2016 MGWIC RESEARCH REPORT

PROPOSAL TITLE: CONTROL AND MANAGEMENT OF SOUR ROT AND VOLATILE ACIDITY IN VINIFERA GRAPES GROWN IN MICHIGAN

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1. Use of the financial support from MGWIC

The funding requested of the MGWIC supported (25%) of technical support costs, 20 trips at 280 miles each (at MSU rate of $ 0.59 per mile) roundtrip from campus to the experimental vineyard at Lemon Creek Winery. Laboratory analyses and undergraduate support (50%, for 2 months at $ 14.00 per hour). The proposal was also submitted to project GREEEN for matching funds in 2016 but not funded. It was submitted again in 2017. Some preliminary data were presented in 2017 (**Vanderweide J., Murad P., Sabbatini P. and J. Lemon 2017**. *Mechanical leaf removal in Pinot gris. Southwest Michigan Horticultural Days*, February 4-5, Lake Michigan College, Mendel Center, Benton Harbor, MI).

2. Summary and objectives of the proposal

In an effort to explore areas of potential and desired research serving Michigan wine industry needs, MSU viticulture researchers were engaged in 2015 with members of the REAC of the MGWIC in two conference calls to ascertain their priorities and those of the industry. Three major topics were suggested and discussed: 1) ***climate change*** (Lee Lutes), 2) ***cold hardiness*** (William Harrison), and 3) ***sour rot*** (Matthew Moersch, Brian Hosmer). All three subjects are extremely important to the Michigan grape and wine industry and offer several research possibilities to the Viticulture program in the Department of Horticulture of Michigan State University. We focused on the industry's limited resources on combating the effects of Sour Rot, an ever-present and persistent problem year-after-year here in our state. We agree with winemaker and grower Matthew Moersch of Round Barn and Free Run wineries who rightly described it as "*…the single most limiting factor to the production of fine quality wine in Michigan".* Our proposal focuses on developing new baseline knowledge of the impact of sour rot on the development of fruit quality at technological maturity. This information will guide us in the development of a new set of environmentally- and economically-sustainable cultural practices as the next key component of an effective solution. Our research was designed to specifically avoid the confounding effects of the interaction between chemical x viticultural x environment effects; often difficult to untangle with a short 3-4 year project.

The primary objective of the study was to characterize the evolution of fruit technological maturity produced in grapes with varying levels of sour rot in order to determine the effect on overall quality at harvest. Secondarily, we expect to be able to judge the effectiveness of varying degrees of leaf removal as a principal strategy for the reduction of sour rot allowing us to make the best recommendations possible to the industry regarding the use of this strategy. We executed the experiment during 2016 in a Merlot and Pinot Grigio vineyard located at the South West Michigan

(Lemon Creek Winery). A factorial experiment was established comparing four LR treatments. The LR treatments will be applied at two phenological stages of grape berry development using the BBCH scale (Lorenz et al. 1995) as follows: PF, LR applied pre-flowering at phenological stage 57; BS (berry set), LR applied at stage 71 and UN (control), LR not applied with untreated vines/leaves. The first four to six basal leaves of all shoots were removed manually as normally carried out for pre-flowering treatments (Sabbatini and Howell, 2010)[1]. Starting at veraison, grape must samples were collected and used to measure and record the following parameters: gray rot index, sour rot index, yeast activity index, lactic bacteria activity index, glucose-fructose content, Brix degree, density, total acidity, volatile acidity, pH, tartaric acid, malic acid, total assimilable nitrogen, anthocyanins, total phenols, and color intensity as reported in Sternad Lemut (2015)[2]. Evaluation of basic viticultural data and grape compactness was performed following the procedure of Sabbatini and Howell (2010). We will also characterize cluster microclimate as reported in Zhuang et al (2014)[3].

