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Monitoring Effects of Ethanol Spray on Cabernet franc and Merlot Grapes and Wine Volatiles Using Electronic Nose Systems

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Abstract: The ability of two electronic nose systems (conducting polymer and surface acoustic wave-based) to differentiate volatiles of grapes and wines treated with an aqueous ethanol spray (5% v/v) at veraison was evaluated. Ethanol spray induced fruit ethylene production immediately posttreatment, which then declined progressively. The electronic nose evaluations of grape volatiles were compared with Cabernet franc and Merlot physicochemistry and with wine gas chromatographic and aroma sensory data. Canonical discriminant and principal component analysis found that both electronic nose systems and the physicochemical measures (Brix, TA, pH, color intensity and hue, total phenols, glycosides, and berry weight) were able to discriminate between ethanol-treated and untreated grapes and wines for both cultivars. Grape physicochemical treatment differences were due mainly to variations in hue, phenolic-free glycosides, and total phenols. Aroma sensory evaluations using a consumer panel differentiated between ethanol treatments and controls for Merlot, but not for Cabernet franc wines.

Key words: electronic nose, volatiles, Merlot, Cabernet franc

Days from bloom to harvest may be an important fruit, and subsequent wine, quality parameter (Haselgrove et al. 2000, Smart 1985). Indeed, it is widely accepted that "hang time" impacts aroma, flavor, and color (Bergqvist et al. 2001, Haselgrove et al. 2000). Additionally, color stability is reportedly impacted by the rate of fruit maturation (Bergqvist et al. 2001).

Ethylene is a plant hormone whose production at the onset of ripening is required to attain maturity for most fruits (Barry and Giovannoni 2007), although grapes are believed to be nonclimacteric (Coombe and Hale 1973). With nonclimacteric fruit, there is neither a burst of endogenous ethylene production nor a consistent growth response to exogenous ethylene. In grapes, the level of endogenous ethylene is low (Shulman et al. 1985) and declines from bloom to ripening (Weaver and Singh 1978), but may increase at veraison, causing a rise in respiration. Ethylene levels have been observed to remain relatively low before and after veraison (Coombe and Hale 1973), suggesting that ethylene plays a minor role in the ripening of grapes.

The effects of exogenous applications of ethylene, or ethylene-generating growth regulators, on grapes depend on rate, timing, cultivar, and region (Chervin et al. 2004, Hale et al. 1970, Powers et al. 1980, Shulman et al. 1985, Weaver and Pool 1971). In Shiraz, there was a delaying or hastening effect on ripening rate by ethylene and/or ethephon (2-chloroethylphosphonic acid, formerly a commercially available growth-regulating compound), depending on pre- or postveraison application (Hale et al. 1970). In Carignane, there were no changes in ripening, but ethephon was effective in increasing color, but only if applied at the start of veraison, not before (Weaver and Pool 1971). It has been suggested that ethephon causes a stress response in grapes that stimulates ethylene production (Weaver and Pool 1971) and that it stimulates phenylalanine ammonia-lyase (PAL) activity (Roubelakis-Angelakis and Kliewer 1986), a key enzyme in anthocyanin production.

Aqueous ethanol sprays may act similarly to ethephon (El Kereamy et al. 2002). Ethanol has been shown to increase anthocyanin concentration in cranberries (Farag et al. 1992), while ethanol vapor has been reported to increase the ripening of tomatoes (Beaulieu and Saltveit 1997). Investigations have shown that ethanol applied to grapes can retard or hasten the ripening process, depending on the concentration and timing (Beaulieu and Saltveit 1997). Spraying aqueous ethanol at 8 to 13 weeks postbloom enhanced grape and wine anthocyanin concentration and decreased acidity (Chervin et al. 2001, El Kereamy et al. 2002). Aqueous ethanol sprayed at 8 to 13 weeks postbloom on Cabernet Sauvignon grape clusters increased the anthocyanin content in berry skins and in subsequent juice and wines (Chervin et al. 2001, 2004). The mode of action is thought to be either an increase in fruit ethylene concentration or a direct effect on anthocyanin biosynthesis (El Kereamy et al. 2002). Ethanol may stimulate production of UDP-glucose flavonoid 3-*O*-glucosyl transferase, a key enzyme for the glycosylation of anthocyanins (El Kereamy et al. 2002) and other secondary metabolites, including aroma and flavor compounds (Hosel 1981).

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A major challenge for the grape and wine industry is to replace time-consuming laboratory analyses with new applications that are fast, precise, and accurate. For example, the analysis of grape and wine volatiles currently represents a significant limitation. Conventional analyses are mostly conducted using gas chromatographic (GC), GC-mass spectrometry (GC-MS), and GC olfactory (GC/O) methods and involve expensive equipment, methods development, and time- and labor-intensive steps, including sample preparation.

Electronic nose (ENose) technology represents a possible alternative to volatile measurement, at least in some applications. These units are multisensor arrays designed to detect volatile compounds. Each sensor type has a greater or lesser affinity for a particular chemical class or group of compounds. The adsorption of volatiles on the sensor surface causes a physical or chemical change in the sensor, allowing a specific pattern or "smellprint" of the volatiles (Mallikarjunan 2005). Using chemometric techniques and multivariate statistical analysis, it is possible to distinguish among groups of samples and, depending on the type of ENose, identify individual sample components. ENose systems are so-named because their methods of operation are analogous to the way the human sense of smell operates, where multiple nerve cells in the olfactory epithelium provide responses so the brain can identify and characterize aromas. ENoses are classified based on sensor type, including conducting polymer (CP) and surface acoustic wave (SAW) systems.

