Early Leaf Removal as a Strategy to Improve Ripening and Lower Cluster Rot in Cool Climate (*Vitis vinifera* L.) Pinot Grigio

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Abstract: Removal of basal leaves early in the vegetative and reproductive development of grapevines is a tool used to decrease fruit set, lower cluster rot severity, and improve fruit quality. However, the considerable time required for implementation limits its use by grapegrowers. Efficient mechanization can potentially mitigate these issues, albeit this practice has not yet been compared to manual application at prebloom and after-bloom stages in a cool and humid growing region where cluster rot is the major limitation for yield and fruit quality. The goal of this study was to compare mechanical leaf removal (ME) with the manual (MA) removal of six leaves at the prebloom (PB) and after-bloom (AB) phenological stages over two seasons in Pinot Grigio (a tight-clustered cultivar). Fruit set was only decreased in 2017 by MA of six basal leaves at PB (PB-MA); however, PB reduced cluster compactness in each season. The loss of fruit to gray mold was lowered by all leaf removal treatments in the drier 2017 season, but only MA treatments mitigated loss from sour rot in that year. This indicates that a clear fruit zone and reduced cluster compactness are both needed to lower the effect of cluster rot disease. Only PB treatments enhanced fruit quality, likely driven by a similar reduction in cluster compactness. The results suggest that ME at PB may be used to decrease fruit loss to gray mold in dry seasons and enhance fruit Brix. Nevertheless, PB-MA can be an effective means to reduce fruit loss to sour rot in drier seasons and enhance ripening in years with high precipitation during veraison. This information provides a single approach to alleviate two prominent issues facing seasonal management strategies in cool climate viticulture.

Key words: *Botrytis cinerea*, canopy management, defoliation, mechanization, sour rot

In cool climates, viticulture inputs differ among cultivars based on growing degree day (GDD) requirements, vine vigor, fruitfulness, risk of disease, and seasonal weather variability (Frioni et al. 2017). Additionally, cluster morphology can dictate the approach to managing a specific cultivar or clone (Poni et al. 2018). The cultivars most important to cool climate regions are typically tight-clustered (e.g., Pinot noir, Riesling, Gewürztraminer, Sauvignon blanc), making them more at risk to cluster rot diseases such as gray mold (*Botrytis cinerea*) or sour rot, especially in seasons with high precipitation and humidity during veraison and fruit ripening (English et al. 1989). Yeast and bacteria involved in the sour rot complex convert glucose and fructose to acetic acid and other metabolites, including glycerol, ethyl acetate, ethanol, acetaldehyde, and galacturonic and gluconic acids (Zoecklein et al. 1995), rendering fruit with high incidence of these as unfit for processing and fermentation. Because of this, incidence of rot in clusters is more likely to be a determinant for harvest date than soluble solids or other fruit quality parameters, further complicating the winemaking process because of lack of desirable metabolite accumulation in fruit (Mosetti et al. 2016). Additionally, sugar accumulation has also been shown to be less homogeneous among berries in compact clusters compared with loose ones (Grimplet et al. 2017), leading to inadequate fruit quality at harvest, particularly in seasons with high fruit set or low seasonal GDDs.

Early leaf removal is a viticulture management practice involving the removal of leaves from selected basal nodes along shoots around the time of bloom, and it has been extensively investigated as a means to simultaneously decrease fruit set, control yield per vine, and reduce cluster rot (Poni et al. 2018). Studies have shown that the percentage of leaves removed (Acimovic et al. 2016) as well as the specific timing of defoliation (Poni et al. 2006) effect fruit quality and cluster rot incidence. When performed at prebloom (PB) (E-L 17;

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Coombe 1995), shoot photosynthesis and carbon allocation to cluster sinks are reduced (Frioni et al. 2018), decreasing the flow of hexoses to inflorescences (Lebon et al. 2008, Vasconcelos et al. 2009), which reduces flower fertility and amplifies the cultivar-specific sensitivity to flower abscission. As a result, fewer berries develop on the cluster, creating a looser cluster morphology (Sabbatini and Howell 2010). This effect, along with improved air flow around clusters (English et al. 1989, Molitor et al. 2011), leads to a reduction in cluster rot for a number of tight-clustered cultivars, including Sauvignon blanc (Mosetti et al. 2016), Pinot noir (Sternad Lemut et al. 2015, Acimovic et al. 2016), Pinot gris (Molitor et al. 2011), Sangiovese (Palliotti et al. 2011, Pastore et al. 2013), Riesling (Molitor et al. 2011), and Vignoles (Sabbatini and Howell 2010). Despite its effectiveness, manual application of leaf removal performed early in the season is expensive and reliant on a diminishing pool of available labor. For this reason, mechanization of leaf removal has become an important option for growers as a means to save time and costs (Hed and Centinari 2018). Mechanical leaf removal could lead to more consistent results because of the short PB phenological stage that spans only five to seven days (Keller 2010). Additionally, Vierra (2005) reported that growers in California spend up to \$260/ha for manual leaf removal, while mechanical leaf removal costs ~\$50/ha. There are two primary mechanisms for removing leaves: suction to draw leaves into a gird-partitioned rotating blade that cuts the leaves off (Gubler et al. 1991, Percival and Fisher 1994, Intrieri et al. 2008, Kemp et al. 2011), and high-pressure pulsed air directed to the fruit zone to shred leaves (Gubler et. al 1991, Diago et al. 2010, Tardaguila et al. 2012). Machines using the latter mechanism are most suitable for vertical shoot-positioned (VSP) trellis systems (Hed and Centinari 2018).

The effects of early mechanical leaf removal on yield reduction and fruit quality have previously been examined in Sangiovese (Intrieri et al. 2008), Graciano and Carignan (Tardaguila et al. 2010), and Tempranillo (Tardaguila et al. 2012). Additionally, mechanical leaf removal at berry pea-size and veraison (Hed et al. 2015) and at trace-bloom and berry pea-size (Hed and Centinari 2018) were studied previously. However, the effects of early mechanical compared with early manual leaf removal methods under cool, high-rainfall conditions have not been researched. The objective of this research was to determine the interactive effects of two methods of early leaf removal (manual and mechanical) and two application times (PB and after-bloom during fruit set) on fruit quality parameters and bunch rot pressure in Pinot Grigio (*Vitis vinifera* L.), a cultivar characterized by an extremely compact cluster morphology.