3. Plant material and experimental design

The experiment was conducted in Pinot Grigio and Merlot grafted on rootstock 1114 at Lemon Creek Winery in Michigan, USA (41°96' N; 86°44' W). Vines were planted on a spingks loamy fine soil, with a spacing of two meters between vines and two meters between rows, and trained to a double curtain system with vertically divided foliage (Scott Henry system). Vines were spur-pruned during the winter, leaving approximately 40 buds per vine. However, Michigan has spent a hard winter in 2015, most of the previous trunks had been damaged by winter frost (temperature reached -21 to -23 ℃ in mid-January); therefore, majority of the lower cordons were missing. No additional shoot or cluster thinning was performed before treatment application. Recommended crop protection practices were followed, and the pest management program was based on scouting experience and weather condition. No spray was applied during bloom time to avoid potential mechanical damage to flowers by the sprayer. A combination of fungicide and insecticides used for control were rotated to avoid resistance (following the Integrated Pest Management program by Michigan State University). Weather data, including daily temperature and daily precipitation, were recorded during the experiment by an automated weather station in Berrien Spring form the Michigan Automated Weather Network (MAWN) located 10.2 km from the experimental vineyard. Growing Degree Days (GDD) was calculated with the Baskerville-Emin method using a base temperature of 10 ℃ (Baskerville and Emin, 1969). No additional irrigation was applied and standard summer vineyard practices, mowing grass 3 times, sucker cleaning, hedging, no leaf removal and no cluster thinning, were applied. Shoots were trimmed with pruning machine (on July 19, Day 201 of the year).

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[1] Sabbatini, P. and Howell, G.S. (2010) Effects of early defoliation on yield, fruit composition, and harvest season cluster rot complex of grapevines. Hortscience. 45:1804–1808.

[2] Sternad Lemut, M., Sivilotti, P., Butinar, L., Laganis, J., & Vrhovsek, U. (2015). Pre-flowering leaf removal alters grape microbial population and offers good potential for a more sustainable and cost-effective management of a Pinot Noir vineyard. Australian Journal of Grape and Wine Research.

[3] Zhuang, S,, P. Sabbatini, L. Tozzini, A. Green, D. Acimovic, G.S. Howell, and S. Castellarin. (2014). Impact of cluster thinning and basal leaf removal on fruit quality of Cabernet Franc (Vitis vinifera L.) grapevines grown in cool climate conditions. HortScience. 49(6):750-756.

The experiment was conducted in a randomized complete block design with one categorical factor, leaf removal at two different timings and by two different methods: pre-bloom manual leaf removal from six basal leaves and lateral shoots (PB Man, Figure 2.1); pre-bloom mechanical leaf removal (PB Mec, Figure 2.2); after-bloom manual leaf removal from six basal leaves and lateral shoots (AB Man, Figure 2.3); after-bloom mechanical leaf removal (AB Mec, Figure 2.4); and, control without leaf removal treatment(C).

Figure 2.1 Pre-bloom manual leaf removal (PB Man) treatment. Left: before application; Right: after application.

Figure 2.2 Pre-bloom mechanical leaf removal treatment (PB Mec). Left: before application; Right: after application.

Mechanical treatment was carried out by a leaf remover named Collard E2200F (Collard, Bouzy, France, Figure 2.5). The machine releases relatively low pressure air pulse from two to four nozzles of each rotating wheel, positioned on two axes (40 cm each) per side. Unlike manual leaf removal with detaching each single leaf, the machine reduces the leaf area by shattering the leaf into pieces on approximately 60 to 80 cm of canopy. On the mechanical treatment rows, the tractor ran at 1.6 km/h, pulsing air at 0.8 bar from two nozzles (one positioned for the upper cordon while the other for the lower cordon), rotating at 1650 rpm, thus shattering leaves on that 65 cm of canopy which correspond to the six to eight basal leaves of the shoots both on the upper and lower cordon.

Figure 2.5 Left: whole view of the leaf remover; Right: the machine shattering leaf between two rows.

Experimental vines were set up in five blocks, three replicate sub-blocks for each treatment. Within each replicate block, nine vines were tagged for experiment with extra two vines as guard vines (one on each block end). In addition, three target vines were randomly selected in each block and three target shoots were randomly tagged in each target vine to keep track for the detailed measurements of daily shoot length, fruit set percentage, cluster parameters, and fruit chemistry. In summary, each treatment had nine experiment vines (including three tagged vines) and 9 tagged shoots. For space limitations, results are provided as average of the two cultivars.

