Effect of Training Systems on Viognier (*Vitis vinifera* L.) Grape and Wine Glycosides and Volatile Compounds

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ABSTRACT

Viognier (*Vitis vinifera* L.) grapes were grown in Northern Virginia for three seasons using three different training systems in a randomized complete block design consisting of Vertical Shoot Positioned (VSP), Smart Dyson (SD), and Geneva Double Curtain (GDC), and evaluated for the effects on grape and wine glycosides and volatile compounds. Fruit was harvested at the same Brix each season, and differences in berry weights were not observed. VSP-trained vines had the lowest crop load and lowest light levels in the fruit zone. Seventeen volatile compounds were analyzed using headspace solid-phase microextraction, GC-MS. Fruit showed differences in linalool, α-terpineol, β-damascenone, and n-hexanol concentrations among the training systems. Wines showed differences in both grape-derived and fermentation-derived volatiles. SD had the highest concentration for most of the free volatiles quantified in both the juice and wine. VSP had lower phenol-free wine glycosides all three seasons and lower phenol-free juice glycosides one season. Triangle difference sensory testing demonstrated differences between GDC and SD in wine aroma and flavor, and differences between VSP and SD in flavor, for two of three seasons.

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PROJECT SUMMARY/GOALS

The objective of this study was to evaluate Viognier grown in Northern Virginia under three different training systems: Geneva Double Curtain and Smart-Dyson (divided canopy systems) and the Vertical Shoot-Positioned (non-divided, "standard" system). Chemical analyses were performed on the fruit and the wine made from the fruit from each training system to evaluate objectively the flavor chemistry of the fruit and wine. Wine was evaluated by sensory triangle tests to determine sensory differences in the wine aroma and flavor.

The Viognier wine grapes were grown at the Agriculture Research and Extension Center in Winchester Virginia and evaluated over a period of three years (2001, 2002 and 2003). However in the first year only preliminary data was taken. VSP is the "standard" non-divided training system that is commonly used in the state. Smart Dyson and Geneva Double Curtain are two common divided training systems used in Virginia. Not much research has been done with Viognier to determine how different training systems affect the glycoside and volatile concentrations in Viognier juice and wine or to determine which training systems is optimum for this wine grape in Virginia. The goal of this study is to determine if there are differences between training systems in regard to: 1) total and phenol free glycoside composition in the skins juice and wine; 2) free volatiles in the juice and wine; and 3) aroma and flavor differences between training systems in the wines produced from these grapes. The results of this study will help assist in developing recommendations for training systems that will yield optimal wine quality from Viognier grapes grown in Virginia area. Such recommendations assist the

wine industry in Virginia that has an increasing interest in Viognier with improved grape production and improved wine quality.

Chapter 1: LITERATURE REVIEW

Introduction to Viognier

Viognier is a white grape originating in the northern Rhone region of France. The grape tends to have lower yield due to high susceptibility to fungal disease which has contributed to the initial decrease in grape production in France. The enjoyment of the wine and demand for the grape created a slow rise in planting and production around the world in the last 25 years (Robinson 1994). This grape is typically characterized as having muscat character (Ribéreau-Gayon et al. 2000). Viognier wine noted for being a higher alcohol white wine that tends to be deep yellow in color (Robinson 1994). The aroma of Viognier wine tends to have floral and fruit aroma with characteristics including pear, peach, apricot and blossom (Robinson 1994).

Glycosides

Glycosides are important secondary plant metabolites that develop during grape maturation. These glycosides are important because a correlation has been suggested between increased glycosides and increased wine quality (Abbott et al. 1991). Glycosides include aglycones bound to the disaccharides including α-L-arabinofuranosyl-β-D-glucopyranosides and α-L-rhamnopyranosyl- β-D-glucopyranosides (Williams et al. 1982, Gunata et al. 1985). These aglycones contain groups of compounds including monoterpenes, sesquiterpenes, C-13 norisoprenoids, shikimik acid metabolites, and aliphatic residues (Williams et al. 1980, 1981). Terpenes are a group of compounds that are comprised of 5 carbon isoprene units. Monoterpenes (C10) contain two isoprene units and sesquiterpenes (C15) contain three isoprene units. Monoterpenes are typically

the prominent aroma compound in aromatic or muscat varieties of wine typically giving fruity and floral aromas (Park and Noble 1993). However, these compounds are also present in non-muscat varieties, and are important contributors to the aroma profile (Marais 1983). Bayonove and Cordonnier (1971) showed that certain monoterpenes including linalool, geraniol, citronellol and α-terpineol have 10 to 100 times greater concentration in muscat varieties compared to non-muscat. Geraniol, linalool, and nerol are typically the most common monoterpenes found in muscat grapes and wine (Marais 1983, Park and Noble 1993) with geraniol and linalool collectively comprising 75-80% of the total pool of terpenes (Marais 1983). There is varying distribution of some of the major monoterpenes with in the grape. Geraniol and nerol are typically associated with the skins of the grapes whereas linalool is more evenly distributed between the skins and mesocarp (juice and pulp) (Bayonove et al. 1974, Park and Noble 1993). In Muscat d'Alexandrie 96% of nerol and 94% of geraniol were found in the skins and linalool was found to have 50% in the juice, 26% in the skin and 24% in the cellular debris (Cordonnier and Bayonove 1978, Cordonnier and Bayonove 1981, and Bayonove et al. 1974). During ripening the amount of free and glycosylated monoterpenes can change as well as the distribution within the grape (Park and Noble 1993).

Studies looked at monoterpene glycosides and how to release the bound monoterpenes to better impact the aroma qualities of a wine. Studies by Williams et al. (1981) showed that heat could hydrolyze the glycosidic bond and release the bound terpenes. Usseglio-Tomasset (1981) also showed increases in certain free monoterpenes with heat treatment which increased aroma intensity in the wine and improved the wine

quality. Enzyme and acid hydrolysis have also been shown to break the glycosidic bonds and release bound monoterpenes (Cordonnier and Bayonove 1981, Williams et al. 1981).

C-13 norisoprenoids is another group of aglycones that are produced during carotenoid degradation during grape maturity (Razungles et al. 1988). The carotenoids go through a series of pathways where they eventually break down into various ketones commonly found in grapes and then are broken down to norisoprenoids (Williams et al. 1992). These compounds are typically the dominant component of non-floral varieties of wine grapes including Chardonnay and Semillion where they tend to create aromas of honey, lime, tea, grassy and pineapple. In some floral varieties these compounds contribute to bottle aging characteristics including floral, fruity, and kerosene aromas (Ebeler 2001).

Shikimic acid metabolites are phenolic compounds produced via the shikimic acid pathway. Typically the phenol containing compounds are derived from the aromatic amino acid phenylalanine. Flavonoids are typically composed of two phenols (aromatic rings) and typically contribute to color, especially in the case of flavanols, but do have some aromatic contributions (Zoecklein et al. 1999a). Anthyocyanins, a type of flavanol, are responsible for the red pink and blue colors in the skins. Tannins are typically phenolic polymers and contribute to astringency. Tannins are also noted for their antimicrobial effect (Ribéreau-Gayon et al. 2000). These compounds can impact flavor but more often impact the structure and mouth feel of a wine including body, bitterness and astringency (Ribéreau-Gayon et al. 2000).

Some of these aglycones most importantly the terpenes and norisoprenoids can be aroma/flavor compounds. These bound aroma/flavor molecules are considered potential

aroma/flavor (Abbott et al. 1991). The glycosidic bonds can be hydrolyzed due to enzyme, heat, or acid hydrolysis, or by hydrolysis during fermentation (Marais 1983) and aging (Zoeckein et al. 1999b). This can release the aglycones potentially adding to the free volatile aroma/flavor compounds (Williams et al. 1980, 1981). Grape-derived aroma/flavor compounds can be important to varietal characteristics of wines (Ribereau-Gayon et al. 1975).

