Project Title: Understanding Pinot Noir grape and wine aroma composition as a result of changes in vine balance, year 1 of a 3-year project ID#2014-1507

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Abstract

Crop thinning is commonly implemented in most vineyards of Oregon to regulate crop level, but its practicality has been questioned because of increased production costs and lost yields, as well as lack of knowledge of the relationship between crop thinning and Pinot Noir grape and wine quality. Effects of severity and timing of crop thinning practice on Pinot Noir grape and wine volatile compositions were investigated over three growing seasons (2010, 2011, and 2012) in a commercial vineyard of western Oregon. Crop levels were moderately (35% crop removed) or severely (65% crop removed) thinned at pre-bloom, fruit set, lag phase, or véraison and compared to full crop treatments (control). Grape and wine volatile compositions were analyzed by GC-FID and GC-MS. Results showed that crop thinning practice affect some of the yeast-generated volatile composition. Crop thinning increased levels of certain higher alcohols (e.g., *trans*-3-hexenol, 1-heptanol, and propanol), but decreased levels of some esters (e.g., ethyl hexanoate, ethyl isobutanoate, ethyl 2-methyl butanoate, and isoamyl acetate). However, crop thinning practice had very limited impact on the grape-derived volatile compositions (terpenoids and C_{13}-norisoprenoids).

Keywords: Pinot Noir wine, crop thinning, severity, timing, volatile composition, SBSE-GC-MS, HS-GC-FID, SPME-GC-MS

Introduction

Vine balance is defined as the balance between vegetative growth and crop production in order to not only maintain healthy canopy growth, but also achieve adequate fruit production with optimal fruit quality (e.g., sugar levels, acid balance, and flavor compounds) (Vance and Skinkis, 2013). Considerable studies have shown that vine balance has a significant effect on fruit and wine quality (Howell, 2001; Kliewer and Dokoozlian, 2005; Reynolds et al., 1994; Reynolds and Wardle, 1989; Smart et al., 1990). Crop thinning is a management practice of deliberate removal of selected clusters from the grapevine. It is commonly used in the vineyards to regulate crop level and to achieve desirable vine balance (Kliewer and Dokoozlian, 2005).

Considerable studies have investigated the influence of crop thinning on grape and wine quality; however, results are still inconclusive. It has been reported that crop thinning can effectively reduce fruit yield and increases the berry weight and soluble solids of grapes (Gil et al., 2013; Sun et al., 2012). Grape phenolic and volatile compounds have also been found to be increased by crop thinning. Bureau et al., (2000) showed that crop thinning decreased levels of free-form C_6 compounds and increased levels of bound-form monoterpene alcohols and volatile phenols in Syrah grapes. Gil et al., (2013) reported that crop thinning enhanced Syrah wine quality by elevating anthocyanin and polysaccharide levels. Similarly, Reynolds et al., (1996) found that crop thinning improved color, currant aroma, and astringency of Pinot Noir wine. However, Studies in Cabernet Sauvignon (Bravdo et al., 1985) and Chardonnay Musqué (Reynolds et al., 2007) indicate that differences between wines made from crop-thinned vines compared to full crop vines are not always detected in sensory evaluation. Moreover, variations in vineyard climate and grape cultivar often are found to override the outcomes of crop thinning practice (Andrews et al., 2013; Diago et al., 2010; Keller et al., 2008; Ough and Nagaoka, 1984; Reynolds et al., 2007).

In addition, the timing of the crop thinning practice further complicates impacts of this practice on grape and wine quality. Dokoozlian (1995) reported that cluster thinning before bloom were more effective to improve grape quality through increased total soluble solids and enhanced color. However, they further mentioned that prebloom cluster removal is risky, because berry set and cluster shape were unknown at the time thinning was performed. Crop thinning late in the season (e.g., véraison or later) often has limited impact on fruit quality. Chapman et al., (2004a) reported that different intensities of thinning at véraison in Cabernet Sauvignon resulted in no differences in wine quality. On the contrary, Reynolds et al., (2007) reported that levels of total soluble solids, free-form and bound-form monoterpenes tended to increase with increased delay in thinning time of Chardonnay Musqué grapes and wine. They further stated that crop thinning at later stages prevented berry size compensation. Since small berry size is considered a characteristic of high-quality grapes, berry size compensation is an important consideration for growers when making crop thinning decisions.

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Currently, in Oregon, most commercial vineyard growers perform 2 to 3 crop thinnings (between fruit set and lag phase) during the season, removing 25 to 50% of the vine's total fruit each year (Vance, 2012). However, given the inconsistent data of impacts of crop thinning on grape and wine composition, there emerges the notion that response of berry development to crop thinning may vary differently depending on specific region (e.g., warm climates *vs.* cool climates) and grape cultivar (e.g., high-yielding *vs.* low-yielding cultivars). Furthermore, crop thinning is one of the most expensive management practices in vineyards for Pinot Noir produced in Oregon. It is important to know how this implemented technique might influence the grape quality and if its benefits will offset increased production expenses. The main objective of the present study was evaluate effects of impacts of crop thinning with the focus of severity and timing on Pinot Noir grape and wine volatile composition.

Materials and Methods

Chemicals

Sources of volatile compound standards used in this study are listed in Appendix A. For various reagents, GC grade was purchased, including methanol from EMD (Gibbstown, NJ) and dichloromethane from Burdick & Jackson (Muskegon, MI). Citric acid was purchased from Lancaster (Ward Hill, MA), and 0.2 M citrate buffer solution (pH 3.2) was prepared fresh before usage. Tartaric acid was purchased from Mallinckrodt Inc. (Paris, KY). Macer®8 FJ enzyme solution was purchased from Biocatalysts Limited Inc. (Wales, UK). C_{18} disposable extraction cartridges (500 mg, 6 mL) were obtained from J. T. Baker (Philipsburg, NJ). Tartaric acid was purchased from Mallinckrodt Inc. (Paris, KY). A synthetic wine solution was made by dissolving 3.5 g of L-tartaric acid in 1 L of 12% ethanol solution, and adjusting pH to 3.5 with 1 M NaOH.

