a, b; Keller et al., 2008; Reynolds et al., 2008; Casassa et al., 2015; Trigo-Cordoba et al., 2015). Logically, prolonged DI (from fruitset to harvest) would have effects seen in both early and late deficit treatments.

Much of the effort devoted to examining the influence of deficit irrigation on berry composition has been focused on red varieties and on understanding its effects on total soluble solids, titratable acidity, pH, total phenolics, anthocyanins, and tannins (Robinson et al., 2014; Mirás-Avalos et al., 2017). Available studies on the application of WD or DI to white grape varieties focused on terpene-poor varieties (Mirás-Avalos et al., 2017), were conducted in warm-to-hot climates (Bouzas-Cid et al., 2018a, b), or lacked a comparison to irrigated controls (Reynolds et al., 2008; Vilanova et al., 2019). Furthermore, deficit irrigation effects are highly cultivar-dependent, thus, specific-cultivar studies are required (Mirás-Avalos et al., 2017). No studies exist examining and comparing the response to DI of individual terpenes and related-

genes, yield and canopy growth, and plant physiology (leaf water potential and leaf gas exchange). This study aims to fill this knowledge gap.

2.1.1 Research Hypothesis

In this project, I focused on characterizing how the application of deficit irrigation treatments to field-grown Gewürztraminer grapes affects leaf water potential, photosynthesis, vegetative growth, sugars and acids accumulation, and terpene biosynthesis (analysis of the levels of targeted transcripts) and concentration. Moderate WD was applied via regulated DI from post-fruit set, from veraison, and throughout the season, and was compared to a well irrigated treatment. I have analysed four irrigation treatments: control (CN – well-watered), early deficit (ED – DI applied from post-fruitset to veraison), late deficit (LD – DI applied from veraison to harvest), and prolonged deficit (PD – DI applied from post-fruit set to harvest). Attention was paid to terpenes – as they are associated with cultivar-specific flavors and aromas in Gewürztraminer – as well as associated biosynthetic genes and transcription factors (Guth, 1997; Martin et al., 2012; Savoi et al., 2016). I hypothesized that application of moderate WD would induce the synthesis of quality-determining VOCs with early and prolonged application of moderate WD being mos effective than later application. Overall, this study is novel in the combination of physiological, metabolic, and molecular factors investigated in relation to water stress applied during distinct phenological periods in grapes characterized by a high terpene content.

2.2 Materials and Methods

2.2.1 Vineyard Site

The experiment was conducted throughout 2016-2018 growing seasons in a commercial vineyard in the southern Okanagan Valley (49°14'N, 119°33'W, 420 m a.s.l.), near Oliver, British Columbia. The site hosted field-grown *Vitis vinifera* cv. Gewürztraminer Clone 47 grafted on 3309 rootstock (*V.riparia x V. rupestris*) planted on sandy loam (pH 7.2) in north-south rows at an approximate density of 3,333 vines/ha (2.5 m between rows x 1.2 m within rows; 3 m^2 per vine). Figure S1 depicts site location and experiment setup on-site. Vines were pruned to 8 to 10 buds per cane in a double cane, and trained to a vertical shoot positioning, Guyot trellis system. Pest management, canopy management, and fertilization in the vineyard were applied according to standard local viticultural practice. Approximately 70% of basal (first 4 nodes) leaves were mechanically trimmed ~30 days after anthesis (DAA) on both sides of the canopy. A dripline irrigation system (one line per row) supplied water to vines at a rate of 1.67 L/h/emitter using pressure-compensated emitters (2 emitters per vine) every 7 to 14 days accordingly to the experimental plan as described below.

2.2.2 Experimental Design

Table 1 outlines the start and end dates for treatment application in all three years. Four irrigation treatments were imposed: (i) control irrigation (CN), which matched vineyard standard irrigation practices and implied no water deficit for the vines, where vines were maintained within a range of midday-leaf water potential (Ψ_{leaf}) between -0.5 MPa and -0.9 MPa; (ii) early deficit (ED), where irrigation was withheld in order to maintain Ψ_{leaf} between -1.0 MPa and -1.4 MPa (moderate water stress) before veraison, and after which CN irrigation levels were applied; (iii) late deficit (LD), where pre-veraison irrigation matched CN irrigation and irrigation was

withheld after veraison (starting at 66, 61, 82 DAA for 2016, 2017, and 2018, respectively) in order to mantain Ψ_{leaf} between -1.0 MPa and -1.4 MPa; (iv) prolonged irrigation (PD), where irrigation was withheld before and after veraison in order to maintain Ψ_{leaf} between -1.0 MPa and -1.4 MPa. Figure 2 illustrates the timing of treatment application and the corresponding stages of grape development. The deficit irrigation (DI) treatments were imposed from 25, 24 and 34 DAA in 2016, 2017, and 2018 respectively, and were replicated in four plots of 10-15 vines accordingly to a randomized-block design. Irrigation levels were returned to vineyard standard practice after harvest. Irrigation was applied to ED, LD, and PD treatments only when Ψ_{leaf} was lower than -1.4 MPa in order to avoid severe water stress symptoms (Chaves et al., 2003, 2007; Keller et al., 2008).

Weather conditions (temperature and rainfall) were recorded at an automated meteorological station 500 m from the plot. Growing degree days (GDD) were computed as the sum of the average daily temperature above 10 °C from 1 April until 31 October (Amerine and Winkler 1944).

Table 1. Calendar dates and respective days after anthesis for major phenological and trial-related events

Season	Anthesis		Veraison		Harvest		ED & PD Start		LD Start	
	Date	DAA	Date	DAA	Date	DAA	Date	DAA	Date	DAA
2016	04/06	0	03/08	60	08/09	96	29/06	25	09/08	66
2017	16/06	0	19/08	61	19/09	96	13/07	24	19/09	61
2018	28/05	0	16/08	78	27/09	122	01/07	34	18/09	82

Calendar dates are reported as dd/mm. ED, PD, and LD indicated early deficit, prolonged deficit, and late deficit irrigation treatments, respectively.

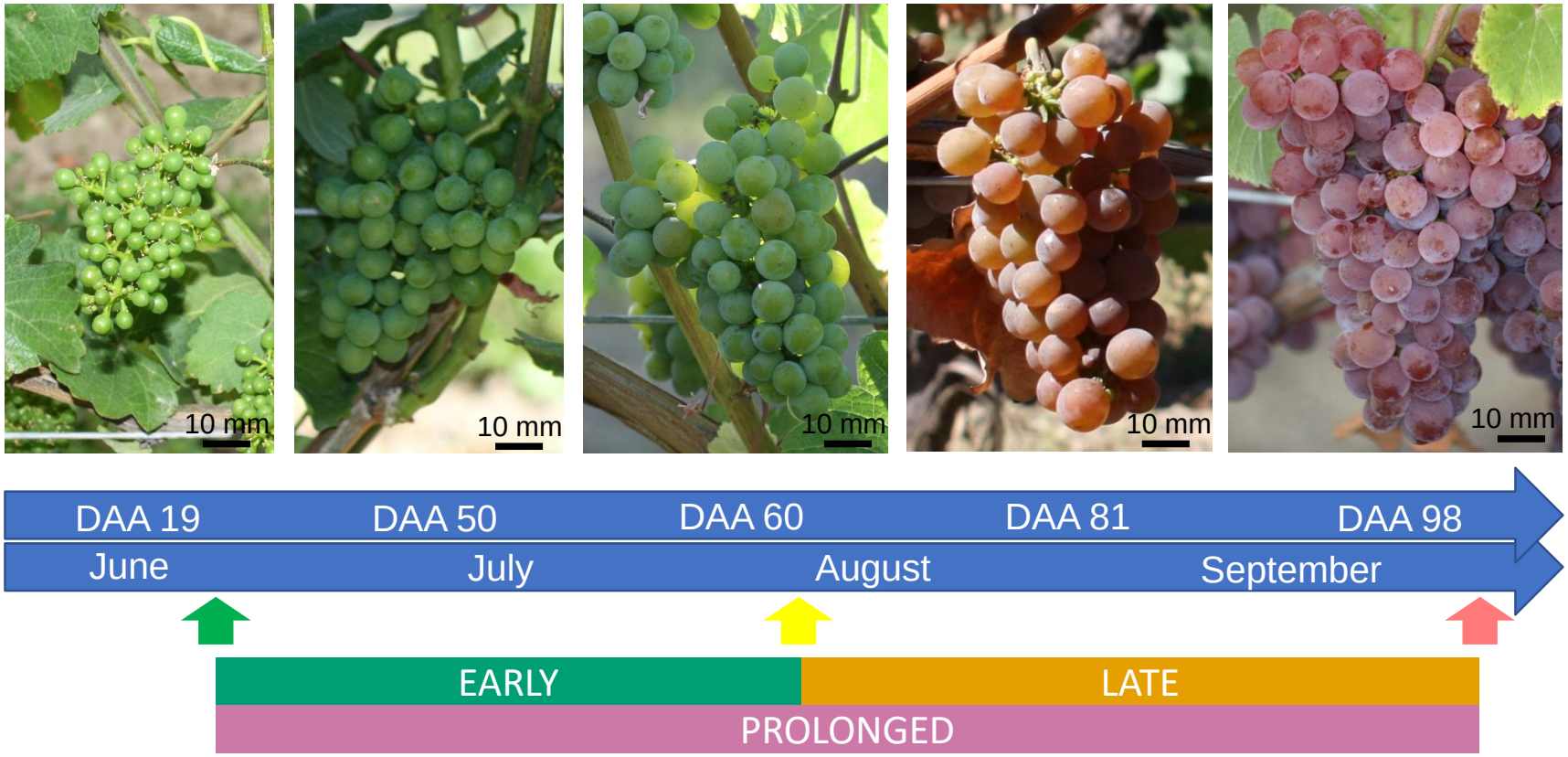

Figure 2: The timing of DI treatment application and corresponding grapeberry development stages, days after anthesis, and calendar months. Green, yellow, and red arrows indicate dates of irrigation treatment application, veraison, and commercial harvest, respectively.

2.2.3 Yield Assessment

Assessment of number of shoots and clusters per vine was completed prior to veraison. Yield per vine, number of clusters per vine, and average cluster mass were determined at commercial harvest (~21 °Brix) on each plot. Pruning weights (the weight of the lignified shoots removed each year via pruning) were collected in the winter following the growing season of DI application, typically in February (Coombe and Dry, 1992).

2.2.4 Point Quadrat Analysis

To assess whether cluster exposure increased due to DI, point quadrat analysis (PQA) measurements were carried out in 2018 before veraison (49 DAA), just after veraison (89 DAA), and within two weeks of harvest (119 DAA). PQA was conducted as in Smart et al. (1982) with some modifications. A 5 mm thick probe was inserted east to west, parallel to ground and perpendicular to the canopy, into the fruit zone every 30 cm for the entire length of each experimental row. Insertions were conducted on the east side of each row. Upon insertion, the type of tissues contacted (leaf, cluster, vegetative, blank/gap) were categorized and recorded. Laminae, leaf veins, and petioles were considered leaf (L). Berries, pedicels, and rachises were recorded as cluster (C). Shoots, tendrils, and canes were recorded as vegetative tissues (V). Blanks or gaps (G) would be recorded when no tissue was contacted upon insertion. Forty-five to seventy insertions per plot were performed. First and last tissues contacted by probe were considered as “sun exposed” tissues.

2.2.5 Leaf Area

Leaf area was measured throughout the growing season, at least once a month and within two weeks from commercial harvest. The method used to quantify primary, secondary, and total leaf area was adapted from Sanchez-de-Miguel et al. (2010) and Williams and Martinson (2003).

Initially, a calibration curve was developed to correlate leaf mid-vein length (L_{mv}, mm) and leaf area (LA, cm^2). Leaves for calibration were collected mid-season (July/August), measured using a line gauge for $L_{mv,}$ and a LI-3100C Area Meter (LI-COR, USA) to measure LA. After graphically assessing the distribution of correlated L_{mv} to LA, a power model (linearized via natural logarithm transformation) was chosen for regression analysis. The following relationship was observed:

$$LA = 0.0098 \cdot L_{mv}^{2.0914}, \text{ or}$$

$$\ln(LA) = 2.0914 \cdot \ln(L_{mv}) - 4.6254; R^2 = 0.9453$$

Utilizing this one-term power model avoided negative LA values at smaller $L_{mv,}$ minimized error at larger $L_{mv,}$ and allowed quick, non-destructive assessment of LA throughout the season. A linear regression with initial logarithmic transformation ($y = b_1x + b_0$) generated a Pearson's correlation coefficient (R^2) of 0.9076 compared to 0.9453 for the linearized power model.

2.2.6 Leaf Water Potential and Gas Exchange

The Ψ_{leaf} was determined with a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA) on bag-covered leaves from four representative vines per plot every 7 to 14 days from ~ 20 DAA to harvest between 1230 h and 1430 h (±1 h from local solar noon). Leaves were assessed from both sides of the row.

Leaf gas exchange parameters (photosynthesis, transpiration, stomatal conductance) were monitored using a LI-COR 6400 (Li-Cor Inc., Lincoln, NE, USA) from + 1 h to + 3 h post-sunrise every 7 to 14 days. Data acquisition was biased towards days with high morning PAR to capture the vines at their photosynthetic maximums. In 2017 and 2018, this proved more challenging with local and prolonged smoke cover from wildfires. A standard leaf chamber

(enclosing 6 cm^2 of leaf area) and a CO_2 injection system (model 6400-01, Li-Cor Inc., Lincoln, NE, USA) adjusted to a constant CO_2 concentration of 400 µmol CO_2 per mol air was used along with a soda lime chamber set to scrub. Light intensity was set to a saturating 1500 µmol m^2 / s (Petrie et al., 2000) and provided red-blue light from an LED source (model 6400-02, Li-Cor Inc., Lincoln, NE, USA). Block temperature was set to 22 °C. Humidity was controlled by the desiccant set to scrub. Two to four exposed leaves per plot were sampled. Leaves were enclosed in the chamber for two to five minutes to allow the photosynthesis rate to stabilize.

2.2.7 Basic Berry Chemical Analysis

Forty to sixty berries (accordingly to the developmental stage) per plot were randomly collected per sampling point (19, 34, 47, 54, 60, 65, 81, and 96 DAA in 2016; 34, 47, 59, 75, 88, and 95 DAA in 2017; 82, 100, and 119 DAA in 2018). Larger amounts of berries were collected for earlier samplings so to ensure enough juice for TSS, pH, and TA analysis. Berry weight, sugar, and acids were determined utilizing the methods reported in Savoi et al. (2016). Total soluble solids were measured with a BRIX Refractometer from LW Scientific™ and expressed in °Brix. The pH was determined by analysing freshly squeezed, clarified juice using a Fisher Scientific™ Accumet Research AR20 pH/conductivity meter and Thermo Fisher Scientific™ Orion™ PerpHecT™ ROSS™ Combination pH/microeletrode. Titratable acidity was determined by acid-base titration using standardized 0.1 M sodium hydroxide (Thermo Fisher Scientific, MA). All analyses were completed on fresh berry juice.

2.2.8 Free Terpene Analysis

Samples of forty to sixty berries (depending on developmental stage) for free terpene analysis were randomly collected from each plot at 19, 34, 47, 54, 60, 65, 81, and 96 DAA in 2016; 34, 47, 59, 75, 88, and 95 DAA in 2017; 82, 100, and 119 DAA in 2018 and immediately

frozen under a cover of dry-ice. Berries were removed from the clusters with scissors by cutting at the pedicel level in order to avoid any damage to the berry tissue. Berry samples were kept in dry ice until stored in the laboratory in a -80 $^{\circ}$C freezer. A subset of samples was analyzed from the total collected. The corresponding DAA to the analyzed subsets were 34, 54, 60, 81, and 96 DAA in 2016; 88 and 95 DAA in 2017; 100 and 119 DAA in 2018. The final two days in each year of samples may be referred to as "pre-harvest" and "harvest" sampling points / dates.

Berries were crushed and deseeded using a motor and pestle under liquid N_2, then powdered using A11 Basic Analytical Mill (IKA, Wilmington, NC, USA). Deseeded, powdered berries were stored in -80 $^{\circ}$C freezer until analysis. Free terpene analysis was adapted from Fedrizzi et al. (2012) and Matarese et al. (2013) with some modifications. In 20 mL headspace analysis vials (Agilent®), 1.50 g ± 0.01 g of NaCl and 5.00 g ± 0.10 g of frozen berry tissue (deseeded, powdered grape pulp and skin) followed by 4.00 mL of citrate-phosphate buffer (0.1 M Na_2HPO_4 and 0.05 M citric acid) at pH 5.0, 100 µL of 200 g L^{-1} ascorbic acid, and 50 µL of 0.12 ppm d3-linalool were combined carefully and promptly. The SPME fiber used was a 50/30 µm x 2 cm DVB/CAR/PDMS Stableflex® (Supelco), the column was a CyclodexB 30 m x 0.25 mm with a 0.25 µm film, and the GC-MS model was an Agilent 5975C with Triple-Axis detector and CTC Combi-PAL autosampler (Zwingen). Volatile adsorption/desorption, GC separation, and MS conditions were performed in accordance with Fedrizzi et al. (2012). Two technical replicates were run per biological sample. Samples were analyzed in a random sequence.

Authentic standards for identification and quantitation of volatiles are outlined in Table S1. Terpenes were identified by comparing the retention times of ion extracted chromatograms (IECs) peaks with the retention times of their reference standards when available, retention indices with published literature, and by identifying the mass spectra using the NIST library.

Stock solutions of standards were prepared in pentane. Calibration curves were generated, akin to Smit et al. (2019), by targeting a final concentration of 0.1 to 5 ppb of authentic standards prepared in 20 mL headspace vials, identical as described above, containing 3 ppb d3-linalool as internal standard. IEC peaks were integrated and normalized to the area of the internal standard. The metabolite concentration in the samples was determined according to the calibration curve of the respective authentic standard. When an authentic standard was unavailable, concentrations were semi-quantitated by using the calibration curve of the compound with the closest molecular structure and functionality. The concentrations are reported as µg/g berry fresh weight (FW) or as µg per berry. Three compounds were observed to have isomers for which no isomer separated authentic standards were available: ocimene, farnesene, and citral. Isomers are referred to in order of retention, "a" being first and "b" being second.

Signal/noise assessment was not possible for most ions, since IECs were used. The definitions of limit of quantitation (LOQ) and limit of detection (LOD) utilized in this study were referenced from the United States Pharmacopoeia (Sangai et al., 2009).

This procedure was semi-quantitative; the values reported are only for comparison proposes between treatments. Full quantitation using HS-SPME methods would require standard addition of each analyte into the sample matrix (frozen berry powder) and more berry tissue than was reasonable to collect.

2.2.9 Glycoside-Bound Terpene Analysis

Bound terpene analysis was adapted from Martin et al. (2012) and Ghaste et al. (2015) with some modifications using the same berry powder described for the free terpene analysis. One gram of frozen, deseeded grape powder was added to 8 mL Milli-Q H_2O in a 15 mL conical vial then vortexed for 10 minutes. Column extraction and extract desiccation was conducted in

accordance with Martin et al. (2012). Once evapourated the eluent was resuspended in a 5.00 pH buffer of 50 mM citrate:100 mM phosphate to about 5 mL. At this point, 30 µL of d_3-linalool (2.8 ppm) was added. The enzyme mix AR2000 (DSM, Netherlands), a mixture of pectinases and glycosidases, was dissolved in the citrate-phosphate buffer at a concentration of 100 mg / mL. The mix was added (0.500 mL) into a GC vial containing the buffer-resuspended methanol eluent. An overlay of 0.5 mL MTBE was added to each vial to capture volatized aglycones. This mixture was incubated at 40 ˚C for 2 h, vortexed for 30 s to extract aglycones, centrifuged for 10 mins at 4000 x g at 4 ˚C, then placed into a -80 ˚C freezer to separate the aqueous and organic phases. The MTBE overlayer was removed carefully and placed into a 2 mL GC amber vial. This was repeated for a total volume of 1.0 mL of MTBE overlayer. The extracts were placed into a glass insert in a capped amber vial for liquid injection GC–MS analysis. This dual 2 h extraction was assessed to be equivalent to a single 24 h extraction (data not shown). The column, GC-MS model, autosampler, and oven regime used were identical to those described for free terpenes. The injection volume was 1.0 µL. Terpenes were identified and quantified as described for free terpenes, however, separate calibration curves were developed using LI-GC-MS methods, as describe above. This procedure was quantitative.

2.2.10 Gene Expression Analysis: RNA extraction to qPCR

Gene expression analyses were conducted only on the 2016 samples at 34, 54, 65, 81, and 96 DAA. Two hundred mg of frozen, deseeded grape powder was utilized for RNA extraction using the Spectrum™ Plant Total RNA Kit (Sigma-Aldrich). RNA quality and quantity were verified using the nanodrop and the integrity was analysed by gel electrophoresis. RNA was stored at -80 ˚C until use. One µg of RNA per reaction was reverse transcribed using RevertAid First Strand cDNA Synthesis Kit (ThermoFisher) to obtain cDNA for downstream applications.

cDNA was diluted 5x using Milli-Q® water in preparation for q-PCR and stored at -20 ˚C. Primers were selected from literature or designed in house for qPCR. In-house primers were designed based on TPS sequences obtained through iterative BLAST searches in NCBI GenBank using members of each of the TPS subfamilies. At arrival of the primers, dilutions were done and 10x working stocks were made and store at -20 ˚C. Table S2 outlines all the sequences of the primers used, their source, and target gene details.

The PowerUp™ SYBR™ Green Master Mix (ThermoFisher) was used for qPCR. The master mix contains buffer, dNTPs, DNA-polymerase and SYBR Green dye. Primer pairs for that gene (0.4 µL of 10 µM), Milli-Q® water (2.2 µL) and SYBR Green Master Mix (5 µL) was combined and 8 µL was loaded to the qPCR plate. In addition to the 8 µL master mix, 2 µL of cDNA sample was added and the plate was sealed with the cover and subsequently centrifuged for 15 sec at 250 rpm. An Applied Biosystems 7500 Real Time PCR System together with the assistance of 7500 Software v2.0.6 was used for the qPCR runs. Primer pairs were tested for efficiency and a 60 ˚C annealing temperature was determined as ideal. Samples were amplified under the following conditions: 50 °C for 2 min, 95 °C for 2 min, followed by 40 cycles of two steps PCR, 95 °C for 15 s and 60 °C for 1 min. The gene AP47 was chosen as the housekeeping gene for this experiment because it is evenly expressed at all stages of *Vitis vinifera* growth and in all tissues. Ubiquitin, another commonly used housekeeping gene for grape berry studies, has been demonstrated to be modulated by water stress, thus was not an ideal housekeeping / reference gene candidate (Savoi et al. 2016). With the Ct values from the housekeeping gene and gene of interest, the $2^{\Delta Ct}$ was calculated to obtain the relative gene expression (Savoi et al. 2016).

2.2.11 Statistics

Basic statistics, analysis of variance (ANOVA), and correlation analyses were undertaken using R software v3.4.4 (R Foundation for Statistical Computing, Vienna, Austria). Two-way ANOVA with treatment and year as main factors and block number as additive random factor, were used to test the main effects and factor interactions on pre-harvest- and harvest-time biochemical, physiological, and vegetative parameters. When an interaction between treatment and year was found, one-way ANOVA without a year main factor was used to assess differences at individual sampling points for biochemical, physiological, and vegetative parameters within years. For multiple comparisons of treatments, standard error, and Tukey's HSD *post-hoc* tests were calculated and reported. Statistically, marginal differences were assumed to be $0.1 > p > 0.05$, while significant differences were assumed for $p < 0.05$. Packages utilized for analysis were tidyverse v1.2.1 (Wickham et al., 2017), moderndive v0.2.0 (Kim et al., 2018), and skimr v1.0.5 (Waring et al., 2019).

2.3 Results

2.3.1 Phenology and Timing of Treatments

The calendar dates of anthesis, veraison, and harvest differed by a maximum of three weeks between the years (Table 1). Considering anthesis-normalized dates, veraison and harvest were nearly identical in 2016 and 2017 but approximately four weeks later in 2018. The application of ED, LD, and PD treatments remained within a two-week period among the years. Initial DI application (ED and PD) occurred on average -39 ± 3 days from veraison; second DI application (LD) and recovery of ED vines occurred on average 3 ± 2 days from veraison. Harvest would occur on average 37 ± 4 days from veraison.

2.3.2 Weather, Irrigation, Canopy Development and Physiology

Heat accumulation (or GDD) for all three years was comparable at harvest (1550–1600 GDD) despite variation in temperature from April to mid-July (Figure 3a). Therefore, the region where the experiment was conducted can be classified as a Winkler Region II (Amerine and Winkler, 1944). There was substantial variation between years in cumulative rainfall for the duration of the experiment (Figure 3b). The 2016 season experienced the largest accumulation of rainfall by Oct. 31 followed by 2018, while in 2017 there was a prolonged drought from June (around fruit-set) until the end of October. However, by the start of treatment application, more precipitation had fallen in 2017 and 2018, than 2016.

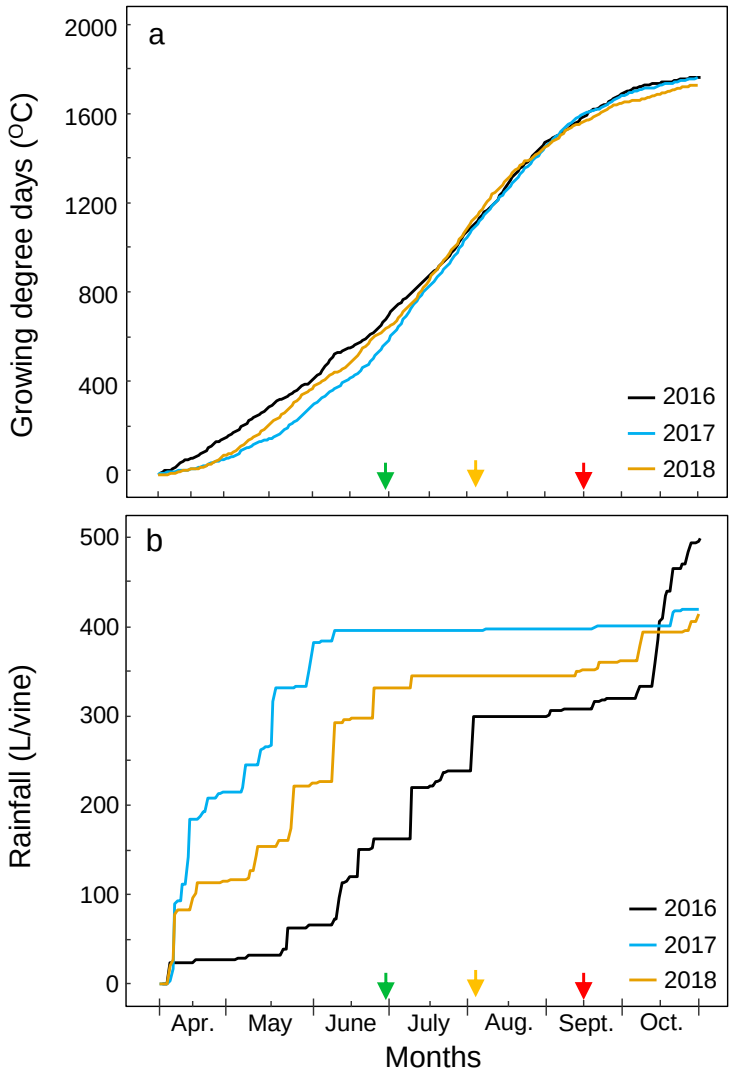

Figure 3: Cumulative GDD (a) and rainfall (b) throughout the 2016 – 2018 seasons. Green arrows indicate average date of initial application of deficit irrigation treatment among all three seasons. Yellow arrows indicate indicate average date of veraison application of deficit irrigation among all three seasons. Red arrows indicate indicate average date of commercial harvest among all three seasons.

Difference in rainfall (or lack thereof) events among seasons had an evident impact on the application of additional irrigation to ED, LD, and PD treatments (Figure 4a-c). In 2017, there was a higher instance of additional irrigation events for the DI treatments, while in 2016, ED and PD vines did not reach moderate stress levels until 3 weeks after the beginning of DI. Less irrigation was generally applied to the vines in 2018, which coincided with lower GDD and periodic rains in July and September.

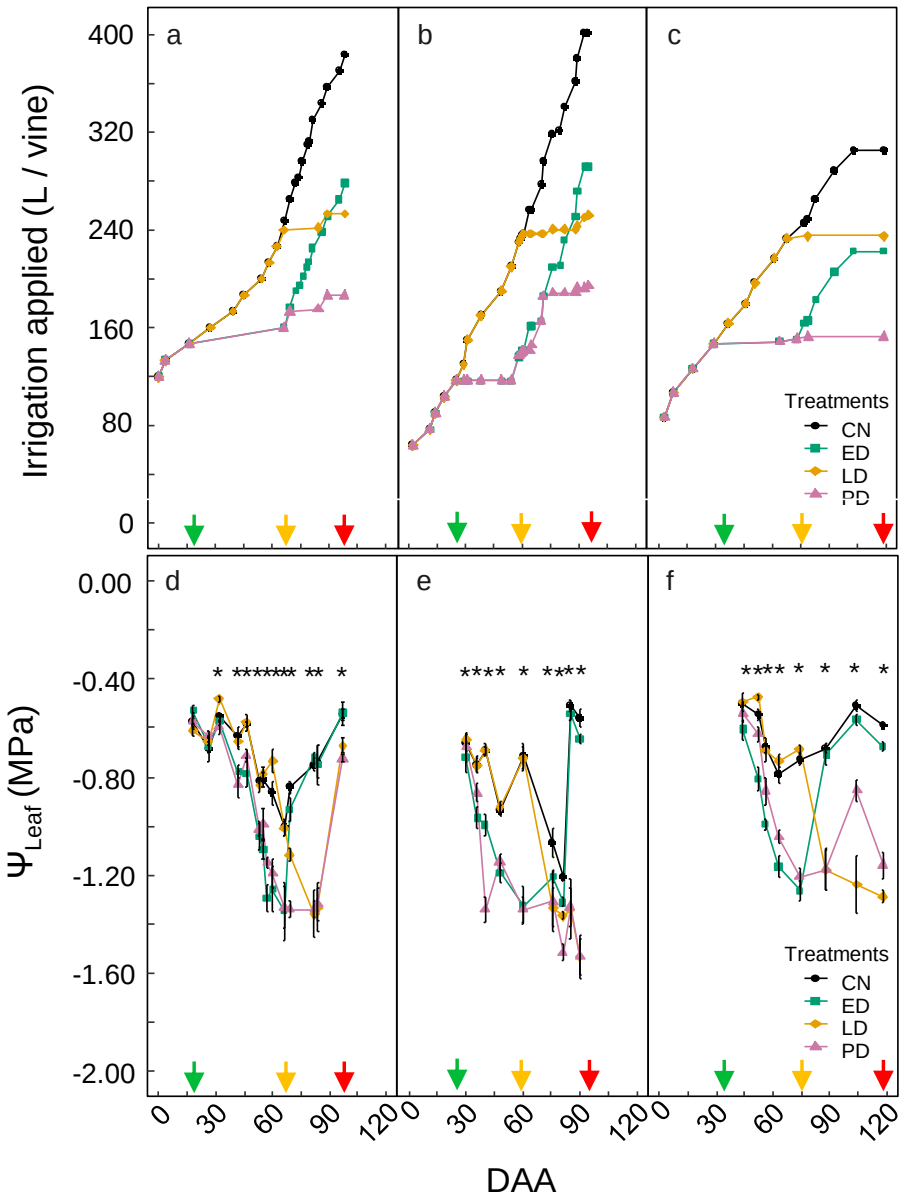

Figure 4: (a-c) Cumulative irrigation applied to grapevines exposed to deficit irrigation treatments throughout 2016 (a), 2017 (b), and 2018 (c) seasons. Green, yellow, and red arrows indicate dates of irrigation treatment application, veraison, and commercial harvest, respectively. (d-f) Mean midday leaf water potential (Ψ_{Leaf}) of Gewürztraminer grapevines exposed to deficit irrigation treatments throughout 2016 (d), 2017 (e), and 2018 (f) seasons. Error bars indicate standard error. Asterisks denote sampling points where the one-way ANOVA test determined significant ($p < 0.05$) difference between the means.

Averaging over the three years, ED, LD, and PD vines received 72.74% ± 0.09%, 68.66% ± 4.29%, and 49.03% ± 0.49% of CN irrigation volumes during the duration of the experiments, respectively. In other words, ED and LD treatments reduced irrigation volumes by

approximately 30% whereas PD irrigation limited water application by 50%. CN vines received comparable amounts of irrigation in 2016 and 2017 (1.278 ML per ha or 384 L per vine and 1.335 ML per ha or 401 L per vine, respectively). In 2018, applied irrigation to CN was reduced by about 25% compared to the former two years (1.017 ML per ha or 305 L per vine). Considering applied irrigation and rainfall together from April 1 to harvest, all treatments received the most water in 2016 and the least in 2018. Irrigation treatments were applied from 27 to 96 DAA, 29 to 95 DAA, and 29 to 119 DAA in 2016, 2017, and 2018, respectively. Final days of treatments coincides with industry harvest day. ED irrigation resumed and LD irrigation ceased at 58, 64, and 77 DAA in 2016, 2017, and 2018 respectively. These days were chosen for treatment application as they were synchronized to 50% colour change of berries or at growth stage 83 on the Biologische Bundesanstalt Bundessortenamt und Chemische Industrie (BBSH) scale for grapes (Lorenz et al., 1994).