A glyosyl-glucose test was developed by Williams et al. (1995) and modified by Iland et al. (1996) to determine the total amounts of bond aglycones present in grape juice and wine. When the glycosidic bonds are hydrolyzed it is broken down to equimolar proportions of aglycone and D-glucose which has been proposed to be a measurement of the bound compounds. A phenol free glycoside measurements was also developed to give a measurement of the total minus the phenol glycosides (shikimic acid metabolites) which is suggested to be a better estimate of the pool of aglycones containing aroma compounds (Zoecklein et al. 2000).

Training Systems

Secondary metabolites including glycosides and grape-derived volatiles can be influenced using training systems which create different canopy architectures and influences the microclimate within the canopy (Smart and Robinson 1991). Three common training systems used in the United States including Virginia are Vertical Shoot Positioned (VSP), Smart Dyson (SD) and Geneva Double Curtain (GDC). Vertical Shoot Positioned is a non-divided canopy and is the most common training and trellising system in the United States as well as many other countries including France and Germany. This

system involves the vine trained up to the cordon wire and the cordons going outward along the cordon wire into the typical T shape with the shoots trained vertically upwards. The fruiting zone is typically 0.9-1.2 meters high. The typical spacing of each row is at least 2 meters apart (Smart and Robinson 1991). The Smart-Dyson (SD) is a vertically divided training system developed by Dr. Richard Smart and Dr. Henry Dyson. The Smart Dyson shoots are alternately trained upwards and downwards along the same cordon (Smart and Robinson 1991). The Geneva Double Curtain (GDC) is a horizontally divided training system developed at the Geneva Experiment Station at Cornell University. This training system involves the vine coming up and splitting out to parallel cordon wires and then the canes split in opposite directions creating two curtains on each side of the base vine. The shoots are trained to grow downward (Smart and Robinson 1991).

Effects of Climate on Glycosides/Free Volatiles in Wine

Different aspects of climate can affect the grape-derived volatiles and glycoside concentration in the grapes. Climate is divided into three classifications, macroclimate, mesoclimate and microclimate. Macroclimate is the climate of the region and mesoclimate is the climate of the vineyard site (Smart and Robinson 1991). Marais et al. (1992b) showed various differences in free and bound monoterpenes and C-13 norisoprenoids in Riesling in different climates suggesting warmer weather increased production. A study by Marais, et al. (1999) found monoterpene development was greater in cooler seasons when grown in a warm climate. Carotenoids in Weisser Riesling and Chenin blanc were shown to have higher concentrations in hotter regions

(Marais et al. 1991) which could be an indicator of more potential C-13 norisoprenoid production in these warmer regions.

Most aspects of macroclimate and mesoclimate cannot be controlled or manipulated, however there are some aspects of the microclimate, which is the climate in the grape vine canopy and around the fruit, including light and temperature that can be influenced by canopy manipulation (Marais 1996). Sunlight is the driving force for photosynthesis which in turn helps sugar production and enzymatic activity responsible for aroma and flavor production. Differences in the canopy light can affect the secondary grape metabolite composition including glycoside concentrations and grape-derived volatile concentrations (Marais et al. 1999, Reynolds et al. 1994, Reynolds et al. 1996a, Zoecklein et. at, 1998). A study by Marais et al. (1999) showed that shaded Sauvignon Blanc grapes gave less monoterpenes and C13 norisoprenoids, which gave floral and fruity aromas and more methoxypyrazines, which gave green and grassy aromas than the grapes with more sunlight. A study by Reynolds and Wardle (1997) showed that increased sun exposure of Gewürztraminer grapes gave more terpenes glycosides and free volatile terpenes than shaded grapes. Bureau et al. (2000 also showed an increase in grape volatiles and glycosides with an increase in sun exposure from 30% to 50% in Syrah berries. Increased sun light has also been shown to cause higher concentrations of C13 norisoprenoids glycosides and volatile C13 norisoprenoids in Reisling (Marais1996, Marais et al. 1992a), although Marais et al. (1991) showed that levels of carotenoids were lower with increased sun exposure indicating less C13 norisoprenoids production. Excessive sunlight exposure, up to 100%, however has been shown to decreased terpene

glycosides compared to 50% possibly due to excessive cluster temperatures (Belancic et al. 1997).

Temperature is important for aroma production because aroma production is an enzyme driven reaction (Kliewer 1977). Optimal rate of grapevine photosynthesis occurs at 23-25°C (Kreidemann 1968). Below this optimum, more sugar is available for production of secondary metabolites (Gladstone 1992). While optimal temperature for secondary metabolite production has not been established (Gladstone 1992), temperatures of 35- 40°C or greater are believed to inhibit production of some secondary metabolites (Kliewer 1970, Spayd et al. 2002). Within the microclimate the leaves and grapes are typically around the same temperature as the air but slight changes can occur because of transpiration, time of day and leaf shading (Smart and Robinson 1991). Increased sun light has been shown to increase the temperature within the grapevine canopy 3-8°C above ambient (Kliewler and Lider 1968, Bergqvist et al. 2001).

Impact of Training System/Vineyard Practices

Viticultural practices can manipulate this microclimate as shown before and in turn the yield and composition of the fruit and wine. Reynolds et al. (1996a) looked at how various training systems/canopy manipulation affected Riesling and showed that two divided canopies gave increased yields (tons/hectare), clusters per vine, as well as lower berry weight and less berries per cluster. Another study by Reynolds and Wardle (1997) showed that divided canopies produced an increase in free volatile terpenes and terpene glycoside concentrations in Riesling grapes. A study by Zoecklein et al. (1998) demonstrated that leaf removal in the fruit zone caused a general increase in total and

phenol free glycosides in the resulting Riesling and Chardonnay juice due to increased light. This is similar to Reynolds et al. (1996a) who showed that basal leaf removal gave increases in terpene glycosides in Riesling grapes.

Training systems can impact yield (Reynolds et al. 1996a, Shaulis et al. 1966, Reynolds et al. 2004) which may in turn impact secondary metabolites (McCarthy et al. 1986). One study demonstrated increased terpene glycoside and volatile tepene concentrations with increased yield: total (tons/acre), clusters per vine, and clusters per shoot (Reynolds et al 1994). However, in another study, doubling yield (tons/hectare) decreased terpene glycosides and no effect on volatile terpenes (McCarthy et al. 1986). Chapman et al. (2004a) observed that a decrease in yield doubled the concentration of grape-derived volatiles.

When climate affects the chemical compounds of the juice and wines, it can also affect the sensory attributes. Reynolds et al. (1996b) who showed that canopy manipulation affected sensory attributes of Gewürztraminer wine. The canopy manipulated wines gave a more floral and muscat aromas and flavor where the control tended to be more vegetative. Wine sensory differences have also been found due to differences in crop yield (Chapman et al. 2004b, Ough and Nagaoka 1984, Sinton et al. 1978)

Many components of microclimate are involved in the aroma and flavor development as well as overall quality of a wine. Changing the microclimate by way of training and trellising system has been shown to produce differences in secondary metabolites in juice and wine.

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Chapter 2: Effect of Training Systems on Viognier (*Vitis vinifera* L.) Grape and Wine Glycosides and Volatile Compounds.

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ABSTRACT

Viognier (*Vitis vinifera* L.) grapes were grown in Northern Virginia for three seasons using three different training systems in a randomized complete block design consisting of Vertical Shoot Positioned (VSP), Smart Dyson (SD), and Geneva Double Curtain (GDC), and evaluated for the effects on grape and wine glycosides and volatile compounds. Fruit was harvested at the same Brix each season, and differences in berry weights were not observed. VSP-trained vines had the lowest crop load and lowest light levels in the fruit zone. Seventeen volatile compounds were analyzed using headspace solid-phase microextraction, GC-MS. Fruit showed differences in linalool, α-terpineol, β-damascenone, and n-hexanol concentrations among the training systems. Wines showed differences in both grape-derived and fermentation-derived volatiles. SD had the highest concentration of most free volatiles quantified in both juice and wines. VSP had lower phenol-free wine glycosides all three seasons, and lower phenol-free juice glycosides one season. Triangle difference sensory testing demonstrated differences

between GDC and SD in wine aroma and flavor, and differences between VSP and SD in flavor, for two of three seasons.