Vineyard experimental design

This trial was conducted from 2010 to 2012 at Stoller Vineyard, Dayton, OR. The experimental block used for 2011 to 2012 was planted in 1998 to *Vitis vinifera* L. Pinot Noir clone 115 grafted to 3309C rootstock at a planting density of 1245 vines/acre (1.5 x 2.1 m/vine). In 2010, a different trial block was used at the same vineyard (16 year old clone 115 PN grafted to 3309). However, due to issues with low yield and a mishap of the vineyard management labor on-site in 2010, trial was moved to a more consistent yielding block that was also more visible to the vineyard manager to avoid treatment losses for 2011 and 2012. A 2x4 + 2 factorial experimental design was used to compare two crop thinning intensities (moderate thinning (MT) and severe thinning (ST)) at four stages of phenology (pre-bloom, fruit set, lag phase and véraison). Moderate thinning was defined as thinning to 1 cluster per shoot, and severe thinning was defined as 1 cluster per every 2 shoots, achieved by a manual thinning scheme of 1-0-1-0. These treatments were compared to a full crop treatment (control) where no clusters were removed. Full crop level on these vines was approximately 1.5 clusters per shoot. Each treatment was replicated five times in a randomized complete block design. There were 8 vines per plot.

Vines were managed with standard practices for the region (e.g., hedging, leaf pulling, and pest management) performed by the grower collaborator with the exception of cluster thinning.

Analysis of volatile compounds in grapes

Randomly selected berry samples (60 g) were frozen in liquid nitrogen and powdered using a blender (Sunbeam products, Inc., Boca Raton, FL). Fifty grams of grape powder was mixed with 30 g of NaCl and 50 mL citrate buffer solution (0.2M, pH 3.2). The mixture was kept under nitrogen in the dark for 24 hr at room temperature, and then centrifuged at 7000 rpm for 30 min (Sorvall RC-5C, Du Pont Company, Wilmington, DE). The supernatant was filtered twice, first through Whatman No. 1 and then VWR 413 filter paper. Filtered juice was used for the analysis of both free- and bound-form volatile compounds.

Free-form volatile compound analysis by SBSE-GC-MS

A stir bar sorptive extraction-gas chromatography-mass spectrometry method (SBSE-GC-MS) was used to analyze the free-form volatile compounds in grape juice as described by Malowicki et al., (2008) with some modifications. Clear grape juice (20 mL) was added in a 20 mL vial, and mixed with 20 μL of internal standard solution (9.4 mg/L of 3-hexanone, 2.4 mg/L of 4-octanol, and 1.3 mg/L of naphthalene). A pre-cleaned polydimethylsiloxane (PDMS) coated stir bar (0.5 mm film thickness, 10 mm length, Gerstel Inc., Baltimore, MD) was placed into the vial and stirred at 1000 rpm for 3 hr at room temperature. After extraction, the stir bar was removed from the sample, rinsed with distilled water, dried with light-duty tissue wipers, and then transferred into a thermal desorption tube for GC-MS analysis.

Analysis of the extracted volatile compounds was carried out using an Agilent 6890 gas chromatograph coupled with a 5973 mass selective detector (Agilent Techonologies, Inc., Wilmington, DE) and a Gerstel MPS-2 multipurpose TDU autosampler with a CIS-4 cooling injection system (Gerstel Inc., Linthicum, MD). The analytes were thermally desorbed at the TDU in splitless mode, ramping from 25 to 250 °C at a rate of 100 °C/min, and held at the final temperature for 2 min. The CIS-4 was cooled to -80 °C with liquid nitrogen during the sample injection, and then heated at 10 °C/s to 250 °C for 10 min. Solvent vent mode was used during the injection with a split vent flow of 50 mL/min. Separation was achieved using a HP-5ms capillary column (60 m × 0.32 mm i.d., 0.25 μm film thickness, Agilent Technologies, Inc.). The oven temperature was programmed at the successive temperature and time points: 40 °C for 2 min, ramped to 220 °C at a rate of 4 °C/min, increased to 250 °C at a rate of 8 °C/min, and held at the final temperature for 6 min. A constant helium flow of 2.5 mL/min was used. A column splitter was used at the end of column, 1 mL/min column flow was introduced to the MS, and the other 1.5 mL/min was vented out. The MS transfer line and ion source temperature were 280 °C and 230 °C, respectively. The mass selective detector was used in full scan mode for acquiring data. Electron ionization mass spectrometric data from m/z 35 to 300 were collected, with an ionization voltage of 70 eV. System software

control, data management, and analysis were performed through Enhanced ChemStation Software (Agilent Techonologies, Inc.). Identifications were made by comparing mass spectral data samples with the Wiley 275 L database and confirmed by authentic pure standards. Internal standard were used for quantification and all analyses were conducted in duplicate.

Bound-form volatile compound analysis by SPE-SBSE-GC-MS

Bound-form volatile compounds in grape were isolated using the C_{18} solid phase extraction method (SPE) described by Williams et al., (1995) with modifications, then enzyme-acid hydrolysis was used to release free-form volatile compounds according to the method described by Du et al., (2010). Each 20 mL of grape juice was loaded onto a C_{18} disposable extraction cartridge (500 mg, J. T. Baker, Philipsburg, NJ) that had been pre-conditioned with 10 mL of methanol and 10 mL of milli-Q water. After sample loading, the cartridge was washed with 10 mL of milli-Q water to remove sugar and organic acids followed by 20 mL of dichloromethane to remove free volatile compounds. The bound-form volatiles were eluted from the cartridge with 6 mL of methanol and concentrated to dryness at 45 °C under vacuum using a rotary evaporator. Twenty milliliters of citrate buffer solution (0.2M, pH 3.2) and 100 μL of Macer®8 FJ enzyme solution were added to the isolates. The mixtures were incubated at 45 °C for 20 hours. The solution was cooled to room temperature, and the released volatiles were analyzed using PDMS-SBSE-GC-MS method described previously. All analyses were conducted in duplicate.

Wine production

After harvest, fruits from field replicates were combined and then randomly subdivided into three lots of equal weight (3 kg) which were used to produce triplicate fermentations for each treatment. Fruits from crop thinning at FLWR and FS stages were combined to form "Early" treatment, and fruit from crop thinning at LAG and VER stages were combined to form "Late" treatment. Fruit was fermented at Oregon State University research winery. Grapes were destemed, pooled and placed into 1 gallon glass micro-scale fermenters that utilize a submerged cap method to maintain skin and juice contact as described by Sampaio et al., (2007) on the day of harvest. Potassium metabisulfite was added to provide a calculated amount of 50 mg/L total sulfur dioxide. Grapes were then inoculated with an active-dry form of *Saccharomyces cerevisiae* RC212 (Lallemand, Montréal, Canada) at approximately 1 x 10^6 cfu/mL after rehydration according to manufacturer's directions. Fermenters were placed in a temperature controlled room set to 27 °C and alcoholic fermentation was monitored by °Brix measurements using an Anton-Paar DMA 35N Density Meter (Graz, Austria). At the completion of alcoholic fermentation (< 0.5 g/L reducing sugar as measured by CliniTest ®), wines were pressed using a small modified basket press that applied a constant pressure of 15 psi for 5 min allowing consistent pressing. Pressed wine was settled in ½ gal glass carboys for 72 hr at 4 °C before being racked into ½ gal glass carboys and an

addition of 50 mg/L of SO_2 was made. Wine was stored at 13 °C until required for analysis. No malolactic fermentation was conducted.

