IMPACT OF CROP LOAD MANAGEMENT ON TERPENE CONTENT IN GEWÜRZTRAMINER GRAPES

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Abstract:

Context and purpose of the study - Crop load management by cluster thinning can improve ripening and the concentration of key metabolites for grape and wine quality. However, little work has been done on testing the impact of crop load management on terpene content of white grapes. The goal of the study was to assess if by reducing crop load via cluster thinning growers can increase terpene concentration of grapes, as well as to test if the timing of thinning application affects terpene concentration.

Material and methods - This study was performed in 2016, 2017, and 2018 in Oliver, British Columbia. Field-grown Gewürztraminer vines were cluster-thinned at two developmental stages, just after fruit-set (Early Thinning) and at veraison (Late Thinning), in order to target three crop levels: Light Crop (7 tons/ha), Moderate Crop (10.5 tons/ha), and High Crop (14 tons/ha). Treatments were replicated on five plots arranged in a randomized block design. The effect of treatments on leaf gas exchanges, vine leaf area, and berry sugar (total soluble solid, TSS), acid (titratable acidity, TA), and terpene concentration was analyzed during ripening and at harvest. Free and glycosylated terpenes were identified and quantified using a SPME-GC-MS and a LI-GC-MS, respectively.

Results - Crop level treatmentsdid not affect leaf gas exchanges and vine leaf area. TSS concentration during ripening and at harvest was higher in Light Crop and Moderate Crop treatments than in High Crop, particularly for Early Thinning treatments. High Crop and Light Crop-Early Thinning determined the highest free terpene concentration at harvest; however, a significant interaction between treatment and year effects was observed. Total glycosylated terpenes at harvest were marginally affected by treatments (P = 0.063), and Light Crop-Early Thinning determined the highest total glycosylated terpene concentration. Interestingly, total free terpenes were significantly affected by the treatments at the sampling before harvest (20-21 Brix), when Light Crop-Early Thinning determined a higher concentration of total free terpenes than High Crop. This result was consistently among the three years. Our study suggests that crop load management can be used as a tool to improve grape terpenes in scenarios (regions and/or seasons) where ripening is impaired and grapes cannot reach relatively high sugar levels.

Keywords: Aroma, Grapevine, Ripening, Thinning, Yield

1. Introduction

Aromatics (also known as volatile organic compounds, or VOCs) impact grape and wine quality. These compounds include terpenes, C13-norisoprenoids, methoxypyrazines, aldehydes, ketones, esters, and alcohols. Terpenes determine the characteristic aroma of grapes and wines of Gewürztraminer, Riesling, and Muscats (Lund and Bohlmann, 2006). Grape terpenes are both free and glycosylated (Robinson et al., 2013). Free terpenes are volatile and can be odorous, while glycosylated (bound) terpenes are odorless. However, the glycoside-terpene bond can be hydrolyzed during winemaking, and the terpene becomes volatile and potentially odorous (Robinson et al., 2013).

The crop load, expressed as the leaf area/crop weight ratio significantly affects grape composition and quality in the vineyard (Kliewer and Dokoozlian, 2005). The reduction of crop size via cluster thinning treatments is a common way to balance leaf area/crop weight ratio to obtain premium grapes (Howell,

2001; Kliewer and Dokoozlian, 2005; Reynolds et al., 2005). Indeed, crop size and grape quality are both relevant economic issues. Reducing crop size can potentially improve the economic value of the grapes but reduces grape yield per vine and per surface unit, another major factor for vineyard returns.
In British Columbia (BC, Canada) vineyards, crop size adjustment via cluster thinning is a common practice that growers adopt to promote fruit ripening and the accumulation in the grapes of key determinants of wine quality – such as pigments and tannins in red varieties, and grape aromatics in white varieties. Although the relationship between crop size and grape quality has been well-studied, a comprehensive understanding of the impact of crop size on grape aromatics is still elusive. Previous studies have indicated that cluster thinning can improve the accumulation of terpenes in white grapes (Reynolds and Wardle, 1989). Also, anecdotally, viticulturists have reported that some white grape varieties, such as Gewürztraminer, are sensitive to over-cropping, meaning that the quality of the fruit and wine would remarkably decreases if large crop sizes are targeted.
This study investigated how crop size manipulation via cluster thinning applied early during berry development and at véraison affects berry terpenes in Gewürztraminer grapes.

