Influence of deficit irrigation and kaolin particle film on grape composition and volatile compounds in Merlot grape (*Vitis vinifera* L.)

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A R T I C L E   I N F O

A B S T R A C T

The effect of deficit irrigation and a kaolin-based, foliar reflectant particle film (PF) on grape composition and volatile compounds in Merlot grapes was investigated over two growing seasons in semi-arid, south-western Idaho. Vines were provided with differential amounts of water based on their estimated crop evapotranspiration (ET_c) throughout berry development, and particle film was applied to half of the vines in each irrigation main plot. Free and bound volatile compounds in grapes were analyzed using stir bar sorptive extraction–gas chromatography–mass spectrometry (SBSE–GC–MS). The concentrations of free C_6 compounds (hexanal, *trans*-2-hexenal, and 1-hexanol) decreased, and bound terpene alcohols (nerol and geraniol) and C_{13}-norisoprenoids (β-damascenone, 3-hydroxy-β-damascenone, 1,1,6-trimethyl-1,2-dihydronaphthalene, and 3-oxo-α-ionol) increased in berries each year in response to severity of vine water stress. Concentrations of C_{13}-norisoprenoids and bound forms of nerol and geraniol were positively correlated with their concentrations in the corresponding wines. Particle film application had minimum effect on free and bound volatile composition in the grapes, and there was no interactive effect between particle film and deficit irrigation. However, particle film application enhanced the total amount of berry anthocyanins.

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1. Introduction

Grape-derived volatile compounds are the secondary metabolites of grape vine, and are considered to be the most important factor for grape and wine quality. These secondary metabolites are found in a wide range of concentrations and chemical classes (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2000; Sefton, 1998). Grape volatiles can exist as free form, but a large percentage exists in the grape as nonvolatile precursors, binding to sugars or amino acids (Ribéreau-Gayon et al., 2000). Bound volatiles are potential aromas, they can be converted to the free volatiles through enzyme or chemical hydrolysis during the vinification and ageing process, and contribute to wine aroma (Ibarz, Ferreira, Hernandez-Orte, Loscos, & Cacho, 2006).

The concentrations of volatile compounds and precursors in grape berries are highly influenced by viticultural practices, such as vine training (Jackson & Lombard, 1993), cluster thinning (Reynolds et al., 2007), leaf removal (Kwasniewski, Vanden Heuvel, Pan, & Sacks, 2010; Reynolds et al., 2007), and water management (Bindon, Dry, & Loveys, 2007; Deluc et al., 2009; Jackson & Lombard, 1993). Among these cultural practices, deficit irrigation,

aimed at improving water use efficiency and reducing canopy vigour (Shellie & Glenn, 2008), is an important practice for sustainable agriculture, especially in arid and semi-arid areas (Chaves et al., 2007; Koundouras, Marinos, Gkoulioti, Kotseridis, & van Leeuwen, 2006).

Imposing a water deficit to the vine during berry development is an important vineyard management strategy to alter grape and wine quality. Previous studies have shown that water deficit influenced physiological parameters of the vine (Iacono, Buccella, & Peterlunger, 1998), changed berry composition (Bindon, Dry, & Loveys, 2008; Esteban, Villanueva, & Lissarrague, 1999), and improved the sensory attribute of wines by increasing fruity aroma and decreasing vegetal aromas (Chapman, Roby, Ebeler, Guinard, & Matthews, 2005). Although many literatures have reported the effect of vine water status on grape derived volatiles and their precursors (Bindon et al., 2007; Koundouras et al., 2006, 2009; Peyrot des Gachons et al., 2005), the results are still inconclusive due to the variations in irrigation regimes, cultivars and other agronomical conditions. In general, water deficit increases C_{13}-norisoprenoids (Bindon et al., 2007; Koundouras et al., 2009) and glycosylated volatile compounds. Although it is possible that water deficit induced volatile increase is merely due to reduction in berry size (Koundouras et al., 2009), it has been demonstrated that increases in the concentration of C_{13}-norisoprenoids were

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independent of berry size (Bindon et al., 2007). Furthermore, it has been shown that water deficit significantly affected the transcripts and pathways associated with the production of volatile compounds (Deluc et al., 2009).

Water deficit reduces canopy size, which subsequently alters the canopy microclimate and increases cluster exposure to sunlight (Intrigliolo & Castel, 2010). Severe water deficit can result in excessive cluster exposure, and lead to supra-optimal berry temperature and sunburn, especially in warm, semi-arid production regions with high solar radiation (Shellie, 2006; Spayd, Tarara, Mee, & Ferguson, 2002), and have a negative effect on grape quality (Peyrot des Gachons et al., 2005).

Kaolin, a white, inert clay mineral, was used as a base to develop a particle film for foliar application to inhibit insect damage in tree fruit and has since been found to reduce heat stress in plants by reflecting infrared (IR) radiation (Glenn & Puterka, 2005). Kaolin particle film (PF) has been reported to reduce leaf and fruit tissue temperature of vines under water deficit (Glenn, Cooley, Walker, Clingeleffer, & Shellie, 2010; Shellie & Glenn, 2008). While PF application did not eliminate heat stress under the most extreme environmental conditions, berry composition data at harvest suggested that the film increased vine capacity (Cooley, Glenn, Clingeleffer, & Walker, 2006; Shellie & Glenn, 2008). In addition, water deficit altered the volatile composition and sensory properties of the wine (Ou, Du, Shellie, Ross, & Qian, 2010). However, it will be advantageous to directly study the volatile and the precursor compositions in the grapes to eliminate the volatile profile modification during vinification. The objective of this study was to investigate effects of vine water deficit and PF on grape quality, so the results can be used to develop a vineyard irrigation guide to conserve water usage in a semi-arid climate.

2. Materials and methods

2.1. Chemicals

Hexanal, *trans*-2-hexenal, heptanal, octanal, nonanal, decanal, 1-hexanol, benzyl alcohol, 2-phenylethanol, 1-octen-3-ol, linalool, geraniol, nerol, 2-methoxy-4-vinylphenol, *cis*-furan-linalool oxide, *trans*-furan-linalool oxide, and β-ionone were purchased from Sigma–Aldrich Chemical Co. (Milwaukee, WI). 1-Heptanol, 1-octanol, and 1-decanol were supplied by Eastman Chemical Products, Inc. (Kingsport, TN). *trans*-2-Octenal was purchased from Compagnie Parento. Inc. (Lenoir, NC). Benzaldehyde was obtained from Polyscience Corp. (Niles, IL). 4-Terpineol was obtained from TCI Japan (Tokyo, Japan). α-Terpineol, 4-octanol were from K & K Laboratories (Jamaica, NY). Geranyl acetone, β-damascenone, and 3-hexanone were supplied by Hoffman-LaRouche (Nutley, NJ), Firmenich (Princeton, NJ) and J.T. Baker Chemical Co. (Phillipsburg, NJ), respectively. Citric acid was purchased from Lancaster (Ward Hill, MA), and Macer® 8 FJ enzyme solution was provided by Biocatalysts Limited (Wales, UK). All standard stock solutions were prepared in methanol individually. The internal standard mixture was composed of 3-hexanone and 4-octanol with concentrations of 9.4, 2.4 mg/l and stored at −15 °C.

