Effects of Frozen Materials Other Than Grapes on Red Wine Volatiles. Mitigation of Floral Taints by Yeast Strains

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Abstract: "Floral taint", a unique sensory defect that has been detected in red wines in North America, is caused by frozen leaves and petioles (materials other than grapes; MOG) introduced during mechanical harvest. Responsible volatiles are likely terpenes, norisoprenoids, and esters. Objectives of this study were to investigate volatile compounds that may cause floral taint and to evaluate yeast strains that could mitigate the problem for a series of different leaf- and petiole-based MOG levels. Replicate Cabernet franc fermentations (2017 to 2019) combined MOG treatments ([wt/wt]: 0, 0.5%, and 2% leaf blades, and 0, 1%, and 5% petioles) with three yeast strains (CSM, EC1118, and FX10). Increases in leaf and petiole levels resulted in primarily linear increases in many aroma compounds, particularly terpenes. Increases in leaf addition increased concentrations of nine (2017), 12 (2018), or 17 terpenes (2019). Increased petioles led to linear increases in 13 (2017), 12 (2018), or 15 terpenes (2019). Norisoprenoids and salicylates were also responsive. Yeast effects varied with leaf and petiole levels and with season. Fermentations with 2% leaves displayed yeast-related differences in nine, four, and 10 terpenes, and four, two, and nine esters in 2017, 2018, and 2019, respectively. Fermentations with 5% petioles displayed yeast-related differences in three, six, and eight terpenes, and three, two, and five esters in 2017, 2018, and 2019, respectively. Yeasts EC1118 and FX10 produced the least terpenes at all leaf and petiole levels, while CSM produced the most. Results suggest that incorporation of frozen MOG to fermentations will result in elevated concentrations of terpenes, norisoprenoids, and salicylates associated with floral taint, and that specific yeast strains may diminish this problem.

Key words: esters, higher alcohols, methoxypyrazines, monoterpenes, norisoprenoids

Mechanized harvesting of winegrapes has become standard practice over the past 50 years, but has inadvertently resulted in more materials other than grapes (MOG) in harvest loads. MOG, e.g., leaves and petioles, can diminish the quality and composition of wines, particularly red wines (Noble et al. 1975, Huang et al. 1988, Guerrini et al. 2018,

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Kilmartin and Oberholster 2022). Recent harvesting technology has largely eradicated this problem, except under unusual circumstances such as frost-damaged canopies (Parenti et al. 2015, Hendrickson et al. 2016, Hendrickson and Oberholster 2017). Consequently, some grape cultivars (e.g., Cabernet franc) are especially prone to a unique sensory defect, locally known as "floral taint," which is widely believed to originate from MOG. Climate change-induced warmer autumns have permitted harvest delays of cultivars such as Cabernet franc into mid- to late-November. The wine industry is concerned that undesirable aroma compounds such as monoterpenes are being introduced by frozen leaves, and that post-frost machine harvesting is a major contributor (Wang et al. 2020, Lan et al. 2022). To address issues of sensory taint and to enable grapegrowers to adapt to the changing climate, the wine industry must know the effects contributed by frozen MOG and identify mitigating strategies to either minimize incorporation of MOG into harvest loads or reduce their undesirable aroma contributions during fermentation.

Aroma compound concentrations in musts and wines are also impacted substantially by MOG. The volatile compound composition associated with the rachis, peduncles, stems, and tendrils is composed mainly of monoterpenes such as geraniol, linalool, and β-citronellol (Matarese et al. 2014). Integration of grape leaves into fermentations can pose a substantial risk to the final wine composition (Noble et al. 1975, Wildenradt et al. 1975, Ward et al. 2015). Leaves are largely composed of C6 compounds like hexanal and 2-hexenal, which are characterized as having a green odor

(Matarese et al. 2014). Other volatile compounds present at lower concentrations include methyl salicylate, benzyl alcohol, benzaldehyde, 2-phenylethanol, norisoprenoids, and eugenol (Gunata et al. 1986, Matarese et al. 2014). Leaf blades (laminae) contain several volatile compounds that could potentially be extracted into wine, including primarily C6 aldehydes and alcohols (e.g., 2-hexanal, 2-hexen-1-ol, *n*-hexanol, and 2-hexenol-1-ol), terpenes (e.g., linalool, citral, citronellol, and geraniol), and norisoprenoids (Wildenradt et al. 1975, Gunata et al. 1986). Petioles have high concentrations of terpenes, especially free forms of citronellol and geraniol, and may serve as storage for free terpenols or for utilization in metabolic pathways, e.g., the geranyl phosphate pathway; hence the higher concentrations of terpenols in petioles than in laminae (Gunata et al. 1986). Juice contact with MOG during fermentation extracts several volatile grapevine compounds into the final wine (Ward et al. 2015, Suriano et al. 2016, Guerrini et al. 2018). Higher alcohols and esters increase in the presence of stems (Suriano et al. 2016, Guerrini et al. 2018), while benzyl alcohol, eugenol, 1-hexanol, methyl salicylate, and ethyl salicylate also increase as a result of MOG incorporation (Ward et al. 2015, Guerrini et al. 2018). Increasing MOG during fermentation results in wine with higher concentrations of several monoterpenes (Ward et al. 2015, Suriano et al. 2016, Guerrini et al. 2018). Characteristic floral monoterpene-based aromas are typical and desirable in white wines such as Muscat, Riesling, and Gewürztraminer (Marais 1983), but are atypical in red table wines (Ferreira et al. 2000). In the presence of high MOG levels, geraniol, linalool, and β-citronellol may be found at concentrations above their detection thresholds, suggesting a potential impact on the perceived sensory profile (Ward et al. 2015, Wang et al. 2020). Less than 5% of petioles was required to substantially alter sensory attributes, particularly increasing terpene-based floral aromas in Cabernet Sauvignon (Ward et al. 2015).

Yeast selection may serve as a mitigating strategy to reduce floral taint. Both *Saccharomyces* and non-*Saccharomyces* yeasts can vary significantly in their potential for producing various odor-active compounds, including esters, higher alcohols, terpenes, and other aroma compounds, as a consequence of their specific enzyme activities (Swiegers et al. 2005, Cordente et al. 2012, Carrau et al. 2017). Esters in wines include the acetate esters, in which the acyl group is derived from acetyl-CoA and the alcohol group is either ethanol or a higher alcohol derived from amino acid metabolism (Swiegers et al. 2005, Cordente et al. 2012, Carrau et al. 2017). Ethyl esters involve ethanol as the alcohol group, while the acyl group is derived from medium-chain fatty acids (Lambrechts and Pretorius 2000, Cordente et al. 2012, Carrau et al. 2017). Ester formation depends on substrate concentrations and on specific yeast-based enzyme activities (Cordente et al. 2012, Carrau et al. 2017). As with esters, specific yeast enzymes and their relative activities are responsible for differences in higher alcohol production (Swiegers et al. 2005, Cordente et al. 2012). Higher alcohols are formed by yeast during fermentation from α-keto acids, involving degradation of an amino acid via the Ehrlich

pathway or from glucose via pyruvate (Cordente et al. 2012). This process involves three steps: transamination resulting in the formation of the α-keto acid, decarboxylation of the keto acid to form a fusel aldehyde, and its subsequent reduction to generate a fusel alcohol. Monoterpenes are considered to be primarily grape-derived and occur as both free aglycones and glycosides (Mateo and Jimenez 2000). Aglycones can be produced by β-glucosidase activity, which cleaves the disaccharide moiety, releasing the free monoterpene (Carrau et al. 2005, Bisotto et al. 2015). Yeasts strains vary in β-glucosidase activity (Tate et al. 2006, Bisotto et al. 2015), although this activity is relatively low at wine pH (Delcroix et al. 1994, Cordente et al. 2012). However, yeasts can also synthesize terpenes through the mevalonic acid pathway and the methylerythritol pathway (Carrau et al. 2005, 2017).

It is perhaps not surprising that research efforts into yeast strain selection have focused on identifying strains with enhanced capabilities for synthesis of esters, higher alcohols, terpenes, thiols, and other aroma compounds. Few to none have focused on identifying yeasts that produce low concentrations of specific odor-active compounds. However, several specific strains that produce comparatively low concentrations of specific compounds, particularly esters and higher alcohols, have been identified (e.g., Soles et al. 1982, Delteil and Jarry 1992, Nurgel et al. 2002, Vilanova et al. 2007, Molina et al. 2009, Vararu et al. 2016); other strains identified have limited enzyme activities (Delcroix et al. 1994, Tate et al. 2006). Fourteen yeast strains produced white wines with different ester concentrations, including ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate, amyl acetate, hexyl acetate, and 2-phenylethyl acetate (Soles et al. 1982). When two Lallemand yeast strains were compared, Chardonnay wines fermented with K1M contained higher concentrations of ethyl dodecanoate, isoamyl acetate, and total esters, while those fermented with D47 contained higher concentrations of ethyl hexanoate and ethyl octanoate (Delteil and Jarry 1992). Subsequent work reported that the EC 1118 strain produced wines with more solvent, fatty, and pineapple aroma attributes attributable to ethyl hexanoate, ethyl octanoate, and phenylethanol, while the VIN 13 strain produced more banana, fruity, yeasty, and green attributes attributable to 3-methyl butyl acetate, 2-methyl propionic acid, and 3-methyl butanoic acid (Molina et al. 2009).