2. Materials and Methods

The experiment was conducted throughout 2016-2018 growing seasons in a Gewürztraminer (Clone 47, SO4 rootstock) commercial vineyard in the Okanagan Valley (49°10'N, 119°32'W, 390 m a.s.l.), near Oliver, British Columbia, Canada. Vine density was 2.4 m between rows x 1.2 m within rows. Vines were cane pruned (four canes of 8-10 buds) and trained in a vertically shoot positioned system. Pest management, canopy management, and fertilization in the vineyard were applied according to standard local agricultural practice.
The cluster thinning treatments included three crop levels: high crop (40 - 50 clusters per vine) – HC, medium crop – MC = 75% cluster number of HC, light crop – LC = 50% cluster number of HC. M and L were imposed at two thinning dates (early thinning – E – post-fruitset; late thinning – L – véraison. In summary, this study considered five treatments: HC, MC-E, MC-L, LC-E, LC-L. Each treatment was imposed onto five plots of 9 to 11 vines organized in a randomized block design.
Yield, number of clusters per vine, and average cluster mass were determined at harvest. Leaf area was measured within two weeks of commercial harvest accordingly to Sanchez-de-Miguel et al. (2010). Gas exchange parameters (photosynthesis, transpiration, stomatal conductance) were monitored using LI-COR 6400 every 14 - 28 days. Light intensity was set to 1500 µmol m^2 s^{-1}. 2 - 4 leaves per plot were sampled on the sun-face. Sugars (TTS), and acids were determined accordingly to Savoi et al. (2017). Free terpene analysis was adapted from Fedrizzi et al. (2012) and Matarese et al. (2013) with some modifications. Berries were collected using scissors and frozen under a cover of dry-ice. Frozen berries were crushed and deseeded using a mortar and pestle under liquid N2, then powdered using A11 Basic Analytical Mill (IKA, Wilmington, NC, USA). 5.00 g ± 0.10 g of frozen, deseeded, powdered grape tissue (pulp and skin) were used for the SPME analysis accordingly to Matarese et al. (2013). 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). Samples were analyzed in a random sequence. Two technical replicates were run per biological sample. Glycosil terpene analysis was adapted from Martin et al. (2012) and Ghaste et al. (2014). One gram of grape tissue was used for the extraction. The column, GC-MS model, autosampler, and oven regime used were identical to those described for free terpenes. Terpenes were identified by comparing the retention times of ion extracted chromatograms (IECs) peaks with the retention times of their reference standards, when available, and by identifying the mass spectra using the NIST library. Concentrations were 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 closest molecular structure and functionality. Concentrations are reported as µg/g berry fresh weight (FW) and µg/berry.
Basic statistics, analysis of variance (ANOVA) and Tukey's HSD post-hoc tests were undertaken using R software v3.4.4 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results and discussion

The treatments sought to restrict vine cluster number and therefore per hectare yield, which was successfully achieved in all three years (Table 1).Cluster number, as well as cluster weight were affected by the thinning treatments and the years (Table 1). LC cluster weights were the largest among the treatments (being heavier than HC clusters), but a significant interaction was observed between the effects of the treatments and the years.

Photosynthesis, transpiration, and stomatal conductance, as well as leaf area was unaffected by the treatments.

Berry TSS accumulation was faster in LC and MC than in the other treatments (Figure 1), but at harvest (~23 Brix), no differences were observed among treatments. Pre-harvest sampling (approx. two weeks before harvest) reveals that MC and LC had an average separation from HC of ~0.5 and ~1 Brix, respectively. At both sampling points, the timing of thinning did not affect the TSS accumulation at LC levels.

Twenty-one terpenoids were consistently found in the free and glycoside form. They comprised monoterpenes (terpinene, phellandrene, ocimene, etc.) and derivative aldehyde (citral), alcohols (terpinol, linalool, geraniol, etc.), acid (geranic acid), ester (methyl geranate), and ether (rose oxide) compounds. Additionally, an isomerable sesquiterpene (farnesene) was detected in the free volatiles at harvest. Along with the farnesene isomers, methyl geranate was not detectable in the bound fraction, while hydroxylinalool was only detectable in the bound fraction.