KEY WORDS: glycosides, volatiles, aroma, training system, Viognier

INTRODUCTION

Total glycosides have been shown to be positively correlated to wine quality (Abbott et al. 1991). Grape glycosides are secondary plant metabolites bound to disaccharides including α-L-arabinofuranosyl- β-D-glucopyranosides and α-L-rhamnopyranosyl- β-D-glucopyranosides (Wiliams et al. 1982, Gunata et al. 1985). These compounds typically comprise aglycones including aliphatic residues, monoterpenes, C13 norisoprenoids, and shikimic acid metabolites. Glycosidic bonds can be broken, releasing aglycones and potentially adding free volatiles, including some aroma/flavor compounds (Williams et al. 1980, 1981). Some grape-derived aroma/flavor compounds are important to varietal characteristics of wines (Ribereau-Gayon et al. 1975).

Grape-derived volatile compounds and glycosides can be influenced by canopy microclimate. Light and heat can be affected by training system architecture (Smart and Robinson 1991), and impacted by sun exposure (Shaulis et al. 1966). Sunlight has been shown to increase the temperature within the grapevine canopy 3-8°C above ambient (Kliewler and Lider 1968, Bergqvist et al. 2001). In one study, increased canopy temperature of 2°C modified terpene glycoside concentration and impacted wine sensory features (Reynolds et al. 1996a).

Secondary metabolite development is enzyme-derived and, therefore, temperature dependent (Kliewer 1977). Optimal rate of grapevine photosynthesis occurs at 23-25°C (Kreidemann 1968). Below this optimum, more sugar is available for production of secondary metabolites (Gladstone 1992). While the optimal temperature for secondary metabolite production has not been established (Gladstone 1992), temperatures of 35-40°C or greater are believed to inhibit production of some secondary metabolites (Kliewer 1970, Spayd et al. 2002).

Training systems have been shown to impact terpene and C-13 norisoprenoid glycosides by influencing fruit light exposure (Razungles et al. 1988, Reynolds and Wardle 1989). For example, sun exposure of 50% increased fruit glycosides, compared to 30% exposure (Bureau et al. 2000). Sunlight exposure up to 100% decreased terpene glycosides, compared to 50%, possibly due to cluster temperature (Belancic et al. 1997).

Volatile compounds in the grape have also been shown to be impacted by sunlight (Belancic et al. 1997). For example, Marais et al. (1995) reported decreases in free volatile 2-methoxy-3-isobutylpyrazine due to increased light. Other studies have shown that increased light exposure, using different training systems, increased free volatiles such as terpenes (Reynolds et al. 1996 a, Marais et al.1999) and C-13 norisoprenoids (Marais et al. 1996).

Training systems can impact the incidence of fungal disease (Zahavi et al. 2001). Dense canopies may have a higher relative humidity in the fruiting zone (English et al. 1989). More open canopies also allow more sunlight (Zahavi et al. 2001) and wind (English et al. 1989), which can reduce moisture through increased evaporation and reduce incidence of mold (English et al. 1993).

Training system may impact yield (tons/hectare), yield per vine (Reynolds et al. 1996a), shoots per vine (Shaulis et al. 1966, Smart et al. 1985), clusters per vine (Reynolds et al. 2004), berry weight and number of berries per cluster (Reynolds et al. 1996a, Reynolds et al. 2004). Yield has been reported to influence secondary metabolites (McCarthy et al. 1986). Reynolds et al. (1994b) demonstrated increased terpene glycosides and volatile terpene concentrations with increases in yield components: weight per vine, clusters per vine, and clusters per shoot. However, when McCarthy et al. (1986) doubled yield (t/ha), terpene glycosides decreased by 33% with no effect on volatile terpenes. Chapman et al. (2004a) observed that a 60% decrease in yield doubled the concentration of grape-derived volatiles. Wine sensory differences have also been found due to differences in crop yield (Chapman et al. 2004b, Ough and Nagaoka 1984, Sinton et al. 1978).

Limited research has been conducted on viticultural practices that affect Viognier grape and wine quality. This study evaluated the impact of training systems on Viognier grape and wine glycosides and free volatile compounds over three seasons.

MATERIALS AND METHODS

Viognier was planted in 1998 on C3309 rootstock at the Agricultural Research and Extension Center in Winchester, Virginia, using three training systems: Geneva Double Curtain (GDC), Smart-Dyson (SD) and Vertical Shoot Positioned (VSP). Vines were utilized in a completely randomized, split-plot experimental design involving the three training systems (main plots) and three cultivars (sub-plots), with three replicates.

Experimental units were three-vine plots. The experimental design was treated as a randomized complete block design for purposes of data analysis.

Vines were planted 2.4 m apart in 3.0-m wide rows. The soil is a Frederick-Poplimento complex with a depth of greater than 1.5 m. Available water was in the rooting profile of 90 to 150 mm/m, and the organic matter was in the range of 0.5 to 2.0% (Soil Survey of Frederick County, Virginia, USDA/Soil Conservation Service, 1987). Vineyard management was typical for commercial operations in the region (Wolf and Poling 1995) and involved row middles maintained with perennial turf-type tall fescue (*Festuca arundinacea* cv. 'Shenandoah'), a 1.2-m weed free under trellis strip, and appropriate pest management.

Canopy light measurements were conducted using an AccuPar model PAR-80 ceptometer (Decagon Devices, Inc., Pulman, WA) ± 2 hours of solar noon on cloudless days on or about véraison each year (6 August 2001, 1 August 2002 and 14 August 2003). Temperature and rainfall were measured at the Campbell Scientific Meteorological Station, Winchester, VA. Yield components including total yield (tons/acre), fruit weight (kg) per vine, clusters per shoot, and berries per cluster were measured, except clusters per shoot were not determined in 2002.

Harvest and Winemaking. Grapes were harvested by hand at approximately the same degrees Brix within each year (14 September 2001, 12 September 2002 and 8 October 2003), and transported to Virginia Tech's research winery (Blacksburg, Virginia) for wine production using standard winemaking methods, as described by Zoecklein et al. (1999a). For two seasons, data from the individual Smart-Dyson up and down canopies were

analyzed separately; however, in 2001 data from the Smart-Dyson training up and down were combined. Fruit was whole-cluster pressed in a Willmes (model TP-100) 100-L bladder press to 1.5 bar, and 16 µL/L pectic enzyme (Cinn-Free, Scott Labs, Petuluma, CA) added. Juice was settled for 24 h at 7°C, racked into 19-L glass carboys in duplicate lots of 9.4 L of must per training system, with 20 mg/L SO_2. Fermentations were conducted using 238 mg/L *Saccharomyces cerevisiae* (VL1 yeast, Laffort Enologie, Bordeaux, Cedex, France), 238mg/L Fermaid K (Lallemand, Montreal, Canada), and 298 mg/L of GO-FERM ® (Lallemand, Montreal, Canada). GO-FERM® and yeast were dissolved in 40°C water for 20 min, mixed and allowed to cool to within 7°C of the must temperature before inoculating. Once dry (≤ 2 g/L of sugar), wines were settled for 4 days at 7°C and then racked with the addition of 35 mg/L sulfur dioxide into 3.78-L glass jugs and stored at 7°C. Wines were subsequently racked from the lees four months post-fermentation.