Wine volatile compound analysis

Higher alcohol and acetate analysis by HS-GC-FID

Headspace gas chromatography method was used to analyze propanol, isobutyl alcohol, isoamyl alcohol, ethyl acetate, and isoamyl acetate. An aliquot (0.5 mL) of wine was diluted with 0.5 mL of milli-Q water in a 20 mL headspace glass vial. Twenty microliter of internal standard solution (2.5 mg/L of methyl propionate) was added into the vial as well. The mixture was incubated at 70°C for 15min to reach the equilibrium between the sample and headspace. An aliquot (1000 mL) of headspace gas was taken by a 2.5 mL of gastight syringe at a rate of 200 µL/s and then injected into the GC injection port.

The analysis of the extracted volatile compounds was carried out by using an Agilent 7890A gas chromatograph coupled with a flame ionization detector (Agilent Techonologies, Inc.). Separation was achieved using a DB-wax capillary column (30 m × 0.25 mm i.d., 0.5 µm film thickness, Agilent Techonologies, Inc.). The oven temperature was programmed at 35 °C for 4 min, then ramped to 150 °C at a rate of 10 °C/min and held at the final temperature for 6 min. A constant helium flow of 2 mL/min was used. The injector temperature was kept at 200 °C and the FID was kept at 250 °C. Peak identification of the volatile components was achieved by comparison with GC retention indices of standards. All analyses were carried out in duplicate.

Volatile compound analysis by SPME-GC-MS

A headspace solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) method was used to analyze majority of volatile compounds in the wine samples. An aliquot (2 mL) of wine was diluted with 8 mL of saturated NaCl solution in a 20 mL glass vial, in which 20 µL of internal standard solution (96 mg/L of 3-heptanone, 109 mg/L of 4-octanol, and 118 mg/L of octyl propionate) were added. A pre-conditioned 2cm 50/30 µm divinylbenzene/Carboxen ^{TM}/Polydimethylsiloxane (DVB/Carboxen TM /PDMS) coated SPME fiber (Supelco, Bellefonte, PA) was inserted into the headspace at 50 °C for 30 min using CTC autosampler (CTC Analytics, Inc., Zwingen, Switzerland). During the extraction, the sample was stirred at 500 rpm. Upon completion of the extraction, the fiber was removed from the sample vial and inserted into the injection port of the GC at 250 °C for 5 minutes at splitless mode.

The analysis of the extracted volatile compounds was carried out on an Agilent 6890N gas chromatograph coupled with a 5973N mass selective detector (Agilent Techonologies, Inc.). Separation was achieved by using a DB-wax capillary column (30 m × 0.25 mm i.d., 0.5 µm film thickness, Agilent Techonologies, Inc.). The oven temperature was initially programmed at 35 °C for 4 min, and then ramped to 230 °C at a

rate of 5 °C/min and held at the final temperature (230 °C) for 10 min. A constant helium flow of 2.5 mL/min was used. The MS transfer line and ion source temperature were 280 °C and 230 °C, respectively. The mass selective detector in the full scan mode was used for acquiring the data. Electron ionization mass spectrometric data from m/z 35 to 300 were collected, with an ionization voltage of 70 eV. System software control and data management/ analysis were performed through enhanced ChemStation software (Agilent Techonologies, Inc.). The unique quantification mass ion and qualifying mass ions were carefully selected to give the highest response and lowest interference for each compound. Internal standard quantification method was performed using the Chemstation software. All analyses were carried out in duplicate.

Statistical analyses

Statistical analyses were conducted by SPSS 16.0 (SPSS, Chicago, IL, USA). The control treatment was compared with thinned treatment using student's t-test, while the timing and severity impacts on grape and wine volatile composition among treatments were performed using two-way ANOVA. Statistical differences among means were evaluated using Tukey's HSD test at the $p < 0.05$ level.

Results and Discussion

Pinot Noir grape volatile composition:

In current study, both grape-derived free- and bound-form volatile compounds, including C_6 aldehydes and alcohols, terpenoids, and C_{13}-norisoprenoids were quantified and presented in Table 6.1-6.3.

Results showed that there was no consistent significant difference in grape volatile composition between treatments, although some variations were observed. In two of three years (2010 and 2011), crop thinned at fruit set had significant higher levels of β-damascenone than other time point ($p<0.05$). It is commonly thought that reducing crop level can produce higher quality grapes. Crop thinning practice has been reported to increase free and potentially volatile terpenes in Chardonnay Musqué grapes (Reynolds et al., 2007) and Gewürztraminer grapes (Reynolds and Wardle, 1989). However, other studies investigating links between crop level and grape secondary metabolites suggest that crop level may not be directly related to any of these compounds (Bowen et al., 2011, Bureau et al., 2000). In fact, the sunlight intensity and temperatures associated with cluster sunlight exposure are more closely correlated with concentrations of these compounds at harvest because of the physiological mechanism by which these compounds are synthesized (Downey et al., 2006; Jackson and Lombard, 1993). In current study, we observed no differences on whole vine leaf area, which indicating cluster sunlight exposure was not affected by crop thinning treatments (Skinkis et al., *in preparation*). Therefore, crop thinning practice in our study was not able to affect the levels of volatile composition in grapes.

In addition, our results showed that reduce crop level did not guaranteed improve grape volatile composition. With consistently high-yielding grapevines, it may be possible for crop thinning practice to reduce vine size and achieve a better ripening and quality of grapes. However, in current study, given the relatively low yields of Pinot Noir without crop thinning, it is not likely that vines are over-cropped or without sufficient canopy to warrant crop removal to support fruit development. Crop thinning practice in this study did not alter the canopy microclimate or vine vigor, therefore, no impact on grape volatile composition.

Pinot Noir wine volatile composition:

The volatile compounds in Pinot Noir wines made from grapes grown with different crop thinning management in 2011 and 2010 vintages were presented in Table 6.4&6.5. Esters, higher alcohols, and fatty acids formed the majority of volatile compounds in the wines. In addition, there were other minor compounds including terpenoids and C_{13}-norisoprenoids presented in Pinot Noir wines.