4. Estimation of fruit set

Clusters on each tagged shoot (n=270) were photographed in the field at developmental stages 20 (onset of bloom) and 31 (pea size berries), after Eichhorn and Lorenz (1977). Samples of twenty clusters at developmental stage 20 and twenty clusters at stage 31 from the guard vines were photographed in the field against a dark background and then separately collected in ziplock bags and transported to the laboratory. Using the same methodology described by Poni et al. (2006), the actual number of florets and berries were destructively counted. The number of florets and berries visible in the photos were counted using ImageJ (Version 1.51e 5 August 2016). The linear relationships between the actual number of florets (Y) and the counted florets (X): Y = 1.421*X, R2 = 0.89 (Figure 2.7); and actual number of berries (Y) and counted berries (X) in the photos: Y = 1.685*X, R2 = 0.92 (Figure 2.8) were used to estimate the initial number of florets and set berries of each basal cluster per tagged shoot. The percentage of fruit set were expressed in two ways: the percentage of fruit set at developmental stage 31(FS-31) and the percentage of fruit set at developmental stage 38, harvest (FS-38). FS-31 was calculated as the ratio between the estimated number of set berries two weeks after bloom and the estimated number of florets. FS-38 was calculated as a ratio between the number of berries at harvest and the estimated number of florets.

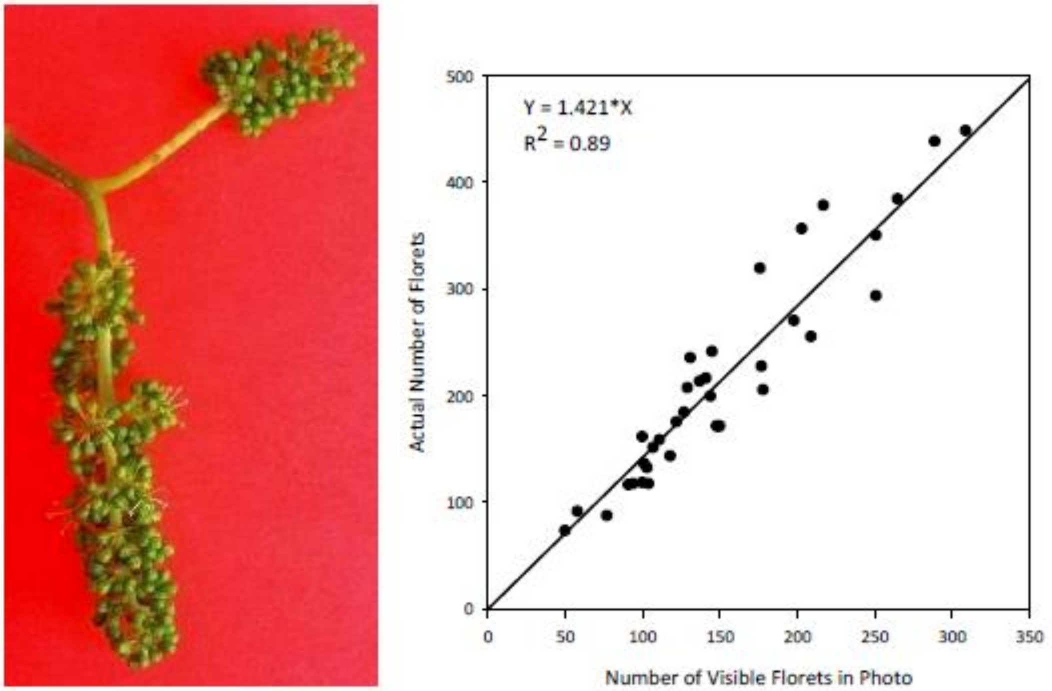

Figure 2.7 Left: a sample cluster from one sample shoot for the number of visible florets; Right: Linear correlation between number of visible florets in photos and number of actual florets per cluster based on a sample of 40 inflorescences collected at developmental stage 20, after Eichhorn and Lorenz (1977).