2.2. Plant material and field procedure

The field study was carried out in a commercial vineyard of *Vitis vinifera* L. cv. Merlot located near Nampa, ID (lat: 43°2′ N, long: 116°42′ W, elevation 810 m) during two consecutive years (2007 and 2008). The own-rooted vines were planted in 1997 on Scism calcareous silt loam with a water-holding capacity of 0.19 cm/cm soil. Vine rows were oriented north to south with row-by-vine spacing of 2.4 by 1.8 m. Vines were cordon trained and spur pruned

on a vertical trellis and managed according to standard practice for the region, as described by Shellie (2006).

The experimental design was a four by two factorial with four amounts of irrigation applied to vines that did or did not receive foliar application of a kaolin-based particle film (PF) (Surround WP; Engelhard Corp. Iselin, NJ) with four replicate blocks. PF was applied as a split-plot within each irrigation main plot. Each main plot contained four rows of 14 vines per row and was equipped with independently controlled water delivery via an above-ground drip [two emitters (2 l/h) per vine]. The vines in one half of each irrigation main plot (23 vines) received a foliar application of PF at a concentration of 60 g/l, as described by Shellie and Glenn (2008). The plots were irrigated weekly from fruit set until harvest with 35%, 70%, or 100% of their estimated evapotranspiration (ET_c) or 35% ET_c until veraison followed by 70% ET_c until harvest. These irrigation regimes are hereafter referred to as 35%, 70%, 100%, and 35–70% ET_c. ET_c was calculated using the Pennman-Monteith model (Allen, Pereira, Raes, & Smith, 1998) and alfalfa as reference evapotranspiration (ET_r), which was obtained weekly from the US Bureau of Reclamation Nampa weather station (http://www.usbr.govgov/pn/agrimet/wxdata.html). PF was not applied to plots under 35% ET_c in 2007 due to cold injury during the previous winter. Vine water status was monitored by measuring leaf water potential at midday (Ψ_{md}) on the sixth day after an irrigation event using a pressure chamber.

2.3. Grape samples

Fruit was harvested from vines in the middle two rows of each trial plot when a randomly collected sample of berries reached approximately 24 °Brix. Two kilograms of harvested clusters were randomly selected from each plot of harvested fruit, chilled to 10 °C during transportation and stored at −23 °C until analysis. Berries used for volatile analyses were randomly hand-picked from frozen clusters on the day of analysis. Samples from vines under 70% ET_c were not analyzed in 2008.

2.4. Determination of berry fresh weight, sugar, pH and titratable acidity in grape juice

Sample preparation and analysis followed the procedures described by Shellie and Glenn (2008). Individual berry fresh weight was measured by the weight of 100 berries. Total soluble solid (TSS) was measured as °Brix using a digital refractometer. Juice pH and titratable acidity (TA) was measured in sequence with an automatic titrator.

2.5. Determination of total anthocyanins (TAC) and total phenols (TP)

Extraction of anthocyanins and phenols of whole berries followed the procedures described by Rodriguez-Saona and Wrolstad (2001). The amount of TP was determined with the Folin–Ciocalteu colorimetric method, and expressed as milligrams of gallic acid per kilogram of berries (Ribéreau-Gayon et al., 2000). The content of TAC was determined using pH differential method (Lee, Durst, & Wrolstad, 2005) and calculated as cyanidin-3-glucoside (molar extinction coefficient of 26,900 l/cm/mol and molecular weight of 449.2 g/mol was used).

2.6. Volatile compounds analysis

2.6.1. Grape free volatile extraction with SBSE

Randomly selected whole berry grape samples (∼60 g) were ground under liquid nitrogen using a blender (Waring Product Div., Dynamics Corp. of America, New Hartford, CT) for 30 s. Fifty grams of the obtained powder was suspended in 50 ml of citrate

buffer solution (0.2 M, pH 3.2) and 30% of NaCl (final concentration) and was allowed to macerate in the dark for 24 h at 25 °C under a nitrogen atmosphere. The mixture was then centrifuged at 7500 rpm for 30 min. The supernatant was filtered through a Whatman No. 1 filter paper (particle retention > 11 μm), followed by a VWR 413 filter paper (particle retention > 5 μm). The filtered clear juice was used for both free and bound volatile analysis.

For free volatile analysis, 20 ml of extracted juice was added into a 20 ml vial that had 20 μl of the internal standard solution. The sample was extracted with a preconditioned stir bar (Twister) coated with polydimethylsiloxane (PDMS) phase (1 cm length, 0.5 mm thickness, Gerstel Inc., Baltimore, MD) for 3 h at a speed of 1000 rpm. After extraction, the Twister bar was rinsed with distilled water, dried with tissue paper, and used for GC–MS analysis.

2.6.2. Bound volatile analysis in grape juice

Bound volatiles were isolated from grape juice according to the procedure described by Du, Finn, and Qian (2010) with minor modification (Du et al., 2010). Twenty millilitres of extract was loaded onto the C_{18} cartridge preconditioned with 10 ml of methanol followed by 10 ml of distilled water. The cartridge was washed with 20 ml of distilled water, followed by 20 ml of dichloromethane, to remove sugar, organic acid, and free volatiles. The bound fraction was eluted from the cartridge into a 40 ml vial with 6 ml of methanol. The eluent was evaporated to dryness in a rotary evaporator at 45 °C under vacuum. Twenty millilitres of citrate buffer solution and 100 μl of Macer® 8 FJ enzyme solution were added to the dried glycoside extract. The mixture was incubated at 45 °C for 20 h. After addition of 6 g NaCl and 20 μl of internal standard solution, the mixture was extracted with a Twister® bar as described previously.

2.6.3. GC–MS analysis

GC–MS analyses were performed using an Agilent 6890 gas chromatograph with a 5973 mass selective detector (Agilent, Wellington, DE) as described previously by Ou et al. (2010). Samples were transferred into a thermal desorption unit (TDU, Gerstel), and the TDU was programmed at a rate of 100 °C/min from 25 °C to a final temperature of 250 °C and held for 2 min. The TDU injection was in splitless mode during thermal desorption, while the programmed temperature vapouriser injector (PTV, Gerstel) was in a solvent vent mode with a venting flow of 50 ml/min for 4.7 min, at a venting pressure of 19.35 psi. After the solvent vent, the PTV was switched to splitless mode. The initial temperature of the PTV was kept at −80 °C for 0.2 min then ramped at a rate of 10 °C/s to a final temperature of 250 °C and held for 10 min.

Compounds were separated with a HP-5 column (60 m × 0.32 mm i.d., 0.25 μm film thickness, (Agilent, Wellington, DE)). The oven temperature was programmed at 40 °C for 2 min

of holding and then increased to 220 °C at a rate of 4 °C/min and to 250 °C at a rate of 8 °C/min and held for 6 min. A constant helium flow of 2.5 ml/min was used. A column splitter was used at the end of column, 1 ml/min column flow was introduced to the MS, and the other 1.5 ml/min was vented out. The MS transfer line and ion source temperature were 280 and 230 °C, respectively. Electron impact mass spectrometric data from m/z 35–350 were collected, with an ionization voltage of 70 eV. LRIs were calculated after analyzing C_7–C_{20} n-alkane series (Supelco, Bellefonte, PA) under the same chromatographic conditions.

Individual standard stock solution was mixed and then diluted with methanol to a serial concentration to set up the calibration curve. Standard solution (20 μl) and internal standard solution (20 μl) were added to 20 ml of citrate buffer with 6 g NaCl added. Volatiles were then extracted using the same procedure as described for grape samples. Standard calibration curves were obtained through Enhanced ChemStation Software (Agilent Technologies Inc.), and were used to calculate the concentrations of volatile compounds in the samples.