Higher alcohols: Higher alcohols (include C_6 alcohols) were quantified in this study, and they were the largest group of the volatile compounds in Pinot Noir wines (Table 6.4&6.5). The impact of crop thinning treatment on other higher alcohols in wine depended on the individual compound and vintage year. *Cis*-3-hexenol, heptanol, and propanol was significantly increased by crop thinning treatment ($p<0.05$). Moreover, severe thinning treatment significantly increased these three higher alcohol levels in wine ($p<0.05$). On the other hand, higher alcohols such as isobutyl alcohol, benzeneethanol, and isoamyl alcohol were significantly decreased by crop thinning practice. Furthermore, the more fruits were removed, lower concentrations of these higher alcohols were found in final wine ($p<0.05$).

Esters: Esters were the major class of aroma-active compounds analyzed in this study, contributing fruity and floral aromas. This group of compounds was composed of ethyl esters of straight-chain fatty acid, higher alcohol acetates, ethyl esters of branched-chain fatty acid, and aromatic esters (Table 6.4). In both years, crop thinning practice significantly reduced levels of ethyl hexanoate, ethyl isobutanoate, ethyl 2-methyl butanoate, and isoamyl acetate in wine ($p<0.05$). Moreover, severe thinning treatment had lower level of these esters than that of moderate thinning treatment ($p<0.05$).

Fatty acids: Four fatty acids, hexanoic acid, octanoic acid, decanoic acid, and dodecanoic acid were identified. At high concentrations, fatty acids are associated with rancid, cheesy and vinegar-like aromas, but they are usually present below their detection thresholds in wines (Louw et al., 2010). The impact of crop thinning on fatty acid composition was dependent on vintages. In 2011, crop thinning had no impact on the levels of fatty acids in wine, but in 2012, crop thinning practice significantly decreased levels of octanoic acid, decanoic acid, and dodecanoic acid in wine ($p<0.05$).

Higher alcohols, esters, and fatty acids are yeast metabolites, and their formation can be affected by many factors such as yeast strain, fermentation temperature, oxygen availability, grape nutrient status, and nitrogen level during fermentation (Bell and

Henschke, 2005; Rapp and Mandery, 1986; Torija et al., 2003; Ugliano and Moio, 2005)..
Therefore, the changes of those compound compositions may attribute to the alteration in grape nutrient status and grape must nitrogen level.

Grape-derived volatile compounds: Terpenoids and C_{13}-norisoprenoids are important volatile compounds, contributing to floral and fruity aromas in wine. However, results showed that there was no consistent or significant difference in these compound compositions between treatments, although some variations were observed. This result is in agreement with our previous observations that crop thinning practice did not alter the volatile composition in Pinot Noir grapes (Table 6.1-6.3).

Wine aroma profiles based on OAVs: By combining the OAV for compounds with similar aroma characteristics, an OAV-based wine aroma profile was obtained (Figure 6.1-6.3). As shown in figures, the aroma of the wine is described as herbaceous, floral, berry fruit, fermented, and fruity. Herbaceous was the term with lowest OAV, while berry fruit, fruity and fermented reached the highest markers. Wines from different treatment had similar aroma profiles indicated that crop thinning practice had very limited impact on the wine aroma profiles.

Summary

Currently, crop thinning practice is applied in most Oregon vineyards with the purpose to reduce crop level and increase grape quality. However, study the grape and wine volatile composition revealed that different from the common belief, crop thinning does not guarantee increased fruit and wine quality. Our results suggest that for low-yielding Pinot Noir cultivar, crop thinning only serve to reduce yields, rather than increase fruit and wine aroma quality.

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(B)
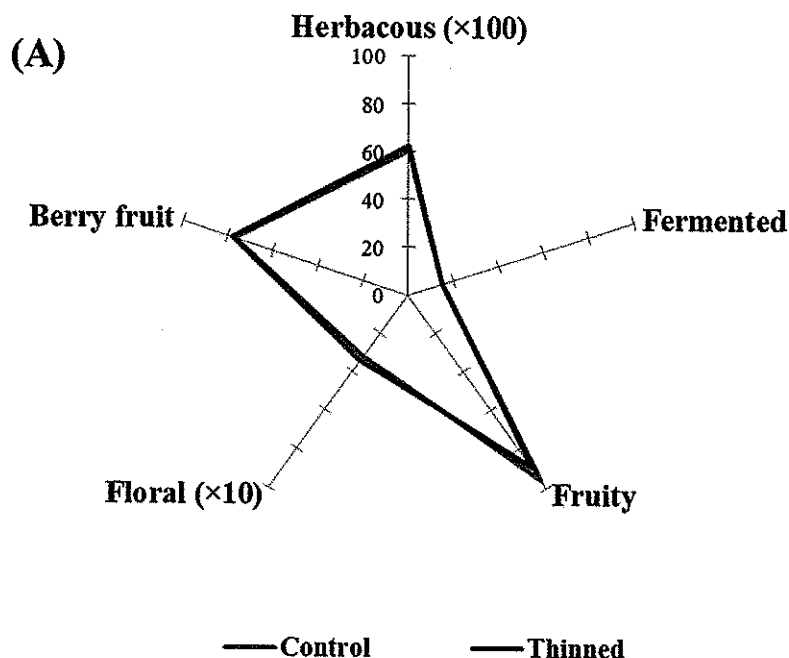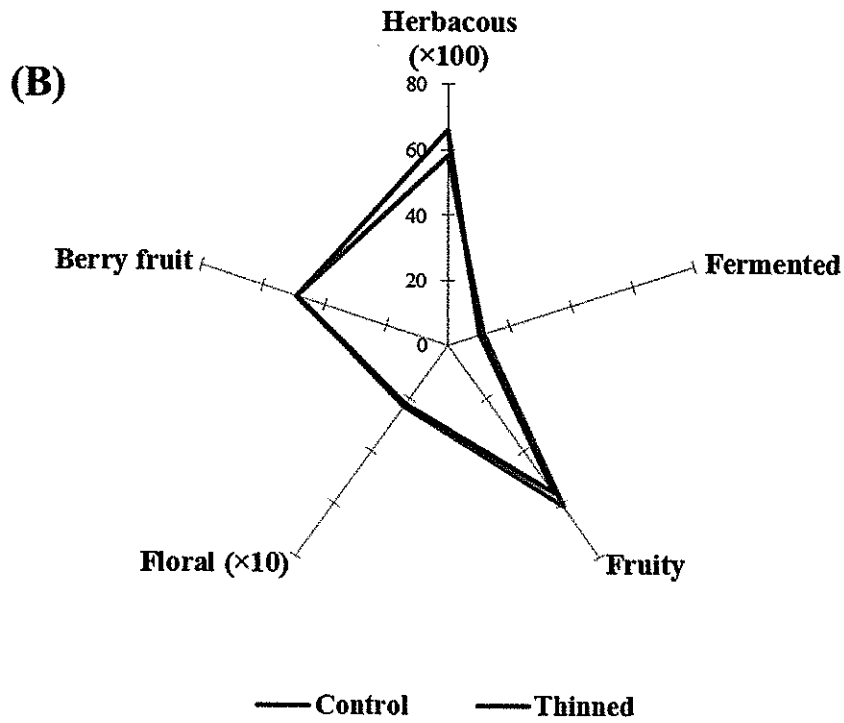
Figure 6.1 Aroma profiles of Pinot Noir wine with crop thinning treatment in (A) 2011 and (B) 2012 based on OAVs.