5. Preliminary results

Following two challenging vintages for Michigan wine industry, 2016 followed a mild winter, no spring frost with average summer heat accumulation. Compared with average growing degree days in southwest of Michigan (1495 GDD), Pinot Grigio gained 1685 GDD in 2016. The lowest temperature (-12.9 ℃) was observed on March 4 (DOY 64) and highest temperature, 34 ℃, was recorded on July 23 (DOY 205). Precipitation was equally distributed from March to July and the highest rainfall happened on August 15 (DOY 228) with 75.4 mm (Figure 3.1). Bud break took place on May 9, 66 days after the coldest day; no extreme temperatures (over 35 ℃) was recorded. Therefore, 2016 was a vintage without extreme weather condition threatening grapevine growth.

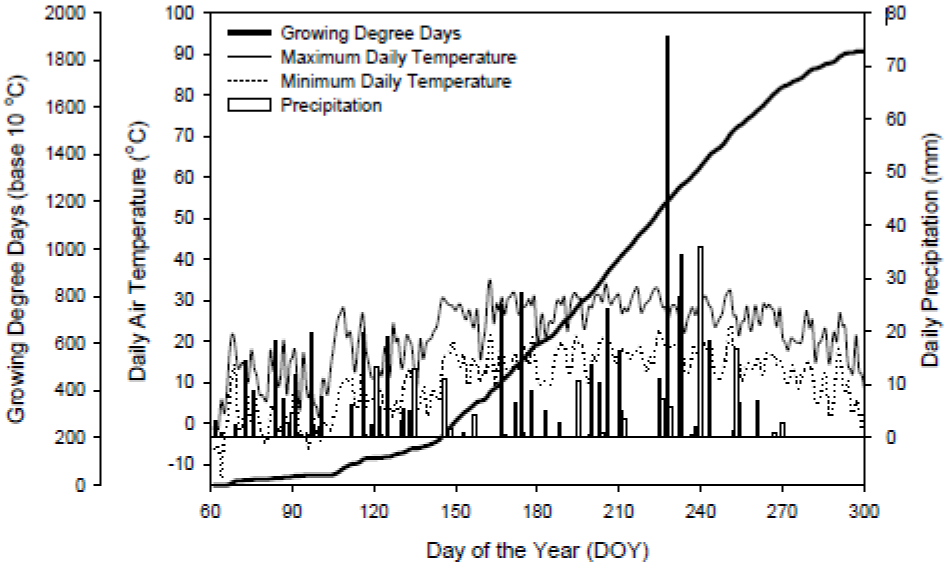

Figure 3.1 Daily precipitation, minimum and maximum daily temperature, and growing degree days from March 1 (DOY 61) to October 26 (DOY 300) in 2016, Berrien Spring.

Using the linear regression between the number of visible flowers and the number of actual flowers per cluster, the estimated flower number was calculated; with the linear regression of the number of visible pea-size berries with actual pea-size berries per cluster, the number of pea-size berry was estimated. Fruit set was expressed in two ways: EL-31 fruit-set, the ratio of estimated pea-size berry number to estimated flower number; and EL-38 fruit-set, the ratio of berry number per cluster at harvest to estimated flower number. As shown in Table 3.2, timing of treatment showed obvious effect on fruit set change. PB Man significantly reduced fruit-set at both phenological stages (EL-31: 50.9 %; EL-38: 43.9 %) with significant difference, in comparison with control (EL-31: 57.7 %; EL-38: 54.6 %). The fruit-set reduction is mainly due to the lower number of pea-size berry as well as the actual berry number at harvest. After-bloom leaf removal did not impact fruit-set percentage, even the manual treatment indicates the trend of fruit-set reduction. Machine did not make significant impact on fruit-set decrease as hand did. However, PB Man significantly reduced the number of florets per cluster to 186 (C: 284), directly resulting in less bunch berry number at harvest (94). AB Mec treatment only decreased number of berries at harvest without changing fruit-set percentage.

