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Research Article

Effect of Early Fruit-Zone Leaf Removal on Canopy Development and Fruit Quality in Riesling and Sauvignon blanc

Brittany L. Komm[1] and Michelle M. Moyer[2]*

[1]Former Graduate Research Assistant, and [2]Assistant Professor; Department of Horticulture, Washington State University-Irrigated Agriculture Research and Extension Center, Prosser, WA 99350.

*Corresponding author (michelle.moyer@wsu.edu; tel: 509-786-9234; fax: 509-786-9370)

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Abstract: Canopy management is vital for quality wine grape (*Vitis vinifera*) production. During the 2012 and 2013 growing seasons, the timing of fruit-zone leaf removal (FZLR) was evaluated in two commercial vineyard blocks (*V. vinifera* 'Riesling' and 'Sauvignon blanc') located north of Prosser, WA. Three different timings of manual FZLR were evaluated relative to a no removal control. Leaf removal consisted of complete removal of all leaves and lateral shoots in the fruiting-zone on both sides of the canopy at the specified times of pre-bloom, bloom and 4 weeks post-bloom. Each vine received the same treatment for both years of the study. No negative implications were observed in total fruit set in either year of the study. When leaf removal was performed, regardless of timing the fruit-zone of the canopy had less lateral shoot development and canopy refill than the control. Leaf removal also improved spray coverage in the fruit-zone in Riesling, but the effect was related to the timing of when leaf removal had occurred relative to the timing of the spray. In 2013, pre-bloom leaf removal resulted in

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significantly reduced Botrytis bunch rot severity in Sauvignon blanc relative to the control (p = 0.01) and the 4 weeks post-bloom leaf removal treatments. In 2013, pre-bloom leaf removal in Riesling increased terpene concentrations in the harvest juice relative to the control (p = 0.03). While in 2012, post-bloom leaf removal in Riesling reduced concentration of acids relative to pre-bloom (p = 0.04) in the harvest juice.

Key words: canopy management, lateral shoot development, early fruit-zone leaf removal, fruit set, spray coverage

Introduction

Fruit-zone leaf removal (FZLR) is a popular canopy management practice employed in wine grape (*Vitis vinifera*) growing regions around the world. This practice is typically carried out between fruit set and veraison (Diago et al. 2010, Percival et al. 1994a). In recent years, the practice of FZLR before fruit set has grown in interest. Pioneering work from around the world has focused on how the timing and degree of FZLR impacts overall vine growth and development, cluster disease severity and fruit composition (Bledsoe et al. 1988, Hunter et al. 1995, Lee and Skinkis 2013, Poni et al. 2006, Sabbatini and Howell 2010, Zoecklein et al. 1992).

Removing leaves in the fruit zone at pre-bloom can reduce the total canopy leaf area by more than 50% (Poni et al. 2008), potentially resulting in insufficient carbohydrates for plant use during bloom and fruit set (Kliewer and Antcliff 1970). This is one factor that may explain why some studies have demonstrated a reduction in both fruit set and yield when FZLR was implemented prior to and during bloom (Palliotti et al. 2012, Percival et al. 1994b, Poni et al. 2009, Sabbatini and Howell 2010, Tardaguila et al. 2008). While a reduction in fruit set might be desired in locations where crop management techniques might be legally restricted, or where loose cluster architecture is desired, it is not always a universal production goal. In eastern

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Washington State, reaching contract-specific yields while maintaining quality is a goal, and a reduction of fruit set may negatively impact their ability to do so and has resulted in a cautionary approach to adopting the practice of early FZLR. Looser clusters as a means to reduce Botrytis bunch rot risk is not always a primary goal either, as the climate conditions in the region are not consistently conducive for disease outbreaks on an annual basis. Growers here focus on ways to improve their pest management programs (e.g., improved coverage of powdery mildew fungicides) and alter components, such as fruit microclimate that can influence wine style and composition in the field.

There are mixed results when reviewing the impacts of early FZLR on harvest juice composition (Percival et al. 1994b, Poni et al. 2006, Staff et al. 1997, Tardaguila et al. 2008). Poni et al. (2009) reported that pre-bloom leaf removal in *V. vinifera* 'Barbera' resulted in fruit with lower TA in harvest juice relative to the untreated control. Zoecklein et al. (1992) reported that FZLR around fruit set in both *V. vinifera* 'Riesling' and 'Chardonnay' did not impact juice pH or TA. In general, however, many pioneering authors reported either unchanged or increased harvest TA. A number of studies saw reductions in powdery mildew (*Erysiphe necator*) and Botrytis bunch rot (*Botrytis cinerea*) disease severity (Diago et al. 2010, Percival et al. 1994b, Sabbatini and Howell 2010, Staff et al. 1997). Both of these diseases can have a negative effect on overall juice composition at harvest.

The objectives of this study were to evaluate the timing of complete, early FZLR on canopy development, fruit set, spray coverage, and fruit composition in *V. vinifera* 'Riesling' and 'Sauvignon blanc' as it relates to optimization of both horticultural and disease management attributes of this cultural practice under the arid conditions in eastern Washington State.

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Materials and Methods

Vineyard description. This trial was conducted in a commercial vineyard located northeast of Prosser, Washington, USA (46°15'36" N, 119°43'12" W) from spring 2012 to winter 2014, for a total of two full growing seasons. Site soils are Warden-Silt loam, are well drained and have a high water-holding capacity. One block each of own-rooted Riesling and Sauvignon blanc were used. Blocks were planted in 2007, with north-south row orientation. Planting distances were 2.7 m x 1.8 m (rows x vines) in Riesling, and 2.7 m x 2.1 m (rows x vines) in Sauvignon blanc. All vines were pruned to 14, three-bud spurs. Water was applied through drip irrigation. In both years, an initial irrigation application was in early to mid-May, with subsequent applications not beginning until after fruit set. Post-fruit set irrigation was designed as a regulated deficit of approximately 80% evapotranspiration (ET_o), and irrigation sets were weekly to biweekly depending on plant response. In both years, a final irrigation set in October was done to replenish the soil moisture profile prior to the dormant season. The canopy was trained using a modification of the vertical shoot positioning (VSP) system. The modification consisted of only training one-third of the shoots to an upright position using fixed catch-wires. The remaining shoots are allowed to sprawl on either side of the canopy. This modification is common in eastern Washington to reduce excessive sun exposure on the fruit. Summer canopy hedging was performed in mid-July, after all leaf removal treatments had been executed. At the time of mechanical hedging, canopies were 1.25 m in height, and the top 8 to 12 cm of 40% of the shoots were removed during the process. Hedging was coupled with regulated deficit irrigation to reduce further canopy development. All other management practices (e.g., insect pest, disease, nutrient) were carried out per the vineyard's standard production procedures.

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Weather. Weather data was collected using Washington State University's AgWeatherNet system (http://weather.wsu.edu). The "WSU Prosser" weather station was used, and was located approximately 1.6 km from the research site. Average daytime high and low temperatures and total precipitation data were recorded, and growing degree days (base of 10°C; 1 April to 31 October) and evapotranspiration (ET_o) were calculated.