Herbacous (×100)

Berry fruit — Fermented

Floral (×10) — Fruity

——Moderate ——Severe
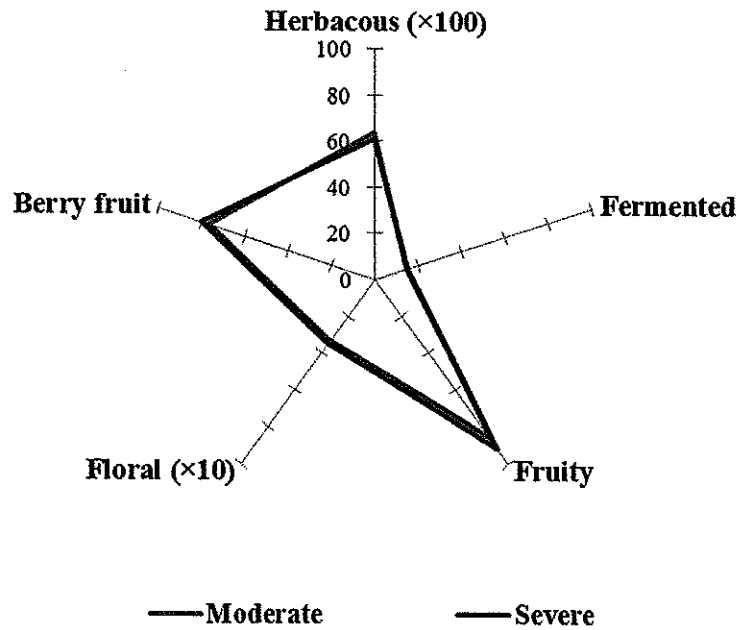
Figure 6.2 Aroma profiles of Pinot Noir wine with different crop thinning severity in (A) 2011 and (B) 2012 with different severity based on OAVs.

(A)

Herbacous (×100)

Berry fruit — Fermented

Floral (×10) — Fruity

——Early ——Later

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(B)

Herbacous (×100)

Berry fruit

Fermented
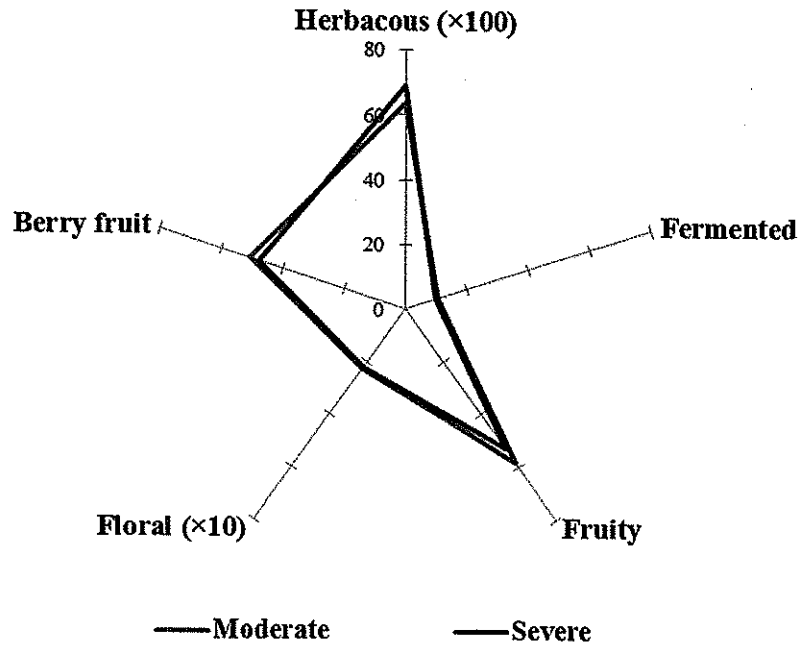
Floral (×10)

