

Effect of early leaf removal on Sangiovese (*Vitis vinifera* L.) under thermal excess and drought conditions

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Abstract. The early defoliation of the fruiting area is a technique that offers considerable advantages both in relation to the quality of grapes and to pest control; on the other hand, when a very warm summer occurs, the risk of grape sunburn may increase. This paper reports the results of a pre-flowering defoliation trial carried out in the province of Arezzo (Italy) in 2017, an exceptionally hot and dry year. The results confirmed the validity of this technique in limiting yield while achieving a concurrent higher concentration of phenolic compounds without increasing the risk of burns and radiative damages of grapes.

1 Introduction

The early defoliation of the cluster-zone (ELF) has been proposed, in the last years, as an easily applicable technique for yield management with several positive effects on grape quality and health status. This procedure can be carried out with comparable results both by hand and by tractor-mounted leaf-plucker units [1, 2].

The grapevine phenological growth stage at defoliation time is a critical factor that influences the type and the intensity of the effects on yield components and grape composition. Moreover, leaf removal after berry set can be a useful tool to control cluster rot complex without impacts on grape composition [3], whereas leaf removal before bloom reduces the fruit set ratio limiting, considerably, the production and the bunch compactness, in turn enhancing the levels of soluble solids and phenolic compounds in musts and grapes [4].

When compared with cluster thinning, early defoliation proved to be similarly effective in reducing the crop load but with better results as concerning grape and wine quality: more polyphenols and color, higher sugar content with no significant effect on the acid profile [5, 6]. If the lateral shoots are removed, clusters are directly exposed to sunlight until harvest time. This circumstance significantly affects the chemical composition of grapes and subsequent wines. For example, in Tempranillo, higher concentrations of hydroxycinnamic acids, flavonols, anthocyanins and resveratrol were found in wines as a consequence of defoliation [7]. In a study conducted on Sangiovese and other cultivars a strong positive correlation was observed between light exposure and increases in the percentage of anthocyanins containing an ortho-dihydroxyl group [8]. Moreover, the direct UV radiation can influence the synthesis of phenolic compounds by altering the expression of the genes involved in their biosynthetic pathways; the UV

rays, in particular, can increase the flavonol glucoside content in all grapevine tissues, grapes included [9].

Despite the countless advantages, leaf removal can present drawbacks such as an increase in grape sunburn damage [10]. The intensity of the damage depends not only on the direct exposure to sunlight but also on many other factors such as vine vigor, row orientation, phenological stage at the heat event, water status, and rootstock drought tolerance [11].

In the present study, the impact of pre-bloom cluster zone leaf removal on yield components, health status, grapes composition was evaluated in central Italy during 2017 summer, characterized by extremely high temperatures and scarce rainfall.

2 Materials and methods

2.1 The vineyard

The trial was conducted in a Sangiovese vineyard (VCR 103 grafted on 1103P), vines were spaced 2.80 m x 0.80 m (between row x within the row) trained to a spur pruned single cordon having 8/10 nodes per vine. The canopy was vertically shoot positioned. The vineyard was located in the farm “Badia di Campoleone”, in the province of Arezzo (Italy, 43°31'38"N, 11°50'02"E). In the experimental field, three distinct areas that differed for row orientation and for an apparently different vigor were identified. Specifically, there were two North-South oriented areas, one with a medium vigor (NS-M) and the other with a low vigor (NS-L). The third block was in an East-West oriented plot characterized by high vigor (EW-H).

Each block was composed of 27 vines per 6 contiguous rows (replicates). Three of these underwent pre-bloom defoliation of the clusters zone (the first 6 basal leaves

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were removed). The leaf removal was performed on 22nd May 2017 at the first signs of the bloom onset (Code 61 of the BBCH scale).

2.2 Weather

The vineyard was equipped with an automatic weather station (Pessl Instruments, Austria) fitted with a data logger connected to the internet and able to record temperature, rainfalls, leaf wetness, wind speed and direction, air relative humidity, etc. The meteorological data were compared with the average values of the last 65 years recorded by the nearest station of the Regional Center of Weather and Hydrological Monitoring (<http://www.sir.toscana.it>).