Yeast strains also vary in their capability to produce monoterpenes. More linalool, nerolidol, and farnesol were found in wines fermented by the *Saccharomyces bayanus* and *Saccharomyces capensis* species (Zea et al. 1995). Five *Saccharomyces cerevisiae* strains (Montrachet 522; CIVC 8130; and the Uruguayan native isolates 881, 882, and KU1) plus a Uruguayan native strain of *Hanseniaspora uvarum* were compared (Carrau et al. 2005). Strain KU1 produced the most linalool, M 522 and KU1 produced the most terpineol, and *H. uvarum* produced the most geraniol (Carrau et al. 2005). Among six yeast strains (QA23, EC 1118, K1M, CEG, S6U, and Flavia) used to ferment Muscat musts, CEG and Flavia produced the most terpenes, while EC 1118 produced the least (Bisotto et al. 2015).

Sensory taints observed in Ontario's commercial red wines have been associated with mechanically-harvested grapes. It was hypothesized that increased concentrations of undesirable aroma compounds such as monoterpenes are being introduced by frozen leaves and petioles. Warmer and prolonged autumn seasons, compared to as little as 10 years ago, can permit substantially later harvests. It is also equally possible that climate change, resulting in harvests occurring later in the season, allows odor-active compounds such as terpenes to develop to undesirable concentrations in mature fruit, which are exacerbated by MOG introduction through post-frost machine-harvesting. Objectives of this trial were to determine whether interactive effects exist between different leaf- and petiole-based MOG levels and yeast strain, and whether these potential postharvest mitigating strategies can counteract undesirable aromas in Cabernet franc.

A preliminary analysis of the impact of frozen MOG has appeared (Wang et al. 2020). This work extends that study to three vintages, 2017, 2018, and 2019, with more robust data analysis.

Materials and Methods

MOG treatments. All Cabernet franc (*Vitis vinifera* L.) grapes were hand-harvested following a killing frost in the 2017 to 2019 seasons from the Andrew Peller Carlton St. vineyards, located in the Niagara Peninsula VQA sub-appellation of Four Mile Creek in Niagara-On-The-Lake, Ontario, Canada. Frozen leaf blades and petioles were collected from the vineyard the day prior to harvest and stored in plastic bags at 4°C. MOG treatments were: (wt/wt): 0, 0.5%, and 2% leaf blades (hereinafter 0L, 0.5L, and 2L, respectively), and 0, 1%, and 5% petioles (hereinafter 0, 1, and 5P, respectively). Following harvest, grapes from each designated MOG level were divided equally into three replicates for subsequent winemaking. Harvest dates were 14 Nov 2017, 11 Nov 2018, and 13 Nov 2019.

Winemaking and yeast treatments. Following destemming, must was treated with 50 mg/L potassium metabisulfite (KMS). Fermentations were performed in triplicates of 40 kg in 46-L plastic fermentation buckets. After addition of various MOG levels, 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 one of three yeast strains: CSM, EC 1118, or Zymaflore FX10 one day after harvest, creating a factorialized trial with three levels of two sources of MOG combined with three yeast strains, replicated three times. Addition of 200 mg/L diammonium phosphate (DAP) was made 24 hrs 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.

Conventional analysis. Conventional chemical analysis (e.g., ethanol, titratable acidity [TA], pH, color, total anthocyanins, and total phenols) was also performed using standard methods. Wine pH was measured with an Accumet pH meter (Model 25). Wine TA was measured with a PC-Titrate autotitrator (Man-Tech Associates) to a pH 8.2 end point. Color intensity and hue were calculated from absorbance at 420 and 520 nm measured on an Ultrospec 2100 Pro UV/VIS spectrophotometer (Biochrom Ltd.). Total anthocyanins were measured by the pH shift method (Fuleki and Francis 1968). Total phenols were determined according to the Folin-Ciocalteu micro method (Waterhouse 2001).

Gas chromatography-mass spectrometry (GC-MS). Wines were analyzed using GC-MS with Gerstel thermal desorption technology as described (Bowen and Reynolds 2012, Moreno Luna et al. 2018). A 30-mL sample was taken from each wine treatment replicate immediately prior to bottling and was kept at 4°C in the presence of inert N_2 gas until analysis. Subsequent extraction details were as described (Lan et al. 2022). An Agilent 6890N/5975B GC-MS equipped with a Gerstel thermal desorption system, cooled injection system, and programmable temperature vaporization was used. Instrument conditions were as described (Bowen and Reynolds 2012, Moreno Luna et al. 2018). Other pertinent details related to GC-MS conditions were as described (Lan et al. 2022).

Scan analysis reflected the presence of more than 100 volatile compounds in wines from all cultivars. For calibration purposes, 41 compounds were chosen for quantification (Lan et al. 2022). Seven-point calibration curves were created for each compound consistent with the literature (Bowen and Reynolds 2012). Sources of aroma compounds are as described (Lan et al. 2022). Model wine was used for calibration curves and prepared based on Bowen and Reynolds (2012), using 12% (v/v) of pure anhydrous ethanol (Commercial Alcohols) diluted in Milli-Q water with 5 g/L tartaric acid. Preparation of aroma standards was as described (Lan et al. 2022). Odor activity values (OAVs) were calculated as the ratio between the concentration obtained by calibration versus its respective threshold. Thresholds were obtained from the literature (Buttery et al. 1968, 1982, 1988, Ruth 1986, Takeoka et al. 1990, Etiévant 1991, Guth 1997, Ferreira et al. 2000, Plotto et al. 2004). These data were used to generate concentrations of aroma compounds for comparative analysis. A list of these compounds and their aroma descriptors is found elsewhere (Lan et al. 2022). Methoxypyrazines were quantified by GC-MS as described (Kotseridis et al. 2008).

Statistical analysis. All data were analyzed using XLStat (Addinsoft). Data were subjected to analysis of variance with MOG levels analyzed using single degree-of-freedom contrasts. Yeast mean separation within each MOG level was accomplished by Duncan's Multiple Range Test, $p \leq 0.05$. All bar plot figures were prepared using the 'ggplot' function in the 'ggplot2' package in an R environment (http://www.r-project.org/).

Results and Discussion

Effects of MOG levels. Increases in leaf and petiole levels resulted in primarily linear increases in many aroma compounds, particularly terpenes (Figures 1 to 6 and Supplemental Tables 1 to 3). Increases in leaf addition led to

concentration increases in nine (2017), 12 (2018), and 17 terpenes (2019) (Figures 1, 3, and 5, respectively). Norisoprenoids and salicylates were also responsive to leaf and petiole additions. Both α- and β-ionone increased relative to leaf level in 2017, while β-damascenone and both α- and β-ionone increased relative to leaf level in 2018 and 2019 (Figures 1, 3, and 5 and Supplemental Tables 1 to 3). Ethyl salicylate

