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ARTICLE

Volatile Compounds and Sensory Attributes of Wine from Cv. Merlot (*Vitis vinifera* L.) Grown under Differential Levels of Water Deficit with or without a Kaolin-Based, Foliar Reflectant Particle Film[†]

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The volatile composition and sensory attributes of Merlot wines produced from vines under differing levels of water stress, with or without a foliar, kaolin-based particle film, were analyzed by stir bar sorptive extraction−gas chromatography−mass spectrometry (SBSE−GC−MS) and sensory evaluation. Vines were irrigated over consecutive vintages with 100, 70, or 35% of their estimated water requirements (ET_c), or 35% until color change then 70% until harvest (35−70% ET_c). Neither of the treatments consistently influenced ester concentrations or their relative amounts, though their concentrations varied from year to year. However, deficit irrigation had an effect on the concentration of terpene alcohols and norisoprenoids. Wines produced from vines under water deficit contained higher amounts of citronellol, nerol, geraniol, and β-damascenone, but linalool and β-ionone were not affected by deficit irrigation. Particle film did not affect volatile composition in the wine. Untrained panelists in 2007 and 2008 distinguished between wines from vines that received 100 or 35% ET_c and between wines from vines that received 35 or 35−70% ET_c. Trained sensory panelists detected differences among wines for aroma, flavor, taste, and mouthfeel; however, significant interactive effects between particle film application and vine water status hindered interpretation of independent main effects.

KEYWORDS: Kaolin; deficit irrigation; Merlot (*Vitis vinifera* L.) wine; volatiles; SBSE

INTRODUCTION

Regulated deficit irrigation is a management practice commonly used to manipulate vegetative growth and enhance desirable fruit components; however, vines under water deficit can experience higher leaf temperatures, reduced leaf gas exchange and reduced transpirational cooling (*1*, *2*). Restricting vine vegetative growth under deficit irrigation regime often alters the microclimate surrounding the developing fruit and may, in some climates, increase the risk of berry exposure to potentially damaging high temperature or solar radiation (*3*). It has been documented that the biosynthetic pathways and reactions of primary and secondary metabolites produced in the grape during development are sensitive to cluster microclimate (*4*, *5*), which impacts the volatile aroma composition of the resulting wine (*6*). To reduce symptoms of heat stress, foliar-applied reflectants such as kaolin have been used successfully with many crops to enhance product quality (*7*).

Kaolin is an inert clay mineral, [$Al_2Si_2O_5(OH)_4$], that reflects potentially damaging ultraviolet and infrared radiation and transmits photosynthetically active radiation (*8*). A commercially available product under the trade name of Surround (Engelhard Corp., Iselin, NJ, USA) has been shown to beneficially mitigate undesirable attributes associated with heat stress on annual crops such as sorghum, tomato, cotton, and pepper (*9*−*12*). A foliar application of kaolin particle film increased leaf carbon assimilation and reduced leaf temperature in apple (*Malus domestica*) (*13*), and reduced sunburn and increased yield and fruit quality in pomegranate (*Punica granatum*) (*14*, *15*). Foliar application of particle film to winegrape under water deficit in an arid climate did not eliminate symptoms of heat stress on the berry but did appear to increase vine carrying capacity (*16*). It is unknown whether a foliar application of kaolin particle film to vines under water deficit during berry development will alter the volatile

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composition or sensory profile of the resulting wine nor whether its influence is related to the severity or timing of the water deficit.

Merlot (*Vitis vinifera* L.) is one of the world's most widely planted red grape cultivars (*17*). Merlot is a principal cultivar in the Snake River Valley American Viticultural Area of southwestern Idaho, a warm, semiarid production region with high solar radiation (*18*). In this area, precipitation during the growing season provides about 25% of the grape's evapotranspiration needs, making irrigation a production necessity. Little is known about how vine water status during berry development influences the resulting wine volatile aroma composition, and no information is available on its interaction with a foliar applied, kaolin-based particle film. The objective of the present study was to investigate the main and interactive effects of water deficit and foliar applied kaolin-based particle film on Merlot wine volatile aroma compounds and sensory attributes.

MATERIALS AND METHODS

Chemicals. Hexyl formate, ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, isoamyl acetate, ethyl hexanoate, hexyl acetate, ethyl phenylacetate, phenylethyl acetate, linalool, nerol and benzeneethanol, citronellol, geraniol, β-ionone, eugenol, and γ-nonalactone were obtained from Sigma-Aldrich Chemical Co. Inc. (Milwaukee, WI). Ethyl isobutyrate, isobutyl acetate, octyl propionate, and 4-octanol were obtained from K & K Laboratories (Jamaica, NY). Ethyl octanoate and ethyl decanoate were purchased from Eastman Chemical (Kingsport, TE). β-Damascenone was obtained from Firmenich (Princeton, NJ). Ethyl cinnamate was from Alfa Aesar (Ward Hill, MA).

Dichloromethane was obtained from Burdick & Jackson (Muskegon, MI), methanol (GC grade) was from EMD (Gibbstown, NJ), and ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY). Tartaric acid was from Mallinckrodt Inc. (Paris, KY). A synthetic wine solution was made by dissolving 3.5 g of L-tartaric acid in 1 L of 12% ethanol solution, and adjusting pH to 3.5 with 1 M NaOH. The internal standard stock solution composed of hexyl formate, octyl propionate, and 4-octanol with concentrations of 103, 108, and 114 mg/L, respectively, was prepared in methanol and stored at −15 °C.

Plant Material and Field Trial Site. Grapes were harvested from ungrafted vines of Merlot (planted in 1997) as part of an irrigation trial begun in 2002 (*2*) and used to produce wines over three consecutive seasons (2006, 2007, 2008). The field plots were composed of four rows of vines, each containing 14 vines per row (56 vines) with four replicates in a randomized block design. A kaolin-based foliar particle film reflectant (Surround WP; Engelhard Cor. Iselin, NJ) was applied as a split-plot within each irrigation main plot, i.e., all vines in a plot received similar irrigation but half of each plot was either sprayed with particle film or left unsprayed (control). Water supply and amount were as described by Shellie (*2*) with plots receiving 35, 70, or 100% of estimated crop evapotranspiration (ET_c) until harvest, or 35% ET_c until berry color change and then 70% ET_c until harvest. These irrigation regimes are hereafter referred to as 35, 70, 100, and 35−70% ET_c. The particle film was applied and irrigation regimes begun just after fruit set. The particle film was applied three times at weekly intervals at a single rate of 60 g L^{-1}. Vine water status was monitored weekly by measuring the midday leaf water potential (Ψ_{md}) of two leaves within each plot throughout the growing season, as described previously (*2*).