2.3 Controls

From fruit set to harvest time (15th Sept), monitorings were scheduled to determine the sanitary status of grapes and in particular to verify the outbreak of *Uncinula necator*, *Plasmopara viticola* and burn damages. The damages were evaluated observing 100 clusters randomly chosen and classifying them according to a 5 class scale of attack (1: healthy; 2: <10% of damaged berries; 3: 10-25% of damaged berries; 4: 26-50% of damaged berries, 5:>50% of damaged berries) [12].

The Diffusion Index of the damage (DI) was expressed as the percentage of ruined bunches and the average Severity Index of the damage (SI) was obtained according to the Townsend–Heuberger's formula [13].

2.4 Yield components and grapes analyses

The grapes from 5 vines were separately collected three times for each repetition and weighted. Samples, made up of small cluster portions from each repetition, were analyzed to determine the commercial ripeness (sugars, total acidity and pH) according to the International Organization of Vine and Wine methods (Compendium of International Methods of Analysis of Wines and Musts, www.oiv.int) and the phenolic maturity indexes, following the method described by Saint-Criq et al.[14]. The remaining grapes were stored at -18°C for further determinations.

For HPLC analysis, the skins of 15 berries were manually separated from seeds and pulps and extracted in 25 mL of a formic acid-methanol solution (50% methanol, 10% formic acid, 40% water) overnight, after which the samples were ground and centrifuged. The resulting pellets were washed twice with the same solution and centrifuged. The supernatants were merged in a 50 mL volumetric flask. During the sample preparations and after a passage on absorbent paper, the weight of the berries, skins, and the number and the weight of the seeds were noted. Before the injection, the extracts were passed through a syringe filter (cellulose acetate, 0.20 µm).

HPLC analyses were carried out by an Agilent 1100 system equipped with an autosampler and diode-array detector. For the anthocyanin profile determination, a

Hypersil 5µm 200x2.1 mm ODS C18 reversed phase HPLC column was used with a guard cartridge (20 × 2.1 mm) packed with the same material (Thermo Fisher Scientific Inc.). Both columns were held at 30°C. The injection volume was 20 µL and the anthocyanins were eluted with a flow rate of 0.225 mL/min by the following gradient of Solvent A (aqueous 10% (w/w) formic acid) and Solvent B (50% (v/v) of methanol, 10% (v/v) of formic acid in water): from 65 to 45% of solvent A in the first 20 minutes, reduced to 40% from minute 20 to 45, then to 5% from minute 45 to 60 and to 1% from minute 60 to 65. Data were collected at the wavelength of 520nm. The separated anthocyanin monomers were identified by their relative retention time and UV-visible absorption spectra. The results are expressed as relative peak area percentages.

The flavonols content was determined with a Luna Omega Polar C18, 100 A, 250x4.6 mm column (Phenomenex) with a guard cartridge of the same material. Both columns were held at 37°C. The injection volume was 20 µL and the phenolic compounds were eluted with a flow rate of 0.750 mL/min by the following gradient of Solvent A (aqueous 2% (v/v) acetic acid) and Solvent B (2% (v/v) of acetic acid in acetonitrile): 95% of Solvent A for the first 5 minutes then from 95% to 82% at minute 35, to 80 % at minute 50, to 57% at minute 60, to 35 at minute 80 to 0% at 95. Chromatograms were collected at the wavelength of 280, 320 and 360nm. The separated phenolic compounds were identified by their relative retention time and UV-visible absorption spectra. The results are expressed as mg/kg of fresh matter.

3 Results

3.1 Climatic trends and disease incidence

Summer 2017 was characterized by quite unseasonal trends. From the data recorded by the weather station placed in the vineyard, it emerged that from June to August the maximum temperatures remained above 30°C for 80 days; over the same period the daily maximum temperature exceeded 35°C in 30 days and 40°C in 5 days.

The summer was characterized by 6 heat waves with temperatures above 35°C and, in particular, at veraison time (the end of July and the first week of August) the temperature raised up to 8°C above the average of the period exceeding 40°C for five consecutive days (Fig. 1). From March to August, rainfall amount was below average and scarce in June, July and August. In this period, the cumulative rain was 268 mm, representing 60% of the average amount over the last 65 years (441 mm) (Fig.2). Low air humidity and continuous ventilation did not allow night dew formation and leaf wetness was limited to short periods during and after the few rainy events. These conditions did not allow the development of any of the typical grapevine parasites (*Plasmopara viticola*, *Uncinula necator*, *Botrytis cinerea*).