increased relative to leaf level in 2018 and both methyl and ethyl salicylate increased in 2019 (Figures 1, 3, and 5 and Supplemental Tables 1 to 3). Esters and alcohols were much less responsive: there were increases relative to leaf addition in ethyl nonanoate (2017), hexyl acetate and isoamyl hexanoate (2018), and five esters (ethyl hexanoate, ethyl heptanoate, hexyl acetate, phenylethyl acetate, and isoamyl hexanoate) and
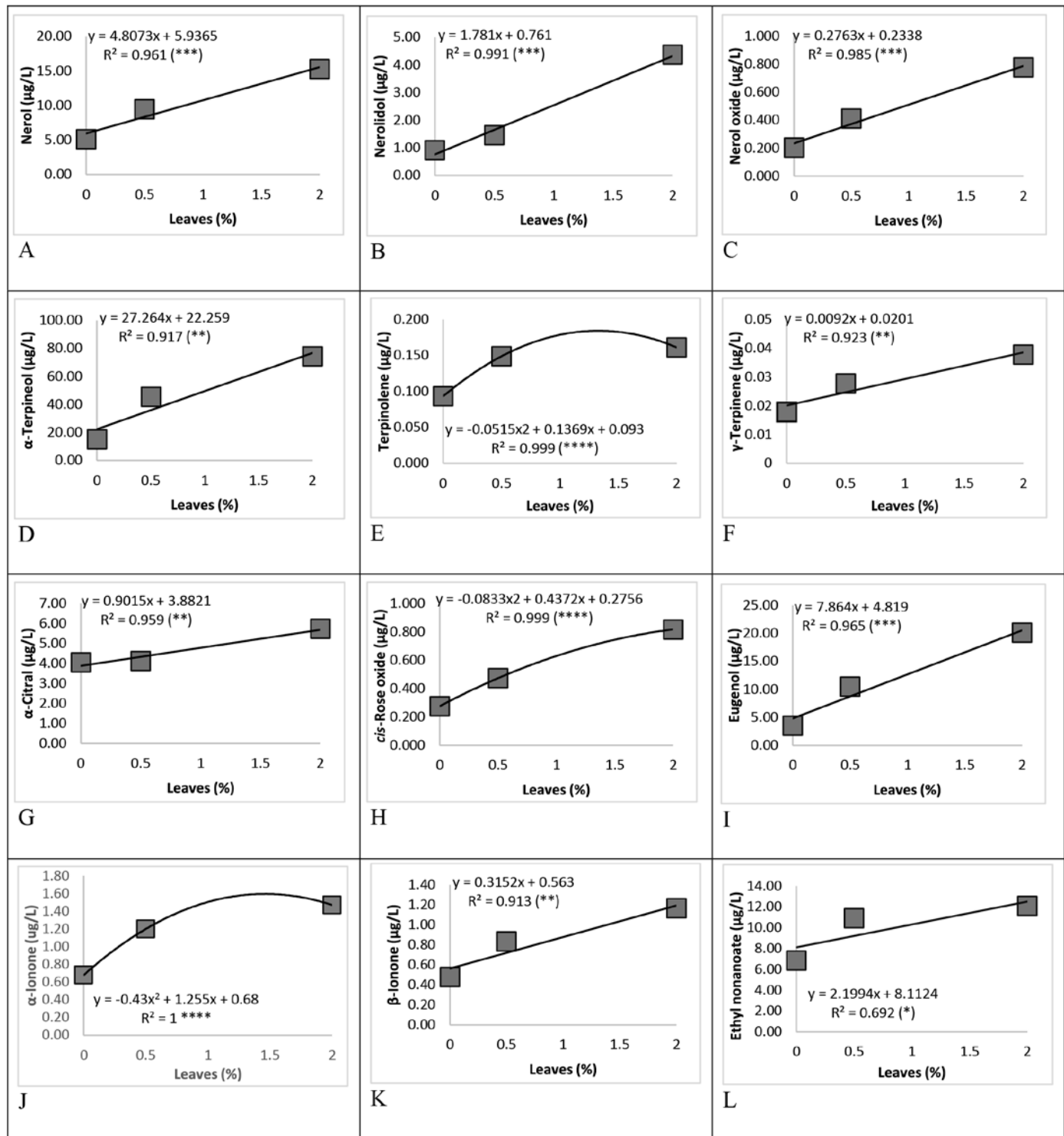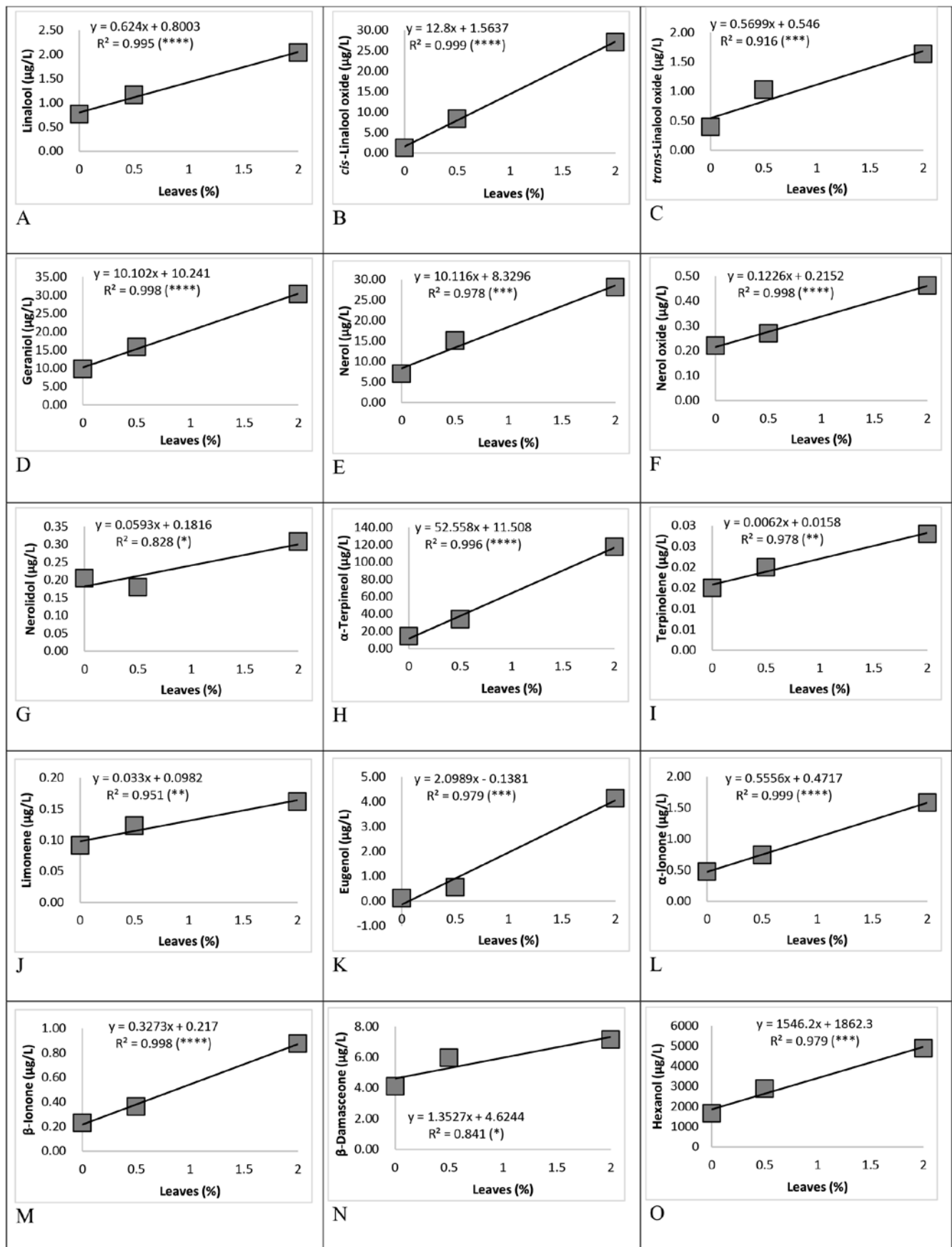

Figure 1 Impact of leaf additions to Ontario Cabernet franc fermentations on aroma compounds across three yeast strains in 2017. *, **, ***, or ****: Significant at $p \leq 0.05$, 0.01, 0.001, or 0.0001, respectively.
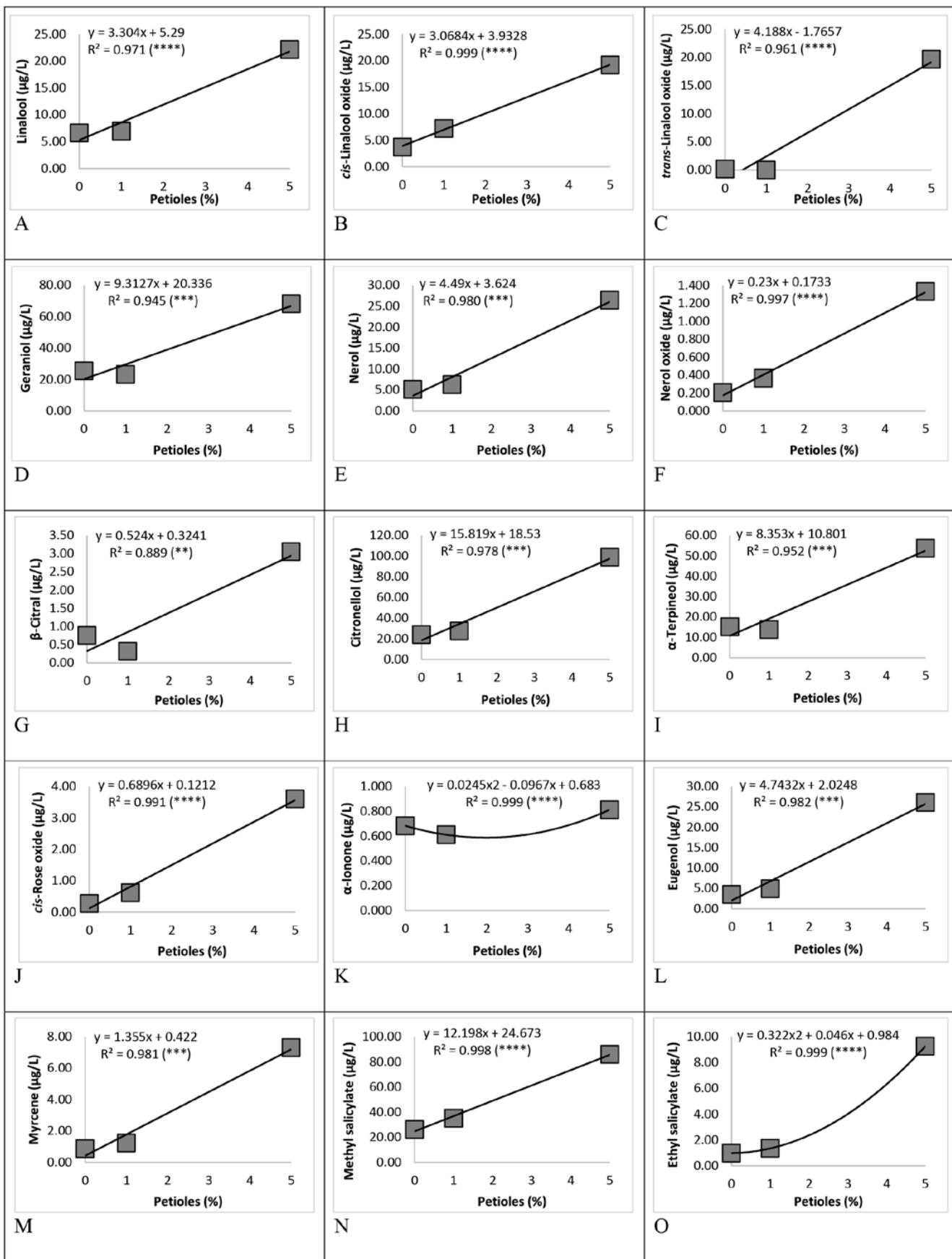
Figure 2 Impact of petiole additions to Ontario Cabernet franc fermentations on aroma compounds across three yeast strains in 2017. **, ***, or ****: Significant at $p \leq$ 0.01, 0.001, or 0.0001, respectively.