Fruity

——Early   ——Later
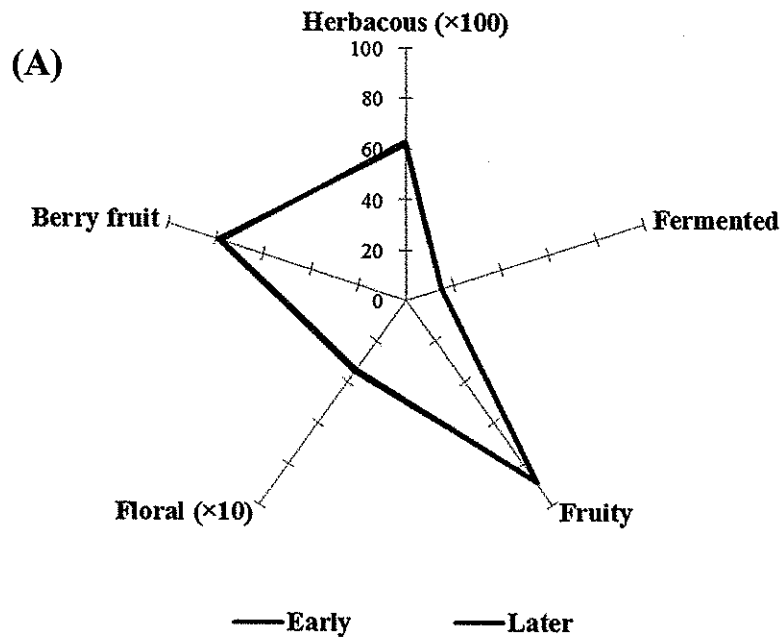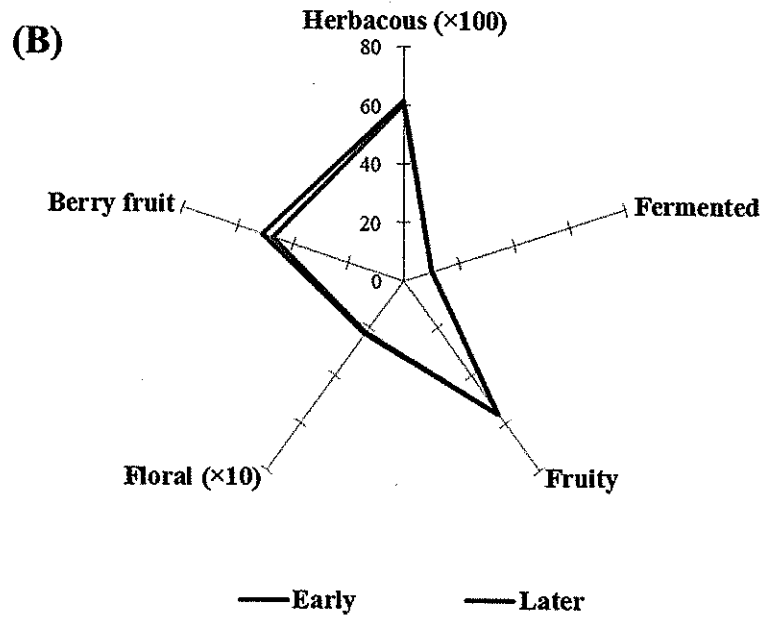
Figure 6.3 Aroma profiles of Pinot Noir wine with different crop thinning timing in (A) 2011 and (B) 2012 with different timing based on OAVs.

Table 6.1 Effect of severity and timing of crop thinning on Pinot Noir grape volatile composition in 2010.

2010 Year	Treatment [a]			Severity [b]			Timing [c]					Severity×Timing interaction
	Ctr	Thinned	p	MT	ST	p	FLWR	FS	LAG	VER	p	
Free form												
hexanal	19.4	18.1	0.734	19.3	16.9	0.475	16.0	21.1	15.8	19.5	0.651	0.496
trans-2-hexenal	420	406	0.780	417	396	0.660	442	424	404	355	0.597	0.710
1-hexanol	145	170	0.282	169	170	0.969	161	205	170	142	0.275	0.154
linalool	0.28	0.28	0.974	0.24	0.33	0.456	0.14	0.34	0.22	0.42	0.319	0.547
citronellol	0.35	0.40	0.638	0.37	0.41	0.699	0.31	0.35	0.34	0.56	0.230	0.947
geraniol	0.30	0.40	0.606	0.32	0.48	0.564	0.19	0.27	0.32	0.83	0.232	0.064
β-damascenone	0.51	0.43	0.515	0.42	0.44	0.927	0.29b	0.68a	0.45ab	0.31b	0.007	0.241
Bound form												
1-hexanol	84.0	84.3	0.936	81.1	85.5	0.402	84.2	75.7	88.5	84.8	0.304	0.233
cis-linalool oxide	25.9	27.6	0.758	30.1	25.1	0.517	22.6	24.1	27.1	36.6	0.599	0.042
trans-linalool oxide	28.7	28.7	0.995	30.5	26.5	0.538	23.9	26.7	27.8	35.6	0.631	0.062
linalool	1.40	1.28	0.462	1.18	1.37	0.327	1.14	1.35	1.52	1.11	0.313	0.000
a-terpineol	1.89	1.99	0.775	1.81	2.21	0.372	1.91	1.72	2.83	1.59	0.096	0.416
geraniol	20.1	18.5	0.232	18.6	19.4	0.696	17.1	19.9	20.2	18.9	0.663	0.159
β-damascenone	5.40	4.92	0.352	5.00	4.92	0.861	4.32	5.20	4.72	5.60	0.066	0.656

Mean are presented (n=5).
Different lowercase letters indicate a statistical difference in means within one year ($p < 0.05$, ANOVA, Turkey's HSD test).
a: Ctr, Control group (without thinning treatment)
b: MT, moderate-thinned treatment (35% crop removed); ST, severe-thinned treatment (65% crop removed)
c: Crop thinning at different timing, pre-bloom (FLWR), fruit set (FS), lag phase (LAG), or véraison (VER).
Table 6.2 Effect of severity and timing of crop thinning on Pinot Noir grape volatile composition in 2011.

2011 Year

	Treatment[a]			Severity[b]			Timing[c]					Severity×Timing interaction
	Ctr	Thinned	p	MT	ST	p	FLWR	FS	LAG	VER	p	
Free form												
hexanal	36.4	35.2	0.505	36.6	34.0	0.062	35.6	34.1	36.5	34.9	0.797	0.823
trans-2-hexenal	207	213	0.263	214	211	0.225	216	212	211	212	0.549	0.144
1-hexanol	211	235	0.098	221	248	0.256	229	245	211	253	0.691	0.411
linalool	2.10	2.24	0.501	2.41	2.06	0.123	2.39	2.30	2.16	2.10	0.870	0.136
citronellol	1.72	1.91	0.294	1.93	1.90	0.880	1.98	1.92	1.62	2.14	0.396	0.392
geraniol	0.96	1.12	0.254	1.21	1.04	0.335	0.98	1.13	1.19	1.19	0.836	0.831
β-damascenone	0.51	0.56	0.059	0.56	0.56	0.995	0.52b	0.58a	0.58a	0.55ab	0.020	0.043
Bound form												
1-hexanol	111	147	0.007	146	148	0.861	129	145	161	153	0.299	0.080
cis-linalool oxide	4.55	4.68	0.719	4.73	4.63	0.758	4.30	5.04	4.98	4.41	0.198	0.069
trans-linalool oxide	5.75	5.55	0.581	5.66	5.43	0.427	5.22	5.80	5.78	5.40	0.557	0.143
linalool	5.53	6.20	0.131	6.08	6.33	0.618	5.85	6.45	6.65	5.85	0.578	0.206
a-terpineol	4.04	4.29	0.391	4.43	4.15	0.364	4.48	4.42	4.12	4.14	0.831	0.155
geraniol	1.21	1.46	0.230	1.21	1.70	0.011	1.65	1.36	1.41	1.41	0.870	0.278
β-damascenone	2.26	2.55	0.275	2.41	2.67	0.282	2.69	2.21	2.91	2.41	0.096	0.376

Mean are presented (n=5).
Different lowercase letters indicate a statistical difference in means within one year ($p < 0.05$, ANOVA, Turkey's HSD test).
a: Ctr, Control group (without thinning treatment)
b: MT, moderate-thinned treatment (35% crop removed); ST, severe-thinned treatment (65% crop removed)
c: Crop thinning at different timing, pre-bloom (FLWR), fruit set (FS), lag phase (LAG), or véraison (VER).

