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Impact of leaf removal after berry set on fruit composition and bunch rot in 'Sauvignon blanc'

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Summary

Leaf removal is a viticultural practice applied to improve cluster microclimate and grape composition. This practice can reduce the incidence of bunch rot but could also promote the degradation of berry methoxypyrazines, key components for the aromatic profile of 'Sauvignon blanc' wines. The influence of cluster-zone leaf removal, applied after berry set, was evaluated on 'Sauvignon blanc' grapevines grown in the Isonzo DOC region (Italy). In 2010 and 2011, yield components and fruit chemical composition were recorded from vines in which the five basal leaves of each single shoot were manually removed at the groat-sized phenological stage, and compared to untreated vines. Our results indicated that leaf removal did not influence yield or fruit composition at harvest, but significantly decreased the incidence and severity of Botrytis bunch rot, while reducing the severity of sunburn damage to the fruit. Increased sunlight cluster exposure decreased 2-methoxy-3-isobutylpyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) concentrations in early stages of berry development, whereas at harvest no significant differences between treatments (defoliated and non-defoliated) were observed. We conclude that leaf removal performed after berry set is a pivotal viticultural management practice to cope with harvest bunch rot complex without negatively affecting fruit composition at harvest.

K e y w o r d s : aroma compounds; *Botrytis cinerea*; cluster exposure; defoliation; sunburn; vineyard; *Vitis vinifera* L.; winegrape.

Introduction

Basal leaf removal is a viticultural technique for grapevine canopy management used worldwide to improve cluster microclimate. It increases fruit exposure to sunlight and improves air penetration in the cluster zone of the canopy (Wolf *et al.* 1986, Austin and Wilcox 2011). Improved fruit exposure to sunlight benefits color and the concentration of skin anthocyanins in red grape cultivars, particularly in cooler viticultural regions where excessive berry temperature is not an issue (Jackson and Lombard 1993, Haselgrove *et al*. 2000, Poni *et al*. 2006, King *et al*. 2012, Sternad Lemut *et al*. 2013, Lee and Skinkis 2013). Moreover, this technique can result in a reduction of bunch rot incidence e.g., *Botrytis cinerea* and sour rot (Zoecklein *et al*. 1992, Percival *et al*. 1994, Molitor *et al.* 2011) and in an improved berry composition at harvest (Jackson and Lombard 1993). Sunlight-exposed clusters by leaf removal are generally higher in sugars, anthocyanins, and phenolics and lower in titratable acidity and malate concentration when compared to shaded fruit (Poni *et al*. 2006, Diago *et al*. 2012). However, the effects of leaf removal on fruit composition at harvest can change dramatically, accordingly with the climate (Wolf *et al*. 1986, Percival *et al*. 1994, Haselgrove *et al*. 2000, Poni *et al*. 2006, Scheiner *et al*. 2010, Austin and Wilcox 2011, King *et al*. 2012, Sternad Lemut *et al*. 2013, Lee and Skinkis 2013, Feng *et al*. 2015). In fact, climatic conditions, such as temperature, atmospheric humidity, solar radiation, water availability (rain or irrigation) have an important impact on vine vegetative growth and physiology, berry chemical composition, and bunch sanitary status (Guidoni *et al.* 2008). In warm climates, leaf removal can also negatively affect fruit composition; overexposed clusters subjected to a combination of high light intensity and high temperature can result in berry sunburn and, in red cultivars, reduce berry anthocyanin accumulation (Price *et al*. 1995, Bergqvist *et al*. 2001, Spayd *et al*. 2002, Greer *et al*. 2006, Chorti *et al*. 2010).

The impact of leaf removal on yield varies depending on timing and severity of the canopy manipulation (Bledsoe *et al*. 1988). Several studies showed that removing leaves between fruit set and veraison or post-veraison stages, yield is not significantly affected (Kliewer and Antcliff 1970, Zoecklein *et al*. 1992, Jackson and Lombard 1993, Bavaresco *et al*. 2008, Lee and Skinkis 2013, Feng *et al*. 2015). On the contrary, pre-flowering leaf removal reduces yield by reducing fruit set (Poni *et al.*

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2006, Intrieri *et al*. 2008, Sabbatini and Howell 2010), an effect associated with carbohydrate deficiency in the flowers at anthesis (Caspari *et al*. 1998, Petrie *et al*. 2003, Vasconcelos *et al*. 2009).