Conducting polymer units consist of an array of polymer sensors which, upon exposure to chemical vapor, expand, causing a change in electrical resistance. Each sensor is comprised of a different conductive material such as polypyrrole, polythiophene, polyaniline, polyacetylene, and polyindole, with a range of properties to allow discrimination among different types of volatiles, although cross-selectivity can occur (Mallikarjunan 2005, Pinheiro et al. 2002). Sensor responses are stored as smellprints and compared with the present readings during identification (Cyrano Sciences Inc., manufacturer guidelines, 2000). Primary problems reported with the use of conducting polymer sensors for wines are the influence of ethanol (Ragazzo-Sanchez et al. 2006) and water vapor. However, several studies used an ethanol baseline with a conducting polymer ENose system to minimize the interference of ethanol with sensor readings and base discrimination primarily on wine aroma and flavor volatiles (Devarajan et al. 2011, Gardner et al. 2011, Santos et al. 2004).

Surface acoustic wave ENose systems perform like a fast-acting GC, using a single uncoated sensor to analyze volatiles quantitatively. Samples are drawn into a trap, the trap is heated to vaporize the sample, and the vapor is passed through a column, where the volatile is identified and quantified (Electronic Sensor Technology, operation manual, 2001). Generally, the sensitivity of various ENose systems varies according to sensor and polymer type. Sensitivity is reported to be in the µg/L to ng/L range (Mallikarjunan 2005).

This study evaluated the effect of aqueous ethanol sprays at veraison on Merlot and Cabernet franc grapes and subsequent wines. Conducting polymer and surface acoustic wave-based electronic nose systems were used to monitor grape and wine volatiles.

Materials and Methods

Field design. The study used Cabernet franc and Merlot (*Vitis vinifera* L.) grapes, grown on a Ballerina training system in central Virginia during the 2008 growing season. Heat summation for the site was 1154.4°C, average monthly rainfall from April through October was 81 mm, and mean relative humidity in September was 75%.

Clone 4 Cabernet franc was planted in 2003 on 101-14 rootstock, with grapevine canopy sides facing east/west (±5°) in a 4040 m^2 plot. Vines were spaced 2.13 x 3.05 m apart. Fruit from the Cabernet franc canopy sides was monitored separately, with Cabernet franc-1 designated as the east side and Cabernet franc-2 as the west side of the canopy. Clone FPS 3 Merlot vines, grafted on 3309C rootstock, were planted in 1998 in a 20,274.76 m^2 plot with 2.13 x 3.05 m spacing. Merlot fruit was not segregated based on canopy side. Both the Cabernet franc and Merlot vineyards were dry-farmed and hedged twice to 15 nodes per shoot.

Ethanol treatment at veraison was carried out on 12 randomly selected Cabernet franc vines from each canopy side and 12 Merlot vines; 12 nonadjacent vines were selected as controls. Treatments consisted of control (water) and aqueous ethanol (5% v/v) spray. Ethyl alcohol (USP 190 proof/95%, AAPER Alcohol & Chemical Co., Shelbyville, KY) was diluted with water and sprayed on each cluster of each treated vine to full coverage. Treatments were applied using a hand-held 3.79 L low-density polyethylene sprayer at a rate of 2 liters per vine, between 0800 and 1000 hr. Fruit sampling was conducted at weeks 14, 15, and 17 postbloom for Cabernet franc, and weeks 12, 13, and 16 postbloom for Merlot.

Cluster ethylene concentrations were determined by extraction of internal atmospheres from control and treated fruit, in triplicate, carried out 1, 3, 6, and 24 hr postspray application. Immediately before analysis, clusters were harvested, adjusted to room temperature, washed with distilled water, weighed, and placed within a funnel, which was inverted and fitted with a 6.3 mm serum cap. The funnel, containing the grape cluster, was completely submerged in a NaCl solution (0.36 g/L granulated food grade salt in distilled water) inside a cylindrical pressure vessel (30.5 x 15.2 cm) as described elsewhere (Maxie et al. 1965, Coombe and Hale 1973). A partial vacuum (686 mm Hg) was applied for 5 min. The internal gases extracted from the fruit and collected from the top of the inverted funnel were sampled with a Hamilton (Reno, NV) Gastight model 1001 syringe for triplicate GC analysis of ethylene. Samples (0.1 mL) were injected directly into a Varian gas chromatograph (model 3740, Palo Alto, CA) (10^{-2} electron capture detector with positive output and an ion detector mode). Nitrogen gas flow rate was 30 mL/min, hydrogen was 30 mL/min, air was 300 mL/min, and a Supelco (Bellefonte, PA) 1% SP-1000 on 60/80 Carbopack B packed column was used. Injection temperature was 150°C, and column temperature 100°C.

Relative humidity was determined by digital hygrometer (Traceable, model 4187; Control Company, Friendswood, TX), and temperature by using an infrared thermometer (model 42529; Extech Instruments, Waltham, MA) within the vine canopy of control and ethanol-treated vines between 0800 and 1000 hr. At harvest, yield components were determined, including shoots per meter, clusters per shoot, clusters per vine, cluster weight, berry weight, berry numbers, and fruit weight per vine. Approximately 57 kg of Merlot fruit and 57 kg from each Cabernet franc canopy side were used for wine production.

Fruit and wine chemistry. Fifty berries per vine were randomly selected, weighed and crushed in 30.97 cm^3 (15.24 x 20.32 x 0.10 cm) plastic sampling bags (Minigrip, Seguin, TX). Juice was filtered through 0.45 µm syringe filters (Whatman, Clifton, NJ). Skins and juice were frozen at -20°C for total glycosyl-glucose (TGG) and phenol-free glycosyl glucose (PFGG) analysis. Brix, pH, and titratable acidity (TA) were analyzed, and color intensity ($A_{420}+A_{520}$), hue (A_{420}/A_{520}), and total phenols (A_{280}) were determined using a Genesys spectrophotometer (Spectronic, Leeds, UK), each as described previously (Zoecklein et al. 1999). Degrees Brix was measured by refractometer (model 10430; AO Scientific Instruments, Southbridge, MA).