Leaf removal treatments. Leaf removal was done manually. On each treatment date, all leaves and lateral shoots (if present at the time of the treatment) from the base of each count shoot up to the distal cluster (the distal cluster was typically present on node four or five in both varieties) were removed. All non-count shoots and cordon suckers were removed prior to the implementation of the first leaf removal treatment in both varieties. Each leaf removal treatment was applied at a key phenological development stage as defined by the BBCH scale (Lorenz et al. 1994). The leaf removal timings evaluated were: no leaf removal (control), pre-bloom (approximately BBCH 57), bloom (BBCH 65; when 50% of the inflorescences were at 50% capfall), and 4 weeks post-bloom (BBCH 75). Dates of leaf removal in Riesling were: 23 May 2012 and 14 May 2013 for pre-bloom; 13 June 2012 and 5 June 2013 for bloom; and 11 July 2012 and 3 July 2013 for 4 weeks post-bloom. Dates of leaf removal in Sauvignon blanc were: 30 May 2012 and 20 May 2013 for pre-bloom; 15 June 2012 and 7 June 2013 for bloom; and 12 July 2012 and 3 July 2013 for 4 weeks post-bloom.

Leaf removal treatments were replicated four times in a randomized complete block design. Each treatment was applied to 24 vines (eight vines per row, in three adjacent rows) in each replicate (block). The six center vines in the center row of each treatment replicate were used for data collection and observation, allowing a 1-row buffer on either side of the treatment,

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and a 1-vine buffer within the center row. The same treatments were imposed on the same vines in both years of the study.

Leaf area removed. In 2013, ten shoots per treatment were arbitrarily collected from vines outside of the experimental design to estimate the approximate leaf area removed during each treatment application. This method was used, as the participating grower had already contracted much of the fruit in the research location and did not want full-vine defoliation at that time of the year. Shoots were collected 10 days and four days after the pre-bloom leaf removal treatment in Riesling and Sauvignon blanc, respectively. The delay in collection of these shoots was a result of delayed vineyard entry due to a combination of timing overlaps relating to pesticide reentry periods. Shoots were collected within two days of the bloom treatment, and within one day of the 4 weeks post-bloom leaf removal treatment in both varieties. To measure leaf area, each shoot was stripped of all leaves and lateral shoots. Once removed, individual leaf area was then estimated for each leaf by multiplying the length of the mid-vein by the width of the leaf at that widest part. To calculate leaf area removed, leaf area of leaves found within the fruiting-zone was compared to total leaf area for each shoot. This was then extrapolated to a whole-vine level.

Summer lateral shoot development. To evaluate the degree of canopy refill in the fruiting-zone, the presence and length of summer lateral shoots that remained after the leaf removal treatments (i.e., were not present at the time of leaf removal), were rated in the fruit-zone on ten shoots in each treatment replicate. In 2012, summer lateral shoot presence and length of those laterals arising between nodes one and four on each main shoot were recorded on 15 August. In 2013, summer lateral shoot presence and length on lateral shoots arising between nodes one and five were recorded on 10 September and 29 August for Riesling and Sauvignon blanc,

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144 respectively. Summer lateral shoot presence and length was rated categorically: i) no lateral
145 shoot present; ii) lateral shoot ≤ 3.0 cm; iii) lateral shoot between 3.1 and 15.0 cm; and iv) lateral
146 shoot >15.0 cm.

147 *Spray coverage.* The impact of fruit-zone leaf removal on fruit-zone spray coverage was
148 also evaluated in 2013. Spray coverage was assessed on 20 June for both varieties (bloom) and
149 again on 30 July for Riesling and 1 August for Sauvignon blanc (just prior to the onset of
150 veraison). In each treatment replicate, one water-sensitive card (Syngenta® Crop Protection AG,
151 Basel, Switzerland) was placed in the vine canopy. Cards were affixed to the node between the
152 basal and secondary clusters using a clothespin, water-sensitive side facing the row middle (i.e.,
153 outside of the canopy). The cards were placed in the vineyard just prior to spraying, and were
154 removed promptly after they had dried (approximately 2 to 3 hr). Coverage was estimated on
155 each card using open-source ImageJ software that calculates pixel areas using color thresholds
156 (Abramoff et al. 2004).

157 *Disease and sunburn severity.* The incidence and severity of Botrytis bunch rot and the
158 severity of sunburn were evaluated. Severity was visually rated as percent cluster surface area
159 affected. Botrytis bunch rot was rated as clusters expressing symptoms of internal berry rot (i.e.,
160 brown discoloration of berries without the presence of fungal sporulation but no acetic acid odors
161 present in order to distinguish it from Sour Rot), or as rot with associated fungal sporulation.
162 Given the dry harvest conditions during the evaluation years, all Botrytis bunch rot was
163 expressed as a non-sporulating, internal berry rot. Ratings were completed on 10 arbitrarily
164 selected clusters within a treatment replicate. Evaluation of clusters in both years and both
165 varieties occurred early to mid-September (pre-harvest, BBCH 89). In both years in both
166 varieties, a dual-action powdery mildew-Botrytis bunch rot fungicide Inspire Super (Syngenta;

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167 difeconazole + cyprodinil) was used during bloom; no additional Botryticide applications were
168 made after bloom in 2012. In 2013, in Sauvignon blanc, an additional Inspire Super application
169 was used at the start of veraison (1 August) and Elevate (Arysta Lifescience; fenhexamid) was
170 applied on 13 August.

171 *Fruit set and berry weights.* Fruit set was evaluated in both years of the study. In 2012,
172 eight and seven basal clusters (Riesling and Sauvignon blanc, respectively) per treatment
173 replicate were used to calculate fruit set, and in 2013, ten basal clusters per treatment replicate in
174 both varieties were used. Calyptras were collected using handmade 15 cm x 12 cm fine mesh
175 white nylon bags (also referred to as "tulle") as previously described by Keller et. al (2001).
176 Bags were affixed to the selected basal clusters at pre-bloom (BBCH 57); and were removed
177 after the completion of bloom (BBCH 71). Caught calyptras were enumerated. After fruit set, the
178 same clusters were destructively sampled to count total berries. Fruit set was calculated by
179 dividing berries per cluster by calyptras per cluster.

180 Berry weights were evaluated in both years of the study. In 2012, fruit was harvested on
181 10 and 18 of September for Sauvignon blanc and Riesling, respectively. In 2013, Sauvignon
182 blanc was harvested on 29 August, and Riesling was harvested on 16 September. Berry weights
183 in 2012 were based on 100 berries per treatment replicate; weights were based on 60 berries per
184 treatment replicate in 2013.

185 *Fruit composition.* Harvest juice soluble solids (Brix), titratable acidity (TA), and pH,
186 from the fruit exposed to the different timing of leaf removal were also evaluated. Data was
187 collected on 18 September 2012 and 16 September 2013 in Riesling; and 10 September 2012 and
188 5 September 2013 in Sauvignon blanc. All evaluations occurred within 10 days of commercial
189 harvest. Three basal clusters per treatment replicate were used in 2012, and five basal clusters

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per treatment replicate were used in 2013. Within a treatment replicate, clusters were pooled and whole-cluster pressed. The resulting juice (approx. 200 mL) was used for analysis. Of this juice, approximately 7.0 mL was used for soluble solids, TA and pH measurements; 50.0 mL was stored at -18°C until it could be transported for volatile and ammonia analysis. Juice soluble solids were measured using a digital refractometer (Quick-Brix 60, Mettler-Toledo©, Schwerzenbach, Switzerland). Juice pH was measured using an electrode (InLab® Versatile 413, Mettler-Toledo©, Schwerzenbach, Switzerland). Juice TA was measured and calculated as described by Iland et al. (2000).