Light exposure and climate seem to have the largest effect on both production and degradation of methoxypyrazines (MPs) in grapes (Sidhu *et al.* 2014). Exposing the cluster to sunlight can result in a reduced methoxypyrazine concentration in the berries (Noble *et al*. 1995, Scheiner *et al*. 2010). The most important MPs found in grapes and wines are 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP). IBMP contributes to the green pepper and asparagus aromas, whereas IPMP imparts earthier aromas (Buttery *et al.* 1969, Allen *et al.* 1991). IBMP and IPMP are present in several wines made from different *Vitis vinifera* L. varieties, especially in Cabernet Sauvignon and 'Sauvignon blanc' (Maga 1992, Koch *et al*. 2010). It has been observed that methoxypyrazine biosynthesis occurs during the early stages of fruit development (i.e. from fruit-set to veraison) and this period might be critical for determining final MP concentration at harvest (Hashizume and Samuta 1999, Sidhu *et al.* 2014). Exposing clusters at pre-veraison reduces the accumulation of IBMP in the berry at pre-veraison stages (Marais *et al*. 1999, Ryona *et al*. 2008, Koch *et al*. 2012, Šuklje *et al*. 2012). Differences in IBMP concentration between exposed and shaded clusters established before veraison persisted until harvest (Koch *et al*. 2012). This is of particular importance in those regions where excessive IBMP concentrations can lead to unpleasant vegetative aromas (Marais and Swart 1999, Falcao *et al*. 2007). However, in the case of 'Sauvignon blanc' green-herbaceous aromas can be appreciated by consumers; for this variety, a reduction of methoxypyrazines concentration due to leaf removal might cause a decrease in desirable fruit and wine aroma traits. Growing grapes in the north-eastern region of Italy Friuli Venezia Giulia (FVG), is challenged by a relatively high amount of rainfall (1990-2014 average: 1500 ± 294 mm/year) in spring and in early summer. Significant rainfall events often occur later during the summer in July, August and September from 1990 to 2014 with 119 ± 52, 131 ± 77, 172 ± 85 mm, respectively. In grape varieties characterized by a compact cluster, such as 'Sauvignon blanc', these weather conditions lead to a high disease pressure for bunch rot near the harvest period and the incidence of bunch rot often determines the harvest date independently of the level of technological maturity of the fruit. In this study, we evaluate the effect of leaf removal, applied after berry set, on the composition of 'Sauvignon blanc' grapes, with the hypothesis that this technique might reduce bunch rot incidence but also reduce the methoxypyrazine concentration and increase sunburn damages.

Material and Methods

Vineyard description and vine management: The field experiment was carried out in 2010 and 2011 season, in a commercial 'Sauvignon blanc' vineyard in Romans d'Isonzo (Italy) (45°89'72'N, 13°45'16'W) within the "DOC Isonzo" premium denomination area. Grapevines were planted in 1991 and grafted onto 'Kober 5BB' rootstock. Rows were oriented north to south with an in-row spacing of 1 m x 2.7 m between rows for a density of 3700 vines·ha^{-1}. Vines were cane-pruned during the winter as a single cane Guyot system with 8-10 buds per cane. Soil type was closely related with the Isonzo river depositions of coarse carbonate material and classified as RhodiCambisolEndoskeletic in the World Reference Base for Soil Resources (WRB) (IUSS Working Group, 2006) with 26 % clay, 45 % silt, 29 % sand and 28 % gravel. Root depth was confined among the first 70 cm by the prevalent presence of gravel in the subsoil. Soil fertilization management included the use of ammonium sulphate, applied pre-anthesis and post-harvest at rates of 40 N kg·ha^{-1} and 15 N kg·ha^{-1}, respectively. Disease and pest control followed a schedule of 13 spray applications during the growing season. Vineyard floor in the vine row consisted of a native mix of weeds. Weeds under the vines were controlled with a broad-spectrum systemic herbicide (glyphosate) applied in the spring. Vine hedging was performed two times during each growing season to regulate canopy height and performed when shoot growth exceeded the top wire of about 30 cm. The weather data were obtained from the Gradisca d'Isonzo weather station (ARPA-OSMER) located 0.6 km from the experimental site. Grapevine phenology was recorded following the BBCH scale (Lorenz *et al*. 1995).

Experimental design: Two treatments were imposed at 25 days after anthesis (DAA), when the berries had reached 3-5 mm diameter corresponding to BBCH-EL stage 73 (groat-sized berries): 1) Leaf removal (LR) in which the five basal leaves of the shoot were manually removed, and 2) Control (C), untreated vines. Each treatment was replicated four times in 10-vines plots that were randomized within the vineyard.