Materials and Methods

Vineyard site and plant material. The experiment was conducted in a commercial vineyard in Michigan (41°96′N; 86°44′W) on 15-yr-old grapevines cv. Pinot Grigio (PG), clone 152 grafted on 3309 rootstock, during two consecutive growing seasons (2016 and 2017). Vines were planted on a Spinks loamy fine soil (U.S. Department of Agriculture, Soil Conservation Service, 1957), with vine × row spacing of 2.33 × 3 m. Vines were trained to a bilateral VSP training system and cane-pruned during the two winters, leaving ~40 buds/vine. Crop level was adjusted four weeks after flowering to ~44 clusters/vine in both years through the removal of tertiary clusters and shoots without clusters, leaving fruit equally distributed between apical and basal clusters. No fungicide or pesticide applications were applied during bloom to avoid potential mechanical and chemical damage to flowers. Recommended crop protection practices were based on scouting experience and weather conditions. Chemicals were rotated to avoid resistance following the Integrated Pest Management program by Michigan State University (MSU) (Wise et al. 2003). Weather conditions, including temperature and precipitation, were recorded during the experiment by an automated weather station in Berrien Springs, MI, from the Michigan Automated Weather Network, located 6.0 km from the experimental vineyard. GDDs were calculated with the Baskerville-Emin method using a base temperature of 10°C (Baskerville and Emin 1969). No irrigation was used, and standard summer vineyard practices were applied, including mechanical hedging on 15 July 2016 and 14 July 2017.

Experimental design. Each year, the experiment was conducted in five rows of vines where treatments were applied to 10-vine plots in a randomized block design with three blocks. The five treatments were: a standard industry practice (control) consisting of manual leaf thinning in the cluster zone at veraison (three to four basal leaves) and a factorial combination of leaf thinning timing (pre- and after-bloom) × method (manual and mechanical; MA and ME, respectively). More specifically, these last four treatments were: manual removal of six basal leaves at PB (PB-MA) and after-bloom (AB-MA), and mechanical leaf removal at PB (PB-ME) and after-bloom (AB-ME). Within each plot, three target vines were arbitrarily selected, and three shoots per vine were randomly selected for taking detailed measurements of shoot growth and leaf area removed by the defoliation treatments. Phenological stages were defined according to the modified Eichhorn and Lorenz (E-L) system (Coombe 1995). MA and ME leaf removal at PB (E-L 17) and AB (E-L 31) were respectively performed on the same dates (6 June 2016 and 27 June 2017) as GDD accumulation was similar in both years. ME leaf removal was performed using a pulsed-air technology (Collard) front-mounted, pneumatic remover, addressed at a zone consisting of six nodes (38 cm). Tractor velocity was 1.6 km/hr, and the remover was pulsing air at 0.8 bar from two nozzles, rotating at 1650 rpm. During the growing season, laterals growing at the defoliated nodes were manually removed. In both years, shoot positioning had been performed prior to the MA and ME leaf removal treatments.

Shoot-length and leaf-area measurements. On each target vine, three representative shoots were tagged, and their shoot length was measured weekly starting two weeks before bloom until the first hedging (28 days after-bloom [DAB] in 2016, and 25 DAB in 2017). Each week, 20 shoots were collected from nonexperimental vines adjacent to the experimental blocks and were returned to the MSU campus in a cooler for measurement of shoot length and leaf area using a leaf area meter (LI-3050AHS, Lambda Instruments Corporation). Regressions between leaf area (y) and shoot length (x), in 2016 ($y = 11.1*x + 358$), $R^2 = 0.92$), and in 2017 ($y = 11.3*x + 219$, $R^2 = 0.91$), were used to estimate total leaf area per shoot from shoot length measurements for control and MA treatments. These formulas were then applied to shoot length measurements from each respective week to estimate the total leaf area per shoot for control and MA treatments. The total leaf area removed by MA treatments was measured by placing the six leaves in a small plastic bag and returning them to the MSU campus in a cooler, where they were subsequently measured using the leaf area meter. Posttreatment application, i.e., leaf area per shoot retained after MA, was then estimated by subtracting the removed leaf area from the total area estimated before MA. Leaf area removed by ME treatment was estimated using 20 shoots (also subjected to ME treatments) collected from nonexperimental vines adjacent to the experimental blocks on each date of treatment application. A regression was created between shoot length and leaf area on ME shoots, and the resulting values were subtracted from values obtained by the abovementioned regression between shoot length and leaf area.

Fruit set, yield, and cluster morphology. The relationships between the actual number of florets and berries, and those counted in the photos taken of tagged inflorescences, were determined and used to estimate floret and berry numbers from photos of clusters taken on tagged shoots at PB (E-L 17) and AB (E-L 31). The clusters on the 20 randomly selected shoots were harvested after photographing, and using the procedure of Poni et al. (2006): 1) the actual number of florets (y) and the counted florets (x) in the photos: 2016: $y = 0.593*x + 23.3$, $R^2 = 0.91$; 2017: $y = 0.426*x + 36.1$, $R^2 = 0.87$; and 2) the actual number of berries (y) and counted berries (x) in the photos: 2016: $y = 0.533*x + 10.1$, $R^2 = 0.93$; 2017: $y = 0.412*x + 15.0$, $R^2 = 0.85$. These regressions were applied to estimate floret (E-L 17) and berry numbers (E-L 27) from field photos of clusters on tagged shoots nondestructively, and consequently, calculate the percentage of fruit set at harvest (E-L 35).