2.7. Statistical analysis

Mean values were calculated and treatment differences were tested by two-way analysis of variance with irrigation amount and PF as the main factors using SPSS (version 16.0; Chicago, IL). Duncan's multiple range test was used for post hoc comparison of significant irrigation treatment means. Linear regression analysis was also carried out between the concentrations of volatile compounds in grapes and those in wines.

3. Results and discussion

3.1. Vintage and berry attributes

Accumulated growing degree days (GDD) in 2007 was higher than in 2008, due to warmer daily average temperature early season prior to veraison (Table 1). Precipitation and ET_r in 2007 was higher than in 2008. Average daily solar radiation postveraison during fruit ripening was higher in 2008 than in 2007. The weather patterns during the years of this study were typical of the climate of southwestern Idaho, which is characterized as a warm, arid to semi-arid, mid-latitude steppe, suitable for cultivation of *V. vinifera* grapes such as Merlot (Shellie, 2006).

The influence of irrigation amount and PF on berry fresh weight, total soluble solids (TSS), pH, and titratable acidity (TA), the common indicators of berry maturity, was presented in Table 2. Irrigation amount significantly affected berry fresh weight, TSS, TA, and pH, whereas PF had no significant main or interactive effects, with the exception of TA in 2007. Fully irrigated vines produced grapes

Table 1
Monthly weather data during the years of this study at the field site location in south-west Idaho.[a]

	Year	April	May	June	July	August	September	October	Total
Growing degree days (°C)	2007	61.7	201.0	285.8	484.3	373.3	195.5	34.1	1636
	2008	23.9	159.3	255.4	141.1	383.1	204.9	60.3	1501
Daily average temperature (°C)	2007	10.6	16.4	19.6	25.2	21.6	16.1	9.8	–
	2008	7.7	14.9	18.4	23.2	22.1	16.4	10.2	–
ET_r April 1–October 31 (mm)[b]	2007	146.1	242.6	245.1	275.8	234.4	154.9	88.4	1387.3
	2008	148.3	212.6	249.9	265.9	207.0	129.5	88.6	1302.0
Precipitation (mm)	2007	19.8	6.9	16.3	0.0	8.9	38.9	12.7	103.4
	2008	4.6	14.7	23.4	7.1	1.0	25.1	7.6	83.6
Daily solar radiation (J/cm²)	2007	2091	2692	2795	2613	2357	1834	1069	–
	2008	2138	2489	2714	2851	2479	1931	1219	–

[a] Growing degree days calculated as the sum of daily average temperature above 10 °C with no upper temperature limit using US Bureau of Reclamation Nampa weather station (http://www.usbr.gov/pn/agrimet/wxdata.html).
[b] ET_r, reference evapotranspiration.

Table 2
Berry composition of Merlot grape harvest in 2007 and 2008 from vines subjected to different irrigation levels with particle film treatment (+PF) or without (−PF) in south-west Idaho.

Compound	PF	Irrigation level				p Value[a]		
		100% ET_c	70% ET_c	35–70% ET_c	35% ET_c	I	PF	I * PF
2007								
Berry fresh weight (g)	+PF	1.02 ± 0.05	0.86 ± 0.10	0.71 ± 0.09		<0.001	ns	ns
	−PF	0.95 ± 0.08	0.87 ± 0.09	0.71 ± 0.10	0.76 ± 0.10			
TSS (°Brix)[c]	+PF	23.4 ± 0.1	24.0 ± 0.5	24.6 ± 0.3	nc[b]	<0.001	ns	ns
	−PF	23.2 ± 0.1	23.6 ± 0.4	24.8 ± 0.2	23.5 ± 0.4			
TA (g/L)[d]	+PF	6.53 ± 0.35	5.10 ± 0.20	4.63 ± 0.31	nc	<0.001	ns	<0.001
	−PF	6.53 ± 0.21	6.17 ± 0.65	4.20 ± 0.10	4.90 ± 0.36			
pH	+PF	3.53 ± 0.06	3.70 ± 0.10	3.87 ± 0.12	nc	<0.001	ns	ns
	−PF	3.50 ± 0.00	3.60 ± 0.00	3.83 ± 0.06	3.67 ± 0.06			
Maturity index[e]	+PF	3.59 ± 0.20	4.71 ± 0.23	5.33 ± 0.42	nc	<0.001	ns	<0.01
	−PF	3.55 ± 0.13	3.86 ± 0.35	5.90 ± 0.18	4.82 ± 0.44			
2008								
Berry fresh weight (g)	+PF	1.25 ± 0.11	nc	0.90 ± 0.11	0.96 ± 0.11	<0.003	ns	ns
	−PF	1.19 ± 0.10	nc	0.88 ± 0.12	0.95 ± 0.08			
TSS (°Brix)	+PF	23.8 ± 0.2	nc	24.8 ± 0.2	25.4 ± 0.3	<0.001	ns	ns
	−PF	23.3 ± 0.5	nc	25.2 ± 1.1	25.4 ± 0.4			
TA (g/L)	+PF	7.40 ± 0.02	nc	4.33 ± 0.25	4.17 ± 0.90	<0.001	ns	ns
	−PF	7.57 ± 0.15	nc	4.23 ± 0.15	3.53 ± 0.06			
pH	+PF	3.51 ± 0.07	nc	3.61 ± 0.02	3.69 ± 0.02	<0.001	ns	ns
	−PF	3.49 ± 0.07	nc	3.68 ± 0.01	3.70 ± 0.07			
Maturity index	+PF	3.21 ± 0.06	nc	5.73 ± 0.12	6.26 ± 1.15	<0.001	ns	ns
	−PF	3.08 ± 0.12	nc	5.95 ± 0.47	7.18 ± 0.22			

[a] p Values for statistical significance according to ANOVA test (I = irrigation; PF = particle film; I * PF = interactive effect; ns: not significant) ($p > 0.05$).
[b] nc, Data not collected.
[c] TSS, total soluble solid.
[d] TA, reported as tartaric acid.
[e] Maturity index = TSS/TA.

Table 3
The concentrations (average ± SD) of total phenols (TP) and total anthocyanins (TAC) of Merlot grape harvested in 2007 from vines subjected to different irrigation levels with particle film treatment (+PF) or without (−PF) in south-west Idaho.

Compound	PF	Irrigation level				p Value[a]		
		100% ET_c	70% ET_c	35–70% ET_c	35% ET_c	I	PF	I * PF
TAC (mg/kg)[b]	+PF	298 ± 30	323 ± 42	314 ± 22	nc[c]	0.038	0.043	ns
	−PF	251 ± 36	292 ± 16	301 ± 20	328 ± 30			
TP (g/kg)[d]	+PF	5.03 ± 0.30	5.69 ± 0.38	5.60 ± 0.08	nc	ns	ns	ns
	−PF	5.37 ± 0.33	5.59 ± 0.47	5.92 ± 0.61	5.64 ± 0.08			

[a] p Values for statistical significance according to ANOVA test (I = irrigation; PF = particle film; I * PF = interactive effect; ns: not significant) ($p > 0.05$).
[b] TAC reported as cyanidin-3-glucoside.
[c] nc, Data not collected.
[d] TP expressed as gallic acid equivalents.

with the largest berry fresh weight, lowest TSS, highest TA, and lowest pH in both years. The 35–70% ET_c or 35% ET_c treatments significantly decreased berry fresh weight by 23–29% in the 2 years, in concordance with the previous results (Koundouras et al., 2009). Higher TSS in grapes under water deficit could be related to the indirect effects of water stress. However, in 2007, vines under severe water deficit (vines that received 35% ET_c) had similar TSS compared with fully irrigated vines. This could be due to cold injury in the deficit irrigated vines that was sustained the previous winter or impaired photosynthesis due to water deficit. Reduced TA and increased pH under water deficit is in agreement with previous reports, and could be attributed to a reduction in malic acid (Esteban et al., 1999; Koundouras et al., 2006). In general, water deficit accelerated grape ripening, while PF application had no effect.