Berry sampling and berry juice analysis: Berry samples were collected every 12-14 days from approximately 50 DAA until harvest. At each sampling date, two sets of berry samples were collected from each plot. A set of 30 berries was harvested and stored in an insulated cooler and then transported to the laboratory within 1 h to measure juice total soluble solids (TSS), pH, and titratable acidity (TA). Another set of 30 berries was harvested by carefully cutting the berries at the pedicel level, snap frozen with liquid nitrogen and brought to the laboratory where it was stored at -80 °C until analyzed for IBMP and IPMP concentration. Berries for juice measurements were weighed and manually pressed at room temperature and the juice was used to determine TSS using a manual refractometer (ATC-1, Atago, Japan); the pH by a pH-meter (HI2211 Hanna Instruments, USA); and TA by titration with NaOH 0.1N until pH = 8.2 (Iland *et al.* 2004). Malic and tartaric acid concentration (g·L^{-1}) was also measured using an enzymatic assay kit (Megazyme, Ireland) following producer instructions.

At harvest, the yield and the number of clusters per vine were measured and cluster weight was calculated dividing the yield by the number of clusters per vine. Incidence and severity of *Botrytis* and sour rot damages were also assessed on 100 randomly selected clusters per plot. Incidence was determined by the presence or the absence of damages on each single cluster, while severity was visually rated in individual clusters on a continuous 0 to 100 % scale, based on the proportion of cluster tissue damaged as indicated by EPPO guideline PP1/17 (Molitor *et al.* 2011). Similar procedure was applied for assessing the incidence and severity of sunburn damages on the same clusters (Hulands *et al.* 2014).

Methoxypyrazines analysis: The concentration in the berry of 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) was analyzed during ripening. Frozen berries were thawed and homogenized at room temperature using an Ultra-Turrax (T25, Ika, Staufen, Germany) and centrifuged at 15,000 rpm for 5 minutes. Subsequently, 8 mL of the supernatant were transferred to a 10 mL volumetric flask and 50 µL of 5 $\mu g \cdot L^{-1}$ deuterated 3-isobutyl-2-methoxypyrazine ($[^2H_3]$-IBMP) standard (Sigma-Aldrich, St. Louis, MO, USA) was added to reach the final concentration of 25 $ng \cdot L^{-1}$ of $[^2H_3]$-IBMP as internal standard. Then the flask was made up to the volume with additional supernatant. IBMP and IPMP were extracted *via* solid phase micro-extraction and analyzed by gas chromatography with mass spectrometric detection (SPME-GC-MS) (Agilent Technologies 7890A, Shanghai, China). The SPME-GC-MS was equipped with a Gerstel MPS2 multipurpose sampler (Gerstel, Mülheim an der Ruhr, Germany) and two successively connected columns, an HP 1 MS (Agilent Technologies, 30 m, 0.32 mm i.d., 0.25 µm film thickness) and an HP INNOWAX (Agilent Technologies, 30 m, 0.32 mm i.d., 0.25 µm film thickness), with a constant flow of helium at 1.5 $mL \cdot min^{-1}$. Procedure of analysis was performed as described in Šuklje *et al*. (2012). For qualitative determination, retention time and mass spectrum in selective ion monitoring mode (SIM) were used. The mass channel was m/z 124 and 151 for IBMP, m/z 137 and 152 for IPMP, and m/z 127 and 154 for [2H3]-IBMP. Ions 137, 124, and 127 were the target ions used for quantification, whereas 152, 151, and 154 were used as qualifier ions. Calibration was performed with calibration standards in sugar solution (Šuklje *et al*. 2012). Linearity was verified by using spiked samples of dearomatized must (Šuklje *et al*. 2012). Concentrations of methoxypyrazines were expressed as ng/L of grape juice.

Statistical analysis: Two-way analysis of variance was performed to examine defoliation treatment and season effects on yield components and grape composition using JMP 7.0 (SAS Institute Inc.). When season x treatment interaction was significant, then data were analyzed separately by year. Results of the seasonal evolution of methoxypyrazines are shown as means ± standard error. Mean separation was performed using the Tukey HSD test.