The treatments matched prescribed Ψ_{leaf} targets and remained above severe deficit levels (Figure 4d-f; Table 2). DI application significantly affected Ψ_{leaf} when applied before and after veraison. Later season Ψ_{leaf} was typically lower than pre-veraison values. Also, water deficit was more severe in 2017 than in other years. No interaction was observed between DI treatments and years signaling that DI application was equal across all growing seasons. Rain events in July reduced water deficit in ED and PD in 2016 and 2018, however, this did not inhibit achieving targeted Ψ_{leaf} values. Due to extended environmental drought in 2017 – none to minute precipitation events from June to October – severe water deficit had to be avoided by the application of additional irrigation to the DI treatments.

Table 2. Two-way ANOVA of the effects of DI treatments and years on midday leaf water potential of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada.

	Midday Leaf Water Potential (MPa)					
DI Treatment	**Before Veraison**[†]			**After Veraison**[‡]		
p - value	$< 2.00 \cdot 10^{-16}$			$< 2.00 \cdot 10^{-16}$		
	Mean	SE		Mean	SE	
CN	-0.74	0.02	a	-0.73	0.04	a
ED	-1.07	0.03	b	-0.78	0.04	a
LD	-0.72	0.02	a	-1.25	0.04	b
PD	-1.03	0.03	b	-1.24	0.04	b
Year p - value	$8.95 \cdot 10^{-9}$			$1.17 \cdot 10^{-4}$		
2016	-0.88	0.03	a	-0.94	0.04	a
2017	-0.96	0.03	b	-1.14	0.05	b
2018	-0.83	0.03	a	-0.89	0.04	a
DI x Year Interaction p - value	n.s.			n.s.		

[†]*Before* Veraison *is defined as the three to four* Ψ_{Leaf} *measurements preceding* veraison *(three-year average: -22 to -2 days from* veraison*) of the respective year*

[‡] *After* Veraison *is defined as the three to four* Ψ_{Leaf} *measurements proceeding* veraison *(three-year average: 11 to 35 days from* veraison*) of the respective year*

Leaf area remained consistent through all the trial years (Table S3). Averaging across treatments and years, LA per vine was ~6 m^2. Treatments had no effect on LA during any point of the season, when considering all three seasons together. However, there was a significant interaction effect between the treatments and years. Separating the harvest time point by seasons reveals that in 2016 there was a significant effect of the treatment (Figure 5). *Post-hoc* testing revealed CN maintained larger LA (~ +50%) than any DI treatment at harvest in 2016. Notably, 2017 and 2018 seasons demonstrated no significant effect from treatments on LA at harvest.

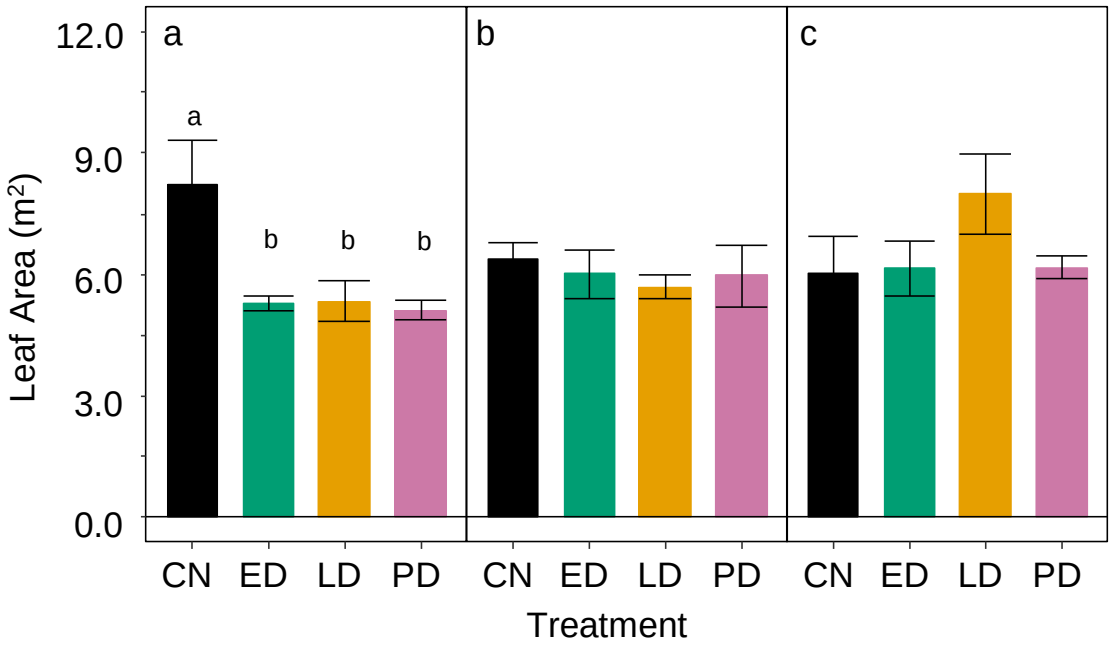

Figure 5: Total vine leaf area of Gewürztraminer grapevines exposed to deficit irrigation treatments in 2016 (a), 2017 (b), and 2018 (c) seasons. Leaf area was measured within two weeks prior to harvest (92, 81, and 113 DAA in 2016, 2017, 2018, respectively). Error bars indicate standard. Different letters above bars denote significance ($p < 0.05$) among treatments, within years, as determined by *post-hoc* Tukey's HSD.

In 2018, the canopy structure (leaf layers, proportion of exposed fruit, proportion of gaps) was estimated by point quadrat analysis via horizontal probe insertion at the fruit zone level (Figure 6a-c). Leaf layer number was similar among treatments at all sampling dates (Figure 6a). Fruit was differentially exposed at 89 DAA (Figure 6b) among treatments; however, only marginal differences were observed between LD and ED ($p = 0.050$), as well as LD and PD ($p = 0.100$). No other differences were observed. Otherwise, the proportion of exposed clusters increased across all treatments from the early season (~40% of exposed tissues at 49 DAA) to the late season (~70% of exposed tissues at 119 DAA). Additionally, treatments had no effects on the proportion of gaps in the fruit zone (Figure 6c).

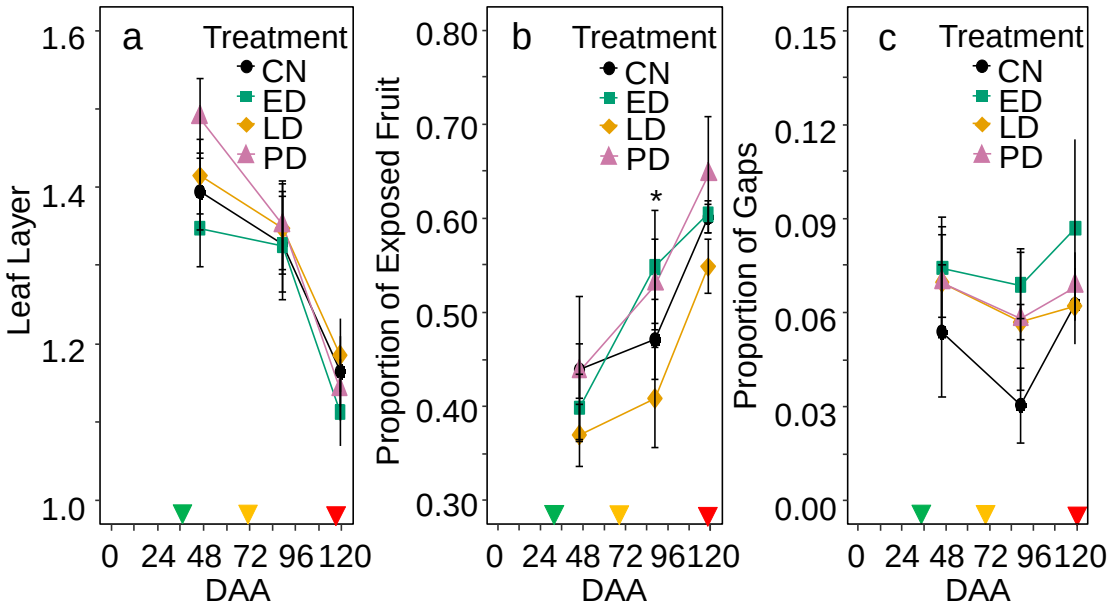

Figure 6: Average (a) leaf layer number, (b) proportion of exposed fruit, and (c) proportion of gaps in Gewürztraminer grapevine fruit-zone canopy. Error bars indicate the standard error. Green, yellow, and red arrows indicate dates of irrigation treatment application, veraison, and commercial harvest, respectively. Asterisks indicate significant ($p < 0.05$) differences among treatments according to one-way ANOVA within sampling dates.

Regardless of the year, photosynthesis, transpiration, and stomatal conductance values (Table 3) closely mirrored Ψ_{leaf} trends throughout the season. This is most evident when comparing Figure 4a-c with Figure 7a-c. DI application reduced these gas exchange parameters by 25-50%. Photosynthetic rates of DI treatments recovered to CN levels with substantial rain or irrigation (for ED after veraison) mirroring the Ψ_{leaf} recoveries. Significant interactions between the DI treatments and years were observed for transpiration and stomatal conductance. Photosynthesis rates demonstrated a marginal interaction effect. These interactions likely arose from differing environmental conditions among the years such as variable early season temperature/heat accumulation and rainfall.

Table 3. Two-way ANOVA of the effects of DI treatments and years on photosynthesis rate, transpiration rate, and stomatal conductance of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada.

	Photosynthesis Rate (μmol CO_2 per m^2 per s)						**Transpiration Rate (mmol H_2O per m^2 per s)**				**Stomatal Conductance (mol H_2O per m^2 per s)**			
	Before Veraison[†]			**After Veraison**[‡]			**Before Veraison**		**After Veraison**		**Before Veraison**		**After Veraison**	
DI Treatment p – value	$< 2.00 \cdot 10^{-16}$			$2.56 \cdot 10^{-13}$			$< 2.00 \cdot 10^{-16}$		$< 2.00 \cdot 10^{-16}$		$< 2.00 \cdot 10^{-16}$		$1.23 \cdot 10^{-14}$	
	Mean	SE		Mean	SE		Mean	SE	Mean	SE	Mean	SE	Mean	SE
CN	11.52	0.49	a	10.80	0.67	a	4.18	0.21	3.73	0.20	0.20	0.02	0.17	0.01
ED	5.29	0.46	b	10.51	0.56	a	1.73	0.13	3.60	0.19	0.07	0.01	0.16	0.01
LD	12.95	0.53	a	5.79	0.37	b	4.91	0.28	1.73	0.14	0.24	0.02	0.09	0.01
PD	6.69	0.62	b	5.70	0.48	b	2.16	0.25	1.73	0.14	0.09	0.01	0.08	0.01
Year p – value	$3.16 \cdot 10^{-4}$			n.s.			$5.03 \cdot 10^{-7}$		$3.59 \cdot 10^{-7}$		$< 2.80 \cdot 10^{-10}$		$< 2.00 \cdot 10^{-16}$	
2016	9.28	0.69	a	7.61	0.66		3.83	0.32	3.25	0.28	0.15	0.02	0.14	0.01
2017	7.99	0.68	b	8.09	0.37		2.74	0.29	2.52	0.14	0.11	0.01	0.11	0.01
2018	10.46	1.10	a	8.90	0.78		2.84	0.30	2.33	0.20	0.22	0.03	0.12	0.01
DI x Year Interaction p - value	0.0788			0.0864			0.0492		$7.71 \cdot 10^{-5}$		0.0232		$4.02 \cdot 10^{-4}$	

[†] *Before* Veraison *is defined as the three to four* Ψ_{Leaf} *measurements preceding* veraison *(three-year average: -14 to -5 days from* veraison*) of the respective year.*

[‡] *After* Veraison *is defined as the three to four* Ψ_{Leaf} *measurements proceeding* veraison *(three-year average: 17 to 36 days from* veraison*) of the respective year.*

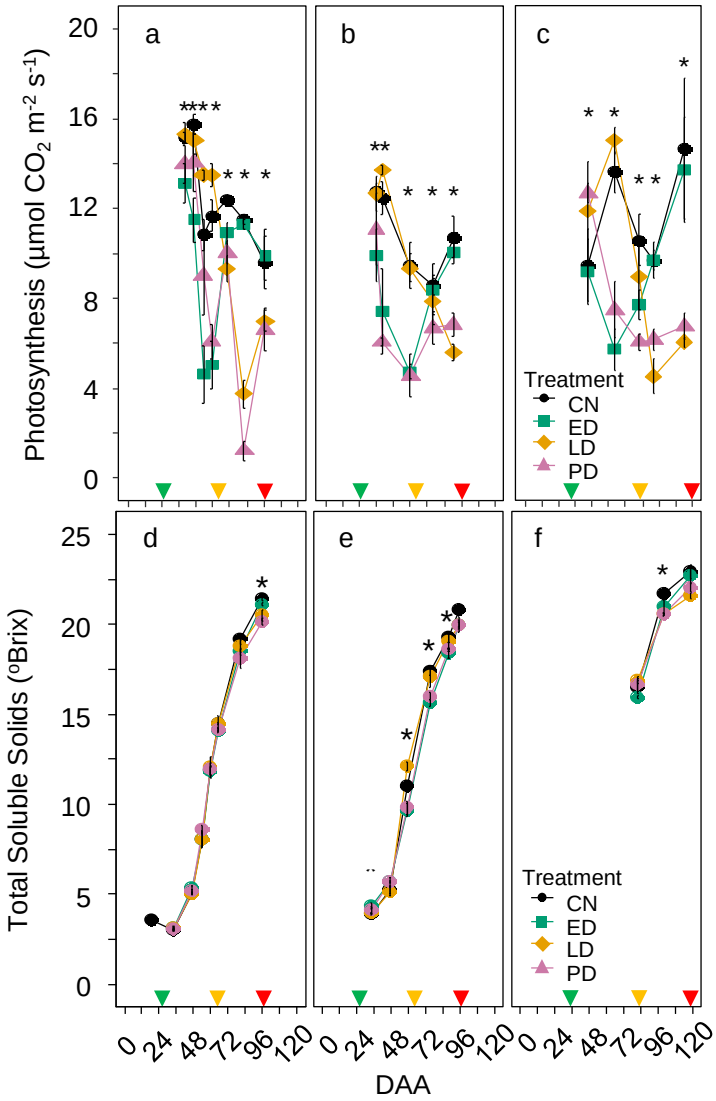

Figure 7: (a-c) Leaf photosynthetic rates of Gewürztraminer grapevines exposed to deficit irrigation treatments throughout 2016 (a), 2017 (b), and 2018 (c). (d-f) Total soluble solids in berry juice of Gewürztraminer grapevines exposed to deficit irrigation treatments throughout 2016 (d), 2017 (e), and 2018 (c) seasons. Error bars indicate the standard error. Green, yellow, and red arrows indicate dates of irrigation treatment application, veraison, and commercial harvest, respectively. Asterisks indicate significant ($p < 0.05$) differences among treatments according to one-way ANOVA within years and sampling dates.

2.3.3 Yield Parameters

Treatment and year effects were significant on cluster weight, berry weight, and yield (Table 4). The number of clusters per vines and berries per cluster were unaffected by DI, but the year had a significant effect. No interactions were observed for any of the yield-related

parameters, except for berry weight. In 2016, relative to CN berry weights (1.55 ± 0.04 g), ED and LD were reduced by ~10% and PD by ~20% (1.35 ± 0.04 g, 1.35 ± 0.03 g, and 1.26 ± 0.06 g for ED, LD, and PD, respectively). In 2017 and 2018, LD had similar weights to CN (1.32 ± 0.04 g and 1.29 ± 0.02 g in 2017; 1.57 ± 0.04 g and 1.60 ± 0.02 g in 2018 for LD and CN, respectively), while ED and PD (1.00 ± 0.06 g and 1.00 ± 0.07 g in 2017; 1.30 ± 0.04 g and 1.30 ± 0.04 g in 2018 for ED and PD, respectively) had the berry weight reduced by ~20% compared to CN.

Table 4. Two-way ANOVA of DI treatments and year effects on yield parameters of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada.

	Berries per Cluster			**Berry Weight (g)**			**Clusters per Vine**			**Cluster Weight (g)**			**Yield (t/ha)**			**Pruning Weights (kg)**		
DI Treatment p – value	0.0745			$9.03 \cdot 10^{-10}$			n.s.			$6.80 \cdot 10^{-6}$			$2.35 \cdot 10^{-6}$			n.s.		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
CN	105	5		1.48	0.04		25	2		154.0	7	a	12.40	0.70	a	1.16	0.15	
ED	92	4		1.21	0.05		24	1		110.8	5	b	8.82	0.53	b	1.08	0.11	
LD	97	3		1.41	0.04		26	1		136.8	6	ab	11.68	0.75	a	1.12	0.13	
PD	103	6		1.19	0.05		23	1		120.9	6	b	9.36	0.59	b	1.05	0.14	
Year p – value	$3.03 \cdot 10^{-5}$			$2.61 \cdot 10^{-16}$			$7.54 \cdot 10^{-6}$			$3.39 \cdot 10^{-5}$			$3.64 \cdot 10^{-7}$			$7.70 \cdot 10^{-15}$		
2016	86	2	b	1.38	0.03		21	1	c	118.2	5	b	8.39	0.37	b	0.79	0.03	b
2017	108	4	a	1.15	0.05		28	1	a	123.9	6	b	11.43	0.68	a	1.67	0.07	a
2018	104	4	a	1.44	0.04		24	1	b	149.8	7	a	11.89	0.55	a	0.85	0.03	b
DI x Year Interaction p – value	n.s.			0.0364			n.s.			n.s.			n.s.			n.s.		

Yield and cluster weight were not reduced by LD (relative to CN), while treatments that generated early WD (ED and PD) had yield and cluster weight reduced by ~25%. Additionally, the yields across all treatments were smaller in 2016 compared to 2017 and 2018. However, cluster weights were similar between 2016 and 2017 and were larger in 2018. The average cluster number was lowest in 2016 followed by 2018 and 2017. The number of berries per

cluster mirrored yield trends from year-to-year. Both berries per cluster and yield were lower in 2016, than in 2017 and 2018.

Pruning weights (that are considered proxy for canopy growth) were unaffected by DI treatments, but were affected by the year. No interaction effect was observed. Pruning weights were equal in 2016 and 2018, while they were twice as large in 2017. This did not align with trends in LA or patterns in environmental factors.

2.3.4 Berry Composition

The effect of DI treatments on sugar accumulation (TSS) throughout the season was small, but evident close to and at harvest (Figure 7 d-f; Table S4). Later application of deficit irrigation (LD and PD) reduced TSS in all seasons at harvest (Table 5). Compared to CN vines, LD and PD reduced TSS accumulation by 1 °Brix (4%). ED treatments did not reduce TSS accumulation relative to CN. TSS accumulation was highest in 2018 likely due to a commercial harvest date that was two weeks later than the former years.

Table 5. Two-way ANOVA of DI treatments and year effects on berry composition at harvest of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada.

	Total Soluble Solids (°Brix)			**Titratable Acidity (g/L)**			**Juice pH**		
DI Treatment p - value	$9.06 \cdot 10^{-5}$			0.00800			0.0436		
	Mean	SE		Mean	SE		Mean	SE	
CN	21.71	0.30	a	5.64	0.15	a	3.38	0.03	
ED	21.25	0.37	ab	5.86	0.14	ab	3.44	0.02	
LD	20.68	0.26	b	5.86	0.18	ab	3.43	0.03	
PD	20.73	0.30	b	6.24	0.20	b	3.38	0.03	
Year p - value	$2.67 \cdot 10^{-12}$			$7.49 \cdot 10^{-8}$			$8.28 \cdot 10^{-8}$		
2016	20.79	0.15	b	6.22	0.10	a	3.44	0.02	a
2017	20.18	0.15	c	6.20	0.13	a	3.31	0.02	b
2018	22.31	0.20	a	5.29	0.09	b	3.48	0.01	a
DI x Year Interaction p - value	n.s.			n.s.			n.s.		

The later harvest date also affected harvest TA, but not pH. Titratable acidity in 2018 was ~1 g/L less than 2016 and 2017 (Table 5). Averaging over the three years, PD had significantly higher TA than CN. Berry pH was affected by the treatments, but differences among treatments were small. All treatments had a pH of ~3.4 at harvest. However, pH was lower in 2017 compare to the other years by ~0.1 pH. No interactions between the treatment and the year were observed for any berry composition parameter. Within each season, pH and TA only varied occasionally among treatments (Table S5 and S6).

2.3.5 Berry Terpene Composition

2.3.5.1 Detection and Identification of Terpenes in Gewürztraminer Berries

In all the years, free and bound terpenes were analysed at harvest (96, 96, and 115 DAA in 2016, 2017, and 2018, respectively) and 7-15 days before harvest (81, 88, and 100 DAA in 2016, 2017, and 2018, respectively). Twenty-two terpenoids were consistently observed in detectable and quantifiable amounts between freely volatile and glucoside bound fractions. The 22 compounds observed comprised of monoterpenes and monoterpenoid derivative alcohol, aldehyde, acid, ester, and oxide (Table S1). Additionally, an isomerable sesquiterpene (farnesene) was detected in the free fraction at harvest. Along with the farnesene isomers, methyl geranate was not detectable in the bound fraction. Hydroxylinalool was only detectable in the bound fraction.

Certain compounds were above LOD as confirmed by comparison to specific ion peaks of authentic standard IECs but were below LOQ. Citral-a was below LOQ in all three years in both free and bound fractions. In the free fraction, the only other compounds observed below LOQ were α-terpinol (2018) and γ-terpinene (2017 and 2018). More compounds were below LOQ in the bound fraction. The number of “quantifiable” compounds changed year-to-year in

the bound fraction. In 2016, of the 22 tracked compounds, 19 were detectable in the bound fraction of which 10 were below LOQ. In 2017 and 2018, 4 of 19 and 10 of 19 compounds were below LOQ, respectively. Citronellol, geraniol, hydroxylinalool, limonene, linalool, myrcene, and nerol were the only compounds above LOQ in all three years in the bound fraction.

2.3.5.2 Free Terpenes

In 2016 total free terpenes were tracked across the growing season (Figure 8); Figure 8a and 8b illustrate the evolution of total free terpene content (ng per berry) and concentration (ng / g FW).

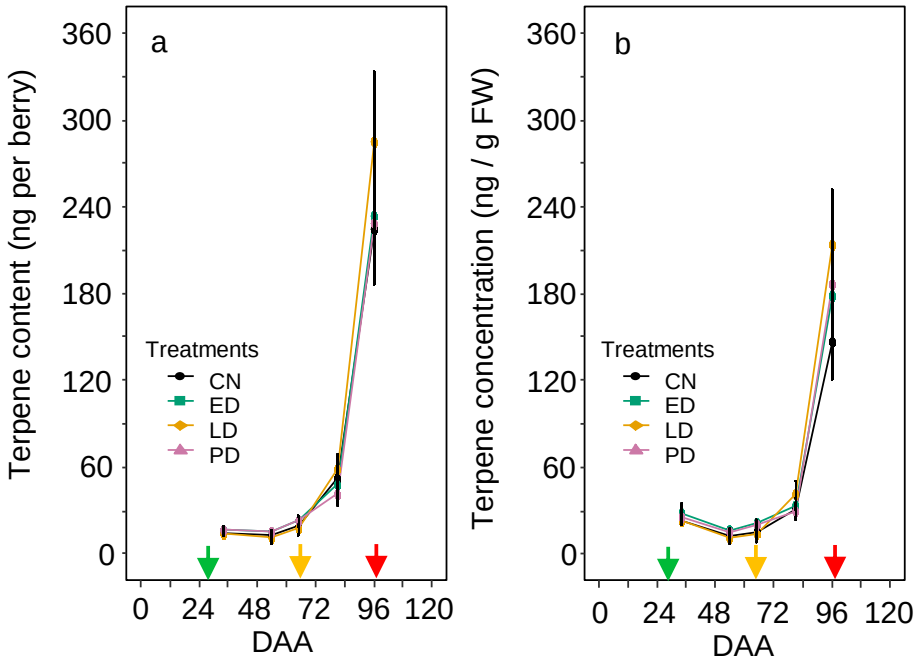

Figure 8: Evolution of total free terpene expressed as per berry (a) and per g of berry fresh weight (b) in Gewürztraminer throughout the 2016 season. Error bars indicate the standard error. Green, yellow, and red arrows indicate dates of irrigation treatment application, veraison, and commercial harvest, respectively. Asterisks indicate significant ($p < 0.05$) differences among treatments according by one-way ANOVA within years and sampling dates. No significant differences were observed among treatments.

In all treatments, the terpenes were approximately 3- to 5-fold less during the season than at harvest (96 DAA). LD had highest levels of free terpenes at harvest, but differences were not statistically significant. Two-way ANOVA considering treatment and year as factors revealed no

effect from treatments on the total content (ng per berry) and concentration (ng /g berry FW) at harvest (Table 6); p = 0.0827 and p = 0.124, respectively.

Table 6. Two-way ANOVA of DI treatments and year effects on free and glycoside bound terpenes of field-grown Gewürztraminer berries at harvest (96, 96, and 115 DAA in 2016, 2017, and 2018, respectively) in the Okanagan Valley, BC, Canada.

	Free Volatile Terpenes						**Glycoside Bound Terpenes**					
	[Total] (ng/g berry FW)			**Total per Berry (ng)**			**[Total] (ng/g berry FW)**			**Total per Berry (ng)**		
DI Treatment p – value	n.s.			0.0827			n.s.			n.s.		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE	
CN	177.09	26.75		245.07	45.80		5144.92	517.86		7041.84	658.85	
ED	179.15	23.14		216.57	30.17		6433.35	799.19		7327.85	918.75	
LD	223.21	22.93		288.49	39.40		6264.91	824.25		7983.99	941.82	
PD	203.21	28.90		243.19	39.97		7250.01	828.65		7706.88	809.56	
Year p – value	$8.22 \cdot 10^{-10}$			$1.09 \cdot 10^{-12}$			$1.33 \cdot 10^{-4}$			$3.80 \cdot 10^{-4}$		
2016	181.19	13.82	b	214.53	16.07	b	8336.47	623.37	a	9853.97	690.86	a
2017	118.27	9.50	c	128.88	11.35	c	5801.32	679.57	b	6152.16	667.22	b
2018	287.53	15.84	a	401.59	23.23	a	4682.11	211.95	b	6539.30	320.37	b
DI x Year Interaction p - value	n.s.			n.s.			n.s.			n.s.		

Year-to-year variation in free terpenes was large and significant ($p = 1.09 \cdot 10^{-12}$). This is supported by principal component analysis (PCA) (Figure S3a, c) which illustrates how variation in the terpene content is more influenced by the year factor than the treatment factor. Free terpene values were lowest in 2017 and highest in 2018. This was observed for both the total concentration and total content. No interaction effect was observed, so a year-by-year analysis is not necessary: however, Figure 9 illustrates total free terpene content and concentration as assessed at harvest for all three years (96, 96, and 115 DAA in 2016, 2017, and 2018, respectively). Neither free terpene concentration or content displayed statistically significant differences among treatments on a year-by-year basis. Total free terpenes at pre-harvest (81, 88, and 100 DAA in 2016, 2017, and 2018, respectively) were similar among treatments, but large differences were observed from year-to-year (Table 7).

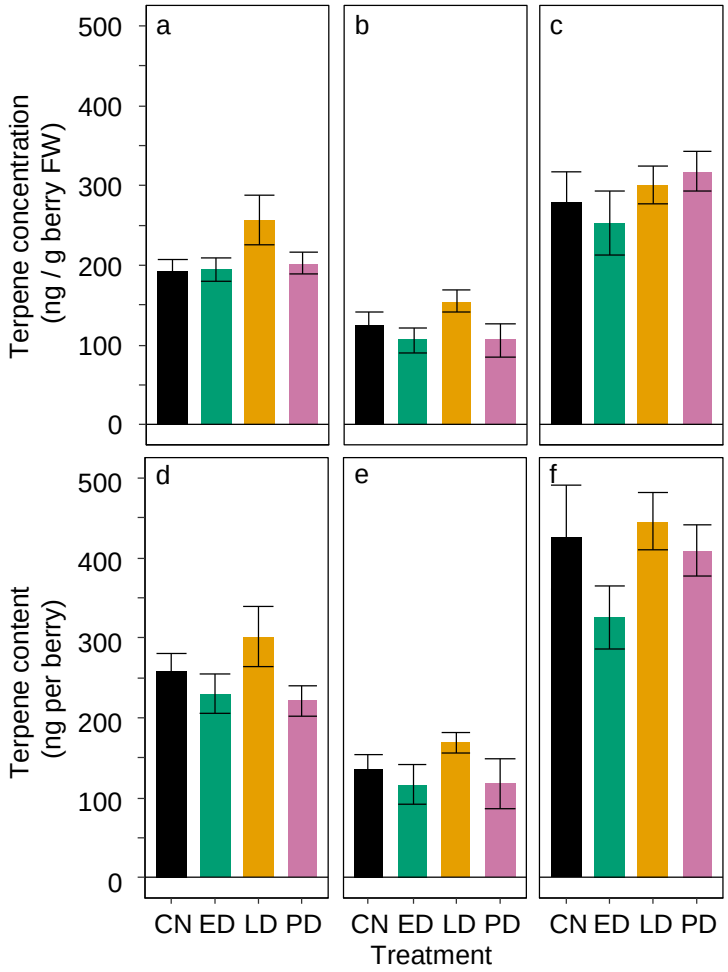

Figure 9: Total free terpenes expressed as per g of berry FW (a-c) and as per berry (d-f) at harvest in berries of Gewürztraminer grapevines exposed to deficit irrigation treatments in 2016 (a,d), 2017 (b,e), and 2018 (c,f) seasons. Error bars indicate the standard error. Two-way ANOVA utilizing treatment and year as factors revealed differences (Table 6), however, no significant differences were observed among treatments, within years according to one-way ANOVA test.

Table 7. Two-way ANOVA of DI treatments and year effects on free and glycoside bound terpenes of field-grown Gewürztraminer berries at pre-harvest (81, 88, and 100 DAA in 2016, 2017, and 2018, respectively) in the Okanagan Valley, BC, Canada.

	Free Volatile Terpenes						**Glycoside Bound Terpenes**					
	[Total] (ng/g berry FW)			**Total per Berry (ng)**			**[Total] (ng/g berry FW)**			**Total per Berry (ng)**		
DI Treatment p – value	n.s.			n.s.			0.0137			n.s.		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE	
CN	159.57	34.35		221.11	48.97		3857.18	257.14	b	5212.95	394.74	
ED	158.53	29.63		183.20	35.34		5291.89	287.07	a	5926.43	309.29	
LD	164.87	33.48		218.38	47.73		4870.58	371.73	ab	6351.94	542.94	
PD	138.31	27.08		159.88	33.63		4725.23	238.68	ab	5283.93	374.08	
Year p – value	$5.80 \cdot 10^{-12}$			$6.64 \cdot 10^{-12}$			n.s.			0.0684		
2016	33.77	2.99	c	43.48	3.85	c	-	-		-	-	
2017	179.92	17.73	b	203.17	24.49	b	4859.60	252.08		5330.00	277.13	
2018	252.27	14.04	a	340.28	21.33	a	4512.84	221.27		6057.63	308.57	
DI x Year Interaction p - value	n.s.			n.s.			n.s.			n.s.		

General trends in the free terpene profile are revealed by PCA (Figure S3a, c). As stated above, year-to-year effects had more influence on the terpene profile than the treatments when averaged over three years. The terpene profile in berries from 2016 maintained larger concentrations of α-terpinol and γ-terpinene and lower geranic acid concentrations. 2017 and 2018 berries had the opposite relationship. Rose oxide, nerol, citronellol, geraniol, and limonene concentrations were higher in 2016 and 2018, and lower for 2017 berries. All other compounds were to be present in higher concentrations in berries in 2018 and lower for 2017 berries, whereas middling values were found in 2016 samples.

Despite a larger influence from the year factor, DI treatments did display significant effects on some terpenes. The Figure 10 illustrates concentrations of individual terpenes at harvest plotted as z-scores scaled among treatments and within the compounds and years. Two-way ANOVA revealed significant treatment effects on free geraniol and citronellol without treatment-year interaction (Table S7 and S8).