Yield components, cluster morphology, and rot infection. In the spring of 2016 and 2017 when inflorescences became visible (E-L 12), the number of shoots and inflorescences (to become clusters) per vine were counted, and the potential treatment carryover effects estimated through the calculation of bud fertility (cluster number per shoot). Yield and number of clusters per vine were determined at harvest (26 Sept 2016 and 18 Sept 2017) when berry juice soluble solids reached ~20 Brix. Tagged clusters were harvested and stored at -20°C until detailed measurements were made, including the rachis weight and length and the number and total weight of berries. Cluster compactness index was calculated as (number of berries/cluster)/(rachis length) according to Acimovic et al. (2016). Sour rot (SR) and gray mold (GM, *B. cinerea*) incidence and severity were measured. The number of berries infected with GM and SR per cluster were recorded separately and factored into "rot severity" (percentage of berries per cluster affected with GM or SR). A cluster was assessed as rot infected at harvest if the percent of infected berries was >5%, previously determined to be the threshold for decreasing wine quality (Ky et al. 2012). The number of clusters assessed as rot infected was used to estimate rot incidence per vine. Qualitative loss per cluster and per vine due to SR or GM were estimated at harvest by the following equation:

$$[\text{Quantitative loss from SR or GM}] = [\text{cluster weight (g) or vine yield (kg)} \times SR_i \text{ or } GM_i \times SR_s \text{ or } GM_s] \quad \text{Eq. 1}$$

where SR_i or GM_i is the percentage of clusters having >5% of berries with SR or GM symptoms, and SR_s or GM_s is the percentage of berries with SR or GM symptoms in affected clusters (i.e., 5% or more berries infected).

Basic fruit composition. Berries from tagged clusters were crushed within Ziploc (SC Johnson) bags at room temperature, and the free-run juice was collected into 100 mL beakers. Total soluble solids (TSS; Brix) was determined using a digital refractometer (ATA-3810 PAL-1, Pulse, Inc.), and pH was measured with a 370 Thermo Orion pH meter (Thermo Fisher Scientific, Inc.). Total acidity (TA) was analyzed using a Multi-T 2.2 digital titrator (Laboratory Synergy, Inc.) with 10 mL juice diluted with water to 100 mL and titrated with 0.1 M sodium hydroxide to pH 8.2 using an equation to yield the TA (g/L).

Statistical analysis. The effect of treatment, year, and treatment × year interaction was evaluated by a one- (treatment) or two-way (treatment, year) analysis of variance (ANOVA) using IBM SPSS software (SPSS, Inc.). If one or more assumptions for ANOVA were not met, a linear mixed model was utilized. Total leaf area and basic fruit quality were determined separately for the two years of the trial and were subjected to a mixed model repeated measures with treatment and time as factors using SAS statistical software 9.3 (SAS Institute, Inc.). When the treatment × time interaction was significant, means were separated by Tukey's honest significant difference test at $\alpha = 0.05$. Figures were created with Sigma Plot ver. 11.0 (Systat Software, Inc.). Disease incidence and severity data were arcsin transformed to improve variance homogeneity before ANOVA was conducted. The arcsin values were then used in a one-way ANOVA to test the effects of treatments.

Results

Climate differences between experimental years. Heat unit accumulation at development stages was similar between seasons, especially early in the season, but in 2016, 16% more GDD accumulated between pea-size berry and veraison and between veraison and harvest (Table 1). Harvest date was four days earlier when calculated from the days after budbreak in 2016 when compared to 2017 (Table 1). There was only 5% more total rainfall in 2016 than in 2017. However, in 2016 ~30% of the rain fell before budbreak, whereas in 2017 almost 50% fell during that period. Rainfall from the pea-size berry stage to veraison was approximately two times higher in 2017 than in 2016, whereas the period between veraison and time

Table 1 Dates of phenological stages expressed as calendar date, days after budbreak (DABB), associated growing degree days (GGD, base 10°C), and cumulative precipitation in 2016 and 2017.

Phenological stage	2016 Date	2016 DABB	2016 GDD	2016 Precipitation (mm)	2017 Date	2017 DABB	2017 GDD	2017 Precipitation (mm)
Budbreak	3 May	0	106	7.4	2 May	0	126	12.2
Bloom	11 June	39	384	10.8	11 June	40	381	14.4
Pea-size berry	27 June	55	576	12.4	27 June	56	562	15.5
Veraison	5 Aug	95	1052	15.1	1 Aug	93	971	21.5
Time of harvest	22 Sept	142	1663	25.0	25 Sept	146	1497	24.9

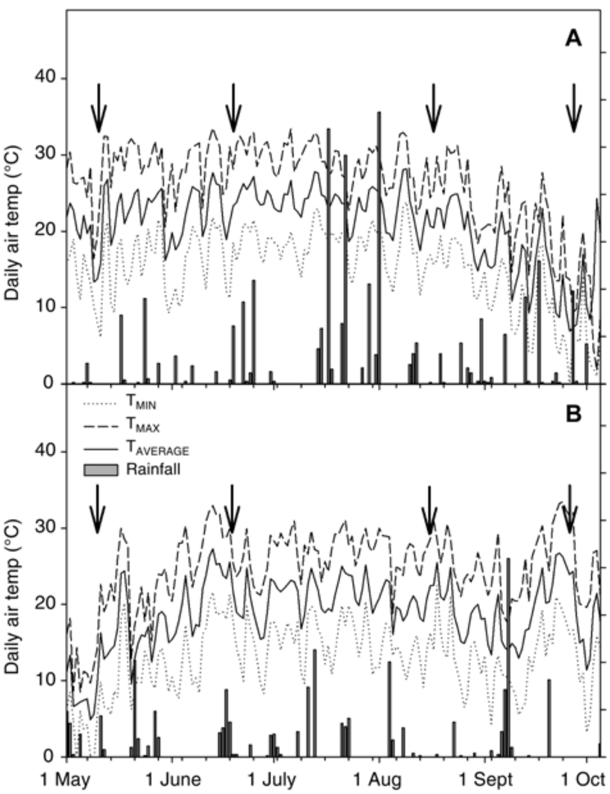

Figure 1 Ambient temperature and rainfall during the 2016 (**A**) and 2017 (**B**) growing seasons in southwest Michigan (Berrien Springs). Daily Minimum (T_{MIN}), Maximum (T_{MAX}), and Average Temperature ($T_{AVERAGE}$) are also reported. Arrows indicate budbreak, bloom, veraison, and harvest.