HC and LC-E displayed the highest free terpene concentration at harvest (Table 2); however, a significant interaction between treatment and year effects was observed. In 2016, free terpenes were assessed throughout the season to understand the general trend of terpene development. Notably, commercial harvest was not the peak in terpene content in all treatments; peak content was two weeks prior to harvest in MC and LC treatments. Interestingly, total free terpenes were significantly affected by the treatments two weeks before harvest (~20-21 Brix), when LC-E berries had a higher concentration (+49.1%) of free terpenes than HC. This result was also confirmed by a two-way ANOVA where effects of treatments across the three seasons were compared.

At harvest LC-E had a higher concentration of terpene glycosides than LC-L (Table 2). However, differences were marginally significant. None of the major bound terpenes (geraniol, nerol, linalool, citronellol) were affected by thinning treatment. At pre-harvest, LC-E had a significantly higher per berry content (+ 20.4% than HC) of terpene glycosides.

Our results confirmed that the reduction of crop load via cluster thinning can be used to accelerate ripening (increase in sugar level). These results confirmed the ones of previous studies in the same viticultural regions (Hannam et al. 2014). When relatively high sugar levels are achieved (harvest sampling), reduction of crop load did not improved terpene concentration in berries. However, the reduction of crop load at early stages of berry development determined a higher terpene concentration at pre-harvest (~20-21 Brix).

4. Conclusion

Our study suggests that crop load management can be used as a tool to accelerate fruit ripening and improve grape terpenes in scenarios where ripening is impaired and where grapes cannot reach relatively high sugar levels. In this study, the reduction of crop load determined a faster sugars and terpene accumulation during ripening. This allows growers to harvest the grapes earlier in seasons when climate conditions at harvest are challenging, and to improve the grape terpenes in those regions and/or season characterized by cooler climates that can impair fruit ripening. However, our results also suggest that in regions and seasons that allow late harvest, optimal sugar and terpene levels can be achieved also with high crop levels.

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6. Literature cited

FEDRIZZI B., CARLIN S., FRANCESCHI P., VRHOVSEK U., WEHRENS R., VIOLA R., MATTIVI F.. 2012. D-Optimal Design of an Untargeted HS-SPME-GC-TOF Metabolite Profiling Method. Analyst. 137(16), 3725–31.

GHASTE M., NARDUZZI L., CARLIN S., VRHOVSEK U., SHULAEV V., MATTIVI F.. 2015. Chemical Composition of Volatile Aroma Metabolites and Their Glycosylated Precursors That Can Uniquely Differentiate Individual Grape Cultivars. Food Chemistry. 188, 309–19.

HANNAM K.D., NEILSEN G.H., NEILSEN D., BOWEN P.. 2015. Cluster thinning as a tool to hasten ripening of wine grapes in the Okanagan Valley, British Columbia. Canadian Journal of Plant Science, 2015, 95(1), 103-113.

HOWELL G.S.. 2001. Sustainable Grape Productivity and the Growth-Yield Relationship: A Review. American Journal of Enology and Viticulture. 52(3), 165–74.

KLIEWER W.M., DOKOOZLIAN N.K.. 2005. Leaf Area/Crop Weight Ratios of Grapevines: Influence on Fruit Composition and Wine Quality. American Journal of Enology and Viticulture. 56(2), 170–81.

LUND S.T., BOHLMANN J. 2006. The Molecular Basis for Wine Grape. Science. 311, 804–5.

MARTIN D.M., CHIANG A., LUND S.T., BOHLMANN J.. 2012. Biosynthesis of Wine Aroma: Transcript Profiles of Hydroxymethylbutenyl Diphosphate Reductase, Geranyl Diphosphate Synthase, and Linalool/Nerolidol Synthase Parallel Monoterpenol Glycoside Accumulation in Gewürztraminer Grapes. Planta. 236(3): 919–29.

MATARESE F., SCALABRELLI G., D'ONOFRIO C.. 2013. Analysis of the Expression of Terpene Synthase Genes in Relation to Aroma Content in Two Aromatic Vitis Vinifera Varieties. Functional Plant Biology. 40(6), 552–65.

REYNOLDS A.G., MOLEK T., DE SAVIGNY C.. 2005. Timing of Shoot Thinning in Vitis Vinifera : Impacts on Yield and Fruit Composition Variables. American Journal of Enology and Viticulture. 4, 343–56.

REYNOLDS, A.G., WARDLE D.A.. 1989. Impact of Various Canopy Manipulation Techniques on Growth, Yield, Fruit Composition, and Wine Quality of Gewürztramine. American Journal of Enology and Viticulture. 40(2), 121–29.