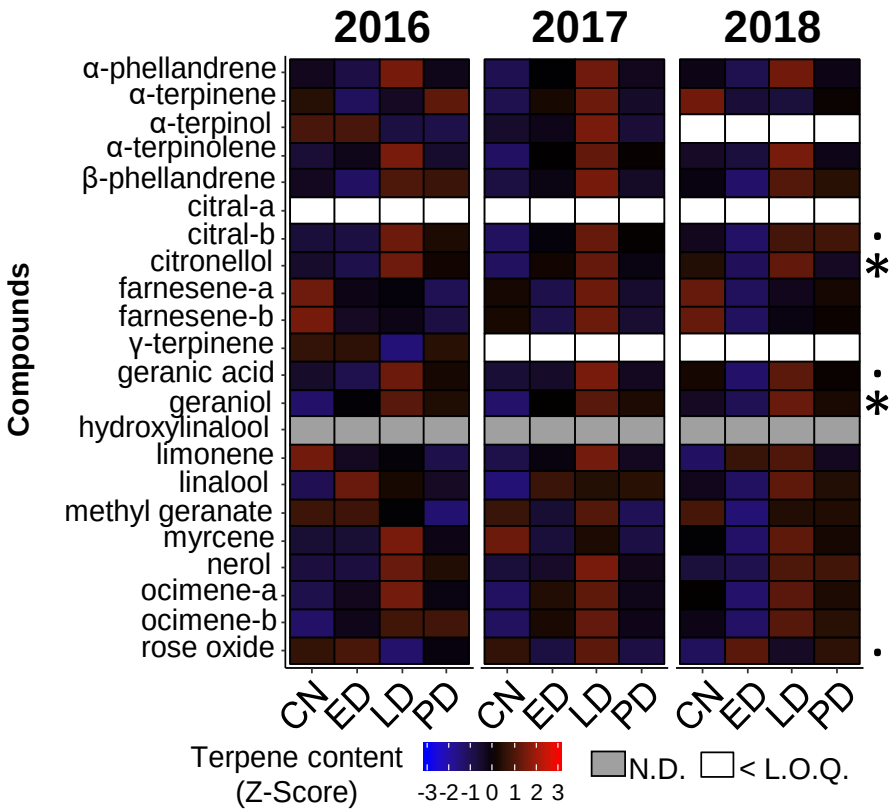

Figure 10: Heatmap of free terpene concentration at harvest in berries of grapevines exposed to deficit irrigation treatments in 2016, 2017, and 2018. Data are plotted as Z-scores of terpene concentration calculated for each terpene among treatments and within each year. Z-scores range from red (+3σ) through black (0σ) to blue (-3σ) and indicate the highest, average, and lowest values respectively. Two-way ANOVA results are indicated by " * " for $p < 0.05$ and " . " for $0.05 < p < 0.1$. Only compounds for which no interaction term between treatments and years was observed are reported as significantly affected by the deficit irrigation treatments.

Specifically, LD increased the concentration of both compounds relative to CN. A marginal effect on citral-b, rose oxide, and geranic acid concentrations from DI treatments without accompanying interaction was observed ($p = 0.0687$, $p = 0.0698$, and $p = 0.0622$, respectively) (Table S7). Farnesene-a and -b, α-terpinol, and methyl geranate were significantly affected by DI treatments but displayed an interaction effect between the treatment and year factors (Table S7 and S8). Due to interaction effects, analysis was completed with data separated by years for these four compounds (Table S7 and S8). The 2016 season demonstrated the most

differences among treatments. For both farnesene isomers, CN berries had a higher concentration than the other treatments. Methyl geranate was significantly decreased by PD compared to all other treatments and increased by ED compared to LD and PD. Concentration of α-terpinol was decreased in LD and PD berries relative to CN and ED. The 2017 season demonstrated little differences between the treatments, farnesene-a and -b were marginally affected while methyl geranate and α-terpinol were increased by LD relative to PD. In 2018, only methyl geranate concentration was decreased by ED relative to CN and PD.

2.3.5.3 Bound Terpenes

Total bound terpene concentration at harvest was similar between 2017 and 2018 (~5000 ng / g berry FW) and generally higher in 2016 (~8000 ng / g berry FW) (Figure 11; Table 6). The year factor was significant ($p = 1.33 \cdot 10^{-4}$), while treatment factor had no effect on both the concentration and the amount per berry of total bound terpenes. This was corroborated by PCA (Figure S3 b, d). In general terms, berries from 2016 correlated with higher concentrations of α-terpinol and rose oxide and with lower concentrations of hydroxylinalool, geranic acid, geraniol, nerol, citronellol, and linalool relative to other years. 2018 berries had the opposite relationship. All other compounds (myrcene, ocimene, limonene, etc.) showed higher concentrations in berries from 2017 and lower concentrations for 2018. Interaction effects between treatment and year factors were present in compounds significantly affected by DI treatments (α-terpineol, ocimene-a, and γ-terpene) with *post-hoc* tests only revealing higher γ-terpene and ocimene-b in CN compared to ED (Table S9; Figure 12).

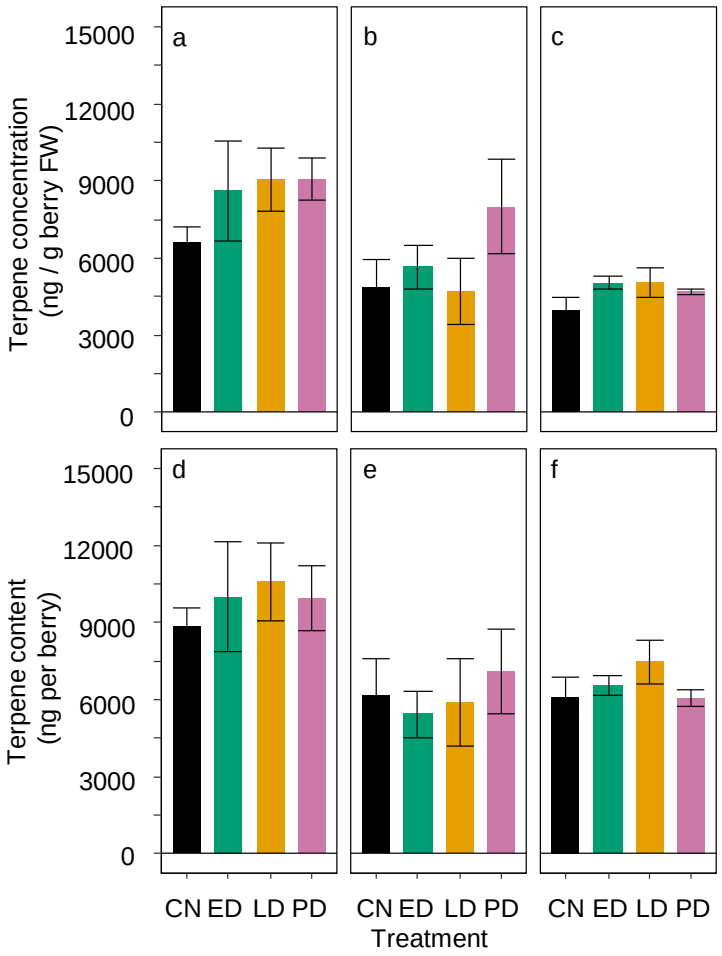

Figure 11: Total glycoside bound terpenes expressed per g of berry FW (a-c) and per berry (d-f) at harvest in berries of Gewürztraminer grapevines exposed to deficit irrigation treatments in 2016 (a,d), 2017 (b,e), and 2018 (c,f) seasons. Error bars indicate the standard error. No significant differences were observed among treatments, within years accordingly to one-way ANOVA tests.

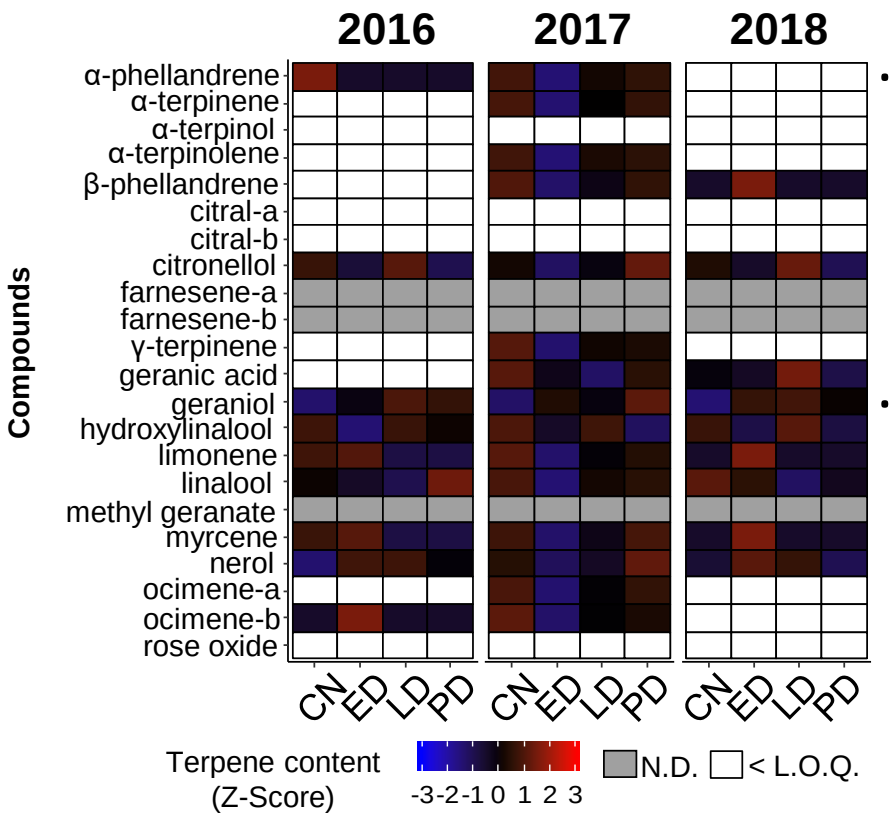

Figure 12: Heatmap of glycoside bound terpene concentration at harvest in berries of grapevines exposed to deficit irrigation treatments in 2016, 2017, and 2018. Data are plotted as Z-scores of terpene concentration calculated for each terpene among treatments and within each year. Z-scores range from red (+3σ) through black (0σ) to blue (-3σ) and indicate the highest, average, and lowest values respectively. Two-way ANOVA results are indicated by " * " for $p < 0.05$ and " . " for $0.05 < p < 0.1$. Only compounds for which no interaction term between treatments and years was observed are reported as significantly affected by the deficit irrigation treatments

Total bound terpenes at pre-harvest were analysed only for 2017 and 2018 samples. Unfortunately, the frozen powder from 2016 pre-harvest samples was damaged via thawing and refreezing prior to analysis, thus analysis was not conducted on these compromised samples (Table 7). Total bound terpenes were significantly affected by DI treatments ($p = 0.0137$) with *post-hoc* tests showing that bound terpenes were more concentrated in ED than in CN berries. Total bound terpenes were unaffected between years at pre-harvest (88 and 96 DAA in 2017 and 2018, respectively), however, most individual bound terpenes at pre-harvest were significantly affected between years and demonstrated interactions between treatment and year factors (Table S11 and S12). Only γ-terpinene demonstrated significant differences among treatments on year-

separated data in 2017 showing that γ-terpinene was higher in CN berries than ED berries. Comparisons among treatments in other years or for other compounds with significant interactions were equivalent (Table S11 and S12).

As for individual terpenes at pre-harvest, all detected compounds were affected by year effects. The geraniol concentration (ng per g FW) was not affected by DI treatments; however, the p-value was marginally significant ($p = 0.0527$) without interaction (Table S13). The geraniol content (ng per berry) was unaffected by DI treatments (Table S14). All compounds with significant interactions were tested on their year-separated data with one-way ANOVA followed by *post-hoc* TukeyHSD (Table S13 and S14). Only γ-terpinene demonstrated significant differences among treatments on year-separated data, specifically in 2017 showing that ED berries were more concentrated than CN berries in γ-terpinene. All other comparisons were equivalent.

2.3.6 Gene Expression

2.3.6.1 MEV Pathway and GPP Synthase

Expression profiles of terpene related genes were investigated only in 2016 samples. All examined genes were assessed throughout the growing season with RNA extracted from the same berry samples from which terpene profiles were analyzed. Early terpene biosynthesis genes – *DXS1*, *DXS3*, and *HDR* – displayed three different profiles (Figure 13). *DXS1* decreased and plateaued as the season progressed (Figure 13a). *DXS3* expression initially decreased and then did not vary during the season (Figure 13b). *HDR* expression steadily increased with berry ripening (Figure 13c). *GPPS* expression levels did not vary largely throughout the season and among treatments (Figure 13d). *HDR*, *DXS1*, *DXS3*, and *GPPS* expression were not affected by treatments; only marginal effects were observed in HDR and DXS3 at 81 and 96 DAA and 96

DAA respectively. Moreover, the expression of these genes did not appear to strongly (R^2 < 0.90) correlate with the level of any free or bound terpene nor with any other gene (Figure S7). For example, *DXS1* expression was observed to be negatively correlated with free methyl geranate and bound nerol concentration (R = -0.25 and -0.51, respectively) (Figure S7b).

2.3.6.2 Monoterpene-Related Genes

Monoterpenes are primarily synthesized by TPSs belonging to two subfamilies, TPS-b and TPS-g (Martin et al. 2010). In this study, the expression of specific TPS-b genes (*TPS20, TPS27*, *TPS31, TPS34/35, TPS38, TPS44*, *TPS45*, and *TPS47),* and TPS-g genes (*TPS52*, *TPS54, TPS56*, *TPS57, TPS58, TPS61*, *TPS63*, and *TPS69)* was assessed in preliminary tests at three time points (34, 65, and 96 DAA in 2016) from pooled cDNA from all treatments (Table S15).

Genes that were expressed in the mid to late season were selected and their expression was assessed at all time points on all treatments as the patter of expression of these genes would likely correlate with the monoterpene accumulation. *TPS14, TPS20, TPS27, TPS31*, *TPS44, TPS47*, *TPS52, TPS58, TPS61,* and *TPS69* demonstrated none to low (relative expression < 0.0005) relative expression (Table S15).

TPS45, TPS56, *TPS57,* and *TPS63* were moderately (relative expression ~ 0.1) expressed just after anthesis but had no or low expression during ripening and at harvest (Table S15). *TPS34/35*, *TPS38*, and *TPS54* were the only genes that increased in expression at veraison or approaching harvest. The expression profile of *TPS34/35*¸ genes that codifies for β-ocimene synthase, was low early in the season and increased after veraison (Figure 13e). At 81 and 96 DAA, all DI treatments were down-regulated relative to CN. The expression profile of *TPS34/35* correlated negatively with the concentration of ocimene-a and ocimene-b isomers (R = -0.26 and R = -0.27, respectively). Gene expression of *TPS38*, a gene that codifies for a (*E*)-β-ocimene and

myrcene synthase, generally unvaried until 96 DAA when it increased in expression; however, some marginal differences among treatments were observed at 54 DAA (Figure 13f). *TPS38* correlated negatively with bound geraniol, citronellol, and nerol concentrations (R = -0.73, -0.52, and -0.60, respectively) (Figure S7b).

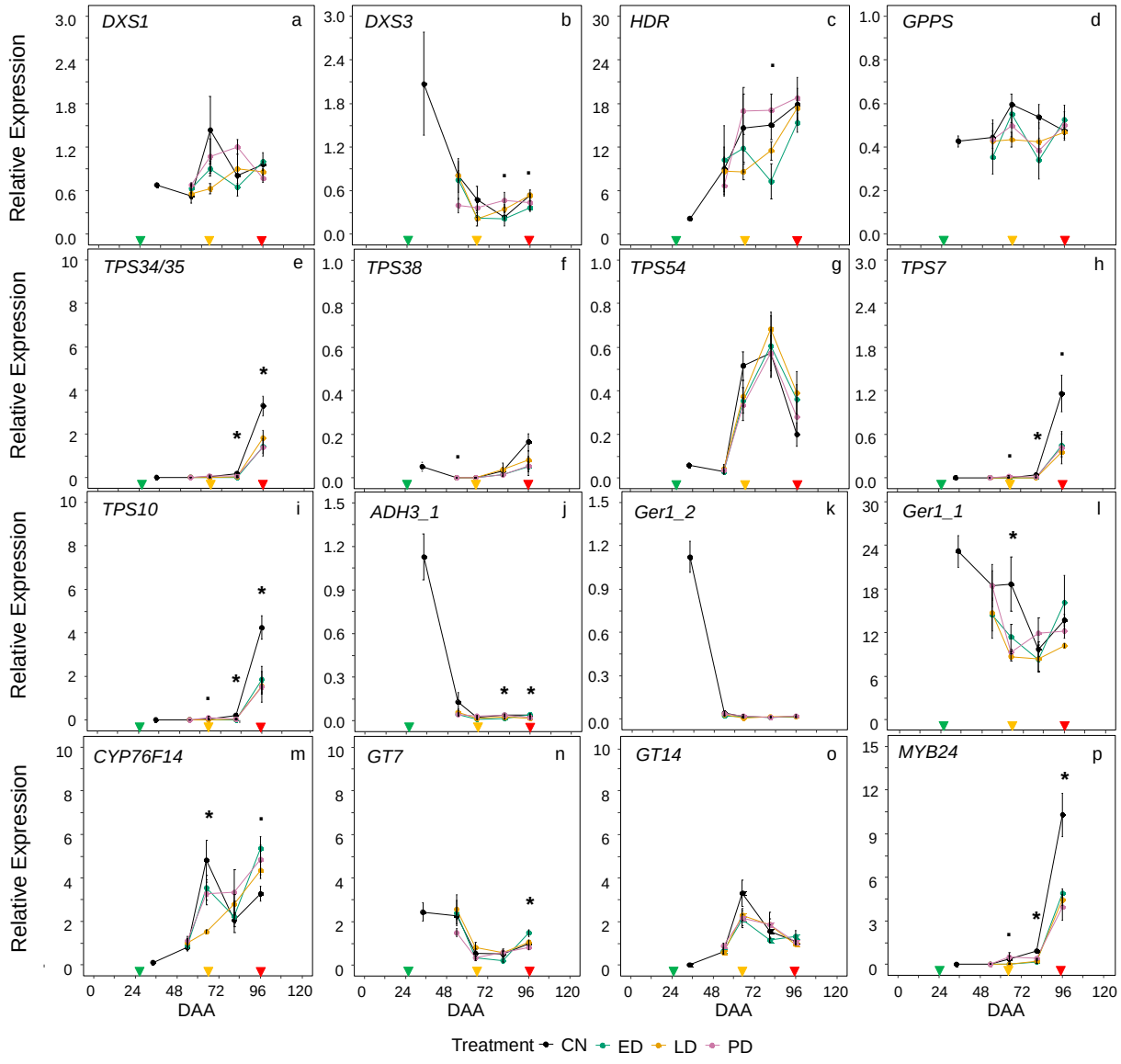

Figure 13: Evolution of select terpene biosynthetic gene expression in Gewürztraminer berry relative to AP47 expression throughout the 2016 season under various DI treatments. Standard error is indicated by error bars. Green, yellow, and red arrows indicate dates of early treatment application, application at veraison and commercial harvest. Legend at the bottom of the figure indicates coding of colours of point and lines to treatments. Asterisks and dots above mean relative expression values indicate significant ($p < 0.05$) and marginal ($0.05 < p < 0.1$) differences between means as determined by one-way ANOVA within genes, dates and between treatments.

The expression of *TPS54*, a gene that codifies for (3S)-linalool/(*E*)-nerolidol synthase, peaked during ripening, at 81 DAA, and no differences among DI treatments were observed (Figure 13g). *TPS54* did not correlate with the accumulation of any free terpene (Figure S7a).

2.3.6.3 Sesquiterpene-Related Genes

The subfamily TPS-a has been characterized to primarily generate sesquiterpenes. Two TPS-a genes were examined in this study; *TPS07* and *TPS10* which were characterized by Martin et al. (2010) as recombinant germacrene D and (*E*)-α-bergamotene, respectively. These were chosen as they were expressed to high level in the berry and had been shown to be modulated under drought conditions in Tocai Friulano (Savoi et al., 2016). The expression of these genes strongly increased during the late stages of ripening. *TPS07* was negatively modulated at pre-harvest ($p = 0.00949$; 81 DAA) but not at harvest ($p = 0.0634$; 96 DAA) by DI treatments (Figure 13h). *Post-hoc* testing revealed DI berries had a significantly lower expression than CN at 81 DAA. Similarly, *TPS10* was modulated negatively by DI treatments at 81 and 96 DAA (Figure 13i). Both genes demonstrated higher expression in CN, which correlates with higher free farnesene-a and -b concentration at harvest in CN berries. Furthermore, the expression profiles of these TPS genes and the accumulation of farnesene isomers correlated positively ($p < 0.05$; $R = 0.28$ and 0.37 and $R = 0.31$ and 0.38 for *TPS07* and *TPS10*, respectively).

TPSs such as *TPS47, TPS54, TPS56, TPS57, TPS58, TPS61*, and *TPS63* have been shown to generate sesquiterpenes when incubated with FPP despite being primarily monoterpene synthases (Martin et al., 2010). Preliminary tests on *TPS47, TPS58*, and *TPS61* at three time points from pooled cDNA from all treatments demonstrated none to low (< 0.0005-fold) relative expression at all stages. *TPS56*, *TPS57*, and *TPS63* only showed expression at 0.01 to 0.1 levels at 34 DAA with no expression during ripening and at harvest. As a result, these genes were not

tested further. *TPS54* was examined in this study as seen above. *TPS54* did not correlate with the concentration of farnesene-a or farnesene-b (Figure S7a).

2.3.6.4 Other Related Genes

Following terpene generation, several genes are known to act upon the initial terpenes causing further modification through isomerization, reduction/oxidation, dehydrogenation, and glycosylation. Several of these genes were examined: predicted geraniol dehydrogenases *ADH3_1* (XM_002279796.4; Wong et al., 2017) and *ADH3_2* (XM_002279682.2; Wong et al., 2017), predicted geranial reductases *Ger1_1* (XM_002285116.2; Wong et al., 2017) and *Ger1_2* (XM_002272235.3; Wong et al., 2017), glycoside transferases *GT7* (XM_002276510.2; Li et al., 2017) and *GT14* (XM_002285734.2; Wen et al., 2015), and *CYP76F14* (XM_010659727.2; Ilc et al., 2017). The predicted genes were identified based on homology with *CpADH3* and *CpGER1* genes which were highly expressed in the flowers of the orchid species *Caladenia plicata* (Wong et al., 2017). These genes are proposed to catalyze the conversion of geraniol into β-citronellol (Xu et al., 2017). Additionally, a predicted regulator of terpene biosynthesis, *MYB24* (NP_001268062.1; Carbonell-Bejerano et al., 2014; Savoi et al., 2016), was tested for expression.

The predicted gene *ADH3_2* showed little to no expression in the later part of the season and is not shown in Figure 13 (see Table S15). *ADH3_1* and *GER1_2* had similar expression profiles through the 2016 season (Figure 13 j, k), however only *ADH3_1* was differentially expressed among treatments during 2016, at 81 DAA ($p = 0.0499$) and 96 DAA ($p = 0.0263$) but *post-hoc* testing revealed marginal pairwise differences among treatments. *GER1_1* was highly expressed throughout the season (Figure 13l) and differentially expressed at 65 DAA with CN demonstrating higher expression than LD and PD (Table S16).

CYP76F14 steadily increased from 34 DAA until 96 DAA (Figure 13m); differences among treatments were observed at 65 DAA with CN berries showing a higher *CYP76F14* expression than LD (Table S16).

GT7 expression was high at 34 and 54 DAA, decreased at 65 and 81 DAA, and increased again at harvest, 96 DAA (Figure 13n). At harvest, *GT7* expression was higher in ED than PD berries (Table S16). Conversely, *GT14* was not expressed prior to 40 DAA then peaked in expression and at 65 DAA, decreasing steadily until harvest; no differences among treatments were observed (Figure 13o).

The expression of *MYB24* correlated with the expression profile of *TPS07*, *TPS10*, *TPS14*, *TPS34/35*, and *TPS38* (Figure 13p, Figure S7a). As with some of these TPS genes, *MYB24* expression was higher in CN berries than in the ones of the DI treatments at 81 DAA and harvest. Methyl geranate and both farnesene accumulation patterns were significantly correlated ($p < 0.05$, $R = 0.27$, 0.37, and 0.41) with *MYB24* expression pattern (Figure S7a).

2.4 Discussion

The effects of deficit irrigation have not been thoroughly explored in white grape varieties grapes and this study provides key observation into one of the most cultivated white grape varieties in Canada and BC. Water deficit and deficit irrigation on Gewürztraminer vines were investigated in the context of effects on shoot / vegetative growth (Hardie and Martin, 2000; Reynolds et al., 2005), yield, berry composition, and total free/potentially volatile terpenes (Reynolds et al., 2005). However, these parameters were investigated in context of DI applied ~30 to 60 DAA (ED), ~60 – 100 DAA (LD), and ~30 – 100 DAA (PD) but not in comparison with a well irrigated treatment (Reynolds et al. 2005), that well represents a standard irrigation strategy for white grape production. Moreover, this is the first study that combines terpene profiling, gene expression analysis, and physiological measurements such as leaf gas exchange.

DI reduced water inputs compared to well irrigated vines: 50% reduction with PD and 30% reduction with ED and LD. Similar irrigation ratios were seen in irrigation experiments on Cabernet Sauvignon (Bravdo et al., 1985), Cabernet Franc (Matthews and Anderson, 1988), Syrah (Ojeda et al., 2002), Sauvignon Blanc (Wample and Smithyman, 2002), Thompson Seedless (Williams et al., 2010), and Tocai Friulano (Savoi et al., 2016). Tracking and reporting irrigation volumes – especially in field-grown studies – provides the industry with relevant information as well as tracks a parameter than can be considered for economic assessments.

Photosynthesis was affected by DI application and generally unaffected from year-to-year. Measurements being prioritized to days with more clear weather. This was not always possible in 2017 and 2018 as evident by PAR values (Figure S5). Application of DI treatments reduced the rates of gas exchange (photosynthesis rate, transpiration rate, stomatal conductance); the reduction significantly correlated to the reduction in Ψ_{leaf} (Figure S6). This was expected as

similar results were documented in studies by Poni et al. (1993) in greenhouse Pinot Noir, Schultz (2003) and Soar et al. (2006) in Grenache and Syrah, and Martinez-Lüscher et al. (2015) in Tempranillo. Overall, physiological parameters were affected as expected from DI with lower photosynthesis, transpiration, and stomatal conductance being associated with lower midday-leaf water potentials.

Sugar accumulation and acid degradation were affected by DI treatment. A loss of ~1 Brix by harvest was observed in LD and PD berries. These results reflect the diminished rates of photosynthesis seen in DI vines. Since sugar accumulation mainly occurs after the onset ripening (Thomas et al., 2006), sugars in ED berries were unaffected relative to CN. The effects of water stress on sugar concentration are known to be cultivar-dependent. Poni et al. (1993) reported less °Brix in Pinot Noir musts produced from grapes subjected to LD relative to CN. Schultz (1998) reported larger decreases in TSS for Grenache compared to Syrah when they were subjected to identical water stress conditions. In Muscat varieties and Castelão, Santos et al. (2003, 2005, 2007) observed no differences in sugar between full irrigation and various irrigation regimes (no irrigation/rain fed, partial rootzone drying, and deficit irrigation). Similarly, Wample and Smithyman (2002) saw no differences in TSS with Sauvignon Blanc under ED, LD, and PD conditions compared to CN. Post-veraison is when phloem input into the berry is dominant over xylem influx (Greenspan et al., 1996) and if phloem sap itself receives reduced sugar input from stunted photosynthesis, as in DI vines, the berry intake of sugar may be impaired.

Berry juice TA at harvest was primarily affected by PD application. This suggests that DI did not influence berry temperature during the season, since higher berry temperatures are undoubtedly demonstrated to correlate with decreased malic acid content and thus lower TA (Ruffner et al., 1976). In this study, PD berries maintained ~10% higher TA than CN ones at

harvest. The pattern of lower sugar accumulation and higher acid content suggests that ripening may be slowed in Gewürztraminer berries under PD, counter to observations in red varieties (Castellarin et al., 2007a, b). If shown to be a consistent result in similar conditions and for similar cultivars, this could prove useful for growers that may wish to extend their growing season while reducing irrigation volumes by 50%.

Yield parameters, berry weight, cluster weight, and vine yield were negatively affected by DI treatments (Table 4). Generally, DI applied before veraison reduced berry size and thus cluster weight and vine yield relative to DI applied after veraison and the well-watered control. It was unlikely that the DI treatments negatively impacted berry cell division due to the timing of application (~30 DAA and ~ 60 DAA) and the level of stress that was not severe. Water deficit before 30 DAA is believed to affect berry cell number within the grape which would limit berry size (Hardie and Considine, 1976; Considine and Knox, 1981; Matthews and Anderson, 1989; Coombe and McCarthy, 2000). Thus, it is more likely that reduced yield from DI treatments arose from reduced berry water content relative to CN. Regardless, the differences in berry weight and yield of ED and PD compared to CN and LD likely arose from the lower water input into the berry before veraison and the inability of the vines to recover water content into the berry after veraison due to altered vine-to-berry hydraulic conductivity (Greenspan et al., 1996; Knifper et al., 2015). Cluster weight and vine yield were affected by DI treatments (Table 4). Cluster weight was reduced by ED and PD, relative to CN and LD, reflecting the effect on berry weight. Similar results were seen in two of six years of experiments by Reynolds et al. (2005). PD conditions on various grafted and own-rooted Syrah vines demonstrated 25- 60% reduced vine yield in Barossa Valley vineyards (McCarthy et al., 1997). ED but not LD treatments on Syrah grapes also determined a reduction of yields relative to CN (Ojeda et al., 2002). Similar

results were obtained by Wample and Smithyman (2002) in Sauvignon Blanc, but this effect was present in three of the five years of research. Overall, these data suggest DI treatments hindered berry water content and vine yield when applied before veraison as seen in previous studies mentioned above.

Differences in free terpenes among treatments were realized at harvest, but only for specific terpenes. Generally, the gradient of their concentration was LD > PD > ED = CN, but differences were significant only between LD and CN (LD > CN). Other pairwise comparisons were not significant. Reynolds et al. (2005) found similar results in Gewürztraminer berries at harvest with vines stressed from veraison onwards (LD-like) maintaining higher free terpene concentration compared to vines stressed from post-bloom. Furthermore, similar results to my study were observed in Merlot vines exposed to PD and ED water deficit treatments and compared to CN (Song et al., 2012). In Chardonnay, a microarray study by Deluc et al. (2011) demonstrated increased expression of one terpene synthase from grapes under WD suggesting the terpene synthesis might also be affected by WD. Moreover, in Tocai Friulano grapes, transcript and metabolite profiling suggested that prolonged severe WD applied boosted free monoterpene content (Savoi et al., 2016). However, counter to results from this present study, in Malbec grapes, Alonso et al. (2015) also demonstrated that moderate WD applied from veraison onwards did not affect monoterpene or sesquiterpene content. It is evident from literature and this study that the effect of WD on free terpene content is generally unclear and might depend on the variety and the type of terpene considered. In my study, I demonstrate that the LD total terpene levels are equal to those of CN, while specific terpenes were increased in concentration in LD relative to CN, and, hence, LD allows maximizing terpenes while saving irrigation water.

No effect was observed on bound terpenes at harvest; however, two weeks prior harvest, there was a higher concentration in ED than CN berries. A concentration effect due to the decreased berry size was likely the reason for the increase in concentration observed in ED berries, as they were smaller than CN and bound terpenes were not different between the two treatments when they were expressed as a content per berry (ng per berry). In Reynolds et al. (2005) study on Gewürztraminer, water deficit from veraison onwards boosted total bound terpenes compared to water deficit from post-bloom or lag phase. In Merlot, Song et al. (2012), observed no effect of DI treatments on bound terpene concentration. This study and literature suggest that the effectiveness of DI treatments possibly depends on the variety and experimental or seasonal conditions.

The profile and concentration of free and bound terpenes detected were largely similar compared to profiles previously reported (Girard et al., 2002, Skinkis et al., 2008, Martin et al., 2012, Ghaste et al., 2015). The limited differences in reported terpene profile between this study and literature may have arisen from methodological differences (e.g., compounds not consistently appearing in over LOD/LOQ in this study), or environmental factors influencing the biosynthesis. Examples of methodological differences include studies using grape juice obtained from berries collected at an undefined time from commercial harvest, crushed and pressed after thawing to -20 $^{\circ}$C (Girard et al., 2002, Skinkis et al., 2008) to using grape or wine distillates with or without prior extraction (Girard et al., 2002, Ghaste et al., 2015). In this study, whole berries were bagged and frozen on site with dry ice (-78.5 $^{\circ}$C) inside 5 cm thick polystyrene foam coolers. Frozen berries were then transported back to the lab within 24 h and stored in -80 $^{\circ}$C freezers until analysis. To prepare for analysis by GC-MS and for qPCR, berries with pedicles removed were crushed and deseeded under liquid N_2. Our processing method prioritized

minimizing berry oxidation, endogenous grape enzyme activity, and volatile vaporization, which ideally preserves and allows accurately assessment of berry volatiles. Furthermore, harvest samples were collected as close to commercial harvest to capture berries at an industry relevant and expertly assessed timepoint. Commercial harvest was not always at the same developmental timepoint as measured by DAA between years since external environmental factors (temperature, rainfall, light exposure) alter berry composition and development (Haselgrove et al., 2000; Bergqvist et al., 2001; Spayd et al., 2002; Gambetta et al., 2017; Noestheden et al., 2017).

The only free terpenes significantly influenced by DI (LD specifically) without interaction were geraniol and citronellol. LD berries maintained ~30% higher citronellol and ~40% higher geraniol than CN berries. These compounds are crucial for Gewürztraminer-characterisitic wine aroma. Higher levels of free terpenes in LD grapes does not necessarily translate to higher levels in wine, however terpene content in grape juice and skin has been suggested to strongly correlate with wine content (Slegers et al., 2015); further study will be required to assess treatment affect on Gewürztraminer wine. No bound terpenes were influenced by the DI treatments, without significant DI treatment and year interaction. All terpenes (free and bound) were highly affected year-to-year suggesting that seasonal variations associated to environmental factors other than water availability are the dominate determinant of terpene content. This is supported by the high incidence of significant interaction between treatment and year terms.

The observation of monoterpenes such as ocimene, phenllandrene, terpinene, and myrcene in the bound profile was notable. For terpenes to be directly glycosylated, they require a glycoside acceptor which tend to be free -OH groups in terpenes (Schwab and Wüst, 2015; Schwab, Fischer, and Wüst, 2015). However, due to rearrangement reactions following

deglycosylation – especially in acidic environments – measured aglycones may include non-oxygenated terpenes (Schwab, Fischer, and Wüst, 2015). This may explain the presence of oxygen-lacking monoterpenes in the bound profiles.