Figure 3 Impact of leaf additions to Ontario Cabernet franc fermentations on aroma compounds across three yeast strains in 2018. *, **, ***, or ****: Significant at $p \leq$ 0.05, 0.01, 0.001, or 0.0001, respectively.
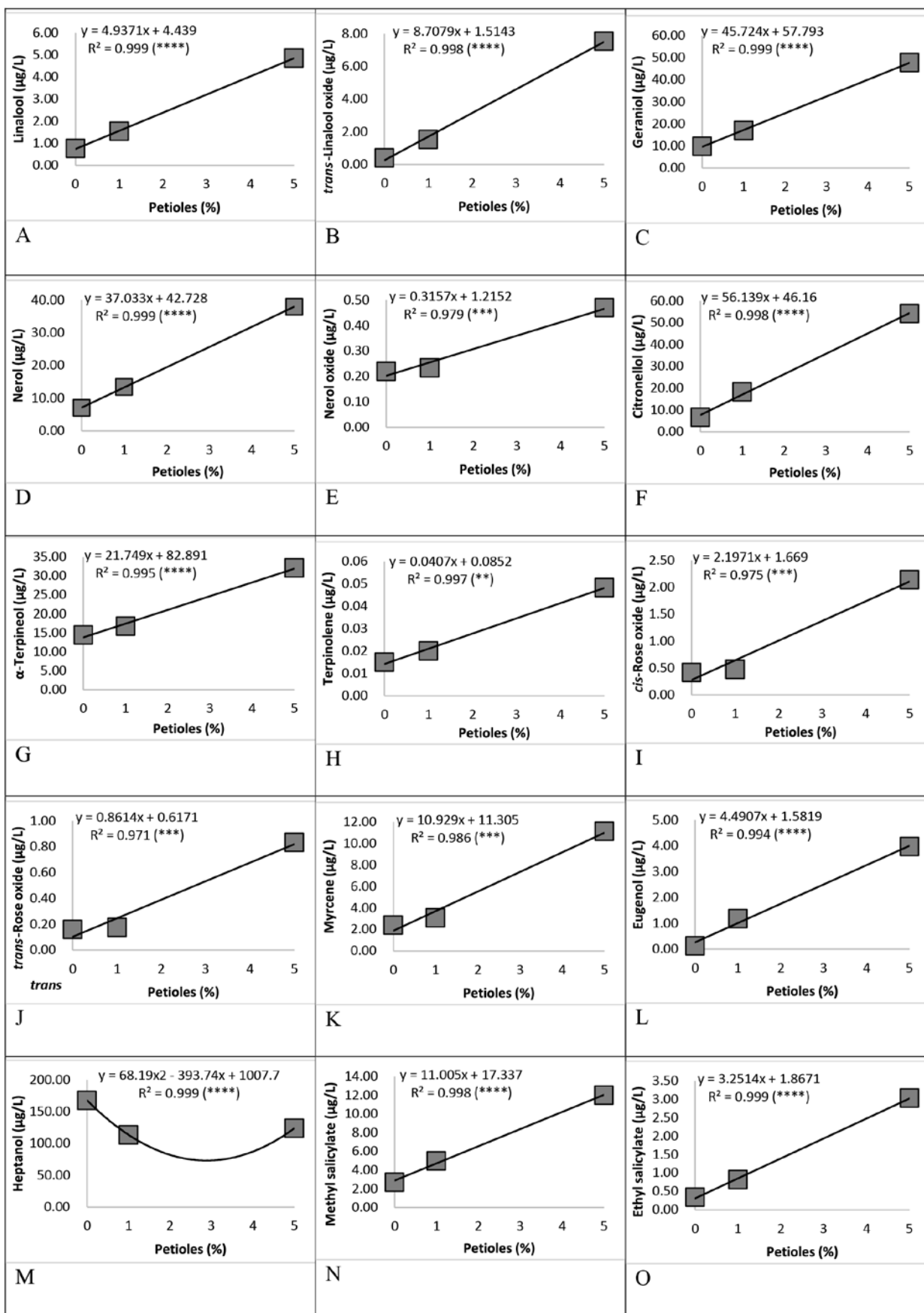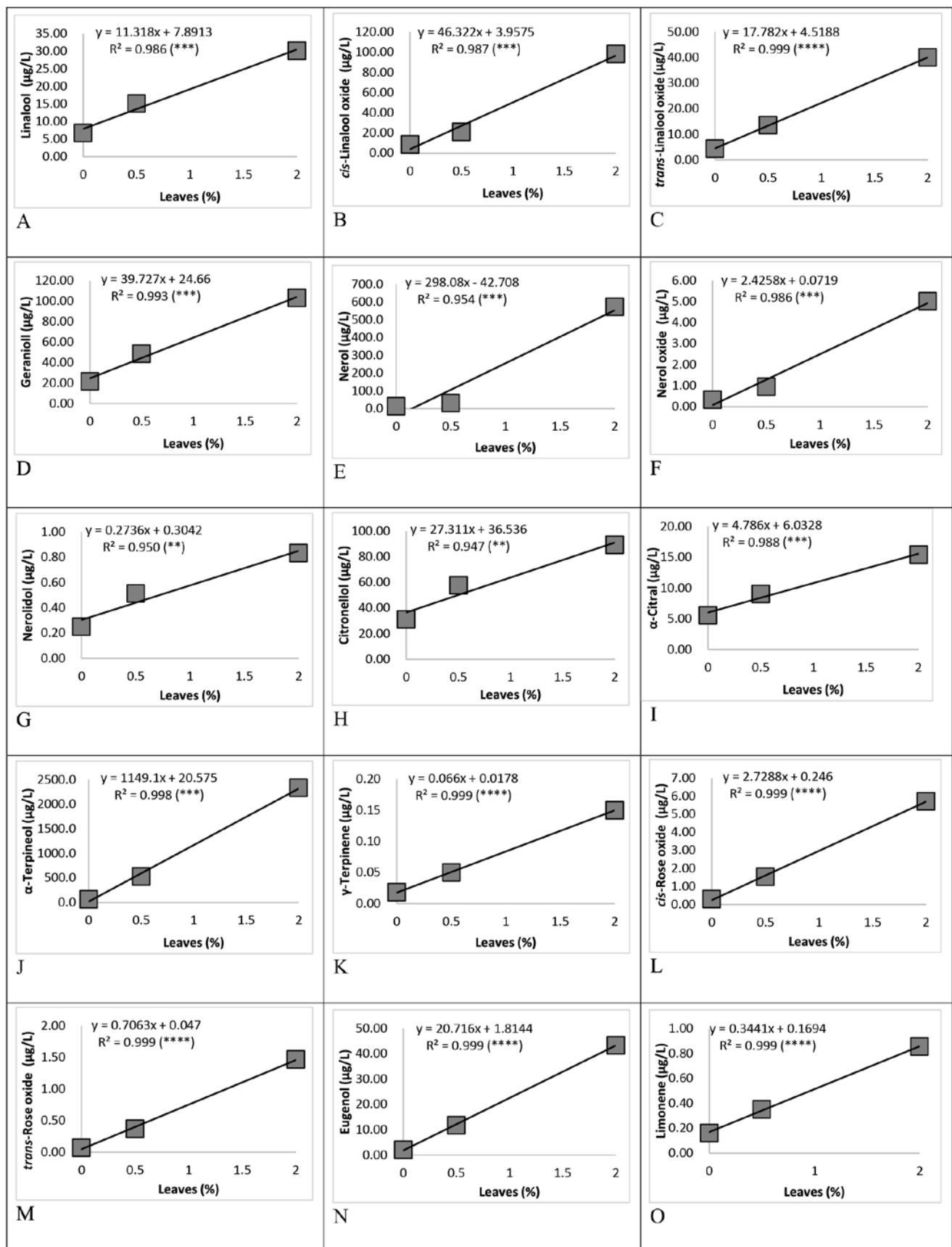
Figure 4 Impact of petiole additions to Ontario Cabernet franc fermentations on aroma compounds across three yeast strains in 2018. **, ***, or ****: Significant at $p \leq 0.01$, 0.001, or 0.0001, respectively.

