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Early leaf removal applied in warm climatic conditions: Impact on Tempranillo wine volatiles

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ABSTRACT

Defoliation is a cultural practice with demonstrated benefits in grape and wine quality. The objective of this study was to evaluate the effect of early leaf removal applied in warm climatic conditions on volatile composition of Tempranillo wines. During three consecutive vintages (2009–2011) wine volatile compounds (alcohols, C_6-compounds, ethyl esters, acetates, volatile acids, lactones and carbonyl compounds) from defoliated and non-defoliated vines were identified and quantified by GC–MS. Early leaf removal induced the increase of the concentration of all families of volatile compound quantified with exception of lactones. Significant increase was observed for 23 out 34 volatile compounds analyzed. The vintage effect also was shown, where the highest effect of defoliation was exhibited in 2009 vintage. Principal component analysis (PCA) showed a good separation of defoliation, non-defoliation and vintage according to wine volatile composition. The analysis of odour activity value (OAV) exhibited an increase of fruity and floral odour on Tempranillo wines when early defoliation was applied.

1. Introduction

Aromatic composition has an important impact on grape and wine quality. In the last years have been demonstrated that modifications in the canopy management could be an important tool to improvement the aromatic grape quality.

Several works have shown management practices in the canopy increasing grape light exposure, has an important influence in the accumulation of secondary metabolites, like aroma compounds (Diago, Ayestarán, Guadalupe, Garrido, & Tardaguila, 2012; Poni, Casalini, Bernizzoni, Civardi, & Intrieri, 2006; Poni et al., 2013; Risco, Pérez, Yeves, Castel, & Intrigliolo, 2014). Leaf removal is usually applied on grapevines, whether manual or mechanical, from fruit set to veraison to improve light exposure, increase air circulation around the clusters penetration of fungicide sprays and decrease disease incidence, especially rot (Bledsoe, Kliewer, & Marois, 1988; Reynolds, Yerle, Watson, Price, & Wardle, 1996; Smart, 1985; Smart & Robinson, 1991). These effects were more pronounced in pre-bloom leaf-pulled vines than in those defoliated at fruit set (Vilanova, Diago, Genisheva, Oliveira, & Tardaguila, 2012). Poni et al. (2006) reported that defoliation near bloom improved grape composition because controlled cropping of high-yielding cultivars by reducing fruit set.

Volatile composition of grapes is affected by this canopy manipulation increasing several free and bound compounds, important components of fruit quality (Jackson, 2000; Smith & Codrington, 1988; Zoecklein, Wolf, Marcy, & Jasinki, 1998). In this regard, several authors have shown the increased of volatile composition in different cultivars and wines like Gewürztraminer, Bachus, Pearl of Csaba, Schönburger and Siegerrebe cultivars from Valley of British Columbia (Reynolds & Wardle, 1989), Muscat grape cultivars (Belancic et al., 1997), Riesling from Virginia (Zoecklein et al., 1998), Chardonnay Musqué from Ontario (Roberts, Reynolds, & De Savigny, 2007), Traminette from Indiana (Skinkis, Bordelon, & Butz, 2010), Tempranillo from La Rioja, Spain (Vilanova, Diago, et al., 2012) and Ciliegiolo from Umbria region, Italy (Palliotti, Gardi, Berrios, Civardi, & Poni, 2012). Research performed by Ristic et al. (2007) established that bunch shading of Shiraz grapes decreased the levels of norisoprenoids in wine and other compounds may be altered inducing changes in wine aroma and flavor. Leaf removal in Sauvignon Blanc resulted in a greater reduction in vegetal aromas and flavors when is applied after fruit set, compared to veraison (Arnold & Bledsoe, 1990). It was described that early defoliation reduce berry weight and consequently fruit yield (Diago, Vilanova, & Tardaguila, 2010; Poni et al., 2006), whereas defoliation after veraison affects primary and secondary metabolite synthesis (Hunter & Visser, 1989;

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Zoecklein, Wolf, Duncan, Judge, & Cook, 1992; Zoecklein et al., 1998). Therefore, the lack of information on the effects of pre-bloom basal leaf removal on the wine volatile composition opens an important field of research.