Wine Production. Wines were produced with fruit harvested from field trial subplots utilizing a standard protocol as described by Qian et al. (*6*). An equal weight of fruit was harvested from the interior vines of each field subplot. Fruit from field replicates was combined and then randomly subdivided into three lots of equal weight that were used to produce triplicate fermentations for each treatment level. Fruit was fermented at Willow Crest Winery (Prosser, WA, USA) in 2006 using 55 kg of fruit per fermentation that was treated with or without particle film and grown under 35 or 100% ET_c. In 2007, triplicate fermentations were performed at the USDA-ARS research facility (Parma, ID USA) each using 59 kg of fruit grown under 35−70, 70 or 100% ET_c with or without particle film or grown under 35% ET_c without particle film. In 2008, triplicate fermentations were produced at the University of Idaho Food Technology Center (Caldwell, ID, USA) each using 64 kg of fruit grown under 35, 35−70, or 100% ET_c with or without particle film. The grapes were crushed and stems removed on the day of harvest, and must degrees Brix (°Bx), pH, and titratable acidity (TA) were measured prior to fermentation. Potassium metabisulfite was added after crush, and the must was inoculated 24 h later with Premier Cuvée yeast (Davis 796). The must fermented at 23 °C for 7 days until 0 °Bx (Densito 30PX, Mettler Toledo), was pressed, and was racked 3 days later; sulfur dioxide was added prior to transfer into screw-cap glass bottles, and then the samples were stored at 22 °C. The wine samples were analyzed within three months.

Wine Volatile Analysis. Selected volatile compounds were analyzed using a stir bar sorptive extraction−gas chromatography−mass spectrometry (SBSE−GC−MS) method described previously (*6*, *19*, *20*) with minor modification. Esters, terpene alcohols, norisoprenoids and a few other compounds of importance to wine aroma (*21*, *22*) were selected for quantification. A 10 mL wine sample was diluted with 10 mL of water in a 20 mL vial, in which 20 μL of internal standard solution and 6 g of sodium chloride were added. A stir bar (Twister) coated with a poly(dimethylsiloxane) (PDMS) phase (1 cm length, 0.5 mm thickness, Gerstel Inc., Baltimore, MD) was washed with solvent (methanol:dichloromethane 1:1), then dried with air and conditioned for 30 min at 300 °C. The sample was extracted with the stir bar for 2 h at a speed of 1000 rpm. After extraction, the stir bar was rinsed with distilled water, dried carefully with paper and placed into a sample holder for GC−MS analysis.

GC−MS analyses were performed using an Agilent 6890 gas chromatograph with a 5973 mass selective detector (Agilent, Santa Clara, CA). Samples were loaded into a thermal desorption unit (TDU) by a multipurpose autosampler (Gerstel). A cooled injection system (CIS4, Gerstel) was used in the GC−MS system. The TDU had an initial temperature of 25 °C. After the sample was loaded, the TDU was heated at a rate of 300 °C/min to a final temperature of 250 °C and held for 1 min. The TDU injection was in splitless mode during thermal desorption, while the CIS4 was in a solvent vent mode with a venting flow of 50 mL/min for 4.0 min, at a venting pressure of 22.8 psi. After the solvent vent, the CIS4 was switched to splitless mode for 3.0 min, then changed to split mode with a venting flow of 50 mL/min. The initial temperature of the CIS4 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 DB-WAX column (30 m length, 0.25 mm i.d., 0.25 μm film thickness, Phenomenex, Torrance, CA). The oven temperature was programmed at 40 °C for a 1 min holding, then to 190 °C at a rate of 3 °C min^{-1}, and to 230 °C at a rate of 8 °C min^{-1} with 5 min holding. A constant helium column flow of 2.5 mL/min was used. A column splitter was used at the end of the column, 1 mL min^{-1} column flow was introduced to the MS, and the other 1.5 mL min^{-1} column flow was vented out. The MS transfer line and ion source temperature were 280 and 230 °C, respectively. Electron ionization mass spectrometric data from m/z 35−350 were collected using a scan rate of 5.27 s^{-1}, with an ionization voltage of 70 eV.

Calibration Curves and Quantification Method. The individual stock solution containing around 10,000 mg L^{-1} of the target compound was prepared in methanol and stored at −15 °C. The standard solutions were prepared by diluting the stock solution in a synthetic wine to give a range of concentrations with eight levels (ranging from 0.05 to 1020 μg L^{-1} depending upon the compound). The standard solutions were analyzed using the same procedure as described for wine samples. The calibration curve for each target compound was built up by plotting the selected mass ion abundance ratio of target compound with their respective internal standard against the concentration ratio. Standard calibration curves were obtained through Chemstation software for all compounds except as indicated and were used to calculate the concentrations of volatile compounds in wine samples. Duplicate analysis was performed, and the average values are reported. Data were analyzed by irrigation regime and subjected to a t test (with or without particle film) and by analysis of variance (ANOVA) using a fixed model with irrigation as the main effect. Fisher's least significant difference (LSD) or Duncan's multiple range test was used for comparison of means at 95% confidence level using the statistical software S-PLUS Version 7.0 (Insightful Corp., Seattle, WA).

Sensory Difference Testing. Preliminary testing determined no detectable difference between replicate fermentations, and thus those were combined. A difference test (triangle test) with 24 (2007) and 35 (2008) panelists was used in 2007 and 2008 to compare irrigation treatment levels 35 and 100% ET_c and 35 and 35−70% ET_c. Untrained panelists ranged in age from 21 to 65 and indicated on a demographic questionnaire that they consumed wine at least once per week. A minimum amount of information on the nature of the study was provided to panelists to reduce potential bias. All test sessions were conducted in the sensory laboratory under red lights to mask possible color differences. Wine samples were presented in two flights, in random order using a balanced block design. Twenty-five milliliter aliquots of wine were served at room temperature in ISO/INAO wine glasses covered with a small Petri dish. Each panelist was provided deionized filtered water and unsalted crackers for cleansing the palate between flights. Additional difference tests and trained sensory evaluations were made in 2008 between wines from vines with or without particle film for each irrigation level. Data were statistically analyzed using Compusensefive software (release 4.6, Compusense Inc., Guelph, ON) according to the method of Roessler et al. (*23*). Level of significance for treatment differences was established at $p < 0.1$ or $p < 0.05$.

