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Pinot noir wine volatile and anthocyanin composition under different levels of vine fruit zone leaf removal

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ABSTRACT

The impacts of fruit zone leaf removal on volatile and anthocyanin compositions of Pinot noir wine were investigated over two growing seasons. Wine volatiles were analyzed by multiple techniques, including headspace solid phase microextraction-GC-MS (HS-SPME-GC-MS), headspace-GC-FID (HS-GC-FID) and stir bar sorptive extraction-GC-MS (SBSE-GC-MS). Fruit zone leaf removal affected the concentration of many grape-derived volatile compounds such as terpene alcohols and C_{13}-norisoprenoids in wine, although the degree of impact depended on the vintage year and severity of leaf removal. Fruit zone leaf removal resulted in greater concentrations of linalool, α-terpineol and β-damascenone but had no impact on other terpene alcohols or β-ionone. Fruit zone leaf removal had no consistent impact on C6 alcohols, volatile phenols, lactones, fermentation-derived alcohols, acids, or most esters. Fruit zone leaf removal increased anthocyanins in final wine.

1. Introduction

Grape vine fruit zone leaf removal is a common vineyard management practice used to remove select leaves around the clusters during grape development. The primary objectives of this viticultural practice are to increase sunlight exposure of the grapes and to improve airflow in the vineyard to reduce disease pressure (Austin & Wilcox, 2011).

Fruit zone leaf removal directly affects the microclimate of the canopy, and may influence the primary and secondary metabolites in grape berries (Pereira et al., 2006; Sternad Lemut, Sivilotti, Franceschi, Wehrens, & Vrhovsek, 2013). Many studies have investigated the impact of leaf removal on titratable acidity, soluble solids, and anthocyanins in grapes (Kemp, Harrison, & Creasy, 2011; King, McClellan, & Smart, 2012; Lee & Skinkis, 2013), but the results were inconsistent due to differences in cultivars and other factors used in research.

Wine aroma is a complex mixture of volatile compounds from multiple origins. Hundreds of volatile compounds have been identified in wine, and their concentrations vary tremendously (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2000). Although the majority of volatile compounds in wine are formed during alcoholic fermentation, grape-derived volatile compounds are very important to wine quality and varietal characteristics (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006b). The influence of leaf removal on volatile composition of grape and wine varies with cultivar and climate. Zoecklein, Wolf, Marcy, and Jasinski (1998) reported that leaf removal increased bound-form terpenoids in Riesling grapes. On the contrary, Kozina, Karoglan, Herjavec, Jeromel, and Orlic (2008) reported that leaf removal had no impact on Riesling grape but had an influence on both free- and bound-form terpenoids in Sauvignon Blanc. In addition, Lee et al. (2007) reported that leaf removal conducted at the fruit set stage increased vitispirane and 1,1,6-trimethyl-1,2-dihydronaphthalen (TDN) concentrations in Cabernet Sauvignon grape and wine. However, Kwasniewski, Vanden Heuvel, Pan, and Sacks (2010) did not find any increase in TDN with leaf removal in Riesling grape or wine. The discrepancy across these studies may come from more than just the climate and grape cultivar; the differences in how leaf removal was managed should also be considered.

Fruit zone leaf removal is commonly used in Pinot noir production in Oregon's Willamette Valley due to the cool climate, high spring soil moisture and high vegetative growth. However, there

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is no published research to date that defines the level of leaf removal required to enhance wine composition. The objectives of this study were to investigate fruit zone leaf removal on Pinot noir wine volatile and anthocyanin composition, and to develop better canopy management guidelines related to wine quality.

2. Materials and methods

2.1. Chemicals and reagents

Standards of the volatile compounds were purchased from commercial sources: Sigma-Aldrich (Milwaukee, WI), TCI America (Portland, OR), K & K Laboratories (Jamaica, NY), Alfa Aesar (Ward Hill, MA), Firmenich (Princeton, NJ), and J & T Baker (Phillipsburg, NJ). GC grade methanol was obtained from EMD (Gibbstown, NJ) and ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY). Tartaric acid was purchased from Mallinckrodt Inc. (Paris, KY). A synthetic wine solution was made by dissolving 3.5 g of L-tartaric acid in 1 L of 12% ethanol solution and adjusting pH to 3.5 with 1 M NaOH.

2.2. Plant material and field trial site

Leaf removal field experiment was conducted at Oregon State University's Woodhall research vineyard located in Alpine, OR during 2011 and 2012. This vineyard was planted in 2006 with a vine density of 3417 vines/ha. Pinot noir clone Pommard was grafted to 101-14 rootstock. The rows were north to south oriented. Vines were pruned to a bilateral Guyot system, with shoots vertically positioned. Four cluster zone leaf removal treatments were applied for the duration of the season, including: 1) 100% leaf removal: all leaves removed from the base of the shoot to the node just above the apical cluster, 2) 50% leaf removal: leaves removed from alternating nodes from the base of the shoot to the node just above the apical cluster, 3) industry standard (IS) treatment: leaves removed to expose clusters on only the morning-sun side of the canopy (east), and 4) 0% (None): no leaf removed in the cluster zone. When the 100%, 50%, and IS treatments were applied, all lateral shoots in the cluster zone were also removed. Leaf removal treatments were imposed at the pea-sized stage of the berry development. Each treatment plot had 6 vines in a randomized complete block design with five field replicates for each treatment. Other vineyard management practices were kept the same.