Figure 5 Impact of leaf additions to Ontario Cabernet franc fermentations on terpene aroma compounds across three yeast strains in 2019. **, ***, or ****: Significant at $p \le$ 0.01, 0.001, or 0.0001, respectively.
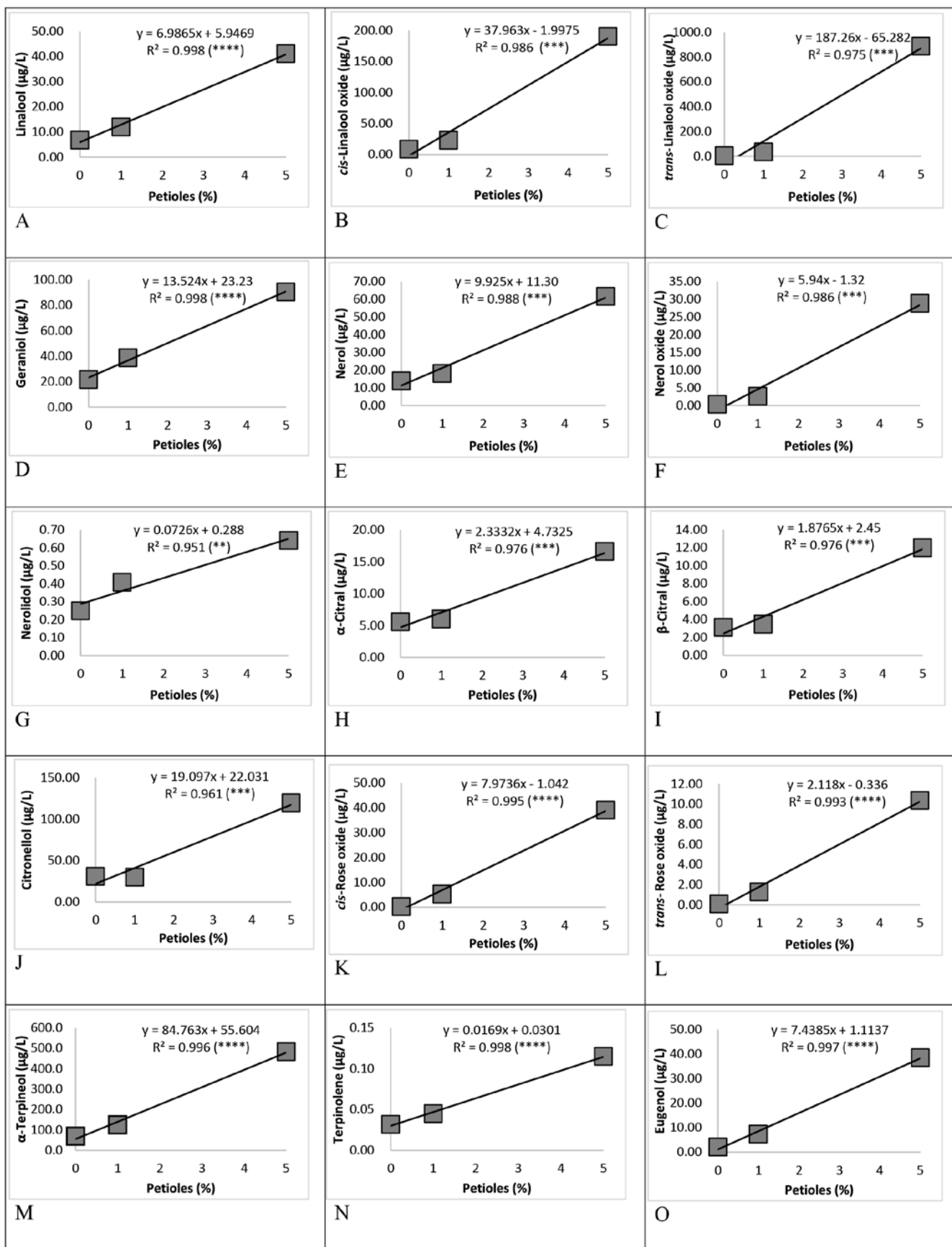
Figure 6 Impact of petiole additions to Ontario Cabernet franc fermentations on aroma compounds across three yeast strains in 2018. **, ***, or ****: Significant at $p \leq 0.01$, 0.001, or 0.0001, respectively.

two alcohols (heptanol and octanol) in 2019 (Figures 1, 3, and 5 and Supplemental Tables 1 to 3). Increased petioles led to linear increases in 13 (2017), 12 (2018), and 15 terpenes (2019) (Figures 2, 4, and 6 and Supplemental Tables 1 to 3). Other responses included increases in α-ionone (2017 and 2019), both salicylates (all three seasons), heptanol (2018 and 2019), and octanol (2019) (Figures 2, 4, and 6 and Supplemental Tables 1 to 3).

A consideration of potential odor-active values (sensory threshold/concentration) is crucial to determine those compounds that are likely of sensory significance. Among responsive aliphatic compounds, hexyl acetate had OAV values >1 (2018, 2019), as did ethyl hexanoate and ethyl heptanoate (2019), heptanol (2018, 2019), and octanol (2019), but phenylethyl acetate (2019) did not (Figures 3 to 6 and Supplemental Tables 1 to 3). Norisoprenoids have relatively low sensory thresholds, and thus α-ionone (responsive in all three seasons) and β-damascenone (2018, 2019) were detected at above-threshold concentrations across all MOG levels (Figures 1 to 6 and Supplemental Tables 1 to 3). β-Ionone was likely below threshold in 2017 and 2018, but had an OAV >1 for the 2 L level only in 2019 (Figure 3 and Supplemental Table 3). Both isopropylmethoxypyrazine (IPMP) and *sec*-butylmethoxypyrazine (SBMP) were consistently above threshold in 2018 across all MOG levels (Figures 3 and 4 and Supplemental Table 2). Methyl and ethyl salicylates have high odor thresholds (Leffingwell and Leffingwell 1991) and although responsive to MOG levels in all three seasons, they were likely not odor-active. Ethyl nonoate (responsive in 2017) and isoamyl hexanoate (2018) have no published sensory thresholds.

Among responsive terpenes were many with relatively low sensory thresholds, such as linalool, geraniol, α- and β-citral, *cis*-rose oxide, limonene, myrcene, and eugenol (Figures 1 to 6 and Supplemental Tables 1 to 3). Those responsive terpenes with OAV values that were consistently >1 across all MOG levels were *cis*-rose oxide only (all three seasons). Those with OAV values <1 in some seasons included linalool (2018), *cis*- and *trans*-linalool oxide (all three seasons), nerol (2017, 2018), nerolidol and nerol oxide (all three seasons), citronellol (2018), α- and β-citral (2017), α-terpineol (2017, 2018), terpinolene (2017, 2018), γ-terpinene (2017, 2019), limonene (2018, 2019), myrcene (2017, 2018), eugenol (2018), and *trans*-rose oxide (2018, 2019) (Figures 1 to 6 and Supplemental Tables 1 to 3). Perhaps of greatest interest are those responsive terpenes that became potentially odor-active (OAV >1) at increased MOG levels. Responsive compounds with OAV values >1 at specific treatment levels included linalool (5 P in 2017; 0.5 L, 2 L, and 5 P in 2019), geraniol (0.5 L, 2 L, 1 P, and 5 P in 2019), citronellol (5 P in 2017 and 2019), α-citral (2 L and 5 P in 2019), β-citral (2 L in 2019), nerol (2 L in 2019), α-terpineol (0.5 L, 2 L, and 5 P in 2019), and eugenol (0.5 L, 2 L, and 5 P in 2017; 0.5 L, 2 L, 1 P, and 5 P in 2019).

Differences between leaf and petiole level were observed for aroma compound composition, particularly for terpenes and norisoprenoids. In general, the addition of petioles had a greater impact on the concentration of monoterpenes. These

results are consistent with previous MOG studies, which reported significant impacts on the profile of aroma compounds in MOG-affected wines (Ward et al. 2015, Guerrini et al. 2018, Wang et al. 2020). Increased terpene concentrations were found as a result of petiole additions (Ward et al. 2015). Furthermore, an increase in the concentration of terpenes, higher alcohols, and esters were found in the presence of stems (Suriano et al. 2016, Guerrini et al. 2018). The results of this study therefore support the initial hypothesis that the addition of frozen MOG would result in extraction of specific aroma compounds, mainly monoterpenes, into the wine. Grapevine leaves and petioles have a significant number of aroma compounds, including terpenes, esters, aldehydes, and higher alcohols (Joslin and Ough 1978, Gunata et al. 1986, Matarese et al. 2014, Wang et al. 2020). Contact of grape leaves with juice during fermentation can result in the extraction of several aroma compounds from the leaves (Wildenradt et al. 1975). The composition of aroma compounds in leaves and petioles can include a large percentage of monoterpenes, including geraniol, linalool, nerol, α-terpineol, and citronellol (Gunata et al. 1986, Matarese et al. 2014, Wang et al. 2020).

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.) (Joslin and Ough 1978). The aroma compound concentrations in MOG-affected wine can be substantially impacted by yeast strains used during fermentation (Joslin and Ough 1978, Carrau et al. 2005). Some strains of *S. cerevisiae* can specifically impact the accumulation of monoterpenes such as linalool and citronellol in wine (Carrau et al. 2005). Furthermore, yeast strains can impact the reduction of *trans*-2-hexenol into *n*-hexanol, thus increasing the level of grassy aromas (Joslin and Ough 1978).

The wines differed very little in basic composition. There were tendencies for TA to decrease relative to increasing MOG levels in two instances, and five instances (one significant) in which pH increased relative to increased MOG. Color (four instances) and anthocyanins (two instances) showed a tendency to decrease relative to increased MOG. Increased petioles led to decreased ethanol in one instance. These findings are not completely in agreement with other studies (Ward et al. 2015, Pascual et al. 2016, Guerrini et al. 2018), the latter of whom reported increased pH and decreased ethanol and TA in wines exposed to more stems. Similarly, high-petiole wines were reported as having a higher pH, but no differences in TA or ethanol (Ward et al 2015). Several studies also found higher concentrations of anthocyanins and phenols in wines made with petioles, leaves, and/or stems (Noble et al. 1975, Huang et al. 1988, Pascual et al. 2016). Additionally, the presence of MOG during fermentation can allow transfer of flavonoids and other phenolic compounds, especially tannins, into the wine (Spranger et al. 2004, Suriano et al. 2016). MOG components such as stems can absorb anthocyanins from the must during fermentation, resulting in an overall decrease in color intensity (Pascual et al. 2016, Suriano et al. 2016). The overall impact of MOG on basic variables is affected by

several factors, including maceration time, components of MOG, interactions with other compounds, and the degree of berry damage prior to fermentation (Joslin and Ough 1978, Spranger et al. 2004, Suriano et al. 2016).