Sensory Trained Panel Evaluation. The trained sensory panel was composed of 8 trained wine panelists (7 females and 1 male). All panelists had previously participated in at least 20 h of wine evaluation training and were given four additional hours of training to describe attributes including dried fruit, fresh fruit, woody, earthy, canned vegetal and spicy. Sourness, bitterness and astringency were also evaluated. The standards used to train the panelists are shown in **Table 1**. Standards were prepared at a moderate intensity level (assigned a 7.5−9 on the 15 cm line scale). Panelists evaluated the intensity of each attribute using a 15 cm unstructured line scale, anchored with extremely low (= 1) and extremely high (= 14). Each treatment was assigned a 3-digit code, served in random order and evaluated in replicate as described previously for difference tests except samples were served under white lights. Intensity data were expressed as cm along the 15 cm line scale as perceived by the panelist.

Table 1. Standards Used by a Trained Sensory Panel (n = 8) To Evaluate 2008 Merlot Wines

sensory attribute	standard preparation
Aroma and Flavor	
fresh fruit	5 mL of raspberry, 10 mL of cherry jam[a]
dried fruit	2 dried prunes, cut into pieces[a]
canned vegetal	5 mL of juice from canned asparagus[a]
woody	10 mL of oak wood shavings[a]
earthy	10 mL of potting soil[a]
spicy	6 clove buds, soak for 5 min, remove 4[a]
Taste	
bitter	20 mg of quinine sulfate[a]
sour	3.5 g of tartaric acid[a]
Mouthfeel	
drying	2 g of tannic acid, 0.875 g of alum[b]
weight	0.38 g of carboxymethylcellulose[b]
alcohol/hot	6.4% ethanol (v/v)

[a] Standards prepared in 100 mL of base wine (Livingston Red Rose). [b] Standards dissolved in 750 mL of base wine (Livingston Red Rose).

Data were analyzed using two-way analysis of variance (panelist, wine and replicate as the main effects), with mean separation by Fisher's LSD ($p < 0.05$) (XLSTAT, Addinsoft, Paris). Principal components analysis on the means of the significant attributes was performed using XLSTAT.

RESULTS AND DISCUSSION

Seasonal temperature during the three years of this study was warmer in 2006 and 2007 than the 30-year average of 1553 °C growing degree days (http://www.wrcc.dri.edu/) and cooler than average in 2008 (**Table 2**). No difference in leaf Ψ_{md} was observed between vines with or without kaolin. Preveraison vine water status was lowest in 2006, and this preveraison period was the only occasion where vines under different irrigation regimes had similar leaf Ψ_{md}. In all other occasions, vines under 100% ET_c had the highest leaf Ψ_{md}. In 2007 and 2008, leaf Ψ_{md} of preveraison under each irrigation regime was higher than postveraison, with values ranging from −0.88 to −1.39 MPa for preveraison and from −0.99 to −1.63 MPa for postveraison for vines under 100 or 35% ET_c, respectively. The values of leaf Ψ_{md} in this study were similar to well-watered, moderately, or severely stressed grapevines grown in Spain and California (*24*−*27*). Low preveraison leaf Ψ_{md} in 2006 was most likely due to higher evaporative demand associated with warmer ambient temperature and an inadequate amount of irrigation.

The presence of particle film during berry development had no effect on must °Brix, pH, or titratable acidity; however, these attributes were affected by the irrigation treatment level (**Table 3**). Alleviating water stress at veraison by increasing irrigation amount from 35 to 70% ET_c enhanced fruit maturity as reflected in higher must °Brix, higher pH, and lower titratable acidity. Fruit from vines under 100% ET_c were the least mature. Reduced titratable acidity and increased °Brix under water deficit was also reported by Shellie (*2*), and has been attributed to a reduction in malate (*27*, *28*).

Wine Volatile Compounds. Wine aromas are derived from a complex interaction of diverse volatile compounds that include esters, terpenoids, C_{13} isoprenoids, methoxypyrazines and thiols. The relative importance of these compounds to wine varietal aroma character varies with the type of grape cultivar (*21*, *22*, *29*), growing conditions and management practices (*30*, *31*).

Esters. Esters are the most abundant class of volatile compounds present in wine except alcohols and acids. Ethyl esters and acetates are important contributors to wine flavor because they are present in high concentrations and have low sensory thresholds (*21*, *22*). The most abundant esters in the wines were ethyl isobutyrate, ethyl butanoate, 3-methylbutyl acetate, ethyl hexanoate, and ethyl octanoate (**Tables 4**, **5**, and **6**). Their concentrations ranged from 200 to 2000 $\mu g\ L^{-1}$. Although the concentrations of branched-chained esters, including ethyl isobutyrate, isobutyl acetate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and 3-methylbutyl acetate, were low, their aroma contribution may not be ignored because the sensory thresholds of these esters

Table 2. Seasonal Heat Unit Accumulation from April 1 through October 31 and Average Midday Leaf Water Potential (Ψ_{md}) of Vines with and without Foliar Particle Film Prior to (Preveraison) and after (Postveraison) Berry Color Change That Received Differential Amounts of Their Estimated Transpirational Requirements (ET_c)[a]

		Ψ_{md} (MPa)							
		preveraison				postveraison			
	heat units (°C)	100[b]	70[b]	35[b]	35−70[b]	100[b]	70[b]	35[b]	35−70[b]
2006	1595	−1.30 (0.03)[c]	−1.36 (0.02)	−1.38 (0.02)	−1.37 (0.02)	−1.25 (0.02)	−1.48 (0.02)	−1.61 (0.02)	−1.49 (0.02)
2007	1636	−1.05 (0.04)	−1.14 (0.04)	−1.35 (0.01)	−1.39 (0.01)	−1.28 (0.04)	−1.45 (0.03)	−1.62 (0.03)	−1.41 (0.04)
2008	1501	−0.88 (0.02)	−1.09 (0.02)	−1.33 (0.02)	−1.29 (0.02)	−0.99 ((0.02)	−1.37 (0.03)	−1.63 (0.03)	−1.25 (0.04)

[a] Heat units were calculated as the sum of daily average temperature above 10 °C. Vine water status was measured weekly at midday by determining leaf water potential (Ψ_{md}) with a pressure chamber and expressed in megapascals (MPa). [b] % ET_c. [c] Standard error of treatment mean.