The analyses of TGG and PFGG were performed as described (Williams et al. 1995) and modified (Zoecklein et al. 2000). L-Malic acid and yeast assimilable nitrogen (YAN) were determined enzymatically (R-Biopharm AG, Darmstadt, Germany, and Megazyme, Bray, Ireland, respectively), wine alcohol content by Fourier transform infrared (Foss WineScan FT 120, Eden Prairie, MN), and residual sugar concentration was estimated by Clinitest (Bayer, Pittsburgh, PA).

Wine samples for GC-MS analysis were prepared using a 4 mL sample with NaCl (1.0 g) in 10 mL clear glass vials sealed with septa (MicroLiter Analytical Supplies, Suwanee, GA), as published elsewhere (Whiton and Zoecklein 2000). Vials were preincubated for 30 sec at 30°C with agitation at 250 rpm. A CAR/DVB/PDMS grey SPME fiber (Supelco, Sigma-Aldrich, St. Louis, MO) was used to penetrate vials to a 32 mm depth into the headspace and was equilibrated for 30 min. A GC-MS system (model 6890N; Network GC System, 5975B inert MSD; Agilent Technologies, Santa Clara, CA) was used, with injector temperature at 250°C, DB-Wax column (30 m x 0.25 mm), and helium carrier gas with a flow rate of 1 mL/min. Oven temperature was 40°C with a ramp rate of 6°C/min to 230°C. Thirty-two compounds were manually integrated and quantified using standard solutions.

Processing and fermentation. Cabernet franc and Merlot grapes were crushed and destemmed using a Wottle (Anton, Poysdorf, Austria) destemmer-crusher to ~50% berry breakage, estimated visually. Berries were distributed into three open-top 60 L Nalgene fermenting bins of equal height and volume for each variety and treatment. Each bin was treated with 250 mg/L Velcorin (dimethyl-dicarbonate; Scott Laboratories, Petaluma, CA), followed by addition of 25 mg/L potassium metabisulfite. Grapes were cold soaked for six days at 7°C, with daily punching of must caps.

Juice analysis was performed on day six of cold soak. Titratable acidity adjustments were made using tartaric acid, and YAN adjustments (12 g/100 L) using FermAid K (Lallemand, Blagnac Cedex, France). Go-Ferm (Lallemand) yeast nutrient was prepared according to manufacturer's recommendations and added during yeast rehydration. *Saccharomyces cerevisiae* ICV-D254 (Lallemand) yeast (24 g/100 L) was hydrated according to the manufacturer's recommendation and inoculated after cold soak. Caps were punched three times daily. Fermentation was monitored by hydrometry and carried out at 23 ± 2°C (liquid temperature) until dryness (<1% residual sugar). Following fermentation, wines were dejuiced and pressed using a Willmes bladder press (model 100; Bensheim, Germany) to 0.5 bar. Free-run and press-run fractions were combined in sanitized glass carboys. Wine was kept at 7°C for 24 hr, then racked into carbon dioxide-filled 3.80 L glass bottles, and stored at 12°C.

Electronic nose analysis. Two ENose systems were used: conducting polymer-based (CP) Cyranose 320 (Smiths Detection, Pasadena, CA) and surface acoustic wave-based (SAW) ZNose 7300 (Electronic Sensor Technology, Newbury Park, CA). The CP ENose optimization method for fruit (Athamneh et al. 2008) evaluated the impact of field variables, such as heat and humidity, in our climatic region. The wine optimization method for CP ENose was that of Gardner (2009). Optimizations involved evaluations of sample temperature, incubation time, pump speed, purge, and individual sensor response. Canonical projection plots showed no separations between replications, indicating minimum sensor drift. Optimum sample temperature was determined to be 30°C. Ethanol standard solutions (three standards per treatment) were used to create an ethanol baseline to evaluate the impact on polymer sensor response and to minimize effects of differences in alcohol concentrations among wine samples while using the ENose. Concentrations for ethanol standards were based on the alcohol concentration of each wine treatment. For the SAW ENose, the default DB-5 system settings were used, except for the sensor temperature (30°C). The system was calibrated using C6 to C14 alkane standards each day.

For field analysis, five ethanol-sprayed clusters per vine were randomly selected. Clusters were bagged with an HDPE bag (Interplast, Livingston, NJ) and equilibrated for 45 min, followed by CP ENose evaluation of headspace volatiles. Fruit temperature, ambient temperature, and relative humidity were measured.

For laboratory analysis, five replicates of 20 mL juice or wine samples were placed in 40 mL GC clear glass vials sealed with Teflon/silicone 3 mm septa (MicroLiter Analytical Supplies, Suwanee, GA). ENose analysis of the samples occurred after equilibrating in a water bath at 30°C for 20 min. Wines were analyzed twice, once immediately postfermentation and again 6 months postfermentation.

Sensory analysis. A triangle difference test was conducted on wine aroma under standard conditions 6 months postfermentation, as described (Meilgaard et al. 2007), comparing control and ethanol treatments. Wines were prescreened for sulfurlike off odors. All wines were identified

with a randomized three-digit code. Standard ISO glasses were filled with 20 mL wine and covered with petri dishes. Wines were presented to the panelists at 19 ± 1.0°C under red light in separate booths in a controlled environment. Thirty-two panelists aged 21 to 27 years, with a gender ratio of 1:1, were chosen ($\alpha = 0.05$, $\beta = 0.30$, $\rho d = 40\%$, 16 correct responses for significant difference), with a prerequisite of wine consumption at least once a week. Written instructions were provided and each panelist received two 20-min training sessions outlining the procedures. Each panel member smelled two sets of samples at different time periods.