Total phenol (TP) and total anthocyanins (TAC) in grapes harvested in 2007 were presented in Table 3. Neither irrigation amount nor PF effected TP content, which was in agreement with the result of Bindon et al. (2008), while a previous study showed a clear positive effect of water deficit on berry phenolic composition (Koundouras et al., 2006). The content of TAC in grape berry from vines with PF treatment was higher than that from vines without PF treatment within each irrigation regime. In particular, PF application under 100% ET_c treatment resulted in 19% higher TAC in grape berries than those without PF application. The increased TAC could be due to the reduction of fruit tissue temperature that resulted from PF treatment (Glenn et al., 2010; Shellie & Glenn, 2008) since it has been demonstrated that high temperature of fully exposed berries can reduce or even inhibit the synthesis of anthocyanins (Spayd et al., 2002). Furthermore, irrigation level had a significant effect on TAC with or without PF applications. The grape berries from vines that received 70%, 35–70%, or 35% ET_c had 12%, 12%, and 19% higher concentrations of TAC as compared to fully irrigated vines, respectively, and a significant difference was observed between 100% and 35% ET_c treatments ($p < 0.05$). As anthocyanins are mainly located in berry skins, smaller berries resulted from water stress, having a larger surface to volume ratio, could be in part responsible for the higher TAC content (Roby, Harbertson, Adams, & Matthews, 2004). The increased TAC might also be attributed to higher levels of synthesis caused by water deficit (Koundouras et al., 2009).

Table 4
Free volatile compounds (μg/kg ± SD) in Merlot grapes harvested in 2007 from vines subjected to different irrigation levels with particle film treatment (+PF) or without (−PF) in south-west Idaho.

Compound	LRI[b]	PF	Irrigation level				p Value[a]		
			100% ET_c	70% ET_c	35–70% ET_c	35% ET_c	I	PF	I ∗ PF
C_6 compounds									
Hexanal	801	+PF	45.45 ± 4.78	37.96 ± 8.27	37.32 ± 2.14	nc[c]	0.050	ns	ns
		−PF	59.52 ± 13.91	36.75 ± 6.64	35.19 ± 5.89	37.75 ± 5.51			
trans-2-Hexenal	855	+PF	79.62 ± 9.14	51.89 ± 16.42	50.54 ± 6.71	nc	<0.001	ns	ns
		−PF	86.67 ± 16.25	48.72 ± 5.12	45.98 ± 8.25	50.38 ± 1.69			
1-Hexanol	869	+PF	23.75 ± 8.53	12.19 ± 9.19	9.44 ± 5.64	nc	0.013	ns	ns
		−PF	18.56 ± 11.80	5.22 ± 2.18	3.80 ± 0.77	8.03 ± 2.44			
Aldehydes and alcohols									
Heptanal	903	+PF	0.63 ± 0.05	0.56 ± 0.07	0.60 ± 0.08	nc	ns	ns	ns
		−PF	0.59 ± 0.04	0.55 ± 0.05	0.61 ± 0.06	0.60 ± 0.05			
Octanal	1003	+PF	0.04 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	nc	ns	ns	ns
		−PF	0.03 ± 0.02	0.04 ± 0.04	0.03 ± 0.02	0.03 ± 0.01			
trans-2-Octenal	1061	+PF	1.44 ± 0.17	1.85 ± 0.40	1.44 ± 0.14	nc	0.030	ns	ns
		−PF	1.42 ± 0.14	1.65 ± 0.06	1.39 ± 0.27	1.24 ± 0.16			
Nonanal	1106	+PF	0.40 ± 0.26	0.34 ± 0.14	0.23 ± 0.07	nc	ns	ns	ns
		−PF	0.34 ± 0.18	0.27 ± 0.03	0.24 ± 0.05	0.37 ± 0.22			
Decanal	1207	+PF	0.71 ± 0.41	0.80 ± 0.54	0.46 ± 0.08	nc	ns	ns	ns
		−PF	0.64 ± 0.21	0.67 ± 0.17	0.53 ± 0.14	0.62 ± 0.16			
1-Octen-3-ol	979	+PF	1.21 ± 0.10	1.54 ± 0.13	1.30 ± 0.05	nc	ns	0.049	ns
		−PF	1.94 ± 0.42	1.51 ± 0.43	1.42 ± 0.15	1.41 ± 0.31			
1-Octanol	1071	+PF	0.38 ± 0.11	0.54 ± 0.09	0.34 ± 0.06	nc	ns	ns	ns
		−PF	0.31 ± 0.05	0.35 ± 0.18	0.40 ± 0.10	0.37 ± 0.07			
Terpenoids									
Limonene	1034	+PF	Trace	Trace	Trace	nc	–	–	–
		−PF	Trace	Trace	Trace	Trace			
Linalool	1102	+PF	Trace	Trace	Trace	nc	–	–	–
		−PF	Trace	Trace	Trace	Trace			
α-Terpineol	1200	+PF	0.14 ± 0.05	0.12 ± 0.08	0.07 ± 0.06	nc	ns	ns	ns
		−PF	0.09 ± 0.05	0.09 ± 0.05	0.08 ± 0.05	0.09 ± 0.01			
Geranyl acetone	1457	+PF	0.95 ± 0.02	0.95 ± 0.09	0.99 ± 0.04	nc	ns	ns	ns
		−PF	0.94 ± 0.04	0.97 ± 0.04	1.03 ± 0.11	0.98 ± 0.005			
C_{13}-norisoprenoids									
Vitispirane[d,e]	1293	+PF	0.02 ± 0.01	0.02 ± 0.004	0.01 ± 0.003	nc	ns	ns	ns
		−PF	0.01 ± 0.01	0.01 ± 0.002	0.01 ± 0.0003	0.01 ± 0.003			
β-Damascenone	1396	+PF	0.83 ± 0.01	0.85 ± 0.03	0.87 ± 0.04	nc	0.040	ns	ns
		−PF	0.82 ± 0.02	0.83 ± 0.02	0.87 ± 0.03	0.86 ± 0.02			
β-Ionone[f]	1498	+PF	13.66 ± 3.48	15.35 ± 6.53	11.16 ± 2.40	nc	ns	ns	ns
		−PF	10.76 ± 5.35	12.66 ± 0.33	12.72 ± 1.51	10.22 ± 5.23			
Dihydroactinidiolide[d,g]	1542	+PF	0.22 ± 0.01	0.19 ± 0.02	0.18 ± 0.01	nc	ns	ns	ns
		−PF	0.18 ± 0.02	0.22 ± 0.04	0.19 ± 0.01	0.17 ± 0.02			
3-Hydroxy-β-damascenon[e,g]	1631	+PF	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	nc	ns	ns	ns
		−PF	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.002	0.03 ± 0.01			

[a] p Values for statistical significance according to ANOVA test (I = irrigation; PF = particle film; I ∗ PF = interactive effect; ns: not significant) ($p > 0.05$).
[b] LRI, linear retention index calculated on HP-5 capillary column.
[c] nc, Data not collected.
[d] Tentatively identified by using mass spectra data (Wiley 275) and comparison of LRI data to literature values (http://webbook.nist.gov/).
[e] μg/kg Equivalents of β-damascenone.
[f] ng/kg.
[g] Tentatively identified by using mass spectra data.