Table 3. Merlot Juice Composition Prior to Fermentation from Vines with or without a Foliar Application of Kaolin-Based Particle Film (PF) That Received Differential Percentages of Their Estimated Transpirational Requirements (ET_c)

		2006			2007			2008		
% ET_c	PF[a]	°Brix	pH	TA[b]	°Brix	pH	TA	°Brix	pH	TA
100	+	24.8 a	3.7 a	4.6 a	23.4 bc	3.5 c	6.5 a	24.0 c	3.4 c	7.4 a
100	−	25.0 a	3.8 a	4.2 a	23.2 c	3.5 c	6.5 a	23.3 d	3.3 d	7.6 a
70	+	*c*	*c*	*c*	24.0 b	3.7 b	5.1 b	*c*	*c*	*c*
70	−	*c*	*c*	*c*	23.6 bc	3.6 bc	6.2 a	*c*	*c*	*c*
35	+	24.6 a	3.8 a	3.5 a	*c*	*c*	*c*	25.7 a	3.6 b	3.7 c
35	−	24.7 a	3.8 a	3.4 a	23.5 bc	3.7 b	4.9 bc	25.5 a	3.7 a	3.5 c
35−70	+	*c*	*c*	*c*	24.6 a	3.9 a	4.6 bc	25.0 b	3.6 b	4.3 b
35−70	−	*c*	*c*	*c*	24.8 a	3.8 a	4.2 c	24.9 b	3.7 ab	4.2 b

[a] Same letter within a column indicates no significant difference by Duncan's multiple range test at $P \leq 0.05$. [b] TA = juice titratable acidity (g L^{-1}) to pH 8.2 using 0.1 N sodium hydroxide. [c] Wines were not produced from treatment.

Table 4. Volatile Concentrations (μg $L^{-1} \pm$ SD) in Merlot Wines Produced in 2006 from Vines with or without a Foliar Application of Kaolin-Based Particle Film (PF) That Received Differential Percentages of Their Estimated Transpirational Requirements (ET_c)

	100% ET_c		35% ET_c	
compound[a]	−PF	+PF	−PF	+PF
		Esters		
ethyl 2-methylpropanoate	420 ± 40 a	394 ± 2 a	460 ± 60 a	466 ± 2 a
isobutyl acetate	152 ± 1 a	145 ± 3 a	130 ± 40 a	130 ± 30 a
ethyl butanoate	310 ± 30 a	300 ± 6 a	300 ± 50 a	330 ± 30 a
ethyl 2-methylbutanoate	35 ± 2 a	32 ± 1 a	41 ± 1 a	43 ± 4 a
ethyl 3-methylbutanoate	54 ± 9 a	50 ± 4 a	61 ± 2 a	68 ± 6 a
isoamyl acetate	2030 ± 5 a	2100 ± 100 a	1790 ± 60 a	1800 ± 200 a
ethyl hexanoate	1200 ± 200 a	1300 ± 200 a	1200 ± 300 a	1500 ± 300 a
hexyl acetate	120 ± 30 a	123 ± 8 a	57 ± 7 a	100 ± 40 a
ethyl octanoate	830 ± 100 a	870 ± 60 a	710 ± 250 a	940 ± 190 a
ethyl decanoate	110 ± 20 a	120 ± 20 a	130 ± 40 a	150 ± 50 a
ethyl 2-phenylacetate	7.7 ± 1.9 a	7.0 ± 0.1 a	8.1 ± 1.2 a	9.1 ± 0.9 a
2-phenylethyl acetate	150 ± 40 a	150 ± 10 a	78 ± 4 a	120 ± 40 a
ethyl cinnamate	0.5 ± 0.02 a	0.5 ± 0.4 a	0.6 ± 0.2 a	0.5 ± 0.2 a
		Terpene Alcohols		
linalool	9.5 ± 2.6 a	8.8 ± 0.7 a	9.9 ± 0.9 a	9.1 ± 0.7 a
citronellol	13.4 ± 1.5 a	13.1 ± 1.5 a	15.0 ± 0.9 a	12.5 ± 1.4 a
nerol	0.50 ± 0.09 a	0.47 ± 0.03 a	0.52 ± 0.02 a	0.34 ± 0.04 a
geraniol	4.5 ± 0.7 a	4.1 ± 0.4 a	4.7 ± 0.1 a	3.4 ± 0.6 a
nerolidol	3.9 ± 0.3 a	3.9 ± 0.8 a	5.8 ± 0.1 a	4.4 ± 1.3 a
		Norisoprenoids		
β-damascenone	2.6 ± 0.1 a	2.4 ± 0.2 a	5.7 ± 0.6 b	5.7 ± 0.1 b
β-ionone	0.13 ± 0.01 a	0.12 ± 0.01 a	0.13 ± 0.01 a	0.13 ± 0.03 a
		Others		
benzeneethanol[b]	18 ± 1 a	17.1 ± 0.2 a	20 ± 2 a	19 ± 1 a
eugenol	1.30 ± 0.03 b	1.31 ± 0.04 b	0.94 ± 0.06 ab	0.9 ± 0.2 a
γ-nonalactone	4.9 ± 0.02 a	5.4 ± 0.4 a	5.8 ± 0.1 a	5.2 ± 0.3 a

[a] Same letter within a row indicates no significant difference by least significant difference at $P \leq 0.05$. [b] mg L^{-1}.

are very low (*21*, *22*). Aromatic esters, including ethyl phenylacetate, 2-phenylethyl acetate, and ethyl cinnamate, were the least present in the wines. The concentration of 2-phenylethyl acetate ranged from 19 to 150 μg L^{-1}, below its sensory threshold of 1.8 mg L^{-1} and within the range reported in literature for red wines (*21*) . Similarly, ethyl cinnamate had a concentration range of 0.5 to 2 μg L^{-1}, which is also below its sensory threshold of 160 μg L^{-1} (*21*). Neither of the treatments evaluated in this study consistently influenced ester concentrations or their relative amounts, though their concentrations varied from year to year. The annual variability in concentration may reflect differences in wine making and yeast fermentation behavior.