Statistical analysis. The physicochemistry, CP, and SAW ENose data from grapes and wines were analyzed and compared using univariate (one-way analysis of variance, ANOVA), least significant difference (LSD), multivariate (canonical discriminate analysis, CDA) and principal component analysis (PCA) statistical methods using JMP software, version 7 (SAS Institute, Cary, NC). The SAW ENose data was analyzed using the GC chromatographic approach as described (Lammertyn et al. 2004). For the sensory data, the number of correct responses was counted.

Results and Discussion

Internal ethylene concentrations illustrated the impact of aqueous ethanol on ethylene production. Cabernet franc-1 (canopy east side) fruit demonstrated a pattern typical of this study. One-hour postspray, treated fruit had a mean ethylene concentration of 70 ppb, compared to 10 ppb in control fruit. Three hours postspray, treated fruit averaged 50 ppb, while controls remained at 10 ppb. There were no differences between treatment and control Cabernet franc-1 fruit at 6- and 24-hr postspray, a consistent pattern for both cultivars.

Components of yield at harvest (shoots per meter, clusters per shoot, clusters per vine, cluster weight, berry weight, berry numbers, and fruit weight per vine) did not differ among control and ethanol treatment for either grape variety (data not shown). Fruit weight per vine averaged 3.1 kg and 2.47 kg for Cabernet franc-1 and -2, respectively, and 5.27 kg for Merlot.

Physicochemical (Brix, berry weight, pH, titratable acidity, color, total phenols, and total and phenol-free glycosides) differences between treatments for both varieties were determined (Table 1). Berry weights were not affected by treatment, consistent with results elsewhere (Chervin et al. 2001). Hue was the only Cabernet franc grape index that demonstrated a

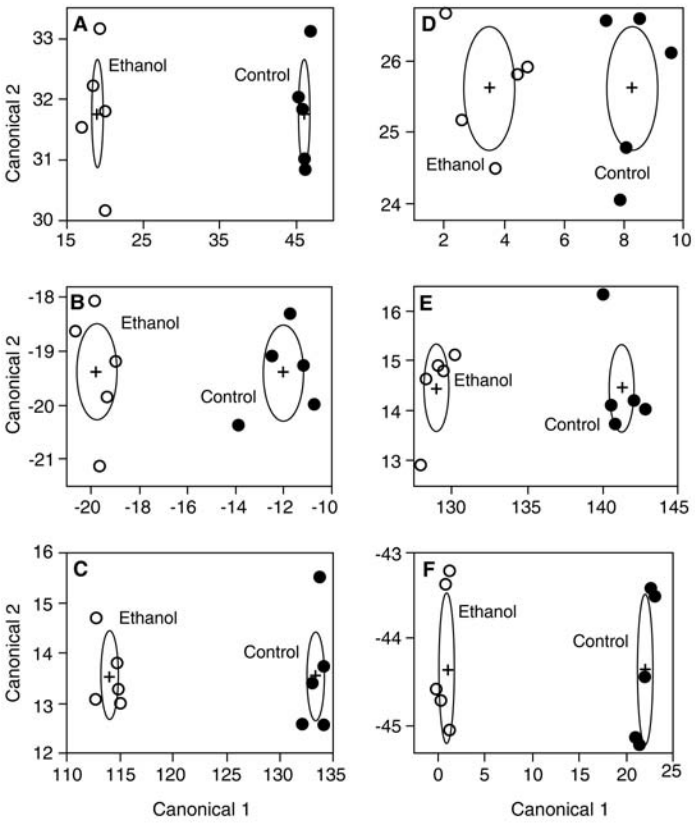

Figure 1 Canonical distribution of physicochemical analyses of ethanol treatment and control juice sampled at weeks (**A**) 14, (**B**) 15, and (**C**) 17 postbloom for Cabernet franc-1 (east-facing canopy side), and weeks (**D**) 14, (**E**) 15, and (**F**) 17 postbloom for Cabernet franc-2 (west-facing side). Significant differences at $\alpha = 0.05$ level indicated by nonintersecting circles.

Table 1 *p* Values comparing physicochemical indices between ethanol treatment and control fruit, for three sampling periods for Cabernet franc-1 (east side of canopy), Cabernet franc-2 (west side) (weeks 14, 15, and 17 postbloom), and Merlot (weeks 12, 13, and 16 postbloom).

Physicochemical indices	Cabernet franc-1			Cabernet franc-2			Merlot		
	Week 14	Week 15	Week 17	Week 14	Week 15	Week 17	Week 12	Week 13	Week 16
Brix	0.79	0.81	0.22	0.97	0.69	0.27	0.72	0.86	0.19
Berry wt (g)	0.24	0.31	0.94	0.71	0.10	0.08	0.71	0.95	0.63
pH	0.96	0.62	0.00*[a]	0.82	0.61	0.01*	0.80	0.93	0.44
Titratable acidity (g/L)	0.20	0.14	0.12	0.26	0.48	0.41	0.27	0.78	0.70
Color intensity ($A_{420}+A_{520}$)	0.05*	0.15	0.38	0.05*	0.15	0.11	0.66	0.79	0.05*
Hue (A_{420}/A_{520})	0.67	0.00*	0.03*	0.04*	0.00*	0.01*	0.88	0.77	0.28
Total phenols (A_{280})	0.00*	0.08	0.71	0.03*	0.04*	0.34	0.07	0.21	0.01*
PFGG (μ*M*)	nd[b]	nd	0.34	nd	nd	0.00*	nd	nd	0.03*
TGG (μ*M*)	nd	nd	0.09	nd	nd	0.85	nd	nd	0.94

[a]Asterisk indicates significant difference ($p < 0.05$).
[b]nd indicates that no data points are available for those samples.

consistent pattern between ethanol-treated and control samples (five out of six sampling dates). Fruit pH values for both Cabernet franc plots demonstrated a treatment effect on the last sampling date. Color intensity differences have been reported as a result of ethanol treatment (El Kereamy et al. 2002). Treated Cabernet franc fruit did not consistently have higher color intensities. At harvest, Merlot ethanol-treated grapes did show a significant difference in color intensity. Phenol-free glycosides, which are, in part, aroma and flavor precursors, illustrated treatment differences for Merlot and for Cabernet franc-2 grapes at harvest.