Effects of yeast strain. Preliminary data for 2.5% leaves and 5% petiole additions in 2017 were published previously (Wang et al. 2020). Effects of yeast strains over three seasons for all MOG levels were determined (Supplemental Tables 4 to 8 [2017], 9 to 13 [2018], and 14 to 18 [2019]). Fermentations without MOG addition displayed yeast-related differences that included seven (2017), one (eugenol; 2018), or six terpenes (linalool, cis-linalool oxide, citronellol, α-citral, γ-terpineol, and limonene; 2019), and three esters (ethyl heptanoate, ethyl nonanoate, and phenylethyl acetate; 2017), one ester (ethyl nonanoate; 2018), or nine esters (2019) (Figures 7 to 9 and Supplemental Tables 4, 9, and 14). Additionally, yeast strain affected diethyl succinate (2019), IPMP and SBMP (2018), and five alcohols (2019 only; isobutanol, hexanol, heptanol, octanol, and phenylethanol) (Figures 7 to 9 and Supplemental Tables 4, 9, and 14). As with MOG additions, many of those terpenes, esters, and alcohols have low thresholds and were potentially odor-active.

The yeasts EC 1118 and FX10 usually produced the fewest terpenes among all leaf and petiole addition treatments, while CSM usually produced the most terpenes, but yeast effects varied considerably with leaf and petiole level and season (Figures 7 to 9 and Supplemental Tables 4 to 18). Examination of the data without MOG addition provides a useful baseline to evaluate the potential for these yeasts to produce wines with low terpene, norisoprenoid, ester, and alcohol concentrations in high-MOG musts. Among compounds that varied significantly among yeast strains, CSM yeast produced wines with the highest concentrations of five of 10 compounds in 2017, of which three were terpenes (linalool, geraniol, and nerol oxide) (Supplemental Table 4). In 2018, CSM yeast produced wines with the highest concentrations for only two methoxypyrazines (Supplemental Table 9). In 2019, wines produced with CSM yeast had the highest concentrations of nine of 20 compounds, including six esters (ethyl isobutyrate, ethyl hexanoate, ethyl heptanoate, isobutyl acetate, isoamyl acetate, and isoamyl hexanoate), one alcohol (isobutanol), and two terpenes (linalool and *cis*-linalool oxide) (Supplemental Table 14).

Fermentations with relatively low MOG additions also showed yeast-related differences. Fermentations with 0.5% leaves displayed yeast-related differences in zero (2017), one (eugenol; 2018), and nine (2019) terpenes and in two (ethyl heptanoate and isoamyl acetate; 2017), two (ethyl isobutyrate and isobutyl acetate; 2018), and nine (2019) esters (Figures 7 to 9 and Supplemental Tables 5, 10, and 15). Additionally, two alcohols (hexanol and heptanol) were affected by yeast strain in 2017 and four (isobutanol, isoamyl alcohol, octanol, and phenylethanol) in 2019 (Figures 7 to 9 and Supplemental Tables 5, 10, and 15). Other compounds impacted included α-ionone (2019) and both IPMP and SBMP (2018) (Figures 7 to 9 and Supplemental Tables 5, 10, and 15). Fermentations with 1% petioles displayed yeast-related differences in one

(β-citral; 2017), six (linalool, cis-linalool oxide, geraniol, citronellol, α-citral, and β-citral; 2018), and 13 terpenes (2019), and in three (ethyl isobutyrate, ethyl nonanoate, and isoamyl hexanoate; 2017), seven (2018), and nine (2019) esters (Figures 7 to 9; Supplemental Tables 7, 12, and 17). Additionally, two alcohols (heptanol and phenylethanol) were affected by yeast strain in 2017, four (isobutanol, isoamyl alcohol, octanol, and phenylethyl alcohol) in 2018, and five (isobutanol, isoamyl alcohol, heptanol, octanol, and phenylethyl alcohol) in 2019 (Figures 7 to 9 and Supplemental Tables 7, 12, and 17). Diethyl succinate was impacted in 2017 and 2018; methyl and ethyl salicylate, in 2019; and both IPMP and SBMP in 2018 (Figures 7 to 9 and Supplemental Tables 7, 12, and 17).

In fermentations containing 0.5% leaves, considering compounds that varied significantly between yeast strains, CSM yeast produced wines with the highest concentrations of two of four compounds in 2017, both of which were esters (ethyl heptanoate and phenylethyl acetate) (Supplemental Table 5). In 2018, CSM yeast produced wines with the highest concentrations of two of five compounds, both of which were esters (ethyl isobutyrate and isobutyl acetate) (Supplemental Table 10). In 2019, wines produced with CSM yeast had the highest concentrations of 14 of 23 compounds, including seven esters, two alcohols (isobutanol and isoamyl alcohol), one norisoprenoid (α-ionone), and four terpenes (linalool, geraniol, nerol, and α-terpineol) (Supplemental Table 15). In fermentations containing 1% petioles, CSM yeast produced wines with the highest concentrations of two of seven compounds (ethyl isobutyrate and β-citral) in 2017 (Supplemental Table 7). In 2018, CSM yeast produced wines with the highest concentrations of 12 of 20 compounds, including six esters (ethyl isobutyrate, ethyl hexanoate, ethyl heptanoate, isobutyl acetate, isoamyl acetate, and phenylethyl acetate), three alcohols (isobutanol, isoamyl alcohol, and phenylethyl alcohol) and three terpenes (geraniol, terpinolene, and myrcene) (Supplemental Table 12). In 2019, wines produced with CSM yeast had the highest concentrations of 19 of 29 compounds, including eight esters, two alcohols (isobutanol and isoamyl alcohol), and seven terpenes, plus methyl and ethyl salicylate (Supplemental Table 17).