Volatiles were analyzed from pressed grape juice using a modification of the methods of Francioli (1999) and Howard et al.(2005). Frozen juice samples described above were allowed to gradually thaw, and then adjusted to a pH of 6.4 with 0.5% phosphoric acid (H_3PO_4) for acid hydrolysis of glycosides. A total of 3.0 mL juice was added to a 15.0 mL sample vial (Supelco, Bellefonte, PA, USA) with 30% w/v sodium chloride (NaCl) and sealed with a silicon septa cover. The sample was magnetically stirred at 1200 rpm and heated to 50°C for 2 min prior to exposure of the solid-phase micro-extraction (SPME) fiber. A 60-µm polydimethylsiloxane divinylbenzene (PDMS/DVB) SPME fiber (Supelco, Bellefonte, PA, USA) was exposed to the headspace (HS) of the sample for 60 min at 50°C with constant stirring. After extraction, fibers were desorbed in the injection port of a gas chromatography–mass spectrometry system (Hewlett Packard 5890II/5970, Agilent Technologies, Santa Clara, CA, USA) with a (60 m X 0.32 uM) DB1 capillary column (Phenomenex, Torrance, CA, USA). The mass spectrometer (ion source maintained at 250°C) used electron impact with electron energy of 70 eV. The SPME fiber then was desorbed in the injection port for 5 min at 200°C using splitless injection. The capillary column was set at 33°C and held for 5 min before ramping to 50°C at a rate of 2°C/min. Mass

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spectral results were viewed using Chemstation G1803C software (Agilent Technologies, Santa Clara, CA, USA). Compounds observed as chromatographic peaks were identified comparing their mass spectra and retention times with monoterpene standards: linalool (≥95% GC, Fluka, Switzerland), geraniol (98% GC, Sigma-Aldrich, St. Louis, MO, USA), *cis*-rose oxide (>99% GC, Fluka), nerol (≥90% GC, Fluka), 2- and 3-carene (95% GC, Sigma-Aldrich), and α-terpineol (mix of isomers 95%, Sigma-Aldrich), as well as hexanal, nonanal, decanal, hexanoic acid, and decanoic acid (≥95% (Sigma-Aldrich). Identifications were also made using published retention times and mass spectra of the Wiley and NIST library spectra database, as well as the listed standards. There were four replicate extractions per treatment. The quantitations were achieved by running compounds as mixtures from minimum detection to above the highest detected amounts in the study. The R^2 of standard curve was ≥85%.

The 50.0 mL juice samples used for volatile analysis were also used in the ammonia analyses. Frozen samples were allowed to gradually thaw prior to total ammonia analysis. To conduct juice ammonia analysis, a standard curve was created following manufacturer's instructions (Ammonia Combination Electrode, Denver Instruments, Bohemia, NY, USA) using 1, 5, 10 or 100 mg/L single-concentration standard solutions of ammonium (NH_4^+) and 0.2 mL allotments of 10 M NaOH. The standard curve was created twice. To evaluate ammonia in the juice samples, the same protocol for developing the standard curve was used, but with 10.0 mL of juice rather than the standard, and NaOH added in 0.4 mL allotments.

Skin and seed tannin and phenolic content. Tannin and phenolic content in berry skins and seeds were evaluated in both years of the study using 30 berries per treatment replicate. Berries, with pedicels attached, were removed from ten and five (2012 and 2013, respectively) clusters at harvest. Berries were collected from various locations throughout each cluster.

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Removed berries were then placed into plastic storage bags and stored at -35°C (2012) and -80°C (2013) until analysis could be conducted. Immediately prior to analysis, pedicels were removed from the frozen berries and the pool of 30 berries per treatment replicate were weighed. The skin of each berry was removed from the flesh by hand, and seeds were extracted from the flesh and counted. Both skins and seeds were dried separately at room temperature for 4 hours and weighed. After drying, skins and seeds were placed into separate plastic vials and stored at -35°C (2012) or -80°C (2013) until tannin and phenolic extraction could be completed.

To extract seed tannins and phenolics, seeds were ground to a fine powder using liquid nitrogen and a sterile mortar and pestle. The powder was dissolved in 30 mL of 70% acetone. To extract skin tannins and phenolics, skins were transferred directly to a vial containing 30 mL of 70% acetone. Both skin and seed sample solutions were shaken for 12 to 24 hours at 100 rpm (SCILOGEX SK-330-Pro-Shaker, Berlin, CT, USA). After agitation, samples were centrifuged at 5000 rpm for 5 min (Eppendorf 5804 R, Hamburg, Germany), and decanted into polyvac vials (PB3002-S, Mettler-Toledo©, Schwerzenbach, Switzerland). Samples were heated to 40°C and agitated under a vacuum at 300 rpm (Buchi Syncore® Polyvap, Switzerland) until 13.0 to 15.0 mL of the sample remained. Post evaporation, samples were weighed and transferred to plastic vials for storage at -80°C until total tannins and phenolics could be measured. Tannin and phenolic measurements were done using protocols developed by Hagerman and Butler (1978) and Harbertson et al. (2003).

Statistical analyses. Statistical analysis was completed using JMP® statistical analysis software (JMP® 9.0.0, SAS Institute Inc., Cary, NC, USA). Data were tested for normality by Shapiro-Wilk test and homogeneity of variance by Levene's test. No variables required transformation.

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Data were subjected to analysis of variance (ANOVA), and means were separated using Tukey's HSD at $\alpha \leq 0.05$.

Results

Weather. In 2012 and 2013, the Yakima Valley American Viticultural Area accumulated 1468 and 1589 (°C) growing degree day units, respectively. The historical average for the area is 1406 (°C). Average monthly temperatures and average monthly minimum and maximum temperatures for May to September in 2012 and 2013 are reported in Table 1. In addition, monthly total solar radiation, evapotranspiration, and precipitation are reported in Table 1.

Assessment of leaf area removed. Total leaf area (TLA) was assessed in 2013. The pre-bloom, bloom, and 4 weeks post-bloom leaf removal in Riesling removed 59.6%, 49.7%, and 22.4% of the TLA, respectively (Fig. 1A). Riesling pre-bloom and bloom treatments resulted in a significantly higher proportion of the canopy removed at the time of treatment than the post-bloom leaf removal, as expected. Pre-bloom, bloom and 4 weeks post-bloom leaf removal in Sauvignon blanc removed 52.6%, 35.0% and 18.3% of the TLA, respectively (Fig. 1B). Sauvignon blanc pre-bloom treatment had significantly higher proportion of the canopy removed than the bloom and post-bloom treatment, and the bloom treatment had significantly more canopy removed than the post-bloom treatment. Additionally, with pre-bloom leaf removal assessments occurring several days after the treatment was implemented, it is expected that actual total leaf area removed may be higher than recorded here.

Summer lateral shoot development. In both years and on both varieties, leaf removal, regardless of timing, reduced the incidence of lateral shoot development in the fruit-zone and the incidence of lateral shoots of between 0 and 3 cm (Fig. 2). Leaf removal also reduced the incidence of lateral shoots of intermediate length in Sauvignon blanc when compared to the

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control. There were also significant effects of leaf removal timing. For example in 2013, leaf removal at bloom was more effective at keeping the fruit-zone free of lateral shoots than the pre-bloom leaf removal in both Riesling and Sauvignon blanc (Fig. 2C and 2D). In Riesling specifically, both bloom and the post-bloom leaf removal significantly lowered the incidence of lateral shoots of intermediate length in comparison to the control, whereas pre-bloom leaf removal did not (Fig. 2C).