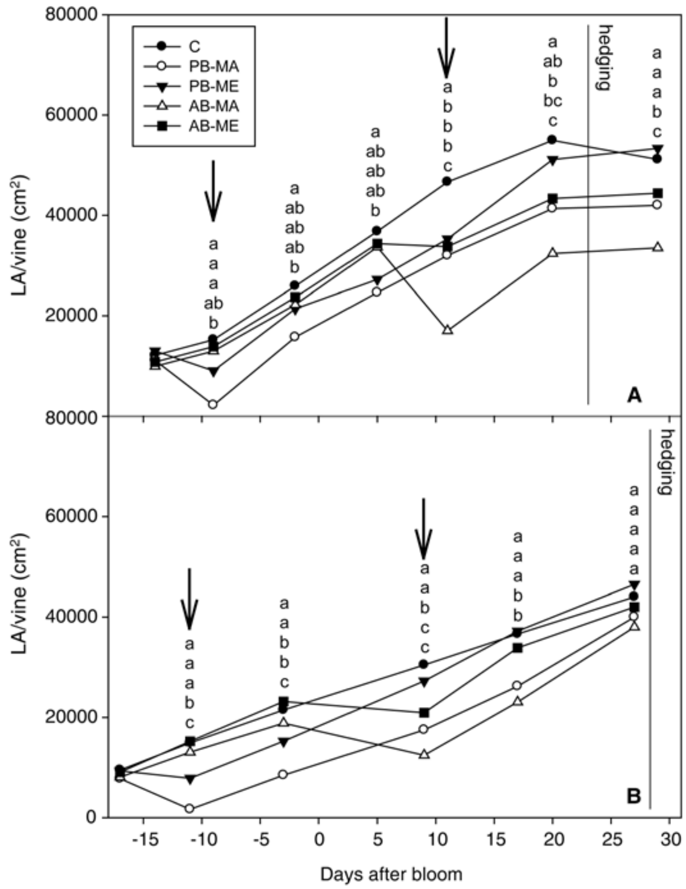

Figure 2 Effects of leaf removal treatments on vine leaf area (LA) development in 2016 (**A**) and 2017 (**B**) from approximately one week prior to E-L 17 (prebloom) to hedging. Arrows identify the dates when prebloom and after-bloom leaf removal treatments were applied. Data were analyzed by repeated measures linear mixed model, and when the differences were statistically significant, means were separated with Tukey's honest significant difference test ($p < 0.05$). Different letters identify significantly different means at each time point. C = control with cluster zone veraison leaf removal; PB-MA = prebloom manual removal of six basal leaves; PB-ME = prebloom mechanical removal of six basal leaves; AB-MA = after-bloom manual removal of six basal leaves; AB-ME = after-bloom mechanical removal of six basal leaves.

of harvest experienced a three-fold increase in precipitation in 2017 compared to 2016 (Figure 1).

Vine leaf area dynamics in response to defoliation. The growth of vine leaf area was different between seasons, with 2016 having more growth around bloom (Figure 2). However, measurements at harvest (averaged across treatments) were similar between years (2016: 44.9 m^2/vine; 2017: 42.1 m^2/vine). Removal of leaves, regardless of mode or timing, had a significant effect on the growth of vine leaf area. In each year, MA treatments at both times of application removed more leaf area than ME treatments (Table 2). The subsequent growth of leaf area by MA vines resulted in no difference between MA and ME treatments in either year at the time of hedging (Figure 2). Interestingly, the leaf area growth for the control group (C) is slightly lower also during this same period, although more prominently in 2016 than 2017.

Fruit set, cluster architecture, and yield components. Floret number determined before bloom was similar between the two experimental years (Table 3). Although the PB treatments had no effect on fruit set in 2016 and only in PB-MA in 2017, when compared with AB and C treatments, they produced less compact clusters in both years (Table 4). Additionally, cluster weight was reduced by PB-ME in 2016 and by both PB treatments in 2017. Clusters were also less compact in response to AB-ME in 2016 but not in 2017 (Table 4). Interestingly, cluster compactness was also reduced in response to AB-ME in 2016 but not in 2017. In this instance, neither fruit set nor cluster weight values were different from the C. Berry weight was affected by a year × treatment interaction, and it was higher in 2017 compared to 2016.

Yield, vine balance, and basic fruit quality parameters. Yield was not affected by vine balance in either year (Table 5). Vine balance, indexed as the ratio between leaf area measured at hedging and yield (LA/yield), was similar between years (Table 5). In the 2016 season, both PB treatments led to enhanced TSS accumulation in berries, with PB-ME having the highest TSS at harvest and being significantly greater than AB treatments (Table 5). Similarly, in 2017, PB-ME resulted in the highest TSS concentrations, while the AB-MA reported a reduction in TSS level compared to C, PB-MA, and AB-ME. Figure 3 reveals that the rate of TSS accumulation was relatively similar among treatments in 2016. PB-ME reported the highest TSS concentration as early as 57 days after bloom when TSS for the C was ~10. Despite PB-ME having the highest TSS from 60 days after bloom in 2017, it only exceeded that of the control during mid-ripening (66, 87, and 96 DAB).

Berry pH was not affected by treatments in 2016; however, pH was higher in response to both PB treatments and AB-ME than to C and AB-MA in 2017 (Table 5). In 2016, although pH was higher for PB-ME than for C during early and mid-ripening (68, 77, 82, and 89 DAB), at harvest it was only higher for PB-MA (Figure 3). In 2017, only pH in PB-ME was greater than C during mid-ripening and harvest (66 to 99 DAB); TA was lower for PB-ME than C. In 2017, TA was increased by all ME treatments compared with C (Table 5), however differences were observed during maturation (Figure 3). Early in

Table 3 Floret number and the effects of leaf removal treatments on fruit set in 2016 and 2017.

	Florets/cluster (E-L 17)[b]		Berries/ cluster		Fruit set (%)[c]	
Treatment[a]	2016	2017	2016	2017	2016	2017
C	284	258	149 a[d]	139 a	53	44 ab
PB-MA	253	250	107 bc	82 b	42	33 c
PB-ME	196	229	94 c	94 b	48	41 bc
AB-MA	274	248	139 a	125 a	51	47 a
AB-ME	250	248	125 ab	129 a	48	48 a
p value (trt)[e]	0.116	0.282	<0.001	<0.001	0.075	<0.001
p value (year)	0.773		<0.001		<0.001	
p value (trt*year)	0.663		0.082		0.436	

[a]C = control with cluster zone veraison leaf removal; PB-MA = prebloom manual removal of six basal leaves; PB-ME = prebloom mechanical removal of six basal leaves; AB-MA = after-bloom manual removal of six basal leaves; AB-ME = after-bloom mechanical removal of six basal leaves.
[b]E-L 17 = separated inflorescence.
[c]Calculated as berries per cluster (E-L 35)/florets per cluster (E-L 17).
[d]Different letters identify significantly different means within each column.
[e]Data were analyzed by two-way analysis of variance (ANOVA) with treatment as a fixed factor, and year as a random factor, and if one or more assumptions for ANOVA were not met, a linear mixed model was utilized. In both cases, if the differences were statistically significant, means were separated with Tukey's honest significant difference test ($p < 0.05$).

