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Posted Date: 20 June 2024

doi: 10.20944/preprints202406.1438.v1

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Article

The Impacts of Frozen Material-Other-Than-Grapes (MOG) on Aroma Compounds of Cabernet Franc and Cabernet Sauvignon

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Abstract: An undesirable sensory attribute (“floral taint”) has been recently detected in red wines in some winegrowing jurisdictions in North America (e.g. Ontario, British Columbia, Washington), caused by introduction of frost-killed leaves and petioles [materials-other-than-grapes (MOG)] during mechanical harvest and winemaking. It was hypothesized that terpenes, norisoprenoids, and higher alcohols would be the main responsible compounds. Objectives were to investigate causative volatile compounds for floral taint, and explore threshold concentrations for this problem. Commercial wines displaying varying intensities of floral taints were subjected to GC-MS and sensory analysis. Several odor-active compounds were higher in floral-tainted wines, including terpenes (geraniol, citronellol, *cis*- and *trans*-rose oxide), norisoprenoids (β -damascenone, β -ionone), five ethyl esters, and three alcohols. Thereafter, fermentations of Cabernet franc (CF) and Cabernet Sauvignon (CS) (2016, 2017) were conducted. MOG treatments were (wt/wt): 0, 0.5%, 1%, 2% and 5% petioles, and 0, 0.25%, 0.5%, 1%, and 2% leaf blades. Terpenes (linalool, geraniol, nerol, nerolidol, citronellol, citral, *cis*- and *trans*-rose oxides, eugenol, myrcene), norisoprenoids (α - and β -ionone), and others (e.g. hexanol, octanol, methyl and ethyl salicylate) increased linearly/quadratically with increasing MOG levels in both cultivars. Principal components analysis separated MOG treatments from the controls with 5% petioles and 2% leaves as extremes. Increasing MOG levels in CF wines increased floral aroma intensity, and were primarily associated with terpenes, higher alcohols, and salicylates. Increased leaf levels in CF were associated with higher vegetal and earthy attributes. Increased petioles in CS were not correlated with floral aromas, but increased leaves increased floral, vegetal and herbaceous attributes. Overall, petioles contributed more to floral taint than leaves through increased terpenes and salicylates (floral notes), while leaves contributed norisoprenoids and C₆ alcohols (green notes).

Keywords: monoterpenes; norisoprenoids; esters; higher alcohols; sensory analysis; mechanical harvesting

1. Introduction

Mechanical grapevine harvest has been standard practice worldwide since the 1960's [1], but can inadvertently result in the occurrence of materials other than grapes (MOG) in harvest loads. Mechanized harvest is normally considered equal to hand harvesting in terms of ultimate wine quality [2-6]. The first mechanical harvesters were unable to expel all the MOG produced during harvest, but more recent harvesting technology has largely eliminated the problem except under

anomalous circumstances such as with frost-damaged canopies [7,8]. New mechanical harvesting technologies with optical sorting capabilities can eject leaves, petioles and unsound or unripe fruit [8]. The presence of MOG, such as stems and petioles, can be an important deterrent for wine composition and quality, particularly for red wines [9-11].

Of particular concern are “floral taints” in red wines, which can develop following mechanical harvest that has occurred following killing frosts [12-15]. Reports from Ontario suggested that the compounds responsible were primarily monoterpenes and norisoprenoids [12-14], whereas recent research in Washington State suggested involvement of 6-methyl-5-hepten-2-ol, p-menth-1-en-9-al, and 6-methyl-3,5-heptadien-2-one [15]. Some red wine cultivars (e.g. Cabernet franc, Cabernet Sauvignon) are specifically prone to floral sensory taints introduced by these materials [12-15]. Climate change has permitted harvest delays of cultivars such as Cabernet Sauvignon into late November, when foliage has usually been killed by frost. There is a concern that undesirable aroma compounds are being introduced by the presence of frozen leaf blades and petioles, and that post-frost machine-harvesting is a significant contributor to this problem. To address these concerns of sensory taints and enable grape growers to adapt to changing environmental conditions, the wine industry has expressed a need to understand effects contributed by frozen MOG.

Grape leaves and petioles have a high potential for being integrated into the fermentation following mechanical harvesting, therefore they pose a substantial risk to the final wine composition [9,16-18]. In particular, MOG may impact concentrations of aroma compounds in musts and wines. The concentration and composition of volatile compounds vary depending on the organ of the grapevine, especially between the vegetative tissues (stems, rachis, peduncles, etc.), and flowers/green berries [13,19]. Volatile compounds associated with vegetative tissues of grapevines, especially the rachis, peduncles, and stems, are mainly monoterpenes such as geraniol, linalool, α -terpineol, nerol, and α -citronellol [19]. Remaining organs including leaf blades (laminae) contain several volatile compounds that may be extracted into wine, particularly C_6 compounds such as aldehydes and alcohols (e.g. hexanal, 2-hexenal, 2-hexen-1-ol, and *n*-hexanol), and other aliphatic compounds (e.g. 2,4-heptadienal, 1-octen-3-ol) that are associated with green odors [19-21], as well as terpenes and norisoprenoids (e.g. linalool, citronellol, geraniol, ionone) [16,22,23]. The C_6 compounds responsible for the ‘grassy’ characteristics associated with grape leaves originate from fatty acids in leaf cellular structures via the lipxygenase pathway [16]. Petioles contain high concentrations of free terpenes, especially citronellol and geraniol [23]. The petiole may act as a storage vehicle for free terpenols prior to transportation to other parts of the vine, or for utilization in metabolic pathways such as the geranyl phosphate pathway, hence the high concentrations of terpenols in petioles vs. laminae [23]. Other volatile compounds in leaves and petioles include benzenic compounds (e.g. methyl salicylate, benzyl alcohol, benzaldehyde, and 2-phenylethanol), norisoprenoids, and eugenol [19,23].

Pre-fermentation juice contact with MOG during fermentation results in extraction of numerous volatile grapevine compounds into the wines, and the presence of increasing MOG during fermentation leads to in wine with higher concentrations of several monoterpenes and other aroma compounds [11,17,18,24]. In situations of high MOG concentrations, geraniol, linalool, and β -citronellol are found at concentrations above their detection thresholds, suggesting a potential sensory impact [17]. Higher alcohols and esters increase if stems are included [11,24]. Other compounds such as benzyl alcohol, eugenol, 1-hexanol, methyl salicylate, and ethyl salicylate also increase with MOG incorporation [11,17]. However, contrary to other aroma compounds and other studies [25], methoxypyrazines can be reduced with high petiole concentrations, possibly through adsorption by petioles in fermenting wines [17].

Minimal sensory differences were initially found in wines from hand-harvested and mechanically-harvested treatments, with no differences in volatile compounds [9]. There were likewise no increases in the concentration of leaf volatiles, such as *trans*-3-hexenal and *cis*-3-hexenol in machine-harvested must, and no sensory differences between hand-harvested and machine-harvested wines [26]. However, studies comparing hand-harvest or various mechanized harvest methods with/without post-harvest optical berry sorting demonstrated positive chemical and/or

sensory impacts of both mechanical harvest method and post-harvest optical sorting [5,8,14]. Pinot noir grapes had higher concentrations of linalool, β -myrcene, α -terpinene, and β -damascenone, presumably caused by glycosidic hydrolysis initiated by berry maceration during mechanical harvest [8]. Considering that the aforementioned sensory taints observed in Ontario's red wines have been associated exclusively with mechanically-harvested grapes, it was surmised that increased concentrations of undesirable aroma compounds such as monoterpenes were being introduced by frozen MOG. Floral terpene-based aromas are typical and desirable in white wines, such as Muscat, Riesling, and Gewürztraminer [27]. However, monoterpenes are atypical compounds in red table wines [28,29]. More than 5% petiole content was reported to significantly alter sensory qualities, particularly increasing terpene-based floral aromas in Cabernet Sauvignon [17].

Initial hypotheses, based on largely anecdotal evidence, were that MOG would be associated with: Increased terpenes and other odorants; bitter taste compounds; malic acid increases; and, decreased anthocyanins and color intensity. It was also hypothesized that there might be breakdown of glycosides in leaves, petioles, and fruit—and subsequent release of terpene and norisoprenoid aglycones—due to light freezing of grapes that could occur with late harvesting. To investigate the impact of MOG on wine composition and quality, two main goals were addressed: 1) Identify and quantify key odor-active compounds in several commercial Ontario red wines (Cabernet franc, Cabernet Sauvignon, blends) produced from MOG and non-MOG-affected grapes. These initial sensorial and chemical analyses were ultimately used by participating wineries to identify the source material in terms of variety and vineyard location; 2) Use controlled fermentations to investigate threshold frozen MOG levels that result in undesirable sensory characteristics of wines. It is also possible, MOG notwithstanding, that late-season harvests allow odor-active compounds to develop to undesirable concentrations in mature fruit in varieties such as Cabernet Sauvignon and Cabernet franc [29], or, that they are introduced by MOG through post-frost machine-harvesting.

Reports of the impact of frozen MOG have been previously published [12-14]. This current work was in fact the initial component of the overall investigation, and extends the aforementioned studies to include two different vintages (2016, 2017), two varieties (Cabernet franc, Cabernet Sauvignon) and sensory evaluation of wines, complete with a more comprehensive data set involving five levels of leaves and petioles and a single yeast strain. Related work addressed mitigating effects of yeast strains on Cabernet franc, and the impacts of harvest technologies [13,14].

2. Materials and Methods

2.1. Analysis of Commercial Wines

Identification of undesirable odor-active compounds in wines from post-frost harvested grapes. To initially identify and ascertain concentrations of key odor-active compounds associated with floral taint, MOG-affected (machine harvested) and comparable non-affected (hand harvested) replicate samples of commercial Cabernet franc and/or Cabernet Sauvignon wines from the 2015 and 2016 vintages were acquired from two different wineries (Andrew Peller and Arterra). These wines were initially qualitatively assessed in tank by winemakers to rate the intensity of floral taint, to relate this to the harvesting method and amount of MOG present at harvest. Replicate samples of all affected wines and comparable samples of hand-harvested wines from the same vineyard blocks were obtained. In total, three replicates each of 12 samples were collected.

2.2. Gas Chromatography-Mass Spectrometry

Wines were analyzed using GC-MS with Gerstel thermal desorption technology according to previous methods [29,30]. A 30 mL sample was taken from each wine treatment replicate immediately prior to bottling and was kept at 4 °C in presence of N₂ inert gas until analysis. The prepared sample was transferred into a 10 mL Gerstel extraction vial. A 10 mm stir bar ("Twister"; Gerstel, Baltimore, MD) coated with polydimethylsiloxane (0.5 mm film thickness) was added to the sample and stirred for 1 hr at 1000 g for extraction at room temperature. Other details are described in Lan, Wang, Aubie, Crombleholme, and Reynolds [13,14].

2.3. GC-MS Conditions; Conditioning of Material

An Agilent 6890N/5975B GC-MS equipped with a Gerstel TDU cooled injection system, and programmable temperature vaporization was used. Columns were: Agilent 19091S-433 HP-5MS 5% phenyl methylsiloxane, nominal length 30.0 m, nominal diameter 250.00 μm , nominal film thickness 0.25 μm ; J&W 122-7032 DB-WAX nominal length 30.0 m, nominal diameter 250.00 μm , nominal film thickness 0.25 μm . Instrument conditions were identical to Moreno Luna, di Profio and Reynolds [29] and Bowen and Reynolds [30]. MS information: Solvent delay: 3 min, SCAN acquisition method for identification compounds, low mass: m/z 30, high mass: m/z 400, threshold: 150, and SIM/SCAN mode for quantification of aroma compounds. Other relevant details are described in Lan et al. [13,14].

2.4. Calibration Compounds and Odor Activity Values

Scan analysis identified more than 100 volatile compounds in wines from both cultivars. For calibration purposes, 41 compounds were chosen for quantification (Supplemental Table S1). Seven-point calibration curves were created for each compound consistent with literature [29,30]. Acquisition of aroma standards was as described in Lan et al. [13,14]. Calibration samples were analyzed in selective ion monitoring/scan mode using the same conditions as described previously with the use of the same internal standard. Odor activity values (OAVs) were calculated as a ratio between each concentration obtained by calibration vs. their respective threshold. Other details of calibration standard preparation are in Lan et al. [13,14]. Thresholds were obtained from literature [31-37]. These data were used to generate concentrations of aroma compounds for comparative analysis. These compounds and their aroma descriptors are listed in Supplemental Table S1.