Spray coverage. On both assessment dates in Riesling, bloom leaf removal resulted in improved spray coverage relative to the control (Fig. 3A). On 20 June, pre-bloom leaf removal had significantly higher coverage relative to the control. Unexpectedly, coverage in the 4 weeks post-bloom leaf removal treatment, which had not occurred at the time of this spray application, was not different from the other leaf removal treatments, but as expected, it was also not different from the control. High variability in spray coverage results in late June may not be entirely unusual as canopies have not reached full size or density by this time. On 30 July, a time when all leaf removal treatments had been completed, 4 weeks post-bloom leaf removal had significantly higher coverage than the control, and pre-bloom leaf removal was not different than the control but it was also not different from the other treatments (Fig. 3A).

There were no differences in spray coverage as a result of leaf removal timing on either assessment date (p = 0.75 and 0.08) in Sauvignon blanc (Fig. 3B). While the 20 June assessment date had a similar coverage pattern (Fig. 3B) as seen in Riesling, high level of coverage variability resulted in no difference between treatment means. Interestingly, on the 1 August assessment date, the control had the overall lowest average coverage (10.5%), whereas the different timing of leaf removal ranged between 3 and 4X more coverage than the control (34.0-41.4%) following a similar trend seen in Riesling.

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Disease and sunburn severity. Botrytis bunch rot severity in Riesling was not influenced by leaf removal in either year of the study (p = 0.28 and 0.15, respectively, for 2012 and 2013). Severity ratings in 2012 were 17.8, 29.5, 20.0 and 20.3%, for the control, pre-bloom, bloom and post-bloom leaf removal timings respectively. Severity ratings for Botrytis bunch rot in Riesling in 2013 were 19.5, 13.0, 6.8, and 6.3% for the control, pre-bloom, bloom and post-bloom leaf removal timings, respectively.

Botrytis bunch rot severity in Sauvignon blanc was not influenced by leaf removal in 2012 (p = 0.43). Total disease severity was 19.8, 20.3, 19.0, and 22.5% for the control, pre-bloom, bloom, and post-bloom leaf removal, respectively. However, in 2013, the Sauvignon blanc pre-bloom leaf removal had lower disease severity (4.7%) relative to both the control (12.0%) and 4 weeks post-bloom leaf removal (11.8%) (p = 0.01 and 0.01, respectively). Bloom leaf removal had an intermediate level of disease (6.8%) relative to the pre-bloom and other two treatments.

The timing of leaf removal did not influence sunburn severity in both years and varieties. In 2012, sunburn severity for the control, pre-bloom, bloom, and 4 weeks post-bloom timings were 5, 7, 6 and 12% for Riesling (p = 0.36), and 5, 8, 14, and 16%, for Sauvignon blanc (p = 0.09), respectively. In 2013, sunburn severity for the control, pre-bloom, bloom, and 4 weeks post-bloom timings were 7, 10, 8, and 20% Riesling (p = 0.12), and 6, 8, 12, and 14% for Sauvignon blanc (p = 0.29), respectively.

Fruit set and berry weight. Fruit-zone leaf removal, regardless of timing, did not impact overall fruit set (Fig. 4) in either year for Riesling (p = 0.60 and 0.05, respectively, for 2012 and 2013) or Sauvignon blanc (p = 0.65 and 0.30, respectively, for 2012 and 2013). Overall, 2012

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had higher fruit set in both varieties than 2013, likely do to the more moderate temperatures and evaporative demands during bloom (Table 1).

Average berry weights for Riesling in 2012 were 1.27, 1.20, 1.23 and 1.23 g, respectively, for the control, pre-bloom, bloom, and 4 weeks post-bloom treatments, and were not significantly different from each other (p =0.73). Average berry weights in 2013 were 1.24, 1.23, 1.20 and 1.22 g, respectively, for the control, pre-bloom, bloom, and 4 weeks post-bloom treatments, and were not different from each other (p =0.89). Average berry weights for Sauvignon blanc in 2012 were 1.27, 1.22, 1.23 and 1.23 g, respectively, for the control, pre-bloom, bloom, and 4 weeks post-bloom treatments, and were not different from each other (p =0.74). Average berry weights in 2013 were 1.18, 1.17, 1.16 and 1.10 g, respectively, for the control, pre-bloom, bloom, and 4 weeks post-bloom treatments, and were not different from each other (p =0.62).

Fruit composition. Leaf removal did not influence harvest soluble solids, TA, or pH in either variety in either year (Table 2). In addition, the timing of leaf removal did not influence total aromatic alcohols and terpenes in both varieties in 2012 (Table 2), nor did it influence composition (all volatiles assessed) in Sauvignon blanc in 2013. However, bloom leaf removal in Riesling in 2012 did result in reduced total aromatic aldehyde concentrations relative to the control (p = 0.05); specifically hexanal was reduced (p = 0.03). Pre-bloom leaf removal in Riesling in 2012 resulted in higher total acid concentrations relative to 4 weeks post-bloom (p = 0.04).

In 2013, the timing of leaf removal did not influence total aromatic aldehydes and acids in Riesling (Table 2). However, pre-bloom leaf removal did result in increased total terpene concentrations relative to the control (p = 0.03), specifically, an increase in α-ionone relative to

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the control and bloom leaf removal (p = 0.003 and 0.05, respectively). Nerol oxide concentrations were significantly reduced in the control relative to pre-bloom and 4 weeks post-bloom leaf removal (p = 0.02 and 0.02, respectively). The timing of leaf removal influenced total ammonia (NH_3) in Riesling in 2012 (Fig. 5). Bloom and 4 weeks post-bloom leaf removal resulted in reduced ammonia relative to the control (p = 0.003 and 0.0006; respectively). The 4 weeks post-bloom leaf removal also had reduced ammonia relative to pre-bloom (p = 0.02). Leaf removal did not influence total ammonia in 2013.

Leaf removal did not influence aromatic volatiles in Sauvignon blanc in either year of the study (Table 2). It did, however, influence total ammonia (NH_3) (Fig. 5). In 2012, bloom and 4 weeks post-bloom leaf removal resulted in lower total ammonia relative to the control and pre-bloom leaf removal (p = 0.0002) (Fig. 5). In 2013, bloom leaf removal reduced total ammonia relative to 4 weeks post-bloom leaf removal (p = 0.02). In both varieties, pre-bloom leaf removal was the only leaf removal timing that did not reduce total ammonia relative to the control.

Skin and seed tannin and phenolic content. The timing of leaf removal in Riesling did not alter total skin or seed tannins or phenolics in either year (Table 3).

In Sauvignon blanc, the timing of leaf removal influenced seed total phenolics, and skin tannins and phenolics in 2012 (Table 3), and seed tannins, and skin tannins and phenolics in 2013. In 2012, pre-bloom and 4 weeks post-bloom leaf removal increased total seed tannins relative to the control (p = 0.001 and 0.001, respectively), as well as skin phenolic content relative to the control (p = 0.01 and 0.001, respectively). In 2013 bloom leaf removal had higher skin phenolic content and seed tannin content relative to the control (p = 0.008 and 0.005, respectively) as well has higher skin tannins (p = 0.005). Bloom leaf removal also resulted in higher skin tannins than 4 weeks post-bloom leaf removal (p = 0.005).