Results

Environmental condition: Contrasting climate conditions between the two seasons of experimentation were observed. In 2010, mean temperatures during the summer were very similar to the most recent historic 10-year mean temperatures in the same period (Fig. 1). On the contrary, in 2011, mean monthly temperatures during the summer were higher, with a peak at 25.1 °C in August. In 2011, seven consecutive days with maximum air temperature between 35 to 38 °C were registered before harvest in August (data not shown). Overall, yearly rainfall was higher in 2010 than in 2011 with 1845 mm and 839 mm, respectively, as well as the cumulative precipitation measured from April to October that was 924 mm and 625 mm in 2010 and 2011, respectively (Fig. 2). Growing Degree Days (GDD) during the period April 1 – October 1 were calculated using the 10 °C-base model. GDD were higher in 2011 with 2078 GDD than 2010 (1697 GDD), a 20 % difference between the warmer (2011) and the cooler season (2010).

Grapevine phenology, yield components, bunch health and berry composition: Anthesis (BBCH 65) was recorded on June 5 and May 28 in 2010 and 2011, respectively. Veraison (BBCH 83) was noted on August 5 (61 DAA) and July 21 (54 DAA) in 2010 and 2011, respectively. Harvest (BBCH 89) occurred on August 29 (85 DAA) and August 19 (83 DAA) in 2010 and 2011, respectively.

In both seasons, number of clusters per vine, mean cluster weight, mean berry weight, and yield were not affected by leaf removal (Tab. 1). However, variability between seasons was observed; in general, a lower yield was

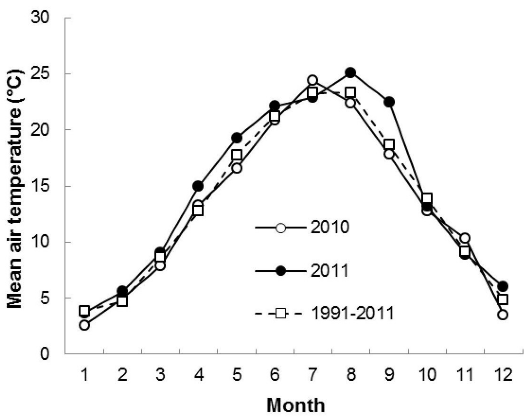

Fig. 1: Monthly mean air temperature during 2010 and 2011 as compared to the 1991-2011 mean.

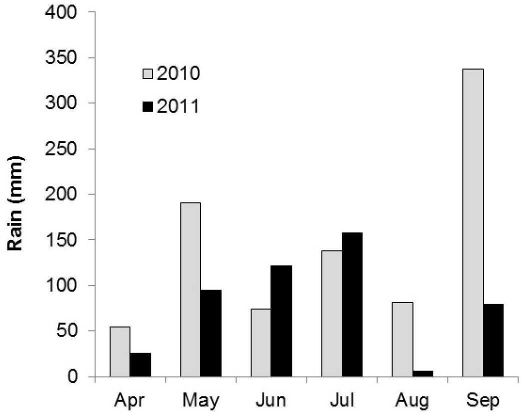

Fig. 2: Monthly cumulate rain (mm) during the growing season.

Table 1

Yield components in 'Sauvignon blanc' grapevines subjected to leaf removal (LR) and control (C) in 2010 and 2011. Data averaged over treatments and seasons in the absence of significant interactions

Parameter	Treatment		Season		Treat x season
	C	LR	2010	2011	
Yield/vine (kg)	2.77 a	2.69 a	3.19 a	2.27 b	ns
Clusters/vine	21.6 a	19.6 a	22.5 a	18.7 b	ns
Cluster weight(g)	127.3 a	135.9 a	141.6 a	121.6 b	ns
Berry weight (g)	1.69 a	1.69 a	1.78 a	1.59 b	ns

Different letters within the same row denotes significant differences ($p < 0.05$) between treatments.

observed in 2011 than in 2010, due to a reduced number of clusters per vine, reduced mean cluster weight, and slightly reduced berry weight (Tab. 1).

Botrytis bunch rot and sour rot were observed in both seasons. Interaction between treatment and season was significant and thus data are presented separately for each season. Seasonal differences, mainly related to rain events, stimulated a higher incidence and severity of the diseases in 2010 when compared to 2011 (Tab. 2). In 2010, these incidence and severity of *Botrytis* bunch rot were significantly lower in LR grapevines: incidence was 73.8 % and 43.8 % in C and LR, respectively, while severity was 25.2 % and 4.6 % in C and LR, respectively. In 2011, the incidence was 38.8 % and 27.7 % in C and LR, respectively; and severity was 6.3 % and 2.2 % in C and LR, respectively; but differences were not significant. Sour rot incidence was low in both seasons and ranged from 0 % to 8.3 %. In 2010, LR prevented any sour rot damage; in contrast, 5.5 % of the C clusters were attacked by sour rot. In 2011, some clusters were damaged with sour rot (8.3 % and 5.6 % in C and LR, respectively) but there were no statistical differences between the two treatments. The severity of sour rot was always below 1 %.