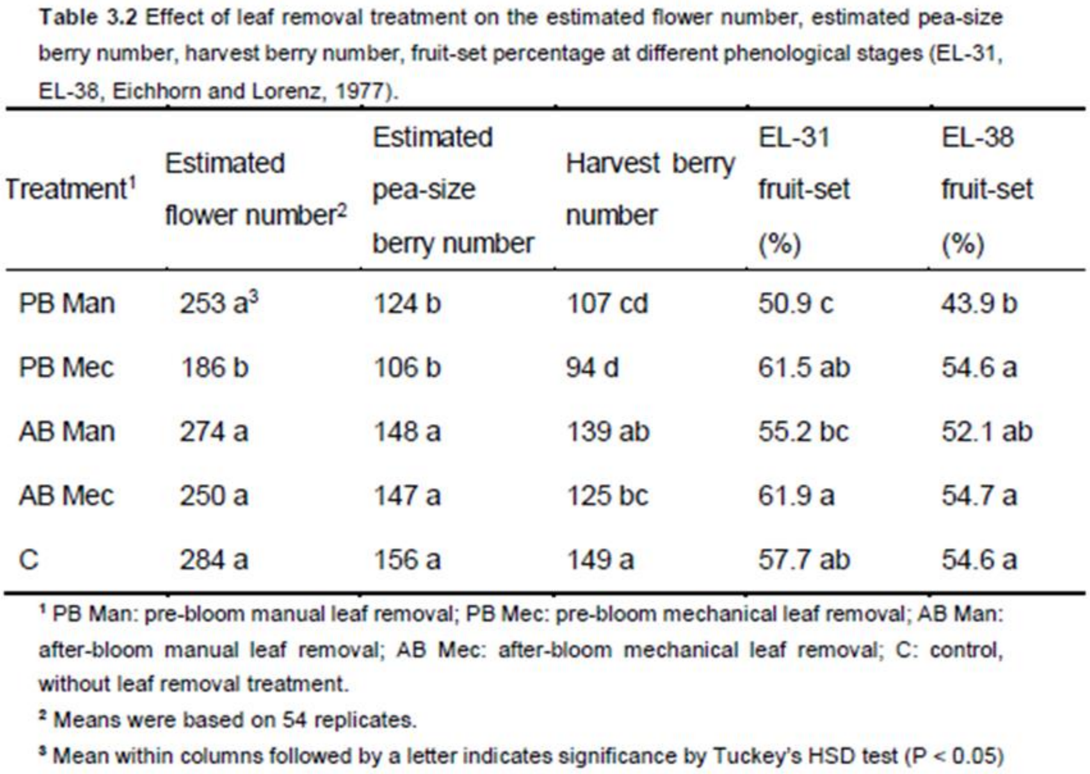

Table 3.2 Effect of leaf removal treatment on the estimated flower number, estimated pea-size berry number, harvest berry number, fruit-set percentage at different phenological stages (EL-31, EL-38, Eichhorn and Lorenz, 1977).

Treatment[1]	Estimated flower number[2]	Estimated pea-size berry number	Harvest berry number	EL-31 fruit-set (%)	EL-38 fruit-set (%)
PB Man	253 a[3]	124 b	107 cd	50.9 c	43.9 b
PB Mec	186 b	106 b	94 d	61.5 ab	54.6 a
AB Man	274 a	148 a	139 ab	55.2 bc	52.1 ab
AB Mec	250 a	147 a	125 bc	61.9 a	54.7 a
C	284 a	156 a	149 a	57.7 ab	54.6 a

[1] PB Man: pre-bloom manual leaf removal; PB Mec: pre-bloom mechanical leaf removal; AB Man: after-bloom manual leaf removal; AB Mec: after-bloom mechanical leaf removal; C: control, without leaf removal treatment.

[2] Means were based on 54 replicates.

[3] Mean within columns followed by a letter indicates significance by Tuckey's HSD test ($P < 0.05$)

All leaf removal treatments did not have impact on TA but PB Man showed the trend of TA reduction. As for grape pH, only pre-bloom treatments significantly increased pH, 3.92 and 3.86 for PB Man and PB Mec respectively, in comparison with C (3.69) (Table 3.3). Pre-bloom leaf removal increased grape total soluble solids by 2.2 ^{0}Brix by PB Mec and 1.1 ^{0}Brix by PB Man, compared with control (20.0 ^{0}Brix). After-bloom leaf removal showed different impact on sugar accumulation as AB Mec slightly increased 0.5 ^{0}Brix while AB Man (19.5 ^{0}Brix) stayed similar to control. Unlike manual leaf removal, mechanically treated vines consistently produced grapes with higher ^{0}Brix.