Table 2 Leaf area (LA) per vine and the amount removed by manual and mechanical treatments applied prebloom (E-L 17) and after-bloom (E-L 31) in 2016 and 2017.

	Phenological stage E-L 17[a]				Phenological stage E-L 27[a]			
	Treatment[b]				Treatment[b]			
2016	C	PB-MA	PB-ME	*Signif. F*[c]	C	AB-MA	AB-ME	*Signif. F*
Total LA/vine (m^2)	10.5	10.2	10.5	*0.802*	27.7	28.2	26.7	*0.511*
Removed LA/vine (m^2)	0 c[d]	8.68 a	4.80 b	*<0.001*	0 c	16.8 a	8.48 b	*<0.001*
Removed LA/vine (%)	0 c	85.0 a	45.7 b	*<0.001*	0 c	59.7 a	31.7 b	*<0.001*
	Phenological stage E-L 17				**Phenological stage E-L 27**			
	Treatment				**Treatment**			
2017	**C**	**PB-MA**	**PB-ME**	***Signif. F***	**C**	**AB-MA**	**AB-ME**	***Signif. F***
Total LA/vine (m^2)	13.7	14.8	14.9	*0.663*	24.6	29.3	29.2	*0.570*
Removed LA/vine (m^2)	0 c	13.2 a	6.57 b	*<0.001*	0 c	11.5 a	8.47 b	*<0.001*
Removed LA/vine (%)	0 c	89.1 a	44.0 b	*<0.001*	0 c	39.3 a	29.0 b	*<0.001*

[a]E-L 17 = separated inflorescence; E-L 27 = fruit set (>2 mm diameter).
[b]C = control with cluster zone veraison leaf removal; PB-MA = prebloom manual removal of six basal leaves; PB-ME = prebloom mechanical removal of six basal leaves; AB-MA = after-bloom manual removal of six basal leaves; AB-ME = after-bloom mechanical removal of six basal leaves.
[c]Data were analyzed by one-way analysis of variance (ANOVA) with treatment as a fixed factor, and if one or more assumptions for ANOVA were not met, a linear mixed model was utilized. In both cases, if the differences were statistically significant, means were separated with Tukey's honest significant difference test ($p < 0.05$).
[d]Different letters identify significantly different means within each sectioned row.

ripening in 2016 (57 and 62 DAB), PB-ME was significantly lower than C, while both ME treatments had values higher than the control (60 and 66 DAB) in 2017.

Cluster rot. SR differed between years, with incidence, severity, as well as loss per cluster and vine being higher in 2016 than 2017, with the exception of SR severity in 2016 and GM incidence in 2017 (Tables 6 and 7). In 2017, GM severity was reduced only by PB-MA compared to C, however, loss per cluster and vine from GM were significantly mitigated by all leaf removal treatments (Tables 6 and 7). SR incidence was reduced by MA in 2017, which translated to a decrease of loss due to rot on a cluster and vine basis. SR severity was only affected by PB-MA in 2016, while in 2017, all leaf removal treatments led to a decrease (Tables 6 and 7) compared with C.

Bud fertility. Shoot and cluster number per vine determined in the spring following the two years that treatments were applied were not affected by the treatments, indicating that bud fertility was not affected by the defoliation treatments. Additionally, floret number per cluster can provide information relating to changes in fertility between years. Here, these values were identical for both the 2016 and 2017 seasons (Table 3).

Discussion

PB-ME enhanced TSS accumulation. Timing and mode of leaf thinning greatly affected the pattern of TSS accumulation. In our experiment, PB-ME reported higher TSS levels than AB and C for both years (Table 5). Tardaguila et al.

Table 4 Cluster morphology parameters before sorting of healthy clusters in response to different leaf removal treatments in 2016 and 2017. CCI, cluster compactness index.

	Cluster wt (g)		Berry wt (g)		Rachis length (cm)		Wing length (cm)		CCI (berry # / cm)[a]	
Treatment[b]	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
C	153 a[c]	156 a	0.927 b	1.35 ab	9.35 ab	8.42	5.69	4.20	16.2 a	13.6 a
PB-MA	134 ab	101 c	1.26 a	1.21 b	8.96 ab	8.49	4.62	3.80	12.1 cd	9.67 b
PB-ME	113 b	120 bc	1.21 ab	1.46 a	8.56 b	7.59	4.46	3.73	10.9 d	11.0 b
AB-MA	139 ab	139 ab	0.947 b	1.15 b	9.65 a	7.99	5.49	4.32	14.5 ab	15.0 a
AB-ME	130 ab	154 a	1.06 ab	1.32 ab	9.40 ab	8.42	4.58	3.90	13.4 bc	13.8 a
p value (trt)[d]	0.061	0.001	0.013	0.004	0.015	0.382	0.049	0.546	<0.001	<0.001
p value (year)	0.032		<0.001		<0.001		<0.001		0.322	
p value (trt*year)e	0.210		0.007		0.379		0.641		<0.001	

[a]Calculated as: (berry number per cluster/rachis length); wing length not factored into equation.
[b]C = control with cluster zone veraison leaf removal; PB-MA = prebloom manual removal of six basal leaves; PB-ME = prebloom mechanical removal of six basal leaves; AB-MA = after-bloom manual removal of six basal leaves; AB-ME = after-bloom mechanical removal of six basal leaves.
[c]Different letters identify significantly different means within each column.
[d]Data were analyzed by two-way analysis of variance (ANOVA) with treatment as a fixed factor and year as a random factor, and if one or more assumptions for ANOVA were not met, a linear mixed model was utilized. In both cases, if the differences were statistically significant, means were separated with Tukey's honest significant difference test ($p < 0.05$).