Sunburn damage was observed only in 2011 (Tab. 2) and the LR treatments showed significantly higher incidence and severity of damage. Sunburn incidence was 9.3 % and 47.9 % in berries from C and LR, respectively, while severity was 0.4 and 3.6 % in C and LR berries, respectively.

Treatments did not affect berry composition at harvest (Tab. 3), except for must pH and malic acid concentration, which decreased under LR. Seasonal variability, mainly linked to differences in mean air temperatures and GDD accumulation, lead to higher TSS concentration in 2011

Table 2

Incidence and severity of *Botrytis* bunch rot, sour rot, and sunburn in 'Sauvignon blanc' grapevines subjected to leaf removal (LR) and control (C) in 2010 and 2011

Parameter	2010		2011	
	C	LR	C	LR
Botrytis bunch rot incidence (%)	73.8 a	43.8 b	38.8 a	27.7 a
Botrytis bunch rot severity (%)	25.2 a	4.6 b	6.3 a	2.2 a
Sour rot incidence (%)	5.5 a	0.0 b	8.3 a	5.6 a
Sour rot severity (%)	0.7 a	0.0 b	0.7 a	0.1 a
Sunburn incidence (%)	0.0 a	0.0 a	9.3 b	47.9 a
Sunburn severity (%)	0.0 a	0.0 a	0.4 b	3.6 a

Different letters within the same row and for each season denotes significant differences ($p < 0.05$) between treatments.

Table 3

Berry juice composition in 'Sauvignon blanc' grapevines subjected to leaf removal (LR) and control (C) in 2010 and 2011. Data averaged over treatments and seasons in the absence of significant interactions

Parameter	Treatment		Season		Treat x season
	C	LR	2010	2011	
Total soluble solids (Brix)	19.5 a	19.6 a	18.2 b	20.9 a	ns
Must pH	3.51 a	3.43 b	3.38 b	3.56 a	ns
Titratable acidity ($g \cdot L^{-1}$)	4.88 a	5.01 a	5.46 a	4.43 b	ns
Malic acid ($g \cdot L^{-1}$)	3.36 a	2.61 b	3.53 a	2.43 b	ns
Tartaric acid ($g \cdot L^{-1}$)	4.88 a	6.30 a	6.87 a	4.30 b	ns

Different letters within the same row denotes significant differences ($p < 0.05$) between treatments.

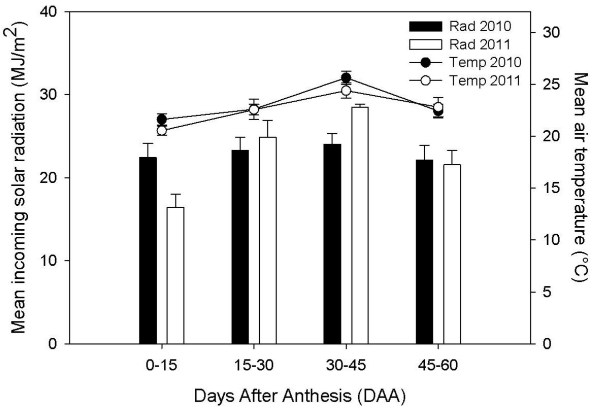

Fig. 3: Incoming solar radiation (MJ/m²) calculated as mean value every 15 d from the day of anthesis (50 % capfall) until veraison, and mean air temperature (°C) in the same period.

than in 2010, together with slightly higher pH, and lower TA, malic and tartaric acid.

Fruit ripening was affected by LR only in 2010 (Fig. 4). In particular, TSS accumulation was slower in the berries of LR vines during the early stages of fruit ripening, but faster shortly after, resulting in no differences between treatments at harvest (Fig. 4). TSS concentration was 10.9 and 9.7 °Brix at 61 DAA (50 % veraison), 13.8 and 15.7 °Brix at 74 DAA, in C and LR, respectively, and 18.2 °Brix in both treatments at harvest (87 DAA). Harvest at such low sugar concentration followed the winery decision based on spread and intensity of bunch rot (Tab. 2) that was similar to our non-defoliated controls within the vineyard. Similarly, TA accumulation was higher in the berries of LR vines during early stages of fruit ripening (29.7 and 33.3 $g{\cdot}L^{-1}$ at 47 DAA for C and LR treatments, respectively) but a faster decrease of TA after veraison resulted in no differences at harvest (5.5 $g{\cdot}L^{-1}$ for both treatments).