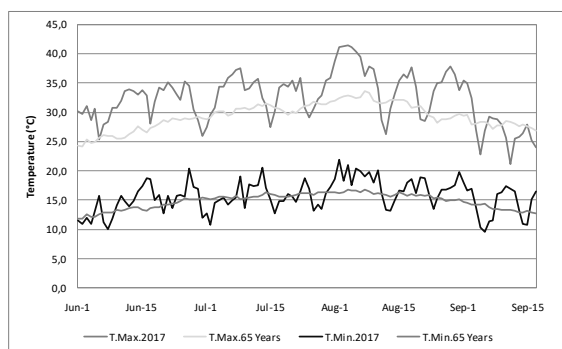


Fig.1. Maximum (T.Max.2017) and minimum (T.Min.2017) temperatures recorded in the vineyard from June to September and the average values of the last 65 years (T.Max.65 Years, T. Min.65 Years)

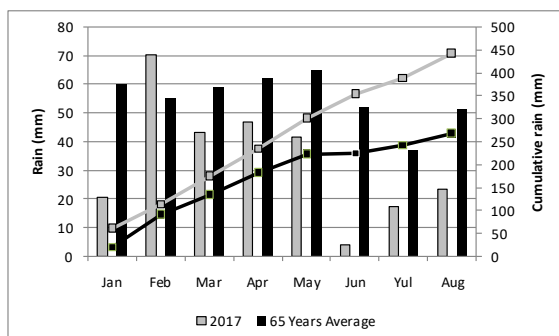


Fig.2. Monthly rainfall and cumulative rain from January to August 2017 and average values over the last 65 years.

3.2 Yield components

The production was higher in the most vigorous vineyard, where defoliation had a minor effect on the crop load. The ELR caused an average drop in production of 9.8%. In the blocks with reduced vigor, defoliation was more impactful at reducing yield per vine (-25%). The effect was related both to the decrease in average berry weight and to a reduced fruit-set rate in the defoliated trials. However, the different vigor influenced the mechanism with which the production decline occurred. In the block with medium and low vigor, the decline in production was mainly due to the decrease in the average berry weight (-19.68% and -9.57% respectively), while a lower rate of fruit set, which reduced the number of berries per cluster, was the determining factor in the decline of production in the most vigorous vineyard (-13.06%) (Tab.1).

3.3 Grapes: maturity indexes

The ELF did not affect the total acidity ($p=0.219$) and pH ($p=0.544$). On the other hand, a positive effect was found on sugar content ($p=0.0145$). However, a significant effect was found only in the tests carried out in the vigorous vineyard, where ELF caused an increase of about 2 °Brix (Tab.1).

The ELF decreased the number of seeds per berry ($p=0.011$) and, again, the effect was significant only in the EW-H block (-12.66%). Skin weight was not affected by defoliation ($p=0.695$) and, in parallel with the concurrent average berry weight, this result suggests

an increase in the thickness of skins. These changes were mirrored to a significant degree on the juice percentage in grapes ($p=0.021$), that was decreased significantly by ELF in the less vigorous plots, as consequence of a variation of the pulp to skin ratio.

In regard to phenolic maturity indexes (Tab.1), significant effects were observed on the amount of anthocyanins, both extractable (+ 9.7%) and potential (+16.6%), and on their extractability (+ 6.4%), only in grapes harvested in the EW-H block. In the less vigorous rows, the results show a decrease, as consequence of defoliation, of anthocyanin content per berry. This result suggests that in the low-vigor tests, the anthocyanin content remained similar only because of the variation in the marc-juice ratio induced by defoliation (Tab.1).

3.4 Anthocyanins profile

The anthocyanin profiles were modified both by vigor and defoliation. The major effects were observed in the EW-H and NS-L tests and malvin and cyanin were the two most involved anthocyanins. The increased exposure to the sun induced a percentage decrease of the malvin equivalent to the increase of cyanin. This, in turn, changed the ratio between di-substituted and three-substituted anthocyanins. In addition, the ELF lowered the percentage of acylated anthocyanins (Tab.1).