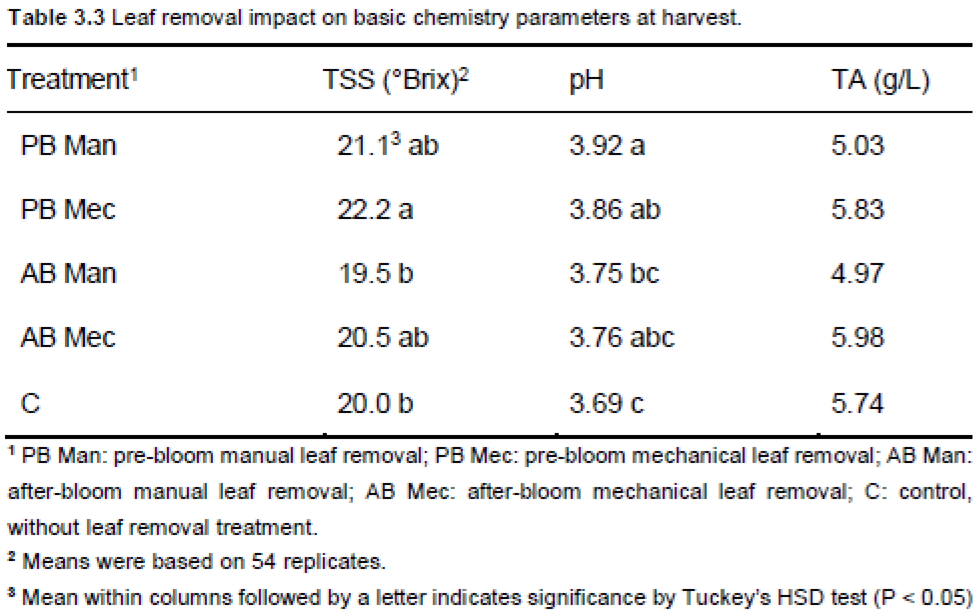

Table 3.3 Leaf removal impact on basic chemistry parameters at harvest.

Treatment[1]	TSS (°Brix)[2]	pH	TA (g/L)
PB Man	21.1[3] ab	3.92 a	5.03
PB Mec	22.2 a	3.86 ab	5.83
AB Man	19.5 b	3.75 bc	4.97
AB Mec	20.5 ab	3.76 abc	5.98
C	20.0 b	3.69 c	5.74

[1] PB Man: pre-bloom manual leaf removal; PB Mec: pre-bloom mechanical leaf removal; AB Man: after-bloom manual leaf removal; AB Mec: after-bloom mechanical leaf removal; C: control, without leaf removal treatment.

[2] Means were based on 54 replicates.

[3] Mean within columns followed by a letter indicates significance by Tuckey's HSD test ($P < 0.05$)

At harvest, sour rot influence was evaluated on each tagged cluster (Table 3.4). Similar to sour rot development process, PB Man resulted in the lowest incidence (41 %), severity (10 %), and quantity loss (13.4g in cluster basis and 0.6 kg in vine basis) while the other three treatments had no significant impact on sour rot in comparison with control (incidence: 65%, severity: 19%, cluster quantity loss: 45.8 g, and vine quantity loss: 2.1 kg). Even without significant reduction on sour rot damage, AB Man, PB Mec, and AB Mec showed the trend of quantity decreasing sour rot damage.