Canonical discriminant analysis (CDA) of the seven physicochemistry indices (plus PFGG and TGG for the final harvest) explained 100% of the variation on all sampling dates, for Cabernet franc (Figure 1) and Merlot (Figure 2). The CDA plots represent the multivariate mean of the data points, with circles indicating the 95% confidence limit for the mean. Nonintersecting circles indicate significant differences. The CDA scores that validated the grouping of the classes indicated that all replicates of each treatment were identified correctly (data not shown).

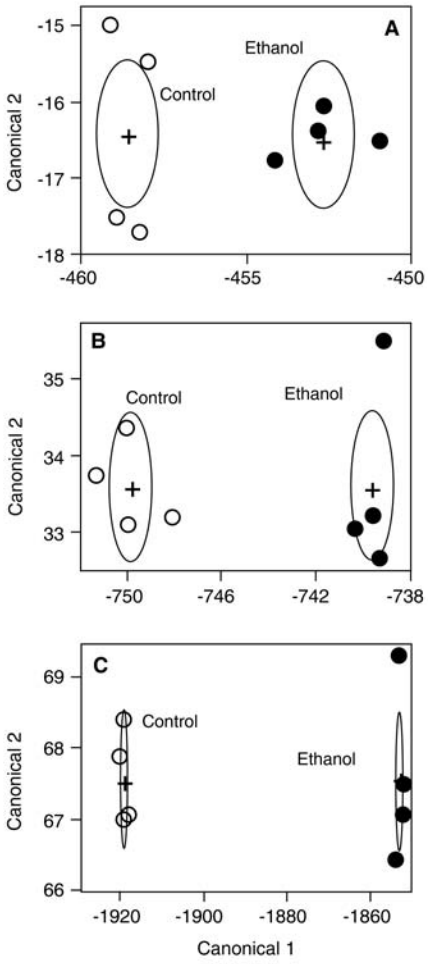

Figure 2 Canonical distribution of physicochemical analyses of control and ethanol treatment Merlot juice sampled at weeks (**A**) 12, (**B**) 13, and (**C**) 16 postbloom. Significant differences at $\alpha = 0.05$ level indicated by nonintersecting circles.

Both the CP and SAW ENose systems were able to explain 100% of the variation with one qualitative/quantitative measurement of clusters (CP) and juice (CP and SAW) for Cabernet franc berries (Figure 3) and Merlot juice (Figure 4). Results for Cabernet franc-2 (west canopy side) showed similar separation (data not shown). From the CP-based system results, #31 was the only sensor (identified by PCA vector plots) that did not help to explain the difference between control and ethanol treatments in both Cabernet franc and Merlot (data not shown).

Cabernet franc wine indices differed between treatment and control, with the exception of TGG and TA for both canopy sides and hue for Cabernet franc-2 (Table 2). Differences in wine alcohol and acidity for canopy sides reflect the differences noted in fruit maturity values. Those differences were also noted in the analysis of total phenols, color intensity, and phenol-free glycosides. Hue and total phenols were differentiated between treatments in Merlot wines (Table 2).

Canonical plots of Cabernet franc-1 (east canopy side) wines, using both CP and SAW ENose systems, demonstrated 100% difference between control and ethanol treatments (Figure 5A, B). These trends were the same for Cabernet franc-2 (data not shown). Merlot wines were also differentiated completely between control and ethanol treatments using both ENose systems (Figure 5C, D).

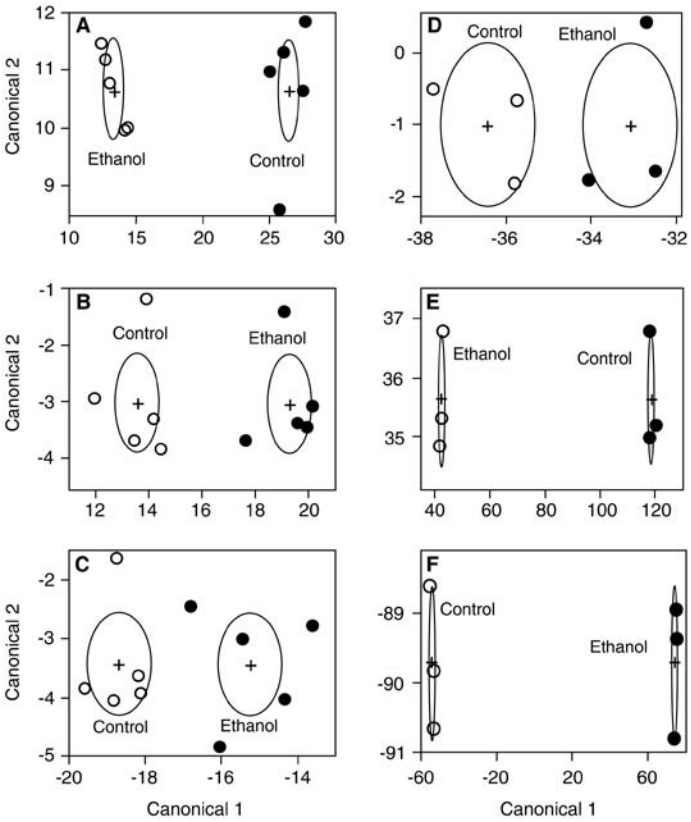

Figure 3 Canonical distribution of analyses of control and ethanol treatment Cabernet franc-1 (east-facing canopy side) berries using conducting polymer-based ENose at weeks (**A**) 14, (**B**) 15, and (**C**) 17 postbloom, and surface acoustic wave-based ENose at weeks (**D**) 14, (**E**) 15, and (**F**) 17 postbloom. Significant differences at $\alpha = 0.05$ level indicated by nonintersecting circles.