Many free terpenes correlated significantly with expression of select-biosynthetic genes. Early pathway genes did not show strong correlation with specific terpenes as their gene products synthesize reagents for many terpenoids. GPP can be utilized by TPSs to generate geraniol, ocimene, linalool, α-terpinol, α-pinene, and limonene directly. Other terpenes arise from subsequent reactions on these immediate products. In the season when gene expression was tested, CN treatment induced the expression of *TPS34/35*, *TPS38*, *TPS07*, *TPS10*, and *MYB24*, but showed insignificant differences from LD in the total free terpene content and was significantly reduced relative to LD in geraniol and citronellol content. Similarly, bound terpene content seemed unrelated to *CYP76F14* and *GT* expression as DI treatments affected *CYP76F14* and *GT7* expression at veraison and harvest, but no differences in the content were observed. Although differences between treatments were not consistent between gene expression and volatile analysis data, trends across treatments mirrored the profiles previously reported in Gewürztraminer (Martin et al., 2012; Ilc et al., 2017; Li et al., 2017) and Muscat varieties (Constantini et al., 2017; Li et al., 2017).

Considering treatment effects on berry weight, increased terpene concentration in late deficit berries was unlikely from berry dehydration and an increased skin:pulp ratio (Ojeda et al., 2002; Roby et al., 2004; Intrigliolo et al., 2016), since differences in the terpene content per berry generally matched the ones observed in the concentration. Additionally, sugar accumulation and terpene content were not necessarily related as CN and ED maintained larger TSS than LD and PD but had similar or lower free terpene concentration. Also, elevated free

terpenes (geraniol and citronellol) in LD cannot be explained by increased light exposure due to a WD-induced basal leaf abscission, since basal leaves were removed on both sides of the canopy and PQA revealed only marginal differences between LD and ED fruit exposure. The impact of DI treatments on gene expression did not appear to directly relate to the impact on total terpene concentration nor the terpene profile. For example, gene expression trends in CN did not match the lower or statistically equivalent terpene concentrations relative to DI treatments. However, overall gene expression did match overall terpene accumulation in all grape samples. Regardless, it appears that from veraison to harvest DI may induce a light-, sugar-, skin:pulp ratio-, transcript-independent response of total free terpenes in Gewürztraminer berries. It is possible that gene expression and terpene biosynthesis are not neatly correlated as tight regulation of terpenoid biosynthesis occurs at the post-transcriptional level involving structural enzymes (Vom Endt et al., 2002; Hemmerlin, 2013; Rodríguez-Concepción and Boronat, 2015). This has been observed in grapevines also (Bönisch et al., 2014; Matarese et al., 2014).

Furthermore, these results did not match the hypothesized outcomes of early and prolonged deficit inducing terpene synthesis more so than late deficit. The rationale stemmed from the fact that berry water status sensitivity to soil and vine water status declines post-veraison when phloem water input dominates (Greenspan et al., 1996). Perhaps, in this study, LD results arose from a combination of LD induced increase in berry abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) concentrations (Niculcea et al., 2014) and a xylem-mediated WD responses (Chaves et al., 2003) as some xylem connectivity remains *post*-verasion (Knifper et al., 2015). SA and JA applications to berries are known to stimulate terpene content (Gómez-Plaza et al., 2012) and many drought-upregulated terpene genes are enriched for promoter elements associated with ABA regulation and drought (Savoi et al., 2016). Perhaps elevated

hormone levels are explicable for higher levels of specific free terpenes in LD compared to CN berries. Hormone level measurements were outside the scope of this study and would be logical next steps in hypothesis testing.

Chapter 3: Viticultural Modification of Grape Aromas via Crop Load Management

3.1 Research Introduction

Crop load management (CLM) is another viticultural practice used to optimize fruit quality in vineyards (Jackson and Lombard, 1993; Keller, 2015). Various CLM techniques exist – leaf removal, shoot thinning, cluster thinning – all of which affect the source-to-sink ratio, and hence the potential of the canopy to ripe the fruit (Jackson and Lombard, 1993; Keller, 2015). Cluster thinning (CT) is CLM that involves removing inflorescences or fruit clusters from vine shoots. Reducing the crop per vine via CT, particularly in situations of high-crop, can increase berry and cluster weight, accelerate ripening, increase VOC and phenolic concentration, and promote berry color (Guidoni et al., 2002; Keller et al., 2005; Peña-Neira et al., 2007; Hannam et al., 2014; Black et al., 2016; Kok, 2016; Condurso et al., 2016; Luna et al., 2017). It is thought that higher source:sink ratios make available more photosynthate to the berry, promoting fruit ripening and vine growth. The timing when CT is applied is also an important factor. Post-fruit set (~30 days after anthesis) CT can promote ripening and grape quality (Guidoni et al., 2002; Ferree et al., 2003; Keller et al., 2005; Diago et al., 2010; Kok, 2011; Hannam et al., 2014). However, CT applied during veraison (~60 DAA) and afterwards (> 60 DAA) shows muted effects compared to CT applied earlier (Kok, 2011). Regardless of timing, CT effectiveness can be influenced by varietal and environmental factors (Keller et al., 2005; Diago et al., 2010; Kok, 2011; Hannam et al., 2014). It is thought that earlier applications of CT (post-fruit set) allow for more photosynthate accumulation in the berry and an earlier triggering of ripening than later applications of CT (veraison and later).

Studies on the effect of CLM on grapes and wines in major wine producing regions have mostly been conducted on red grapes and table grapes (Winkler, 1958; Kliewer and Antcliff, 1970; Kliewer and Weaver, 1971; Poni et al., 1993; Keller et al., 2005; Kliewer and Dokoozlian, 2005; Santesteban and Royo; 2006; Diago et al., 2010; Šuklje et al., 2013; Uriarte et al., 2016; Reeve et al., 2018) and several studies on the impact of CLM on grape and wine quality have been conducted in Canada (Reynolds and Wardle, 1989; Reynolds et al., 1992; Reynolds et al., 1996; Reynolds et al., 2005; Hannam et al., 2014; Black et al., 2016; Luna et al., 2017; Reynolds et al., 2018). Regarding CT studies, generally, our knowledge on the effect of its application to white grape varieties is scant, and little has been done with Gewürztraminer (Reynolds and Wardle, 1989; Reynolds et al., 1996) and terpenes (Reynolds and Wardle, 1989; Reynolds et al., 1996; Kok, 2016). Terpenes are volatile terpenes that are accumulated in the berry either at early developmental stages (sesquiterpenes) (Schwab and Wüst, 2015) or during berry ripening, and particularly close to full maturity (harvest) (Schwab and Wüst, 2015). In Gewürztraminer, terpenes determine the aroma of the wines and, therefore, are considered as major quality compounds (Guth, 1997a, b). No studies exist that comprehensively examined the profiles of individual free and bound terpenes, vine physiology, gene expression, and berry composition in a multi-year experiment. Multi-year experiments are critical for viticultural as vine biology is sensitive to environmental conditions and subtle effects may be enhanced or diluted by seasonal variation.

3.1.1 Research Hypothesis

This study focused on characterizing the effect of CLM via CT on berry sugar, acid, and volatile terpene concentrations in field-grown Gewürztraminer. In order to understand the effect of this viticultural practice on terpenoid metabolism, I also investigated the levels of targeted

transcripts involved in terpenoid synthesis (Guth, 1997; Martin et al., 2012; Savoi et al., 2016). Moreover, the CLM strategies also affect the canopy physiology (Edson et al., 1995; Petrie et al., 2000) and to better characterize the response of the vines investigated to the seasonal climatic conditions, leaf water potential, photosynthesis, and vegetative growth were monitored during the experiment. CLM was applied via cluster thinning in order to achieve three crop levels: light (18 – 21 clusters per vine or 7 to 10 tonnes per ha), moderate (25 – 32 clusters per vine or 11 to 15 tonnes per ha), high crop (35 – 50 clusters per vine or 16 to 20 tonnes per ha). The high crop treatment matched the standard cropping treatment of the commercial vineyard that hosted the experiment and was used as a control. The low and moderate crop treatments were imposed via cluster thinning at two developmental stages, early in the season (just after fruit set, ~30 DAA) and late (at veraison, ~60 DAA) in the season. Since initial cluster number in grapevines depends on the prior year's conditions, crop level differed among seasons. I hypothesized that reducing the crop load via cluster thinning will stimulate ripening and induce the synthesis of VOCs, and particularly terpenes, with early and more severe thinning having a stronger inductive effect than later and moderate thinning.

3.2 Materials and Methodology

3.2.1 Vineyard Site

The experiment was conducted throughout 2016-2018 growing seasons in a commercial vineyard in the southern Okanagan Valley (49°10'N, 119°32'W, 380 m a.s.l.), on the Black Sage Bench near Oliver, British Columbia. Figure S2 depicts site location and experiment setup on-site. The site hosted field-grown *Vitis vinifera* cv. Gewürztraminer Clone 47 grafted on SO4 rootstock (*V.riparia x V. berlandieri*) planted on loamy sand in east-west rows at an approximate density of 3,359 vines/ha (2.4 m between rows x 1.24 m within rows; 2.98 m^2 per vine). Vines were winter-pruned to 8 to 10 buds per cane and to 4 canes. Vines were trained accordingly to a vertically shoot positioned system. Pest management, canopy management, and fertilization in the vineyard were applied according to standard local viticultural practice. Basal leaves were mechanically trimmed ~30 days after anthesis (DAA) on the north side of the canopy according to the standard commercial practice performed in the vineyard. Over-head sprinklers supplied irrigation as necessary, assessed by the vineyard managers.

3.2.2 Experimental Design

The CLM treatments included three crop levels (high crop – HC, medium crop – MC or 75% of HC, light crop – LC or 50% of HC) the latter two which were factorially combined with two thinning dates (early thinning – E, late thinning – L). Crop levels were adjusted by cluster thinning to a designated number of clusters. HC vines were thinned to 35 - 50 clusters per vine (est. 16 - 20 t/ha). MC and LC were thinned to 25 – 32 and 18 – 21 clusters or an estimated 11 – 16 t/ha and 7 – 10 t/ha, respectively. The early thinning (E) was applied ~30 DAA post-fruit set and late thinning (L) was applied at veraison (Table 8; Figure 14). HC vines which maintained >50 clusters at early thinning were thinned to 50 clusters.

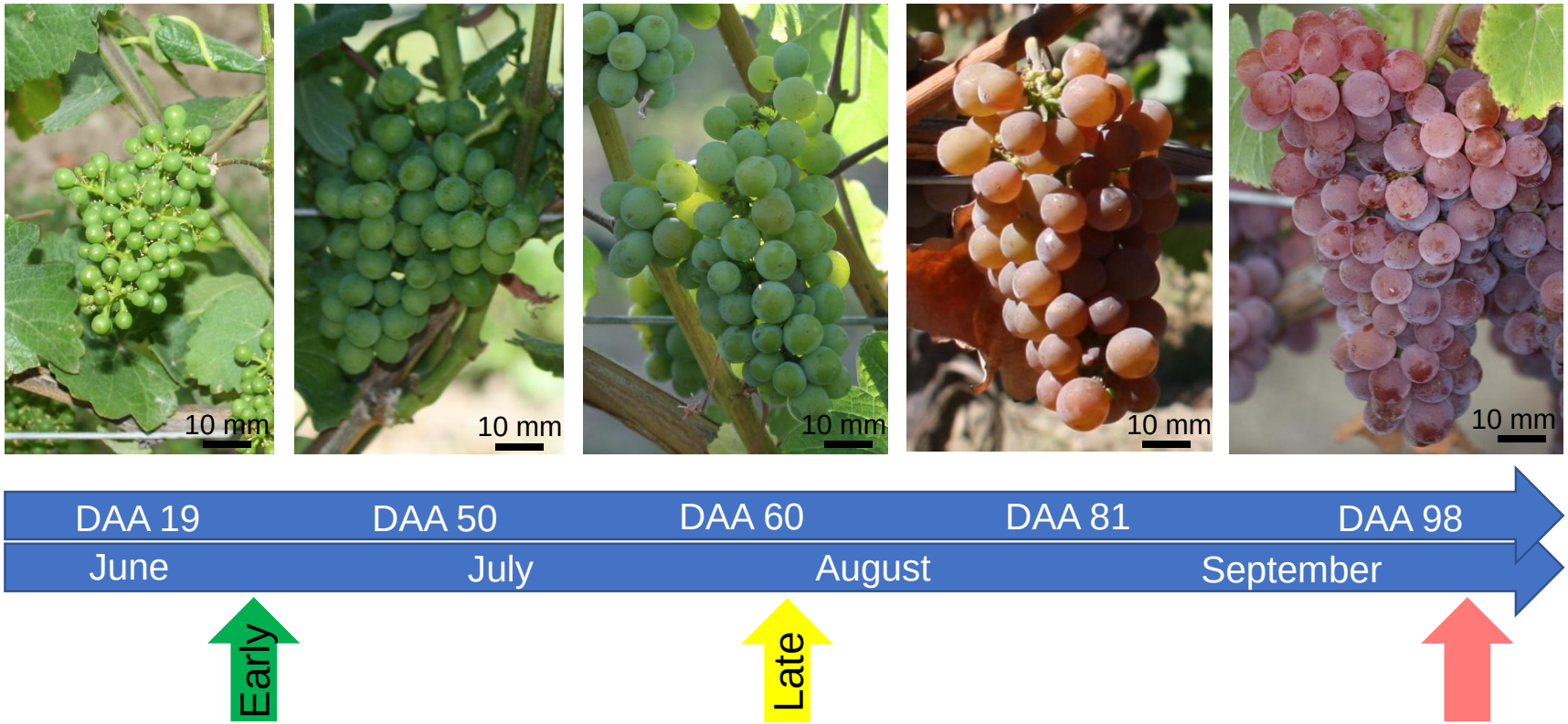

Figure 14: The timing of CLM treatment application and corresponding grapeberry development stages, days after anthesis, and calendar months. Green, yellow, and red arrows indicate dates of irrigation treatment application, veraison, and commercial harvest, respectively.

No thinning to HC vines was applied if they harboured 35 to 50 clusters. Vines in HC plots with <35 clusters were marked and not considered. HC vines were considered as controls (no-to-minimal cluster thinning) relative to light (MC) and severe (LC) cluster thinning. In summary, this study implemented five treatments: HC, MC-E, MC-L, LC-E, and LC-L. Each treatment was imposed onto five plots consisting of 9 to 11 vines per plot, separated into five blocks following a randomized-block design. Prior to thinning, clusters were counted on vines. The same plots were used for all three years.

3.2.3 Yield Assessment

Yield, number of clusters per vine, and average cluster mass were determined at commercial harvest (~22 $^{\circ}$Brix) on each plot. Pre-harvest assessment of number of clusters and shoots were completed prior to veraison. Pruning weights were collected in the winter following the growing season, typically in February (Coombe and Dry, 1992). Vines were pruned to standard vineyard management accordingly to a Guyot training system with four canes per vine.

3.2.4 Leaf Area

Leaf area was measured throughout the growing season, at least once a month and within two weeks from commercial harvest. The method used was identical to what was described in Section 2.2.5.

3.2.5 Water Relations and Gas Exchange

Leaf water potential was determined with a Scholander pressure chamber (PMS Instrument Company, Albany, OR, USA) on bag-covered leaves from two to four representative vines per plot every 14 to 21 days from ~ 20 DAA to harvest between 1230 h and 1430 h (±1 h from local solar noon). Leaves were assessed from both sides of the row.

Leaf gas exchange parameters (photosynthesis, transpiration, stomatal conductance) were monitored using a LI-COR 6400 (Li-Cor Inc., Lincoln, NE, USA) from + 1 h to + 3 h post-sunrise every 14 to 21 days. Data acquisition was biased towards days with high morning PAR to capture the vines at their photosynthetic maximums. The method used was identical to that was described in Section 2.2.6.

3.2.6 Basic Berry Chemical Analysis

Forty to sixty berries (accordingly to the developmental stage) per plot were randomly collected per sampling point (24, 29, 49, 56, 65, 71, 83, 98, 114, and 119 DAA in 2016; 33, 46, 58, 78, 86, 103, and 108 DAA in 2017; 80, 99, and 115 DAA in 2018). The method used was identical to that was described in Section 2.2.7.

3.2.7 Free Terpene Semi-Quantitation

Forty to sixty berries (accordingly to the developmental stage) per plot were randomly collected per sampling point (24, 29, 49, 56, 65, 71, 83, 98, 114, and 119 DAA in 2016; 33, 46, 58, 78, 86, 103, and 108 DAA in 2017; 80, 99, and 115 DAA in 2018). The method used was identical to what was described in Section 2.2.8.

3.2.8 Glycoside-Bound Terpene Quantitation

Forty to sixty berries (accordingly to the developmental stage) per plot were randomly collected per sampling point (24, 29, 49, 56, 65, 71, 83, 98, 114, and 119 DAA in 2016; 33, 46, 58, 78, 86, 103, and 108 DAA in 2017; 80, 99, and 115 DAA in 2018). The method used was identical to what was described in Section 2.2.9.

3.2.9 Gene Expression Analysis: RNA extraction to qPCR

Gene expression analyses were conducted only on the 2016 samples at 49, 65, 83, 98, and 119 DAA. The method used was identical to what was described in Section 2.2.10

3.2.10 Statistics

Basic statistics, analysis of variance (ANOVA), and correlation analyses were undertaken using R software v3.4.4 (R Foundation for Statistical Computing, Vienna, Austria). Two-way ANOVA with treatment and year as main factors and block number as additive random factor, were used to test the main effects and factor interactions on pre-harvest- and harvest-time biochemical, physiological, and vegetative parameters. One-way ANOVA without a year main factor was used to assess differences at individual sampling points for biochemical, physiological, and vegetative parameters within years. For multiple comparisons of treatments, standard error, and Tukey's HSD *post-hoc* tests were calculated and reported. Statistically, marginal differences were assumed to be $0.1 > p > 0.05$, while significant differences were assumed for $p < 0.05$. Packages utilized for analysis were tidyverse v1.2.1 (Wickham et al., 2017), moderndive v0.2.0 (Kim et al., 2018), and skimr v1.0.5 (Waring et al., 2019).

3.3 Results

3.3.1 Phenology and Timing of Treatments

The calendar dates of anthesis, veraison, and harvest occurred within 14-calendar days from year-to-year (Table 8). Veraison occurred at similar DAA in 2016 and 2017 and was one week later in 2018. Harvest dates in DAA were most similar between 2016 and 2018. In 2017, grapes were harvested 8 to 9 DAA before than the other years. The application of treatments post-fruit set and during veraison occured within 15 DAA from year-to-year. Averaging over all three years, early thinning (MC-E and LC-E) occurred on average 29 ± 4 DAA or -37 ± 1 days from veraison; the late thinning (MC-L and LC-L) occurred on average 67 ± 5 DAA or 2 ± 4 days from veraison. Harvest occurred on average 114 ± 6 DAA or 48 ± 6 days from veraison.

Table 8. Calendar dates and respective days after anthesis for major phenological and trial-related events

Year	Anthesis		Veraison		Harvest		Early Thinning		Late Thinning	
	Date	DAA	Date	DAA	Date	DAA	Date	DAA	Date	DAA
2016	31/05	0	04/08	65	27/09	119	29/06	29	08/08	64
2017	15/06	0	17/08	63	01/10	108	13/07	26	19/08	63
2018	01/06	0	12/08	72	25/09	116	06/07	35	18/08	78

Calendar dates are reported as dd/mm

3.3.2 Yield Parameters

The CLM treatments sought to restrict vine cluster number and therefore per hectare yield, which was successfully achieved in all three years (Table 9). No overlap in cluster number and yield per hectare occurred between the cropping levels (HC, MC, and LC), regardless when the treatments were applied. In 2016, approximately eight extra clusters remained in every treatment due to miscounting, however, the desired ratios between the cropping levels remained roughly the same.

Table 9: Crop load management (CLM) strategy and year effects on yield parameters of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada. Effects were tested with a two-way ANOVA and averages were separated by *post-hoc* Tukey's HSD. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

	Berries per Cluster			**Berry Weight (g)**			**Clusters per Vine**			**Cluster Weight (g)**		**Yield (t/ha)**		**Pruning Weights (kg)**			**Ravaz Index (kg/kg)**			**Crop Load (cm^2/g)**		
CLM Treatment p-value	0.0319			n.s.			$< 2.00 \cdot 10^{-16}$			0.00652		$5.49 \cdot 10^{-12}$		n.s.			$2.30 \cdot 10^{-9}$			$1.26 \cdot 10^{-4}$		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE	Mean	SE	Mean	SE		Mean	SE		Mean	SE	
HC	77	2	b	1.58	0.02		43	2	a	121.24	3.36	17.67	1.01	0.97	0.07		5.72	0.39	a	21.21	1.02	b
LC-E	86	3	ab	1.63	0.03		25	1	c	138.24	4.95	11.30	0.40	1.10	0.07		3.18	0.27	bc	29.96	2.03	ab
LC-L	88	4	a	1.63	0.02		24	1	c	142.41	5.02	10.95	0.47	1.15	0.06		2.84	0.13	c	37.30	2.19	a
MC-E	84	3	ab	1.61	0.03		33	1	b	134.28	5.09	14.63	0.55	1.10	0.06		4.05	0.25	b	26.85	1.98	b
MC-L	81	3	ab	1.63	0.03		32	1	b	131.20	5.79	14.08	0.61	1.17	0.08		3.78	0.30	bc	31.83	3.04	a
Year p-value	$3.51 \cdot 10^{-7}$			$6.36 \cdot 10^{-12}$			$1.71 \cdot 10^{-14}$			$1.38 \cdot 10^{-6}$		$6.08 \cdot 10^{-7}$		$1.55 \cdot 10^{-6}$			$1.10 \cdot 10^{-5}$			0.0125		
2016	75	1	c	1.68	0.02	a	36	2	a	126.40	2.11	15.35	0.78	1.28	0.04	a	3.73	0.27	b	28.63	1.99	ab
2017	85	2	b	1.51	0.02	b	29	1	b	128.79	3.22	12.06	0.46	1.09	0.04	b	3.43	0.21	b	32.90	1.95	a
2018	94	3	a	1.64	0.01	a	28	1	b	153.05	4.94	13.79	0.62	0.92	0.04	c	5.02	0.34	a	24.99	1.46	b
CLM x Year Interaction p-value	n.s.			n.s.			n.s.			0.0303		0.0243		n.s.			n.s.			n.s.		

Berry weight and number per cluster as well as cluster weight were affected by the thinning treatments and the years (Table 9). Berry weight and number were smallest in the HC treatment (1.55 ± 0.02 g and 78 ± 2 berries per cluster) compared to all other treatments. However, averaging over all three years, only MC-L and LC-L were significantly larger than HC in berry weight and the number of berries per cluster, respectively. Berry number increased by 10 berries per year from 75 ± 1 in 2016 to 95 ± 3 in 2018, while berry weight was largest in 2016 (1.68 ± 0.02 g, on average across treatments) and smallest in 2017 (1.51 ± 0.01 g) with 2018 falling in the middle (1.61 ± 0.02 g). The combination of berry number and weight resulted in cluster weight being equal among 2016 and 2017 (~125 g per cluster on average across treatments), but significantly larger in 2018 (~155 g per cluster). Cluster weight was unaffected by clustering thinning in 2016 (126.40 ± 2.11 g on average across treatments). In 2017, treatment application affected cluster weight ($p = 0.0136$) and a similar trend was observed in 2018 (Figure 15), but no differences were observed ($p = 0.200$).

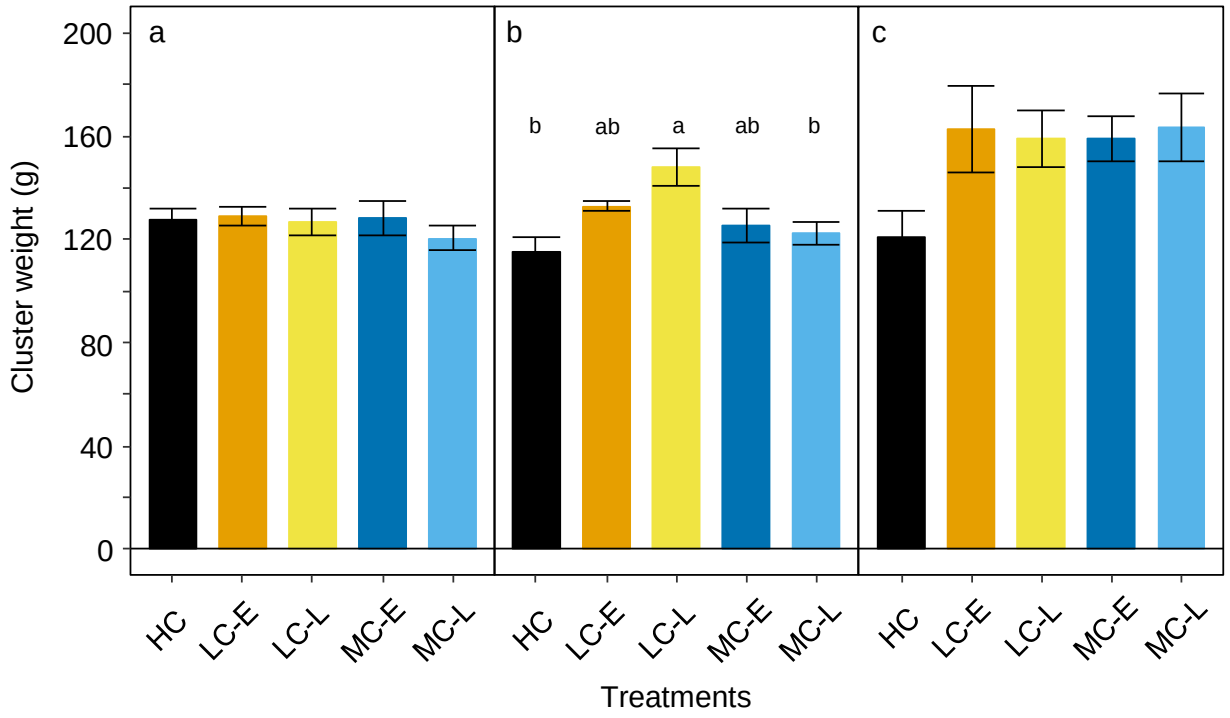

Figure 15: Cluster weight of Gewürztraminer grapevines exposed to different crop load management (CLM) strategies at harvest in 2016(a), 2017(b), and 2018(c). Standard error is indicated by error bars. Different letters above bars denote significance ($p < 0.05$) among CLM treatments, within years, as determined by *post-hoc* Tukey's HSD. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

Pruning weights were unaffected by cluster thinning treatments ($p = 0.152$) but were affected from year-to-year (Table 9). Pruning weights decreased from year-to-year at a rate of -0.20 kg per year. Crop load was affected as intended by treatments ($p = 2.30 \cdot 10^{-9}$) (Table 9). The Ravaz index, a ratio between fruit (measured as vine yield) and vegetative tissues (pruning weights), was used as a measure of crop load. Higher Ravaz index values were observed in HC treatments over MC and LC treatments. The Ravaz index was higher in MC-E over LC-L; however, other pairwise comparisons were equivocal. Similarly, when crop load was expressed as a measure of the ratio between harvest-time leaf area - in cm^2 per vine - and vine yield - in kg per vine - a larger leaf area per unit weight of fruit was available to LC-L and MC-L vines than to HC and MC-E vines (Table 9). LC-E vines were equivocal to all vines in crop load.

3.3.3 Water Relations and Leaf Gas Exchanges

Vines did not experience water stress and remained sufficiently well-watered throughout the seasons as determined by measurements of Ψ_{Leaf}. Photosynthesis, transpiration, and stomatal conductance were not affected by the treatments (Table 10). Furthermore, leaf area was unaffected by CLM (Table S17). Total vine leaf area as well as primary and secondary leaf areas per vine were consistent among all treatments. Averaging over the three-years, the grapevines were observed to go from 11.08 ± 0.34 m^2 per vine to 12.08 ± 0.55 m^2 per vine (~2-3 m^2 per cane). Hedging was applied multiple time during the season accordingly to the standard management of the vineyard and kept back any excess vigor of vines. Leaf area was affected by the year-to-year variation; total vine LA was diminished in 2018 when compared to 2016 and 2017.

Table 10. Crop load management (CLM) strategy and year effects on photosynthesis rate, transpiration rate, and stomatal conductance of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada. Effects were tested with a two-way ANOVA and averages were separated by *post-hoc* Tukey's HSD. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

	Photosynthesis Rate (µmol CO_2 /m^2 /s)						**Transpiration Rate (mmol H_2O/m^2 / s)**						**Stomatal Conductance (mol H_2O/m^2/ s)**					
	July		**Aug.**		**Sept.**		**July**		**Aug.**		**Sept.**		**July**		**Aug.**		**Sept.**	
CLM Treatment p-value	0.0578		n.s.		n.s.		0.0548		n.s.		n.s.		0.0853		n.s.		n.s.	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
HC	12.53	0.62	14.10	0.46	12.56	0.59	5.19	0.31	5.55	0.34	4.16	0.24	0.27	0.02	0.30	0.02	0.21	0.02
LC-E	13.25	0.64	13.83	0.43	12.91	0.57	5.47	0.38	5.34	0.31	4.39	0.24	0.28	0.02	0.28	0.02	0.23	0.02
LC-L	13.80	0.51	13.78	0.42	12.34	0.74	5.91	0.36	5.62	0.33	4.39	0.25	0.34	0.03	0.30	0.02	0.23	0.02
MC-E	13.54	0.46	14.53	0.39	12.32	0.65	5.75	0.35	5.94	0.33	4.32	0.23	0.32	0.02	0.33	0.02	0.22	0.02
MC-L	13.56	0.60	13.96	0.53	12.23	0.68	5.84	0.38	5.72	0.32	4.40	0.24	0.32	0.02	0.31	0.02	0.23	0.02
Year p-value	$< 2.00 \cdot 10^{-16}$		$< 2.00 \cdot 10^{-16}$		$< 2.00 \cdot 10^{-16}$		$< 2.00 \cdot 10^{-16}$		$< 2.00 \cdot 10^{-16}$		$< 2.00 \cdot 10^{-16}$		$3.23 \cdot 10^{-11}$		$1.69 \cdot 10^{-13}$		$< 2.00 \cdot 10^{-16}$	
2016	13.78	0.36 b	15.54	0.19 a	15.37	0.35 a	6.70	0.12 a	6.92	0.10 a	5.35	0.09 a	0.35	0.01 a	0.35	0.01 a	0.31	0.01 a
2017	14.81	0.21 a	13.35	0.23 b	10.86	0.30 b	6.24	0.16 b	4.87	0.21 b	3.86	0.15 b	0.34	0.02 a	0.29	0.02 b	0.18	0.01 b
2018	9.95	0.29 c	10.89	0.43 c	9.90	0.29 c	3.34	0.06 c	3.28	0.08 c	3.19	0.11 c	0.18	0.01 b	0.18	0.01 c	0.14	0.01 c
CLM x Year Interaction p-value	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	

3.3.4 Berry Composition

Berry TSS increased throughout the season (Figure 16 d, e, f). At harvest, HC berries lagged MC and LC fruit by at ~0.5 ^{o}Brix (Table 11). However, the difference was greater in weeks preceding commercial harvest. Pre-harvest sampling (approx. two weeks before harvest) reveals an average separation from HC of ~0.5 and ~1 ^{o}Brix for MC and LC, respectively. The interaction between CLM treatment and year effects was significant at pre-harvest sampling. The timing of thinning application did not affect the sugar levels at harvest and pre-harvest. Within two weeks TSS levels from the late thinning application both LC-L and MC-L treatments caught up to their early thinning equivalents, as well as differences from HC are more pronounced (Figure 16 d, e, f; Table S18).

Table 11. Crop load management (CLM) strategy and year effects on berry composition at harvest and pre-harvest in field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada. Effects were tested with a two-way ANOVA and averages were separated by *post-hoc* Tukey's HSD. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

	Total Soluble Solids ($^{\circ}$Brix)				**Titratable Acidity (g/L)**				**Juice pH**			
CLM Treatment p-value	**Pre-Harvest**		**Harvest**		**Pre-Harvest**		**Harvest**		**Pre-Harvest**		**Harvest**	
	$1.22 \cdot 10^{-8}$		0.00108		0.00144		0.0667		$5.51 \cdot 10^{-8}$		0.000442	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
HC	21.82	0.36	23.38	0.13 b	6.53	0.08	5.44	0.21 ab	3.34	0.02	3.53	0.03 b
LC-E	22.79	0.27	24.01	0.11 a	6.07	0.12	5.14	0.16 b	3.48	0.01	3.65	0.03 a
LC-L	22.71	0.24	24.04	0.12 a	6.44	0.18	5.61	0.12 ab	3.41	0.02	3.56	0.02 b
MC-E	22.36	0.30	23.70	0.12 ab	6.44	0.11	5.44	0.11 ab	3.41	0.01	3.58	0.02 ab
MC-L	22.09	0.29	23.69	0.16 ab	6.58	0.10	5.71	0.13 a	3.39	0.01	3.55	0.02 b
Year p-value	$< 2.00 \cdot 10^{-16}$		0.000655		$1.47 \cdot 10^{-5}$		n.s.		n.s.		$2.63 \cdot 10^{-10}$	
2016	20.93	0.13	23.65	0.10 b	6.51	0.13	5.50	0.09	3.41	0.01	3.67	0.02 a
2017	23.28	0.10	24.07	0.09 a	6.13	0.10	5.32	0.09	3.41	0.02	3.53	0.02 b
2018	22.85	0.11	23.58	0.11 b	6.61	0.11	5.58	0.16	3.40	0.02	3.52	0.02 b
CLM x Year Interaction p-value	0.0028		n.s.		0.00024		n.s.		0.0424		n.s.	