3.5 Flavonols

The content of flavonols in the grapes has been influenced by both defoliation and vine productivity. In non-defoliated thesis, the concentration decreases as the plant vigor increases. The defoliation has significantly increased the synthesis of flavonols. Quercetin 3-O-glucuronide shifted from an average value of 9.2 mg/kg in control tests to about 18 mg/kg in grapes from defoliated vines. The same increase was found for 3-O-glucoside quercetin (from 17 to 28 mg/kg), myricetin glycosides (from 7.6 to 10.5 mg/kg) and kaempferol 3-O-glucoside (from 4.5 to 9.9 mg/kg). Rutin (quercetin-3-O-rutinoside) in non-defoliated tests was found only as a trace, while it reached concentrations close to 0.4 mg/kg in the defoliated trials (Tab.1).

3.6 Sunscald damage

The seasonal climatic trend favored the onset of sunscald damages. Significant differences emerged among the various blocks and were related to the vigor of the vineyard. The damage diffusion index (DI), that measures the percentage of the affected bunches, ranged between a maximum of 42.8% of the NS-L test and 26.5% of EW-H test. The damage severity index, which measures the percentage of crop loss, was related to the diffusion and it was 6.0% in the EW-H test, 9.8% in the NS-M test and 14.5% in the test NS-L.

Table 1. Yield component, grapes maturity indexes, anthocyanins profiles, flavonols content and sunscald damage of vines subjected to ELR (E) compared to non defoliated plants (C) at three different vigor level (V): high (H), medium (M) and low (L).

| Parameter | EW-H | | NS-M | | NS-L | | Average | | Significance | | |
|-----------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|---------|------|--------------|------|------|
| | C | E | C | E | C | E | C | E | E | V | ExV |
| Yield components | | | | | | | | | | | |
| Berry Weight (g) | 1.99 | 1.97 | 1.93 | 1.55 | 1.88 | 1.70 | 1.93 | 1.74 | ** | ** | * |
| Berries per Cluster | 111 | 96 | 104 | 108 | 93 | 83 | 103 | 96 | * | ** | n.s. |
| Cluster weight (g) | 222 | 189 | 209 | 163 | 175 | 141 | 202 | 164 | *** | *** | n.s. |
| Production per plant (kg) | 2.88 | 2.66 | 2.50 | 1.63 | 2.14 | 1.64 | 2.51 | 1.98 | *** | *** | n.s. |
| Seeds per berry | 2.83 | 2.48 | 2.40 | 2.20 | 2.27 | 2.13 | 2.50 | 2.27 | ** | *** | n.s. |
| Skin to berry ratio | 9.6 | 9.7 | 11.4 | 13.3 | 10.2 | 12.4 | 10.4 | 11.8 | * | ** | n.s. |
| Seeds to berry ratio | 6.6 | 5.8 | 6.5 | 8.0 | 5.4 | 6.7 | 6.2 | 6.8 | † | ** | ** |
| Pulp to berry ratio | 86.1 | 86.7 | 84.9 | 82.5 | 86.5 | 84.0 | 85.8 | 84.4 | * | ** | † |
| Grapes maturity indexes | | | | | | | | | | | |
| Sugar (Brix) | 21.7 | 23.4 | 24.6 | 24.7 | 23.5 | 24.2 | 23.3 | 24.1 | ** | *** | n.s. |
| pH | 2.94 | 2.98 | 3.06 | 3.06 | 3.06 | 3.08 | 3.02 | 3.04 | n.s. | * | n.s. |
| Total Acidity (g/L tartaric acid) | 6.99 | 7.23 | 5.80 | 6.13 | 6.17 | 6.01 | 6.32 | 6.46 | n.s. | *** | n.s. |
| Extractable anthocyanins (%) | 588 | 645 | 694 | 705 | 703 | 702 | 662 | 684 | n.s. | *** | n.s. |
| Total Anthocyanins (mg/kg) | 875 | 1050 | 1075 | 1066 | 1070 | 1059 | 1007 | 1058 | n.s. | *** | † |
| Anthocyanins per berry (mg) | 1.71 | 2.02 | 2.02 | 1.56 | 1.90 | 1.68 | 1.88 | 1.75 | n.s. | n.s. | *** |
| Extractability (%) | 32.8 | 38.3 | 35.0 | 33.8 | 34.1 | 33.2 | 34.0 | 35.1 | n.s. | n.s. | † |
| Anthocyanins profile | | | | | | | | | | | |
| Delphinin (%) | 9.4 | 10.7 | 9.8 | 11.9 | 9.2 | 9.7 | 9.5 | 10.7 | ** | n.s. | n.s. |
| Cianin (%) | 16.2 | 22.9 | 26.3 | 28.1 | 28.0 | 33.6 | 23.5 | 28.2 | *** | *** | n.s. |
| Petunin (%) | 16.3 | 17.5 | 15.7 | 16.7 | 14.7 | 14.8 | 15.6 | 16.3 | n.s. | *** | n.s. |
| Peonin (%) | 14.0 | 13.8 | 16.4 | 14.4 | 17.5 | 16.7 | 16.0 | 14.9 | n.s. | *** | n.s. |
| Malvin (%) | 41.0 | 32.7 | 28.8 | 26.2 | 28.2 | 22.7 | 32.6 | 27.2 | *** | *** | n.s. |
| Di-tri substituted ratio | 0.45 | 0.62 | 0.79 | 0.80 | 0.90 | 1.08 | 0.71 | 0.83 | * | *** | n.s. |
| Acilated (%) | 2.8 | 2.3 | 2.7 | 2.5 | 2.3 | 2.2 | 2.6 | 2.4 | ** | ** | n.s. |
| Flavonols (mg/kg) | | | | | | | | | | | |
| Rutin | 0.01 | 0.19 | 0.08 | 0.69 | 0.05 | 0.38 | 0.05 | 0.42 | *** | *** | n.s. |
| Quercetin 3-O-glucuronide | 5.8 | 11.8 | 10.6 | 23.1 | 11.2 | 18.8 | 9.2 | 17.9 | *** | *** | ** |
| Quercetin 3-O-glucoside | 11.9 | 19.8 | 17.7 | 31.8 | 21.4 | 32.6 | 17.0 | 28.1 | *** | *** | n.s. |
| Glycosides of Myricetin | 6.5 | 9.1 | 8.0 | 12.4 | 8.4 | 9.9 | 7.6 | 10.5 | *** | *** | ** |
| Kaempferol 3-O-glucoside | 2.8 | 6.0 | 4.6 | 12.4 | 5.9 | 11.2 | 4.43 | 9.87 | *** | *** | ** |
| Sunscald damage | | | | | | | | | | | |
| Diffusion Index (DI) | 26.1 | 26.6 | 34.1 | 38.1 | 42.6 | 43.1 | 34.3 | 35.9 | n.s. | *** | n.s. |
| Severity Index (SI) | 5.3 | 6.6 | 10.2 | 9.3 | 12.3 | 16.8 | 9.3 | 10.9 | n.s. | *** | n.s. |