Table 5 Commercially acceptable yield (<5% rot), vine balance, and grape composition at harvest in response to different leaf removal treatments in 2016 and 2017. TSS, total soluble solids; LA, leaf area.

	Yield (kg/vine)		LA/yield (m^2/kg)		TSS (Brix)		pH		Titratable acidity (g/L)	
Treatment[a]	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
C	6.17	5.56	8.35	9.17	18.0 c[b]	22.1 b	3.61	3.60 b	5.37	5.94
PB-MA	4.32	5.88	9.73	8.00	20.7 ab	21.5 b	3.87	3.70 a	5.26	5.30
PB-ME	4.60	4.86	11.5	11.3	22.0 a	22.8 a	3.86	3.73 a	5.62	5.83
AB-MA	5.39	4.73	9.37	7.92	18.2 c	19.6 c	3.64	3.59 b	5.42	5.31
AB-ME	4.03	5.47	11.7	8.15	19.6 bc	21.2 b	3.76	3.70 a	5.48	5.94
p value (trt)[c]	0.182	0.467	0.556	0.672	0.003	0.035	0.079	0.043	0.987	0.353
p value (year)	0.453		0.174		0.170		0.773		0.138	
p value (trt*year)	0.170		0.773		0.453		0.174		0.898	

[a]C = control with cluster zone veraison leaf removal; PB-MA = prebloom manual removal of six basal leaves; PB-ME = prebloom mechanical removal of six basal leaves; AB-MA = after-bloom manual removal of six basal leaves; AB-ME = after-bloom mechanical removal of six basal leaves.
[b]Different letters identify significantly different means within each column.
[c]Data were analyzed by two-way analysis of variance (ANOVA) with treatment as a fixed factor and year as a random factor, and if one or more assumptions for ANOVA were not met, a linear mixed model was utilized. In both cases, if the differences were statistically significant, means were separated with Tukey's honest significant difference test ($p < 0.05$).

(2010) found no difference in soluble solids in response to both MA and ME treatments in Carignan for two consecutive years and only in the first year for Graciano grapevines. However, the PB-ME treatment induced a higher soluble solids concentration in the second year in Graciano. Additionally, Intrieri et al. (2008) reported an increase of soluble solids in both PB-MA and PB-ME treatments on Sangiovese, similar to our results. A possible explanation for this is that both Pinot Grigio and Sangiovese are tight-clustered cultivars. It has previously been shown that among clones of the same cultivar, those that were less compact reported more synchronous ripening, improving soluble solids concentration at harvest (Grimplet et al. 2017). A less compact cluster leads to higher light penetration and more uniform temperature within clusters, which is linked to enhanced ripening (Pieri et al. 2016). In addition, the nature of ME treatment application leaves behind fragments of leaves at most nodes and could provide a proximal source for photosynthates capable of instigating ripening sooner than MA treatments (Motomura 1990, VanderWeide et al. 2018).

Precipitation during bunch closure dictated rot form. The presence of GM and SR in clusters reflected patterns of precipitation between both experimental years. The 2016 growing season was warmer and had higher precipitation near the time of bunch closure (late-July to early-August), likely causing higher humidity around clusters, favoring SR development