3.2. C_6 Compounds and other lipid-derived aldehydes and alcohols

Some important free aroma-active compounds, including C_6 aldehydes and alcohols, terpenoids, and C_{13}-norisoprenoids were quantified in Merlot grapes harvested each growing season (Tables 4 and 5). C_6 compounds (hexanal, trans-2-hexenal and 1-hexanol) are the most abundant compounds quantified in this study. Grapes from vines under 100% ET_c had the highest concentrations of C_6 compounds. Deficit irrigation treatments reduced the content of all three C_6 compounds in both of the growing seasons by 23–62%, compared to the 100% ET_c treatment. 1-Hexanol showed the greatest percent reduction in response to water deficit. PF had no main effect, but had an interaction effect with trans-2-hexenal in 2008 of the study where vines with PF under 35% ET_c contained less rather than more trans-2-hexenal.

A reduction in C_6 compounds under water deficit has also been observed by others and has been associated with fruit maturity. It has been demonstrated that the content of C_6 compounds decreased with increasing fruit maturity (Coelho, Rocha, Barros, Delgadillo, & Coimbra, 2007). C_6 compounds are formed by enzymatic oxidation of unsaturated lipids. They are partially responsible for herbaceous and green odours in grape and wine, imparting a negative effect on the final wine (Hashizume & Samuta, 1997). The aldehydes can be transformed to their corresponding alcohols during fermentation (Joslin & Ough, 1978). Other lipid-derived aldehydes and alcohols with chain length of C_8–C_{10} were also quantified (Tables 4 and 5). In general, irrigation amount did not consistently affect the content of these compounds.

The bound form of lipid derived alcohols in the grapes were presented in Tables 6 and 7. Glycosidically bound 1-hexanol had a

Table 5
Free volatile compounds ($\mu g/kg \pm SD$) in Merlot grapes harvested in 2008 from vines subjected to different irrigation levels with particle film treatment (+PF) or without (−PF) in south-west Idaho.

Compound	LRI[a]	PF	Irrigation level			p Value[b]		
			100% ET$_c$	35–70% ET$_c$	35% ET$_c$	I	PF	I * PF
C$_6$ compounds								
Hexanal	801	+PF	173.31 ± 4.64	167.57 ± 14.41	125.74 ± 21.61	<0.01	ns	ns
		−PF	169.07 ± 9.34	161.83 ± 17.12	139.03 ± 0.63			
trans-2-Hexenal	855	+PF	360.97 ± 11.87	311.41 ± 27.93	212.53 ± 36.53	<0.01	ns	0.034
		−PF	341.92 ± 11.69	231.44 ± 13.82	229.50 ± 10.03			
1-Hexanol	869	+PF	62.24 ± 6.99	45.50 ± 3.94	32.31 ± 6.05	<0.01	ns	ns
		−PF	52.41 ± 7.62	37.54 ± 2.07	40.86 ± 12.69			
Aldehydes and alcohols								
Heptanal	903	+PF	1.47 ± 0.34	1.52 ± 0.23	1.57 ± 0.24	ns	ns	ns
		−PF	1.78 ± 0.20	1.58 ± 0.35	1.31 ± 0.15			
Octanal	1003	+PF	0.27 ± 0.03	0.34 ± 0.05	0.34 ± 0.20	ns	ns	ns
		−PF	0.30 ± 0.04	0.42 ± 0.56	0.32 ± 0.05			
trans-2-Octenal	1061	+PF	3.51 ± 0.59	3.11 ± 0.74	3.05 ± 1.34	ns	ns	ns
		−PF	4.41 ± 2.30	2.85 ± 0.24	2.69 ± 0.63			
Nonanal	1106	+PF	1.26 ± 0.12	1.67 ± 0.28	1.00 ± 0.22	0.016	ns	ns
		−PF	1.05 ± 0.13	1.48 ± 0.44	1.17 ± 0.30			
Decanal	1207	+PF	4.74 ± 0.45	6.57 ± 0.86	3.17 ± 1.28	0.018	0.003	0.005
		−PF	3.27 ± 0.19	3.32 ± 0.58	3.68 ± 0.95			
1-Octen-3-ol	979	+PF	3.52 ± 0.63	2.94 ± 0.20	3.90 ± 1.02	ns	0.015	ns
		−PF	3.32 ± 1.49	4.95 ± 0.57	5.11 ± 0.19			
1-Octanol	1071	+PF	0.76 ± 0.12	0.94 ± 0.12	0.69 ± 0.11	0.036	ns	ns
		−PF	0.50 ± 0.27	0.97 ± 0.06	0.96 ± 0.27			
Terpenoids								
Limonene	1034	+PF	4.30 ± 0.10	4.52 ± 0.28	4.53 ± 0.44	ns	<0.001	ns
		−PF	3.68 ± 0.11	3.88 ± 0.04	3.90 ± 0.02			
Linalool	1102	+PF	2.44 ± 0.03	2.38 ± 0.07	2.38 ± 0.18	ns	ns	ns
		−PF	2.39 ± 0.17	2.46 ± 0.19	2.50 ± 0.30			
α-Terpineol	1200	+PF	0.52 ± 0.09	0.43 ± 0.38	0.30 ± 0.21	ns	ns	0.038
		−PF	0.19 ± 0.12	0.53 ± 0.06	0.64 ± 0.06			
Geranyl acetone	1457	+PF	2.27 ± 0.20	2.57 ± 0.20	2.43 ± 0.12	ns	0.001	0.040
		−PF	2.20 ± 0.08	1.98 ± 0.21	2.15 ± 0.02			
C$_{13}$-norisoprenoids								
Vitispirane[c,d]	1293	+PF	0.02 ± 0.003	0.03 ± 0.004	0.08 ± 0.03	0.001	ns	ns
		−PF	0.02 ± 0.003	0.03 ± 0.01	0.06 ± 0.001			
β-Damascenone	1396	+PF	1.52 ± 0.03	1.60 ± 0.01	1.62 ± 0.04	<0.001	ns	ns
		−PF	1.51 ± 0.02	1.63 ± 0.03	1.63 ± 0.004			
β-Ionone[e]	1498	+PF	85.19 ± 25.15	99.50 ± 17.05	63.21 ± 10.13	ns	ns	ns
		−PF	54.01 ± 7.06	82.16 ± 25.33	89.73 ± 13.75			
Dihydroactinidiolide[c,d]	1542	+PF	0.16 ± 0.01	0.17 ± 0.02	0.28 ± 0.10	0.024	ns	ns
		−PF	0.19 ± 0.03	0.19 ± 0.03	0.28 ± 0.005			
3-Hydroxy-β-damascenone[d,f]	1631	+PF	Trace	Trace	Trace			
		−PF	Trace	Trace	Trace			

[a] p Values for statistical significance according to ANOVA test (I = irrigation; PF = particle film; I * PF = interactive effect; ns: not significant) ($p > 0.05$).
[b] LRI, linear retention index calculated on HP-5 capillary column.
[c] Tentatively identified by using mass spectra data (Wiley 275) and comparison of LRI data to literature values (http://webbook.nist.gov/).
[d] $\mu g/kg$ Equivalents of β-damascenone.
[e] ng/kg.
[f] Tentatively identified by using mass spectra data.

much higher content than its free form in the grapes. There was no significant difference for the content of 1-hexanol under water deficit with or without PF in both years. Likewise, other alcohols including 1-heptanol, 1-octanol, and 1-decanol were shown to depend on seasonal climate, with the exception of 1-octen-3-ol. Water deficit, on average, increased the concentration of 1-octen-3-ol by 35% and 83% in 2007 and 2008, compared to full irrigation, respectively.