Fermentations with high-MOG additions showed many yeast-related differences. Fermentations with 2% leaves displayed yeast-related differences in nine (2017), four (citronellol, α-citral, *cis*-rose oxide, and *trans*-rose oxide; 2018), and nine terpenes (2019), and in four (ethyl heptanoate, ethyl decanoate, isobutyl acetate, and isoamyl acetate; 2017), zero (2018), and eight esters (2019) (Figures 7 to 9 and Supplemental Tables 6, 11, and 16). Additionally, yeast impacted three alcohols (isobutanol, isoamyl alcohol, and phenylethyl alcohol; 2019), methyl salicylate (2017), ethyl salicylate (2017, 2019), and diethyl succinate (2017, 2018, and 2019), and both IPMP and SBMP (2018) (Figures 7 to 9 and Supplemental Tables 6, 11, and 16). Fermentations with 5% petioles displayed yeast-related differences in three (2017), four (geraniol, citronellol, γ-terpinene, and eugenol; 2018), and eight terpenes (2019), and three (ethyl heptanoate, isoamyl acetate, and isoamyl hexanoate; 2017), seven (2018), and eight esters (2019) (Figures
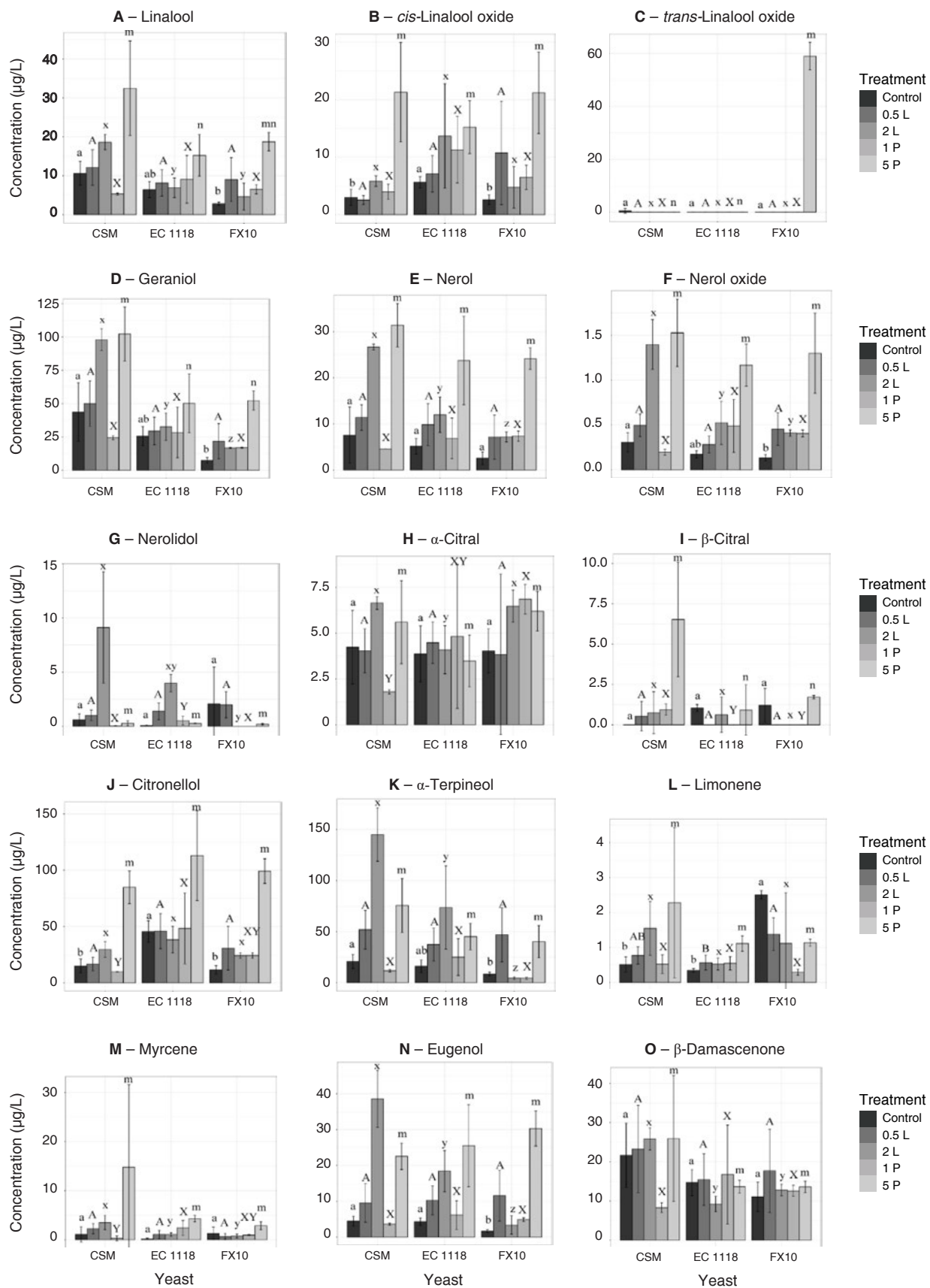
Figure 7 Impact of three yeast strains in combination with five materials other than grapes (MOG) levels on terpene and norisoprenoid concentrations in Cabernet franc wines in 2017. Yeast means within individual MOG levels are significantly different ($p < 0.05$, Duncan's multiple range test) if labeled with different letters. Adapted with permission from Wang et al. 2020.
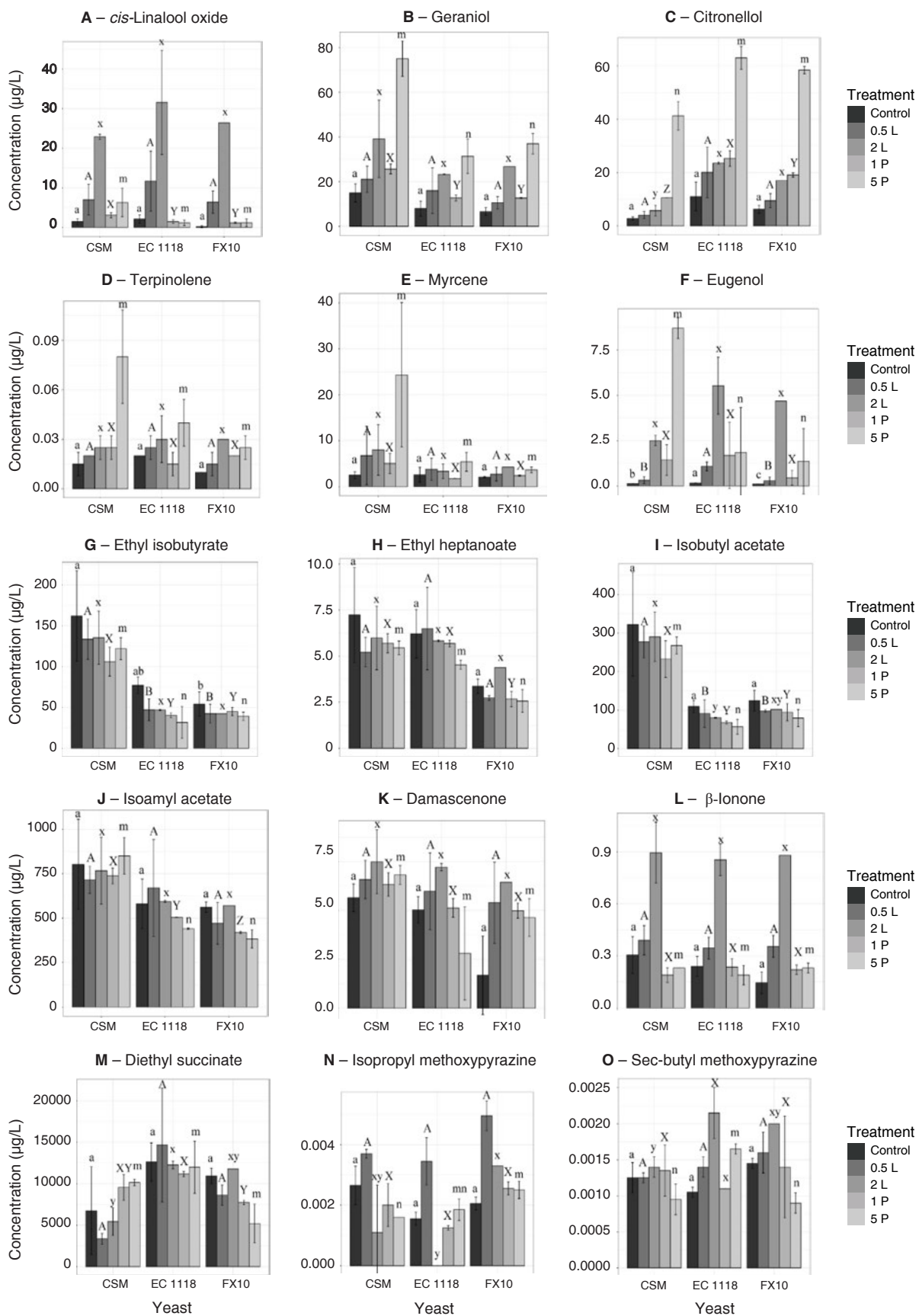
Figure 8 Impact of three yeast strains in combination with five materials other than grapes (MOG) levels on aroma compound concentrations in Cabernet franc wines in 2018. Yeast means within individual MOG levels are significantly different ($p < 0.05$, Duncan's multiple range test) if labeled with different letters.
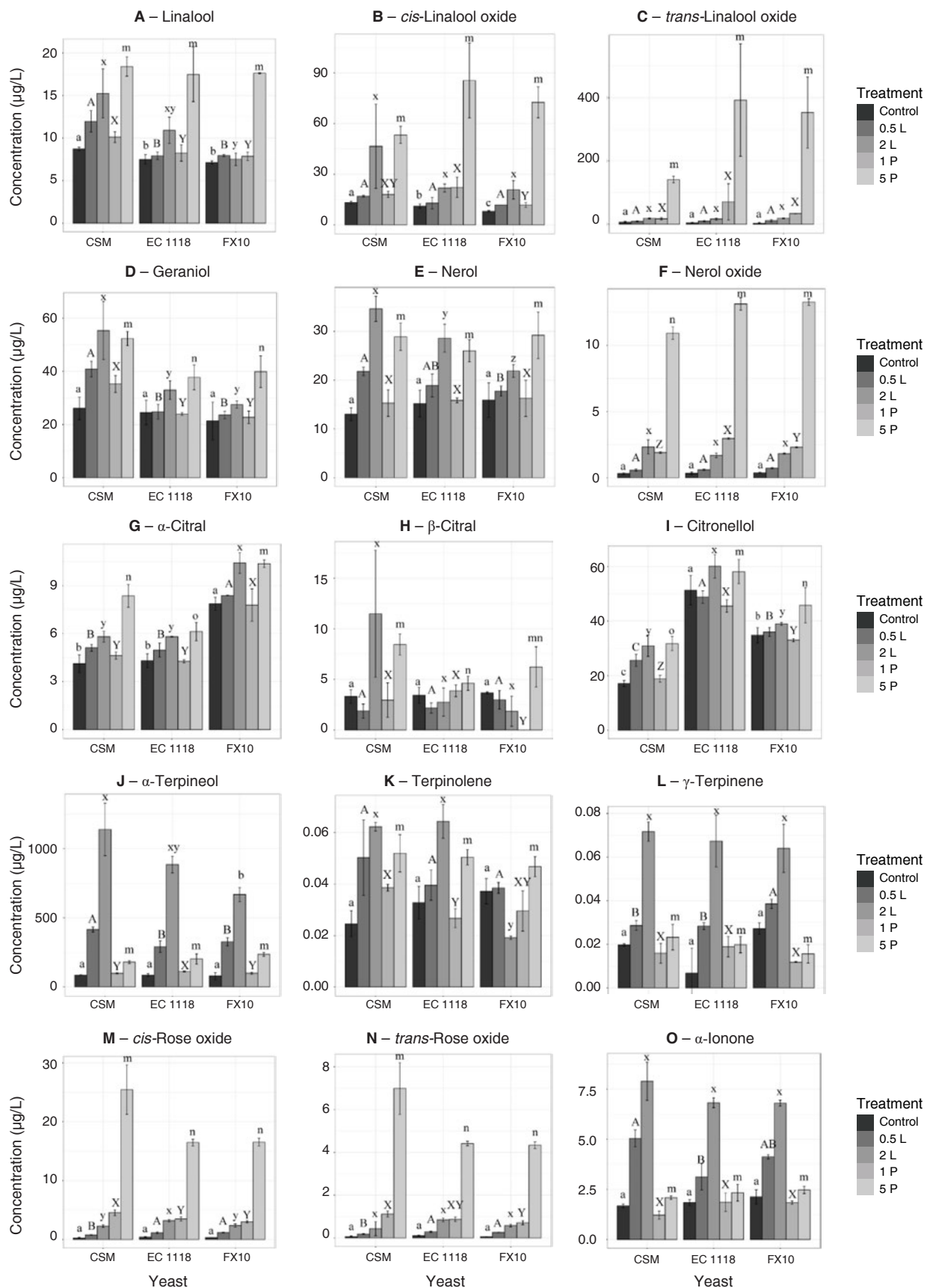
Figure 9 Impact of three yeast strains in combination with five materials other than grapes (MOG) levels on aroma compound concentrations in Cabernet franc wines in 2019. Yeast means within individual MOG levels are significantly different ($p < 0.05$, Duncan's multiple range test) if labeled with different letters.