Terpene Alcohols. Terpene alcohols contribute to the varietal aroma character of a wine. The concentrations of the terpene alcohols including linalool, citronellol, nerol, geraniol, and nerolidol were quantified in this study. In general, wines produced from vines under the water stress had higher concentrations of terpene alcohols, but the trend depended on the individual compound and vintage year. In 2006, there was no differences for any of the terpene alcohols between 100% and 35% treatment ($p < 0.05$) (**Table 4**). Foliar application of kaolin particle film had no significant effect ($p < 0.05$) on any terpene alcohol within any irrigation regime. While the climate conditions may be responsible for the results, it is also the first year that we treated the

Table 5. Volatile Concentrations (μg L^{-1} ± SD) in Merlot Wines Produced in 2007 from Vines with or without a Foliar Application of Kaolin-Based Particle Film (PF) That Received Differential Percentages of Their Estimated Transpirational Requirements (ET_c)

compound[a]	100% ET_c −PF	100% ET_c +PF	70% ET_c −PF	70% ET_c +PF	35−70% ET_c −PF	35−70% ET_c +PF	35% ET_c −PF
				Esters			
ethyl 2-methylpropanoate	170 ± 8 a	190 ± 20 a	150 ± 40 a	150 ± 30 a	130 ± 20 a	160 ± 30 a	
isobutyl acetate	35 ± 5 a	40 ± 3 a	39 ± 4 a	38 ± 5 a	38 ± 7 a	41 ± 8 a	37 ± 7 a
ethyl butanoate	200 ± 30 a	230 ± 20 a	190 ± 40 a	250 ± 10 a	210 ± 20 a	220 ± 30 a	246 ± 7 a
ethyl 2-methylbutanoate	26 ± 1 a	28 ± 1 a	26 ± 6 a	25 ± 4 a	24 ± 3 a	28 ± 4 a	22 ± 5 a
ethyl 3-methylbutanoate	34 ± 3 a	38 ± 2 a	31 ± 7 a	31 ± 4 a	28 ± 3 a	34 ± 7 a	27 ± 5 a
isoamyl acetate	290 ± 40 a	340 ± 70 a	340 ± 20 a	320 ± 80 a	380 ± 50 a	390 ± 90 a	340 ± 90 a
ethyl hexanoate	800 ± 70 a	660 ± 60 a	630 ± 90 a	840 ± 30 a	750 ± 90 a	670 ± 70 a	780 ± 50 a
hexyl acetate	3.4 ± 0.9 a	2.8 ± 0.6 a	3.6 ± 0.6 a	3.4 ± 0.9 a	4.0 ± 0.9 a	3.2 ± 0.7 a	4.0 ± 1.1 a
ethyl octanoate	530 ± 40 a	460 ± 20 a	430 ± 60 a	550 ± 60 a	540 ± 80 a	500 ± 60 a	530 ± 80 a
ethyl decanoate	118 ± 4 abc	160 ± 20 bc	90 ± 10 a	180 ± 20 c	100 ± 20 ab	130 ± 40 abc	110 ± 10 ab
ethyl phenylacetate	8.3 ± 0.7 b	6.6 ± 1.1 ab	7.1 ± 1.9 ab	7.1 ± 0.4 ab	6.3 ± 0.8 ab	6.3 ± 0.3 ab	5.2 ± 0.4 a
2-phenylethyl acetate	23 ± 1 ab	19 ± 3 a	24 ± 4 ab	24 ± 2 ab	29 ± 4 b	25 ± 0.5 ab	23 ± 2 ab
ethyl cinnamate	1.5 ± 0.2 ab	1.2 ± 0.2 a	1.3 ± 0.2 a	1.7 ± 0.1 ab	1.9 ± 0.3 b	1.8 ± 0.07 b	1.6 ± 0.2 ab
				Terpene Alcohols			
linalool	4.8 ± 0.4 a	5.5 ± 0.4 a	5.1 ± 1.0 a	5.1 ± 0.6 a	4.5 ± 0.2 a	5.2 ± 0.6 a	4.6 ± 0.1 a
citronellol	12.0 ± 0.6 a	12.5 ± 0.9 a	13.8 ± 2.0 ab	14.1 ± 1.0 ab	16.7 ± 1.0 b	15.7 ± 1.5 ab	15.5 ± 0.8 ab
nerol	0.43 ± 0.03 a	0.44 ± 0.04 a	0.50 ± 0.02 a	0.71 ± 0.08 ab	1.04 ± 0.13 c	0.90 ± 0.18 bc	0.80 ± 0.05 bc
geraniol	2.8 ± 0.6 a	3.3 ± 0.3 ab	3.1 ± 0.4 a	4.4 ± 0.3 b	4.8 ± 0.5 c	4.8 ± 0.3 c	3.9 ± 0.4 abc
nerolidol	2.6 ± 0.8 a	4.0 ± 0.2 ab	3.5 ± 0.8 ab	4.4 ± 0.3 b	4.2 ± 0.3 b	4.6 ± 0.3 b	3.6 ± 0.6 ab
				Norisoprenoids			
β-damascenone	3.1 ± 0.2 a	3.2 ± 0.3 a	4.3 ± 0.3 b	4.2 ± 0.4 ab	5.9 ± 0.5 c	5.8 ± 0.5 c	5.2 ± 0.4 c
β-ionone	0.19 ± 0.05 a	0.21 ± 0.02 a	0.18 ± 0.02 a	0.24 ± 0.04 a	0.23 ± 0.01 a	0.24 ± 0.02 a	0.21 ± 0.02 a
				Others			
benzeneethanol[b]	32.8 ± 0.8 a	33.1 ± 3.0 a	37.3 ± 5.0 a	35.2 ± 2.1 a	40.4 ± 4.3 a	38.8 ± 3.5 a	31.0 ± 4.5 a
eugenol	1.06 ± 0.13 a	1.17 ± 0.13 a	1.19 ± 0.15 a	1.34 ± 0.12 a	1.02 ± 0.07 a	0.96 ± 0.07 a	1.02 ± 0.05 a
γ-nonalactone	7.4 ± 0.6 a	6.9 ± 0.4 a	7.7 ± 0.6 a	8.2 ± 0.7 ab	9.8 ± 0.4 b	9.6 ± 0.6 b	7.4 ± 0.8 a

[a] Same letter within a row indicates no significant difference by least significant difference at $P \leq 0.05$. [b] mg L^{-1}.