2.5. Conventional Analysis

Conventional chemical analysis (e.g. ethanol, acetic acid, TA, pH, total anthocyanins, total phenols) was performed using standard methods. Wine pH was obtained using standard methods [38]. Wine TA was measured with a PC-Titrate autotitrator (Man-Tech Associates, Guelph, ON) to a pH 8.2 end point. Color intensity and hue were determined using a modified method provided by Mazza, Fukumoto, Delaquis, Girard, and Ewert [39], and were calculated from absorbance values measured at 420 and 520 nm on an Ultrospec 2100 Pro UV/VIS spectrophotometer (Biochrom Ltd., Cambridge, UK). Total anthocyanins were measured by the pH shift method [40]. Total phenols were determined by the Folin-Ciocalteu micro method [41,42].

2.6. Analysis of Controlled Fermentations

Investigation of threshold frozen MOG levels resulting in undesirable wine sensory characteristics. The purpose of these trials was to replicate commercial wine production with variable amounts and types of frozen MOG added, to ascertain odor-active compounds and their respective threshold concentrations [36]. All grapes were harvested in the 2016 and 2017 seasons from the Andrew Peller Carlton St. Cabernet franc and Cabernet Sauvignon vineyards, located in the Niagara Peninsula VQA sub-appellation of Four Mile Creek in Niagara-on-the-Lake, ON.

Following a hard frost, ≈ 1500 kg of fruit were obtained from each cultivar. Cabernet franc grapes were hand-harvested on 7 November 2016 and 14 November 2017. Cabernet Sauvignon grapes were harvested on 21 November 2016 and 17 November 2017. Due to the mild autumn weather in 2016, no frosted leaves and petioles were available when the Cabernet franc was harvested, therefore these conditions were simulated. One day before harvest, leaves and petioles were harvested and frozen at -25°C overnight. The MOG was then laid out in a thin layer to dry for 24 hr. Leaves were collected post-frost for Cabernet franc (2017) and Cabernet Sauvignon (both years). Weight for both leaves and petioles was determined after the freezing and drying process. Leaves and petioles of Cabernet franc (2017) and Cabernet Sauvignon (both years) were harvested on the same day as harvest and the weights reflected the natural drying due to frost in the vineyard.

Following destemming, must was treated with 50 mg/L potassium metabisulfite and stored at 2 °C for one day. Replicated treatments were thereafter imposed including five levels of MOG based upon leaves or petioles only (Supplemental Figure S1). Leaf addition treatments were: 0, 0.25, 0.5, 1, and 2% wt/wt, and petiole additions were 0, 0.5, 1, 2, and 5% wt/wt. Fermentations were performed in triplicates of 40 kg in 46-L plastic fermenters for both cultivars. After addition of MOG, the fermentation vessels were placed in a 24 °C fermentation chamber and allowed to warm-up overnight. Juice samples were taken immediately prior to inoculation and frozen at -25 °C for future analysis. Fermentations were inoculated with 350 mg/L of yeast strain CSM (Lallemand, Montreal, QC) one day after harvest. Additions of 3 g/L of tartaric acid, and 200 mg/L diammonium phosphate (DAP) were made 24 hr after inoculation. Fermentations were hand-plunged twice daily and fermentation kinetics (sugar concentration and temperature) were monitored daily. Seven days after destemming, the must was pressed and the wine was inoculated with malolactic bacteria strain LACTOENOS® SB3 Direct (Laffort, Petaluma, CA).

2.7. Sensory Analysis

2.7.1. Commercial Samples

Sensory analysis of all commercial wine replicates was conducted to assess MOG impacts. Panelists were selected among professional winemakers that were familiar with floral taint. Tasters were initially trained using sensory standards consisting of commercial wines with floral taints [43] for six 1-hour sessions prior to data collection to ensure that all were able to properly identify and detect floral taint. All sensory analyses were conducted under controlled conditions that included individual booths and red lighting. Compusense software (Compusense, Guelph, ON) was utilized for data acquisition.

2.7.2. Controlled Fermentations

Prior to sensory analysis, a bench trial was conducted on representative treatment samples for the 2016 Cabernet franc and Cabernet Sauvignon wines. Descriptive analysis was conducted on the Cabernet franc and Cabernet Sauvignon wine samples from February 2019 until April 2019. Panelists were recruited from students (undergraduate students, graduate students) of the Oenology and Viticulture program at Brock University, as well as staff members from the Cool Climate Oenology and Viticulture Institute. Panelists had varying degrees of experience in descriptive analysis panels. The panel consisted of nine judges, involving six females and three males. All panelists underwent 6 weeks of training consisting of 6 hr total, across six sessions. In the first session, panelists generated a comprehensive list of descriptive attributes found within the wine samples. In the following session, panelists underwent a group discussion to generate a representative list of attributes that best described the wines. During sessions 3 and 4, the panelists generated and adjusted aroma standards and terminology. The attributes selected for the analysis and their corresponding aroma standard recipes are in Supplemental Table S2. In the final 2 weeks of training, panelists were introduced to line scaling and the Compusense cloud software (Compusense Inc., Guelph, ON). The final sensory analysis took place in the sensory lab at Brock University, using Compusense cloud. Panelists evaluated the wines in individual booths, under red light, using clear ISO glasses. The wines were presented to the judges with a three-digit blind code and in a randomized order, using a Williams design (Latin square). The panelists underwent five sensory evaluation sessions in total. The Cabernet franc samples were tested first, followed by Cabernet Sauvignon. A maximum of 12 wines were presented per session, with a maximum of four wines per flight. There was a mandatory 1.5 minute break after each sample with a 5 min break following each flight. Filtered water, unsalted crackers, and spittoons were provided. The aroma standards were available at each session as references. All attributes were scored on a 15 cm intensity scale with anchor terms 0.5 cm from each end. The anchor terms consisted of 'low' or 'absent' on the 0 end, and 'high' on the 15 ends.

2.8. Statistical Analysis

All data were analyzed using XLSTAT (Addinsoft, Paris, France). Statistical analysis of the sensory data was performed using XLSTAT – sensory 2019.2.2 (Addinsoft, New York, NY). Effects of MOG levels were analyzed using regression to ascertain impacts of increasing MOG levels on both aroma compounds and sensory attributes. Data were also subjected to principal components analysis (PCA) and partial least squares analysis (PLS).

3. Results

Commercial red wines from the 2015 vintage that were rated as medium to high in floral taint were associated with several aroma compounds through PCA [12] (Supplemental Figure S2). Several aroma compounds were substantially higher in concentration in the medium/high-rated wines (Table 1 and Supplemental Table S3). These included several terpenes (citronellol, geraniol, *cis*-rose oxide, *trans*-rose oxide, γ -terpinene, limonene), norisoprenoids (β -damascenone, α -ionone, β -ionone), alcohols (heptanol, octanol, phenylethyl alcohol) and esters (ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, phenylethyl acetate). Several were detected at concentrations considered odor-active.

Building on these findings, this study investigated the linear and quadratic relationships between leaf and petiole additions and the concentrations of specific aroma compounds in Cabernet franc and Cabernet Sauvignon wines from the 2016 and 2017 vintages. Additionally, the subsequent multivariate and sensory analyses were examined to understand the broader implications of these viticultural treatments.

Table 1. Summary of means of several aroma compounds (ug/L) of several commercial red wines from Ontario with various levels of MOG-induced floral taint. Adapted, with permission, from Wang [12].

Sample	α -Ionone	β -Ionone	β -Damascenone	Citronellol	Geraniol	<i>cis</i> -Rose oxide	<i>trans</i> -Rose oxide	γ -Terpinene	Limonene
Threshold	2.6	0.09	0.05	100	30	0.2	450	3260	15
Mean low ^a	2.95	0.175	2.47	1.08	5.14	0.027	0.007	0.100	0.185
Range low ^a	1.929-4.859	0.152-0.224	2.06-2.83	0.849-1.413	3.893-7.347	0.016-0.036	0.000-0.014	0.044-0.206	0.145-0.222
Mean med-high ^b	1.40	0.257	3.43	1.20	6.16	0.082	0.031	0.097	0.160
Range med-high ^b	0.662-1.869	0.232-0.291	2.18-5.17	0.922-2.088	4.708-7.716	0.049-0.217	0.007-0.068	0.065-0.138	0.115-0.215
Significance ^c	NS	**	**	*	*	*	**	NS	NS
OAV ^d	0.538	2.86	68.6	0.012	0.205	0.410	0.155	0.00003	0.011
Sample	Ethyl hexanoate	Ethyl heptanoate	Ethyl octanoate	Ethyl nonanoate	Ethyl decanoate	Phenylethyl acetate	Heptanol	Octanol	Phenylethyl alcohol
Threshold	5	2.2	5	---	200	250	3	110	10000
Mean low ^a	176.8	1.17	202.8	0.52	47.1	39.2	33.2	13.56	42310
Range low ^a	130.8-204.0	1.07-1.32	112.9-284.8	0.394-0.770	21.5-69.9	25.58-53.69	29.7-35.7	12.14-15.85	34148-47142
Mean med-high ^b	229.9	1.61	332.5	0.93	100.5	41.6	39.7	15.75	45902
Range med-high ^b	203.6-251.4	1.41-1.79	227.4-399.3	0.661-1.84	60.6-143.0	27.26-55.74	35.5-44.0	13.31-19.09	38734-60569
Significance ^c	**	*	**	*	**	*	*	*	*
OAV ^d	45.98	0.73	66.5	---	0.5	0.167	13.2	0.143	4.59

^a Low sensory floral taint; ^b Medium to high floral taint. ^c *, **, NS: Significant at $p < 0.05$, 0.01, or not significant, respectively. ^d OAV= Odor-activity value for medium-high samples. Boldfaced values indicate those with likely odor-activity.

3.1. Linear and Quadratic Relationships

3.1.1. Cabernet Franc in 2016

Several aroma compounds increased in Cabernet franc, mostly linearly, with increased additions of leaves and petioles in 2016 (Figures 1, 2 and Supplemental Tables S4 and S5). These included terpenes (linalool, geraniol, nerol, citronellol, citral, *cis*-rose oxide, eugenol); norisoprenoids (β -damascenone, α -ionone, β -ionone); higher alcohols and salicylates (hexanol, octanol, methyl salicylate, ethyl salicylate). Specifically, in Cabernet franc, increased leaf addition led to mostly linear increases in terpenes (linalool, geraniol, nerol, nerolidol, citronellol, α -citral, terpinolene, γ -terpinene, *cis*- and *trans*-rose oxide, eugenol, myrcene), norisoprenoids (β -damascenone, α - and β -ionone), hexanol, and methyl and ethyl salicylate (Figure 1 and Supplemental Table S4). Increased petiole levels led to more responses including increases in terpenes (linalool, geraniol, nerol, nerolidol, citronellol, α -citral, terpinolene, γ -terpinene, *cis*- and *trans*-rose oxide, eugenol, myrcene), norisoprenoids (β -damascenone, α - and β -ionone), higher alcohols (hexanol, octanol), and methyl and ethyl salicylate (Figure 2 and Supplemental Table S5). Based exclusively on odor-active values (OAVs) of the highest leaf addition levels, linalool, geraniol, and eugenol were of greatest significance for Cabernet franc compared to Cabernet Sauvignon in 2016 (Figure 1 and Supplemental Table S4). With respect to petiole additions, linalool, geraniol, *cis*-rose oxide, eugenol, and β -damascenone had OAVs > 1 for at least the highest petiole levels in Cabernet franc (Figure 2 and Supplemental Table S5).