Fruit Composition Analyses. A random sample of berries from each 3-vine panel was collected at harvest. The average berry weight was determined by weighing 25 berries. Berry samples were crushed in a Waring commercial laboratory blender (model 31 BL 91) (New Hartford, CT), for 1 sec and placed in a Steward Stomacher® Lab Systems filter bag (model 400) (London, England) and the grape juice expressed. The juice was filtered through a 0.45-µm syringe filter for chemical analyses, and skins were frozen for subsequent analysis. Juice Brix was measured using an American Optical® Scientific Instruments handheld refractometer (model 10430) (Buffalo, NY), pH using an Accumet pH meter, model 20 (Fisher Scientific, Pittsburg, PA) and titratable acidity and percent

alcohol (v/v) were conducted as described by Zoecklein et al. (1999a). Fermentable nitrogen analysis was conducted as described by Gump et al. (2002).

Glycoside Analyses. Total and phenol-free, juice, skin, and wine glycosides were determined using the analysis of glycosyl-glucose as described by Williams et al. (1995), and Zoecklein et al. (2000). Samples were run in triplicate, and the glycosyl-glucose measured using the Boehringer Mannheim glucose/fructose enzyme kit (Mannheim, Germany) and read on a Labsystems Multiskan (model MCC/340) micro-plate reader (Fisher Scientific, Pittsburgh, PA) at 340 nm.

For total wine glycosides, 2 mL of wine was diluted with 2 mL distilled water per sample. Samples were adjusted to a pH of 2.25 with concentrated HCl, and the 4 mL adsorbed to Sep-Pak tC-18 (500 mg) solid-phase extraction cartridges (Waters, Milford, Massachusetts). Phenol-free juice glycoside samples were adjusted to pH 13.0, and 0.5 mL passed through a Strata-X 33 µm polymeric sorbent (Phenomenex, Torrance, CA), using a 96 well (30 mg/well) solid phase extraction plate (Phenomenex, Torrance, CA). For phenol-free wine glycosides, 1.0 mL of wine was diluted with 1.0 mL of distilled water, pH adjusted to 13.0 with 10 N NaOH, and 1.0 mL was passed though a Strata-X 33 µm polymeric sorbent, using a 96 well (30 mg/well) solid phase extraction plate (Phenomenex, Torrance, CA). Samples for total skin glycosides were prepared by removing all pulp and placing the skins (1.0 g) in a Waring commercial laboratory blender, model 31 BL 91(New Hartford, CT), blending for 30 sec with 10 mL 50% ethanol, and 10 mL additional 50% ethanol used to rinse the blender jar. Skins were extracted for 1.0 h, after which the supernatant was removed and filtered through a 0.45-

μm filter. Five mL of supernatant was diluted with 29 mL of distilled water, pH adjusted to 2.25 with concentrated HCl, and passed through a Sep-Pak tC-18 solid-phase extraction cartridges, as described above. Columns were conditioned by rinsing with 10 mL methanol for total glycosides, or 0.5 mL for phenol-free, followed by 10 mL dH_2O for total and 0.5 mL for phenol-free. After entire juice or wine sample was passed through the column, the column was rinsed with dH_2O (10 mL three times for total and 0.5 mL three times for phenol-free), and eluted with ethanol (1.5 mL for total and 0.15 mL for phenol-free) and dH_2O (3 mL for total and 0.35 for phenol-free). Volume of eluate for total was brought up to 5.0 mL. Samples were then heated with 2.25 M H_2SO_4 for 1.0 h. Total glycoside samples were then eluted through the column again. All samples were analyzed enzymatically for glucose as described above.

HS-SPME/GC-MS. Free volatiles were determined using headspace solid-phase microextraction, gas chromatography/mass spectrophotometry as described by Whiton and Zoecklein (2000) with a Hewlett Packard (model 5790) GC (Palo Alto, CA), HP (model 5972) (Palo Alto, CA) mass selective detector and a Carbowax® (Supelco, Bellefonte, Pennsylvania) 65 μm fiber. A CombiPal (model MXY 02-01B) auto-sampler (CTC Analytics, Zwingen, Switzerland) and a DB-WAX (30m x 0.25mm x 0.25μm) column (J.W. Scientific, Folsom, CA) were used with a split injector and helium gas. The sample (4.0 mL or standard (4.0 mL), along with 1.00 g of salt, was placed in a clear 10-mL crimp seal headspace vial (model 20-0050AT) (MicroLiter Analytical Supplies, Inc., Suwanee, GA) with a 20-mm AlumiTin cap with natural teflon/blue silicon septum (model 20-1000) (MicroLiter Analytical Supplies, Inc., Suwanee, GA). Samples were

held 15 sec then agitated 5 sec on and 2 sec off, for 30 min. The fiber was desorbed 15 min in the injection port at 250°C. Oven temperature was 40°C for 5 min, rose 6° per min up to 230°C, and held for 5 min. Samples and standards were run in triplicate. The 2003 wines were analyzed 4-5 months post-fermentation, the 2002 wines 20 months post-fermentation. Six volatile compounds were quantified in the 2002 juice and four compounds in 2003 juice. Sixteen wine volatile compounds were quantified in the 2002 wines and seventeen in 2003. Aroma Units were calculated for wine volatiles by dividing the concentration by the reported aroma detection threshold values (Simpson and Miller 1984, Simpson 1979).

Sensory Analysis. Sensory analyses were conducted 5 to 6 months post-fermentation using a triangle difference test procedure (Meilgaard et al. 1999). Smart Dyson UP was not sensorially tested in 2002 due to the confounding influence of hydrogen sulfide. A consumer panel aged approximately 21-28, underwent three, 2 h training sessions, and evaluated wine aroma and flavor separately under standard sensory testing conditions, as described by Meilgaard et al. (1999). Wines were tested under white light in 2001 and 2002 and red light in 2003 using 20 mL volume at 17°C in clear ISO (International Standards Organization) wine glasses covered by watch glasses. Glasses were labeled with random number codes. Testing was done using 9 groups of 6 panelists each in 2003, 3 groups of 8 in 2002, and 6 groups of 8 in 2001. For each session, panelists were given 10 minutes to determine an aroma difference and 10 minutes to determine a flavor difference, with a 5 minute break in between. Different triangle test combinations were given for aroma and flavor. Panelists were given oral instructions at the beginning of the

session, as well as written instructions during each testing. Sessions were performed every half hour and panelists were not allowed to participate in consecutive sessions. In 2003, the sensory testing had an α-level of 0.05, a β-level of 0.1, p_d = 30%, and a sample size of 54 per testing. In 2002, the sensory testing had an α-level of 0.05, a β-level of 0.2, p_d = 40%, and a sample size of 24 per testing. In 2001, there was an α-level of 0.05, a β-level of 0.2, p_d = 30%, and a sample size of 48 per testing (Meilgaard et al. 1999).

Statistical Analysis. A means comparison between training systems within each year was performed for each analysis using one way ANOVA test and Tukey-Kramer HSD pairwise comparison using the JMPIN version 4.0 statistical software program, SAS Institute (Cary, NC). Sensory statistics were determined using tables in *Sensory Evaluation and Techniques* (Meilgaard et al. 1999)

RESULTS

Yield. Total yield and fruit weight per vine were lowest for Vertical Shoot Positioned (VSP) for each season, while Geneva Double Curtain (GDC) had the greatest total yield for 2002 and 2003 (Table 1). VSP consistently had lower fruit weight per vine and clusters per shoot each season (Table 1). There were no differences in berries per cluster or berry weight among training systems (Table 1).

Light. GDC, in 2001 and 2002, and Smart Dyson Up (SD-UP), in 2001, had a higher percent of photosynthetically active radiation (PAR) than the other training systems (Table 2). In 2003, Smart Dyson Down (SD-Down) had the highest percentage of

canopy light interception among the training systems. VSP had the lowest available light for all three years.

Grape Composition. Grapes were harvested at approximately the same Brix within each year (Table 3). Fruit from each training system differed in pH in 2001 and 2003, and titratable acidity in 2001. No differences were found in berry weight, fruit malic or tartaric acids in 2002 and 2003 (Table 3).

Fruit Glycosides. Total skin glycosides (TGG) were in the greatest concentration in GDC and SD-UP in 2002 and 2003 (Table 4). Phenol-free juice glycosides (PFGG) were lowest in the VSP system one season, and did not differ in the other (Table 4).