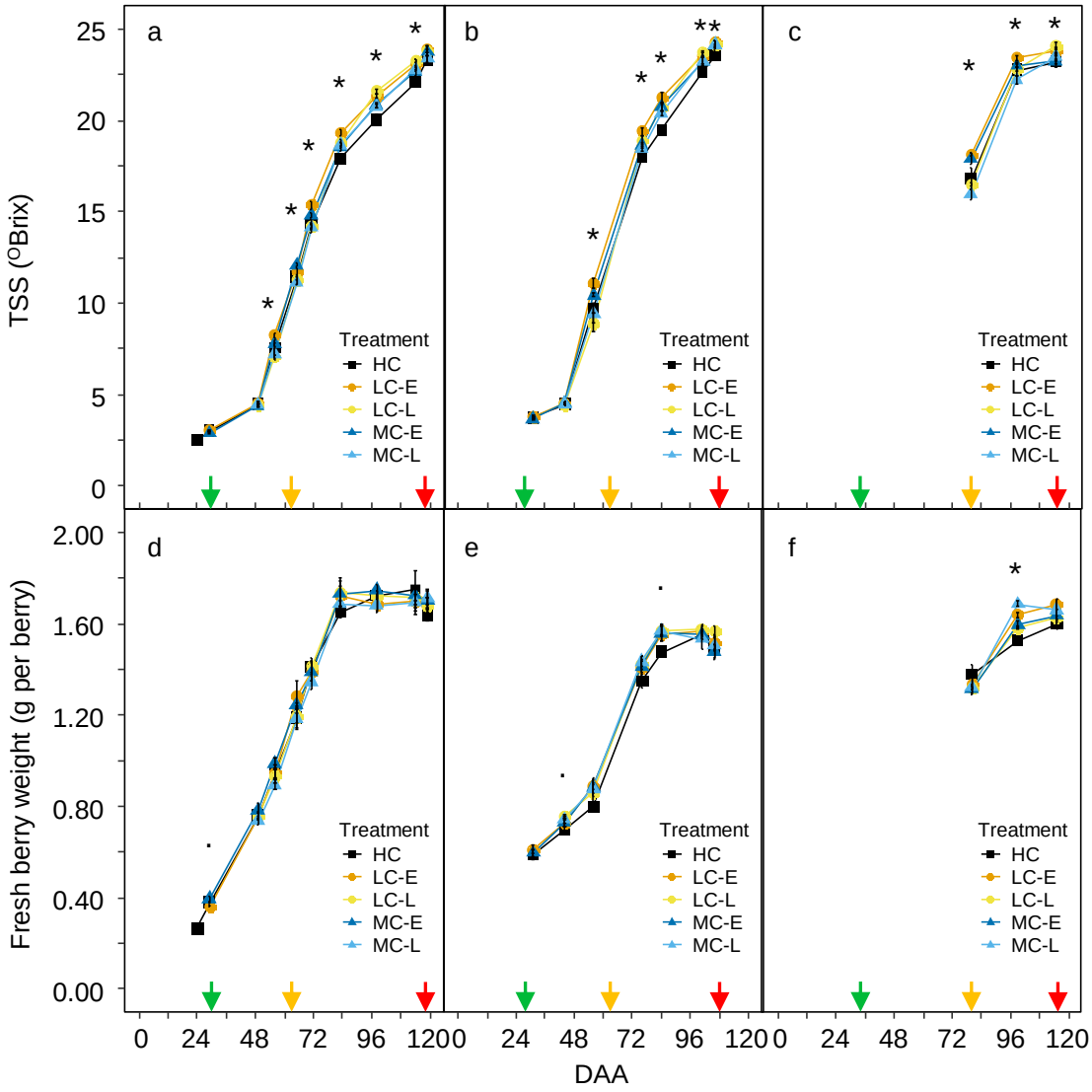

Figure 16: Evolution of total soluble solids (TSS) as ˚Brix (a-c) and berry weight (d-f) in berries of Gewürztraminer vines exposed to different crop load management (CLM) strategies throughout 2016 (a,d), 2017 (b,e), and 2018 (c,f). Standard error is indicated by error bars. Green, yellow, and red arrows indicate dates of early treatment application, application at veraison, and commercial harvest. Asterisks indicate differences ($p < 0.05$) among CLM treatments as determined by one-way ANOVA. Dots indicate marginal differences ($0.10 < p < 0.05$) among CLM treatments. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

Berry titratable acidity was also modulated by the level of crop and the timing of CT application (Table 11). At pre-harvest, LC berries, had a lower concentration of titratable acids than HC and MC berries (Table S19). Timing of application did not affect TA or pH at the pre-

harvest (Table S19 and S20). At harvest titratable acidity was similar among treatments (Table S19). There also appeared to be a year effect on TSS and acidity parameters, but overall values were very similar among years (Table 11).

3.3.5 Berry Terpene Composition

3.3.5.1 Detection and Identification of Terpenes in Gewürztraminer Berries

The profile of terpenes identified in the berries collected at pre-harvest and at harvest in this trial was identical to that described in Chapter 2 (Table S1). In short, a total of 21 terpenoids were identified across both free volatile and glucoside bound fractions. Free and bound profiles were different in that farnesene isomers were found only in the free fraction. Hydroxylinalool was found only in the bound fraction. All the other compounds were found in both fractions.

Regarding inter-season variation of the compounds, citral-a, α-terpinol, α-terpinene, γ-terpinene, and farnesene-b were below LOQ in the free fraction in at least one year, at harvest. For glycoside-bound terpenes at harvest, α-terpinol, citral-a and -b, geranic acid, and rose oxide were below LOQ in at least one year. The 2016 season had more compounds below LOQ (5 of 18 detectable) than 2018 (4 of 18 detectable) and 2017 (3 or 18 detectable).

3.3.5.2 Free Terpenes

Total free terpenes were tracked across the growing season in 2016 (Figure 17). Figure 17a and 17b illustrate the evolution of total free terpene content (ng per berry) and concentration (ng / g FW), respectively, across the 2016 season. All treatments demonstrated greater (2- to 4-fold) terpene concentrations after 90 DAA than before veraison. Additionally, total free terpenes in 2016 peaked prior to 100 DAA. Similar results were observed in 2017 and 2018 with total free terpene levels at pre-harvest (98, 83, and 105 DAA in 2016, 2017, and 2018, respectively) being higher or equivalent to the harvest levels. At harvest (119, 108, and 116 DAA in 2016, 2017, and

2018 respectively), statistical differences were present among treatments ($p = 6.84 \cdot 10^{-5}$) and more so, among years ($p = <2.00 \cdot 10^{-16}$) with an interaction present between year and treatment factors ($p = 0.000771$) (Table 12).

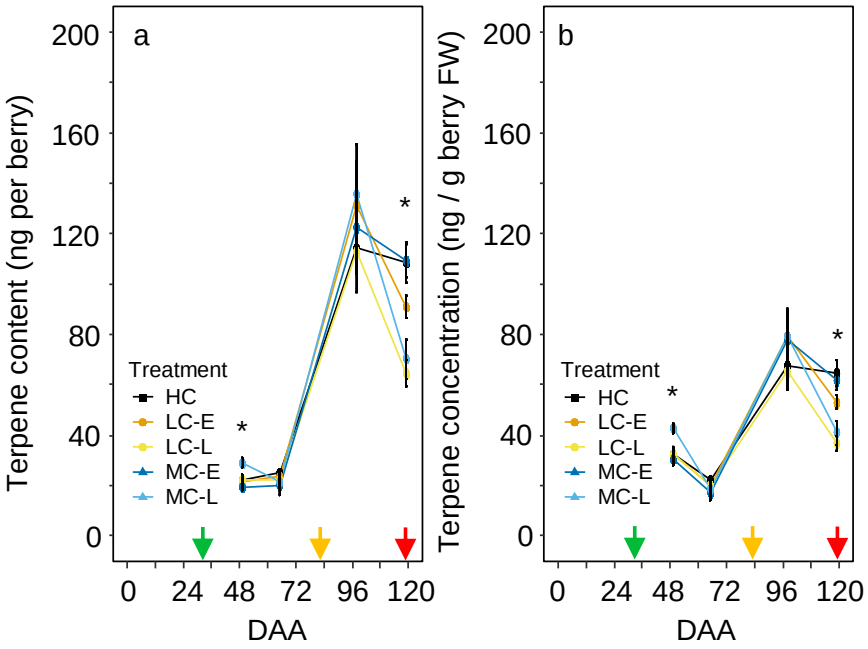

Figure 17: Evolution of total free terpene content (a) and concentration (b) in berries of Gewürztraminer grapevines exposed to crop load management (CLM) strategies throughout the 2016 season. Standard error is indicated by error bars. Green, yellow, and red arrows indicate dates of early treatment application, application at veraison and commercial harvest. Asterisk indicate differences ($p < 0.05$) among CLM treatments as determined by one-way ANOVA. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

Principal component analysis reflects the greater influence of years than treatments on total free terpenes (Figure S4a, c). Total free terpenes values were lowest in 2016 and highest in 2018 (Table 12). Separating data by year revealed significant differences among treatments with MC-L and LC-L reducing total free terpenes relative to LC-E and HC control (Figure 18a-c). The impact of CLM on terpene content was different between 2016 and 2018. In 2016, HC and MC-E maintained higher total free terpenes than MC-L and LC-L, while LC-E showed equivalence to MC-L (Figure 18 a, d). In 2017, LC-E had higher total free terpene concentration than LC-L and MC-E, whereas HC and MC-L were equivalent to all treatments (Figure 16b).

Terpene content in 2017 was similar among all treatments; however, LC-E maintained the largest mean value (Figure 18e).

Table 12. Crop load management (CLM) strategy and year effects on free and glycoside bound terpenes of field-grown Gewürztraminer berries at harvest in the Okanagan Valley, BC, Canada. Effects were tested with a two-way ANOVA and averages were separated by *post-hoc* Tukey's HSD. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

	Free Volatile Terpenes				**Glycoside Bound Terpenes**							
	[Total] (ng/g berry FW)		**Total per Berry (ng)**		**[Total] (ng/g berry FW)**			**Total per Berry (ng)**				
CLM Treatment p-value	$6.82 \cdot 10^{-5}$		$7.45 \cdot 10^{-5}$		n.s.			0.0857				
	Mean	SE	Mean	SE	Mean	SE		Mean	SE			
HC	267.94	63.78	404.23	97.70	6052.04	624.22		9217.80	1034.69			
LC-E	297.29	69.29	470.47	114.06	6082.12	594.34		9519.13	985.00			
LC-L	170.91	38.57	276.88	59.21	5715.45	581.82		9316.09	865.91			
MC-E	228.70	57.01	350.86	87.89	6216.22	796.67		9750.23	1358.89			
MC-L	180.95	34.90	281.95	54.52	4989.70	667.25		7697.26	1041.44			
Year p-value	$< 2.00 \cdot 10^{-16}$		$< 2.00 \cdot 10^{-16}$		$< 2.00 \cdot 10^{-16}$			$< 2.00 \cdot 10^{-16}$				
2016	51.55	2.75	81.23	4.32	8722.66	459.45	a	13781.92	755.52	a		
2017	152.51	12.80	231.49	19.22	3834.17	63.45	c	5863.15	146.86	c		
2018	483.40	32.57	757.91	51.33	4876.48	115.62	b	7655.24	197.58	b		
CLM x Year Interaction p-value	0.000771		0.000126		n.s.			n.s.				

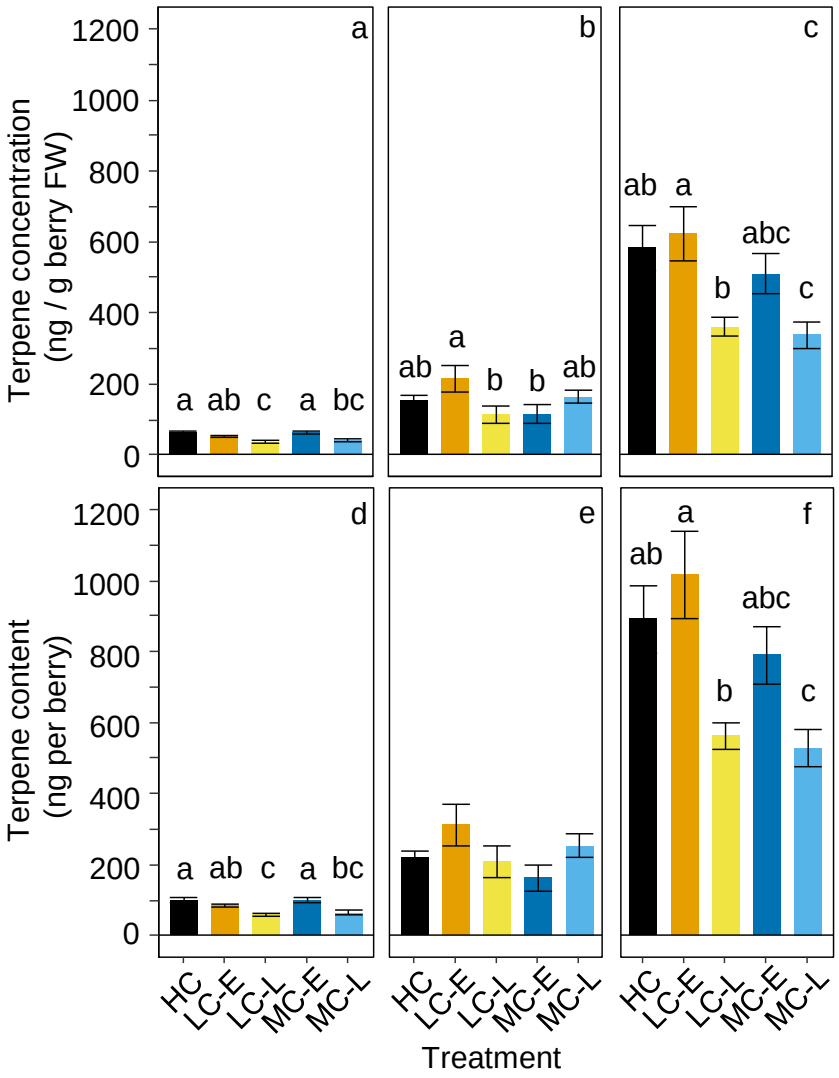

Figure 18: Total free terpene concentration (a-c) and content (d-f) at harvest in berries of Gewürztraminer grapevines exposed to crop load management (CLM) strategies in 2016 (a,d), 2017 (b,e), and 2018 (c,f) seasons. Error bars indicate the standard error. Two-way ANOVA utilizing treatment and year as factors revealed differences but interactions between factors (Table 12). Thus, data were separated by year and tested by one-way ANOVA. Different letters above bars denote significance ($p < 0.05$) among CLM treatments, within years, as determined by *post-hoc* Tukey's HSD after a significant ($p < 0.05$) one-way ANOVA result. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

In 2018, both total free terpene concentration and content were higher in LC-E relative to LC-L and MC-L while HC was only different from MC-L, and MC-E was equivalent to all treatments (Figure 18 c, f). Trends in individual free terpenes mirrored the total free terpene trend. Significant interactions between CLM treatment and year effects were observed for many

terpenes (Table S21 and Table S22). Only citronellol showed no interaction. Citronellol was found in relatively larger concentrations in HC and LC-E than other treatments at harvest dates (Figure 19, Table S21).

Total free terpenes two weeks prior to harvest, averaged over three-years, were higher in LC-E berries than in HC and MC-E berries (Table 13). These terpenes in MC-L and LC-L were equivalent in concentration to all other treatments. Seven compounds were increased in LC-E berries relative to HC: α-phellandrene, citronellol, myrcene, geranic acid, geraniol, nerol, and ocimene-b (Table S23 and S24). Nerol and farnesene-a were increased in LC-E relative to MC-E berries.

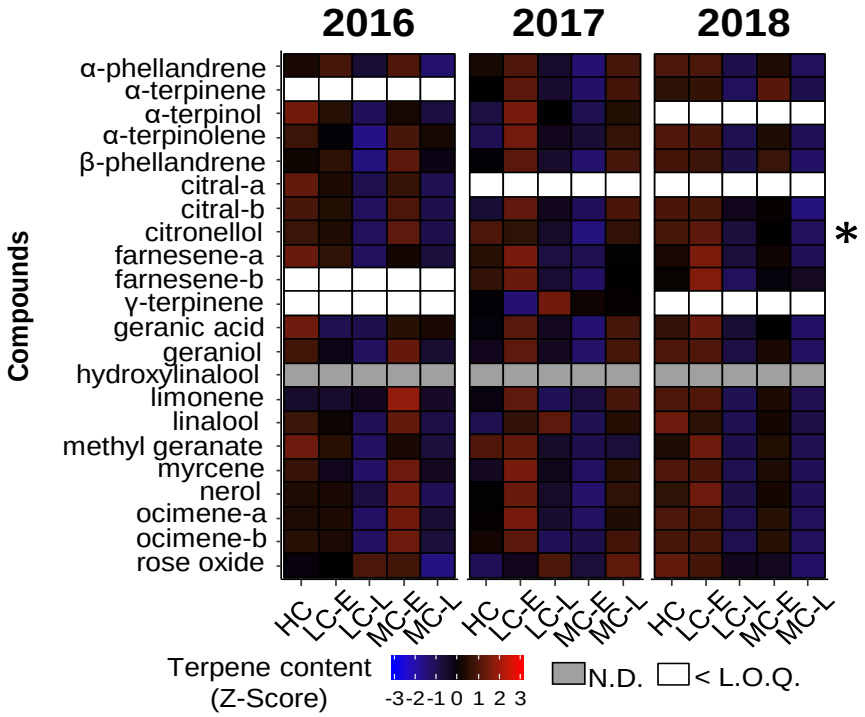

Figure 19: Heatmap of free terpene concentration at harvest in berries of grapevines exposed to crop load management (CLM) strategies in 2016, 2017, and 2018. Data are plotted as Z-scores of terpene concentration calculated for each terpene among treatments and within each year. Z-scores range from red (+3σ) through black (0σ) to blue (-3σ) and indicate the highest, average, and lowest values respectively. Two-way ANOVA results are indicated with asterisks for $p < 0.05$. Only compounds for which no interaction term between treatments and years was observed are reported as significantly affected by CLM strategies. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

Table 13. Crop load management (CLM) strategy and year on free and glycoside bound terpenes of field-grown Gewürztraminer berries at pre-harvest in the Okanagan Valley, BC, Canada. Effects were tested with a two-way ANOVA and averages were separated by *post-hoc* Tukey's HSD. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

	Free Volatile Terpenes						**Glycoside Bound Terpenes**					
	[Total] (ng/g berry FW)			**Total per Berry (ng)**			**[Total] (ng/g berry FW)**			**Total per Berry (ng)**		
CLM Treatment p-value	0.0049			0.00138			0.0248			0.0104		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE	
HC	192.65	33.37	b	284.03	51.49	b	4449.43	344.03	ab	6480.33	613.48	b
LC-E	285.16	53.37	a	441.23	87.37	a	5354.89	465.31	a	8222.77	775.03	a
LC-L	223.82	40.86	ab	336.09	61.66	ab	4426.65	174.97	ab	6564.63	342.58	b
MC-E	205.65	31.23	b	307.88	48.12	b	4698.25	215.28	ab	6940.32	425.43	ab
MC-L	229.85	41.10	ab	342.49	61.86	ab	4372.00	248.75	b	6443.79	459.89	b
Year p-value	$< 2.00 \cdot 10^{-16}$			$< 2.00 \cdot 10^{-16}$			$3.16 \cdot 10^{-6}$			$1.23 \cdot 10^{-7}$		
2016	73.85	3.64	c	114.65	6.18	c	-	-		-	-	
2017	200.70	9.70	b	282.92	13.88	b	4096.03	220.36	b	5810.68	359.60	b
2018	407.73	23.56	a	629.47	38.30	a	5224.45	84.10	a	8050.06	163.21	a
CLM x Year Interaction p-value	n.s.			n.s.			n.s.			n.s.		

3.3.5.3 Bound Terpenes

Total bound terpene concentration at harvest was consistent between 2017 and 2018 (~4000 to 5000 ng / g berry FW); however, it was higher in 2016 (~8000 ng / g berry FW) (Figure 20; Table 12). The year effect was significant ($p = <2.00 \cdot 10^{-16}$), while CLM treatment effect was not significant for both the concentration and the amount per berry of total bound terpenes. This was corroborated by the clustering of points by year in Figure S4b and the sizes of treatment and year vectors in Figure S4d. There was no effect of the treatments on bound terpenes per berry ($p = 0.0857$). The relative concentrations of individual bound terpenes among treatments varied year-to-year (Figure 21). Geranic acid was the only compound affected significantly by the treatments, however this was accompanied by an interaction effect with the year factor (Table S25 and S26). This interaction effect likely arose due to an absence of geranic

acid in 2016 samples. Notably, α- and β-phellandrene, α-terpinene, and ocimene-a were not affected significantly by the year (Table S25 and S26).

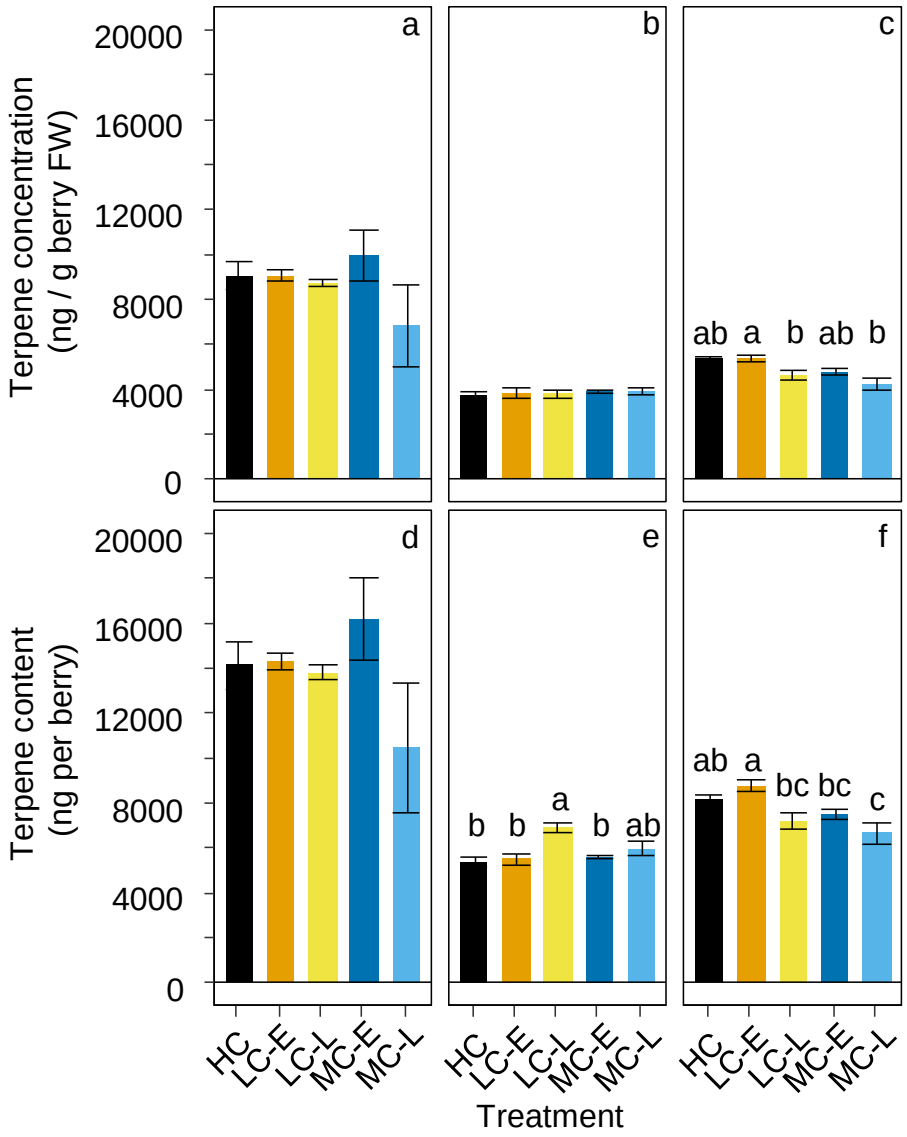

Figure 20: Total bound terpenes expressed as per g of berry FW (a-c) and as per berry (d-f) at harvest in berries of Gewürztraminer grapevines exposed to crop load management (CLM) strategies in 2016 (a,d), 2017 (b,e), and 2018 (c,f) seasons. Error bars indicate the standard error. Two-way ANOVA utilizing treatment and year as factors revealed differences but interactions between factors (Table 12). Thus, data were separated by year and tested by one-way ANOVA. Different letters above bars denote significance ($p < 0.05$) between the treatments, within years, as determined by *post-hoc* Tukey's HSD after a significant ($p < 0.05$) one-way ANOVA result. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

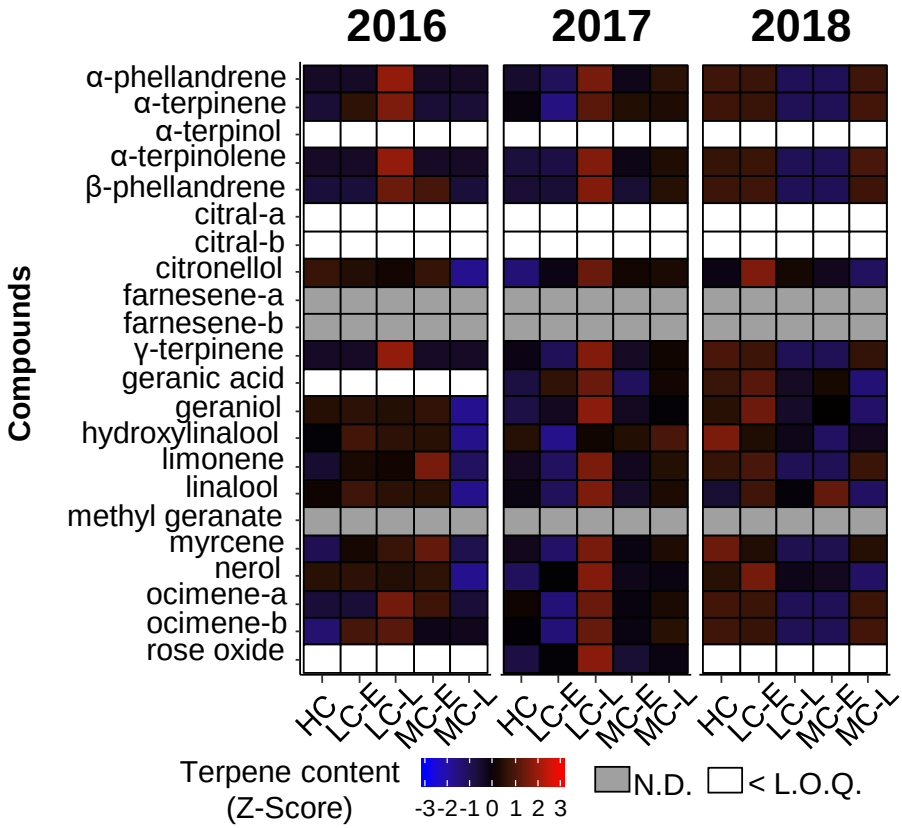

Figure 21: Heatmap of glycoside bound terpene concentration at harvest in berries of grapevines exposed to crop load management (CLM) strategies in 2016, 2017, and 2018. Data are plotted as Z-scores of terpene concentration calculated for each terpene among treatments and within each year. Z-scores range from red (+3σ) through black (0σ) to blue (-3σ) and indicate the highest, average, and lowest values respectively. All compounds with significant treatment effects also demonstrated interaction of those effects with the year factor. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

When comparing the bound terpene profiles among the years, berries from 2016 correlated with higher concentrations of geraniol, nerol, hydroxylinalool, linalool, and citronellol and with lower concentrations of geranic acid (Figure S4b, d). 2018 berries had the opposite relationship (Figure S4b, d). All other terpenes showed higher concentrations in berries from 2017 and were similar between 2016 and 2018 (Figure S4b, d).

Total bound terpenes concentration and per berry content at pre-harvest were significantly affected by CLM treatments ($p = 0.0248$ and $p = 0.0104$, respectively) (Table 13). Generally, the concentration in LC-E and MC-E berries was similar; however, LC-E contained

more total bound terpenes than HC, LC-L, and MC-L. The LC-E treatment increased bound geraniol ($p = 0.00333$) and bound geranic acid ($p = 0.0344$) relative to HC, LC-L, and MC-L and LC-L treatments, respectively (Table S27 and S28). A year effect was observed; with 2018 containing more bound terpenes than 2017. Unfortunately, the frozen powder from 2016 pre-harvest samples was damaged via thawing and refreezing prior to analysis, thus analysis was not conducted on these compromised samples.

3.3.6 Gene Expression

3.3.6.1 MEV Pathway and GPP Synthase

Expression profiles of terpene biosynthesis related genes were investigated only in the 2016 growing season (Figure 22). All examined genes were assessed throughout the growing season with RNA extracted from the same berry samples from which terpene profiles were analyzed. Early terpene biosynthesis genes – *DXS1, DXS3*, and *HDR* – displayed moderate to high relative expression with indications of differences among treatment means at early stages of berry development (49 and 65 DAA) and at harvest (119 DAA) (Figure 22a-c). *Post-hoc* testing revealed differences between LC-L and other treatments with LC-L demonstrating higher expression levels (Table S29). *DXS1* expression was high at early stages of berry development, decreasing at veraison, peaked prior to harvest and reached the minimum expression at harvest (Figure 22a). *DXS3* expression remained relatively constant throughout the season (Figure 22b). Finally, *HDR* was generally high from veraison to pre-harvest, but low at early stages and at harvest (Figure 22c). These expression patterns mostly mirror the ones observed in the DI experiment (Figure 11), except for the last sampling point. *DXS3* expression did not correlate significantly with the pattern of accumulation of any compound (Figure S8). *DXS1* and *HDR* expression correlated negatively to the pattern of accumulation of several terpenes but the R was

low (-0.3 < R <0.3) (Figure S8). *GPPS* expression peaked after veraison with little variation in expression throughout the season (Figure 22d). LC-L had the highest expression levels at 49 and 119 DAA. *GPPS* correlated negatively with free rose oxide (R = -0.32), methyl geranate (R = -0.22), and α-terpinol (R = -0.24) and positively with bound γ-terpinene (R = 0.47), α-phellandrene (R = 0.47), and α-terpinolene (R = 0.47).

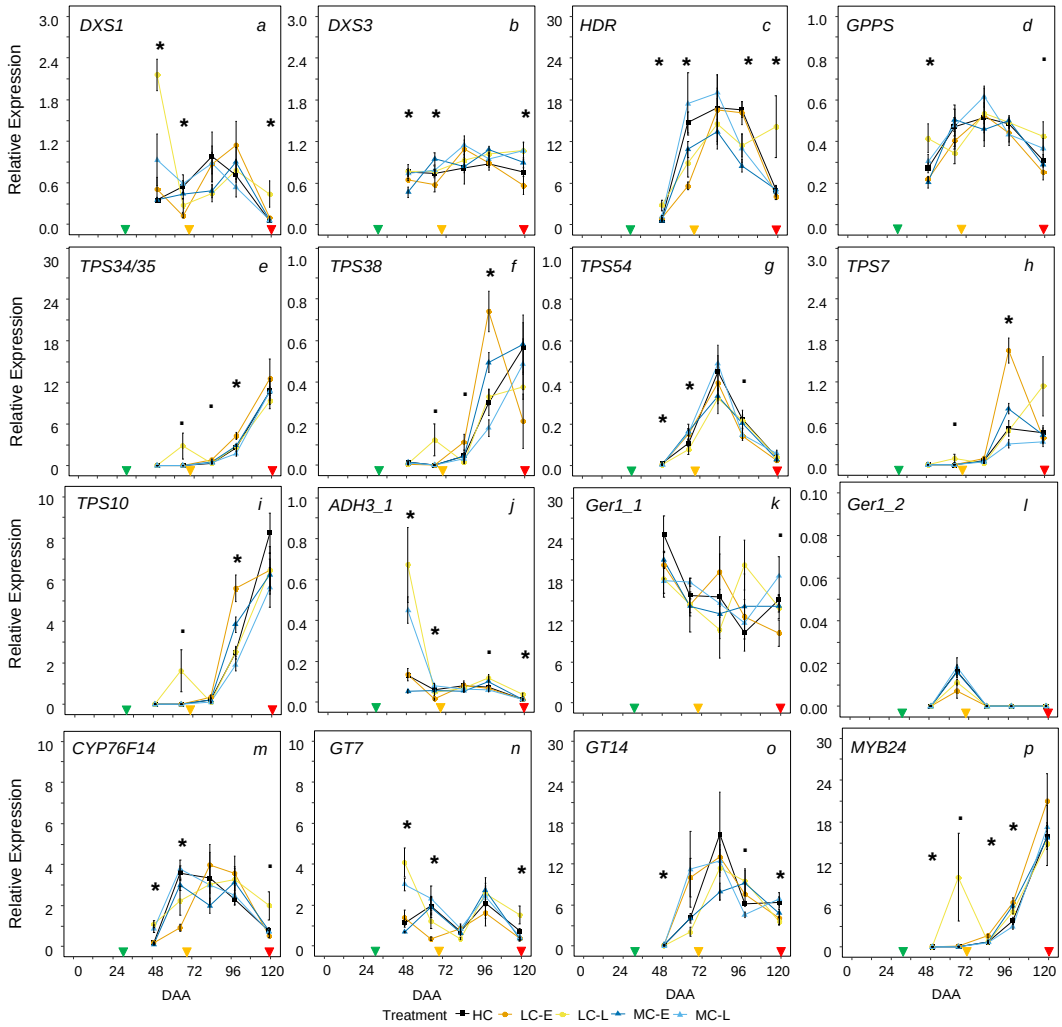

Figure 22: Evolution throughout the 2016 season of the expression of select terpene biosynthetic genes in Gewürztraminer berries exposed to crop load management (CLM) strategies. Standard error is indicated by error bars. Green, yellow, and red arrows indicate dates of early treatment application, treatment application at veraison (late), and commercial harvest. Legend at the bottom of the figure indicates coding of colours of point and lines to treatments. Asterisks and dots above mean relative expression values indicate significant ($p < 0.05$) and marginal ($0.05 < p < 0.1$) differences among means of CLM strategies as determined by one-way ANOVA within genes and sampling dates. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

3.3.6.2 Monoterpene-Related Genes

Monoterpenes are primarily synthesized by TPSs belonging to two subfamilies, TPS-b and TPS-g (Martin et al., 2010). In this study, *TPS20, TPS27, TPS31, TPS34/35, TPS38, TPS44, TPS45, TPS47, TPS52, TPS54, TPS56, TPS57, TPS58, TPS61, TPS63, TPS69* were assessed in preliminary tests at three time points (49, 65, 98 DAA) from pooled cDNA from all treatments (Table S15). Then genes that were highly or preferentially expressed in the mid to late season were selected and tested in the whole set of samples as they were more likely related to the synthesis of terpenes in the berry. *TPS20*, *TPS27*, *TPS44*, *TPS52*, *TPS58*, and *TPS61* genes were expressed at low levels (< 0.001 of relative expression value) throughout the season and showed no expression at veraison or during ripening (65 and 98 DAA, respectively) (Table S15). *TPS31*, *TPS45*, *TPS56*, *TPS63*, and *TPS69* genes showed low expression (0.01 – 0.1 relative expression) at 49 DAA, however had no expression at veraison or during ripening (65 and 98 DAA, respectively) (Table S15). These poorly expressed genes were not tested further. *TPS34/35*, *TPS38*, and *TPS54* were further tested in all the samples since they were expressed from veraison onwards – matching the accumulation of terpenes.