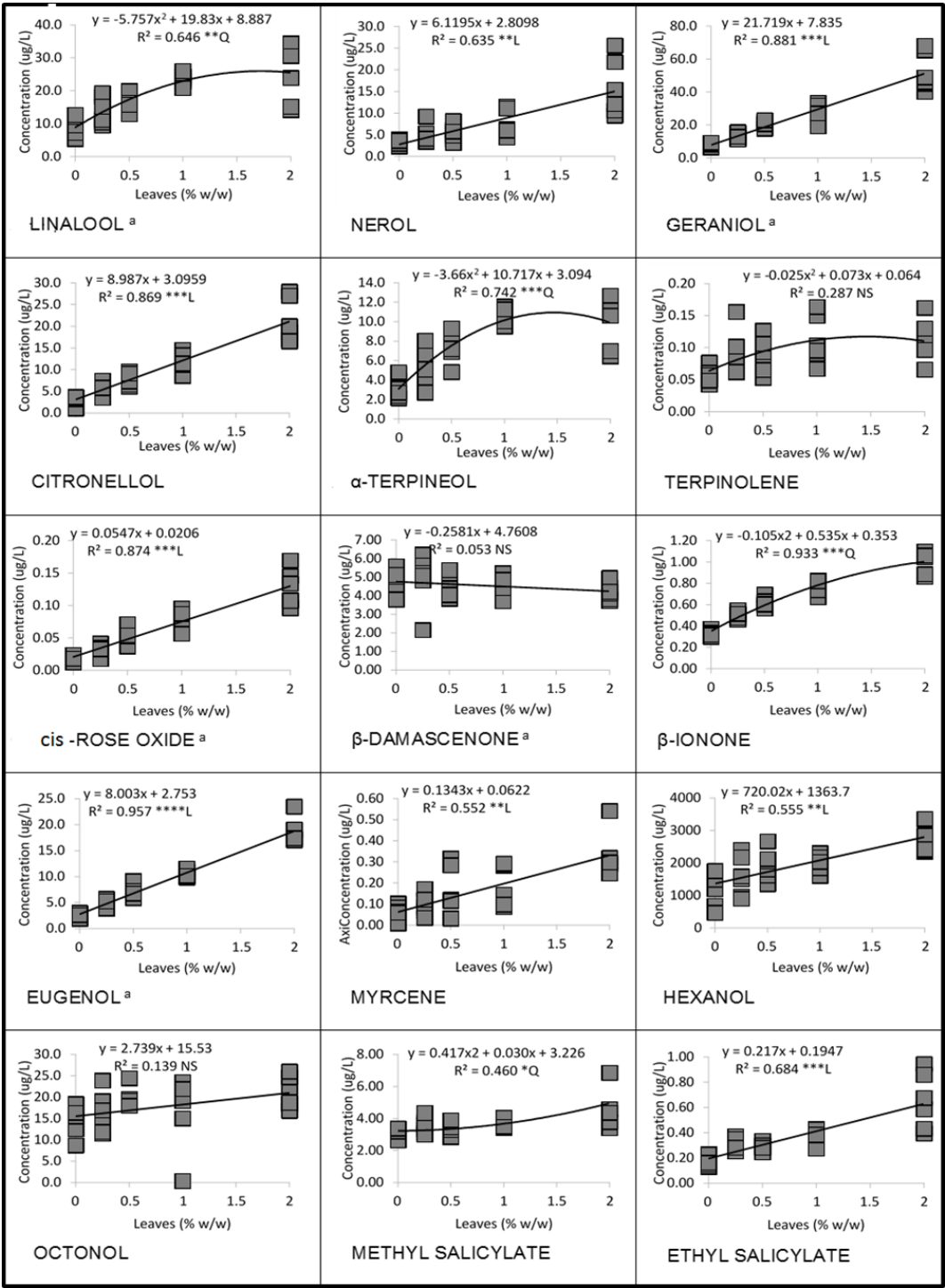


Figure 1. Relationships between several frozen leaf levels (N=30) added to Ontario Cabernet franc wine fermentations vs. aroma compound concentrations, 2016. *, **, ***, ****, NS: Significant at $p \leq 0.05, 0.01, 0.001, 0.0001$, or not significant, respectively. L, Q: Linear or quadratic trends, respectively. ^a Odor-active for at least the highest leaf treatment. Information on other compounds are in Supplemental Table S4.

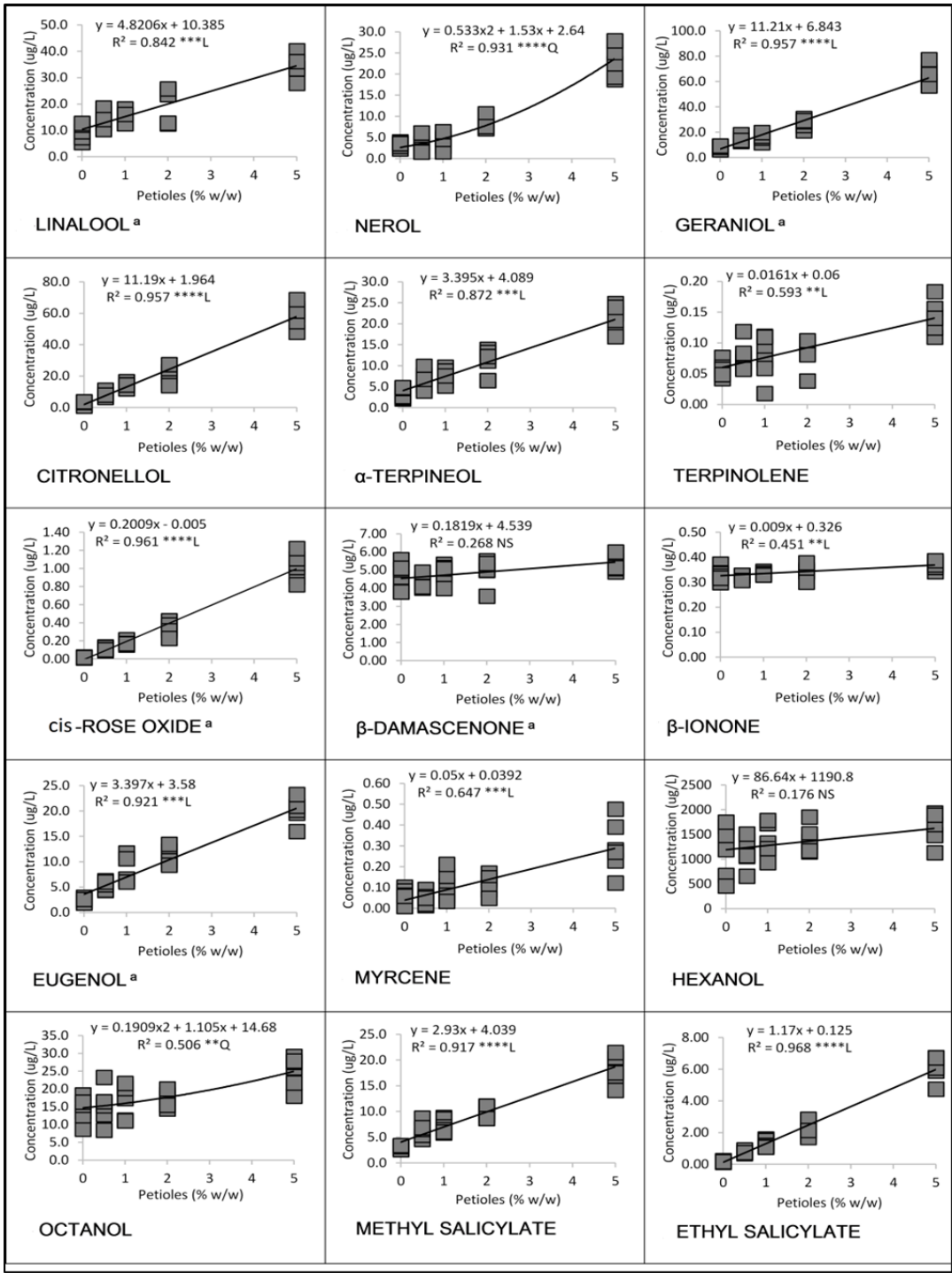


Figure 2. Relationships between several frozen petiole levels (N=30) added to Ontario Cabernet franc wine fermentations vs. aroma compound concentrations, 2016. **, ***, ****, NS: Significant at $p \leq 0.01$, 0.001, 0.0001, or not significant, respectively. L, Q: Linear or quadratic trends, respectively. ^a Odor-active for at least the highest petiole treatment. Information on other compounds are in Supplemental Table S5.

3.1.2. Cabernet Sauvignon in 2016

In Cabernet Sauvignon, increased leaf levels increased concentrations of many of the same terpenes (linalool, *cis*-linalool oxide, geraniol, citronellol, α-terpineol, *cis*-rose oxide, eugenol), higher alcohols (hexanol, octanol), and methyl and ethyl salicylate (Figure 3 and Supplemental Table S6). Increased petiole levels increased concentrations of many of the same terpenes (linalool, *cis*-linalool oxide, geraniol, citronellol, α-terpineol, terpinolene, *cis*-rose oxide, eugenol, myrcene) and methyl

and ethyl salicylate (Figure 4 and Supplemental Table S7). In most circumstances, terpenes were increased more by petiole additions, whereas higher alcohols and norisoprenoids increased more with addition of leaves. Methyl and ethyl salicylate increased to a greater degree by petiole additions than by leaves. Eugenol, *cis*-rose oxide, and β -damascenone were most important for leaf additions in Cabernet Sauvignon with respect to OAVs (Figure 3 and Supplemental Table S6). With respect to petiole additions, linalool, geraniol, *cis*-rose oxide, eugenol, and β -damascenone had OAVs > 1 for at least the highest petiole levels in Cabernet Sauvignon (Figure 4 and Supplemental Table S7).

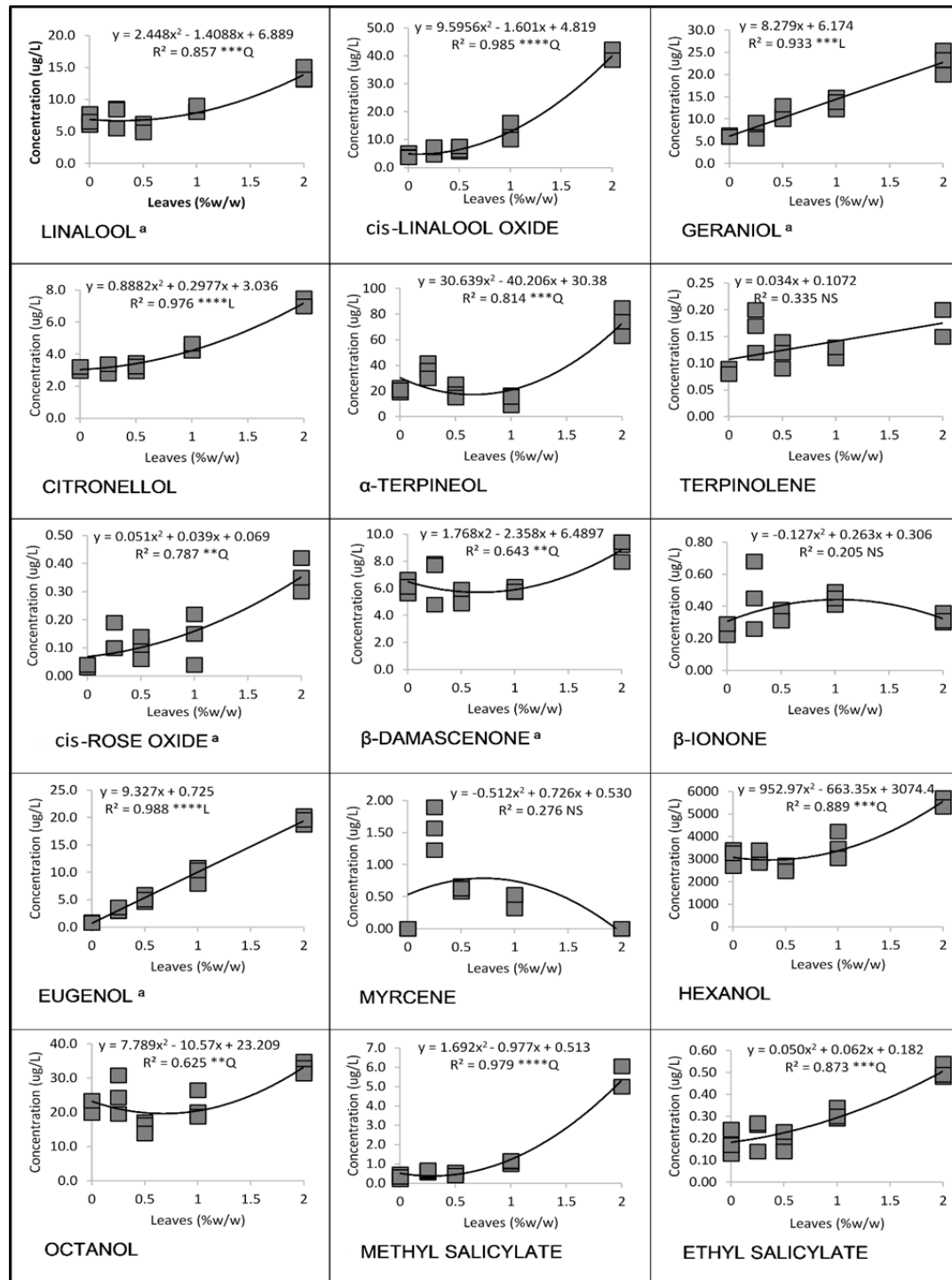


Figure 3. Relationships between several frozen leaf levels (N=15) added to Ontario Cabernet Sauvignon wine fermentations vs. aroma compound concentrations, 2016. **, ***, ****, NS: Significant at $p \leq 0.01, 0.001, 0.0001$, or not significant, respectively. L, Q: Linear or quadratic trends, respectively.