7 to 9 and Supplemental Tables 8, 13, and 18). Additionally, two alcohols (isoamyl alcohol and heptanol) were impacted in 2017 and three (isobutanol, isoamyl alcohol, and phenylethyl alcohol) in 2019. Other responsive compounds included methyl and ethyl salicylate (2019) and both IPMP and SBMP (2018) (Figures 7 to 9 and Supplemental Tables 8, 13, and 18).

For fermentations containing 2% leaves, CSM yeast produced wines with the highest concentrations of 17 of 19 compounds in 2017, including three esters (ethyl heptanoate, isobutyl acetate, and isoamyl acetate) and all terpenes, norisoprenoids, and salicylates quantified (Supplemental Table 6). In 2018, CSM yeast did not produce wines with the highest concentrations of any of the seven affected compounds (Supplemental Table 11). However, in 2019, wines produced with CSM yeast had the highest concentrations of 17 of 22 compounds, including seven esters, two alcohols (isobutanol and isoamyl alcohol), and six terpenes (linalool, geraniol, nerol, α-terpineol, terpinolene, and myrcene), plus methyl and ethyl salicylate (Supplemental Table 16). For fermentations containing 5% petioles, CSM yeast produced wines with the highest concentrations of 4 of 8 compounds in 2017, including two esters (ethyl heptanoate and isoamyl acetate) and two terpenes (geraniol and β-citral) (Supplemental Table 8). In 2018, CSM yeast produced wines with the highest concentrations of 10 of 13 compounds, including six esters (ethyl isobutyrate, ethyl octanoate, isobutyl acetate, isoamyl acetate, hexyl acetate, and isoamyl hexanoate), one alcohol (isobutanol), and three terpenes (geraniol, γ-terpinene, and eugenol) (Supplemental Table 13). In 2019, wines produced with CSM yeast had the highest concentrations of 16 of 21 compounds, including seven esters, two alcohols (isobutanol and isoamyl alcohol), five terpenes (geraniol, β-citral, *cis*-rose oxide, *trans*-rose oxide, and eugenol), and methyl and ethyl salicylate (Supplemental Table 18).

As with differences between MOG levels, the effects of specific yeast strains in mitigating floral taint characteristics is of crucial importance. Yeasts that reduced responsive compounds to concentrations below their sensory threshold are of particular interest. In 2017, compounds with concentrations reduced to below threshold by specific yeasts were ethyl heptanoate (2 L: FX10), isobutyl acetate (2 L: EC 1118 and FX10), linalool (2 L: EC 1118 and FX10), and geraniol (0 L: EC 1118 and FX10; 2 L: FX10) (Figure 7 and Supplemental Tables 4 to 8). In 2018, the reduced compounds were: isobutyl acetate (5 P: EC 1118), hexyl acetate (5 P: EC 1118 and FX10), and SBMP (5 P: EC 1118) (Figure 8 and Supplemental Tables 9 to 13). In 2019, the reduced compounds were: linalool (2 P: EC 1118 and FX10), geraniol (0.5 L and 1 P: EC 1118 and FX10; 2 L: FX10), α-terpineol (0.5 L: EC 1118), and eugenol (1 P: EC 1118 and FX10) (Figure 9 and Supplemental Tables 14 to 18). It is worthy of note that in all cases where concentrations were reduced to below sensory threshold, either EC 1118 or FX10 yeasts, or both, were responsible.

Impacts of both MOG levels and yeast strain on TA, pH, ethanol, color, anthocyanins, and phenols were minimal (Supplemental Table 19). In 2017, there was a tendency for pH to increase and color and anthocyanins to decrease relative to increased leaf level. Increased petioles led to increased pH and decreased ethanol and reduced TA and color. In 2018, there was a tendency towards reduced color relative to leaf level and tendencies for TA to decrease and pH to increase relative to petiole level. In 2019, there were tendencies toward TA and pH increases and color and anthocyanin decreases with increased leaves, and for increased pH and phenols with increased petioles.

Differences among yeasts with respect to ester production are well documented. Specific strains are reported to produce different concentrations of specific esters, organic acids, and higher alcohols (e.g., Soles et al. 1982, Delteil and Jarry 1992, Dumont 1994, Nurgel et al. 2002, Vilanova et al. 2007, Molina et al. 2009). Fourteen yeast strains produced white wines with different ester concentrations, including ethyl hexanoate, ethyl octanoate, ethyl decanoate, isoamyl acetate, amyl acetate, hexyl acetate, and 2-phenylethyl acetate (Soles et al. 1982). Chardonnay wines fermented with K1M (Lallemand) contained higher concentrations of ethyl dodecanoate, isoamyl acetate, and total esters, while those fermented with D47 (Lallemand) contained higher concentrations of ethyl hexanoate and ethyl octanoate (Delteil and Jarry 1992). Lallemand *S. cerevisiae* strains VL1 and CEG (Epernay 2) were compared in Riesling fermentations and EC 1118 versus D47 in Chardonnay, and produced some apparent differences in esters, alcohols, and acids (Dumont 1994). In a subsequent study, the EC 1118 strain led to higher solvent, fatty, and pineapple aroma attributes attributable to ethyl hexanoate, ethyl octanoate, and phenylethanol, while the VIN13 strain led to higher banana, fruity, yeasty, and green attributes attributable to 3-methyl butyl acetate, 2-methyl propionic acid, and 3-methyl butanoic acid (Molina et al. 2009). Mencía wines produced from several indigenous *S. cerevisiae* yeasts varied substantially in ester, alcohol, and acid concentrations (Blanco et al. 2014), as did Bordeaux wines (Gammacurta et al. 2014). In an investigation using synthetic juice media, UCD 522 yeast produced more isobutanol, amyl alcohol, and phenylethanol across several yeast assimilable nitrogen (YAN) and pantothenic acid levels, and hexanoic acid and ethyl hexanoate at lower YAN and pantothenate, while EC 1118 produced more octanoic and decanoic acids (Wang et al. 2003). In a rare instance where yeast strains were selected for low ester production, VIN 13, VIN 7, NT 116, and NT 7 were compared, with the highest ester concentrations produced by NT 116 and the lowest by VIN 7 (Marais 2001). VIN 7 wines were preferred, since the thiol-based Sauvignon blanc varietal typicity was more pronounced due to lack of masking by esters.

Yeast strains also vary in their capacity to produce monoterpenes. Monoterpenes occur as both free aglycones and glycosides and were previously considered to be primarily grape-derived (Mateo and Jimenez 2000). Aglycones can be produced as a result of β-glucosidase activity, which cleaves the disaccharide moiety, releasing the free monoterpene (Carrau et al. 2005). Yeast strains vary in β-glucosidase activity (Bisotto et al. 2015), although this activity is relatively low at wine pH (Delcroix et al. 1994, Tate et al. 2006, Cordente et al. 2012). However, it is noteworthy that yeasts can also

synthesize terpenes, through both the mevalonic acid and methylerythritol pathways (Carrau et al. 2005, 2017). Among three *Saccharomyces* races, more linalool, nerolidol, and farnesol were found in wines fermented by the *S. bayanus* and *S. capensis* races (Zea et al. 1995). Five *S. cerevisiae* strains (Montrachet 522; CIVC 8130; and Uruguayan native isolates 881, 882, and KU1), plus a Uruguayan native strain of *H. uvarum*, were compared (Carrau et al. 2005). Strain KU1 produced the most linalool; M522 and KU1, the most terpineol; and *H. uvarum*, the most geraniol (Carrau et al. 2005). Among eight yeast strains (*S. cerevisiae* strains QA23, EC 1118, K1M, CEG, S6U and DV10; Biodiva [*Torulaspora delbrueckii*]; and Flavia [*Metschnikowia pulcherrima*]) used to ferment Muscat musts, CEG and Flavia produced the most terpenes and EC 1118, the lowest (Bisotto et al. 2015). Only Flavia had noteworthy glycosidase activity.

EC 1118 appeared to be a producer of high concentrations of ethyl nonanoate (five instances across all leaf and petiole levels and three seasons), citronellol (eight instances), and eugenol (four instances). FX10 appeared to be a high α-citral producer (five instances). These differences are likely due to yeast capacity to synthesize terpenes, rather than to their activity on glycosides originating from both fruit and MOG, since (1) these patterns were apparent in the non-MOG controls as well as fermentations to which MOG was added; (2) all fruit was hand-harvested, so release of aglycones due to enzymatic activity in harvested fruit was unlikely; and (3) activity of β-glycosidases at wine pH is relatively low and does not vary substantially among *S. cerevisiae* strains (Delcroix et al. 1994, Tate et al. 2006, Bisotto et al. 2015).

Conclusions

Incorporation of frozen MOG to fermentations elevated concentrations of terpenes, norisoprenoids, and salicylates associated with floral taint. Specific yeast strains may diminish this problem. Yeast strain CSM fermented wines with more terpenes, norisoprenoids, esters, and alcohols than those fermented with either EC 1118 or FX10. However, EC 1118 appeared to be a producer of high concentrations of ethyl nonanoate, citronellol, and eugenol across all leaf and petiole levels and three seasons, while FX10 appeared to be a high α-citral producer. In some cases, EC 1118 and FX10 successfully reduced concentrations of some compounds to below sensory thresholds. It therefore seems possible to select specific yeast strains under these circumstances to reduce the floral taint associated with incorporation of frozen MOG into red wine fermentations.

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