Table 2 Pairwise comparisons of control and ethanol treatment chemistry indices of Cabernet franc-1 (east side of canopy), Cabernet franc-2 (west side), and Merlot wines.

Varietal/treatment	Color intensity ($A_{420} + A_{520}$)	Hue (A_{420}/A_{520})	Total phenols (A_{280})	PFGG (μ*M*)	TGG (μ*M*)	Alcohol (% v/v)	Titratable acidity (g/L)
Cabernet franc							
Control-1	0.72 ± 0.01b[a]	0.47 ± 0.01b	2.67 ± 0.02b	28.18 ± 3.71b	1388 ± 33a	12.4 ± 0.05b	3.74 ± 0.10a
Ethanol-1	0.78 ± 0.01a	0.50 ± 0.01a	2.87 ± 0.02a	62.68 ± 3.71a	1498 ± 33a	13.0 ± 0.07a	3.49 ± 0.10a
Control-2	0.52 ± 0.02b	0.56 ± 0.01a	2.45 ± 0.03b	28.9 ± 2.03b	1323 ± 38a	12.5 ± 0.09b	3.67 ± 0.08a
Ethanol-2	0.72 ± 0.02a	0.57 ± 0.01a	3.02 ± 0.03a	65.55 ± 2.03a	1373 ± 38a	13.2 ± 0.07a	3.56 ± 0.08a
Merlot							
Control	1.31 ± 0.02a	0.43 ± 0.01b	3.65 ± 0.01b	89.99 ± 4.62a	1963 ± 54a	13.7 ± 0.08a	4.90 ± 0.06a
Ethanol	1.29 ± 0.02a	0.47 ± 0.01a	3.81 ± 0.01a	78.49 ± 4.62a	1883 ± 54a	13.8 ± 0.10a	4.86 ± 0.06a

[a]Within a varietal and canopy side, cells in each column marked by different letters indicate 95% significant difference.

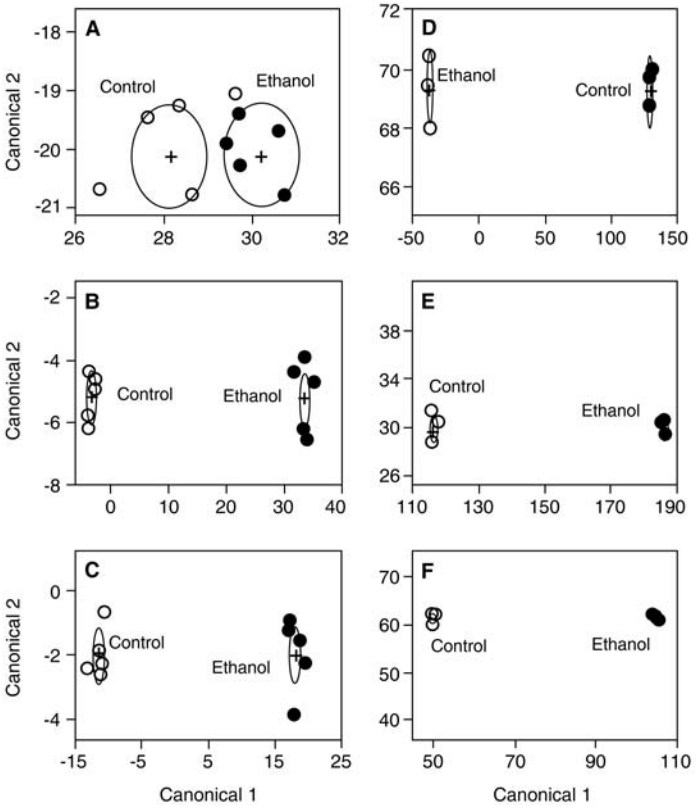

Figure 4 Canonical distribution of analyses of control and ethanol treatment Merlot juice using conducting polymer-based ENose at weeks (**A**) 12, (**B**) 13, and (**C**) 16 postbloom, and surface acoustic wave-based ENose at weeks (**D**) 12, (**E**) 13, and (**F**) 16 postbloom. Significant differences at α = 0.05 level indicated by nonintersecting circles.

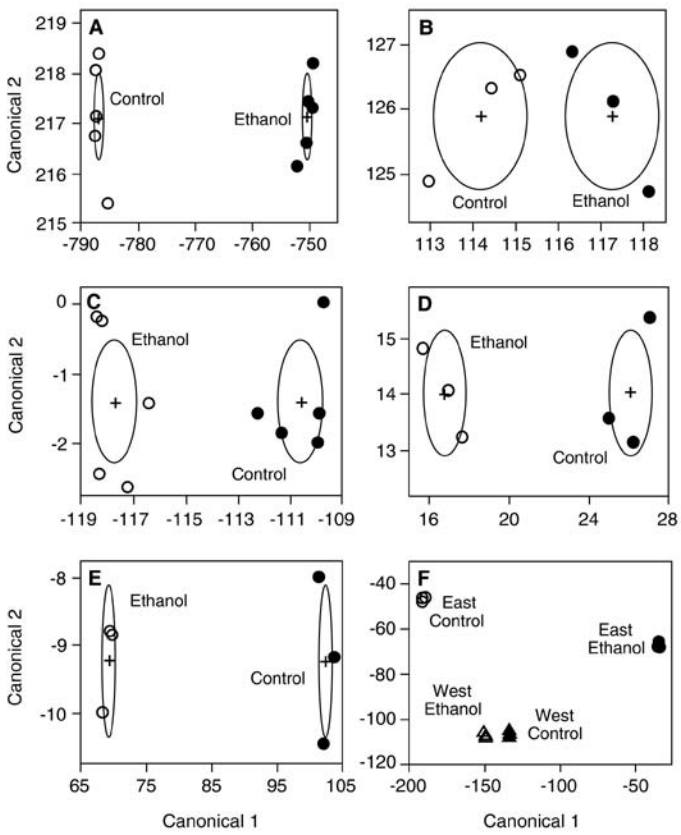

Figure 5 Canonical distribution of analyses of Cabernet franc-1 (east-facing canopy side) wine using (**A**) conducting polymer-based (CP) and (**B**) surface acoustic wave-based (SAW) ENose, Merlot wine using (**C**) CP and (**D**) SAW ENose, and GC-MS analyses of control and ethanol treatment (**E**) Merlot and (**F**) Cabernet franc wines 6 months postfermentation. Significant differences at α = 0.05 level indicated by nonintersecting circles or groups of data.