^a Odor-active for at least the highest leaf treatment. Information on other compounds are in Supplemental Table S6.

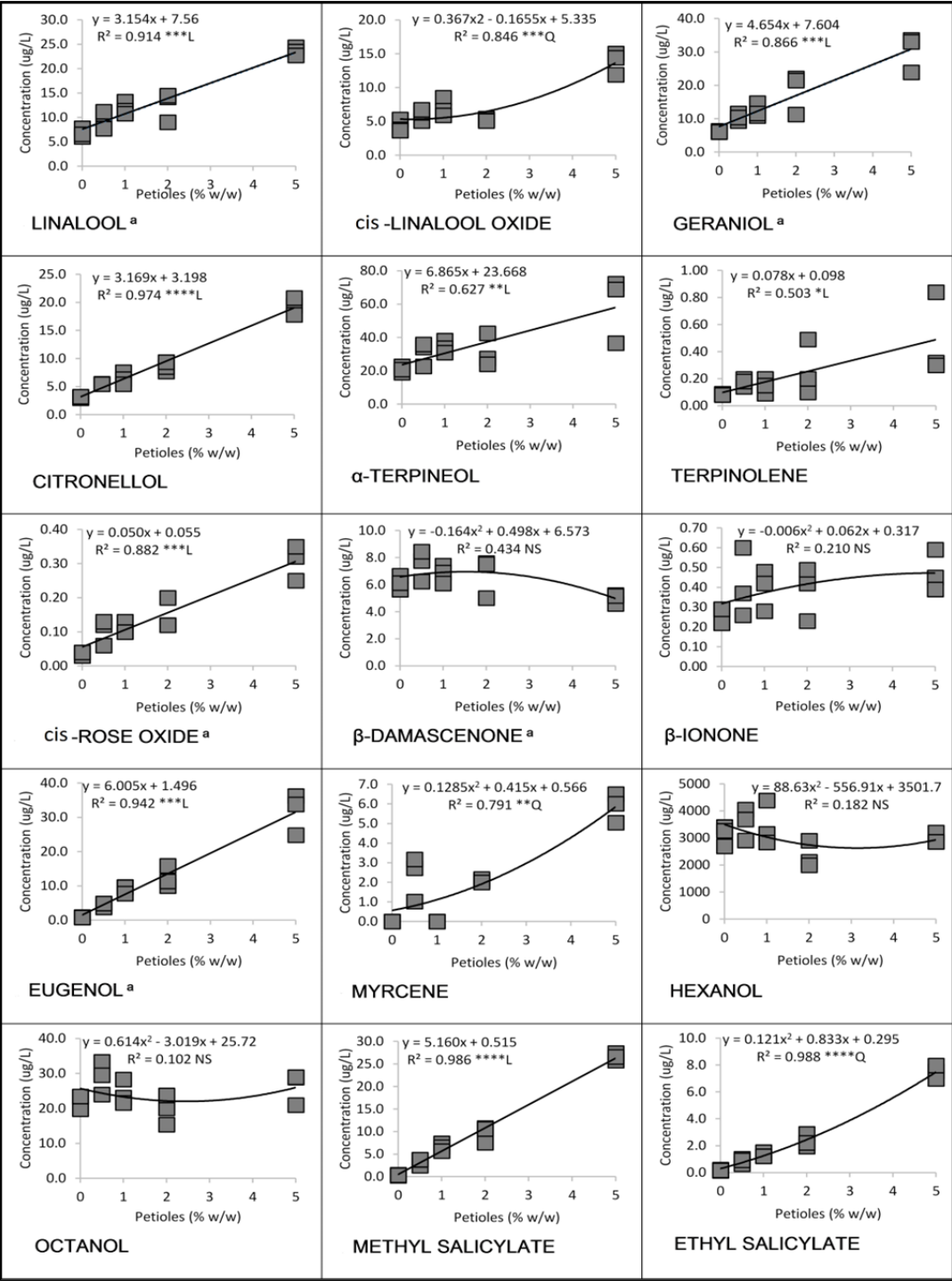


Figure 4. Relationships between several frozen petiole levels (N=15) added to Ontario Cabernet Sauvignon wine fermentations vs. aroma compound concentrations, 2016. *, **, ***, ****, NS: Significant at $p \leq 0.05, 0.01, 0.001, 0.0001$, or not significant, respectively. L, Q: Linear or quadratic trends, respectively. ^a Odor-active for at least the highest petiole treatment. Information on other compounds are in Supplemental Table S7.

3.1.3. Cabernet Franc in 2017

As with 2016, numerous compounds increased linearly with increased leaves or petioles (Supplemental Figures S3, S4 and Supplemental Tables S8, S9). In Cabernet franc, significant linear increases relative to increased leaf addition were observed for several terpenes (linalool, *cis*-linalool oxide, geraniol, citronellol, α -terpineol, *cis*-rose oxide, eugenol) as well as β -ionone (Supplemental Figure S3 and Supplemental Table S8). Additions of petioles produced mostly significant quadratic trends in terpenes (linalool, *cis*-linalool oxide, geraniol, citronellol, α -terpineol, *cis*-rose oxide, eugenol), octanol, and both methyl and ethyl salicylate (Supplemental Figure S4 and Supplemental Table S9). Based on OAVs of the highest leaf levels, linalool, geraniol, *cis*-rose oxide, β -damascenone, eugenol, and hexanol were of greatest significance for Cabernet franc (Supplemental Figure S3 and Supplemental Table S8). With respect to petiole additions, linalool, geraniol, citronellol, *cis*-rose oxide, eugenol, and myrcene had OAVs > 1 for at least the highest petiole levels in Cabernet franc (Supplemental Figure S4 and Supplemental Table S9).

3.1.4. Cabernet Sauvignon in 2017

In Cabernet Sauvignon, linear increases relative to increased leaf addition were observed for several terpenes (*cis*-linalool oxide, geraniol, citronellol, α -terpineol, terpinolene, *cis*-rose oxide, eugenol, myrcene), β -ionone and both methyl and ethyl salicylate (Figure 5 and Supplemental Table S10). Additions of petioles produced mostly quadratic trends in terpenes (linalool, *cis*-linalool oxide, geraniol, citronellol, α -terpineol, terpinolene, *cis*-rose oxide, eugenol, myrcene), β -ionone, and both methyl and ethyl salicylate (Figure 6 and Supplemental Table S11). Based on OAVs, geraniol, *cis*-rose oxide, eugenol, β -damascenone, and hexanol were most important for leaf additions in Cabernet Sauvignon (Figure 5 and Supplemental Table S10). Linalool, geraniol, *cis*-rose oxide, eugenol, β -damascenone, and hexanol had OAVs >1 for petiole treatments in Cabernet Sauvignon (Figure 6 and Supplemental Table S11).

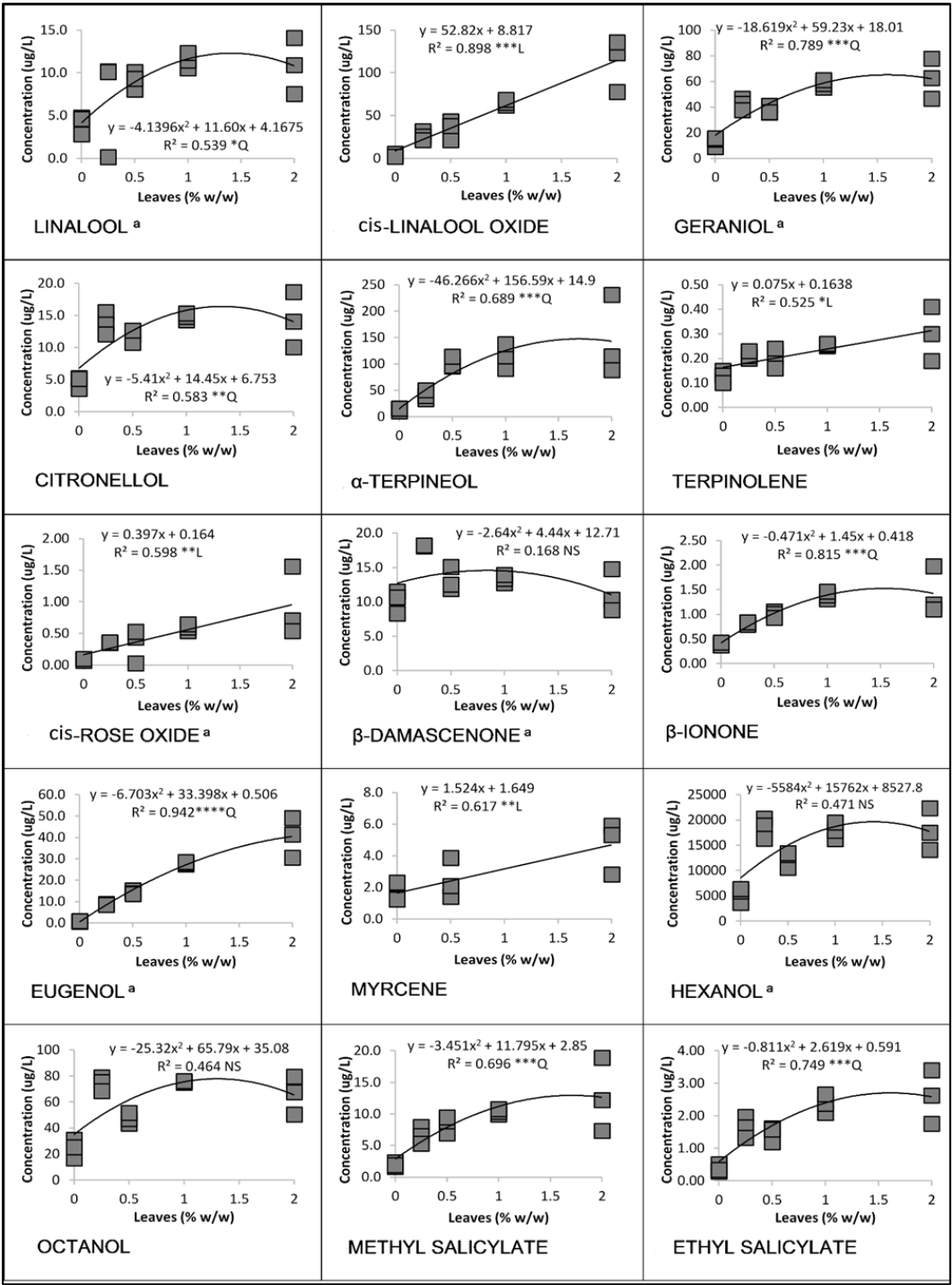


Figure 5. Relationships between several frozen leaf levels (N=15) added to Ontario Cabernet Sauvignon wine fermentations vs. aroma compound concentrations, 2017. *, **, ***, ****, NS: Significant at $p \leq 0.05$, 0.01, 0.001, 0.0001, or not significant, respectively. L, Q: Linear or quadratic trends, respectively. ^a Odor-active for at least the highest leaf treatment. Information on other compounds are in Supplemental Table S10.