2.3. Analysis of berry total soluble solids, pH and titratable acidity

Seven randomly-picked grape clusters were collected at harvest from each plot, transported to the lab and kept cool (6 °C) until analysis. Berries were destemmed manually and pressed to juice immediately. Total soluble solids (TSS), pH and titratable acidity were measured by a digital refractometer (Sper Scientific, Scottsdale, AZ), a pH meter (ThermoFisher Scientific, Waltham, MA), and by titration with 0.1 N NaOH to an end pH of 8.2 according to the procedure described by Zoecklein, Fugelsang, Gump, and Nury (1995), respectively.

2.4. Wine making

Grapes clusters from the same field treatments (5 replicates) were combined and destemmed immediately. Destemmed fruits were randomly divided into three lots of equal weight (3 kg) for triplicate fermentations. Grapes were placed into 1 gallon glass micro-scale fermenter that utilized a submerged cap to maintain skin and juice contact as described by Sampaio, Kennedy, and Vasconcelos (2007). Potassium metabisulfite was added to provide a calculated amount of 50 mg/L total sulfur dioxide. Grapes were then inoculated with *Saccharomyces cerevisiae* RC212 (Lallemand, Montréal, Canada) at approximately 1×10^6 cfu/mL after rehydration according to manufacturer's instructions. Fermenters were placed in a temperature controlled room at 27 °C and alcohol fermentation was monitored by measuring °Brix using an Anton-Paar DMA 35 N Density Meter (Graz, Austria) every day. At the completion of alcoholic fermentation (<0.5 g/L reducing sugar as measured by CliniTest®), wines were pressed using a small modified basket press that applied a constant pressure of 15 psi for 5 min. Pressed wine was settled in 1/2 gal glass carboys for 72 h at 4 °C before being racked into 1/2 gal glass carboys, and 50 mg/L of SO_2 was added. No malolactic fermentation was conducted. Wine was stored at 13 °C and analyzed within 6 months.

2.5. Analysis of wine anthocyanins

Wine anthocyanin composition was analyzed by using reversed-phase HPLC method as described by Lamuela-Raventós and Waterhouse (1994) with some modifications. An aliquot (1 mL) of wine was transferred into a 1.5 mL micro-centrifuge tube and centrifuged at 11,000 rpm for 5 min (Minispin plus, Eppendorf, Hamburg, Germany). Twenty microliters of the supernatant was injected to an Agilent 1100 HPLC system (Palo Alto, CA) consisting of a vacuum degasser, autosampler, quaternary pump, photo-diode array detector, and column heater. The ChemStation software for LC 3D (version A.10.02) (Agilent Technologies Inc., Wilmington, DE, U.S.A.) was used for chromatographic analyses. The separation was carried out on a Prodigy C18 column (100 Å, 5 μm, 250 × 4.6 mm, Phenomenex, Torrance, CA, U.S.A.). The mobile phase consisted of two solvents: solvent A, 5% formic acid in milli-Q water; solvent B, methanol (HPLC grade), with a total flow rate of 1 mL/min. The following gradient was employed: 0–34 min (3–36% B); 34–45 min (36% B); 45–55 min (36–100% B); 55–60 min (100–3% B); 60–70 min (3% B). The absorbance at 520 nm was used for quantification. External calibration was performed using malvidin-3-glucoside standard, and all other compounds were quantified using this calibration curve and reported as malvidin-3-glucoside equivalents.

2.6. Analysis of wine volatile compounds

2.6.1. Analysis of volatile compounds by solid phase micro-extraction (SPME)-GC-MS

Majority of volatile compounds in wine samples was analyzed by SPME-GC-MS method described by Chen, Xu, and Qian (2013) unless specified. For analysis of free-form volatile compounds, a 2 mL aliquot of wine was directly diluted with 8 mL of saturated NaCl solution in a 20 mL glass vial, and 20 μL of internal standard solution (96 mg/L of 3-heptanone, 109 mg/L of 4-octanol, and 118 mg/L of octyl propionate) were added. For analysis of hydrolytically released compounds, wine pH was adjusted to 2.5 with citric acid and the wine was heated to 100 °C for 1 h. Twenty μL of the internal standard solution was added. A pre-conditioned SPME fiber (2 cm 50/30 μm divinylbenzene/Carboxen/Polydimethylsiloxane, Supelco, Bellefonte, PA, U.S.A.) was inserted into the headspace using a CTC autosampler (CTC Analytics, Inc., Zwingen, Switzerland). During the extraction, the sample was equilibrated at 50 °C for 30 min with stirring of 500 rpm. Upon completion of the extraction, the fiber was removed from the sample vial and inserted into the injection port of the GC, and the volatiles were desorbed into the GC at 250 °C for 5 min using splitless mode.

The analysis of the extracted volatile compounds was carried out on an Agilent 6890 N gas chromatograph coupled with a 5973 N mass selective detector (Agilent Technologies, Inc.). Separation was achieved by using a DB-wax capillary column