Differences inside the same block, represented in **bold** type, were identified with a Multiple Range Test, performed according to the Fisher's least significant difference (LSD) with a 95% confidence level. The influence of E, V and their interaction was evaluated with a multifactor ANOVA followed by the Tukey's honestly significance difference (HSD) procedure with a 95% confidence level. *, **, ***, † and n.s. indicates, in the same order, significance at P<0.05, P<0.01, P<0.001, P<0.1 and not significant.

The effect of early defoliation on sunscald damages was negligible in all tests.

4 Conclusions

This study confirms many positive aspects of ELF previously investigated and widely described, such as the reduction in yield per vine and berry weight, the increase of the flavonols synthesis and the change in the pulp to skin ratio. The exceptionally hot and dry 2017 summer, however, made it possible to draw some interesting conclusions. Although temperatures over 40°C were reached, suggesting much higher temperatures on exposed berries surfaces, ELF influenced neither the pH nor the total acidity and increased the sugar content only in the trials with higher vigor. Despite the very harsh conditions, ELF did not increase sunscald damages, confirming the pre-bloom phenological stage as a recommended period to perform a defoliation of the fruiting zone.

From the data, it emerges, finally, the key role of plant vigor. The ELF has drastically reduced productivity in the blocks of the vineyard where production was already low; this implies that is important, to avoid an excessive crop reduction, to assess and study the vineyard productive response to drought on the basis of soil's water stock, since it is not possible to make predictions about the climatic events that may occur between flowering and harvesting.

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