TPS34/35, an (*E*)-β-ocimene synthase, gradually increased in expression from 49 DAA until 119 DAA in all thinning treatments (Figure 22e). The (*E*)-β-ocimene/myrcene synthase *TPS38* demonstrated a similar expression profile (Figure 22f). At 65 and 83 DAA, marginal differences were observed between treatments in both genes. *Post-hoc* testing revealed no further differences at 65 DAA (Table S29). At 83 DAA, *TPS34/35* and *TPS38* expression was marginally higher in LC-E berries than LC-L ones ($p = 0.067$ and $p = 0.075$ for *TPS34/35* and *TPS38*, respectively) (Table S29). These differences became significant at 98 DAA where one-way ANOVA detected a significant difference between treatments ($p = 0.00198$ and $p =$

0.000140 for *TPS34/35* and *TPS38*, respectively) (Table S29). *Post-hoc* testing indicated that *TPS34/35* and *TPS38* expression was higher in LC-E than HC, LC-L, and MC-L treatments (Table S29). Additionally, *TPS34/35* and *TPS38* expression was significantly higher in MC-E than MC-L (Table S29). In summary, at 98 DAA, early application of cluster thinning stimulated the expression of these genes in comparison to late applications of thinning. No differences among treatments were observed at harvest. *TPS54*, a (3S)-linalool/(*E*)-nerolidol synthases, peaked in expression after veraison (83 DAA) in all treatments (Figure 22g). Significant differences between treatments were only observed at 49 and 65 DAA, with the expression being lower in HC berries than LC-E, LC-L and MC-L berries at 49 DAA, and the expression in LC-L berries being lower than LC-E, MC-E, and MC-L at 65 DAA. *TPS54* correlated significantly with *DXS3*, *HDR*, *GPPS*, and *GT14* expression. None of these tested monoterpene synthases strongly correlated ($R > 0.90$) with the ascribed monoterpenes they have been shown to produce (Figure S8).

3.3.6.3 Sesquiterpene-Related Genes

The subfamily TPS-a has been characterized to primarily generate sesquiterpenes (Martin e al., 2010), three of which were examined in this study *TPS07*, *TPS10*, and *TPS14*. Expression generally increases from 49 to 119 DAA with a peak at 65 DAA in LC-L and significant differences between treatments at 98 DAA (Figure 22 h, I; Table S29). The expression was higher in LC-E berries than in the berries of the other treatments at 98 DAA (Table S29). The berries of the MC-E treatment had higher expression of *TPS07* and *TPS10* than the MC-L treatment and marginally higher expression for *TPS14*. This was also seen for *TPS34/35* and *TPS38*. None of the TPS-a subfamily genes tested correlated with sesquiterpene accumulation (Figure S8a).

3.3.6.4 Other Related Genes

Several terpene modification genes were examined: predicted geraniol dehydrogenases *ADH3_1* (XM_002279796.4; Wong et al., 2017) and *ADH3_2* (XM_002279682.2; Wong et al., 2017), predicted geranial reductases *GER1_1* (XM_002285116.2; Wong et al., 2017) and *GER1_2* (XM_002272235.3; Wong et al., 2017), glycoside transferases *GT7* (XM_002276510.2; Li et al., 2017) and *GT14* (XM_002285734.2; Wen et al., 2015), and a cytochrome P450 enzyme, *CYP76F14* (XM_010659727.2; Ilc et al., 2017). The predicted genes were identified based on homology with *CpADH3* and *CpGER1* genes which were highly expressed in the flowers of the orchid species *Caladenia plicata* (Wong et al., 2017). These genes are proposed to catalyze the conversion of geraniol into β-citronellol (Xu et al., 2017). Additionally, a proposed regulator of terpene biosynthesis, *MYB24* (NP_001268062.1; Carbonell-Bejerano et al., 2014; Savoi et al., 2016), was tested for expression.

The two ADHs demonstrated consistent and low to moderate expression throughout the season (Figure 22j). CT treatments had affected *ADH3_1* expression at 65 DAA with LC-E having higher expression than all other treatments; no affect from DI was observed on *ADH3_2* expression (Table S29). *Ger1_2* was not highly expressed also with little to no expression in the later part of the season (Figure 22l). *Ger1_1*, however, was consistently highly expressed throughout the season (Figure 22k). No differences among treatments were detected (Table S29).

At 49 DAA, *GT7* was expressed at higher levels in in MC-L and LC-L berries, compared to other treatments (Figure 22n; Table S29). At harvest, the expression was higher in LC-L berries than in all other treatments except HC (Figure 22n; Table S29). *GT14* was not highly expressed prior to 50 DAA, then peaked in expression around veraison, falling and remaining steady until harvest (Figure 22o). At harvest, differences were observed among treatments;

however, only between MC-L and LC-L and marginally between HC and LC-E, MC-L and LC-E (Figure 22o; Table S29).

CYP76F14 steadily increased from early stages of development to ripening but decreased at harvest (Figure 22m). The expression was higher in HC, MC-E and MC-L berries than in LC-E and LC-L berries at 81 DAA (Table S29).

MYB24 expression (Figure 22p) mirrored the expression profile of *TPS07*, *TPS10*, *TPS14*, *TPS34/35*, and *TPS38* which was confirmed by their correlation values (Figure S8a). As for some of these TPS genes, the expression of *MYB24* was higher in LC-E berries than in the other treatments at pre-harvest (98 DAA) and harvest (119 DAA) (Table S29).

3.4 Discussion

This project explored the physiological and metabolic responses of the white-wine varietal Gewürztraminer to crop load management via cluster thinning, with a focus on terpene accumulation. Compared to previous studies on crop load management in grapevine in similar viticultural regions (Reynolds and Wardle, 1989; Reynolds et al., 1996; Keller et al., 2005; Hannam et al., 2014), this study expands on current literature by examining and linking together yield parameters, basic berry composition, vine physiology, free and glycosidically-bound terpene profiles, and gene expression results. No previous study examined these parameters simultaneously. Linking these parameters allows us to better assess the underlying metabolic and molecular responses to DI in grapes with a confident control for proper comparisons.

Thinning treatments altered vine yield parameters as expected. The number of berries per clusters and clusters per vine were increased and reduced, respectively, similarly to previous studies (Reynolds and Wardle, 1989, Hannam et al., 2014). Berry and pruning weights were unaffected by treatments. Differences among treatments were observed in cluster weights, which are likely explained by differences in berry number. Similar results in yield parameters were observed in early cluster thinned treatments applied to Merlot (Hannam et al., 2014), Cabernet Sauvignon, Riesling, Chenin Blanc (Keller et al.,2005). Increased numbers of berries could be a result of repeated application of thinning treatments to the vines over the three-year study. However, other studies have reported little to no differences on cluster and berry weights from cluster thinning. Mota et al. (2010) demonstrated in Merlot and Cabernet Sauvignon, with applied treatments that matched the cluster numbers of LC and MC in this study, no effects on cluster weight. Keller et al. (2008) showed no effects of cluster thinning on berry weight, berry number, and cluster weight in own-rooted Cabernet Sauvignon under lower crop loads

treatments. Similarly, Luna et al. (2017) showed no difference in berry weight and cluster weight between half crop versus full crop applied at veraison on Pinot Gris, Riesling, Cabernet Franc, and Cabernet Sauvignon vines grown in Niagara, Ontario. Overall, cluster thinning largely reduced yield by cluster removal and not by altering berry number and berry and cluster weights within the season.

Pruning weights did not demonstrate an effect from our treatments as seen in Reynolds et al. (1996), Keller et al. (2005), and Hannam et al. (2014). All treatments experienced a steady reduction in pruning weight by 0.2 kg per year; a likely explanation is that increased wildfire smoke (i.e. less overall photosynthesis) in latter years limited vegetative growth. Pruning weights are used in calculating crop load (Ravaz Index = Yield / Pruning weight) as well as being themselves a measure of vegetative growth of the season just preceding pruning (Coombe and Dry, 1992). In terms of Ravaz Index, HC vines were under-cropped comparatively to the unthinned controls in other studies by Reynolds (1989, 1996). Ravaz Index values of cluster thinned vines from Reynolds and Wardle (1989) were on average 4.6 kg / kg, which falls in-between MC and HC treatments. No studies on Gewürztraminer matched crop loads of LC treatments by Ravaz Index. Values below 4 are uncommon in literature primarily since indices between 4 and 10 are considered "balanced" vines (Bravdo et al., 1985). Another measure of crop load is the available leaf area, in cm^2, per gram of berries. Values between 5 cm^2 and 15 cm^2 leaf area per g of fruit are quoted as required for adequate fruit ripening (Smart and Robinson, 1991; Jackson and Lombard 1993). All treatments maintained more than 21 cm^2 leaf area per g of fruit, likely eliminating source limitation. Differences among treatments exist with later applied treatments possessing greater leaf area per yield than HC and MC-E, but all values are > 21 cm^2 per g.

As expected, no effects were observed on leaf water potential, leaf area, and vine leaf gas exchanges, and leaf water potential. These parameters were only measured to ensure that the reduction of crop load does not affect vine growth and physiology. Likely due to weather variations, the year term had a significant effect on all gas exchange parameters in all months. The year 2018 (final year) had the lowest rates of photosynthesis, transpiration, and stomatal conductance. In literature, crop load studies that report effects on gas exchange considered the effects of severe fruit thinning (0-6 clusters per vine) (Edson et al., 1995; Iacono et al., 1995; Koblet et al., 1996; Naor et al., 1997; Petrie et al., 2000) or from canopy manipulation via leaf removal (Iacono et al., 1995; Petrie et al., 2000; Petrie et al., 2003). Severe fruit thinning appears to decrease photosynthetic rates – when lateral grown is restricted – likely due a build up of carbohydrate content in the leaves from a lack of sink tissues (Iacono et al., 1995; Naor et al., 1997; Petrie et al., 2000). These differences become most apparent after veraison when sink requirements increase (Edson et al., 1995; Petrie et al., 2000). Leaf removal appeared to increase leaf photosynthesis which is thought to result from an increased requirement demand of carbohydrates from source tissues by sink tissues (Edson et al., 1995; Petrie et al., 2000). Evidently, this study did not affect the source-sink relationship as much as to affect vine leaf gas exchange.

Berry sugars and berry acids (TA and pH) were affected by cluster thinning treatments year-over-year at pre-harvest and harvest; however, interactions were observed at pre-harvest dates. Interactions could not have arisen from differences from sampling dates as they were similar (98, 101, and 99 DAA for 2016, 2017, and 2018 respectively). The lighter cropped treatments displayed a faster ripening, as observed by in higher TSS and, pH than higher cropped treatments. These observations matched the hypothesis prediction. Moreover, these results were

previously observed in Gewürztraminer (Reynolds and Wardle, 1989; Reynolds et al., 1996), Cabernet Franc (Luna et al., 2017), Pinot Gris (Reynolds et al., 1994; Luna et al., 2017; Reeve et al., 2018), Riesling (Reynolds, 1989; Luna et al., 2017), and Thompson Seedless (Kliewer and Dokoozlian, 2005). Other studies on Cabernet Sauvignon (Keller et al., 2005; Bowen et al., 2011; Luna et al., 2017), Merlot (Mota et al., 2010; Hannam et al., 2014), Riesling, and Chenin Blanc (Keller et al., 2005) showed little to no effect from cluster thinning on TSS, pH, and TA. This suggests some other factors (experimental conditions, variety) can interact with the crop load effect. TSS and TA were unaffected by the timing of timing, however, there appears to be a small effect of early applied thinning on pH. This matched studies on cluster thinning application timing in Cabernet Franc (Reynolds et al., 2005), Pinot Gris (Reynolds et al., 2005), and Sauvignon Blanc (Kok, 2011). Differences were small at harvest (~0.5 g/L TA and 0.10 pH between LC-E and MC-L), and potentially would not affect wine pH substantially.

Neither thinning severity or timing of application appear to have a consistent effect on berry acidity among years. Berry acidity at harvest is thought to be predominantly determined by temperature and maturity degree (Kliewer 1964; Kliewer et al., 1967; Jackson and Lombard, 1993). Tartaric and malic acids account for 90% of total acids in grapevine berries (Kliewer et al., 1967; Conde et al., 2007). With maturity, overall acidity decreases due to dilution of tartrate and decomposition of malate. Malate concentrations decrease with higher temperatures (Keller et al., 2005, Koundouras et al., 2006, Pereira et al., 2006), while tartrate concentration does not appear to be significantly affected by temperature (Parra et al., 2010). Since leaf area was not affected among treatments, it is likely that temperature/grape exposure were not factors in this study, so differences likely arose from the altered source-sink ratio.

At harvest, a significant interaction between the CLM treatment and year effects was observed for the total free terpenes, but not for the total bound terpenes. The year 2018 was most abundant in total free terpenes across all treatments, while total bound terpenes were 2- to 3- fold higher in 2016 compared to 2017 and 2018. Furthermore, application of cluster thinning at ~30 DAA (early thinning) appeared to consistently increase free terpenes at harvest if compared to the application at veraison (late thinning). At pre-harvest (~21-22 °Brix), no interactions were observed and generally, LC-E berries had the highest total free and bound terpene concentration and content compared to all other treatments. However, there was no observed difference from MC-E in total bound terpene concentration. LC-E also showed higher concentrations of geraniol at pre-harvest relative to HC and later thinning treatments. Overall, bound terpenes at pre-harvest were increased by early thinning in comparison to late thinning with differences among treatments disappearing by commercial harvest (108 to 119 DAA). Free and bound terpenes appeared to accumulate at higher levels and earlier on during development in both early thinned treatments (LC-E and MC-E) – when compared to their later thinned equivalents (LC-L and MC-L). Increased total terpene concentrations from crop load adjustments were observed by Reynolds and Wardle (1989) in Gewürztraminer and Kok (2011; 2016) in Sauvignon Blanc and Muscat Hamburg. Considering total terpene content (free and bound) as well as TSS and acids, LC-E berries at pre-harvest (two-weeks prior to harvest) has similar composition than the berries of the other treatments at harvest. Hence, those berries could have been harvested earlier.

As mentioned in Section 2.4: Discussion, the profile and concentration of free and bound terpenes detected in this study matched the profiles previously reported with few differences. These differences are likely accounted by methodological differences, different environmental

factors influencing biosynthesis conditions, or not reporting compounds that are not the focus of the study (here or in literature).

The observation of monoterpenes such as ocimene, phellandrene, terpinene, and myrcene in the bound profile was notable. This is similar to what was seen in Chapter 2, discussed in detail in Section 2.4.

Earlier terpene pathway genes (*DXS, HDR,* and *GPPS*) did not show strong significant correlation with the individual terpenes, likely as their gene products synthesize precursors for many terpenoids (Schwab and Wüst, 2015). Previous studies outlined the regulatory role of grapevine DXS1 in terpene and isoprenoid production (Battilana et al., 2009; Emanuelli et al., 2010; Costa et al., 2018). Vines engineered to highly express *DXS1* also generated higher levels of total free and bound monoterpenes than controls (Costa et al., 2018). Free and bound geranic acid and bound citronellol were observed in larger amounts in engineered vines than in control vines (Costa et al., 2018). Current literature suggests that grapevine *DXS1* expression can raise the metabolic flux through the MEP pathway and may function in combination with terpene synthases or other genes to affect specific terpene production (Costa et al., 2018). Similar observations have been reported in *Osmanthus fragrans* Lour. (Zeng et al., 2016).

Few terpenes in the free fraction correlated significantly with terpene biosynthetic genes, however, correlations were not observed between terpenes and their biosynthetic TPS gene. For example, expression of *TPS54* and *TPS14* correlated with citral-a and geranic acid concentrations, respectively; however, with *TPS54* and *TPS14* are (3S)- linalool/(*E*)- nerolidol and α-zingiberene synthases (Martin et al., 2010). The bound fraction demonstrated similar trends than the free fraction.

Despite a lack of correlation between genes and individual metabolites, some consistencies were observed among the effect of treatments on total free terpenes and gene expression. Primarily, LC-E induced a significantly higher expression in of *TPS07, TPS10, TPS34/35,* and *TPS38* two-weeks prior to harvest which matched trends in total free terpenes. Additionally, LC-E had induced the expression of *MYB24* at harvest. In conjunction with total free terpene data, these results suggest a response of the terpene biosynthesis biosynthetic pathway to CLM treatments, but only for LC-E relative to HC treatment. Moreover, the timing of cluster thinning appears to interact with the intensity of thinning as LC-E and LC-L (or MC-E and MC-L) did not respond identically. For instance, an early thinning application (MC-E and LC-E) did appear to cause early peaking of *GT14* expression prior to veraison. Otherwise, timing of application had no evident effects on gene expression. Wu et al. (2013) observed 59 differentially expressed proteins (47 down, 12 up) in berry skins from shoots with 2 leaves vs. 12 leaves via 2-DE gels. A protein related to carotenoid degradation/norisoprenoid synthesis – carotenoid cleavage dioxygenase – was down regulated in over-cropped (due to severe limitations of leaf area) vines. Furthermore, many elongation factors were down-regulated indicating that protein synthesis was likely reduced in berry skins under severe over-cropping.

Pastore et al. (2011) observed 108 highly modulated genes involved in secondary metabolism with 25 down-regulated and 83 up-regulated in the berries of Merlot vines exposed to cluster thinning. Only one gene related to sesquiterpene synthesis, (-)-germacrene D synthase, was observed to be down-regulated in cluster thinned vines vs. unthinned vines. This gene demonstrated similar expression profile to *TPS54*. In biannual bearing *Citrus sp*., terpenoid metabolic processes were down-regulated in buds of de-fruited and low-crop-year trees (Shalom et al., 2014). Three genes were commonly down-regulated in buds from heavy cropped trees;

two of these genes were homologous to nerolidol synthases. Over-cropped plants in these studies reduced the expression of terpene genes, mirroring our observations in HC vines at ~95 DAA in this study. My study suggests that the intensity of cluster thinning affected sugar accumulation, berries per cluster, clusters per vine, and crop load metrics over the timing of thinning. The interaction between intensity and timing was most evident on total terpenes and the expression of genes related to terpene synthesis where it was the combination of lighter cropping and early thinning that maintained the highest TPS-gene expression and terpene concentration. These effects are likely due to changes in the source:sink relationship in LC-E berries, possibly due to early exposure to elevated sugar/hormone levels in phloem from these berries. Furthermore, these results mostly coincided with our predictions on the CLM effects on ripening and terpene content with evidence for ripening induction, faster terpene accumulation, and increased TPS expression with severe reductions of crop load and size (-50%).

In summary, this study shows that early applications of cluster thinning in grapevines are more effective in altering berry aroma and ripening than later applied thinning. These results maybe more applicable to similar cooler viticultural regions since warmer viticultural regions can ripen more fruit per vine than cooler regions and in some regions, sugars may accumulate too rapidly creating “ripe” berries by sugar levels, but immature berries by favour and aroma.

Chapter 4: Conclusions

Application of late deficit irrigation increased the concentrations of Gewürztraminer-characteristic terpenes (geraniol, citronellol, citral-b, geranic acid) relative to CN (well irrigated berries) berries at harvest (96-115 DAA). DI treatments did not affect total free or bound terpenes at harvest. However, total bound terpenes were higher in concentration in ED berries than CN berries 15 days prior to harvest. The expression of terpene biosynthesis genes did not explain the observed effects from DI on the terpene accumulation. The initial hypothesis that ED and PD would stimulate terpene synthesis was not confirmed by the gene expression and metabolite analyses. DI treatments reduced irrigation volumes by 25-30% (ED and LD) or 50% (PD) relative to CN, but only ED and PD treatments affected vine yield negatively in relation to CN. Deficit irrigation reduced sugar content (-4%) was likely due to lower photosynthesis rates in the leaves. The LD regime may be most appealing for growers since Gewürztraminer-characteristic aromas can be increased in grapes and wine, while reducing irrigation volumes, and not affecting vine yield, and hence not negatively affecting crop economic output. Wines from LD can be expected to have lower alcohol and possibly higher volatile terpenes than CN wines, however future studies on wines made from similar treatments are necessary to confirm. Limiting water usage in vineyards may prove particularly valuable in future climates as water availability is projected to become more variable due to anthropogenic climate change, especially in the Okanagan basin (Cohen and Kulkarni, 2001).

Reducing yield by 40% in the vineyards via cluster thinning applied early in the season (30 DAA) accelerated ripening as determined by faster sugar and terpene accumulation. This is potentially related to an induction of several terpene genes. In addition to observing the acceleration of ripening by early cluster thinning, it was demonstrated that cluster thinning

applied after 60 DAA had neutral to negative effects on terpene accumulation. Geraniol and total terpenes accumulated more rapidly with early thinning relative to unthinned controls prior to harvest, however, at harvest, individual terpenes and total terpenes were similar among treatments. Our initial hypothesis was confirmed by these results. Wines produced from LC-E grapes can be expected to have higher alcohol and possibly higher volatile terpenes than HC wines (depending on when grapes were harvested). Future studies on wines made from similar grapes are necessary. Growers occasionally choose to apply mid-season cluster thinning – known as "green harvest" – to achieve accelerate ripening on the remaining crop. My study shows that late applications (mid-season) are ineffective. On the contrary, early cluster thinning allows growers to accelerate ripening and terpene accumulation and harvest grapes earlier, which is useful in viticultural regions where the climate does not allow a late harvest as often occurs in Canadian wine regions.

Both studies were limited by not pursuing an analysis of vinification. Analysis of the fermentation, wine composition, and sensory characteristics are clear next step. In a future study, fewer treatments (only the ones that were effectives on berry quality in this study) would be assessed, and fruit would be collected from multiple treated vineyards (larger sample size) and vinified in the same winery using a standardized protocol. This would provide conclusive results on how to manage the wine quality in the vineyard for the BC wine and grape industry.

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Appendix

Table S1: Terpenoids detected consistently and quantified in Gewürztraminer berries and their respectively calibration coefficients, quantitation and base mass spectroscopy fragmentation ions, and retention indices in DB-WAX and HP5 gas chromatography columns.

Terpenoids Detected	Calib. Coefficient		Quant.	Base Ion	DB-WAX Retention Index			HP5 Retention Index		
Monoterpene	**SPME**	**LI**	**Ion**		**A.S.**	**Sample**	**Ref [2]**	**A.S.**	**Sample**	**Ref [1]**
α phellandrene†	0.993	0.335	93	93	1146.5	1149.4	1170[2]	991.9	999.9	1002[1]
myrcene†	14.716	7.323	93	41	1151.8	1151.7	1158[2]	980.9	989.9	990[1]
α terpinene†	3.121	1.821	93	121	1160.2	1160.2	1184[2]	1004.1	1013	1014[1]
limonene†	0.850	1.642	93	68	1180.9	1180.6	1208[2]	1016.1	1024.4	1029[1]
β phellandrene†	1.113	0.393	93	93	1187.9	1188.4		1016.2	1024.4	1029[1]
ocimene a‡	0.437	1.167	93	93	1223.2	1222.5		1029.8	1037.9	1037[1]
γ terpinene†	28.658	17.673	93	43	1230.9	1228.3	1249[2]	1047.6	1055.7	1054[1]
ocimene b‡	0.437	1.167	93	93	1238.5	1243.3	1245[3]	1039.6	1047.9	1050[1]
α terpinolene†	4.609	2.622	93	93	1264.8	1265.2	1297[3]	1076.9	1084.9	1088[1]
Monoterpenol										
linalool†	3.335	0.713	93	71	1544.9	1544.4	1544[2]	1091.2	1100.3	1096[1]
α terpinol†	0.094	0.469	69	59	1682.8	1682.3	1700[2]	1179.5	1188.4	1186[1]
citronellol†	0.326	0.421	69	69	1759.5	1759.7	1768[2]	1222	1228.1	1225[1]
nerol†	1.197	1.742	69	69	1788.2	1788.2		1219	1228.1	1229[1]
geraniol†	2.415	2.207	69	69	1837.9	1837.7	1852[2]	1247.1	1255.7	1252[1]
hydroxylinalool§	na	0.713	71	43		2298.5			1367.8	1619[1]
Monoterpenal										
citral-a‡	1.953	1.683	69	69	1657.9	1657.9		1231.3	1226	1238[1]
citral-b‡	1.953	1.683	69	69	1708.8	1707.8		1261.5		1267[1]
Monoterpene Oxide										
rose oxide†	4.756	75.436	139	139	1339.7	1341.2		1101.2	1110.1	1106[1]
Monoterpenoate										
methyl geranate§	2.415	2.207	69	69	1656.9	1656.2			1319.7	1324[1]
Monoterpenoic Acid										
geranic acid§	2.415	2.207	69	69		2315.4			1357.9	1282[1]
Sesquiterpene										
farnesene-a‡	14.716	7.323	93	41		1709.9			1489	1442[1]
farnesene-b‡	14.716	7.323	93	41		1730.9			1502.7	1456[1]

†*Compounds were identified and calibrated using highly purified authentic standards.*
‡*Compounds were identified and calibrated using configurational isomer mixtures.*

[§]*Compounds were identified matching the mass spectrum and fragmentation pattern against the NIST library. Calibration curve coefficient from a compound with authentic standard and with closest chemical functionality and structure was utilized.*

Table S2: Gene names, identifiers, and forward/reverse primer sequences expression analysis of Gewürztraminer berries from field-grown vines in 2016.

Name	Accesion Number	VIT12Xv1 code	Forward primer	Reverse primer
DXS1	XM_002277883	VIT_05s0020g02130	CTCATTTCCTGCCCATTTTAGC	CTTACTCCTTTGCTGGGATTGG
DXS3	XM_002282392.3	VIT_04s0008g04970	GAAGGCTCTGTTGGAGGGTTT	TCCTCTGGTGATGCCTGTTCT
HDR	XM_002284623.2	VIT_03s0063g02030	TCTTCCTCGTCTGTGGCTGTT	GCGATTCATGAGCTCCAGAGT
GPPS	AY351862	VIT_15s0024g00850	AGAATCTGGGATTGGCATTCC	TGGCGGATGTCAGACAATGA
TPS02	HM807375	VIT_18s0001g04080	AAATCGTGTCAAAGGAGGCCT	CTGATGCTACATGCCCTCTCT
TPS07	HM807377	VIT_18s0001g04280	TTGCTGAGGCCAAATGGTTAC	TCTCCCATTCCCACAAAGGA
TPS10	HM807376	VIT_18s0001g04780	TGGCCTTAATTCTCGCTACCA	GGCTCACGATGAAGGTATTGC
TPS14	HM807405	VIT_18s0001g05240	TCGAGGTGGTGGAAAGACC	ACGCTGTTTATATCCCACCTC
TPS20	HM807379	VIT_19s0014g01060	GGGTGCACGTTGCTTCTAGT	TGGCATCAGCACTGGTGTAG
TPS27	HM807374	VIT_19s0014g04900	TGCCTCAGCTGTTGAATGCT	TGAGGACGGTCATCGGAACA
TPS31	HM807390	VIT_12s0059g02710	TCAAATCCCTCTCACCCTTG	AATTAGCTGATCGCCTTTCG
TPS34/35	HM807385	VIT_12s0134g00020	AGGAAAGTGCTCGTGAACAC	ATGTGCACTCGGAAATTCGC
TPS38	HM807387	VIT_13s0084g00010	GGAACATCACTGGATGAGTTGA	ATCTCCATGCTGATACATGCAC
TPS44	HM807383	VIT_13s0067g03790	TTGGAGAAGCTTAAGGGAGATG	GGTAGCCATGCTGTCTTAGGAG
TPS45	HM807382	VIT_13s0067g03790	TGGAGAATGCTAAGGCTAGAGG	ATTCTCCACCAATCTGTCCCTA
TPS47	HM807388	VIT_00s0361g00060	CCAAAGGAGATCTGCCAATTAC	GCTTCTCTGATCTCCTCCTCAA
TPS52	HM807399	VIT_12s0134g00140	ATCTTCCTTTGTCGCTCCTT	CCGCATGTGGAGATAGAGTT
TPS54	HM807391	VIT_00s0385g00020	GCCGTTAGTTCAGGGGTACA	GGTCATCCCAAAGACGAAGA
TPS56	HM807393	VIT_00s0271g00060	CAGCAATCACGGTGAGAGCA	GCGTGCTGCTGAATCTTTGG
TPS57	HM807394	VIT_00s0372g00040	CAAGGACGAGAATCAGGACG	CAACATTGAGACATGCCTTGG
TPS58	HM807396	VIT_00s0372g00060	AATACATGGAACACGCACAA	TGTTGAAATCCATTTTTGCT
TPS61	HM807397	VIT_00s0372g00070	AAGCGCCTCAACAAGGAATG	TGCTTTCATTGACAAGCAAC
TPS63	HM807395	VIT_00s0266g00010	CGAGAATCAGGATGGGCACG	TGCTGAGGCTCTTCCATGTC
TPS69	XM_010646798	VIT_19s0085g00830	CTTGGTAAAACGACAGCAATGG	TGGTCCCTTCAAAGCCCAGA
ADH3-1	XM_002279796.4	VIT_04s0044g00190	TCCGTTCTCAGAGATCAACAA	ACTCTCTCATCTCAAGATATTCTATGG
ADH3-2	XM_002279682.2	VIT_04s0044g00210	ATTCCAGTCGGCATAAGTGT	TTGCAACTGCATAGACATTGTT
GER1-1	XM_002285116.2	VIT_18s0001g09420	AGAAGCAAAGCATGGTAGAG	ACCCAGGACAAGGTCTATAA
GER1-2	XM_002272235.3	VIT_03s0091g00450	CTAGGCAAGATTGAACCTCAT	CTCACCTCCTCAAACAGTATATC
GT7	XM_002276510.2	VIT_16s0050g01580	TTTAGCACCACCGGAACCGGA	TTCAAGCACTGAGTTCCACCCA
GT14	XM_002285734.2	VIT_18s0001g06060	ACCATGGAGTGGAAGCATAGGG	TGGAAACAAGGCAGGAAAGGTG
CYP76F14	XM_010659734.1	VIT_02s0025g04880	AGCTAGCAGTGATGTGTTAGACGTTC	GTTGTGTCAGTCCCCGCAGC
MYB24	NP_001268062.1	VIT_14s0066g01090	TCAGACACATGATCAAGCAACT	CGAGATTGGCAGGGTAGGA

Table S3: Two-way ANOVA of deficit irrigation treatments and year effects on cane and vine leaf area of field-grown Gewürztraminer grapevines at before and after veraison in the Okanagan Valley, BC, Canada. CN = Well irrigated controls; ED = early deficit; LD = late deficit; PD = prolonged deficit.

	Cane Leaf Area (m^2/cane)				**Vine Leaf Area (m^2/vine)**			
	Before Veraison†		**Harvest‡**		**Before Veraison**		**Harvest**	
DI Treatment	n.s.		n.s.		n.s.		n.s.	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
CN	2.01	0.10	2.56	0.19	5.42	0.30	6.89	0.54
ED	2.48	0.23	2.29	0.12	6.29	0.56	5.82	0.30
LD	2.55	0.17	2.43	0.19	6.64	0.43	6.34	0.49
PD	2.42	0.12	2.18	0.10	6.38	0.32	5.76	0.29
Year	n.s.		n.s.		n.s.		n.s.	
2016	2.31	0.15	2.44	0.13	6.27	0.27	6.00	0.44
2017	2.30	0.09	2.47	0.19	6.47	0.52	6.02	0.25
2018	2.48	0.16	2.18	0.10	5.81	0.26	6.59	0.41
DI x Year Interaction	n.s.		0.0599		n.s.		0.0333	

†Before Veraison is defined as any leaf area measurements in July (42, 30, and 46 DAA in 2016, 2017, and 2018, respectively) of that respective year.
‡ Harvest is defined as any leaf area measurements in September/October (92, 81, and 113 DAA in 2016, 2017, and 2018, respectively) of that respective year.