Table 6.3 Effect of severity and timing of crop thinning on Pinot Noir grape volatile composition in 2012.

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2012 Year

	Treatment[a]			Severity[b]			Timing[c]					Severity×Timing interaction
	Ctr	Thinned	p	MT	ST	p	FLWR	FS	LAG	VER	p	
Free form												
hexanal	24.0	21.7	0.588	19.6	23.9	0.418	25.8	23.7	23.5	23.9	0.351	0.283
trans-2-hexenal	123	112	0.554	123	125	0.549	124	125	126	121	0.699	0.306
1-hexanol	242	260	0.598	261	259	0.952	266	261	291	222	0.664	0.080
linalool	1.58	1.78	0.666	1.78	1.78	0.998	2.03	1.67	1.66	1.75	0.929	0.741
citronellol	1.21	1.10	0.536	1.25	0.95	0.102	1.24	1.27	1.12	0.87	0.607	0.133
geraniol	1.57	1.93	0.388	2.20	1.67	0.228	2.41	1.99	2.00	1.34	0.426	0.559
β-damascenone	0.46	0.41	0.128	0.42	0.40	0.434	0.43	0.44	0.43	0.35	0.135	0.503
Bound form												
1-hexanol	201	216	0.554	215	217	0.430	221	217	232	193	0.239	0.771
cis-linalool oxide	8.56	8.54	0.975	8.53	8.75	0.710	7.21	8.32	9.50	9.51	0.170	0.592
trans-linalool oxide	11.8	11.7	0.928	11.2	11.9	0.379	11.3	11.2	12.1	11.6	0.856	0.767
linalool	4.50	4.41	0.532	4.50	4.54	0.236	4.52	4.54	4.53	4.48	0.107	0.415
a-terpineol	**25.4**	**22.3**	**0.030**	23.8	20.8	0.140	22.1	23.7	24.3	19.0	0.640	0.175
geraniol	1.24	1.26	0.425	1.22	1.27	0.473	1.24	1.27	1.30	1.16	0.572	0.137
β-damascenone	1.87	1.51	0.262	**1.55**	**1.83**	**0.002**	1.79	1.71	1.59	1.57	0.477	0.000

Mean are presented (n=5).
a: Ctr, Control group (without thinning treatment)
b: MT, moderate-thinned treatment (35% crop removed); ST, severe-thinned treatment (65% crop removed)
c: Crop thinning at different timing, pre-bloom (FLWR), fruit set (FS), lag phase (LAG), or véraison (VER).

Table 6.4 Effect of severity and timing of crop thinning on Pinot Noir wine volatile composition in 2011.

2011 Year

	Treatment			Severity			Timing			Severity×Timing interaction
	Ctr	Thinned	p	Moderate	Severe	p	Early	Later	p	
Higher alcohols										
1-hexanol	3947	4156	0.046	4249	4063	0.059	4224	4088	0.183	0.822
trans-3-hexenol	21	23	0.000	23	23	0.767	23	23	0.953	0.560
cis-3-hexenol	20	16	0.057	16.3	15.3	0.132	15.7	15.9	0.741	0.917
trans-2-hexenol	4.00	3.52	0.097	3.65	3.40	0.257	3.63	3.42	0.351	0.453
1-heptanol	23	34	0.026	33.6	33.8	0.959	32.5	34.9	0.585	0.734
1-octanol	27	26	0.581	25.9	26.3	0.707	26.7	25.6	0.312	0.228
1-nonanol	7.05	7.18	0.686	7.05	7.32	0.405	6.91	7.45	0.081	0.823
1-decanol	1.98	1.90	0606	1.91	1.90	0.948	1.99	1.81	0.225	0.967
benzyl alcohol	2289	2364	0.474	2290	2438	0.133	2379	2348	0.764	0.973
benzeneethanol	30.6	32.5	0.143	32.2	32.7	0.666	32	33	0.368	0.874
propanol *	15.4	18.0	0.001	17.8	18.3	0.436	18.2	17.8	0.528	0.404
isobutyl alcohol *	114	105	0.042	105	106	0.792	106	105	0.810	0.629
isoamyl alcohol *	188	187	0.846	183	190	0.389	188	185	0.712	0.439
Esters										
ethyl butanoate	155	154	0.097	145	164	0.004	153	156	0.680	0.649
ethyl hexanoate	263	245	0.024	238	252	0.038	247	242	0.482	0.757
ethyl heptanoate	0.51	0.62	0.013	0.65	0.60	0.276	0.63	0.62	0.885	0.300
ethyl octanoate	256	230	0.023	225	236	0.228	234	227	0.472	0.651

Table 6.4 Effect of severity and timing of crop thinning on Pinot Noir wine volatile composition in 2011 (continued).

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2012 Year	Treatment			Severity			Timing			Severity×Timing
ethyl decanoate	56	62	0.168	64	60	0.253	63.8	60.1	0.385	0.427
ethyl isobutyrate	**119**	**107**	**0.022**	107	107	0.864	104	110	0.179	0.363
ethyl 2-methyl butyrate	4.76	4.53	0.296	4.46	4.60	0.500	4.37	4.69	0.081	0.378
ethyl 3-methyl butyrate	5.91	5.50	0.182	5.44	5.56	0.661	5.25	5.75	0.051	0.141
ethyl acetate *	19.9	21.0	0.188	**20.2**	**21.9**	**0.024**	20.1	21.2	0.627	0.912
isobutyl acetate	**131**	**119**	**0.026**	115	122	0.127	118	119	0.846	0.621
isoamyl acetate	171	197	0.100	**180**	**214**	**0.006**	190	203	0.712	0.672
hexyl acetate	5.01	4.77	0.665	4.66	4.89	0.655	5.18	4.37	0.102	0.920
ethyl phenylacetate	**1.75**	**2.21**	**0.001**	2.21	2.20	0.947	2.17	2.25	0.480	0.384
phenethyl acetate	28.4	28.4	0.997	28.3	28.6	0.737	28.4	28.4	0.950	0.877
diethyl succinate	**813**	**1206**	**0.000**	1148	1265	0.116	1237	1176	0.432	0.240
Terpenoids										
linalool	8.50	8.38	0.662	8.26	8.50	0.382	8.17	8.58	0.113	0.973
α-terpineol	6.64	6.80	0.704	6.86	6.75	0.797	6.85	6.76	0.830	0.878
citronellol	**7.89**	**10.3**	**0.000**	**9.91**	**10.76**	**0.017**	10.28	10.39	0.783	0.570
geraniol	14.1	16.5	0.078	**14.63**	**18.36**	**0.000**	16.6	16.4	0.836	0.199
C13-norisoprenoids										
β-damascenone	3.06	3.11	0.630	**3.02**	**3.19**	**0.042**	3.07	3.14	0.480	0.631
β-ionone	1.43	1.46	0.096	1.47	1.46	0.826	1.45	1.48	0.183	0.966
Fatty acids										
hexanoic acid	171	169	0.772	166	172	0.308	171	167	0.531	0.879
octanoic acid	2554	2477	0.633	2371	2584	0.155	2514	2441	0.642	0.990
decanoic acid	259	249	0.520	246	252	0.694	253	245	0.616	0.551
dodecanoic acid	44.6	45.7	0.454	45.4	46.0	0.663	46.5	44.8	0.203	0.868