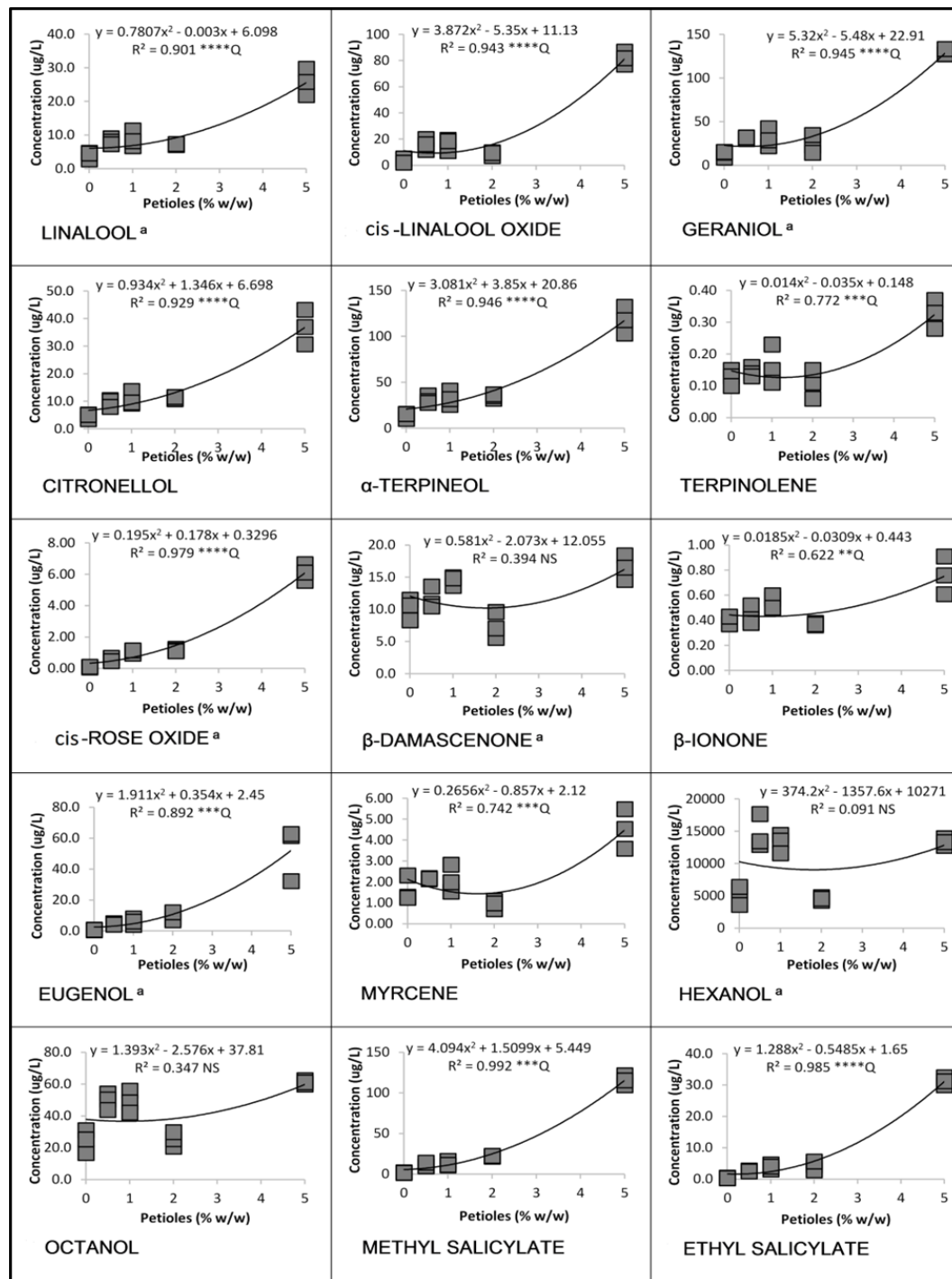


Figure 6. Relationships between several frozen petiole levels (N=15) added to Ontario Cabernet Sauvignon wine fermentations vs. aroma compound concentrations, 2017. **, ***, ****, NS: Significant at $p \leq 0.01, 0.001, 0.0001$, or not significant, respectively. L, Q: Linear or quadratic trends, respectively. ^a Odor-active for at least the highest petiole treatment. Information on other compounds are in Supplemental Table S11.

3.2. Multivariate Statistics

3.2.1.2016. Vintage

PCA likewise showed strong relationships between terpenes, norisoprenoids, higher alcohols, some esters, and methyl and ethyl salicylate and high levels of leaves and petioles (Supplemental Figure S5). In Cabernet franc, the control, 0.25, 0.5, and 1% (w/w) leaves and 0.5 and 1% (w/w) petiole

treatments were located left of PC2 and characterized by ethyl hexanoate, ethyl heptanoate, ethyl octanoate, and α - and β -ionone (Supplemental Figure S5A). The 2% leaf and 2% and 5% petiole treatments were located on or to the right of PC2, and characterized by all remaining compounds, notably all terpenes, β -damascenone, higher alcohols (phenylethyl alcohol, heptanol, octanol), some esters (phenylethyl acetate, isoamyl hexanoate, ethyl nonanoate, ethyl decanoate), diethyl succinate, and methyl and ethyl salicylate. In Cabernet Sauvignon, the control, 0.25, 0.5, and 1% (w/w) leaf and the 1% petiole treatments were located to the left of PC2 and were characterized only by ethyl nonanoate and ethyl decanoate (Supplemental Figure S5B). The 2% leaf and 0.5, 2, and 5% petiole treatments were located to the right of PC2 and were characterized by all remaining compounds.

3.2.2.2017. vintage

In 2017, trends for Cabernet franc were similar (Figure 7). Again, there were strong relationships between terpenes, norisoprenoids, higher alcohols, some esters, and methyl and ethyl salicylate and high levels of leaves and petioles. Most compound eigenvectors were located to the right of PC2, with the exception of isobutanol, ethyl isobutyrate, and isoamyl hexanoate. Those treatments located right of PC2 included 2% leaves and 5% petioles, but also 0.5% leaves and the control, although the latter two treatments were positioned close to PC2. The majority of terpene eigenvectors were located in the lower right quadrant, as was the 5% petiole treatment. In Cabernet Sauvignon, all compound eigenvectors were positioned right of PC2, as were all leaf treatments plus 5% petioles (Figure 8). Once again, the majority of terpene eigenvectors were located in the lower right quadrant, as were the 1% and 2% leaf and 5% petiole treatments.

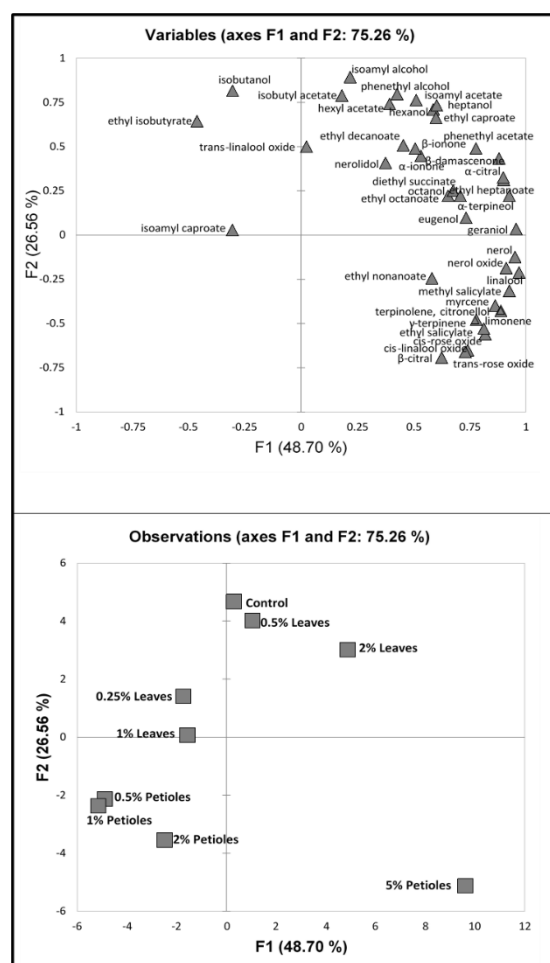


Figure 7. Principal components analysis of aroma compounds of: Cabernet franc wines, Ontario, Canada, 2017. Abbreviations in lower figure: Control: (0 MOG addition); other treatments refer to % w/w addition of frozen leaves or petioles.

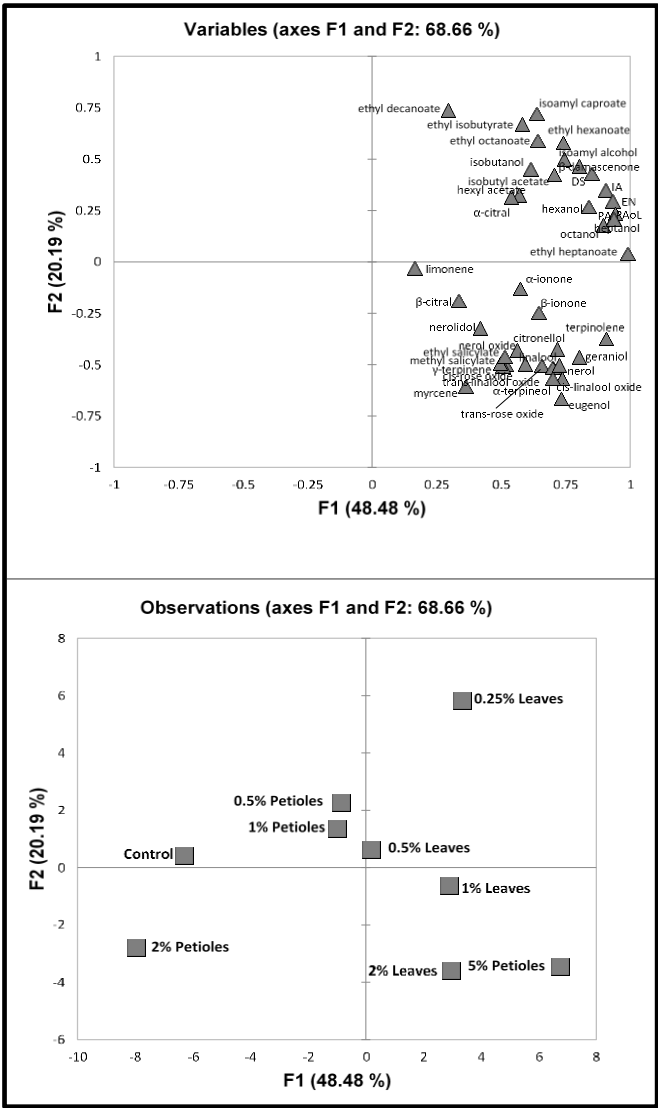


Figure 8. Principal components analysis of aroma compounds of Cabernet Sauvignon wines, Ontario, Canada, 2017. Abbreviations in lower figure: Control (0 MOG addition); other treatments refer to % w/w addition of frozen leaves or petioles. Compound abbreviations in upper figure: DS: diethyl succinate; EN: ethyl nonanoate; IA: isoamyl acetate; PA: phenylethyl acetate; PAol: phenylethyl alcohol.

3.3. Sensory Analysis

3.3.1. Linear and Quadratic Relationships

Linear relationships were observed between the intensity ratings of several sensory attributes and the MOG addition levels (Figures 9 and 10). Both Cabernet franc and Cabernet Sauvignon wines demonstrated either significant linear relationships or strong trends between floral aroma intensity and increasing levels of both leaf and petiole additions (Figures 9 and 10). Cabernet franc wines likewise exhibited increasing levels of vegetal flavor intensity with increasing levels of leaf-based MOG treatments, a trend towards increased tropical fruit aroma relative to increased petioles, and either significant negative linear relationships or strong trends between increased leaf or petiole-based MOG and clarity (Figure 9). In Cabernet Sauvignon, increased intensities of herbaceous and vegetal aromas and herbaceous and dried fruit flavors were associated with increased leaf-based MOG, whereas dark fruit flavor decreased (Figure 10). The color intensity of both varieties was not correlated with the percentage of petioles added, however, there was a negative relationship between leaf-based MOG and the perceived color intensity of the Cabernet Sauvignon wines.