Free Juice Volatiles. SD-UP juice had higher linalool, β-damascenone, and α-terpineol in 2002 and 2003 (Tables 5 and 6) and n-hexanol in 2002, than the three other systems. VSP juice had the highest concentration of n-hexanol in 2003 (Table 6). GDC juice had the lowest geraniol and benzaldehyde in 2002 (Table 5).

Wine Composition. Wine pH differed among training systems (Table 7). SD-Down wines had higher alcohol levels (v/v) in 2002 and 2003, and lower titratable acidity in 2002. Malic acid did not differ among systems, while tartaric acid differed slightly.

Wine Glycosides. Total wine glycosides were greater in the SD-UP and GDC training system than SD-Down in 2002, and greater in SD-UP and VSP than GDC in 2003 (Table

8). Phenol-free wine glycosides were among the highest in the SD-UP for two of three seasons (Table 9). The VSP system consistently produced wines with relatively low phenol-free glycosides (Table 9).

Wine Free Volatiles. SD-UP and SD-Down had higher concentrations of n-hexanol and phenethyl acetate (Table 10) in the wine in 2002, and hexyl acetate in 2002 and 2003 (Tables 10 and 11) than the other two training systems. SD-UP and SD-Down had higher concentrations of isoamyl acetate, 3-methyl butanol, ethyl heanoate, ethyl octanoate, and ethyl decanoate (Table 11) in 2003 than the other training systems. No differences were found among training systems in regard to ethyl hexanoate, octanoic acid and decanoic acid in 2002 (Table 10). Varying differences occurred among training systems with other compounds analyzed.

Aroma units for linalool, 3-methyl butanol, and octanoic acid were greater than 1.0 in wines among all training systems for both years (Table 12). In 2002, isoamyl acetate, ethyl hexanoate, and ethyl octanoate were above the detection threshold (aroma unit greater that 1.0) for all training systems. However, in 2003 these compounds were above the detection threshold in SD-Up and SD-Down but not in the other two training systems. All other compounds had aroma units below 1.0 for each training system both years.

Sensory Analysis. In 2001, sensory analysis showed differences in aroma and flavor between SD and GDC, in flavor between SD and VSP, and in aroma between VSP and GDC (Table 13). No differences were noted between SD-Down, VSP and GDC training

systems in the 2002 wines (Table 14). In 2003, wines showed differences in aroma and flavor between GDC and SD-UP, differences in aroma between GDC and SD-Down, and differences in flavor between VSP and SD-Down (Table 15).

DISCUSSION

This study showed that training systems may impact important secondary metabolites, including glycosides and free volatile compounds. Yield components, such as tons per acre, fruit weight per vine, clusters per shoot, and berry weight may each affect these compounds (Bureau et al. 2000, McCarthy et al. 1986, Reynolds et al. 1996a,b). For example, decreased clusters per shoot have been shown to increase secondary metabolites (Bureau et al. 2000, Reynolds et al. 1994a). Vertical Shoot Positioned (VSP) vines averaged 41% fewer clusters per shoot than Geneva Double Curtain (GDC), for the three seasons of the study. VSP had 23% fewer clusters per shoot than Smart Dyson (SD). In several studies, decreased fruit weight per vine increased secondary metabolites (Chapman et al. 2004a, McCarthy et al. 1986, Reynolds et al. 1996a.). VSP averaged 47% lower fruit weight per vine than GDC, and 42% lower fruit weight per vine than the SD system. Berry size can impact yield due to its influence on cluster weight and total weight. Lower berry weight has been shown to increase glycosides localized in the skins on a per gram basis, due to the impact on the surface to volume ratio (Iland et al. 1996). Berry weights among treatments did not differ in this study.

Training system architecture can impact the light in the grapevine canopy (Smart and Robinson 1991). In this study, VSP averaged 12% less available light than GDC,

and 6% less than Smart Dyson Down (SD-Down). Increased canopy light has been shown to increase secondary metabolites (Marais et al. 1992, Razungles et al. 1996). Over exposure has also been shown to decrease secondary metabolites possibly due to higher temperatures (Belancic et al. 1997). Secondary metabolite production is a temperature-dependent enzyme reaction (Kliewer 1977) that has been shown to be inhibited by temperatures of 35-40°C or greater (Kliewer 1970, Spayd et al. 2002).

Grape skin contains the majority of the fruit's glycosides (Bayonove et al. 1974, Wilson et al. 1986). Higher skin glycosides in the GDC and SD-UP may indicate a higher concentration of phenolic glycosides. For example, Price et al. (1995) and Spayd et al. (2002) reported increases in skin-derived quercetin glycosides on sun-exposed berries and less on the interior, shaded fruit. This group of phenolic compounds contributes to wine bitterness and astringency (Price et al. 1995). GDC had a higher percentage of light interception in the canopy in 2002, which could have impacted the concentration of skin glycosides, including quercetin glycosides. However, this group of phenolic glycosides was not quantified in this study. Although light measurements were taken at véraison each season, it is possible that the relative light exposures changed post-véraison. Relative canopy densities can change throughout the season (Gladstone and Dokoozlian 2003, Smart et al. 1985). SD-UP had lower relative light interception at véraison in 2002 and 2003, and higher skin glycoside concentration than VSP and SD-Down. SD-UP had 46% fewer clusters per shoot than GDC. Bureau et al. (2000) showed that a 50% reduction in clusters per shoot increased total glycosides by 10%. Thus, increased total glycosides could have been impacted by both light and yield.

The total glycoside analysis used in this study included an estimate of all glycosidically bound components. Phenol-free glycoside analysis estimated the non-phenolic glycosides. Zoecklein et al (2000) showed that phenol-free glycosides (PFGG) account for about 76% of the total glycosides in white grape juice. This group represents a smaller pool of compounds, which may be more representative of aroma/flavor precursors (Williams et al. 1996, Zoecklein et al. 2000). In this study, training systems had limited impact on phenol-free juice glycosides, with the exception of VSP in 2002. In 2002, VSP had a 30% lower PFGG concentration than SD-UP and SD-Down. While the percentage of light in canopy was similar among treatments that year, VSP had 35% fewer clusters per shoot than SD. This may have impacted the PFGG concentration. For example, Reynolds et al. (1994b) demonstrated a 63% decrease in concentration of terpene glycosides with a 22% decrease in clusters per shoot.

Aroma descriptors are associated with various volatile compounds found in juice. However, sensory detection thresholds of juice volatiles have not been determined. The C13-norisoprenoid β-damascenone is associated with rose-like aromas (Flavors and Fragrance 2003-2004 catalog, Sigma-Aldrich, St. Louis, MO). GDC averaged 29% less β-damascenone than SD-UP for two seasons analyzed and 49% lower monoterpene volatiles (α-terpineol and linalool) than SD-UP. Monoterpenes contribute to the varietal character of Viognier (Ribéreau-Gayon et al. 2000). The monoterpene linalool is typically associated with citrus aromas (Flavors and Fragrance 2003-2004 catalog, Sigma-Aldrich, St. Louis, MO), while α-terpineol is described as lilac (Flavors and Fragrance 2003-2004 catalog, Sigma-Aldrich, St. Louis, MO). Belancic et al. (1997) found 30% lower canopy light interception resulted in 43% lower total free volatile

terpenes. In this study, lower volatile terpenes were found with higher light in the GDC canopy. Higher yields in the GDC may have impacted the lower concentration of free volatiles for this system. Increased yields have been shown to decrease grape-derived free volatiles (Chapman et al. 2004a). For example, Reynolds et al. (1996a) showed 45% increase in yields (tons/hectare) gave 10% decrease in free volatile terpenes.