LR treatment affected seasonal levels of methoxypyrazines in the berries (Fig. 5). Levels of IBMP were lower in LR vines during early stages of development in both seasons. However, at harvest, the levels of IBMP were below the threshold of instrumental detection (0.6 $ng{\cdot}L^{-1}$) for both treatments (Fig. 5). In both seasons, levels of IPMP concentration in the berries were lower in LR than in C at the first sampling date; then IPMP concentration steeply decreased and no further differences between treatments were noticed. At harvest, IPMP concentration levels were below the threshold of instrument detection in both seasons.

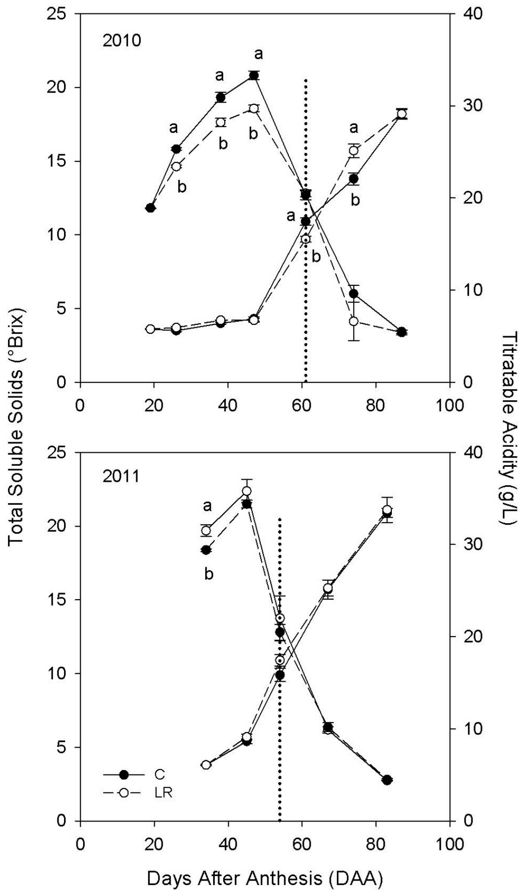

Fig. 4: Total soluble solids (Brix) and titratable acidity ($g{\cdot}L^{-1}$) concentration during berry development in 'Sauvignon blanc' grapevines subjected to leaf removal (LR) and control (C). At each sampling date, different letters denote statistical significance at $p < 0.05$. Dotted line indicates the veraison date.

Discussion

The FVG region is often characterized by relatively high rain events during the growing season, which triggers high disease pressure both on canopy and clusters. In this study, the 2010 season was more rainy than 2011 (Fig. 2), particularly during August and September, which typically herald the end of ripening and the harvest of most varieties. For viticulturists, this period is of critical strategic concern. The harvest date is often largely dependent on the presence of bunch rot and the intensity and pace of the fungal attack. Accordingly, in 2010 season, harvest were performed when berries had reached only 18 °Brix because *Botrytis* bunch rot was widely spread and with great intensity within the vineyard. Early harvest to avoid potential winemaking issues with *Botrytis* and sour rot infections is often decided at the expense of more standard maturity parameters based on TSS, TA and pH. Thus, applying viticultural practices, such as basal leaf removal, for controlling bunch rot is of great interest and applicability in FVG. However, viticulturists are concerned with whether the introduction of such techniques could alter wine grape composition at harvest, as bunch-zone leaf removal has been associated with sunburn and a lower varietal aroma of grapes (Chorti *et al.* 2010, Lohitnavy *et al.* 2010, Pastore *et al.* 2013, Šuklje *et al.* 2014). Our results show that in two seasons of experiments, leaf removal applied after fruit set (at the groat-size stage of development) did not affect yield (Tab. 1) and fruit technological composition (TSS, TA, pH) at harvest (Tab. 3) in 'Sauvignon blanc'. Scheiner *et al.* (2010) did not find statistical differences in the yield and grape composition of 'Cabernet franc' and 'Merlot' vines when different severities and timings of leaf removal treatments were imposed after anthesis. King *et al.* (2012) also showed the same result on 'Merlot' vines cultivated in a cool climatic region. However, a slight delay in ripening was observed in