In previous research we have studied the effects of the manual pre-flowering early defoliation on phenolic composition of Tempranillo grapes in the semiarid *terroir* of western Spain (Moreno et al., 2015). The aim of the present study, as a continuation of previous studies, was to identify and quantify the main differences in volatile composition of Tempranillo wines affected by manual basal leaf removal at pre-flowering stage during three vintages (2009–2011).

2. Materials and methods

2.1. Experimental design and climatic conditions

This experiment was carried out in a *Vitis vinifera* L. cv Tempranillo vineyard located in Extremadura (western Spain) (lat. 38°51′N; long. 6°40′W; altitude 198 m) over the years 2009–2011. The vineyard was planted in 2001 on Richter 110 rootstock at a spacing of 2.5 by 1.2 m (3333 vines/ha). Row orientation was north-south and vines were trained to a bilateral cordon and vertical trellis. Vineyard soil had a silt-loam texture with 37.3% sand, 25.5% clay, 6.1% silt and 1.1% of organic matter (average depth 0.0–1.6 m).

The experimental design was a split-plot with four replicates by treatment accounted 108 plants per plot distributed in 18 plants across 6 rows. The experiment comprised 864 plants in total. The two treatments were: (a) manual leaf removal of the first seven basal leaves at pre-bloom, at stage 19 (Coombe, 1995) (ED) and control or non-defoliated (C). In 2010 and 2011 secondary shoots were also removed from ED vines. Drip irrigation was based on replacing 100% of crop evapotranspiration. Water consumption was calculated with a weighing lysimeter installed in the experimental vineyard. By the end of the season, the total applied irrigation was 666.6, 597.3 and 483.6 mm in 2009, 2010 and 2011, respectively.

The climate is continental semiarid and annual rainfall is 351 mm, of which ~70% falls during dormancy. Climatic conditions were measured by an automatic meteorological station (CR10X datalogger; Campbell Scientific, Shepshed, UK) located in the plot. Rainfall registered from vegetative period (April–September) was 101.8, 123 and 150.4 mm for 2009, 2010 and 2011 respectively. The mean temperatures were 16.6, 16.3 and 16.4 °C for 2009, 2010 and 2011 respectively.

2.2. Agronomic determinations

The leaf area removed (LAR) was measured by LI-3000 area meter (LI-COR Biosciences, Lincoln, NE). The percentage of LAR represented 36%, 50% and 40% of the total leaf area at defoliation date in 2009, 2010 and 2011 respectively. In 2010 and 2011, the intensity of this practice was increased by removing of secondary shoots, if exist, from the first seven nodes from ED vines.

Vine total leaf area (TLA) was estimated using a plant canopy analyzer (LAI-2000; LI-COR Biosciences, Lincoln, NE) as described Moreno et al. (2015). TLA measured as the average from veraison to harvest shown 9.1 and 7.2 m^2/vine for C and ED respectively in 2009 ($p < 0.05$), 7.1 and 6.7 m^2/vine for C and ED respectively in 2010 ($p > 0.05$) and 11.1 and 8.7 m^2/vine for C and ED respectively in 2011 ($p < 0.05$). In 2011 vine TLA was, for the same phenological stage, higher than in 2010, which explains the lower percentage of LAR.

Bloom stage was estimated from photographs of four clusters per vine for each experimental unit as described (Poni et al., 2006). Photographs were taken perpendicular to the cluster on a dark background just before anthesis and a linear regression between actual number of flowers and flowers counted in the digital photographs was used. The number of flowers per cluster was 349 and 341 for C and ED respectively in 2009 ($p > 0.05$), 565 and 560 for C and ED respectively in 2010 ($p > 0.05$) and 712 and 599 for C and ED respectively in 2011 ($p < 0.05$).