ROBINSON A.L., BOSS P.K., SOLOMON P.S., TRENGOVE R.D., HEYMANN H., EBELER S.E.. 2014. Origins of Grape and Wine Aroma. Part 1. Chemical Components and Viticultural Impacts. American Journal of Enology and Viticulture 65(1), 1–24.

SAVOI S., WONG D.C.J., ARAPITSAS P., MICULAN M., BUCCHETTI B., PETERLUNGER E., FAIT A., MATTIVI F., CASTELLARIN S.D.. 2016. Transcriptome and Metabolite Profiling Reveals That Prolonged Drought Modulates the Phenylpropanoid and Terpenoid Pathway in White Grapes (Vitis Vinifera L.). BMC Plant Biology 16(1), 67.

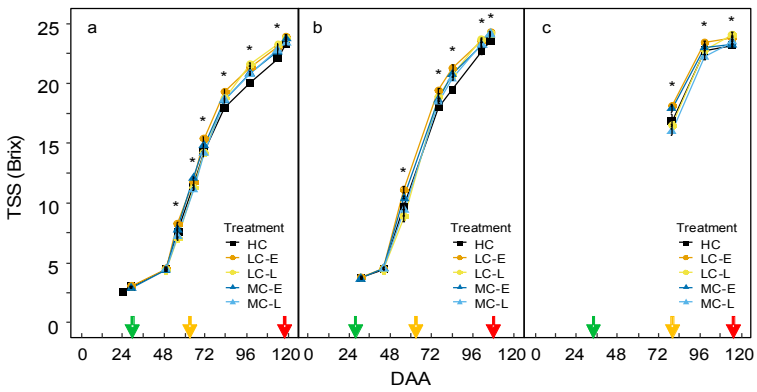

Figure 1 - Evolution of total soluble solids (TSS) in Gewürztraminer berries throughout (a) 2016, (b) 2017, and (c) 2018. Standard error is indicated by error bars. Green, yellow, and red arrows indicate dates of early (E) treatment application, late (L) treatment application, and commercial harvest, respectively. Asterisks indicate significant differences among treatments as indicated by a one-way ANOVA.

Table 1 - Two-way ANOVA of crop load treatments, year, and crop load treatment x year interaction effects on yield parameters. Letters indicate differences among treatments according to a post-hoc Tukey HSD test.

	Cluster/Vine		Cluster Weight (g)		Yield/Vine (kg)		Crop Load (cm^2/g)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
HC	43 a	2	121.24	3.36	5.260	0.301	21.21 b	1.02
LC-E	25 c	1	138.24	4.95	3.364	0.119	29.96 ab	2.03
LC-L	24 c	1	142.41	5.02	3.260	0.140	37.3 a	2.19
MC-E	33 b	1	134.28	5.09	4.355	0.164	26.85 b	1.98
MC-L	32 b	1	131.2	5.79	4.192	0.182	31.83 a	3.04
Two-way ANOVA								
Treat. *P*	< 0.001		0.007		< 0.001		< 0.001	
Year *P*	< 0.001		< 0.001		< 0.001		< 0.001	
Treat. x Year *P*	0.321		0.0303		0.0243		0.735	

Table 2 - Percent change from control (high crop, HC) of crop load management treatments. Bold values indicate significant difference from HC according to post-hoc Tukey HSD test. Two-way ANOVA of crop load management treatment, year, and treatment x year interaction effects are reported.

	Pre-harvest				Harvest			
	Free Terpenes		Terpene Glycosides		Free Terpenes		Terpene Glycosides	
	µg/g berry	µg/berry	µg/g berry	µg/berry	µg/g berry	µg/berry	µg/g berry	µg/berry
	%	%	%	%	%	%	%	%
LC-E	**49.3**	**56.8**	**20.4**	**27.1**	11.2	17	8.46	11.7
LC-L	16.2	18.6	-0.511	1.82	**-42.4**	**-41.2**	-12.3	-9.24
MC-E	6.75	7.56	5.59	7.33	33.92	27	2.21	-0.783
MC-L	19.3	20.7	-1.74	-0.493	**-21.2**	-19.5	2.11	0.725
Two-way ANOVA								
Treat. *P*	0.00329	< 0.001	0.0248	0.0109	0.001	< 0.001	0.0633	0.29
Year *P*	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treat. x Year *P*	0.266	0.132	0.111	0.202	< 0.001	< 0.001	0.0691	0.16