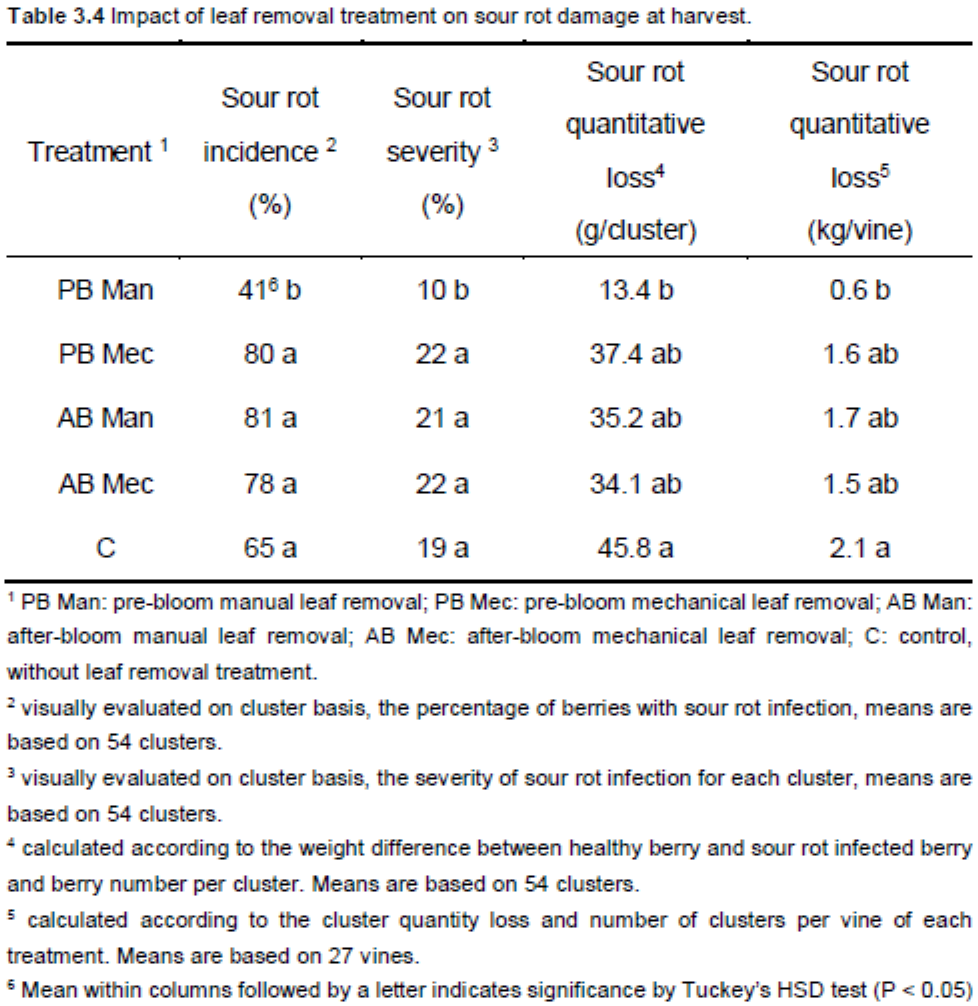

Table 3.4 Impact of leaf removal treatment on sour rot damage at harvest.

Treatment [1]	Sour rot incidence [2] (%)	Sour rot severity [3] (%)	Sour rot quantitative loss[4] (g/cluster)	Sour rot quantitative loss[5] (kg/vine)
PB Man	41[6] b	10 b	13.4 b	0.6 b
PB Mec	80 a	22 a	37.4 ab	1.6 ab
AB Man	81 a	21 a	35.2 ab	1.7 ab
AB Mec	78 a	22 a	34.1 ab	1.5 ab
C	65 a	19 a	45.8 a	2.1 a

[1] PB Man: pre-bloom manual leaf removal; PB Mec: pre-bloom mechanical leaf removal; AB Man: after-bloom manual leaf removal; AB Mec: after-bloom mechanical leaf removal; C: control, without leaf removal treatment.

[2] visually evaluated on cluster basis, the percentage of berries with sour rot infection, means are based on 54 clusters.

[3] visually evaluated on cluster basis, the severity of sour rot infection for each cluster, means are based on 54 clusters.

[4] calculated according to the weight difference between healthy berry and sour rot infected berry and berry number per cluster. Means are based on 54 clusters.

[5] calculated according to the cluster quantity loss and number of clusters per vine of each treatment. Means are based on 27 vines.