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Discussion

Fruit-zone leaf removal, starting as early as pre-bloom, did not result in an overall negative impact of key production parameters in Riesling and Sauvignon blanc as demonstrated in this study. Growers initially feared a reduction in fruit set as a result of complete leaf removal in the fruit-zone prior to fruit set, as production goals in eastern Washington are aimed at optimal yield rather than a reduction in cluster compactness to combat diseases. This focus on yield rather than disease management is expected in a location where environmental conditions are not conducive for wide-spread incidence of various harvest rots (Table 1). However, these same conditions that reduce disease pressure, are also often associated with increases in sunburn and decreases in fruit quality (Spayd et al. 2002). As a partial result, there has not been a wide-spread adoption of early fruit-zone leaf removal, and leaf removal on both sides of the canopy. For the years of 2012 and 2013, which were considered "average" and "above average" in terms of heat, neither a reduction in fruit set nor a loss of overall fruit quality were seen as a result of fruit-zone leaf removal, suggesting that early leaf removal, on both sides of the canopy in a modified VSP system, might be an appropriate cultural practice for eastern Washington grape growers.

The lack of response in fruit set may be related to the environmental conditions in eastern Washington, or it might be related to the level of leaf removal severity used in the present study. The level of leaf removal severity presented here (complete removal of leaves at 4 to 5 basal nodes), while more severe than current grower standards (i.e., leaf removal on the east or north sides of the canopy only), was likely not severe enough to induce significant changes in plant source-sink relationships given the typical growing conditions and season length seen in Washington (Table 1). In studies where the severity of leaf removal was higher, data showed that leaves from the medial area of the main shoot upwards had a higher photosynthetic capacity and

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were able to compensate for the loss of older basal leaves (Poni et al. 2006). Poni et al. (2008) also found that removing the first six basal leaves on shoots at pre-bloom resulted in higher net canopy CO_2 exchange rates compared to vines without FZLR, with the compensation peaking approximately 15 days post defoliation. Coinciding with the increased CO_2 exchange rates, carbohydrate content also increased.

While the degree of fruit-zone leaf removal presented here may not be as severe as imposed in other studies, it still improved spray coverage during the critical period of fruit susceptibility to diseases such as powdery mildew and Botrytis bunch rot (Ficke et al. 2003, McClellan et al. 1973) which occurs around bloom. In Riesling, leaf removal at pre-bloom and bloom resulted in significantly higher spray coverage than that of the control or post-bloom leaf removal. While spray coverage after veraison was not assessed in this study, leaf removal, regardless of timing, had significantly fewer instances of summer laterals in the fruit-zone relative to the control (Fig. 2C), which would result in improved air circulation and sunlight penetration, reducing the microclimate favorability for Botrytis bunch rot (English et al. 1990). Interestingly, in our study, there was only one variety-year combination with leaf removal that resulted in a significant reduction in Botrytis bunch rot, and that was pre-bloom leaf removal in 2013 in Sauvignon blanc. This also happened to be a variety and year without significant differences in spray coverage. Botrytis bunch rot incidence is low to nil in most years in eastern Washington, due to the lack of conducive environmental conditions during fruit ripening. In this situation, during the bloom-time pesticide application for Botrytis bunch rot control both pre-bloom and bloom leaf removal had a more exposed fruit-zone for reduced environmental favorability for *B. cinerea* colonization, despite a lack of difference in spray coverage. At the end of the season, however, the pre-bloom leaf removal timing still had a more open fruit-zone as

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compared to the bloom treatment (Fig. 2D), thus potentially allowing for improved coverage for the post-veraison Botryticide applications made in that year. The reduced Botrytis severity seen as a result of pre-bloom leaf removal in Sauvignon blanc may be due to improved coverage during those later applications (data not collected), or related to specific alterations in canopy microclimate at key time points thus reducing initial colonization by the fungus. The authors speculate that years with more conducive environmental conditions for Botrytis bunch rot development during veraison, early fruit-zone leaf removal would result in improved rot control. Additionally, a more severe application of fruit-zone leaf removal at pre-bloom or bloom (i.e., to above the fruit-zone) might be sufficient to reduce fruit set resulting in looser clusters that have a reduced risk for Botrytis bunch rot disease severity.

Past studies also demonstrated that removing the six basal leaves on shoots during rachis elongation (BBCH 57) resulted in strong lateral shoot growth (Kriedemann 1968, Reynolds and Wardle 1989). Canopy hedging, which results in the loss of apical dominance and also promotes the development of lateral shoots. In certain environments this may require additional vineyard passes to maintain an open fruit-zone. In eastern Washington, more than 1 pass is not desirable by growers due to the increased vineyard management costs. At the same time, a completely exposed fruit-zone that might result in sunburn of fruit is also a concern. As such, some lateral shoot growth in the fruit-zone is desired, provided the other benefits of leaf removal, such as reduced disease pressure and improved spray coverage, remain optimal. Interestingly, in eastern Washington, grapevine canopy size is predominately controlled through the use of regulated deficit irrigation, which can limit the development of lateral shoots, even when coupled with hedging. In our study, if lateral shoots were present at the time of leaf removal, they were also removed. As a consequence, leaf removal, regardless of timing, resulted in fewer laterals in the

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fruit-zone (Fig. 2). However, in 2013, pre-bloom leaf removal appears to have occurred prior to the initial (albeit small) development of lateral shoots, as this timing in both varieties resulted in more lateral shoots developing than the bloom timing (i.e., fewer instances of a "no lateral shoot present" rating) (Fig. 2). In order to obtain a partial canopy refill, but still maintain the benefits of doing FZLR, these current results suggest that leaf removal at pre-bloom (Fig. 2) is likely the optimal timing under the environmental conditions presented here.

One challenge in the production of aromatic varieties such as Riesling and Sauvignon blanc, in warm climates can be the loss of varietal characteristics due to excessive heating of the fruit. Compounding the macroclimate effects would be practices such as fruit-zone leaf removal that expose clusters to sunlight, and thus, resulting in higher fruit temperatures (Spayd et al. 2002). Fruit-zone leaf removal has been shown to increase accumulation of these aromatic compounds (Vilanova et al. 2012, Zoecklein et al. 1998). In this study bloom leaf removal in Riesling increased terpene concentrations (specifically, α-ionone and nerol oxide), likely due to the increase in sun exposure (Zoecklein et al. 1998). Pre-bloom leaf removal in Riesling reduced aldehydes; past studies have shown high levels of aldehydes in shaded fruit (Lohitnavy et al. 2010), and thus, the reduction in aldehydes seen here may be due to increased exposure of the fruit. Aldehydes are often associated with an herbaceous or grassy aroma; while terpenes are associated with floral aromas (Rapp and Mandery 1986, Ristic et al. 2007, Simpson 1978). Our results indicate that the timing of leaf removal may influence the aromatic character of the fruit, and thus, growers may tailor their timing to meet their needs and preferred wine styles; pre-bloom leaf removal improved floral character, while bloom leaf removal reduced grass character.

Washington grapes are characteristically low in free ammonia relative to grapes from other regions (Spayd and Andersen-Bagge 1996). This can pose problems for yeast nutrition

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during fermentation. Typically, levels from 150 to 400 mg/L of free ammonia are required for successful fermentation, but the number can range based on grape variety and yeast strain used (Ugliano et al. 2007). The present study indicated that the timing of fruit-zone leaf removal can impact total free ammonia content. While all treatments (control included), were at the low levels of free nitrogen typically seen in eastern Washington, only the pre-bloom leaf removal timing consistently did not result in lower free ammonia than the control. However, a reduction in total ammonia as a result of leaf removal may not be a concern for conventional growers, as they would still likely require ammonia additions in the winery for successful fermentation. The reduction in free ammonia as a result of early leaf removal might be a factor to consider for organic wine production where sources for nitrogen additions for yeast are more limited.