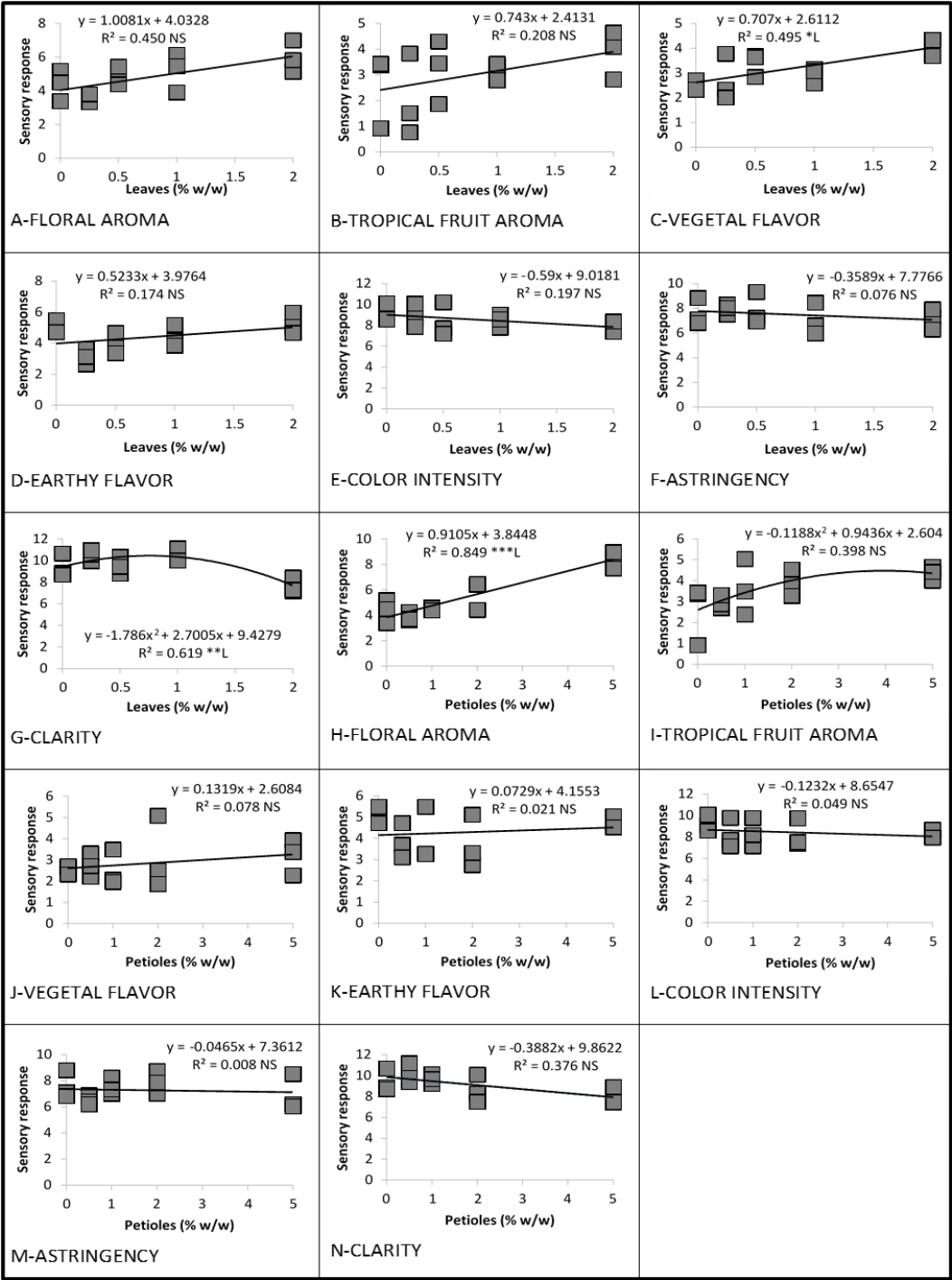


Figure 9. Sensory response of Ontario Cabernet franc wines in relation to frozen leaf (A to G) and petiole (H to N) additions, 2016. *, ***, NS: Significant at $p \leq 0.05$, 0.001, or not significant, respectively. L, Q: Linear or quadratic trends, respectively.

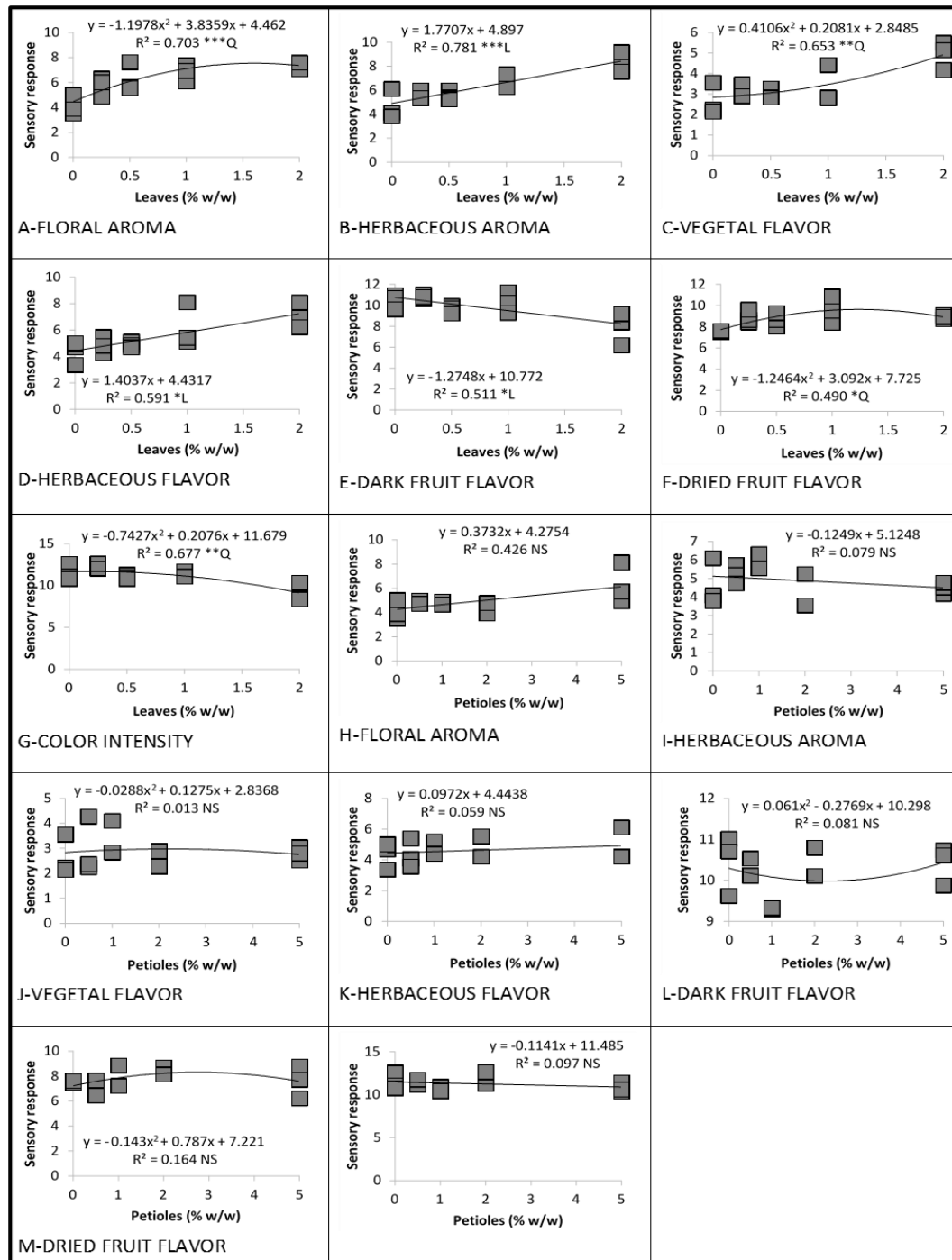
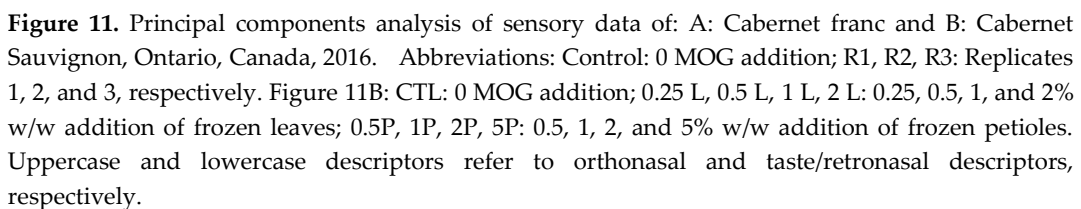


Figure 10. Sensory response of Ontario Cabernet Sauvignon wines in relation to frozen leaf (A to G) and petiole (H to N) additions, 2016. *, **, ***, NS: Significant at $p \leq 0.05$, 0.01, 0.001, or not significant, respectively. L, Q: Linear or quadratic trends, respectively.

3.3.2. Multivariate statistics

PCA analysis. The relationships among significant sensory attributes and the MOG treatments for both cultivars were analyzed using PCA (Figure 11). The attributes of color intensity, clarity, floral and tropical fruit aromas, vegetal flavor, and astringency in the Cabernet franc MOG treatments (Figure 11A) described 58.8% of the variance (F1 34.3%, F2 24.6%). The 2% leaf and 5% petiole treatments were clustered to the right of PC2 and on or below PC1 in the lower right quadrant. Most remaining treatments were clustered to the left of PC2; two of three control replicates plus three others (1% and 2% petioles; 0.5% leaves) were located in the upper right quadrant. Floral aroma and



The PCA performed on the Cabernet Sauvignon wines (Figure 11B) explained 69.5% of the variance (F1 57.8%, F2 11.7%). The 2% leaf treatments (and one 1% leaf replicate) were clustered in the lower right quadrant and associated with floral and herbaceous aromas, and vegetal, herbaceous, and dried fruit flavors, as well as lower ratings for color intensity, clarity and dark fruit flavor. Remaining treatments were positioned left of PC2 and were associated with color intensity, clarity and dark fruit flavor. The 5% petiole treatment replicates were left of PC2 but further from the intersection of PC1 and PC2 than the other treatments.

PLS analysis. PLS was used to examine correlations between the sensory attributes, analytical components, and the MOG treatments (Figures 12 and 13). Both the Cabernet franc and Cabernet Sauvignon MOG treatments were well distinguished on the PLS plot. For both cultivars, the high petiole (5%) and high leaf (2%) wines were clearly separated from the control and lower MOG treatments. For Cabernet franc, floral and tropical fruit aromas were positioned in the upper left quadrant and positively correlated with the aroma compounds of octanol, several terpenes (linalool, geraniol, nerol, citronellol, nerolidol, *cis*- and *trans*-rose oxides, terpinolene, eugenol), as well as methyl and ethyl salicylate (Figure 12). The earthy aroma and vegetal flavor attributes were positioned in the lower left quadrant and correlated with higher levels of γ -terpinene, hexanol, β -ionone, myrcene and anthocyanins. The higher petiole additions (2% and 5%) were associated with floral aroma and the correlating aroma compounds. In contrast, the highest leaf additions (1% and 2%) were positively correlated with high earthy and vegetal flavor intensity ratings, higher ethanol, anthocyanins and increased concentrations of γ -terpinene, hexanol and β -ionone. Remaining treatments were clustered in the center of the plot. Color intensity and clarity were negatively correlated with high leaf additions, and positively correlated with lower MOG treatments.

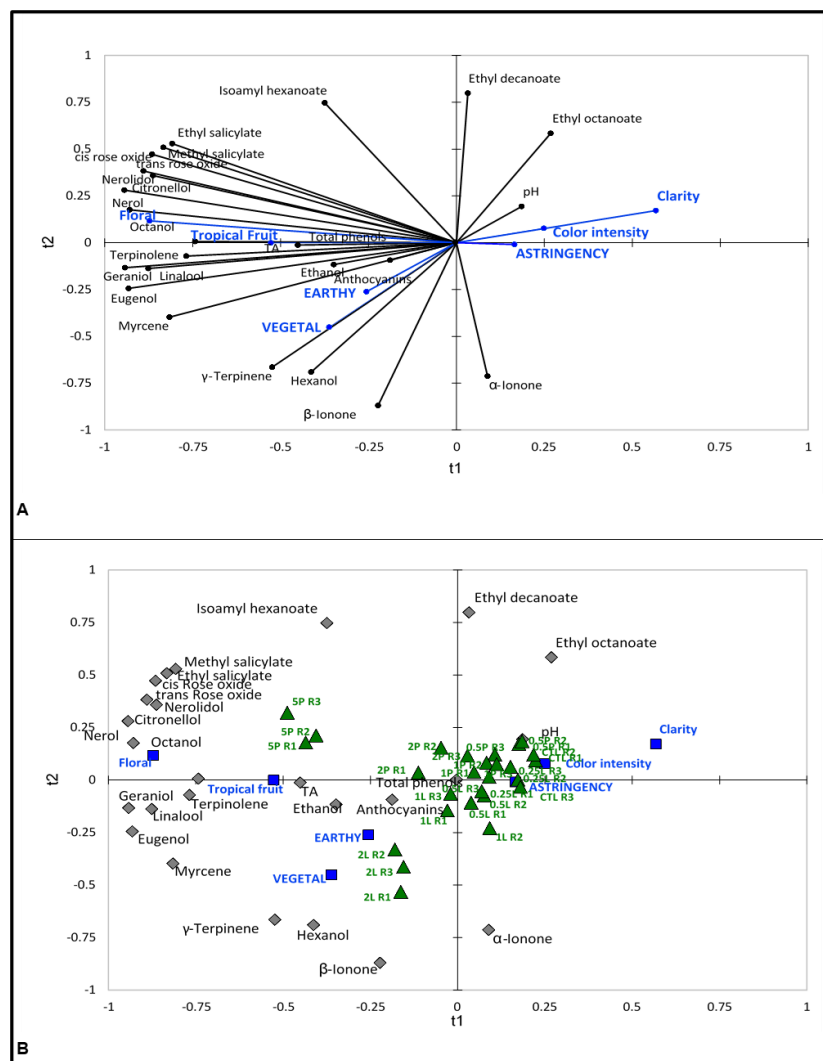


Figure 12. Partial least squares analysis of sensory data of: Cabernet franc, Ontario, Canada, 2016. Variability of X: 45.2%, Y: 23.3%. A: Aroma compounds and sensory descriptors; B: Aroma compounds, sensory descriptors, and treatments. Abbreviations (Figure 12B): CTL: 0 MOG addition; R1, R2, R3: Replicates 1, 2, and 3, respectively; 0.25L, 0.5L, 1L, 2L: 0.25, 0.5, 1, and 2% w/w addition of frozen leaves; 0.5P, 1P, 2P, 5P: 0.5, 1, 2, and 5% w/w addition of frozen petioles. Uppercase and lowercase descriptors refer to orthonasal and taste/retronasal descriptors, respectively.