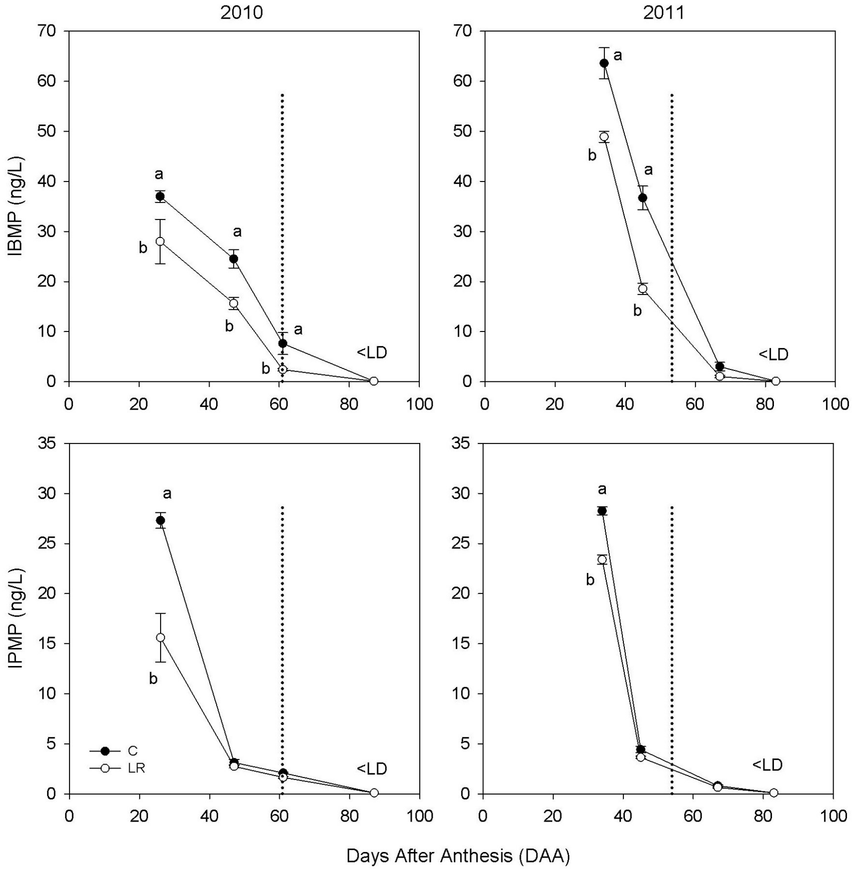

Fig. 5: 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) concentration during berry development in 2010 and 2011 in 'Sauvignon blanc' grapevines subjected to leaf removal (LR) and control (C). At each sampling date, different letters denote statistical significance at $p < 0.05$. Dotted line indicates the veraison date. LD = limit of detection.

fruit of LR vines (lower TSS and higher TA at early stages of ripening) in 2010. This transient effect might have been related to a lower leaf area of the main shoots of LR vines – due to the removal of five basal leaves per shoot – and/or to a higher competition for sugars between lateral shoots and clusters of LR vines. LR treatment normally increases the growth of lateral shoots (Caspari *et al.* 1998) and lateral shoots are strong sink competitors for developing clusters until the leaves of lateral shoots fully mature (Candolfi-Vasconcelos and Koblet 1990). Later during the season, the loss of leaf area due to leaf removal might have been compensated by the increases of photosynthetic efficiency of the remaining leaf and an increase of the lateral leaf area (Caspari *et al.* 1998). In this study, leaf area was measured only in 2011 (data not shown) and the data confirmed that differences in total leaf area were observed in LR vines just after the treatment was applied, but they disappeared during fruit ripening.