Table S4: One-way ANOVA and *post-hoc* testing of the effect of deficit irrigation (DI) treatments on the berry sugars (in °Brix) in berries of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada from 2016 to 2018. CN = Well irrigated controls; ED = early deficit; LD = late deficit; PD = prolonged deficit.

	One-Way ANOVA				
	Tukey's HSD				
Total Soluble Solids (°Brix)	**CN**	**ED**	**LD**	**PD**	**p-value**
2016 Season					
DAA 34					$2.30 \cdot 10^{-1}$
DAA 47					$5.68 \cdot 10^{-2}$
DAA 54					$7.18 \cdot 10^{-1}$
DAA 60					$9.79 \cdot 10^{-1}$
DAA 65					$5.75 \cdot 10^{-1}$
DAA 81					$2.31 \cdot 10^{-1}$
DAA 96	a	ab	bc	c	$4.35 \cdot 10^{-3}$
2017 Season					
DAA 33	b	a	b	ab	$2.39 \cdot 10^{-2}$
DAA 46					$1.42 \cdot 10^{-1}$
DAA 58	ab	b	a	b	$1.04 \cdot 10^{-3}$
DAA 74	b	a	ab	ab	$1.37 \cdot 10^{-2}$
DAA 87					$1.65 \cdot 10^{-1}$
DAA 94					$1.16 \cdot 10^{-1}$
2018 Season					
DAA 85					$7.92 \cdot 10^{-2}$
DAA 103	b	ab	a	a	$3.39 \cdot 10^{-2}$
DAA 122					$6.41 \cdot 10^{-2}$

Table S5: One-way ANOVA and *post-hoc* testing of the effect of deficit irrigation (DI) treatments on the berry titratable acidity (in g/L) in berries of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada from 2016 to 2018. CN = Well irrigated controls; ED = early deficit; LD = late deficit; PD = prolonged deficit.

	One-Way ANOVA				
	Tukey's HSD				
Titratable Acids (g/L)	**CN**	**ED**	**LD**	**PD**	**p-value**
2016 Season					
DAA 34	b	a	b	b	$2.32 \cdot 10^{-3}$
DAA 47					$6.91 \cdot 10^{-1}$
DAA 54					$3.36 \cdot 10^{-1}$
DAA 60					$6.53 \cdot 10^{-2}$
DAA 65					$8.49 \cdot 10^{-1}$
DAA 81					$1.00 \cdot 10^{0}$
DAA 96					$1.81 \cdot 10^{-1}$
2017 Season					
DAA 33					$6.18 \cdot 10^{-2}$
DAA 46					$1.59 \cdot 10^{-1}$
DAA 58	b	a	c	ab	$1.95 \cdot 10^{-3}$
DAA 74					$5.37 \cdot 10^{-1}$
DAA 87					$6.28 \cdot 10^{-1}$
DAA 94					$1.55 \cdot 10^{-1}$
2018 Season					
DAA 85					$4.04 \cdot 10^{-1}$
DAA 103					$3.22 \cdot 10^{-1}$
DAA 122					$3.51 \cdot 10^{-1}$

Table S6: One-way ANOVA and *post-hoc* testing of the effect of deficit irrigation (DI) treatments on the berry juice pH in berries of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada from 2016 to 2018. CN = Well irrigated controls; ED = early deficit; LD = late deficit; PD = prolonged deficit.

	One-Way ANOVA				
	Tukey's HSD				
Juice pH	**CN**	**ED**	**LD**	**PD**	**p-value**
2016 Season					
DAA 34					$8.17 \cdot 10^{-1}$
DAA 47	b	ab	a	ab	$4.64 \cdot 10^{-2}$
DAA 54					$4.11 \cdot 10^{-1}$
DAA 60					$1.99 \cdot 10^{-1}$
DAA 65					$5.98 \cdot 10^{-2}$
DAA 81					$4.84 \cdot 10^{-1}$
DAA 96					$5.21 \cdot 10^{-1}$
2017 Season					
DAA 33					$4.07 \cdot 10^{-1}$
DAA 46					$1.16 \cdot 10^{-1}$
DAA 58					$7.81 \cdot 10^{-1}$
DAA 74					$3.84 \cdot 10^{-1}$
DAA 87					$5.24 \cdot 10^{-1}$
DAA 94					$3.31 \cdot 10^{-1}$
2018 Season					
DAA 85	b	a	ab	a	$6.74 \cdot 10^{-3}$
DAA 103					$7.69 \cdot 10^{-1}$
DAA 122					$3.28 \cdot 10^{-1}$

Table S7: Two- way ANOVA of DI treatments and year effects on free terpenes (in ng per g FW) of field-grown Gewürztraminer berries at harvest (96, 96, and 115 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data.[†]

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data											
				2016				2017				2018			
	Treatment	Year	Interaction	CN	ED	LD	PD	CN	ED	LD	PD	CN	ED	LD	PD
farnesene-a	**	***	*	a	c	c	b								
farnesene-b	**	***	**	a	b	b	b								
α-phellandrene		***													
α-terpinene		***													
α-terpinol	***	***	***	a	a	b	b	ab	ab	a	b				
α-terpineolene		***													
citronellol	*	***													
myrcene		**													
β-phellandrene		***													
ocimene-a		***													
rose oxide	•	***													
citrol-b	•	**													
γ-terpinene		***													
geranic acid	•	***													
geraniol	*	***													
limonene		***													
linalool		**													
methyl geranate	***	***	***	ab	a	b	c	ab	b	a	b	a	b	ab	a
nerol		***													
ocimene-b		***													
citrol-a															

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test. Blank cells indicate no interaction or no data present if an interaction was present.

Table S8: Two-way ANOVA report on deficit irrigation (DI) treatment and year effects on free terpenes (in ng per berry) of field-grown Gewürztraminer berries at harvest (96, 96, and 115 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data.†

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data											
				2016				2017				2018			
	Treatment	Year	Interaction	CN	ED	LD	PD	CN	ED	LD	PD	CN	ED	LD	PD
farnesene-a	**	***	**	a	b	b	c					a	b	ab	ab
farnesene-b	**	***	***	a	b	b	b					a	b	ab	ab
α-phellandrene		***													
α-terpinene		***													
α-terpinol	***	***	***	a	a	b	b								
α-terpineolene		***													
citronellol	*	***													
myrcene		***													
β-phellandrene		***													
ocimene-a		***													
rose oxide		***													
citrol-b		***													
γ-terpinene		***	•												
geranic acid	*	***													
geraniol		***													
limonene		***	•												
linalool		***													
methyl geranate	***	***	***	a	a	ab	b					a	b	a	a
nerol		***													
ocimene-b		***													
citrol-a															

†*Post-hoc* tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test. Blank cells indicate no interaction or no data present if an interaction was present.

Table S9: Two-way ANOVA report on deficit irrigation (DI) treatment and year effects on bound terpenes (in ng per g FW) of field-grown Gewürztraminer berries at harvest (96, 96, and 115 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data.†

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data											
				2016				2017				2018			
	Treatment	Year	Interaction	CN	ED	LD	PD	CN	ED	LD	PD	CN	ED	LD	PD
α-phellandrene	•	***	•												
α-terpinene		***	•												
α-terpinol															
α-terpineolene	*	***	**	a	a	a	a	a	a	a	a	a	a	a	a
citronellol		***													
myrcene		***	*	a	a	a	a	a	a	a	a	a	a	a	a
β-phellandrene	•	***	**	a	a	a	a	a	a	a	a	a	a	a	a
ocimene-a	*	***	**	a	a	a	a	a	a	a	a	a	a	a	a
rose oxide															
citrol-b															
γ-terpinene	*	***	**	a	a	a	a	a	a	a	a	a	a	a	a
geranic acid		***													
geraniol	•	***													
limonene		***	*	a	a	a	a	a	a	a	a	a	a	a	a
linalool		***													
nerol		***													
ocimene-b		***	•												
citrol-a															
hydroxylinalool		***													

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "**•**" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test. Blank cells indicate no interaction or no data present if an interaction was present.

Table S10: Two- way ANOVA of DI treatments and year effects on bound terpenes (in ng per berry) of field-grown Gewürztraminer berries at harvest (96, 96, and 115 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data.[†]

Compounds	**Two-Way ANOVA of Three-Years' Data**			**Tukey's HSD *post-hoc* test of Year-Separated Data**											
				2016				2017				2018			
	Treatment	Year	Interaction	CN	ED	LD	PD	CN	ED	LD	PD	CN	ED	LD	PD
α-phellandrene	•	***													
α-terpinene		***													
α-terpinol															
α-terpineolene	*	***	**	a	a	a	a	a	a	a	a	a	a	a	a
citronellol		***													
myrcene		***	•												
β-phellandrene	•	***	*	a	a	a	a	a	a	a	a	a	a	a	a
ocimene-a	*	***	*	a	a	a	a	a	a	a	a	a	a	a	a
rose oxide															
citrol-b															
γ-terpinene	**	***	***	a	a	a	a	a	b	ab	ab	a	a	a	a
geranic acid		***													
geraniol	•	***													
limonene		***	•												
linalool		***													
nerol		***													
ocimene-b		***	*	a	a	a	a	a	b	ab	ab	a	a	a	a
citrol-a															
hydroxylinalool		***													

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "**•**" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test. Blank cells indicate no interaction or no data present if an interaction was present.

Table S11: Two-way ANOVA report on deficit irrigation (DI) treatment and year effects on bound terpenes (in ng per g FW) of field-grown Gewürztraminer berries at pre-harvest (88 and 96 DAA in 2017 and 2018, respectively) and corresponding Tukey's HSD results on year-separated data.†

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data							
				2017				2018			
	Treatment	Year	Interaction	CN	ED	LD	PD	CN	ED	LD	PD
α-phellandrene	•	***	•								
α-terpinene	*	***	*	a	a	a	a	a	a	a	a
α-terpinol											
α-terpineolene	**	***	**	a	a	a	a	a	a	a	a
citronellol		***									
myrcene	•	***	•								
β-phellandrene	*	***	*	a	a	a	a	a	a	a	a
ocimene-a	*	***	*	a	a	a	a	a	a	a	a
rose oxide		***									
citrol-b											
γ-terpinene	**	***	**	a	b	ab	ab	a	a	a	a
geranic acid		***									
geraniol	•	***									
limonene		***									
linalool	*	**	**	a	a	a	a	a	a	a	a
nerol		***									
ocimene-b	*	***	*	a	a	a	a	a	a	a	a
citrol-a											
hydroxylinalool		***									

†*Post-hoc* tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.
Symbols represent different p-values: "**•**" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test. Blank cells indicate no interaction or no data present if an interaction was present.

Table S12: Two-way ANOVA report on deficit irrigation (DI) treatment and year effects on bound terpenes (in ng per berry) of field-grown Gewürztraminer berries at pre-harvest (88 and 96 DAA in 2017 and 2018, respectively) and corresponding Tukey's HSD results on year-separated data.†

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data							
				2017				2018			
	Treatment	Year	Interaction	CN	ED	LD	PD	CN	ED	LD	PD
α-phellandrene		***									
α-terpinene	*	***	*								
α-terpinol											
α-terpineolene	*	***	*	a	a	a	a	a	a	a	a
citronellol		***									
myrcene		***									
β-phellandrene	•	***	•								
ocimene-a		***									
rose oxide		***									
citrol-b											
γ-terpinene	**	***	**	a	b	ab	ab	a	a	a	a
geranic acid		***									
geraniol		***									
limonene		***									
linalool		***	*	a	a	a	a	a	a	a	a
nerol		***									
ocimene-b		***									
citrol-a											
hydroxylinalool	*	***									

†*Post-hoc* tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.
Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test. Blank cells indicate no interaction or no data present if an interaction was present.

Table S13: Two- way ANOVA of DI treatments and year effects on free terpenes (in ng per g FW) of field-grown Gewürztraminer berries at pre-harvest (81, 88, and 96 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data.[†]

Compounds	**Two-Way ANOVA of Three-Years' Data**			**Tukey's HSD *post-hoc* test of Year-Separated Data**											
				2016				2017				2018			
	Treatment	Year	Interaction	CN	ED	LD	PD	CN	ED	LD	PD	CN	ED	LD	PD
farnesene-a	***	***	***					a	b	a	b				
farnesene-b	***	***	***					a	b	ab	b				
α-phellandrene		***													
α-terpinene		***													
α-terpinol															
α-terpineolene		***													
citronellol		***													
myrcene		***													
β-phellandrene		***													
ocimene-a		***													
rose oxide		***													
citrol-b		***													
γ-terpinene		***													
geranic acid		***													
geraniol		***													
limonene	**	***													
linalool		***													
methyl geranate	**	***													
nerol		***													
ocimene-b		***													
citrol-a															

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "**•**" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test. Blank cells indicate no interaction or no data present if an interaction was present.

Table S14: Two- way ANOVA of DI treatments and year effects on free terpenes (in ng per berry) of field-grown Gewürztraminer berries at pre-harvest (81, 88, and 96 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data.†

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data											
				2016				2017				2018			
	Treatment	Year	Interaction	CN	ED	LD	PD	CN	ED	LD	PD	CN	ED	LD	PD
farnesene-a	***	***	***					a	b	ab	b				
farnesene-b	***	***	***					a	b	ab	b				
α-phellandrene		***													
α-terpinene		***													
α-terpinol															
α-terpineolene		***													
citronellol		***													
myrcene		***													
β-phellandrene		***													
ocimene-a		***													
rose oxide		***													
citrol-b		***													
γ-terpinene		***													
geranic acid		***													
geraniol		***													
limonene	***	***													
linalool		***													
methyl geranate	**	***													
nerol		***													
ocimene-b		***													
citrol-a															

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test. Blank cells indicate no interaction or no data present if an interaction was present.

Table S15: Preliminary qPCR results of terpene-related biosynthetic genes expression in Gewürztraminer berry relative to AP47 expression throughout the 2016 season (before veraison, at veraison, and after veraison) on pooled deficit irrigation (DI) and crop load strategy (CLM) samples.

Genes	Days After Anthesis in 2016 (DAA)					
	Deficit Irrigation			Cluster Thinning		
	34 DAA	65 DAA	96 DAA	49 DAA	65 DAA	98 DAA
AP47	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
DXS1	2.0741	0.4783	0.5363	0.8610	0.3951	0.8135
DXS3	0.6792	1.4332	0.9630	0.6809	0.7658	0.9657
HDR	2.1463	14.6338	17.8864	1.2567	11.5244	12.7463
GPPS	0.4277	0.5960	0.4756	0.2834	0.4403	0.4708
MYB24	0.0045	0.3753	10.2907	0.0073	2.0602	4.8202
TPS02	0.0002	0.0000	0.0000	0.0000	0.0000	0.0004
TPS07	0.0004	0.0045	1.1650	0.0027	0.0193	0.7609
TPS10	0.0031	0.0483	4.2322	0.0029	0.3309	3.2748
TPS14	0.0000	0.0000	0.0005	0.0001	0.0004	0.0004
TPS20	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TPS27	0.0054	0.0007	0.0001	0.0286	0.0036	0.0001
TPS31	0.0056	0.0001	0.0001	0.0092	0.0001	0.0000
TPS34/35	0.0013	0.0389	3.2982	0.0014	0.5714	2.8221
TPS38	0.0528	0.0011	0.1648	0.0042	0.0210	0.2794
TPS44	0.0000	0.0001	0.0002	0.0009	0.0000	0.0000
TPS45	0.1100	0.0006	0.0001	0.1150	0.0002	0.0001
TPS46/47	0.0003	0.0003	0.0001	0.0000	5.2467	0.0000
TPS52	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000
TPS54	0.0588	0.5141	0.1999	0.0060	0.1323	0.1867
TPS56	0.0249	0.0003	0.0003	0.0922	0.0002	0.0003
TPS57	0.1223	0.0000	0.0000	0.0120	0.0000	0.0000
TPS58	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000
TPS61	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TPS63	0.1331	0.0000	0.0000	0.0102	0.0000	0.0000
TPS69	0.0001	0.0015	0.0030	0.0013	0.0011	0.0010
CYP76F14	0.1204	4.8168	3.2702	0.4867	2.7054	2.9493
ADH3-1	1.1267	0.0215	0.0389	0.2893	0.0531	0.0852
ADH3-2	0.0000	0.0015	0.0025	0.0000	0.0037	0.0021
Ger1-1	23.1773	18.6902	13.7583	20.3963	15.2813	13.7883
Ger1-2	1.1224	0.0183	0.0155	0.0000	0.0138	0.0000
GT14	0.0014	3.3046	1.0795	0.1055	6.2871	7.4205
GT7	2.4452	0.5544	1.0014	2.0648	1.5565	2.2932

Table S16: One-way ANOVA and *post-hoc* testing of the effect of deficit irrigation (DI) treatments on the expression of terpene-related genes in berries of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada in 2016. CN = Well irrigated controls; ED = early deficit; LD = late deficit; PD = prolonged deficit.

	One-Way ANOVA Results					Tukey's HSD *post-hoc* test																			
						34 DAA				54 DAA				65 DAA				81 DAA				96 DAA			
***V. vinifera* Genes**	**34 DAA**	**54 DAA**	**65 DAA**	**81 DAA**	**96 DAA**	**CN**	**ED**	**LD**	**PD**	**CN**	**ED**	**LD**	**PD**	**CN**	**ED**	**LD**	**PD**	**CN**	**ED**	**LD**	**PD**	**CN**	**ED**	**LD**	**PD**
DXS1																									
DXS3				•	•																				
HDR				•																					
GPPS																									
TPS07			•	**	•													a	b	b	b				
TPS10			•	**	*													a	b	b	b	a	a	a	a
TPS14					•																				
TPS34/35				***	*													a	b	b	b	a	b	ab	b
TPS38		•																							
TPS44		*	•							a	a	a	a												
TPS54																									
Ger1_1			*											a	a	a	a								
Ger1_2																									
ADH3_1				*	*													a	a	a	a	a	a	a	a
ADH3_2																									
MYB24			•	**	**													a	b	b	b	a	b	b	b
CYP76F14			*		•									a	ab	b	ab								
GT14																									
GT7					*																	ab	a	ab	b

Table S17: Two-way ANOVA of crop load management (CLM) strategy and year effects on cane and vine leaf area of field-grown Gewürztraminer grapevines at before and after veraison in the Okanagan Valley, BC, Canada. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

	Cane Leaf Area (m^2/cane)					**Vine Leaf Area (m^2/vine)**				
	Before Veraison†		**Harvest‡**			**Before Veraison**		**Harvest**		
CLM Treatment	n.s.		n.s.			n.s.		n.s.		
	Mean	**SE**	**Mean**	**SE**		**Mean**	**SE**	**Mean**	**SE**	
HC	1.99	0.13	2.19	0.13		9.58	0.58	10.55	0.62	
LC-E	1.91	0.14	2.14	0.12		9.03	0.72	10.06	0.57	
LC-L	2.00	0.20	2.38	0.16		9.74	0.96	11.46	0.78	
MC-E	1.93	0.14	2.28	0.11		9.41	0.71	11.09	0.58	
MC-L	2.25	0.13	2.55	0.22		11.03	0.67	12.23	1.07	
Year	n.s.		0.00525			0.0503		0.000389		
2016	2.10	0.11	2.59	0.15	a	12.44	0.68	10.15	0.57	a
2017	2.04	0.09	2.29	0.10	ab	11.44	0.52	10.19	0.44	a
2018	1.77	0.13	2.05	0.07	b	9.34	0.32	7.97	0.57	b
CLM x Year Interaction	n.s.		n.s.			n.s.		n.s.		

†Before Veraison is defined as any leaf area measurements in July (48, 27, and 52 DAA in 2016, 2017, and 2018, respectively) of the respective year

‡ Harvest is defined as any leaf area measurements in September/October (118, 78, and 115 DAA 2016, 2017, and 2018, respectively) of the respective year

Table S18: One-way ANOVA and *post-hoc* testing of the effect of crop load management (CLM) treatments on the berry total soluble solids (in °Brix) in berries of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada from 2016 to 2018. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

	One-Way ANOVA					
	Tukey's HSD					
Total Soluble Solids (°Brix)	**HC**	**LC-E**	**LC-L**	**MC-E**	**MC-L**	**p-value**
2016 Season						
DAA 29[†]						$9.56 \cdot 10^{-2}$
DAA 49						$7.17 \cdot 10^{-1}$
DAA 56	ab	a	b	ab	ab	$2.37 \cdot 10^{-2}$
DAA 65	ab	ab	b	a	b	$5.24 \cdot 10^{-3}$
DAA 71	b	a	b	ab	b	$3.01 \cdot 10^{-3}$
DAA 83	b	a	a	ab	ab	$1.10 \cdot 10^{-3}$
DAA 98	c	ab	a	b	b	$7.83 \cdot 10^{-6}$
DAA 114	b	a	a	ab	ab	$3.02 \cdot 10^{-3}$
DAA 119						$3.11 \cdot 10^{-1}$
2017 Season						
DAA 33						$4.44 \cdot 10^{-1}$
DAA 46						$8.25 \cdot 10^{-1}$
DAA 58	ab	a	b	ab	b	$6.52 \cdot 10^{-3}$
DAA 78	b	a	a	ab	b	$5.17 \cdot 10^{-4}$
DAA 86	b	a	a	a	a	$1.31 \cdot 10^{-4}$
DAA 103	b	a	a	ab	ab	$3.96 \cdot 10^{-3}$
DAA 108						$6.30 \cdot 10^{-2}$
2018 Season						
DAA 80	ab	a	b	a	b	$1.09 \cdot 10^{-3}$
DAA 99	ab	a	ab	ab	b	$6.12 \cdot 10^{-3}$
DAA 115	a	a	a	a	a	$2.89 \cdot 10^{-2}$

[†]Later CT treatments were not applied at this time point and could be considered equivalent to HC/unthinned vines

Table S19: One-way ANOVA and *post-hoc* testing of the effect of crop load management (CLM) treatments on the berry titratable acidity (in g/L) in berries of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada from 2016 to 2018. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

	One-Way ANOVA					
	Tukey's HSD					
Titratable Acidity (g/L)	**HC**	**LC-E**	**LC-L**	**MC-E**	**MC-L**	**p-value**
2016 Season						
DAA 29[†]	b	b	-	a	-	$1.41 \cdot 10^{-2}$
DAA 49						$1.02 \cdot 10^{-1}$
DAA 56	ab	b	ab	b	a	$9.52 \cdot 10^{-3}$
DAA 65	b	a	a	b	b	$2.16 \cdot 10^{-5}$
DAA 71						$6.66 \cdot 10^{-1}$
DAA 83	ab	b	a	ab	ab	$2.26 \cdot 10^{-2}$
DAA 98						$2.58 \cdot 10^{-1}$
DAA 114	a	b	ab	ab	a	$4.93 \cdot 10^{-4}$
DAA 119						$4.54 \cdot 10^{-1}$
2017 Season						
DAA 33						$6.09 \cdot 10^{-1}$
DAA 46						$6.81 \cdot 10^{-1}$
DAA 58						$1.66 \cdot 10^{-1}$
DAA 78						$3.25 \cdot 10^{-1}$
DAA 86	ab	b	ab	ab	a	$4.05 \cdot 10^{-2}$
DAA 103	a	a	a	a	a	$4.67 \cdot 10^{-2}$
DAA 108	ab	b	ab	ab	a	$1.40 \cdot 10^{-2}$
2018 Season						
DAA 80	c	b	a	b	bc	$4.05 \cdot 10^{-11}$
DAA 99	bc	c	ab	b	b	$3.82 \cdot 10^{-4}$
DAA 115						$5.62 \cdot 10^{-1}$

[†]Later CT treatments were not applied at this time point and could be considered equivalent to HC/unthinned vines

Table S20: One-way ANOVA and *post-hoc* testing of the effect of crop load management (CLM) treatments on the berry pH of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada from 2016 to 2018. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

	One-Way ANOVA					
	Tukey's HSD					
Juice pH	**HC**	**LC-E**	**LC-L**	**MC-E**	**MC-L**	**p-value**
2016 Season						
DAA 29[†]						$9.05 \cdot 10^{-1}$
DAA 49	ab	ab	ab	b	a	$3.86 \cdot 10^{-2}$
DAA 56						$1.41 \cdot 10^{-1}$
DAA 65						$5.84 \cdot 10^{-1}$
DAA 71						$6.56 \cdot 10^{-2}$
DAA 83	ab	a	b	b	ab	$2.70 \cdot 10^{-2}$
DAA 98	b	a	a	ab	b	$4.91 \cdot 10^{-4}$
DAA 114	b	a	ab	b	b	$5.62 \cdot 10^{-4}$
DAA 119						$8.48 \cdot 10^{-2}$
2017 Season						
DAA 33	ab	a	-	b	-	$3.84 \cdot 10^{-2}$
DAA 46	c	a	abc	ab	b	$6.35 \cdot 10^{-4}$
DAA 58						$1.63 \cdot 10^{-1}$
DAA 78						$6.21 \cdot 10^{-1}$
DAA 86	b	a	b	b	b	$2.94 \cdot 10^{-4}$
DAA 103	b	a	ab	ab	ab	$2.89 \cdot 10^{-2}$
DAA 108	b	a	ab	ab	ab	$1.25 \cdot 10^{-2}$
2018 Season						
DAA 80	a	b	c	ab	c	$1.19 \cdot 10^{-9}$
DAA 99	b	a	b	ab	b	$2.41 \cdot 10^{-4}$
DAA 115						$7.74 \cdot 10^{-2}$

[†]Later CT treatments were not applied at this time point and could be considered equivalent to HC/unthinned vines

Table S21: Two-way ANOVA report on CLM treatment and year effects on free terpenes (in ng per g FW) of field-grown Gewürztraminer berries at harvest (119, 108, and 115 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data														
				2016					2017					2018				
	Treatment	Year	Interaction	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L
farnesene-a	***	***	*	a	ab	b	ab	ab	a	a	a	a	a	ab	a	b	ab	b
farnesene-b		***																
α-phellandrene	**	***	**	a	a	a	a	a	ab	a	b	b	ab	a	ab	b	ab	b
α-terpinene		***																
α-terpinol	***	***	***	a	ab	b	b	b	a	a	a	a	a	a	a	a	a	a
α-terpineolene	•	***	•															
citronellol	*	***																
myrcene	**	***	**	a	ab	b	a	ab	a	a	a	a	a	a	a	b	a	b
β-phellandrene	•	***	•															
ocimene-a	**	***	**	ab	ab	c	a	bc	b	a	b	b	ab	a	a	a	a	a
rose oxide		***																
citrol-b	*	***	*	a	ab	b	a	b	ab	a	b	b	a	a	a	a	a	a
γ-terpinene		***	•															
geranic acid	*	***	*	a	b	b	ab	ab	ab	a	ab	b	ab	a	a	a	a	a
geraniol	***	***	***	a	b	b	a	b	ab	a	b	b	ab	a	ab	b	ab	b
limonene	***	***	***	a	a	a	a	a	a	a	a	a	a	a	a	b	ab	b
linalool	**	***	***	a	ab	b	a	b	b	a	b	b	ab	a	ab	b	ab	b
methyl geranate	***	***	***	a	b	b	b	b	a	a	a	a	a	ab	a	b	ab	b
nerol	***	***	***	ab	ab	b	a	b	ab	a	b	b	ab	ab	a	ab	ab	b
ocimene-b	*	***	*	ab	ab	b	a	ab	ab	a	b	b	ab	a	a	a	a	a
citrol-a	***	***	***	a	ab	b	a	b	a	a	a	a	a	a	a	a	a	a

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test.

Table S22: Two-way ANOVA report on CLM treatment and year effects on free terpenes (in ng per berry) of field-grown Gewürztraminer berries at harvest (119, 108, and 115 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data														
				2016					2017					2018				
	Treatment	Year	Interaction	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L
farnesene-a	***	***	**	a	ab	b	ab	ab	a	a	a	a	a	ab	a	b	ab	b
farnesene-b		***																
α-phellandrene	**	***	***	a	a	a	a	a	a	a	a	a	a	ab	a	b	abc	c
α-terpinene		***																
α-terpinol	***	***	***	a	ab	b	ab	b	a	a	a	a	a	a	a	a	a	a
α-terpineolene	•	***	*	a	a	a	a	a	b	a	ab	ab	ab	a	a	a	a	a
citronellol	•	***																
myrcene	**	***	**	ab	abc	c	a	bc	a	a	a	a	a	a	a	a	a	a
β-phellandrene		***	•															
ocimene-a	**	***	**	ab	ab	b	a	b	ab	a	b	b	ab	a	a	a	a	a
rose oxide		***																
citrol-b	*	***	*	a	ab	b	a	b	ab	a	ab	b	ab	a	a	a	a	a
γ-terpinene	•	***	*	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
geranic acid	**	***	**	a	b	b	ab	ab	ab	a	ab	b	ab	ab	a	ab	ab	b
geraniol	***	***	***	ab	bc	c	a	c	a	a	a	a	a	a	a	b	ab	b
limonene	***	***	***	a	a	a	a	a	a	a	a	a	a	a	a	b	ab	b
linalool	•	***	***	a	ab	b	a	b	bc	ab	a	c	abc	a	ab	b	ab	b
methyl geranate	***	***	***	a	ab	b	ab	b	a	a	a	a	a	ab	a	b	ab	b
nerol	***	***	**	ab	ab	b	a	b	ab	a	ab	b	ab	ab	a	b	ab	b
ocimene-b	*	***	**	ab	ab	b	a	b	a	a	a	a	a	a	a	a	a	a
citrol-a	***	***	***	a	ab	b	a	b	a	a	a	a	a	a	a	a	a	a

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test.

Table S23: Two-way ANOVA report on CLM treatment and year effects on free terpenes (in ng per g FW) of field-grown Gewürztraminer berries at pre-harvest (98, 83, and 115 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data														
				2016					2017					2018				
	Treatment	Year	Interaction	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L
farnesene-a	***	***																
farnesene-b	*	***	*	a	a	a	a	a	a	a	a	a	a	ab	a	b	ab	ab
α-phellandrene	*	***																
α-terpinene		***																
α-terpinol	**	***	***	b	b	ab	b	a	a	a	a	a	a	a	a	a	a	a
α-terpineolene		***																
citronellol	*	***																
myrcene	*	***																
β-phellandrene	*	***																
ocimene-a		***																
rose oxide		***																
citrol-b		***																
γ-terpinene		***	*	a	a	a	a	a	a	a	a	a	a	b	a	ab	b	ab
geranic acid	*	***	•															
geraniol	*	***																
limonene	•	***																
linalool		***	•															
methyl geranate	***	***	*	a	a	a	a	a	a	a	a	a	a	ab	a	b	ab	b
nerol	**	***																
ocimene-b	•	***																
citrol-a	**	*	*	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test.

Table S24: Two-way ANOVA report on CLM treatment and year effects on free terpenes (in ng per berry) of field-grown Gewürztraminer berries at pre-harvest (98, 83, and 115 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data														
				2016					2017					2018				
	Treatment	Year	Interaction	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L
farnesene-a	***	***																
farnesene-b	**	***	*	a	a	a	a	a	a	a	a	a	a	ab	a	b	ab	ab
α-phellandrene	*	***																
α-terpinene	•	***																
α-terpinol	**	***	***	b	b	ab	b	a	a	a	a	a	a	a	a	a	a	a
α-terpineolene		***																
citronellol	**	***																
myrcene	*	***																
β-phellandrene	**	***	•															
ocimene-a		***																
rose oxide		***																
citrol-b		***																
γ-terpinene		***	*	a	a	a	a	a	a	a	a	a	a	b	a	ab	b	ab
geranic acid	*	***	•															
geraniol	*	***																
limonene	*	***																
linalool	*	***	•															
methyl geranate	***	***	*	a	a	a	a	a	ab	a	ab	b	ab	ab	a	b	ab	b
nerol	**	***																
ocimene-b	*	***																
citrol-a	**	*	*	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test.

Table S25: Two-way ANOVA report on CLM treatment and year effects on bound terpenes (in ng per g FW) of field-grown Gewürztraminer berries at harvest (119, 108, and 115 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data														
				2016					2017					2018				
	Treatment	Year	Interaction	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L
α-phellandrene																		
α-terpinene																		
α-terpinol																		
α-terpineolene		•																
citronellol		***																
myrcene		***	*	a	a	a	a	a	ab	b	a	ab	a	a	a	a	a	a
β-phellandrene																		
ocimene-a																		
rose oxide		***	•															
citrol-b																		
γ-terpinene		•																
geranic acid	***	***	**	a	a	a	a	a	a	a	a	a	a	a	a	ab	a	b
geraniol		***																
limonene		***																
linalool		***																
nerol																		
ocimene-b		***																
citrol-a	•	***	**	b	ab	a	ab	ab	b	a	b	ab	b	a	a	a	a	a
hydroxylinalool																		

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "**•**" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test.