The GC-MS wine analysis results, evaluated by ANOVA and pairwise comparison, showed the differences in volatile concentrations between ethanol treatment and control for both grape varietals (Table 3). In Cabernet franc-1 and -2, treatment and control wines showed differences in 26 of 31 volatiles quantified, perhaps consistent with the differences noted in fruit maturity and resultant alcohol concentration. Merlot wines demonstrated differences in 11 of 32 volatiles as a result of treatment, despite no differences in alcohol concentration between treated and control wines. As shown by CDA, GC-MS was able to 100% discriminate the ethanol treatment from control wines for both varietals (Figure 5E, F). The GC-MS data support the ENose results, which also showed differences between wines as a result of treatment.

Under the set conditions of the sensory evaluations (α = 0.05, β = 0.30, ρd = 40%), panelists were able to differentiate the ethanol-treated and control Merlot, but not the Cabernet franc wines (Table 4). Changing the test power by using an alpha level of 0.20 would result in significant differences for all sensory evaluations. The GC-MS data suggested significant

Table 3 Effect of control and ethanol treatments on Cabernet franc-1 (east side of canopy) and -2 (west side) and Merlot wine volatiles as determined by GC-MS analysis.

	Cabernet franc concn (µg/L)					Merlot concn (µg/L)		
Compound	**Control-1**	**Ethanol-1**	**Control-2**	**Ethanol-2**	***p* value**	**Control**	**Ethanol**	***p* value**
Ethyl acetate	24.88 ± 0.34bc[a]	25.71 ± 0.34a	24.13 ± 0.34c	29.13 ± 0.34a	0.00*[a]	28.88 ± 0.66a	30.28 ± 0.66a	0.21
1-Propanol	nd[b]	nd	nd	nd	na[c]	39.14 ± 0.80a	38.53 ± 0.80a	0.62
2-Methyl propanol	23.07 ± 0.65bc	25.09 ± 0.65b	22.44 ± 0.65c	27.33 ± 0.65a	0.00*	21.04 ± 0.68a	20.83 ± 0.68a	0.84
Isoamyl acetate	3004.95 ± 65.3b	3557.83 ± 65.3a	2781.96 ± 65.3c	3393.67 ± 65.3a	0.00*	5571.85 ± 179.78a	5157.57 ± 179.78a	0.18
n-Butanol	9.12 ± 0.01c	9.18 ± 0.01b	9.13 ± 0.01c	9.24 ± 0.01a	0.00*	9.30 ± 0.02a	9.34 ± 0.02a	0.24
3-Methyl butanol	75.26 ± 1.76bc	80.12 ± 1.76b	71.77 ± 1.76c	90.37 ± 1.76a	0.00*	85.83 ± 2.81a	86.62 ± 2.81a	0.85
Ethyl hexanoate	216.73 ± 4.29b	213.06 ± 4.29b	194.05 ± 4.29c	246.09 ± 4.29a	0.00*	342.71 ± 10.09a	351.06 ± 10.09a	0.59
Hexyl acetate	4.17 ± 0.07a	3.78 ± 0.07b	1.47 ± 0.07d	2.48 ± 0.07c	0.00*	10.25 ± 0.21a	6.82 ± 0.21b	0.00*
Ethyl heptanoate	5.01 ± 0.10c	9.66 ± 0.10a	5.05 ± 0.10c	5.81 ± 0.10b	0.00*	7.79 ± 0.19a	8.38 ± 0.19a	0.09
n-Hexanol	1.54 ± 0.04b	1.49 ± 0.04b	1.69 ± 0.04a	1.71 ± 0.04a	0.01*	1.13 ± 0.04a	1.02 ± 0.04a	0.09
Ethyl octanoate	109.93 ± 2.06c	117.37 ± 2.06b	109.39 ± 2.06c	155.68 ± 2.06a	0.00*	168.08 ± 3.07a	175.48 ± 3.07a	0.16
2-Ethyl-1-hexanol	6.60 ± 0.23c	9.60 ± 0.23a	8.14 ± 0.23b	9.59 ± 0.23a	0.00*	5.98 ± 0.22b	7.48 ± 0.22a	0.01*
Benzaldehyde	nd	nd	nd	nd	na	17.01 ± 0.08b	18.73 ± 0.08a	0.00*
Ethyl nonanoate	24.13 ± 0.04c	24.79 ± 0.04a	24.29 ± 0.04b	24.69 ± 0.04a	0.00*	24.80 ± 0.07b	25.41 ± 0.07a	0.00*
1-Octanol	106.82 ± 3.01d	129.47 ± 3.01c	255.91 ± 3.01a	220.85 ± 3.01b	0.00*	24.52 ± 0.84a	26.11 ± 0.84a	0.25
Terpinene-4-ol	13.17 ± 0.76d	27.37 ± 0.76c	41.86 ± 0.76b	50.93 ± 0.76a	0.00*	2.11 ± 0.05b	2.91 ± 0.05a	0.00*
Ethyl decanoate	59.04 ± 1.70c	80.74 ± 1.70b	82.00 ± 1.70b	130.36 ± 1.70a	0.00*	121.60 ± 1.85a	113.14 ± 1.85b	0.03*
Isoamyl octanoate	36.21 ± 0.05d	36.80 ± 0.05b	36.57 ± 0.05c	37.46 ± 0.05a	0.00*	38.71 ± 0.05a	37.46 ± 0.05b	0.00*
Nonanol	7.92 ± 0.25b	8.55 ± 0.25b	12.01 ± 0.25a	8.69 ± 0.25b	0.00*	8.34 ± 0.24b	9.31 ± 0.24a	0.05*
Isovaleric acid	2.23 ± 0.05b	2.27 ± 0.05b	2.05 ± 0.05c	2.51 ± 0.05a	0.00*	2.05 ± 0.05a	2.12± 0.05a	0.41
Diethyl succinate	341.11 ± 11.13b	369.07 ±11.13b	275.90 ±11.13c	515.46 ± 11.13a	0.00*	264.11 ± 7.97a	278.10 ± 7.97a	0.28
Methionol	1.56 ± 0.04a	1.60 ± 0.04a	1.57 ± 0.04a	1.63 ± 0.04a	0.59	2.05 ± 0.12a	2.11 ± 0.12a	0.78
Citronellol	10.17 ± 1.16b	10.28 ± 1.16b	19.82 ± 1.16a	22.74 ± 1.16a	0.00*	3.81 ± 1.25a	2.81 ± 1.25a	0.60
Phenethyl acetate	93.22 ± 2.42ab	98.47 ± 2.42a	88.60 ± 2.42b	89.15 ± 2.42b	0.07	147.30 ± 4.19a	119.46 ± 4.19b	0.01*
β-Damascenone	24.91 ± 0.47b	22.61 ± 0.47c	26.85 ± 0.47a	17.84 ± 0.47d	0.00*	6.39 ± 0.20a	6.27 ± 0.20a	0.71
Hexanoic acid	1.04 ± 0.05a	1.00 ± 0.05a	0.98 ± 0.05a	1.14 ± 0.05a	0.22	nd	nd	na
Ethyl dodecanoate	26.42 ± 0.64b	27.84 ± 0.64b	27.09 ± 0.64b	39.78 ± 0.64a	0.00*	54.89 ± 0.76a	46.28 ± 0.76b	0.00*
Benzyl alcohol	137.62 ± 2.87c	149.97 ± 2.87b	150.74 ± 2.87b	166.07 ± 2.87a	0.00*	176.51 ± 5.27a	175.22 ± 5.27a	0.87
Phenethyl alcohol	21.12 ± 0.63b	21.86 ± 0.63b	20.96 ± 0.63b	26.31 ± 0.63a	0.00*	24.00 ± 0.88a	25.37 ± 0.88a	0.33
γ-Nonalactone	0.08 ± 0.00b	0.08 ± 0.00ab	0.09 ± 0.00a	0.09 ± 0.00a	0.01*	0.06 ± 0.00a	0.06 ± 0.00a	0.37
Ethyl myristate	41.18 ± 0.16a	39.70 ± 0.16b	40.02 ± 0.16b	39.66 ± 0.16b	0.00*	41.84 ± 0.29a	41.22 ± 0.29a	0.20
Octanoic acid	1.63 ± 0.14a	1.32 ± 0.14a	1.63 ± 0.14a	1.22 ± 0.14a	0.16	2.08 ± 0.16a	1.06 ± 0.16b	0.01*
Ethyl palmitate	89.01 ± 0.53a	87.12 ± 0.53b	87.61 ± 0.53ab	89.05 ± 0.53a	0.07	104.78 ± 3.56a	111.72 ± 3.56a	0.24