3.3. Terpenoids in Merlot grapes

The free terpenoids quantified in this study were limonene, linalool, α-terpineol and geranyl acetone. Among the terpenoids, linalool is probably one of the most important compounds, contributing to a floral note, because of its low sensory threshold. Trace amounts of nerol and geraniol were also detected in the grape berries. In general, water deficit had no consistent effect on

free terpenoids over the 2 years. Similarly, PF treatment affected the individual terpenoid in a different way, but the trend was not consistent for the two experimental years. Regardless, free terpenoids are present in small portion in grapes, and majority of terpenoids are presented as glycosides in grapes.

The concentrations of bound terpenoids including geraniol, nerol, and linalool, *cis*-furan-linalool oxide, *trans*-furan-linalool oxide, α-terpineol and 4-terpineol were listed in Tables 6 and 7. The content of bound-form geraniol increased as water stress increased both years. Compared with the 100% ET$_c$ treatment, the 35% ET$_c$ treatment resulted in 21% and 12% higher levels of geraniol in 2007 and 2008, respectively. Likewise, the water deficit increased the concentration of bound nerol in both years, although significant increases did not occur in 2007. Increased sun exposure to the canopy has been associated with increased monoterpene glycosides (Belancic et al., 1997; Bureau, Razungles, & Baumes, 2000). Thus, the increased terpenoids (nerol, geraniol) could be due to the higher

Table 6

Glycosidically bound volatile compounds (μg/kg ± SD) in Merlot grapes harvested in 2007 from vines subjected to different irrigation levels with particle film treatment (+PF) or without (−PF) in south-west Idaho.

Compound	LRI[b]	PF	Irrigation level				p Value[a]		
			100% ET_c	70% ET_c	35–70% ET_c	35% ET_c	I	PF	I ∗ PF
C₆ compound									
1-Hexanol	869	+PF	76.62 ± 7.84	86.67 ± 21.00	94.73 ± 5.65	nc[c]	ns	ns	ns
		−PF	76.40 ± 8.81	94.89 ± 7.77	89.92 ± 13.01	95.66 ± 10.68			
Alcohols									
1-Heptanol	970	+PF	1.21 ± 0.17	1.45 ± 0.61	2.12 ± 0.28	nc	ns	ns	ns
		−PF	1.07 ± 0.29	1.49 ± 0.65	1.53 ± 0.54	1.66 ± 0.63			
1-Octen-3-ol	979	+PF	3.14 ± 0.55	4.19 ± 0.54	4.83 ± 0.54	nc	0.005	ns	ns
		−PF	3.13 ± 0.22	3.35 ± 0.27	4.42 ± 0.55	4.24 ± 1.18			
1-Octanol	1071	+PF	1.20 ± 0.43	2.13 ± 0.82	2.10 ± 0.53	nc	ns	ns	ns
		−PF	1.40 ± 0.52	0.76 ± 0.64	2.40 ± 0.37	2.07 ± 1.31			
1-Decanol	1273	+PF	0.39 ± 0.12	0.59 ± 0.14	0.38 ± 0.15	nc	ns	ns	ns
		−PF	0.39 ± 0.11	0.33 ± 0.19	0.57 ± 0.11	0.54 ± 0.23			
Shikimic acid derivatives									
Benzaldehyde	971	+PF	9.07 ± 1.24	6.17 ± 0.05	7.08 ± 1.29	nc	ns	ns	0.018
		−PF	7.13 ± 0.60	8.85 ± 1.58	7.76 ± 0.42	7.41 ± 2.05			
Benzyl alcohol[e]	1040	+PF	1.08 ± 0.16	0.81 ± 0.18	1.14 ± 0.22	nc	ns	ns	ns
		−PF	0.83 ± 0.07	0.86 ± 0.27	1.10 ± 0.25	1.14 ± 0.07			
2-Phenylethanol	1121	+PF	220.93 ± 58.94	124.45 ± 35.62	193.67 ± 60.04	nc	ns	ns	ns
		−PF	141.99 ± 32.99	173.77 ± 36.19	193.86 ± 61.36	175.80 ± 6.52			
2-Methoxy-4-methylphenol[f,g]	1320	+PF	3.14 ± 0.75	3.90 ± 0.25	4.09 ± 0.78	nc	ns	ns	ns
		−PF	3.27 ± 1.16	3.26 ± 0.29	3.32 ± 0.39	4.07 ± 1.78			
2-Methoxy-4-vinylphenol	1328	+PF	50.05 ± 1.51	44.24 ± 5.68	46.41 ± 4.70	nc	ns	ns	ns
		−PF	47.90 ± 1.54	48.06 ± 2.02	46.16 ± 3.32	45.89 ± 3.53			
Terpenoids									
cis-Furan-linalool oxide	1078	+PF	5.77 ± 0.36	3.54 ± 0.29	3.65 ± 0.53	nc	0.005	ns	0.048
		−PF	4.79 ± 0.37	4.27 ± 0.30	4.30 ± 1.00	4.11 ± 0.93			
trans-Furan-Linalool oxide	1094	+PF	13.89 ± 0.46	7.14 ± 0.72	7.78 ± 1.78	nc	<0.001	ns	0.003
		−PF	10.28 ± 0.35	8.52 ± 0.54	9.41 ± 2.05	9.41 ± 1.38			
Linalool	1102	+PF	1.65 ± 0.02	1.53 ± 0.03	1.58 ± 0.05	nc	ns	ns	ns
		−PF	1.61 ± 0.11	1.55 ± 0.03	1.64 ± 0.06	1.71 ± 0.19			
4-Terpineol	1187	+PF	2.49 ± 0.18	2.28 ± 0.14	2.24 ± 0.23	nc	ns	ns	ns
		−PF	2.66 ± 0.19	2.22 ± 0.10	2.46 ± 0.15	2.65 ± 0.47			
α-Terpineol	1200	+PF	1.73 ± 0.11	1.25 ± 0.17	1.49 ± 0.22	nc	ns	ns	ns
		−PF	1.86 ± 0.50	1.39 ± 0.05	1.33 ± 0.11	1.63 ± 0.47			
Nerol	1233	+PF	0.58 ± 0.10	0.67 ± 0.02	0.69 ± 0.09	nc	ns	ns	ns
		−PF	0.64 ± 0.15	0.61 ± 0.13	0.73 ± 0.09	0.76 ± 0.20			
Geraniol	1257	+PF	1.71 + 0.10	1.68 + 0.07	1.82 + 0.20	nc	0.027	ns	ns
		−PF	1.64 + 0.26	1.69 + 0.02	1.93 + 0.35	2.03 + 0.27			
C₁₃-norisoprenoids									
Vitispirane[d,h]	1293	+PF	0.44 ± 0.09	0.60 ± 0.09	0.50 ± 0.05	nc	ns	ns	ns
		−PF	0.47 ± 0.15	0.47 ± 0.05	0.49 ± 0.04	0.57 ± 0.19			
β-Damascenone	1396	+PF	2.38 ± 0.09	2.89 ± 0.21	3.09 ± 0.31	nc	<0.001	ns	ns
		−PF	2.56 ± 0.11	2.61 ± 0.14	3.41 ± 0.08	3.31 ± 0.45			
1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)[f,h]	1598	+PF	0.15 ± 0.01	0.21 ± 0.03	0.22 ± 0.03	nc	0.009	ns	ns
		−PF	0.16 ± 0.03	0.18 ± 0.02	0.23 ± 0.03	0.22 ± 0.06			
3-Hydroxy-β-damascenone[f,h]	1631	+PF	0.34 ± 0.03	0.35 ± 0.02	0.40 ± 0.01	nc	0.010	ns	ns
		−PF	0.34 ± 0.05	0.32 ± 0.02	0.44 ± 0.08	0.37 ± 0.03			
3-Hydroxy-7,8-dihydro-β-ionol[f,h]	1643	+PF	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	nc	ns	ns	ns
		−PF	0.05 ± 0.01	0.05 ± 0.002	0.08 ± 0.03	0.06 ± 0.01			
3-Oxo-α-ionol[f,h]	1666	+PF	0.42 ± 0.09	0.38 ± 0.03	0.49 ± 0.03	nc	0.035	ns	ns
		−PF	0.36 ± 0.05	0.33 ± 0.03	0.53 ± 0.17	0.43 ± 0.07			