Table S26: Two-way ANOVA report on CLM treatment and year effects on bound terpenes (in ng per berry) of field-grown Gewürztraminer berries at harvest (119, 108, and 115 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data														
				2016					2017					2018				
	Treatment	Year	Interaction	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L
α-phellandrene																		
α-terpinene																		
α-terpinol																		
α-terpineolene																		
citronellol																		
myrcene		***																
β-phellandrene		***	*	a	a	a	a	a	bc	c	a	b	b	a	a	a	a	a
ocimene-a																		
rose oxide	***	***	***	a	a	a	a	a	b	b	a	b	b	a	a	a	a	a
citrol-b																		
γ-terpinene		•																
geranic acid	*	***	***	a	a	a	a	a	a	a	a	a	a	a	a	ab	ab	b
geraniol		***																
limonene		***																
linalool		***																
nerol		***																
ocimene-b	*	***	**	b	ab	a	ab	ab	bc	c	a	bc	ab	a	a	a	a	a
citrol-a																		
hydroxylinalool		***	*	a	a	a	a	a	a	b	ab	a	a	a	a	a	a	a

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test.

Table S27: Two-way ANOVA report on CLM treatment and year effects on bound terpenes (in ng per g FW) of field-grown Gewürztraminer berries at pre-harvest (98, 83, and 105 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data															
				2016					2017					2018					
	Treatment	Year	Interaction	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L
α-phellandrene		***																
α-terpinene		***																
α-terpinol																		
α-terpineolene	•	***	•															
citronellol	•	***																
myrcene		***																
β-phellandrene	*	***	*	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
ocimene-a	•	***	•															
rose oxide																		
citrol-b																		
γ-terpinene		***																
geranic acid	*	***	•															
geraniol	**	***																
limonene	•	***	•															
linalool		***	***	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
nerol	***	***	*	a	a	a	a	a	b	a	ab	ab	ab	a	a	a	a	a
ocimene-b		***																
citrol-a																		
hydroxylinalool	•	**																

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test.

Table S28: Two-way ANOVA report on CLM treatment and year effects on bound terpenes (in ng per berry) of field-grown Gewürztraminer berries at pre-harvest (98, 83, and 105 DAA in 2016, 2017, and 2018, respectively) and corresponding Tukey's HSD results on year-separated data

Compounds	Two-Way ANOVA of Three-Years' Data			Tukey's HSD *post-hoc* test of Year-Separated Data															
				2016					2017					2018					
	Treatment	Year	Interaction	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L	HC	LC-E	LC-L	MC-E	MC-L
α-phellandrene		***																
α-terpinene	•	***	•															
α-terpinol																		
α-terpineolene	•	***	•															
citronellol	*	***																
myrcene		***																
β-phellandrene	*	***	*	a	a	a	a	a	b	a	ab	ab	ab	a	a	a	a	a
ocimene-a	•	***	•															
rose oxide																		
citrol-b																		
γ-terpinene		***																
geranic acid	*	***																
geraniol	**	***																
limonene	*	***	*	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
linalool		***	**	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
nerol	***	***	•															
ocimene-b		***																
citrol-a																		
hydroxylinalool	•	**																

†Post-hoc tests were performed on year-separated data only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.

Symbols represent different p-values: "•" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the DI treatments, within years, as determined by *post-hoc* test.

Table S29a: One-way ANOVA results of the effect of crop load management (CLM) strategies on the expression of terpene-related genes in the berries of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada.

	One-Way ANOVA Results				
***V. vinifera* Genes**	**49 DAA**	**65 DAA**	**83 DAA**	**98 DAA**	**119 DAA**
DXS1	***	**			*
DXS3	*	*			*
HDR	**	*		**	*
GPPS	*				•
TPS07		•		***	
TPS10		•		***	
TPS14				***	
TPS34/35		•	•	**	
TPS38		•	•	***	
TPS44				*	
TPS54	*	**		•	
Ger1_1					•
Ger1_2					
ADH3_1	**	**		•	*
ADH3_2	*				•
MYB24	*	•	*	***	
CYP76F14	***	***			•
GT14	*			•	*
GT7	***	*			*

Table S29b: *Post-hoc* testing of the effect of crop load management (CLM) strategies on the expression of terpene-related genes in berries of field-grown Gewürztraminer grapevines in the Okanagan Valley, BC, Canada in 2016. HC = high crop; LC-E = light crop, early thinning; LC-L = light crop, late thinning; MC-E = medium crop, early thinning; MC-L = medium crop, late thinning.

	Tukey's HSD *post-hoc* test																								
	49 DAA					**65 DAA**					**83 DAA**					**98 DAA**					**119 DAA**				
***V. vinifera* Genes**	**HC**	**LC-E**	**LC-L**	**MC-E**	**MC-L**	**HC**	**LC-E**	**LC-L**	**MC-E**	**MC-L**	**HC**	**LC-E**	**LC-L**	**MC-E**	**MC-L**	**HC**	**LC-E**	**LC-L**	**MC-E**	**MC-L**	**HC**	**LC-E**	**LC-L**	**MC-E**	**MC-L**
DXS1	b	b	a	b	b	a	b	ab	ab	a											a	a	a	a	a
DXS3	a	a	a	a	a	ab	b	ab	a	ab											a	a	a	a	a
HDR	b	b	a	b	b	ab	ab	b	ab	a						a	a	ab	b	ab	ab	b	a	ab	ab
GPPS	ab	b	a	b	ab																				
TPS07																bc	a	bc	b	c					
TPS10																bc	a	bc	ab	c					
TPS14																b	a	b	b	b					
TPS34/35																b	a	b	ab	b					
TPS38																bc	a	bc	ab	c					
TPS44																a	a	a	a	a					
TPS54	b	a	a	b	a	ab	b	a	b	b															
Ger1_1																									
Ger1_2																									
ADH3_1	b	b	a	b	a	b	a	b	b	b											a	a	a	a	a
ADH3_2	a	a	a	a	a																				
MYB24	ab	ab	b	a	ab						a	a	a	a	a	bc	a	ab	a	c					
CYP76F14	b	b	a	b	a	b	a	ab	b	b															
GT14	ab	ab	b	a	b																ab	ab	b	ab	a
GT7	bc	bc	a	c	ab	ab	b	ab	ab	a											ab	b	a	b	b

†Post-hoc tests were performed only when two-way ANOVA revealed significant interaction ($p < 0.05$) between treatment and year factors.
Symbols represent different p-values: "**•**" indicates $0.05 < p < 0.1$, "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$. Different letters denote significance ($p < 0.05$) among the CLM strategies as determined by *post-hoc* test.

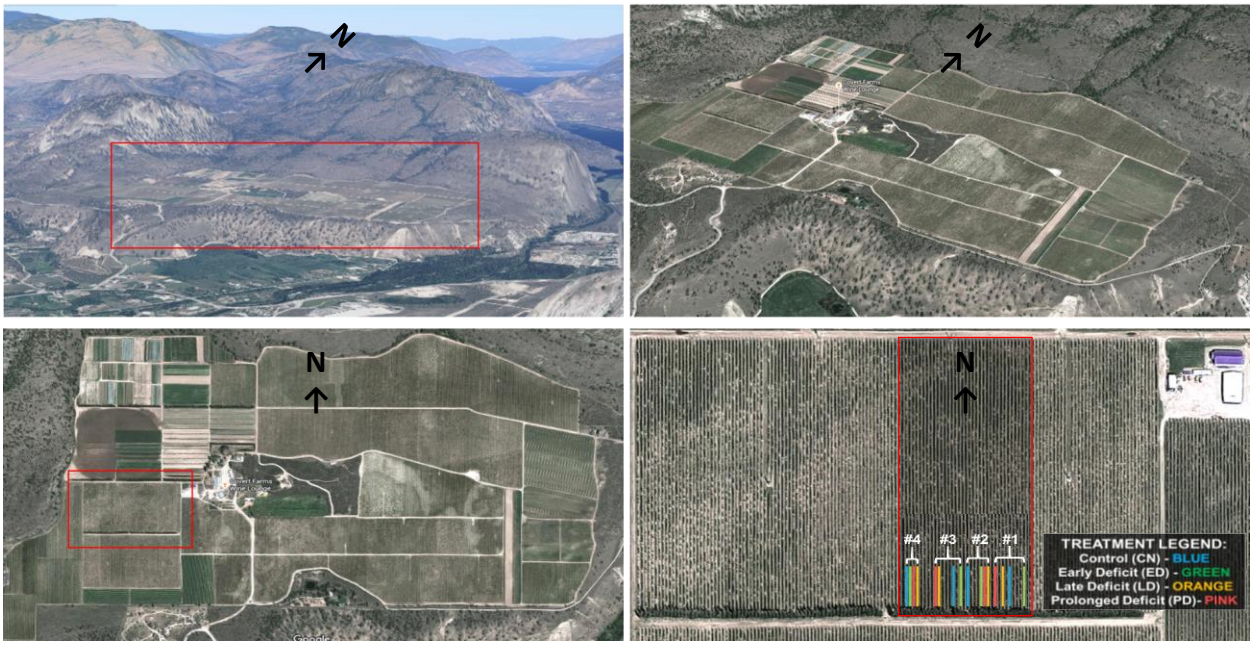

Figure S1: Vineyard sitemap and layout of randomized block design of deficit irrigation study. Coloured bars outline to scale rows included in the study. Vine rows (thin dark green vertical lines) extend ~200 m north to south from road to road (thick light brown straight horizontal lines).

49°10’21.3”N 119°31’27.4”W 380 m

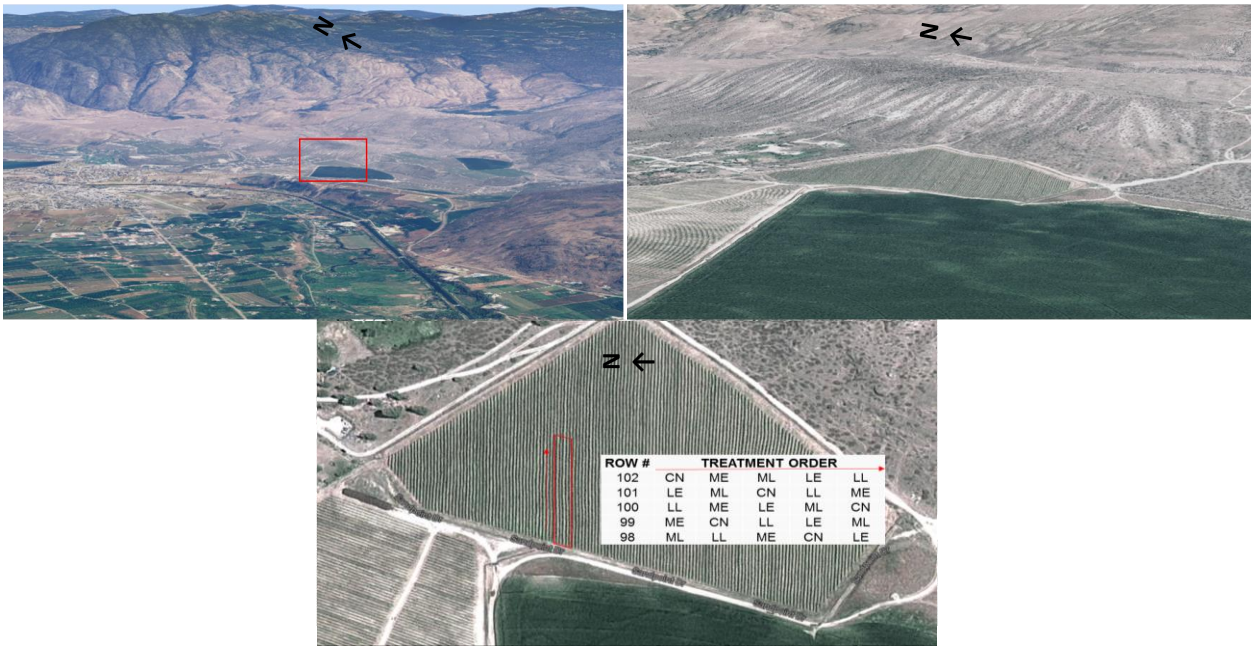

Figure S2: Vineyard sitemap and layout of randomized block design of crop load management study. Red arrow on map and in table outline the layout of experimental design. Vine rows (thin dark green vertical lines) extend ~100 to 200 m east to west from road to road (thick light brown straight diagonal lines).

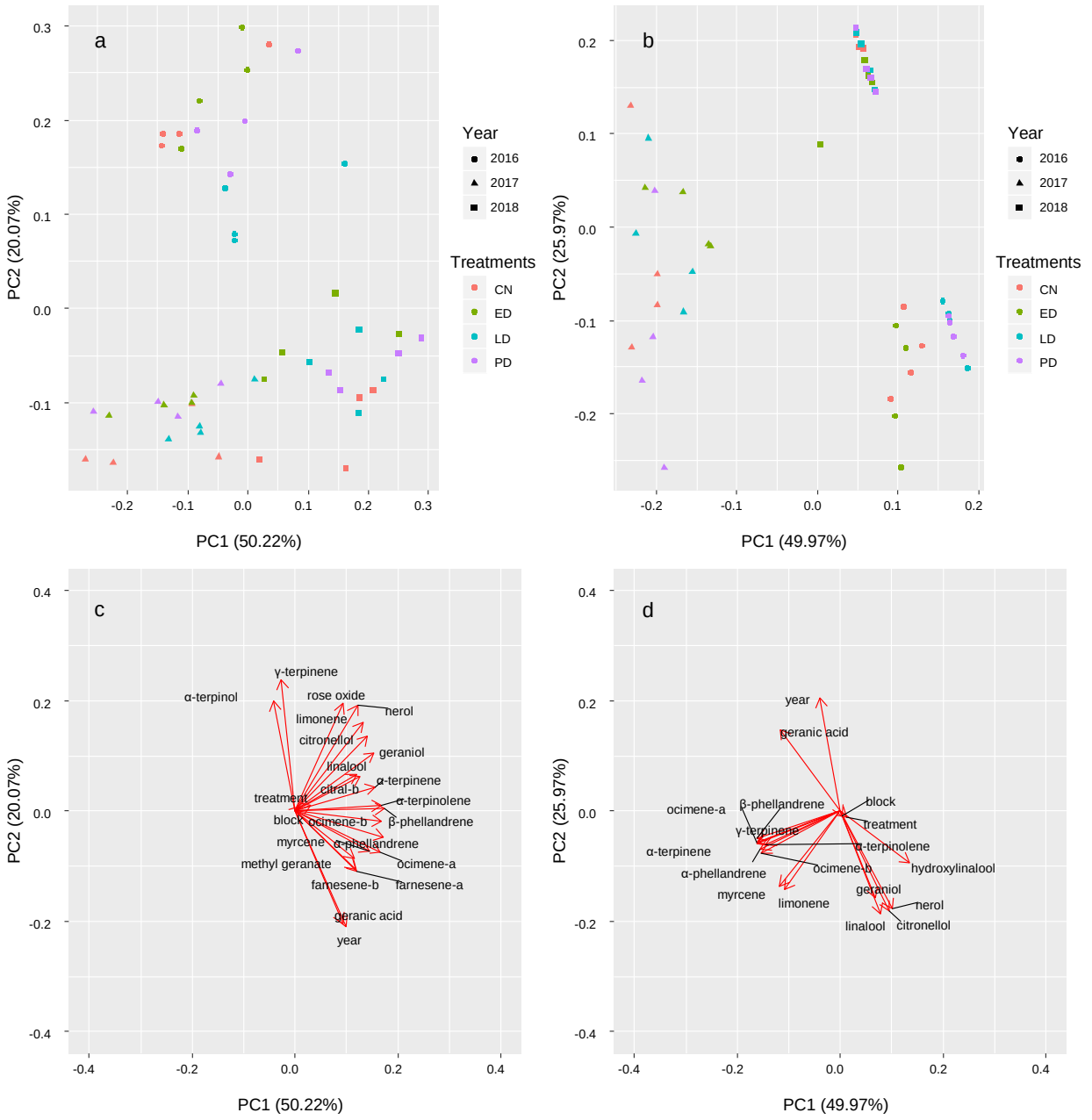

Figure S3: Principal component analysis of free (a,c) and bound (b,d) terpene profiles at harvest in berries from field-grown Gewürztraminer grapevine exposed to deficit irrigation (DI) treatments. Points outline individual biological replicates (experimental units) from each DI treatment from each year (2016, 2017, 2018). Red arrows illustrate loadings / influences of individual compounds, DI treatments, years, and blocks on the measurements. Angles between loadings illustrate correlation between those vectors, i.e. 0º, 90º, 180º can be interpreted as 1,0, -1 = R. Distance between points indicates similarity in terpene concentration.

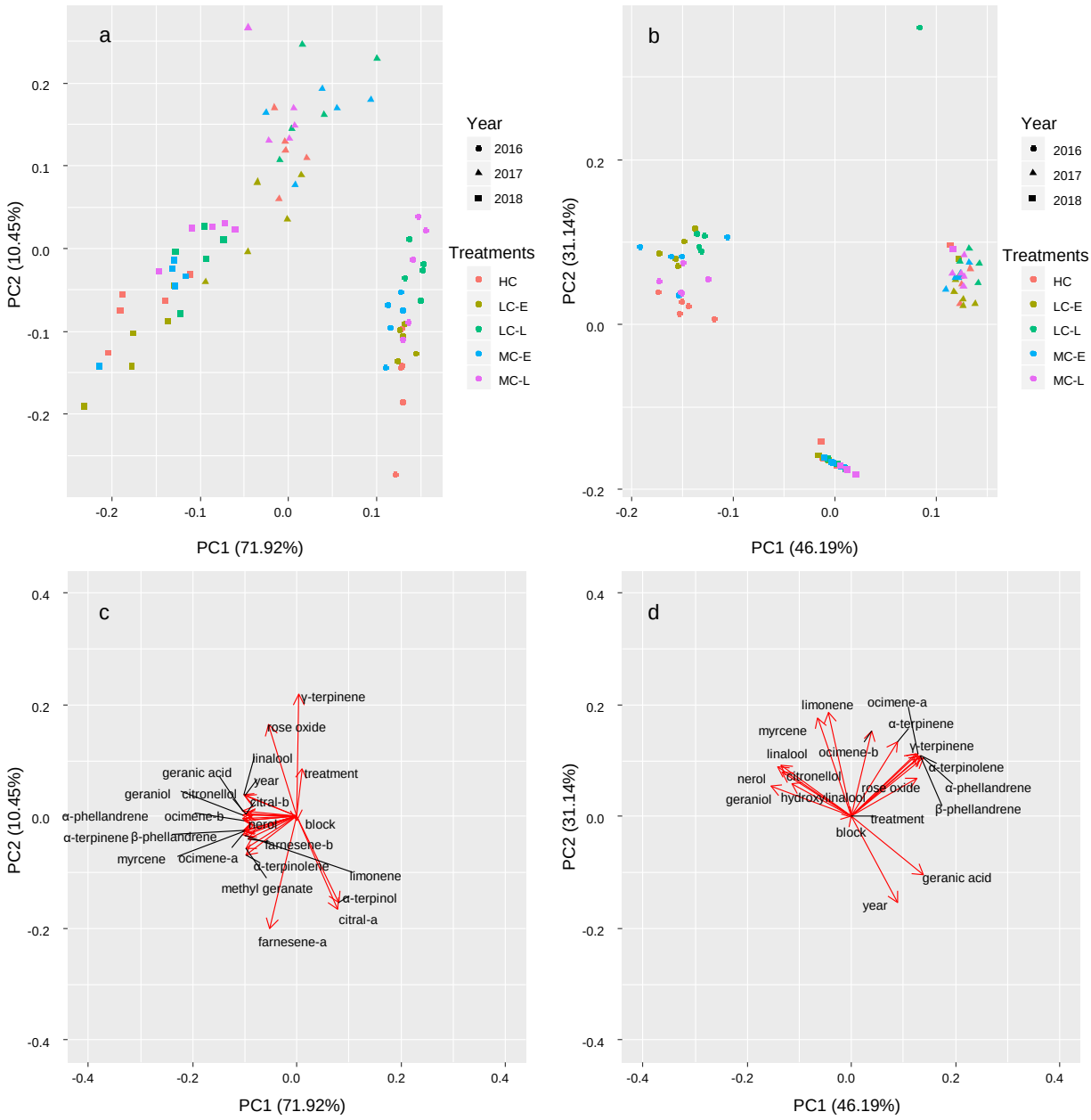

Figure S4: Principal component analysis biplot of free (a,c) and bound (b,d) terpene profiles in berries at harvest of field-grown Gewürztraminer grapevines exposed to crop load management (CLM) strategies. Points outline individual biological replicates (experimental units) from each CLM treatment from each year (2016, 2017, 2018). Red arrows illustrate loadings / influences of individual compounds, CLM strategies, years, and blocks on the variation between EUs. Angles between vectors on biplot illustrate correlation between those vectors, i.e. 0º, 90º, 180º can be interpreted as 1,0, -1 = R. Distance between points indicates similarity in terpene concentration.

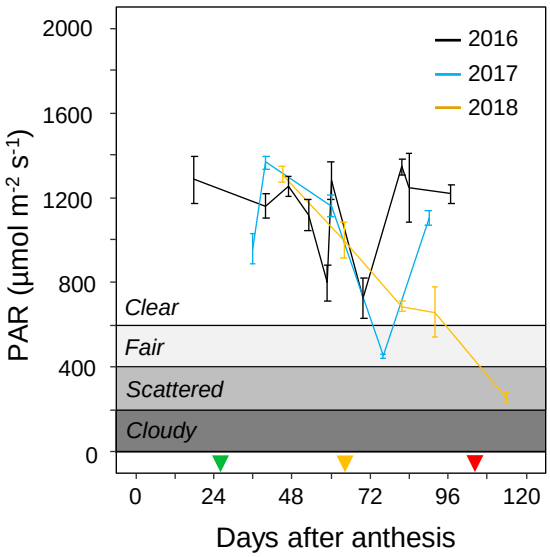

Figure S5: Seasonal photosynthetic active radiation (PAR) throughout growing seasons 2016, 2017 and 2018, respectively. Clear, Fair, Scattered, and Cloudy labels sunshine duration values during typical cloud cover as defined by World Meteorological Organization (2010). This was adopted for the measured, instantaneous PAR values. Values above 0, 200, 400, and 600 PAR as indicated by horizontal lines indicate the Clear, Fair, Scattered, and Cloudy equivalents. Standard error is indicated by coloured error bars. Green, yellow, and red arrows indicate average dates of early treatment application (DI and CLM), application at veraison (DI and CLM), and commercial harvest (DI and CLM).

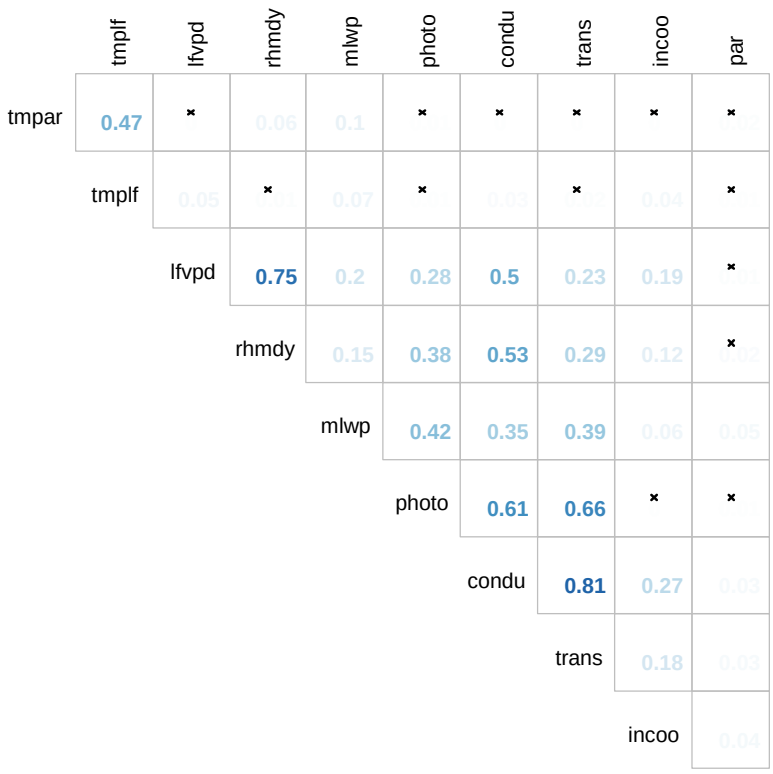

Figure S6: Correlation table of week-averaged leaf water potential and gas exchange parameters from field-grown Gewürztraminer leaves under deficit irrigation (DI) treatments throughout the growing season in 2016, 2017, 2018. Numbers within squares indicate significant ($p < 0.05$) correlation between measured values. Correlation values indicated here are R^2 values with colour added to illustrate no correlation (white) for strong correlation (blue). Black "X" in square signify non-significant relationships between parameters. Tmpar = air temperature (ºC), tmplf = leaf temperature (ºC), lfvpd = vapour pressure differential based on leaf temperature (kPa), rhmdy = relative humidity of air (%), mlwp = midday leaf water potential (MPa), photo = photosynthesis rate (µmol CO_2 m^{-2} s^{-1}), condu = stomatal conductance (mol H_2O m^{-2} s^{-1}), trans = transpiration rate (mmol H_2O m^{-2} s^{-1}), incoo = leaf internal CO_2 concentration (µmol CO_2), par = photosynthetic active radiation (400 to 700 nm).

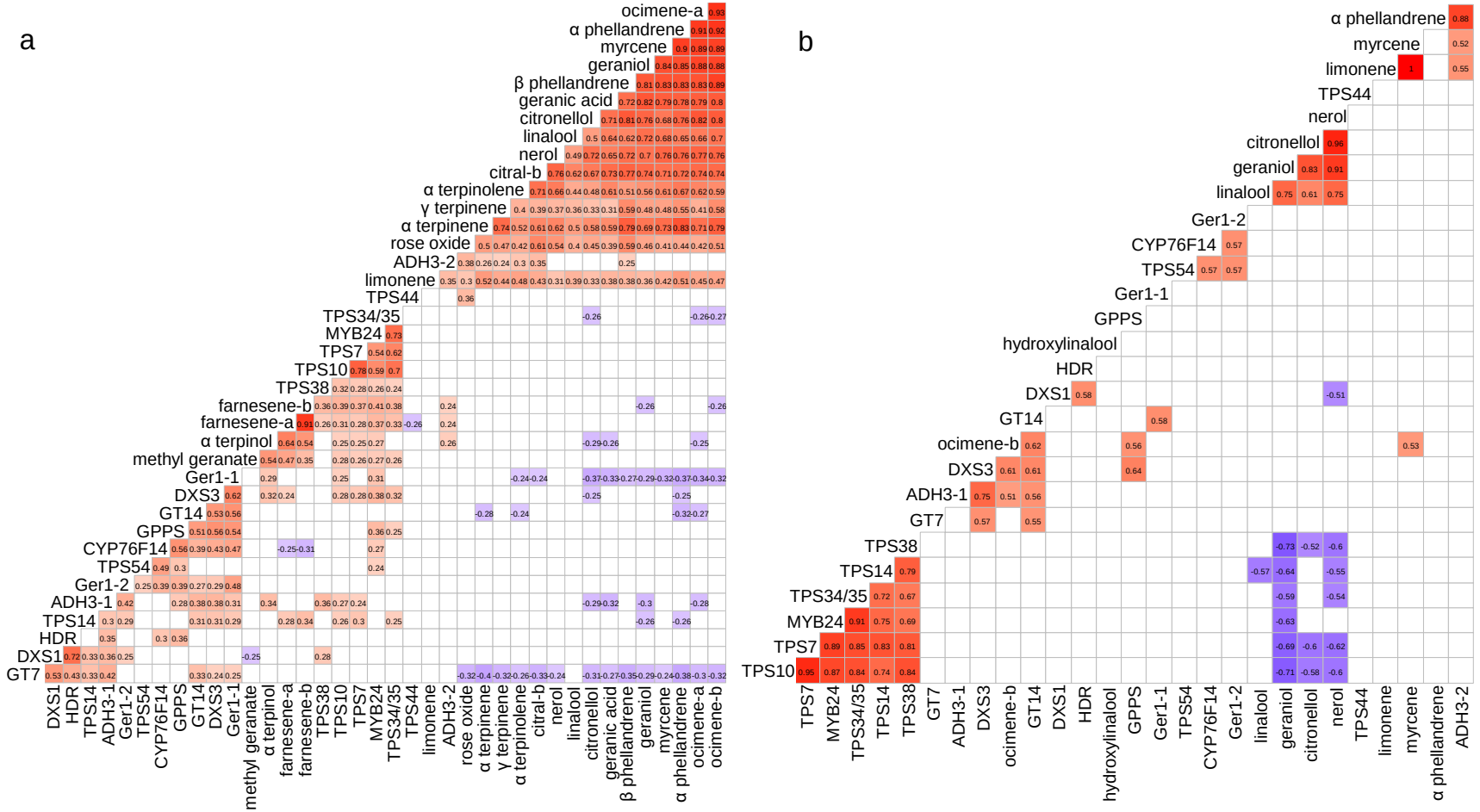

Figure S7: Correlation table of date-matched free (a) and bound (b) terpene concentrations, gene expression, and berry tissues of berries from field-grown Gewürztraminer grapevine exposed to deficit irrigation (DI) treatments throughout 2016 season. Numbers within squares indicate significant ($p < 0.05$) Pearson coefficients between measured values. Correlation values indicated here are R values with colour added to illustrate no correlation (white) for strong positive correlation (red) and for strong negative correlation (blue). Blank squares indicate no significant correlation. R^2 values discussed in text were calculated from R values plotted.

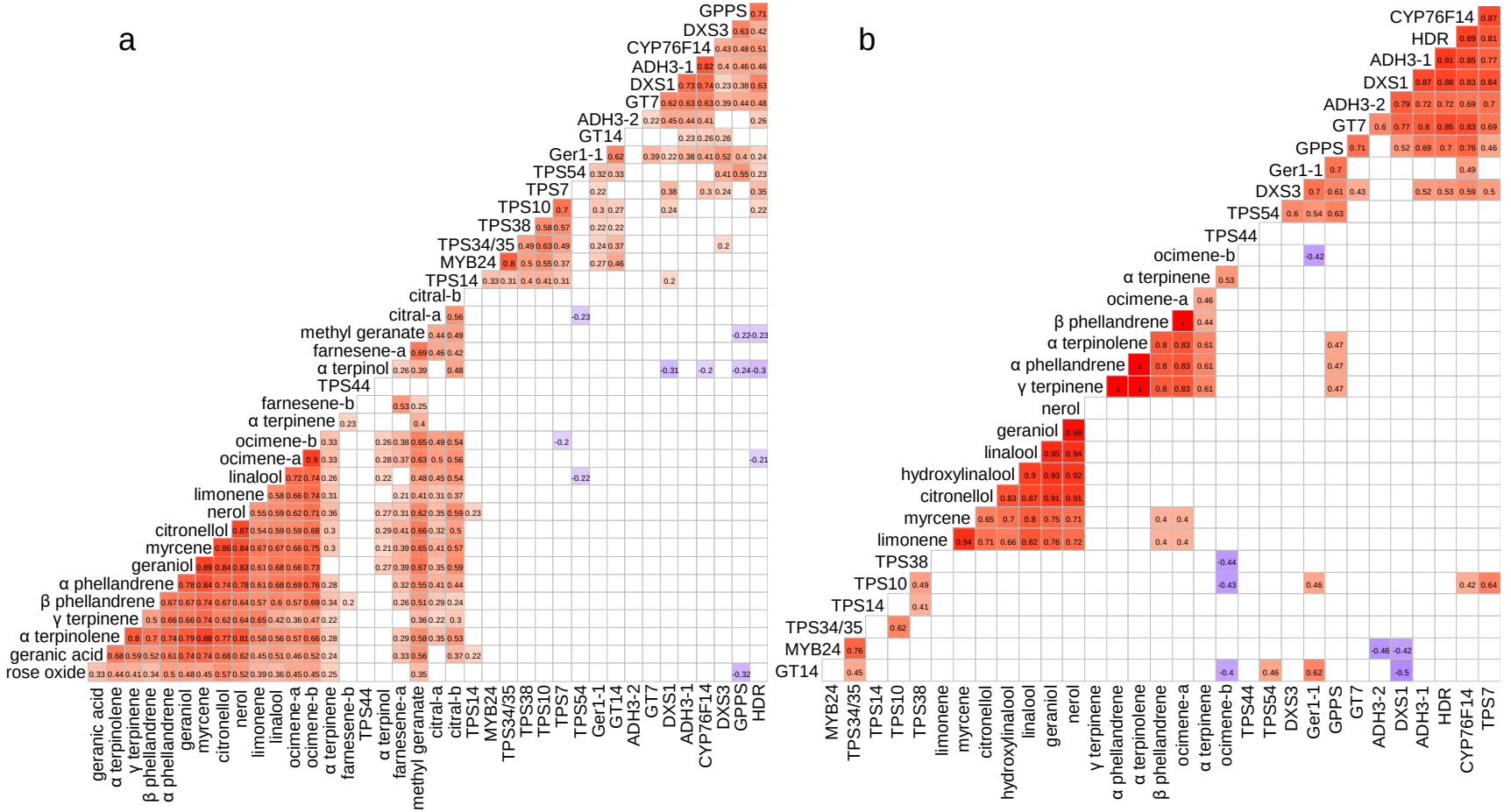

Figure S8: Correlation table of date-matched free (a) and bound (b) terpene concentrations and gene expression in berries from field-grown Gewürztraminer grapevines exposed to crop load management (CLM) strategies throughout 2016 growing season. Numbers within squares indicate significant ($p < 0.05$) Pearson coefficients between measured values. Correlation values indicated here are R values with colour added to illustrate no correlation (white) for strong positive correlation (red) and for strong negative correlation (blue). Blank squares indicate no significant correlation. R^2 values discussed in text were calculated from R values plotted