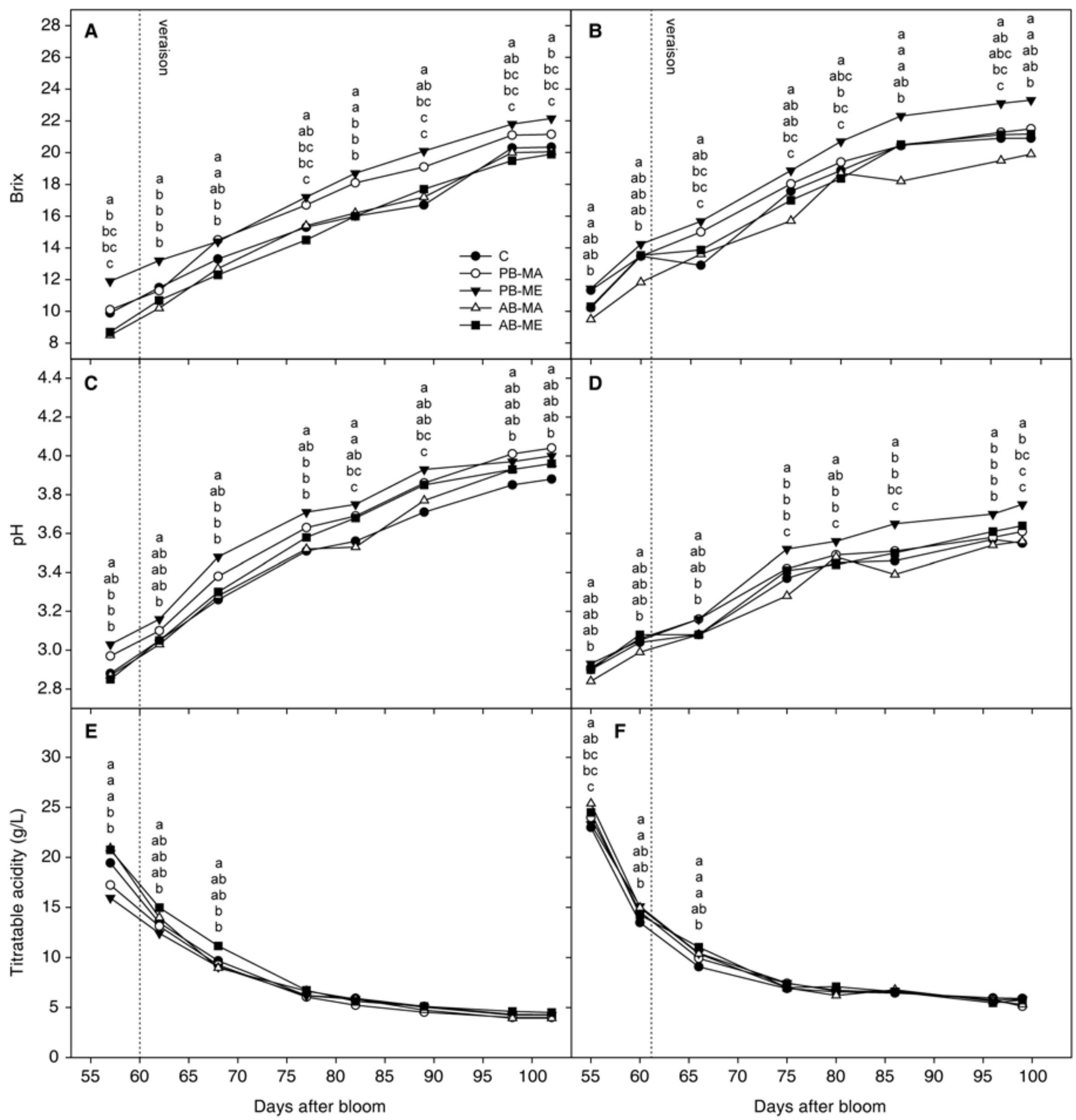

Figure 3 Seasonal evolution of Brix in (**A**) 2016 and (**B**) 2017, pH in (**C**) 2016 and (**D**) 2017, and titratable acidity in (**E**) 2016 and (**F**) 2017 in Pinot Grigio from veraison to harvest. Data were analyzed by repeated measures linear mixed model, and when the differences were statistically significant, means were separated with Tukey's honest significant difference test ($p < 0.05$). Different letters identify significantly different means at each time point. C = control with cluster zone veraison leaf removal; PB-MA = prebloom manual removal of six basal leaves; PB-ME = prebloom mechanical removal of six basal leaves; AB-MA = after-bloom manual removal of six basal leaves; AB-ME = after-bloom mechanical removal of six basal leaves.

(Sternad Lemut et al. 2015). In 2017, which was cooler than 2016, precipitation was lower, especially during bunch closure. As a result, cluster rot microflora led to a higher incidence of GM instead of SR, often reported in dry-cool climates (Molitor et al. 2011). Warmer temperatures favor SR over GM, which thrives better under lower temperatures (Hausinger et al. 2015), potentially explaining the differences found between the warm (2016) and cool (2017) growing seasons.

Cluster rot prevention involves both cluster compactness and fruit zone porosity. Despite the lack of consistent treatment effects on rot incidence and severity in 2016, PB-MA, as compared with C, reduced loss due to bunch rot by 20% per cluster and 26% per vine. Early leaf removal limits assimilate availability for flower fertilization, decreasing fruit set, and in turn reduce cluster compactness (Frioni et al. 2018). Reduced cluster compactness allows rainfall to drip freely through clusters and evaporate from berry surface (Percival and Fisher 1994), increasing the overall evaporative potential of the fruit zone (Dokoozlian and Hirschfelt 1995). While all leaf removal treatments led to lower GM in 2017 than did C, only MA treatments reduced SR per cluster, meaning that reduction of GM relies more on an open canopy than reduced cluster compactness (Molitor et al. 2011). With this in mind, the presence of both GM and SR appears to be related to two factors, cluster compactness and fruit-zone porosity. MA treatments were the most effective at reducing rot in the dry growing season because of their effects on cluster compactness and the complete removal of leaf fragments

Table 6 Incidence and severity of gray mold (*Botrytis cinerea*) and sour rot in response to different leaf removal treatments in 2016 and 2017.

	Gray mold incidence (%)[a]		Gray mold severity (%)[b]		Sour rot incidence (%)[a]		Sour rot severity (%)[b]	
Treatment[c]	2016	2017	2016	2017	2016	2017	2016	2017
C	0.00	39.1	0.667	2.82 a[d]	61.7	55.7 a	23.0 a	8.11 a
PB-MA	6.67	2.5	1.00	0.00 b	54.0	0.00 b	8.67 b	0.203 b
PB-ME	15.3	25.9	2.67	1.39 ab	65.0	25.7 ab	19.7 ab	1.78 b
AB-MA	8.33	11.1	0.33	1.61 ab	70.0	11.0 b	20.7 ab	1.56 b
AB-ME	11.0	22.2	2.33	1.24 ab	64.3	29.3 ab	20.0 ab	3.30 b
p value (trt)[e]	0.279	0.081	0.521	0.073	0.955	0.003	0.056	0.001
p value (year)	0.022		0.983		0.017		<0.001	
p value (trt*year)	0.035		0.227		0.214		0.185	

[a]Percentage of clusters with at least 5% of infected berries.
[b]Percentage of infected berries per cluster.
[c]C = control with cluster zone veraison leaf removal; PB-MA = prebloom manual removal of six basal leaves; PB-ME = prebloom mechanical removal of six basal leaves; AB-MA = after-bloom manual removal of six basal leaves; AB-ME = after-bloom mechanical removal of six basal leaves.
[d]Different letters identify significantly different means within each column.
[e]Data were analyzed by two-way analysis of variance (ANOVA) with treatment as a fixed factor and year as a random factor, and if one or more assumptions for ANOVA were not met, a linear mixed model was utilized. In both cases, if the differences were statistically significant, means were separated with Tukey's honest significant difference test ($p < 0.05$).

Table 7 Quantitative measure of fruit loss due to gray mold (*Botrytis cinerea*) and sour rot in response to different leaf removal treatments in 2016 and 2017.