[6] Mean within columns followed by a letter indicates significance by Tuckey's HSD test ($P < 0.05$)

Compared with yield in C (6.2 kg/vine), pre-bloom leaf removal reduced yield per vine, with PB Man slight reduction (0.3 kg/vine) and PB Mec significant decrease (1.4 kg/vine). After-bloom treatments did not have significant impact on yield and only AB Mec (0.5 kg/vine) indicated the trend of yield reduction while AB Man showed no difference. Cluster number per vine was not affected by leaf removal treatment (Table 3.5). At harvest, the ratio between total leaf area per vine and yield was calculated. Pre-bloom leaf removal significantly increased the leaf area per yield unit (PB Man: 0.78; PB Mec: 1.11) while after-bloom treatments caused the reduction of leaf area to yield ratio, in comparison with C (0.64). Comparing the impact between hand and machine, mechanically treated vines had higher leaf area to support one yield unit than manual leaf removal. A significant increase was observed when a mechanical treatment was conducted earlier during growing season.

Table 3.5 Leaf removal impact on yield components.

Treatment [1]	Yield [2] (Kg/vine)	Number of clusters per vine	Cluster weight (g)	Total Leaf Area/Yield[3] (m^2/kg)
PB Man	5.9[4] ab	43	136.3 a	0.78 b
PB Mec	4.8 b	43	111.9 b	1.11 a
AB Man	6.2 a	47	131.6 ab	0.47 d
AB Mec	5.7 ab	44	129.6 ab	0.56 cd
C	6.2 a	46	135.3 ab	0.64 c

[1] PB Man: pre-bloom manual leaf removal; PB Mec: pre-bloom mechanical leaf removal; AB Man: after-bloom manual leaf removal; AB Mec: after-bloom mechanical leaf removal; C: control, without leaf removal treatment.

[2] Means are based on 27 replicate vines.

[3] Calculated based on vine total leaf area divided by yield per vine, 27 replicate vines.

[4] Mean within columns followed by a letter indicates significance by Tuckey's HSD test ($P < 0.05$)

Figure 3.8 Cluster pictures for each treatment at harvest. Treatment: PB Man: pre-bloom manual leaf removal; PB Mec: pre-bloom mechanical leaf removal; AB Man: after-bloom manual leaf removal; AB Mec: after-bloom mechanical leaf removal; C: control, without leaf removal treatment.

6. Preliminary conclusions

Pre-bloom leaf removal largely reduced fruit set percentage by earlier carbon resource limitation than after-bloom treatment, reducing berry number per cluster with heavier berry weight and decreasing cluster compactness. Yield was reduced by leaf removed applied before bloom, especially by machine. Besides, fruit quality was consistently enhanced by leaf removal treatments conducted prior to bloom, higher total soluble solids and uniformity by machine.

As for bunch rot control, pre-bloom leaf removal by hand was proved to be the most effective treatment with the least qualitative loss because of higher spray efficiency as well as better microclimate created in fruit zone. Vines treated with pre-bloom leaf removal compensated leaf area loss by intense lateral growth and achieved similar canopy size as control after hedging. However, after-bloom leaf removal (particularly by hand) posed slightly excessive carbon source stress, thus vines were less capable of compensating. Compared with hand, machine removed less leaf area when treatment was applied; therefore, less carbon source limitation led to lower degree of fruit set reduction. Due to air blowing effect of machine, some flowers were blown away at pre-bloom, which result in lower berry number per cluster. In addition, mechanical treatments increased berry weight as well, even in a slight extent. Significant yield reduction was observed when mechanical treatment was conducted before bloom. In regard to fruit quality, mechanical leaf removal favored berries to reach higher ^{0}Brix uniformity at harvest. As for bunch rot management, mechanical treatments had no impact on diminishing sour rot incidence but increased spray efficiency was observed. With leaf residue left on petioles after treatment, pre-bloom mechanically treated vines ended with higher leaf area to yield ratio.

In summary, pre-bloom turned out the better timing for leaf removal than after-bloom and machine had shown its potential to replace hand treatment. However, the amount of leaf area removed by machine should be optimized as well. Therefore, field machine calibration needs further research to achieve the match between timing and method.