plants, and it may take some time for the vines to response to the treatment. The total terpene alcohols were the highest in 2007 vintage among the three years. In the 2007, the concentration of citronellol, nerol, geraniol, and nerolidol increased an average of 15%, 150%, 50%, 50%, and 300% in vines under water deficit (the 70%, 70% + PF, 35−70%, 35−70% + PF and 35% ET_c treatments) relative to the 100% ET_c control (**Table 5**). These results support that of Reynolds et al. (*32*), who reported increased formation of terpenes in Gewürztraminer wine produced from vines under a water deficit during berry development. A similar increasing trend of terpene alcohol with deficit irrigation was observed in 2008 though the increase was less dramatic (**Table 6**). The average increase in citronellol, nerol, geraniol, and nerolidol in wine from vines under water deficit (the 70%, 70% + PF, 35−70%, 35−70% + PF, 35%, and 35% + PF ET_c treatments) in 2008 was 20%, 10%, 10%, and 10%, respectively. Surprisingly, the concentration of linalool did not respond to the deficit irrigation treatments in either 2007 or 2008. The influence of viticulture practices on terpene alcohol composition in wine is complex because terpene alcohols are largely present as glycoside precursors that can be hydrolyzed by enzymes and acids during wine making. In addition, terpene alcohols can rearrange under acidic conditions in wine to form other terpene alcohols. Terpene alcohols such as linalool, geraniol, ho-trienol, nerol, and α-terpineol have very low sensory thresholds, with citrus, floral, rosy, and geranium aroma notes, and are particularly important to aromatic wines such as Muscat, Gewürztraminer, and Morio Muskat, which typically contain these terpene alcohols at concentrations that greatly exceed their sensory thresholds. The concentrations of these terpene alcohols are typically much lower in nonfloral wines such as Merlot and other reds, but their aroma contribution could have an important synergistic effect.

Norisoprenoids. Grape-derived C_{13} norisoprenoids are a very diverse group of natural compounds, which are putatively derived from carotenoid degradation. Norisoprenoid compounds such as β-damascenone, vitispirane, β-ionone, and TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) contribute complex aromas, including honey, berry, rosy, and fruity sensory notes to red and white nonfloral varieties. These compounds generally have very low sensory thresholds (*33*).

In this study, only β-damascenone and β-ionone were quantified because the concentrations of other norisoprenoids are below the detection limits of the SBSE−GC−MS method. Particle film application to the vine had no significant effect ($p < 0.05$) on the concentrations of β-damascenone and β-ionone in the wines produced in any of the years in this study. However, in each year of the study, the concentration of β-damascenone in the wines from vines under 35−70% ET_c was 50−100% higher than that in wines made from well-watered vines (100% ET_c). These results support findings from our previous study (*6*). The concentration of β-ionone in the wines did not differ among irrigation treatments in any of the three study years, though in 2008, the wines from deficit-irrigated vines tended to have lower concentrations than well-watered vines.

Other Compounds. Although benzeneethanol exists in grapes, it is largely produced during fermentation by wine yeast.

Table 6. Volatile Concentrations (μg L^{-1} ± SD) in Merlot Wines Produced in 2008 from Vines with or without a Foliar Application of Kaolin-Based Particle Film (PF) That Received Differential Percentages of Their Estimated Transpirational Requirements (ET_c)

	100% ET_c		35−70% ET_c		35% ET_c	
compound[a]	−PF	+PF	−PF	+PF	−PF	+PF
			Esters			
ethyl 2-methylpropanoate	40 ± 3 a	38 ± 4 a	36 ± 4 a	37 ± 1 a	37 ± 2 a	34 ± 2 a
isobutyl acetate	60 ± 10 ab	73 ± 9 b	43 ± 1 a	41 ± 2 a	50 ± 10 a	42 ± 3 a
ethyl butanoate	240 ± 40 a	290 ± 20 a	250 ± 30 a	250 ± 20 a	290 ± 50 a	258 ± 4 a
ethyl 2-methylbutanoate	5.4 ± 0.4 a	5.9 ± 0.5 ab	7.1 ± 0.7 abc	7.8 ± 0.3 bc	7.7 ± 0.6 bc	8.1 ± 1.3 c
ethyl 3-methylbutanoate	7.7 ± 0.3 a	8.4 ± 0.5 ab	8.7 ± 0.6 ab	9.4 ± 0.4 ab	9.6 ± 1.0 ab	10.0 ± 1.2 b
isoamyl acetate	550 ± 90 a	660 ± 20 a	490 ± 30 a	520 ± 40 a	660 ± 180 a	680 ± 160 a
ethyl hexanoate	1440 ± 500 a	1680 ± 170 a	1030 ± 100 a	1160 ± 160 a	1130 ± 70 a	1090 ± 170 a
hexyl acetate	8.2 ± 1.2 ab	9.7 ± 0.5 b	5.3 ± 0.4 a	6.4 ± 1.2 ab	7.3 ± 1.1 ab	8.1 ± 1.8 ab
ethly octanoate	1180 ± 380 a	1380 ± 140 a	950 ± 90 a	1020 ± 120 a	1070 ± 150 a	1050 ± 90 a
ethyl decanoate	180 ± 60 a	220 ± 20 a	170 ± 20 a	180 ± 20 a	210 ± 50 a	190 ± 20 a
ethyl phenylacetate	5.9 ± 0.8 ab	6.2 ± 0.1 b	4.3 ± 0.5 a	5.1 ± 0.8 ab	4.7 ± 0.4 ab	4.9 ± 0.6 ab
2-phenylethyl acetate	36 ± 10 a	42 ± 0.3 a	28 ± 4 a	34 ± 5 a	36 ± 1 a	37 ± 6 a
ethyl cinnamate	2.2 ± 0.4 c	2.0 ± 0.2 bc	1.3 ± 0.2 a	1.4 ± 0.1 ab	1.4 ± 0.1 ab	1.4 ± 0.1 ab
			Terpene Alcohols			
linalool	4.1 ± 0.3 a	3.9 ± 0.1 a	4.0 ± 0.3 a	4.0 ± 0.3 a	3.7 ± 0.4 a	4.3 ± 0.6 a
citronellol	18 ± 1 ab	16 ± 0.4 a	21 ± 1 ab	20.6 ± 0.7 ab	22 ± 3 b	21 ± 2 b
nerol	1.3 ± 0.2 a	1.2 ± 0.1 a	1.4 ± 0.03 a	1.4 ± 0.4 a	1.4 ± 0.2 a	1.2 ± 0.3 a
geraniol	7.6 ± 2.0 a	8.7 ± 0.5 a	8.4 ± 1.0 a	8.3 ± 0.9 a	9.4 ± 0.9 a	8.9 ± 0.5 a
nerolidol	2.9 ± 0.5 a	2.2 ± 0.6 a	3.3 ± 0.03 a	3.4 ± 0.1 a	3.8 ± 0.2 a	4.3 ± 1.5 a
			Norisoprenoids			
β-damascenone	4.1 ± 0.1 a	3.6 ± 0.1 a	6.0 ± 0.2 b	5.9 ± 0.4 b	6.1 ± 0.9 b	5.8 ± 0.1 b
β-ionone	0.40 ± 0.01 bc	0.43 ± 0.02 c	0.34 ± 0.01 ab	0.32 ± 0.01 a	0.29 ± 0.02 a	0.28 ± 0.02 a
			Others			
benzeneethanol[b]	31 ± 6 a	34 ± 4 ab	41 ± 2 b	46 ± 9 bc	45 ± 3 bc	49 ± 4 a
eugenol	0.84 ± 0.16 b	0.74 ± 0.10 b		0.71 ± 0.04 ab		0.35 ± 0.05 ab
γ-nonalactone	5.8 ± 0.2 ab	5.7 ± 0.1 a	7.3 ± 0.3 ab	7.9 ± 0.9 b	7.4 ± 0.5 ab	7.5 ± 1.4 ab