The PLS for Cabernet Sauvignon (Figure 13), indicated that the sensory attributes of floral and vegetal aromas, and herbaceous, vegetal and dried fruit flavors were positioned in the upper left quadrant to the left of PC2. These were positively correlated with numerous terpenes (linalool, geraniol, nerol, citronellol, α -citral, *cis*-linalool oxide, nerolidol, *cis*- and *trans*-rose oxide, eugenol), norisoprenoids (β - damascenone, α -ionone), higher alcohols (hexanol, heptanol, octanol), ethyl heptanoate, and methyl salicylate. The 2% leaf addition wines were positioned in the upper left quadrant and hence associated with higher levels of floral aroma, vegetal flavor and herbaceous attributes, and the correlating aroma compounds. The 5% petiole addition wines were positioned in the lower left quadrant (one replicate excepted) and hence also positively correlated with many of the aforementioned terpenes, norisoprenoids, and higher alcohols. In contrast, there was a negative correlation between floral aroma, vegetal flavor and herbaceous attributes with several esters (ethyl hexanoate, ethyl octanoate, ethyl nonoate, ethyl decanoate), β -citral, myrcene, ethyl salicylate, anthocyanins, and ethanol, as well as the attributes clarity, color and dark fruit flavor. The remaining low MOG treatments were clustered near the center of the plot and associated with lowest concentrations of the aroma compounds.

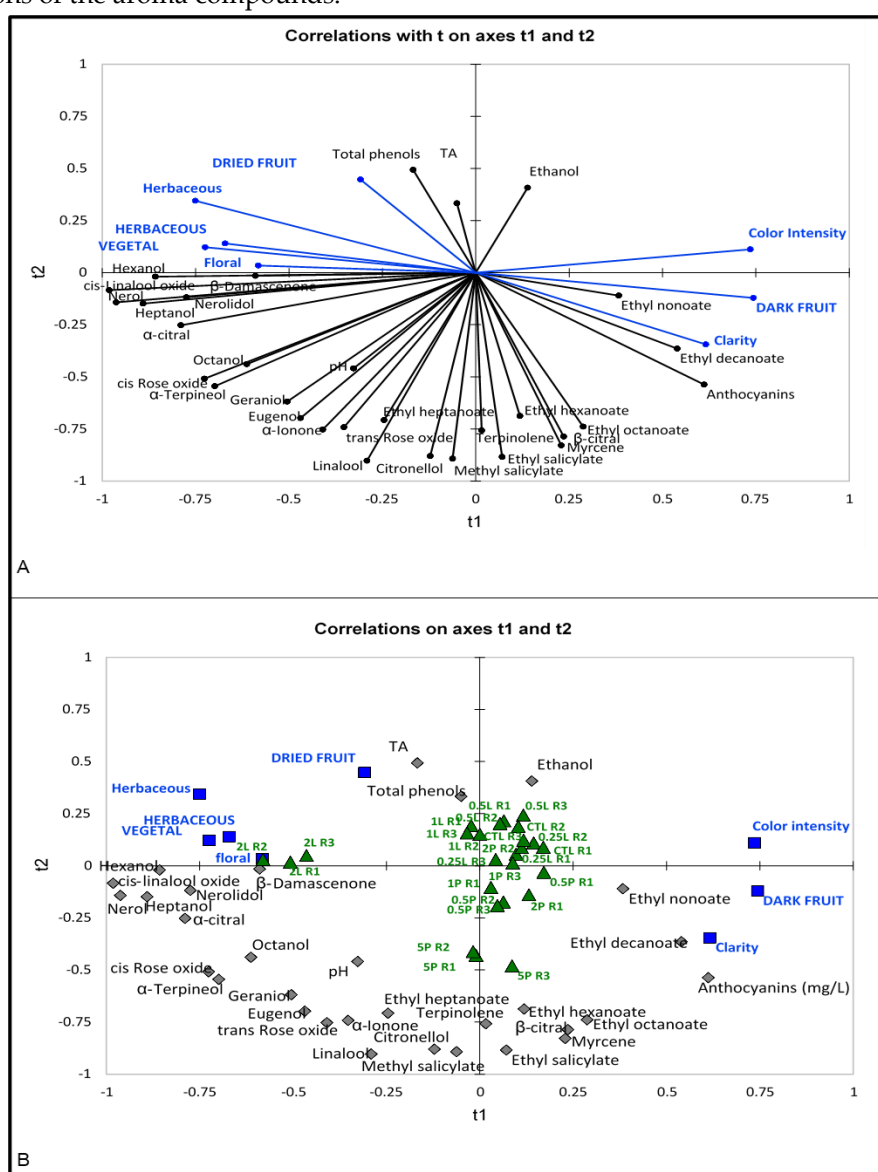


Figure 13. Partial least squares analysis of sensory data of: Cabernet Sauvignon, Ontario, Canada, 2016. Variability of X: 43.1%, Y: 27.4%. A: Aroma compounds and sensory descriptors; B: Aroma compounds, sensory descriptors, and treatments. Abbreviations (Figure 13B): CTL: 0 MOG addition; R1, R2, R3: Replicates 1, 2, and 3, respectively; 0.25L, 0.5L, 1L, 2L: 0.25, 0.5, 1, and 2% w/w addition of frozen leaves; 0.5P, 1P, 2P, 5P: 0.5, 1, 2, and 5% w/w addition of frozen petioles.

4. Discussion

4.1. Chemical Composition

Differences were observed in both the aroma compound composition and basic variables (Supplemental Table S12). The Cabernet franc and Cabernet Sauvignon wines differed in several aroma compounds, including terpenes, esters, norisoprenoids, higher alcohols, and other aliphatic compounds. In general, the addition of petioles had a greater impact on the concentration of monoterpenes. Overall, Cabernet Sauvignon was more responsive in terms of aroma compounds than Cabernet franc, further supporting the potential of varietal differences among frozen MOG-impacted wines. These results are consistent with previous MOG studies, which reported significant impacts on the profile of aroma compounds in MOG-affected wines [11,15,17]. Increases in terpene concentrations were found as a result of petiole additions [17]. Furthermore, an increase in the concentration of terpenes, higher alcohols, and esters in the presence of stems were shown previously [11,13,24].

This study confirms that the addition of frozen MOG results in the extraction of specific aroma compounds, mainly monoterpenes, into the wine [12-14]. Leaves and petioles of grapevines contain a wide range of aroma compounds, such as terpenes, esters, aldehydes, and higher alcohols [19-21,23,26]. Contact of grape leaves with the juice during fermentation can result in the extraction of several aroma compounds from the leaves [16]. The composition of aroma compounds in leaves and petioles can include a large percentage of monoterpenes, including geraniol, linalool, nerol, α -terpineol, and citronellol [19,23]. Frost, Fox, Keller, Collins and Harbertson [15] also identified three “frost taint” marker compounds: 6-methyl-5-hepten-2-ol, *p*-menth-1-en-9-al, and 6-methyl-3,5-heptadien-2-one based on treatments that ranged from 0, 0.5, 2 and 8 g/kg (0, 0.05, 0.2, 0.8%). Terpenes and norisoprenoids were not implicated. It was nonetheless interesting that anomalous compounds were present in wines into which MOG additions were considerably lower than in this trial. The concentration of aroma compounds in wine can also be impacted by yeast strains used during fermentation [13,26,44]. Some yeast strains of *Saccharomyces cerevisiae* can specifically impact the accumulation of monoterpenes such as linalool and citronellol in wine [44]. Furthermore, yeast strains can impact the reduction of *trans*-2-hexenol into *n*-hexanol, thus increasing the level of grassy aromas [26]. Overall, the concentration of aroma compounds extracted from MOG is influenced by several factors including enzymatic activity, maceration time, cultivar, leaf maturity, temperature, pH, yeast strain, and the condition of the MOG content (damage, withering etc.) [26].

The wines also differed in several basic wine variables. Common differences across both varieties include pH, TA, total anthocyanins, and ethanol (Supplemental Table S12). Although the Cabernet Sauvignon wines differed in total phenols, there were no differences in total phenols among the Cabernet franc samples. This is in agreement with other studies [9-11,17,45]. Basic composition of musts and wines can be adversely affected by MOG incorporation, including alcohol, titratable acidity (TA), pH, malic acid, phenols, and color [10,11,17]. Inclusion of MOG in fermentations can result in wines with higher pH and lower TA, but with higher malic acid [11,17]. Ward, Petrie, Johnson, Boss and Bastian [17] found higher pH levels in high-petiole wines; however, there were no differences in TA or ethanol. Wine ethanol concentration has likewise been reduced with high levels of MOG [11,17]. MOG-induced changes in elemental composition (e.g. Fe, K, Mg, and Na) can affect both overall wine quality and individual sensory attributes; e.g., increased ion concentrations can modify yeast metabolism during fermentation, thus altering the composition of volatile compounds [17].

The incorporation of MOG into fermenting must also has an adverse effect on color and phenolic concentration [9-11]. Flavonoids and phenolic compounds, e.g. tannins, can transfer from MOG into fermenting wines [24,45,46]. Petioles can likewise absorb anthocyanins from the must during fermentation, resulting in decreased color intensity [24,45]. Frost et al. [15] reported reduced concentration of anthocyanin, tannin, and iron-reactive phenolics in Cabernet Sauvignon wines to which frozen MOG was added at rates from 0 to 8 g/kg. However, increased MOG is normally associated with an increase in total phenols, attributable to elevated flavonoid concentrations

observed in MOG-affected wines [9-11]. High MOG levels can also lead to increased anthocyanin concentrations in wines, and consequently, adversely impact hue and color intensity [10,11]. Others found higher concentrations of both anthocyanins and phenols in wines made with petioles, leaves and/or stems [9,10,45]. In some circumstances, high levels of MOG can likewise cause increases in both wine color intensity and hue [11]. These alterations in phenolic composition can impact wine sensory profile and overall quality [9,11].

Overall alterations in basic wine variables in this study suggest that both petioles and leaves can impact the basic chemical composition of wine. These results have been verified by previous studies that focused upon the effects of MOG constituents on wine [24,45,46]. The overall impact of MOG on basic variables is likely due to several factors, including maceration time, components of MOG, interactions with other compounds, and the level of berry damage prior to fermentation [24,26,46].

4.2. Sensory profiles

The objectives of this study were to determine the impact of frozen MOG on both the chemical composition and also sensory profiles of the two cultivars. Descriptive analysis indicated that both Cabernet franc and Cabernet Sauvignon wines differed in several sensory attributes when contaminated with post-frost MOG (both petioles and leaves). These results supported the initial hypothesis that frozen MOG would have an impact on the sensory profile of red wine cultivars. MOG treated Cabernet franc wines differed in terms of color intensity, clarity, floral and tropical fruit aromas, vegetal and earthy flavors, and astringency. Similar to the Cabernet franc wines, Cabernet Sauvignon wines differed in color intensity, clarity, floral and herbaceous aromas, and dried fruit, dark fruit, vegetal, and herbaceous flavors. These results are consistent with Ward, et al. [17], who found differences in several sensory descriptors of Cabernet Sauvignon MOG wines, including vegetal, floral, earthy, and leafy aromas, as well as bitterness, acidity and body. Although there were some consistencies in the sensory profile of the two cultivars, the Cabernet Sauvignon and Cabernet franc differed in several treatment-related flavor attributes, indicating that there is a potential varietal difference in the perception of wines contaminated with frozen MOG.