At harvest, the percentage of fruit set was carried out by the ratio between the number of inflorescences, previously estimated, and the manual count of the total number of berries per cluster (Poni et al., 2006). Fruit set in 2009 was 60% and 52% for C and ED respectively ($p > 0.05$), in 2010 was 52% and 33% for C and ED respectively ($p < 0.05$) and in 2011 was 42 and 35% for C and ED respectively ($p > 0.05$) (Moreno et al., 2015).

2.3. Microvinifications, must and wine composition

Vines were manually harvested when total soluble solids concentration (TSS) reached 23–24 °Brix (common harvesting criterion for this variety in this area). According to that criterion, control vines were harvest at September 01st, September 13th and August 31st in 2009, 2010 and 2011 respectively, while ED vines were harvest at August 20th, 31st and 18th in 2009, 2010 and 2011 respectively. The grapes were transported to the Technological Institute of Food and Agriculture experimental winery from INTAEX. Must were analyzed for TSS, pH, titratable acidity (TA), malic acid and tartaric acid. Analysis for density, TSS, pH and TA was according to the official methods of the OIV (1990). Malic acid and tartaric acid were, respectively, analyzed enzymatically (European Commission 1990) and according to the Rebelein method Blouin (1972), using a multidetector Easychem system (Systea S.p.a., Anagni, Italy). Yield weight at harvest was determined on ten marked vines per experimental plot.

The must from each experimental block was fermented in 50-L steel tanks at 22–24 °C. Total SO_2 was added to the musts at 50 mg/L and commercial yeast *Saccharomyces cerevisiae* Viniferm from Agrovin Company (Spain) was inoculated at 25 g/hL. Fermentation was monitored daily by measure of density and total phenolic index (TPI). The must were drawn off when a maximum in the TPI values was recorded during two consecutive days. Once fermentation was completed, the wines were settled at 4 °C and sulphur content was adjusted to 35 mg/L (as free SO_2). Finally, the wines were bottled and stored at 15 °C until analysis.

Wine analysis was carried out four months after bottling. Wine alcoholic degree (% v/v) was analyzed according to OIV methods (1990), pH, total acidity (TA), tartaric and malic acids were analyzed like musts determinations. Potassium was determined by an atomic absorption spectrophotometer (AA 240 FS, Varian, California, USA) by official method (OIV, 1990). All analyses were carried out in triplicate.

2.4. Wine volatile composition

In a 10 mL culture tube (Pyrex, ref. 1636/26MP), 8 mL of wine, 2.4 μg of internal standard (4-nonanol, Merck ref. 818773, Darmstadt, Germany) and a magnetic stir bar were added. Extraction of volatiles was done by stirring the sample with 400 mL of dichloromethane (Merck, ref. 1.06054; Darmstadt, Germany), according to the method of Oliveira, Fari, Sa, Barros, and Araujo (2006). After cooling at 0 °C for 10 min, the magnetic stir bar was removed and the organic phase was detached by centrifugation (RCF = 5118, 5 min, 4 °C) and the extract was recovered into a vial, using a Pasteur pipette. The aromatic extract (200 μg/L) was dried with anhydrous sodium sulphate (Merck, ref. 1.06649; Darmstadt, Germany) and placed in a new vial. Extractions of volatile compounds from each of the respective wines were performed in triplicate.

Gas chromatographic analysis of volatile compounds was performed using a GC–MS system constituted by an Agilent Technologies 6890N Chromatograph and an ion-trap mass spectrometer Agilent 5975C. A 1 μL injection was made into a capillary column, coated with CP-Wax 52 CB (50 m × 0.25 mm i.d., 0.2 μm film thickness, Chrompack). The temperature of the injector (7683) was programmed from 20 °C to 250 °C, at 180 °C min^{-1}. The oven temperature was held at 40 °C, for 5 min, then programmed to rise from 40 °C to 250 °C, at 3 °C min^{-1},