Total wine glycosides averaged 56% less than the total skin glycosides over two seasons. Wines produced by whole cluster pressing of the fruit presumably have lower total glycosides due to limited skin extraction. Bureau et al. (2000) showed an increase in total glycoside concentration with a 20% increase in light. SD-UP had lower light than GDC with increased glycosides, which could be due to fewer clusters per shoot and lower fruit weight per vine. Smart Dyson averaged 9% lower fruit weight per vine and 23% reduction in clusters per shoot than GDC. Decreases in clusters per shoot and total yield (tons/acre) have been shown to increase glycosides (Bureau et al. 2000, McCarthy et al. 1986).

Wines averaged 83% lower concentration of phenol free glycosides than juice, possibly due to hydrolysis during fermentation (Laffort et al. 1989) and aging (Zoecklein et al 1999b). VSP averaged 10% lower phenol-free wine glycosides than GDC and 15-16% lower than SD-UP and SD-Down, respectively. VSP had 12% lower light in canopy than GDC and 6% less than SD-Down. Zoecklein et al. (1998) saw a 5.3-17.7% increase in phenol free glycosides with increased light exposure. Macaulay and Morris (1993) observed 15% lower concentration of terpene glycosides in wine with 10% lower light in the fruit zone.

Approximately 300 volatile compounds have been identified in wine (Schreier et al. 1976). Various categories of fermentation volatiles were analyzed in this study, including ethyl esters, acetate esters, fatty acids, and higher alcohols. Ethyl esters are typically associated with fruity, apple and wine-like characters, acetate esters with fruity, floral and banana notes and fatty acids with rancid and off odors. Higher alcohols typically give negative traits at higher concentrations, but can contribute to complexity at levels below 300 mg/L. GDC had 33% less ethyl esters than SD-UP and 20% lower than SD-Down. GDC had 63% lower acetate ester concentration than SD-UP and 44% less than SD-Down. GDC had 15% lower concentration of higher alcohols than SD-UP and 16% less than SD-Down. Fatty acid concentrations were 36% less in GDC than SD-Down, but not lower than SD-UP. GDC typically had less concentration of wine volatiles than SD-UP and –Down, possibly due to higher fruit weight per vine. Sinton et al. (1978) showed lower fermentation volatiles (acetate esters) with higher fruit weight per vine. Of the wine volatiles that had aroma units greater than 1.0, isoamyl acetate, ethyl hexanoate and ethyl octanoate would typically give fruity/banana aromas which may positively impact the wine aroma, and 3-methly butanol, the higher alcohol, would contribute to complexity at its level. Octanoic acid which typically associated with off odors, may negatively impact wine aroma.

Sensory differences in 2003 could be partially due to variation in volatile concentrations as noted by the aroma unit calculations. Many volatile compounds may affect the sensory character of wine. Sensory results could possibly change over time due to the aging of the wines. Glycosides has been shown to decrease with storage (Zoecklein et al. 1999b) Volatile compounds, including hexyl acetate and ethyl

octanoate, have been shown to increase during 8 months aging (Spirov and Granov 1977). Isoamyl acetate, hexyl acetate and 2-phenylethyl acetate have been shown to decrease with aging (Marais and Rapp 1998). Differences in aroma units could also be due to seasonal variation. The 2003 wines tended to have less concentration of volatiles than 2002, which could be due to less ripe berries indicated by lower Brix in the 2003 berries. If the berries had been riper, the aroma units may have been higher in 2003. Sensory differences between SD-Down and GDC, and SD-Down and VSP, may have also been impacted by higher alcohol in SD-Down.

CONCLUSIONS

Training system impacted the glycoside and volatile components of Viognier juice and wine in this study. The Smart Dyson and GDC systems tended to have the highest total and phenol free glycosides. Additionally, the Smart Dyson consistently gave higher free volatile and glycoside concentrations than the non-divided VSP. The increased glycosides and free volatiles, along with higher yields may make Smart Dyson an economical system for use in Virginia. However, additional study may be needed to determine all character impact compounds for this important variety. Qualitative descriptive analysis throughout the aging period would be desirable.

Table 1 Viognier total yield (tons per acre), fruit weight (kg) per vine, clusters per shoot, berries per cluster and berry weight (g) for Vertical Shoot Positioned (VSP), Smart Dyson (SD) and Geneva Double Curtain (GDC) for years 2001, 2002 and 2003.

Measure	VSP	SD	GDC
Total yield (tons/acre)			
2001	2.6 c	5.4 a	5.1 b
2002	5.0 c	7.7 b	8.5 a
2003	3.0 c	5.3 b	6.7 a
Fruit weight (kg)/vine			
2001	4.4 b	9.0 a	8.4 a
2002	8.3 b	12.8 a	14.1 a
2003	5.0 b	8.9 a	11.2 a
Clusters/shoot			
2001	0.9 b	1.3 a	1.0 b
2002	ND	ND	ND
2003	1.1 c	1.3 b	2.4 a
Berries/cluster			
2001	54 a	65 a	67 a
2002	121 a	128 a	121 a
2003	74 a	88 a	83 a
Berry weight (g)			
2001	1.9 a	1.7 a	1.8 a
2002	1.7 a	1.6 a	1.7 a
2003	1.8 a	1.8 a	1.9 a

[a,b,c] different letters within row indicate significance at $p \leq 0.05$
ND indicates not determined

Table 2 Effect of Viognier training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) on % available light (Photosynthetically Active Radiation, PAR) in 2001, 2002 and 2003.

Training System	2001	2002	2003
VSP	12.6 c	2.2 b	5.9 c
SD-UP	29.2 a	3.6 b	7.4 c
SD-Down	18.4 b	3.8 b	18.6 a
GDC	29.3 a	15.5 a	12.6 b

[a,b,c] different letters within column indicate significance at $p \leq 0.05$

Table 3 Effect of Viognier training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) on berry weight, pH, titratable acidity, malic acid, and tartaric acid at harvest in 2001, 2002 and 2003.

Year	Training System	Berry Weight (g)	Berry Brix	pH	Titratable Acidity (g/L)	Malic acid (g/L)	Tartaric acid (g/L)
2001	VSP	1.86 a	24.3 b	3.67 b	5.90 a	ND	ND
	SD	1.73 a	25.0 a	3.61 c	5.75 b	ND	ND
	GDC	1.76 a	25.0 a	3.71 a	5.13 c	ND	ND
2002	VSP	1.78 a	22.6 a	3.96 a	5.13 a	5.48 a	1.25 a
	SD-UP	1.75 a	23.8 a	3.88 a	4.54 a	4.71 a	1.55 a
	SD-Down	1.65 a	23.1 a	3.89 a	5.15 a	5.03 a	1.36 a
	GDC	1.61 a	22.7 a	3.92 a	4.99 a	5.01 a	1.41 a
2003	VSP	1.78 a	19.9 a	3.85 ab	6.28 a	6.56 a	1.50 a
	SD-UP	1.79 a	20.1 a	3.74 b	5.73 a	5.84 a	1.64 a
	SD-Down	1.76 a	20.3 a	3.86 ab	5.58 a	5.84 a	1.45 a
	GDC	1.79 a	20.2 a	3.94 a	5.94 a	5.92 a	1.34 a

[a] different letters within column indicate significance at $p \leq 0.05$
ND indicates not determined

Table 4 Effect of Viognier training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) on total skin glycosides (TGG) (μ*M*) and phenol-free juice glycosides (PFGG) (μ*M*) in 2002 and 2003.

Training System	2002 TGG (μ*M*)	2003 TGG (μ*M*)	2002 PFGG (μ*M*)	2003 PFGG (μ*M*)
VSP	426 ab	206 b	299 b	336 a
SD-UP	525 a	338 a	436 a	330 a
SD-Down	312 b	197 b	418 a	309 a
GDC	566 a	326 a	404 ab	330 a

[a,b] different letters within column indicate significance at $p \leq 0.05$

Table 5 Effect of Viognier training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) on juice volatiles in 2002.