[a] Same letter within a row indicates no significant difference by least significant difference at $P \leq 0.05$. [b] mg L^{-1}.

Table 7. *F*-Ratio Results from Analysis of Variance on Trained Panelists' Evaluations of Aroma Attributes in Merlot Wines Produced in 2008 from Vines with or without a Foliar Application of Kaolin-Based Particle Film That Received Differential Percentages of Their Estimated Transpirational Requirements[a]

		aroma			
source of variation	df	fresh fruit	dried fruit	canned vegetal	spicy
panelist (P)	7	15.621*	15.143*	18.950*	10.962*
wine (W)	5	2.259*	2.241*	4.683*	2.186*
replication	1	0.212	0.567	1.457	1.086
P × W	35	0.845	1.201	1.234	1.302

[a] Asterisk (*) indicates significance at $p \leq 0.05$.

Table 8. Trained Panelists (n = 8) Mean Values for Ratings of Aroma Attributes of Merlot Wines Produced in 2008 from Vines with or without a Foliar Application of Kaolin-Based Particle Film (PF) That Received Differential Percentages of Their Estimated Transpirational Requirements (% ET_c)[a]

		aroma			
% ET_c[b]	PF	fresh fruit	dried fruit	canned vegetal	spicy
100	+	5.75 abc	7.20 a	6.41 ab	4.56 ab
100	−	4.70 a	5.03 c	7.38 a	6.01 a
35	+	6.77 c	6.87 ab	3.92 c	5.82 a
35	−	5.17 ab	6.05 abc	4.86 bc	5.84 a
35−70	+	5.57 abc	5.65 bc	6.08 ab	4.98 ab
35−70	−	6.22 bc	5.91 abc	4.84 bc	3.96 b

[a] Evaluations were made in replicate along a 15 cm unstructured line scale. [b] Same letter within a column indicates no significant difference by Fisher's LSD ($p \leq 0.05$).

Benzeneethanol could contribute floral and rosy aroma notes; however, the threshold for benzeneethanol in wine is in the range of 7−200 mg L^{-1} (*21*). In this study, benzeneethanol was present in wines at concentrations that ranged from 20 to 50 mg L^{-1}. Neither particle film application nor irrigation regimen influenced its concentration in the wines in 2006 (**Table 4**) or 2007 (**Table 5**); however, in 2008, wines from vines that received 35−70% ET_c or 35% ET_c had higher concentrations of benzeneethanol than the wines from vines that received 100% ET_c (**Table 6**). These results suggest that vine water deficit during berry development may enrich the content of aromatic amino acids in the grapes that results in an increased concentration of benzeneethanol in the wine.

Eugenol is a phenolic compound present in the grape berry but can also be extracted from oak during aging. Eugenol has a sensory threshold of 10 μg L^{-1} in wine (*21*), and contributes clove and balsamic aroma to the wine. In this study, the concentration of eugenol was 1 μg L^{-1}, which is below its sensory threshold. Neither treatment factor had any measurable influence on wine eugenol concentration.

γ-Nonalactone is present in the grape berry and can also be extracted from oak during aging. γ-Nonalactone has a sensory threshold of 30 μg L^{-1} in wine (*21*), and is associated with a coconut odor. The concentrations of γ-nonalactone in the wines in this study were below its sensory threshold. There was no measurable influence of particle film of its concentration in the wine; however, wines produced from vines under water deficit tended to contain a higher concentration of γ-nonalactone.

Sensory Attributes: Untrained and Trained Panelists. Difference testing revealed that panelists were able to significantly distinguish between the 35 and 100% ET_c irrigation regimes in 2007 and 2008 and between the 35 and 35−70% ET_c treatments in 2007 ($p < 0.05$). These findings support the observed differences in wine volatile composition and demonstrate that the water status of the vine during berry development has an influence that can be detected in volatile character and sensory properties of the resulting wine.

Aroma, flavor, taste and mouthfeel were sensory attributes found by trained panelists to significantly differ between wines shown in **Tables 7**, **8**, **9**, and **10**. The aroma attributes that differed among treatments were fresh fruit, dried fruit, canned vegetal and spicy; the flavor attributes were dried fruit and spicy; bitter taste and drying mouthfeel ($p < 0.05$). Trained panelists detected a significant influence of particle film application in wines made from vines grown under 35% ET_c ($p < 0.05$), suggesting an interaction effect between particle film and vine water status. Particle film application significantly increased the fresh fruit aroma and decreased the spicy flavor and bitter taste of wines from the most water deficit vines (35% ET_c) ($p \leq 0.05$). The only other significant influence of particle film application within an irrigation treatment level was an increase in the dried fruit aroma of wines from vines under 100% ET_c. Wines produced from the 35% ET_c treatment without particle film had the most intense spicy flavor. Canned vegetal aroma was associated with higher amount of

Table 9. *F*-Ratio Results from Analysis of Variance on Trained Panelists Evaluations of Flavor, Taste and Mouthfeel Attributes of Merlot Wines Produced in 2008 from Vines with or without a Foliar Application of Kaolin-Based Particle Film That Received Differential Percentages of Their Estimated Transpirational Requirements[a]

		flavor		taste	mouthfeel
source of variation	df	dried fruit	spicy	bitter	drying
panelist (P)	7	30.133*	20.993*	7.176*	4.454*
wine (W)	5	2.095*	3.863*	2.849*	2.402*
replication	1	4.187*	5.274*	4.323*	0.546
P × W	35	3.051*	0.815	2.043*	1.225

[a] Asterisk (*) indicates significance at $p \leq 0.05$.