Specifically, the wines made from higher percentages of MOG had higher intensity ratings of floral aroma, indicating a positive linear relationship between the amounts of frozen MOG and the perceived floral intensity, which supports the original hypothesis. This is also in agreement with Huang, Cash and Santerre [10], who reported an increase in the floral aroma of Cabernet Sauvignon wines when exposed to increasing levels of petioles. It is likewise in agreement with recent results from Washington State Cabernet Sauvignon, in which addition of MOG increased the intensity of floral aroma, herbaceous/straw aroma, artificial fruit aroma, and floral aftertaste, while decreasing intensity of dark fruit aroma and astringency [15]. The Cabernet franc and Cabernet Sauvignon wines in the present study also displayed a positive linear relationship between the perceived vegetal intensity and the concentration of frozen leaves. However, increasing levels of petioles to the wines had no correlations with perceived intensity of vegetal characteristics. This contradicts Ward et al. [17], who found the perceived vegetal and leafy intensities were lower in Cabernet Sauvignon wines made from higher additions of petioles. They hypothesized that petioles adsorbed methoxypyrazines from the fermentation, thus lowering vegetal characteristics [10]. However, they examined the impact of fresh petioles rather than post-frost petioles, which may explain in differences in results [17]. Furthermore, a significant negative correlation was found in this study between color intensity and the leaf-treated Cabernet Sauvignon wines; however, no correlation was found for the other wine treatments.

4.3. Correlation between Sensory Attributes, Chemical Profile and MOG Content

4.3.1. Correlation of Sensory Attributes among Wines

Use of PCA permitted relationships to be visualized between significant sensory attributes, as well as with the MOG treatments. Both cultivars had strong associations between the vegetal attribute and high additions of leaves. Relationships were apparent between floral aroma and the high

Cabernet franc petiole treatment; however, no association was observed for the Cabernet Sauvignon 5% petiole wines. Instead, an elevated floral aroma in Cabernet Sauvignon was associated with the 1% and 2% leaf treatments. High leaf additions in Cabernet Sauvignon wines were inversely correlated with color intensity, whereby increases in leaf content resulted in lower perceived intensity in color. In terms of Cabernet franc, no relationships were evident between the higher MOG treatments and color intensity. Overall, PCA results demonstrated that MOG impacted wines could be differentiated based on their sensory profiles. These results help support the initial hypothesis, and observations made by local wineries, that the incorporation of frozen MOG results in higher floral, and vegetal attributes, as well as a lower color intensity.

4.3.2. Correlation of Sensory Attributes, Aroma Compounds and Basic Variables

The PLS regression indicated correlations among the levels of MOG with the significant sensory attributes, aroma compounds and basic wine variables. For Cabernet franc, there were strong positive correlations of perceived floral aroma with several aroma compounds including octanol, nerol, citronellol, nerolidol, geraniol, linalool, *cis*- and *trans*-rose oxides, ethyl salicylate and methyl salicylate. Floral aroma and correlating aroma compounds were associated with the higher petiole additions (2% and 5%). In contrast, high leaf additions (1% and 2%), and the correlating high earthy and vegetal flavor intensities, were associated with greater concentrations of α -terpinene, hexanol, β -ionone, and myrcene. These results assisted in establishing those aroma compounds that were likely involved sensorially. Overall, the PLS regression results are supported by several studies on the associated aromas of volatile compounds. For example, citronellol, ethyl salicylate, geraniol, linalool, *cis*- and *trans*-rose oxide, are correlated with floral and perfume-like aromas [8,11,17]. In contrast, hexanol is often described as green/grassy [11,16,24]. The association between the vegetal attribute and the aroma compounds of γ -terpinene, β -ionone, and myrcene was surprising, as those compounds are often described as having floral aromas [8,17]. A possible explanation for this is that those compounds were below their odor detection thresholds.

Furthermore, the PLS plots showed that higher leaf and petiole wines were well distinguished, both from one another, as well as from the lower MOG treatments. This indicates that the level of MOG in wines can be differentiated based on the sensory and chemical profiles. These results further support the hypothesis that frozen MOG has an impact on both the sensory and chemical profiles of wine.

Moreover, a varietal difference in the panel's perception of MOG was observed using PLS. Cabernet Sauvignon had positive correlations between the high leaf additions and herbaceous and vegetal sensory attributes. In contrast, Cabernet franc had a positive correlation between high floral aroma and the higher leaf treatments, rather than the high petiole wines. However, when comparing the aroma compounds and the amount of MOG, similar correlations to the Cabernet franc wines were observed. The Cabernet Sauvignon 2% leaf samples were associated with higher concentrations of hexanol, heptanol, linalool oxide, nerol, nerolidol, and α -damascenone, while the 5% petiole samples were positively correlated with octanol, *cis*- and *trans*-rose oxide, α -terpineol, geraniol, eugenol, linalool, citronellol, myrcene, terpinolene, ethyl salicylate and methyl salicylate. Some of these aroma compounds can decrease in concentration with age [8]. Therefore, considering the wines were tasted 2 yr after bottling, it could be that there were dissimilarities between the two varieties due to aging differences. Overall, the monoterpenes seemed to have a greater impact on the perception of floral aroma in frozen MOG wines, whereas higher alcohols were more associated with the herbaceous and vegetal characteristics.

This study is among the first to look at the sensory and chemical impact of frozen MOG on red wine cultivars [12-15]. This research can provide the industry with valuable information in terms of how the incorporation of frozen MOG might impact wine quality. Overall, the incorporation of 1% or 2% leaves resulted in wines with higher herbaceous and vegetal attributes. These characteristics are generally considered undesirable and are often associated with significantly lower quality wines [16], who found that the incorporation of more than 5% leaves resulted in unpleasant grassy aromas. However, several previous studies [8,9,26] found no differences in the quality of mechanically

harvested wines due to the inclusion of MOG. Although there were several differences in the chemical composition, the differences did not translate into detectable sensory alterations [8]. This study also found that high levels of petioles were largely associated with a higher perception of floral aroma. Red wine cultivars, such as Cabernet Sauvignon are generally considered terpene neutral, and lack floral attributes as a defining characteristic [17]. However, they suggested that the presence of floral attributes in wine may not necessarily be considered undesirable or unusual to consumers if they have been associated with specific red wine cultivars, and they further described the potential advantage of petiole additions as a means to obtain a desired wine style. In Shiraz, for example, fresh grape leaves (1% wt/wt) resulted in wines with increased confectionary aromas, fruity flavors, and astringency, but inclusion of rachis (2.6% wt/wt) and peduncles (1.5% w/w) increased “green” aromas and flavors [18]. With machine-harvested fruit reported to have concentrations as high as 4.7% wt/wt MOG, well above detectable treatment levels, there is potential for results such as these to translate to an industrial setting [10].

Another important observation of this study is that there is a potential varietal difference in the impact on the chemical composition, and perceived sensory profile of MOG wines. Some cultivars may be more susceptible to MOG-associated sensory aromas [17]. Thus, this study can help in providing information on how greater care can be taken in the harvesting and production method of more MOG-sensitive cultivars.

5. Conclusions

The inclusion of petioles and leaves into fermentations had a significant impact on the chemical and sensory profiles of Cabernet franc and Cabernet Sauvignon wines. High levels of MOG were associated with higher levels of floral and vegetal attributes, as well as several aroma compounds including terpenes, esters, norisoprenoids, and higher alcohols. Increased leaf levels in Cabernet Sauvignon were also negatively correlated with color intensity, indicating a potential decrease in color at greater levels of MOG. Increases in floral aroma ratings were associated with the addition of petioles as well as leaves. In contrast, increases in vegetal and herbaceous characteristics were associated only with the inclusion of leaves. Increased floral aromas were detectable at levels of 2% petioles for Cabernet franc and 1% leaves for Cabernet Sauvignon. Similarly, increased vegetal and herbaceous attributes were associated with 2% leaves for Cabernet franc and 1% leaves for Cabernet Sauvignon. Thus, there appeared to be a cultivar-dependent threshold for the amount of MOG required to cause perceivable sensory differences. The sensory attributes associated with the increase in frozen MOG are generally considered undesirable in Cabernet franc and Cabernet Sauvignon wines. However, as no preference testing was conducted, no conclusions regarding wine quality could be made.

This study provided a sound basis for the impact of post-frost MOG; however, there are several future components that could allow for a greater understanding of the impact of frozen MOG during red wine fermentations. Recent trials addressed the effects of different yeast strains, as well as the impact of different harvester technologies, to evaluate these as possible mitigating strategies. The data presented here are overwhelmingly indicative of the significance and role played by terpenes and norisoprenoids in the determination of MOG-induced floral taint in late-harvested red wine varieties. “Floral taint” associated with frozen MOG is due primarily to several terpenes (linalool, geraniol, *cis*- and *trans*-rose oxide, citronellol, nerol), methyl and ethyl salicylate, and β -ionone. Several esters and other aliphatic compounds also appeared related—including esters (ethyl heptanoate, octanoate, nonoate, decanoate) and alcohols (hexanol, octanol, phenylethyl alcohol). However—some of these, although very responsive, may occur below sensory threshold; however, many sensory thresholds measured in water could be much lower in an alcohol-based medium, and there are several interactions between chemicals that are not fully understood. Regardless, it would be useful to quantify wider range of aroma compounds, including methoxypyrazines, to assess if there are any masking effects. It would also be valuable to conduct trials on combinations of leaves and petioles, as well as different maceration times. Sensory analysis should also include preference testing to determine any potential impacts on consumer perception of quality.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Overview of representative fermentations of treatments involving various leaf and petiole additions to Cabernet franc, 2017; Figure S2: Principal components analysis of several commercial Ontario red wines with various levels of MOG-induced floral taint, 2015; Figure S3: Relationships between several frozen leaf levels (N=15) added to Ontario Cabernet franc wine fermentations vs. aroma compound concentrations, 2017; Figure S4: Relationships between several frozen petiole levels (N=15) added to Ontario Cabernet franc wine fermentations vs. aroma compound concentrations, 2017; Figure S5: Principal components analysis of aroma compounds of: A: Cabernet franc and B: Cabernet Sauvignon, Ontario, Canada, 2016; Table S1: Volatile standards for quantification of aroma compounds in Ontario Cabernet franc and Cabernet Sauvignon wines, 2015 to 2017; Table S2: Aroma reference standards used during descriptive analysis of Ontario Cabernet franc and Cabernet Sauvignon wines with varying contents of MOG; Table S3: Concentrations of several aroma compounds ($\mu\text{g/L}$) in Ontario red wines, with their odor activity values; Table S4: Additional compounds (not depicted in Figure 1) in Cabernet franc wines impacted by various leaf additions, Ontario, Canada, 2016; Table S5: Additional compounds (not depicted in Figure 2) in Cabernet franc wines impacted by various petiole additions, Ontario, Canada, 2016; Table S6: Additional compounds (not depicted in Figure 3) in Cabernet Sauvignon wines impacted by various leaf additions, Ontario, Canada, 2016; Table S7: Additional compounds (not depicted in Figure 4) in Cabernet Sauvignon wines impacted by various petiole additions, Ontario, Canada, 2016; Table S8: Additional compounds (not depicted in Supplemental Figure S3) in Cabernet franc wines impacted by various leaf additions, Ontario, Canada, 2017; Table S9: Additional compounds (not depicted in Supplemental Figure S4) in Cabernet franc wines impacted by various petiole additions, Ontario, Canada, 2017; Table S10: Additional compounds (not depicted in Figure 5) in Cabernet Sauvignon wines impacted by various leaf additions, Ontario, Canada, 2017; Table S11: Additional compounds (not depicted in Figure 6) in Cabernet Sauvignon wines impacted by various petiole additions, Ontario, Canada, 2017; Table S12: Composition of Cabernet franc and Cabernet Sauvignon wines impacted by various leaf and petiole additions (N=15), Ontario, Canada 2016 and 2017.

Author Contributions: Conceptualization, M.C. and A.R.; methodology, Y.B.L. and X.X.Y.; software, Y.B.L.; validation, Y.B.L., X.X.Y., and J.M.W.; formal analysis, Y.B.L. and X.X.Y.; investigation, J.M.W.; resources, Y.B.L.; data curation, X.X.Y. and J.M.W.; writing—original draft preparation, Y.B.L. and J.M.W.; writing—review and editing, E.A. and A.R.; visualization, Y.B.L. and E.A.; supervision, A.R. and A.R.; project administration, A.R.; funding acquisition, A.R. All authors have read and agreed to the published version of the manuscript.

Funding: The project is financed by the program of the Ontario Grape and Wine Research Inc. (project number OGWRI 002000) and by the National Science and Engineering Research Council of Canada (NSERC) program.

Data Availability Statement: Data are contained within the article and supplementary materials.

Acknowledgments: We gratefully acknowledge the following partners: Ontario Grape and Wine Research Inc. and Ontario Centres of Excellence (funding); Andrew Peller Ltd. and Arterra Wines Canada (wine samples and fruit); DIAM (corks).

Conflicts of Interest: The authors declare no conflicts of interest.

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