Compounds	VSP	SD-UP	SD-Down	GDC
linalool (µg/L)	75.4 b	89.4 a	72.6 b	59.5 c
α-terpineol (µg/L)	30.1 b	57.6 a	32.9 b	15.2 c
β-damascenone (µg/L)	1.7 b	10.1 a	3.8 b	ND
n-hexanol (mg/L)	1.0 c	2.0 a	1.5 b	1.0 c
geraniol (µg/L)	4.1 a	6.7 a	7.9 a	1.4 b
benzaldehyde (µg/L)	13.8 a	13.0 ab	11.9 b	10.0 c

[a,b,c,] different letters within row indicate significance at $p \leq 0.05$
ND indicates not detected

Table 6 Effect of Viognier training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) on juice volatiles in 2003.

Compounds	VSP	SD-UP	SD-Down	GDC
linalool (µg/L)	42.4 ab	54.1 a	47.9 ab	31.5 b
α-terpineol (µg/L)	15.5 b	26.2 a	20.1 ab	10.1 b
β-damascenone (µg/L)	11.5 bc	14.8 a	12.6 ab	8.8 c
n-hexanol (mg/L)	2.9 a	2.2 b	2.3 b	2.3 b

[a,b,c] different letters within row indicate significance at $p \leq 0.05$

Table 7 Effect of Viognier training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) on wine alcohol level, pH, titratable acidity, malic acid, and tartaric acid in 2002 and 2003.

Year	Training System	% Alcohol (v/v)	pH	Titratable Acidity (g/L)	Malic acid (g/L)	Tartaric acid (g/L)
2002	VSP	14.4 ab	3.3 c	6.5 a	2.7 a	2.1 a
	SD-UP	13.9 b	3.4 b	6.2 ab	2.4 a	2.3 a
	SD-Down	15.0 a	3.5 a	5.6 b	2.5 a	1.6 b
	GDC	14.3 ab	3.3 c	6.1 ab	2.5 a	2.0 a
2003	VSP	11.8 c	3.5 c	7.7 a	4.5 b	1.7 a
	SD-UP	12.1 b	3.5 c	7.3 a	4.4 b	1.7 a
	SD-Down	12.5 a	3.6 b	7.0 a	4.3 b	1.6 a
	GDC	12.0 bc	3.7 a	6.9 a	5.0 a	1.4 b

[a,b,c] different letters within column indicate significance at $p \leq 0.05$

Table 8 Effect of Viognier training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) on total wine glycosides (TGG) (μM) in 2002 and 2003.

Training System	2002 TGG (μM)	2003 TGG (μM)
VSP	129 ab	185 a
SD-UP	151 a	189 a
SD-Down	123 b	179 ab
GDC	145 a	164 b

[a,b] different letters within column indicate significance at $p \leq 0.05$

Table 9 Effect of Viognier training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down [Smart Dyson collectively for 2001]; and Geneva Double Curtain, GDC) on phenol-free wine glycosides (PFGG) in 2001, 2002 and 2003.

Training System	2001 PFGG (μM)	2002 PFGG (μM)	2003 PFGG(μM)
VSP	56 b	55 b	43 b
SD-UP	56 b	69 a	58 a
SD-Down		74 a	51 ab
GDC	64 a	55 b	59 a

[a,b] different letters within column indicate significance at $p \leq 0.05$

Table 10 Effect of Viognier training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) on wine volatiles in 2002.

Compounds	VSP	SD-UP	SD-Down	GDC
2-methyl propanol (mg/L)	33.2 b	34.6 ab	44.0 a	35.4 ab
isoamyl acetate (µg/L)	450.9 b	2858.8 a	2465.9 b	2209.4 b
3-methyl butanol (mg/L)	167.4 ab	172.6 a	179.0 a	141.4 b
ethyl hexanoate (µg/L)	1112.3 a	1195.0 a	1182.5 a	1096.2 a
hexyl acetate (µg/L)	27.0 c	65.1 a	44.0 b	30.7 c
n-hexanol (mg/L)	1.0 b	1.3 a	1.2 a	0.9 b
ethyl octanoate (µg/L)	1162.7 ab	1041.1 b	1168.2 a	1095.4 ab
acetic acid (mg/L)	283.7 b	265.0 b	509.2 a	292.5 b
linalool (µg/L)	26.3 ab	31.8 a	31.1 ab	22.8 b
ethyl decanoate (µg/L)	386.4 a	295.0 b	429.9 a	323.0 b
diethyl succinate (µg/L)	3188.0 ab	3618.8 a	3258.0 ab	2937.3 b
α-terpineol (µg/L)	18.2 ab	22.0 a	16.8 b	14.9 b
phenethyl acetate (µg/L)	184.2 c	207.0 b	225.6 a	183.5 c
β-damascenone (µg/L)	ND	ND	ND	ND
2-phenylethanol (mg/L)	12.7 ab	10.7 b	14.3 a	13.4 a
octanoic acid (mg/L)	10.1 a	9.0 a	10.3 a	8.4 a
decanoic acid (mg/L)	3.2 a	2.6 a	3.0 a	2.2 a

[a,b,c] different letters within row indicate significance at $p \leq 0.05$
ND indicates not detected

Table 11 Effect of Viognier training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) on wine volatiles in 2003.

Compounds	VSP	SD-UP	SD-Down	GDC
2-methyl propanol (mg/L)	16.5 b	26.4 a	21.4 ab	21.3 ab
isoamyl acetate (µg/L)	273.6 c	6227.0 a	3024.6 b	303.2 c
3-methyl butanol (mg/L)	85.5 d	145.0 a	130.0 b	115.2 c
ethyl hexanoate (µg/L)	160.5 c	1423.7 a	605.6 b	175.5 c
hexyl acetate (µg/L)	23.5 c	286.7 a	135.0 b	19.7 c
n-hexanol (mg/L)	0.9 c	1.1 a	1.1ab	1.0 bc
ethyl octanoate (µg/L)	702.3 c	1885.9 a	1172.5 b	727.3 c
acetic acid (mg/L)	78.3 b	146.9 a	159.2 a	126.7 a
linalool (µg/L)	20.0 b	24.6 a	21.0 ab	25.1 a
ethyl decanoate (µg/L)	263.5 b	445.3 a	418.8 a	266.7 b
diethyl succinate (µg/L)	134.3 b	209.3 a	177.2 ab	133.6 b
α-terpineol (µg/L)	6.7 b	9.6 a	10.9 a	11.4 a
phenethyl acetate (µg/L)	986.9 b	991.8 b	1146.8 a	1187.8 a
β-damascenone (µg/L)	3.3 b	5.0 a	4.4 ab	4.1 ab
2-phenylethanol (mg/L)	13.1 b	13.9 b	16.6 a	14.4 b
octanoic acid (mg/L)	12.3 b	12.9 b	16.3 a	13.7 ab
decanoic acid (mg/L)	3.1 b	4.2 a	4.4 a	3.5 a

[a,b,c] different letters within row indicate significance at $p \leq 0.05$

Table 12 Aroma units by training system (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) for Viognier wine volatiles in 2002 and 2003.