[a]Within a varietal, values in each row marked by different letters, and *p* values with asterisks, indicate 95% significant difference.
[b]nd indicates that the volatile was not detected.
[c]na indicates that a *p* value is not applicable for those samples.

Table 4 Triangle difference aroma sensory results of control versus ethanol spray (EtOH) on Cabernet franc-1 (east side of canopy) and -2 (west side), and Merlot wines.

Varietal/treatment	Correct responses	Total responses	Signif[a]
Cabernet franc			
Control-1 vs EtOH-1	9	32	No
Control-2 vs EtOH-2	14	32	No
Merlot			
Control vs EtOH	19	32	Yes

[a]$\alpha = 0.05$, $\beta = 0.10$, $\rho_{max} = 40\%$, 16 or more correct responses corresponds to a significant difference.

differences in some wine volatiles analyzed between treatments and control, indicating a treatment effect. The fact that ENoses can evaluate both aroma and nonaroma volatiles (Haugen and Kvaal 1998) may help to explain the variations between their discrimination abilities and some of the sensory evaluations. The sensory analyses were conducted using essentially untrained panelists. Evaluations using experienced, trained judges may have provided different results, as suggested elsewhere (Lattey et al. 2006).

Conclusions

Changes in grape and wine volatiles caused by spraying Cabernet franc and Merlot fruit with an ethanol/water solution at veraison were evaluated using conducting polymer-based and surface acoustic wave-based electronic nose systems. Both of the ENose systems were able to separate ethanol-treated grapes and wine from controls. Wine aroma sensory evaluation showed differences between treatments for Merlot only. Some individual physicochemistry indices did not show differences as a result of treatment for either variety. However, when all indices except TGG and PFGG were analyzed together (multivariate), ethanol treatments were distinguished from the controls 100% of the time. Although multivariate

statistical results indicated the usefulness of traditional grape physicochemistry indices, the general inconsistency in detecting differences and the amount of time required to conduct these measurements suggests the need for alternatives. Electronic nose analysis (conducting polymer ENose) is a rapid and nondestructive technology that may supplement or replace the existing methods for evaluating select vineyard management practices. With only one measurement, the electronic noses demonstrated greater discrimination between ethanol-treated and control fruit and wines than did conventional assays.

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