The level of desired tannin and phenolic content differ between wine styles, but in general, higher levels are desirable in red wines, and lower levels in white wines. In the present study, pre-bloom and bloom leaf removal increased skin tannin and phenolic content in Sauvignon blanc, depending on the year. This increase in skin tannins is likely a result of increased cluster exposure (Ristic et al. 2007). In 2012, the pre-bloom timing resulted in increased tannin and phenolic content relative to the control, whereas the bloom timing had these same effects in 2013. Interestingly, in both years, when comparing the pre-bloom and bloom timing to each other, they did not differ in either skin tannin or phenolic content, likely indicating little distinct differences between these two. However, it is not likely that this increase in skin tannin and phenolic content as a result of leaf removal would translate through to the wine based on standard white wine processing procedures (i.e., little to no skin and seed contact).

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Conclusion

The results of the present study indicate that leaf removal prior to and during bloom, is an appropriately-timed cultural practice for eastern Washington wine grape production for the region's flagship white varieties Sauvignon blanc and Riesling. Fruit-zone leaf removal during this time optimized both horticultural and disease management attributes of the practice, without reductions in fruit set or increases in sunburn. In some cases, additional enological properties can be altered, such as increased terpenes and a reduction in aldehydes, depending on the timing selected. Overall, this study suggests that the current practice of fruit-zone leaf removal between fruit set and bunch closure may be improved if the implementation window was advanced to earlier in the growing season. However, with the current vineyard technology available, this cultural practice would most likely be implemented using hand-labor, and the costs of such should be weighed against the potential improvements in spray coverage or juice aromatic characteristics.

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Table 1 The monthly average maximum, minimum and average temperatures, monthly total solar radiation, and monthly total reference evapotranspiration for 2012 and 2013 in Prosser, Washington. Data from AgWeatherNet (www.weather.wsu.edu); weather station used was "WSU-Prosser."

Month	Average maximum (°C)	Average minimum (°C)	Daily average (°C)	Monthly total solar radiation (MJ/m^2)	Monthly total evapotranspiration[a] (mm)	Monthly precipitation (mm)
2012						
May	21.7	6.6	14.8	764	147.8	6.6
June	24.1	9.8	17.3	741	148.7	41.1
July	31.6	13.4	22.8	887	193.8	7.4
August	31.7	12.4	22.1	826	179.9	1.3
September	26.6	8.4	17.1	588	114.4	0.0
2013						
May	23.2	8.4	16.3	778	148.9	32.5
June	26.1	11.7	19.2	787	156.4	40.1
July	33.4	13.5	24.0	954	214.4	0.0
August	31.0	14.3	22.5	736	159.7	9.8
September	25.5	11.3	18.1	492	104.5	14.7

[a]Evapotranspiration as calculated for grass; ET_o.

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Table 2 Juice composition as a result of leaf removal treatments in *Vitis vinifera* Riesling and Sauvignon blanc for the 2012 and 2013 vintages.

Riesling												
Leaf removal treatment	Soluble solids (°Brix)		Titratable acidity (g/L)		pH		Aldehydes[b] (µg/mL)		Terpenes[c] (µg/mL)		Acids[d] (µg/mL)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Control	19.8	21.9	10.37	6.91	2.95	3.24	5.51 a	1.51	1.96	0.52 b	0.40 ab	0.11
Pre-bloom	19.6	22.3	9.71	6.43	2.91	3.29	4.35 ab	1.19	2.15	1.05 a	0.53 a	0.22
Bloom	20.0	21.5	9.21	7.17	2.91	3.22	3.34 b	1.29	2.37	0.82 ab	0.30 ab	0.16
4 weeks post-bloom	19.6	20.9	9.19	7.09	2.88	3.09	4.18 ab	1.13	1.71	0.93ab	0.21 b	0.12
ANOVA *p-value*[a]	*0.74*	*0.10*	*0.43*	*0.31*	*0.90*	*0.10*	*0.05*	*0.47*	*0.67*	*0.03*	*0.04*	*0.12*
Sauvignon blanc												
	Soluble solids (°Brix)		Titratable acidity (g/L)		pH		Aldehydes[e] (µg/mL)		Terpenes[f] (µg/mL)		Acids[g] (µg/mL)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Control	21.6	21.8	7.49	6.89	3.65	3.33	2.40	0.56	0.67	0.02	0.71	0.02
Pre-bloom	21.9	21.9	7.16	6.45	3.57	3.33	2.99	0.55	1.02	0.09	0.72	0.05
Bloom	21.6	22.6	7.63	6.99	3.57	3.29	2.40	0.41	0.97	0.03	0.79	0.07
4 weeks post-bloom	21.4	21.0	6.83	6.74	3.56	3.27	1.26	0.50	1.09	0.10	0.93	0.02
ANOVA *p-value*[a]	*0.97*	*0.60*	*0.88*	*0.67*	*0.57*	*0.56*	*0.11*	*0.17*	*0.06*	*0.15*	*0.68*	*0.76*

[a]Values within a column not connected by the same letter(s) indicate significant differences between treatment means using Tukey's HSD at α=0.05.
[b]Aldehyde compounds consisted of: Hexanal and (E)-2-hexenal.
[c]Terpene compounds consisted of: Linalool oxide, Linalool, Nerol oxide, L-a-terpineol, trans-Geraniol, α-ionone, β-Damscenone.
[d]Acids consisted of: Octanoic acid, Hexanoic acid, Decanoic acid.
[e]Aldehyde compounds consisted of: Decanal, Nonanal, Hexanal, (E)-2-hexenal.
[f]Terpene compounds consisted of: Nerol oxide, L-a-terpineol, α-ionone, β-Damascenone.
[g]Acids consisted of: Hexanoic acid.

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Table 3 Skin and seed tannins and total phenolics as a result of leaf removal treatments in *Vitis vinifera* Riesling and Sauvignon blanc for the 2012 and 2013 vintages.

Riesling								
Leaf removal treatment	Seed tannins (mg/g)[b]		Seed total phenolics (mg/g)[b]		Skin tannins (mg/g)[b]		Skin total phenolics (mg/g)[b]	
	2012	2013	2012	2013	2012	2013	2012	2013
Control	3.67	4.19	6.17	7.02	0.24	0.18	0.34	0.34
Pre-bloom	3.57	4.18	5.20	6.24	0.23	0.19	0.36	0.38
Bloom	3.99	4.34	5.55	6.93	0.24	0.19	0.40	0.37
4 weeks post-bloom	3.57	3.75	4.94	6.11	0.23	0.30	0.39	0.56
ANOVA *p-value*[a]	*0.25*	*0.32*	*0.15*	*0.06*	*0.98*	*0.09*	*0.65*	*0.08*
Sauvignon blanc								
	Seed tannins (mg/g)[b]		Seed total phenolics (mg/g)[b]		Skin tannins (mg/g)[b]		Skin total phenolics (mg/g)[b]	
	2012	2013	2012	2013	2012	2013	2012	2013
Control	2.25	2.19 b	3.78 ab	4.38	0.28 b	0.22 b	0.39 c	0.37 b
Pre-bloom	2.18	2.27 ab	3.65 b	4.50	0.40 a	0.28 ab	0.55 ab	0.48 ab
Bloom	2.13	2.56 a	3.75 ab	4.76	0.35 ab	0.32 a	0.46 bc	0.57 a
4 weeks post-bloom	2.55	2.30 ab	4.48 a	4.49	0.44 a	0.26 b	0.61 a	0.45 ab
ANOVA *p-value*[a]	*0.15*	*0.05*	*0.03*	*0.52*	*0.001*	*0.005*	*0.001*	*0.008*

[a]Values within a column not connected by the same letter(s) indicate significant differences between treatment means using Tukey's HSD at α=0.05.
[b]As mg/g fresh berry weight.