Means are presented (n=3).

Table 6.5 Effect of severity and timing of crop thinning on Pinot Noir wine volatile composition in 2012

	Ctr	Thinned	p	Moderate	Severe	p	Early	Later	p	interaction
Higher alcohols										
1-hexanol	2279	2451	0.099	2372	2530	0.093	2458	2444	0.894	0.204
trans-3-hexenol	55	64	0.003	61.2	66.8	0.021	43.3	44.3	0.840	0.147
cis-3-hexenol	43	44	0.229	44	44	0.927	32.8	36.1	0.454	0.110
trans-2-hexenol	20.9	34.5	0.000	30.3	38.7	0.001	43.1	44.3	0.262	0.274
1-heptanol	89	110	0.025	100	119	0.007	110	109	0.892	0.022
1-octanol	915	998	0.001	977	1019	0.023	1000	995	0.795	0.283
1-nonanol	12.4	12.5	0.982	11.9	13.0	0.045	12.4	12.5	0.932	0.007
1-decanol	1.34	0.84	0.003	0.86	0.81	0.105	0.86	0.81	0.068	0.537
benzyl alcohol	1323	1375	0.409	1333	1417	0.159	1419	1332	0.142	0.003
benzeneethanol	19.1	17.9	0.014	18.2	17.6	0.118	17.8	18.1	0.537	0.859
propanol *	17.3	20.4	0.002	19.7	21.2	0.086	20.7	20.2	0.515	0.028
isobutyl alcohol *	53	44	0.000	46.7	40.8	0.000	43.1	44.3	0.449	0.095
isoamyl alcohol *	166	142	0.002	149	136	0.026	140	145	0.613	0.618
Esters										
ethyl butanoate	130	124	0.402	125	122	0.666	125	123	0.727	0.670
ethyl hexanoate	230	206	0.050	219	194	0.014	210	202	0.503	0.465
ethyl heptanoate	1.87	1.84	0.827	1.85	1.83	0.861	1.97	1.70	0.042	0.128
ethyl octanoate	127	124	0.690	130	117	0.043	121	126	0.457	0.978
ethyl decanoate	42.5	43.9	0.055	44.3	43.5	0.672	43.3	45.1	0.051	0.514
ethyl isobutyrate	54.3	42.2	0.003	47.2	37.1	0.000	43.2	41.2	0.583	0.636
ethyl 2-methyl butyrate	8.44	6.92	0.006	6.79	7.05	0.521	7.49	6.34	0.000	0.985

Table 6.5 Effect of severity and timing of crop thinning on Pinot Noir wine volatile composition in 2012 (continued).

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ethyl 3-methyl butyrate	**7.39**	**5.51**	*0.000*	**5.96**	**5.05**	*0.008*	5.66	5.35	*0.412*	*0.049*
ethyl acetate *	19.9	21.1	*0.102*	**20.4**	**21.9**	*0.019*	21	21.3	*0.701*	*0.326*
isobutyl acetate	44.9	40.5	*0.156*	41.6	39.5	*0.407*	40.3	40.7	*0.893*	*0.587*
isoamyl acetate	**122**	**98**	*0.000*	100	96	*0.373*	98	98	*0.988*	*0.420*
hexyl acetate	**1.97**	**1.58**	*0.003*	1.54	1.63	*0.384*	1.66	1.51	*0.146*	*0.797*
ethyl phenylacetate	**1.56**	**1.10**	*0.000*	**1.19**	**1.02**	*0.001*	1.13	1.08	*0.384*	*0.710*
phenethyl acetate	**12.5**	**9.12**	*0.000*	9.37	8.88	*0.051*	9.21	9.03	*0.510*	*0.492*
diethyl succinate	1971	1929	*0.487*	1921	1937	*0.787*	1918	1940	*0.704*	*0.421*
Terpenoids										
linalool	**7.53**	**7.06**	*0.038*	7.15	6.97	*0.374*	7.12	7.01	*0.575*	*0.291*
α-terpineol	3.18	3.02	*0.134*	3.00	3.03	*0.726*	**3.12**	**2.91**	*0.022*	*0.151*
citronellol	7.33	6.81	*0.123*	6.94	6.68	*0.380*	6.98	6.64	*0.239*	*0.341*
geraniol	15.9	16.6	*0.483*	16.8	16.4	*0.646*	16.0	17.1	*0.209*	*0.862*
C13-norisoprenoids										
β-damascenone	2.28	2.31	*0.800*	2.37	2.25	*0.251*	**2.21**	**2.41**	*0.039*	*0.679*
β-ionone	**0.33**	**0.27**	*0.015*	**0.29**	**0.26**	*0.061*	0.28	0.26	*0.130*	*0.160*
Fatty acids										
hexanoic acid	860	822	*0.122*	837	806	*0.096*	824	819	*0.820*	*0.385*
octanoic acid	**1065**	**930**	*0.014*	956	904	*0.087*	920	940	*0.543*	*0.188*
decanoic acid	**189**	**142**	*0.000*	**150**	**133**	*0.004*	142	141	*0.856*	*0.012*
dodecanoic acid	65.2	64.5	*0.291*	65.5	63.5	*0.061*	65.2	63.9	*0.138*	*0.108*

Means are presented (n=3)