[a] p Values for statistical significance according to ANOVA test (I = irrigation; PF = particle film; I ∗ PF = interactive effect; ns: not significant) ($p > 0.05$).

[b] LRI, linear retention index calculated on HP-5 capillary column.

[c] nc, Data not collected.

[d] Tentatively identified by using mass spectra data (Wiley 275) and comparison of LRI data to literature values (http://webbook.nist.gov/).

[e] Benzyl alcohol, mg/kg of grape.

[f] Tentatively identified by using mass spectra data.

[g] μg/kg Equivalents of 2-methoxy-4-vinylphenol.

[h] μg/kg Equivalents of β-damascenone.

percent canopy light transmission due to water deficit treatments (Bureau et al., 2000; Shellie, 2006). However, water deficit had no significant effect on the contents of linalool glycosides. Water deficit decreased *trans*-furan-linalool oxide content for both years, and an interaction effect on the concentration of *trans*-furan-linalool oxide was detected in the cooler year (2008). In general, PF

application had no effect on the concentrations of bound form terpenoids, with the exception of 4-terpineol in 2008.

The concentrations of bound nerol and geraniol in grapes were positively correlated with their concentrations in wines over the two experimental years (Ou et al., 2010) (nerol, $r = 0.57$ versus 0.54, $p < 0.05$; geraniol, $r = 0.58$ versus 0.58, $p < 0.05$), and could

Table 7

Glycosidically bound volatile compounds (µg/kg ± SD) in Merlot grapes harvested in 2008 from vines subjected to different irrigation levels with particle film treatment (+PF) or without (−PF) in south-west Idaho.

Compounds	LRI[b]	PF	Irrigation level			p Value[a]		
			100% ET$_c$	35–70% ET$_c$	35% ET$_c$	I	PF	I * PF
C$_6$ compound								
1-Hexanol	869	+PF	357.71 ± 89.01	399.09 ± 42.90	370.67 ± 27.96	ns	ns	ns
		−PF	338.57 ± 38.94	344.97 ± 12.11	356.86 ± 48.18			
Alcohols								
1-Heptanol	970	+PF	2.15 ± 0.93	3.87 ± 0.91	4.05 ± 0.56	<0.001	ns	ns
		−PF	2.15 ± 0.73	3.22 ± 0.36	4.90 ± 0.51			
1-Octen-3-ol	979	+PF	3.99 ± 0.28	6.65 ± 1.18	8.17 ± 0.61	<0.001	ns	ns
		−PF	4.27 ± 0.07	6.75 ± 0.38	8.58 ± 1.20			
1-Octanol	1071	+PF	8.58 ± 0.62	10.77 ± 1.04	7.05 ± 0.54	0.004	<0.001	<0.001
		−PF	5.36 ± 1.09	2.32 ± 0.70	3.23 ± 0.75			
1-Decanol	1273	+PF	1.76 ± 0.16	1.92 ± 0.26	1.26 ± 0.29	0.005	<0.001	0.023
			1.50 ± 0.21	0.85 ± 0.31	0.88 ± 0.24			
Shikimic acid derivatives								
Benzaldehyde	971	+PF	24.11 ± 7.04	15.20 ± 2.19	22.10 ± 3.97	ns	ns	ns
		−PF	16.97 ± 4.33	17.46 ± 4.85	21.81 ± 3.06			
Benzyl alcohol[d]	1040	+PF	1.65 ± 0.31	1.71 ± 0.34	1.49 ± 0.07	ns	ns	ns
		−PF	1.76 ± 0.18	1.71 ± 0.14	1.85 ± 0.29			
2-Phenylethanol	1121	+PF	237.01 ± 55.28	247.19 ± 56.11	166.66 ± 28.27	0.018	ns	ns
		−PF	214.27 ± 26.02	265.18 ± 32.80	196.51 ± 26.66			
2-Methoxy-4 methylphenol[e,f]	1320	+PF	1.98 ± 0.35	4.24 ± 1.13	7.10 ± 0.21	<0.001	ns	ns
		−PF	2.13 ± 0.29	3.46 ± 0.01	7.22 ± 0.71			
2-Methoxy-4 vinylphenol	1328	+PF	52.42 ± 6.29	49.81 ± 1.88	51.97 ± 2.67	ns	ns	ns
		−PF	54.32 ± 0.90	48.96 ± 3.37	51.20 ± 5.24			
Terpenoids								
cis-Furan-linalool oxide	1078	+PF	4.78 ± 0.12	7.06 ± 1.54	5.19 ± 0.46	ns	ns	ns
		−PF	5.47 ± 1.18	4.51 ± 2.15	5.80 ± 2.15			
trans-Furan-Linalool oxide	1094	+PF	12.47 ± 1.90	11.84 ± 1.58	9.15 ± 0.65	0.04	ns	ns
		−PF	14.33 ± 1.74	9.20 ± 2.87	9.11 ± 2.30			
Linalool	1102	+PF	2.98 ± 0.03	3.00 ± 0.29	3.12 ± 0.05	ns	ns	ns
		−PF	2.90 ± 0.05	3.09 ± 0.16	3.20 ± 0.10			
4-Terpineol	1187	+PF	2.44 ± 0.10	2.27 ± 0.21	3.00 ± 0.14	<0.001	0.050	0.001
		−PF	3.04 ± 0.02	2.43 ± 0.08	2.70 ± 0.24			
α-Terpineol	1200	+PF	2.71 ± 0.18	2.35 ± 0.23	2.88 ± 0.12	0.001	ns	ns
		−PF	2.71 ± 0.27	2.10 ± 0.16	2.90 ± 0.35			
Nerol	1233	+PF	0.56 ± 0.17	0.94 ± 0.73	1.75 ± 0.13	0.001	ns	ns
		−PF	0.50 ± 0.05	1.14 ± 0.02	1.52 ± 0.22			
Geraniol	1257	+PF	2.93 + 0.21	3.14 + 0.14	3.17 + 0.03	0.008	ns	ns
		−PF	2.76 + 0.16	2.98 + 0.12	3.19 + 0.21			
C$_{13}$-norisoprenoids								
Vitispirane[c,g]	1293	+PF	0.35 ± 0.05	0.69 ± 0.15	1.05 ± 0.05	<0.001	ns	ns
		−PF	0.39 ± 0.04	0.67 ± 0.01	1.09 ± 0.14			
β-Damascenone	1396	+PF	3.61 ± 0.11	5.00 ± 0.33	4.58 ± 0.14	<0.001	ns	ns
		−PF	3.35 ± 0.05	5.11 ± 0.02	4.76 ± 0.29			
1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN)[e,g]	1598	+PF	0.13 ± 0.02	0.27 ± 0.07	0.35 ± 0.03	<0.001	ns	ns
		−PF	0.11 ± 0.02	0.24 ± 0.05	0.32 ± 0.03			
3-Hydroxy-β-damascenone[e,g]	1631	+PF	0.57 ± 0.05	0.90 ± 0.04	0.74 ± 0.07	<0.001	0.003	0.049
		−PF	0.54 ± 0.04	0.71 ± 0.05	0.68 ± 0.06			
3-Hydroxy-7,8-dihydro-β-ionol[e,g]	1643	+PF	0.24 ± 0.03	0.23 ± 0.17	0.14 ± 0.02	ns	ns	ns
		−PF	0.24 ± 0.02	0.31 ± 0.04	0.19 ± 0.13			
3-Oxo-α-ionol[c,g]	1666	+PF	0.28 ± 0.05	0.42 ± 0.10	0.53 ± 0.09	<0.001	ns	ns
		−PF	0.30 ± 0.03	0.37 ± 0.04	0.49 ± 0.05			