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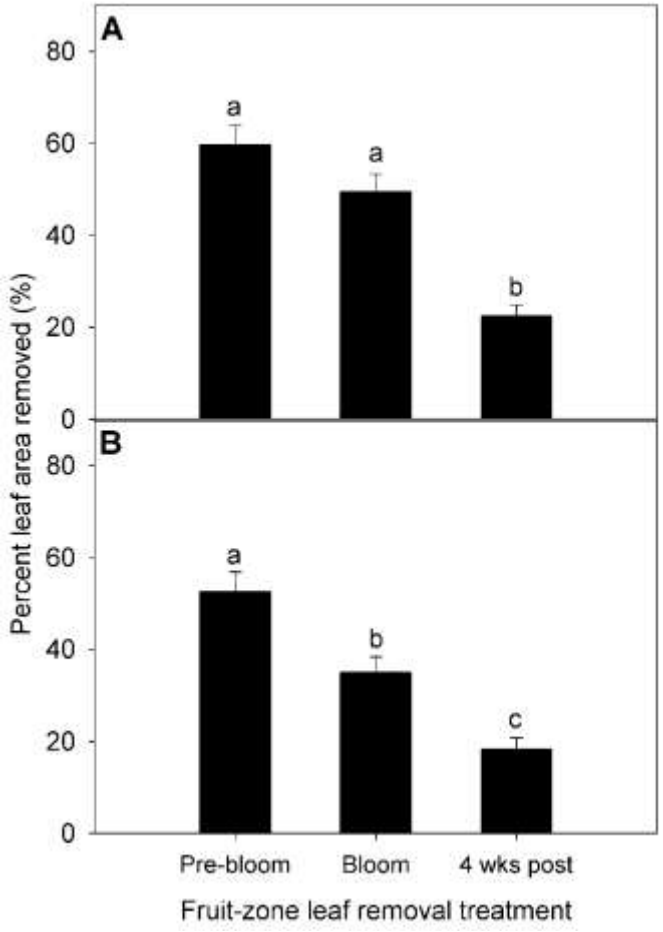

Figure 1 Total leaf area removed as a percent of the existing canopy at pre-bloom, bloom and 4 weeks post-bloom in 2013 for (**A**) *Vitis vinifera* Riesling and (**B**) Sauvignon blanc. Ten shoots per treatment were used to determine total leaf area removed. Letters denote significant differences between treatments within each year, using Tukey's HSD at $\alpha = 0.05$. Bars denote standard error of the mean.

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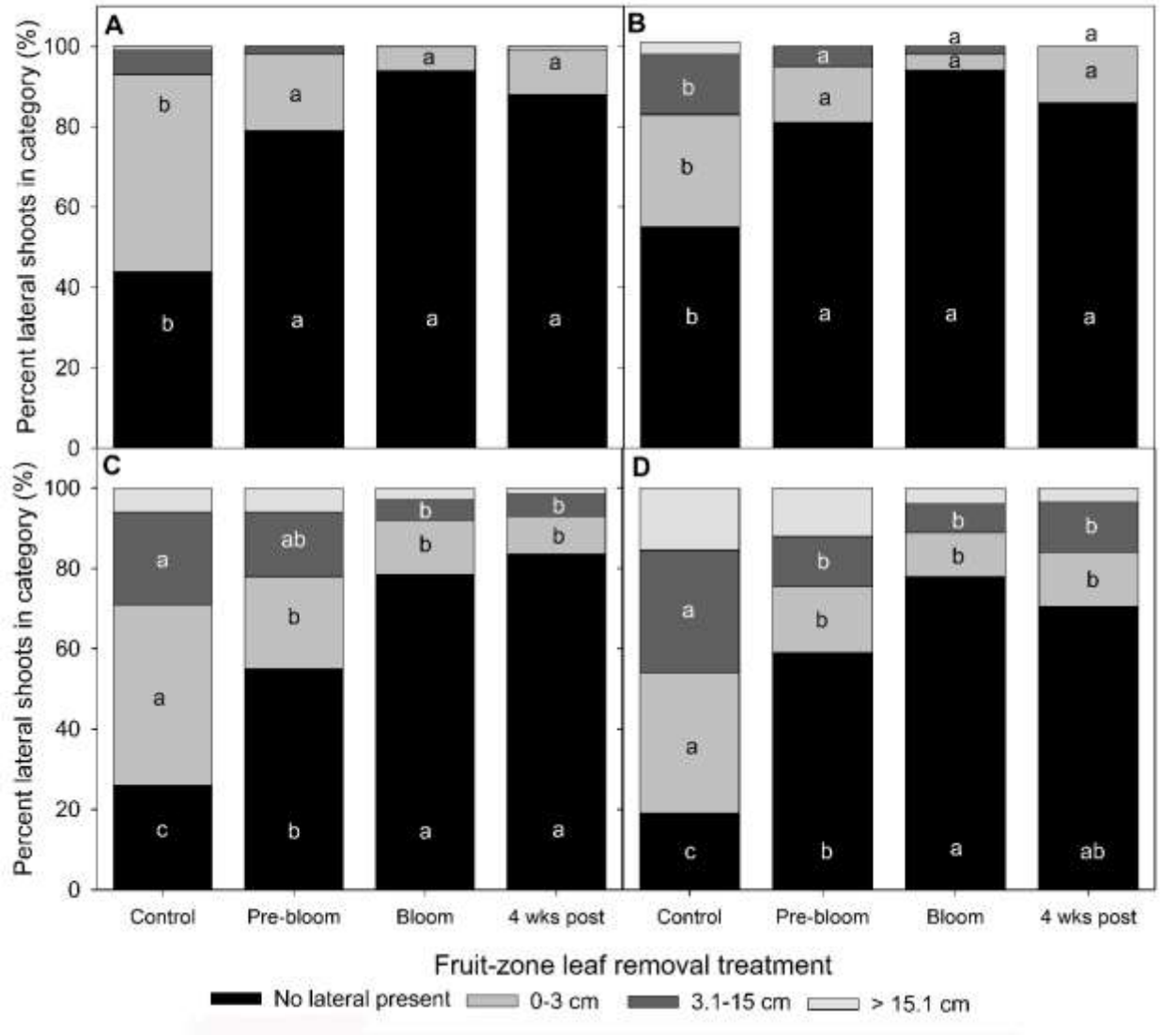

Figure 2 Lateral shoot development in the fruit-zone (first 4 to 5 nodes on count shoots) of *Vitis vinifera* 'Riesling' and 'Sauvignon blanc' undergoing different fruit-zone leaf removal treatments. In 2012, lateral shoot growth was assessed and categorized on 15 August in (**A**) Riesling and (**B**) Sauvignon blanc. In 2013, lateral shoot growth was categorized on (**C**) 10 September in Riesling and (**D**) 29 August in Sauvignon blanc. Categories for shoot development included: 1) no laterals present; 2) laterals between 0 and 3 cm; 3) laterals greater than 3 cm but at or less than 15 cm; 4) and laterals greater than 15 cm. Letters denote significant differences between treatments within each year, using Tukey's HSD at $\alpha = 0.05$.