Table 10. Trained Panelists ($n = 8$) Mean Values for Ratings of Flavor, Taste and Mouthfeel Attributes of Merlot Wines Produced in 2008 from Vines with or without a Foliar Application of Kaolin-Based Particle Film (PF) That Received Differential Percentages of Their Estimated Transpirational Requirements (% ET_c)[a]

		flavor		taste	mouthfeel
% ET_c[b]	PF	dried fruit	spicy	bitter	drying
100	+	6.09 ab	5.47 a	6.93 ab	7.68 abc
100	−	6.89 a	5.07 a	7.02 ab	6.87 a
35	+	5.96 ab	5.64 a	6.30 a	7.18 ab
35	−	5.84 ab	7.77 b	8.15 bc	8.25 abc
35−70	+	5.20 b	5.47 a	8.73 c	8.98 c
35−70	−	5.59 b	5.97 a	7.92 bc	8.75 bc

[a] Evaluations were made in replicate along a 15 cm unstructured line scale. [b] Same letter within a column indicates no significant difference by Fisher's LSD ($p \leq 0.05$).

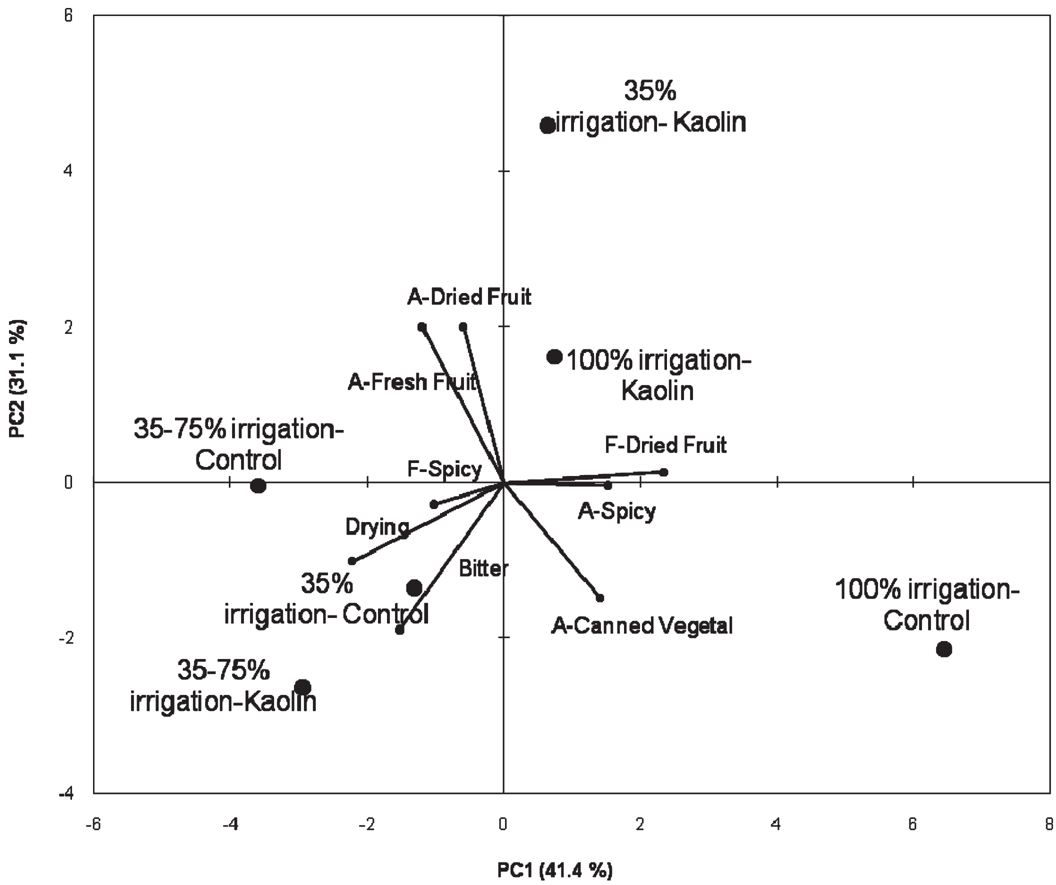

Figure 1. Principal component analysis of Merlot wines produced in 2008 from vines with or without (Control) a foliar application of kaolin-based particle film that received differential percentages of their estimated transpirational requirements (irrigation) with separation based on attributes of aroma (A), flavor (F), taste and mouthfeel.

irrigation (100% ET_c). Differences in spicy aroma, dried fruit flavor and drying mouthfeel were inconsistent among treatment levels.

Principal components analysis was used to illustrate the differences between wines based on the significant sensory attributes determined using ANOVA (**Figure 1**). Factor 1 was used to describe 41.4% of the variation in the data and was a contrast between drying with dried fruit flavor and spicy aroma. Factor 2 was primarily a function of dried fruit and fresh fruit aroma. Located in the same quadrant were 35% ET_c without particle film and 35−70% ET_c with and without particle film. The 35−70% ET_c without particle film was defined by its higher spicy flavor and drying attributes. The treatment 35% ET_c without particle film and 35−70% ET_c with particle film were defined by their more intense drying, bitter and spicy flavor sensory attributes. The 100 and 35% ET_c with particle film had high intensities of dried fruity flavor and spicy aroma, with the 35% ET_c having a higher intensity of dried fruit and fresh fruit aroma. The 100% ET_c without particle film was also high in dried fruit flavor, spicy aroma and canned vegetal aroma.

In a study of the sensory attributes of Cabernet Sauvignon wines made from vines with different water status, Chapman et al. (*34*) found that minimal irrigation resulted in wines with the highest fruity attributes. These fruity attributes were lower in the wines made from standard or double irrigation. The standard irrigation treatment resulted in wines with the highest rating in vegetal aroma and astringency and bitterness. A similar trend was found in the present study, with fresh fruit aroma being highest in the 35% ET_c wines, canned vegetal aroma being highest in the 100% ET_c wines and drying mouthfeel being highest in the 35−70% ET_c.

Few studies have investigated how foliar particle film application during berry development influences the composition and quality of the resulting wine (*35*, *36*). Results from this study with Merlot, grown in a warm, arid climate, provides further evidence that vine water status during berry development influences the sensory and volatile profile of the resulting wine and provides new information about affected volatile compounds and sensory attributes. Particle film application did not alter wine volatile composition and had an inconsistent influence on fruit harvest maturity and wine sensory attributes. The interactive effect between particle film application and vine water status hinders a straightforward prediction of response to particle film, especially under warm, arid growing conditions.

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