Compound	Detection threshold	VSP 2002	VSP 2003	SD-UP 2002	SD-UP 2003	SD-Down 2002	SD-Down 2003	GDC 2002	GDC 2003
n-hexanol	4 (mg/L)	0.24	0.24	0.32	0.29	0.30	0.27	0.22	0.26
linalool	6 (µg/L)	4.38	3.33	5.30	4.10	5.19	3.50	3.80	4.19
α-terpineol	330 (µg/L)	0.06	0.02	0.07	0.03	0.05	0.03	0.05	0.03
β-damascenone	50 (µg/L)	ND	0.07	ND	0.10	ND	0.09	ND	0.08
3-methyl butanol	60 (mg/L)	2.79	1.42	2.88	2.41	2.98	2.16	2.36	1.92
2-phenylethanol	50 (mg/L)	0.25	0.26	0.21	0.28	0.29	0.33	0.27	0.29
octanoic acid	3 (mg/L)	3.36	4.11	2.99	4.31	3.44	5.55	2.79	4.56
decanoic acid	6 (mg/L)	0.53	0.52	0.43	0.71	0.49	0.74	0.37	0.58
2-methyl propanol	200 (mg/L)	0.17	0.08	0.17	0.13	0.22	0.11	0.18	0.11
isoamyl acetate	1000 (µg/L)	2.45	0.27	2.86	6.23	2.47	3.02	2.21	0.30
ethyl hexanoate	300 (µg/L)	3.71	0.54	3.98	4.75	3.94	2.02	3.65	0.59
ethyl octanoate	800 (µg/L)	1.45	0.88	1.43	2.36	1.46	1.47	1.37	0.91
hexyl acetate	2400 (µg/L)	0.04	0.01	0.10	0.12	0.07	0.06	0.05	0.01
acetic acid	1200 (mg/L)	0.24	0.07	0.21	0.12	0.42	0.13	0.24	0.11
ethyl decanoate	510 (µg/L)	0.76	0.52	0.58	0.87	0.84	0.82	0.63	0.44

(Simpson and Miller 1984, Simpson 1979)

Table 13 Summary of Viognier wine triangle difference testing among training systems (Vertical Shoot Positioned, VSP; Smart Dyson, SD; and Geneva Double Curtain, GDC) in 2001.

Aroma comparison	Total N	Correct	N Needed	Significance
GDC v VSP	90	43	38	Y*
GDC v SD	48	26	22	Y*
VSP v SD	48	13	22	N

Flavor comparison	Total N	Correct	N Needed	Significance
GDC v VSP	90	29	38	N
GDC v SD	48	23	22	Y*
VSP v SD	48	22	22	Y

* Indicates significance at $p \leq 0.05$
Total N = number of responses
N needed = number of responses needed for significance

Table 14 Summary of Viognier wine triangle difference testing among training systems (Vertical Shoot Positioned, VSP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) in 2002

Aroma comparison	Total N	Correct	N Needed	Significance
GDC v VSP	24	6	13	N
GDC v SD-Down	24	7	13	N
VSP v SD-Down	24	8	13	N

Flavor comparison	Total N	Correct	N Needed	Significance
GDC v VSP	24	11	13	N
GDC v SD-Down	24	7	13	N
VSP v SD-Down	24	7	13	N

* Indicates significance at $p \leq 0.05$
Total N = number of responses
N needed = number of responses needed for significance

Table 15 Summary of Viognier wine triangle difference testing among training systems (Vertical Shoot Positioned, VSP; Smart Dyson Up, SD-UP; Smart Dyson Down, SD-Down; and Geneva Double Curtain, GDC) in 2003.

Aroma comparison	Total N	Correct	N Needed	Significance
GDC v VSP	54	15	25	N
GDC v SD-UP	54	26	25	Y*
GDC v SD-Down	54	25	25	Y*
VSP v SD-Down	54	20	25	N
VSP v SD-UP	54	23	25	N
SD-UP v SD-Down	54	18	25	N

Flavor comparison	Total N	Correct	N Needed	Significance
GDC v VSP	54	18	25	N
GDC v SD-UP	54	27	25	Y*
GDC v SD-Down	54	23	25	N
VSP v SD-Down	54	33	25	Y*
VSP v SD-UP	54	23	25	N
SD-UP v SD-Down	54	22	25	N

* Indicates significance of $p \leq 0.05$
Total N = number of responses
N needed = number of responses needed for significance

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APPENDIX A

Virginia Polytechnic Institute and State University
Informed Consent for Participation in Sensory Evaluation

Title of Project: Wine triangle sensory evaluation

Principal Investigators: Dr. Bruce Zoecklein, Lindsay Millard

I. THE PURPOSE OF THIS PROJECT

You are invited to participate on a sensory evaluation panel about white/red wine aroma and flavor.

II. PROCEDURES

There will be sessions throughout the semester involving 30 minutes. You will be presented with six samples of wine. Should you find a sample unpalatable or offensive, you may choose to spit it out and continue to other samples. YOU MUST EXPECTORATE ALL SAMPLES.

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergies, list them in the following space.

_____

III. BENEFITS/RISKS OF THE PROJECT

Your participation in the project will provide the following information that may be helpful:

- Quantification of differences in wine aroma and flavor caused by differing growing and processing methods.

You may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have an unknown food allergy, are a steroid-dependent asthmatic, or suffer from alcohol flush syndrome.

IV. EXTENT OF ANONYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. COMPENSATION

Participants in this research tasting will receive no monetary compensation.

VI. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology.

VIII. SUBJECT'S RESPONSIBILITIES

I know of no reason I cannot participate in this study that will require the evaluation of 6 wine samples.

_____
Printed name

_____
Signature/Date

Please provide address and phone number so investigator may reach you in case of emergency or schedule changes.

e-mail_____

Address _____

Phone _____

----------(Tear off)----------

IX. SUBJECT'S PERMISSION (tear off and keep)

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study that will require: (list sessions to be attended or other requirements.)

_____
Signature Date

Should I have any questions about this research or its conduct, I should contact:

Dr. Bruce Zoecklein / Lindsay Millard
Investigators

231-5325 / 231-9843
Phone

APPENDIX B

Aroma Triangle Difference Test

BOOTH ONE

Name: _____ Seat Number: _____ Date: _____

Type of Sample: Wine

Instructions:
Smell the samples on the tray from left to right in the order provided. Two samples are the same and one is different. Select the sample with the **ODD OR DIFFERENT** aroma and indicate your choice by **circling** the code of the odd sample below.

Samples:

236 658 974

If you wish to comment on the reasons for your choice or if you wish to comment on the product characteristics, you may do so in the space below.

THANK YOU FOR YOUR PARTICIPATION IN THIS SENSORY EVALUATION!

Aroma Triangle Difference Test

BOOTH TWO

Name: _____ Seat Number: _____ Date: _____

Type of Sample: Wine

Instructions:
Smell the samples on the tray from left to right in the order provided. Two samples are the same and one is different. Select the sample with the **ODD OR DIFFERENT AROMA** and indicate your choice by **circling** the code of the odd sample below.

Samples:

296 537 278

If you wish to comment on the reasons for your choice or if you wish to comment on the product characteristics, you may do so in the space below.

THANK YOU FOR YOUR PARTICIPATION IN THIS SENSORY EVALUATION!

APPENDIX C

Flavor Triangle Difference Test

BOOTH ONE

Name: _____ Date: _____

Type of Sample: Wine

Instructions:

Taste the samples on the tray from left to right in the order provided. Two samples are the same and one is different. Select the **ODD OR DIFFERENT** sample and indicate your choice by **circling** the code of the odd sample below.

Samples:

236 658 974

If you wish to comment on the reasons for your choice or if you wish to comment on the product characteristics, you may do so in the space below.

THANK YOU FOR YOUR PARTICIPATION IN THIS SENSORY EVALUATION!

Flavor Triangle Difference Test

BOOTH TWO

Name: _____ Date: _____

Type of Sample: Wine

Instructions:

Taste the samples on the tray from left to right in the order provided. Two samples are the same and one is different. Select the **ODD OR DIFFERENT** sample and indicate your choice by **circling** the code of the odd sample below.

Samples:

296 537 278

If you wish to comment on the reasons for your choice or if you wish to comment on the product characteristics, you may do so in the space below.

THANK YOU FOR YOUR PARTICIPATION IN THIS SENSORY EVALUATION!

VITA

Lindsay Millard was born in Germany and grew up in the life of an "Army Brat" until her family settled in Bel Air, MD. She attended Virginia Tech for her B.S. degree. While she was working toward her degree she also worked as an undergraduate research assistant in meat science and went to New York for a summer to work as an intern in Pepsi Cola's research and development facility. After graduating with her undergraduate degree, Lindsay continued on at Virginia Tech for her M.S. in Food Science and Technology and worked for a semester at Villa Appalaccia Winery.