[a] p Values for statistical significance according to ANOVA test (I = irrigation; PF = particle film; I * PF = interactive effect; ns: not significant) ($p > 0.05$).
[b] LRI, linear retention index calculated on HP-5 capillary column.
[c] Tentatively identified by using mass spectra data (Wiley 275) and comparison of LRI data to literature values (http://webbook.nist.gov/).
[d] Benzyl alcohol, mg/kg of grape.
[e] Tentatively identified by using mass spectra data.
[f] µg/kg Equivalents of 2-methoxy-4-vinylphenol.
[g] µg/kg Equivalents of β-damascenone.

be responsible for the increased fruity, floral aromas perceived by sensory evaluation (Ou et al., 2010).

3.4. C$_{13}$-norisoprenoids in Merlot grapes

C$_{13}$-norisoprenoids are from degradation products of carotenoids, and some are important aroma-contributing compounds in wine (Mendes-Pinto, 2009; Ribéreau-Gayon et al., 2000). Plant water status has been shown to correspond with the content of C$_{13}$-norisoprenoid precursors (Bindon et al., 2007). Under water stress, plants will dissipate excess energy from photosynthesis in the form of heat using the xanthophylls cycle. Potentially, this could increase carotenoids that are the precursors of C$_{13}$-norisoprenoids (Bindon et al., 2007). Water deficit could increase carotenoid cleavage dioxygenase (CCD) (Deluc et al., 2009).

The free C$_{13}$-norisoprenoids detected in this study were β-damascenone, β-ionone, vitispirane, dihydroactinidiolide, and 3-hydroxy-β-damascenone. Among them, β-damascenone was the most abundant. Water deficit (35–70% ET$_c$) significantly increased the concentration of β-damascenone as compared to full

irrigation. It is not clear whether the effect is due to the synthesis or breakdown of carotenoids, because water stress can indirectly affected the light environment, and cluster microclimate (Shellie, 2006), leading to the change of β-damascenone (Lee et al., 2007). The sensory perception of β-damascenone is "cooked apple/floral/quince" with an extremely low odour threshold of 2 ng/l in water (Winterhalter & Rouseff, 2002). It can enhance fruity notes while masking herbaceous aroma (Pineau, Barbe, van Leeuwen, & Dubourdieu, 2007). The levels of vitispirane and dihydroactinidiolide were not affected by any water stress level in 2007, although grape fruit from vines that received 35% ET_c had the highest concentrations of vitispirane and dihydroactinidiolide in 2008 ($p < 0.05$). This might be due to the higher daily solar radiation during grape harvest in 2008 (Lee et al., 2007). PF application had no effect on the free C_{13}-norisoprenoids.

C_{13}-norisoprenoids can also occur as nonvolatile precursors, including glycosides, which are all monoglucosides (Ribéreau-Gayon et al., 2000). Bound form C_{13}-norisoprenoids involves not only enzyme hydrolysis, but also acid-catalyzed rearrangements during fermentation (Kwasniewski et al., 2010). The bound form β-damascenone was up to 3-fold higher than the free form. Increased water deficiency level was associated with increased bound β-damascenone content in the grape berries in the two experimental years. In each year, the 35% ET_c treatment increased the concentration of bound β-damascenone by 34% relative to the 100% ET_c control. The result was in agreement with Bindon et al. (2007), who found that water stress increased the content of bound C_{13}-norisoprenoids, including β-damascenone. However, Marais, van Wyk, and Rapp (1992) reported that hydrolytically released β-damascenone concentration was not affected by sunlight levels (Marais et al., 1992). When both free and bound form β-damascenone were considered, and their concentrations in grapes were positively correlated to their contents in wines ($r > 0.61$, $p < 0.01$). The higher concentrations of both free and bound β-damascenone under water deficit correlated well with our previous data that higher β-damascenone was in wines from deficit irrigated grapes (Ou et al., 2010).

The concentration of bound 3-hydroxy-β-damascenone, a precursor of β-damascenone (Ribéreau-Gayon et al., 2000), also increased under water deficit and had an interaction effect with PF application in the cooler year (Table 7). The content of 3-hydroxy-β-damascenone was increased by PF application only when vines were deficit irrigated. 3-Hydroxy-7,8-dihydro-β-ionol, a precursor of damascenone under acidic conditions (Sefton, Francis, & Williams, 1993), was not affected by either irrigation treatment or PF application. With the increase of water deficit level, the bound 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) concentrations were increased. As compared to 100% ET_c treatment, the 35% ET_c treatment significantly increased TDN content by 46% in 2007 and 1.8-fold in 2008. TDN is associated with a kerosene-like character of some Reisling wines, and it has also been related to a honey attribute in wines (Francis, Kassara, Noble, & Williams, 1999). The concentration of bound vitispirane increased significantly under water deficit in the cooler year ($p < 0.01$) (Table 7). Vitispirane has a "camphoraceous/eucalyptus" descriptor (Mendes-Pinto, 2009). These two compounds, potentially derived from neoxanthin, could contribute to bottle-aged wines (Winterhalter & Rouseff, 2002). In addition, the 35–70% or 35% ET_c treatment had higher concentrations of bound 3-oxo-α-ionol than the 100% ET_c treatment over both of the years.

3.5. Shikimic acid derivatives in Merlot grapes

Benzaldehyde, benzyl alcohol and 2-phenylethanol, and 2-methoxy-4-vinylphenol were identified in the glycosidically bound fraction. There was no consistent effect of water deficit or PF

application on these compounds over the 2 years, indicating that the behaviour of these compounds depended on vintage climate.

4. Conclusions

In conclusion, water deficit alters grape maturity and composition. Deficit irrigation decreases the concentration of negative compounds, and increases the concentration of positive compounds. The kaolin based particle film had minimum effect.

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