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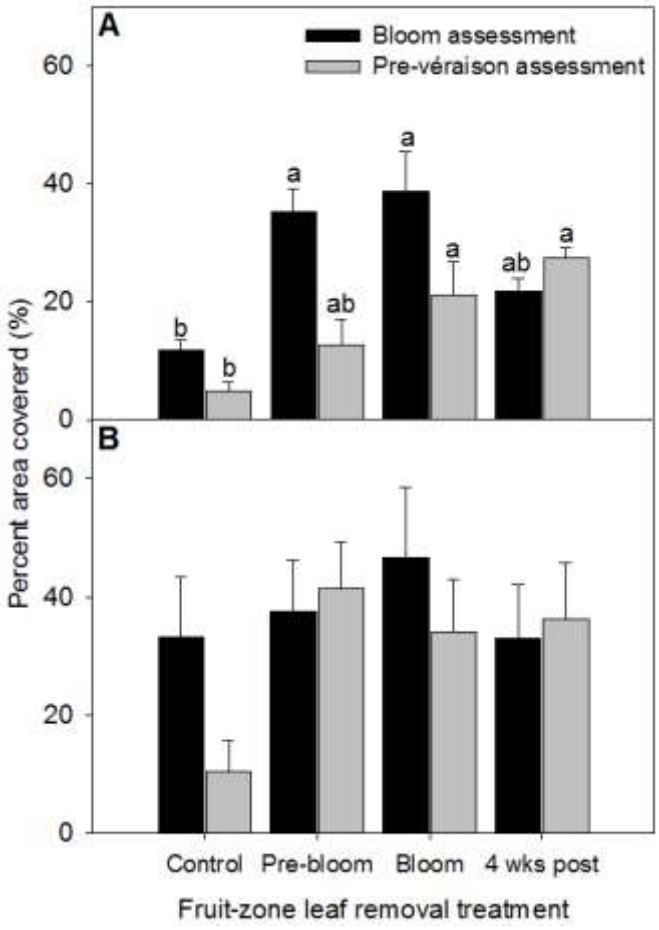

Figure 3 Spray coverage at bloom and pre-veraison between different fruit-zone leaf removal treatments in *Vitis vinifera* (**A**) Riesling and (**B**) Sauvignon blanc in 2013. Spray coverage near bloom in Riesling was assessed on 20 June; the pre-veraison spray coverage assessment was on 30 July. Spray coverage near bloom in Sauvignon blanc was on 20 June 2013; the pre-veraison spray coverage assessment was on 1 August 2013. At the time of the bloom assessment, only the pre-bloom and bloom leaf removal treatments had been implemented. Water sensitive cards were placed between basal and secondary clusters on count shoots just prior to spray application. Treatment means within an assessment date not connected by the same letter(s) denote significant differences using Tukey's HSD at $\alpha = 0.05$. Bars denote standard error of the mean.

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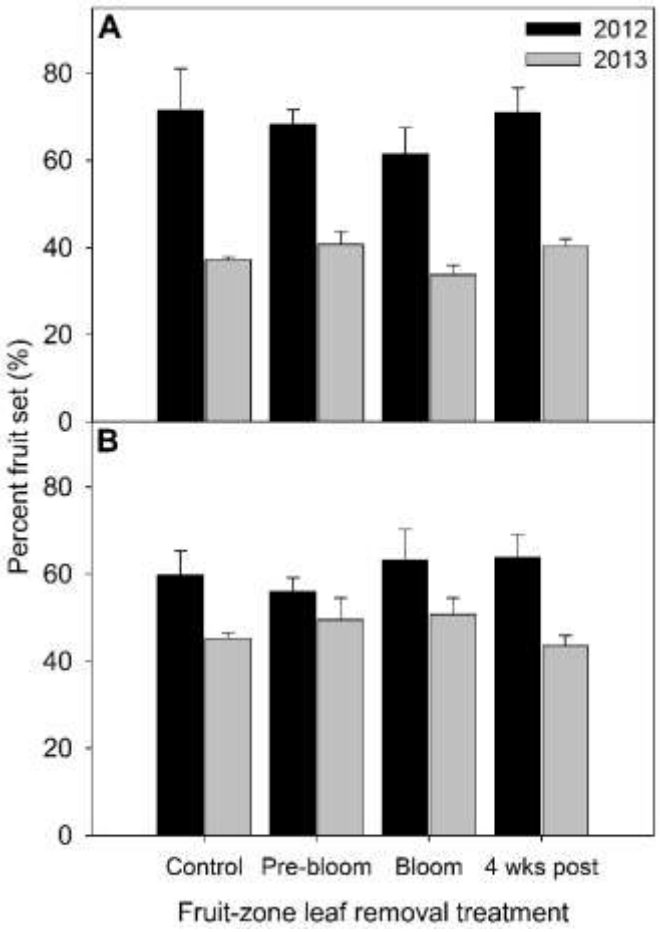

Figure 4 Fruit set, expressed as percent of total flowers (estimated through calyptras counts) setting to berries for the different fruit-zone leaf removal treatments in *Vitis vinifera* (**A**) Riesling and (**B**) Sauvignon blanc in 2012 and 2013. Bars denote standard error of the mean. No significant differences were seen between treatments in both varieties and both years.

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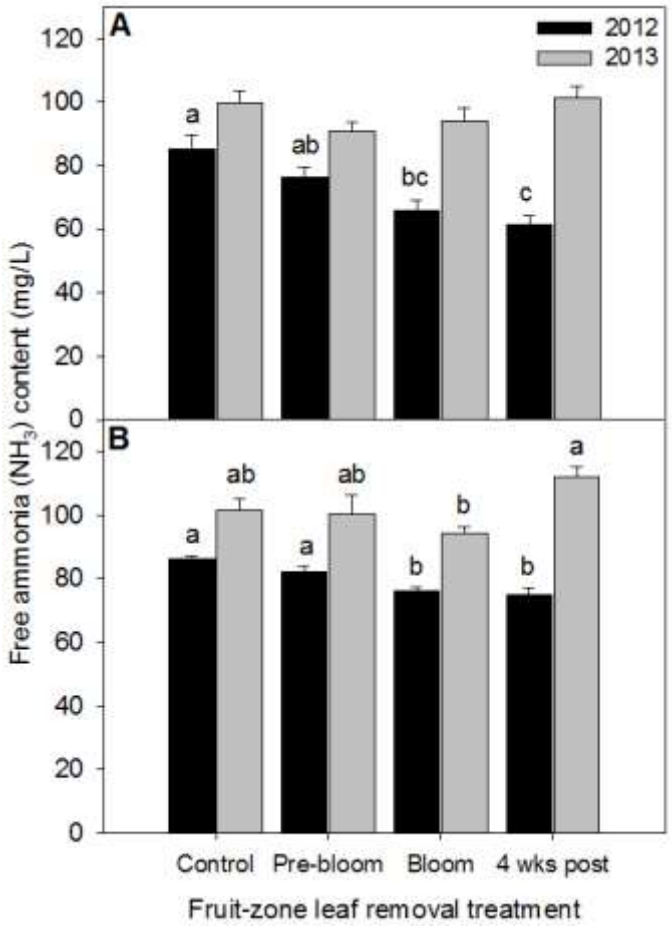

Figure 5 Free ammonia (NH_3) content in juice from *Vitis vinifera* (**A**) Riesling and (**B**) Sauvignon blanc subjected to different fruit-zone leaf removal treatments in 2012 and 2013. Treatment means within a year and variety not connected by the same letter(s) denote significant differences using Tukey's HSD at $\alpha = 0.05$. Bars denote standard error of the mean.