	Quantitative loss from gray mold (g/cluster)[a]		Quantitative loss from gray mold (kg/vine)[a]		Quantitative loss from sour rot (g/cluster)[a]		Quantitative loss from sour rot (kg/vine)[a]	
Treatment[b]	2016	2017	2016	2017	2016	2017	2016	2017
C	0.000	8.67 a[c]	0.000	0.463 a	16.4	6.87 a	0.817	0.373 a
PB-MA	0.803	0.00 b	0.037	0.000 b	12.3	0.00 b	0.570	0.000 b
PB-ME	2.26	1.42 b	0.104	0.075 b	17.6	3.72 ab	0.751	0.200 ab
AB-MA	1.56	1.85 b	0.076	0.110 b	22.0	0.78 b	1.08	0.050 b
AB-ME	2.71	2.99 b	0.098	0.167 b	24.0	3.48 ab	0.887	0.197 ab
p value (trt)[d]	0.568	0.001	0.564	0.002	0.626	0.014	0.694	0.023
p value (year)	0.056		0.025		<0.001		<0.001	
p value (trt*year)	0.143		0.139		0.545		0.558	

[a]Quantitative loss = rot incidence × rot severity of rotten clusters.
[b]C = veraison leaf removal; PB-MA = prebloom manual leaf removal; PB-ME = prebloom mechanical leaf removal; AB-MA = after-bloom manual leaf removal; AB-ME = after-bloom mechanical leaf removal.
[c]Different letters identify significantly different means within each column.
[d]Data were analyzed by two-way analysis of variance (ANOVA) with treatment as a fixed factor and year as a random factor, and if one or more assumptions for ANOVA were not met, a linear mixed model was utilized. In both cases, if the differences were statistically significant, means were separated with Tukey's honest significant difference test ($p < 0.05$).

by the MA treatment. This is similar to previous studies on early manual leaf removal in Chardonnay (Hed et al. 2015), Sauvignon blanc (Komm and Moyer 2015), and Pinot noir (Acimovic et al. 2016). Growers could take advantage of the reduced cluster compactness with PB-ME treatments and operate additional MA or ME passes after heavy precipitation events to further remove leaves around the cluster zone, thus reducing the potential for SR while reducing chemical inputs.

Mitigation of SR, but not GM, improved fruit quality. It is likely that the presence of either SR or GM in fruit had an influence on fruit TSS concentration. Quantitative loss from SR (kg/vine) was 30% and 8% lower for PB-MA and PB-ME, respectively, than the C and AB treatments in 2016, in part because of decreased compactness (Table 4). Coincidently, TSS was significantly higher in PB-MA and PB-ME in 2016, whereas no difference existed in AB treatments. Yeast and bacteria such as Gluconobacter and Acetobacter involved in the SR complex convert glucose and fructose into acetic acid and other metabolites including glycerol, ethyl acetate, ethanol, acetaldehyde, and galacturonic and gluconic acids, lowering berry TSS in compact clusters and compromising fruit quality for fermentation (Zoecklein et al. 1995). This implies that the presence of SR may have limited sugar concentrations in the fruit of compact clusters. Interestingly, GM incidence was strongly correlated with TSS ($R^2 = 0.553$, $p < 0.01$) and TA ($R^2 = 0.396$, $p < 0.01$), and GM severity was correlated with TSS ($R^2 = 0.513$, $p < 0.01$) in 2016, implying that drier conditions around clusters of PB-treated vines increased GM rather than SR. In 2017, dry weather favored the formation of GM (Table 5). In contrast to SR, GM concentrates hexoses in berries, which suggests that GM may have raised TSS in the treatments with more compact clusters. In 2017, when GM was more prevalent than SR, all leaf removal treatments led to a reduction in GM loss per cluster and vine. As a result, TSS was poorly correlated with GM incidence ($R^2 = 0.083$) and severity ($R^2 = 0.074$). GM and SR were partially mitigated by leaf removal treatments. This was not associated with a yield reduction (Table 5), which is consistent with previous studies (VanderWeide et al. 2018) but not others (Intrieri et al. 2008, Tardaguila et al. 2010). In the wetter year, 2016, percentages of infected fruit were above the threshold (5%) for reduction of wine quality (Ky et al. 2012) in all treatments, when calculated on a whole vine basis. In contrast, only the C treatment resulted in infection above the threshold (8%), while all the other treatments were efficient in maintaining the percentage of fruit infected at 2% or below. This displays the potential of PB-MA to improve wine quality, especially in drier years.

Early leaf removal did not affect bud fertility after two years. Results from this experiment indicated that leaf removal treatments did not affect bud fertility after two consecutive years of implementation. Similarly, no effect was reported in Sangiovese after three years of leaf removal at PB (Palliotti et al. 2011) or in Riesling after removal of leaves manually or mechanically for two seasons at AB (Percival and Fisher 1994). While the data presented here is consistent with previous studies measuring this parameter, the potential effects of long-term (2+ years) utilization of early leaf removal cannot be fully repudiated. In hybrid cultivars, Sabbatini and Howell (2010) found that fruitfulness was severely decreased after removing four to six main and lateral leaves at the PB stage. Likewise, Silvestroni et al. (2019) also found a long-term effect of PB manual leaf removal after four seasons. Differences in cultivars, viticulture practices, and vine physiological characteristics among studies may explain the differences in their results and those reported here. Many of the studies reporting no carryover effects were conducted with cane-pruned vines. Following cold events, damaged cordons are renewed, which could replace canes having some levels of carbohydrate depletion with healthy ones. In addition, the yield per vine in control vines reporting a carryover effect was very high (Sabbatini and Howell 2010), due to large cluster size. If these large clusters are a strong sink for carbohydrates (because of their larger weight), root starch is typically allocated to aid in fruit growth, as this is the largest repository of stored carbohydrates (Rossouw et al. 2017). Larger clusters may require a longer growing season to reach maturity, leaving less time for woody tissues to store carbohydrates after harvest, which are vital resources for early vegetative and reproductive growth the following season.

Conclusions

There was 66% more precipitation between veraison and harvest in 2016 than in 2017, which favored the development of SR compared to GM in fruit. Loss of fruit to GM was not affected by treatments in 2016 but was mitigated by all leaf removal treatments in the drier 2017 season. PB-MA reduced SR severity in both seasons; however, the loss of fruit to SR was only significantly prevented by MA treatments in 2017. This implies that it is necessary to have both more open vine canopy and cluster to reduce bunch rot and suggests PB-MA as the best option for growers to mitigate SR. While PB-MA produced higher TSS compared to C in 2016, we report that only PB-ME led to increased TSS accumulation in 2016 and 2017. Given that the two seasons experienced vastly different precipitation from veraison to harvest and that treatments did not significantly modulate vine balance, this consistent enhancement of TSS may be attributed to enhanced light exposure to fruit or to the closer photosynthate source attributed to leaf fragments retained by the machine. In conclusion, PB manual leaf removal was the best strategy to mitigate fruit loss to bunch rot, while PB mechanical leaf removal was shown to be an important tool for addressing inadequate ripening for cultivars grown in cool climates.

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