LR treatment significantly decreased IBMP and IPMP concentration during berry development (Fig. 4). Viticultural practices that favor bunch exposure to solar radiation normally decrease methoxypyrazines concentration during berry development and at harvest (Scheiner *et al.* 2010, Ryona *et al.* 2008). Methoxypyrazines are biosynthesized in the berry at early stages of development and decrease from pre-veraison stages to harvest (Roujou de Boubée *et al.* 2002, Ryona *et al.* 2008, Koch *et al.* 2012, Scheiner *et al.* 2010). In this experiment, the accumulation of these compounds followed the same trend. Interestingly, IBMP concentration early in the season was higher in 2011 than in 2010 independently of the treatments. This result is probably related to the lower solar radiation and lower temperatures recorded in 2011 from the end of May to beginning of June (Fig. 3). This period is concomitant with berry-set and first stages of fruit development and thus with MP accumulation (Hashizume and Samuta 1999); the impact of light exposure during this period on the quantity of MPs seems to be a balance of two effects: biosynthesis and photodecomposition (Sidhu *et al.* 2014). Leaf removal reduced IBMP and IPMP in both seasons. IBMP is the major MP detected in 'Sauvignon blanc'. The relationship between IBMP and TSS concentration reported in Fig. 6 indicates that during ripening the trend of IBMP degradation was similar between LR and C berries. Hence, the major effect of LR on IBMP concentration in the berry was established before ripening as demonstrated in previous studies (Koch *et al.* 2012). In contrast to what reported in other studies that considered 'Merlot', 'Cabernet Sauvignon', and 'Cabernet franc' grapes (Koch *et al.* 2012, Scheiner *et al.* 2010, Ryona *et al.* 2008), in this study, IBMP concentration at harvest was below the sensory detection threshold – 2 $ng \cdot L^{-1}$ in water solution and of 8 $ng \cdot L^{-1}$ in 'Sauvignon blanc' wines (Allen *et al.* 1991, Kotseridis *et al.* 1998, Pickering *et al.* 2007) – and below the instrument limit of detection that was LD = 0.6 $ng \cdot L^{-1}$. Fast IBMP degradation occurred before and at veraison regardless of the treatment imposed. This stresses the difficulty of obtaining 'Sauvignon blanc' wines characterized by green pepper, as-

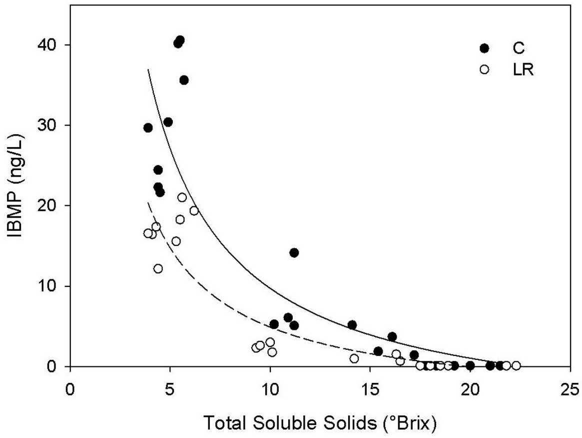

Fig. 6: Relationship between total soluble solids (°Brix) accumulation and 2-methoxy-3-isobutylpyrazine (IBMP) (ng·L^{-1}) concentration during berry development in 'Sauvignon blanc' grapevines subjected to leaf removal (LR) and control (C). Two seasons data are plot together.

paragus, grassy and vegetative aromas (Allen *et al*. 1991, Pickering *et al*. 2007) in similar climatic conditions.

The season of 2010 was mostly rainy, particularly during ripening (Figure 2). Sour rot damages were negligible while the *Botrytis* bunch rot was remarkable and LR treatment was successful for controlling incidence and severity of both diseases (Tab. 2). These results are in accordance with previous work on the efficiency of leaf removal for *Botrytis* control (Percival *et al*. 1994, Intrieri *et al.* 2008, Molitor *et al.* 2011, Palliotti *et al.* 2011). The lower incidence of *Botrytis* bunch rot due to the leaf removal treatment most likely determined the higher cluster weight (+ 15 %) in LR vines in 2010. However, in a drier season (2011), the lack of *Botrytis* resulted in no effects of LR on cluster weight or other yield components.

Bunch sunburn was observed only in 2011, the season that registered higher summer temperatures. Previous studies have reported that in warm viticultural areas, the combination of high solar radiation and temperature can lead to sunburn in berries exposed to sunlight via leaf removal at berry set, a phenological stage close to the one considered in this study (Chorti *et al.* 2010). However, in our study, despite a high incidence of sunburn, the severity of the damage was very limited and did not determine any significant impact on the compositional parameters analyzed in this study (although may have altered parameters not analyzed here) neither a loss in the production.

Conclusion

Basal leaf removal applied after berry set (groat-sized) in 'Sauvignon blanc' was effective at decreasing the incidence and severity of *Botrytis* bunch rot, while showing no significant impact on yield and fruit technological composition in 'Sauvignon blanc' grapes. Cluster exposure decreased IBMP accumulation at early stages of berry development, but not at harvest when values of both the treatments, C and LR, were not detectable. Therefore, leaf removal can be considered a suitable practice in similar wine growing regions, particularly during wet and cool seasons, to cope with bunch diseases without impacting grape composition. However, leaf removal can also result in sunburn during seasons with high temperatures and, therefore, its implementation should take into account the climate and other agronomic factors such as row orientation.

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