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# Effect of Leaf Area Size on the Main Composition in Grape Must of Three Varieties of *Vitis vinifera* L. in an Organic Vineyard

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**Abstract:** The concentrations of sugars and acids are very important for the quality and the stability of wines. In addition, the proportion of the two main acids, i.e., tartaric acid and malic acid, is a significant factor for wine taste and stability. Over a period of three seasons in an organic vineyard, the influence of leaf area on the concentration of total soluble solids (TSS), pH, titratable acidity (TA), the concentration of tartaric acid and malic acid, and their mutual proportions were monitored. Vines of three varieties ('Rhine Riesling', 'Pinot Gris', 'Sauvignon Blanc') were treated using three different treatments (proportion of leaves removed 0%, 40%, and 70%). All varieties exhibited positive correlations between leaf area and TSS. In terms of relationships between TA and leaf area, 'Sauvignon Blanc' was the most sensitive variety. The highest differences between the individual variants were found for this variety. The tartaric to malic acid ratio displayed a significant seasonal effect, which was mostly more important than leaf area reduction. The size of the leaf area mainly affected the accumulation of sugars in the grapes, while content and ratio of acids was not affected so significantly. Therefore, leaf area regulation is one of the ways to optimize the composition of grapes in organic vineyards.

**Keywords:** grapevine; organic; leaf area; tartaric acid; malic acid; pH

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## 1. Introduction

The aim of organic viticulture is to introduce a production system that minimizes the occurrence of diseases and pests and reduces the use of pesticides, so that the final product is not affected by a large number of interventions [1]. Organic grapevine production carries certain risks, such as unreliable yields, disease and pest control problems, as well as reduced yields of 8–16% [2–4]. Organic grape production systems differ from conventional production and may, therefore, have an impact on the quality and composition of wine [5]. In organic vineyards, the grapes have a longer ripening time because the release of the supplied nutrients is slower [6]. No differences in sugar or acid contents were found when comparing conventional and organic grapes production [7].

Organic acids in wine grapes include tartaric acid, malic acid, citric acid, gluconic acid, mucic acid, and more. Of these, tartaric acid and malic acid are most prevalent. The proportion of the two is important, not only in terms of the manner the wine expresses its taste, as it also determines the pH of the must and is the key criterion for determining the ripeness of the grapes [8]. Wines with a higher concentration of malic acid have an unbalanced taste because malic acid has a sharper taste than tartaric acid. Tartaric acid, i.e., dihydroxysuccinic acid, occurs in the grapes in the L(+) form and exists in all parts of the grape and in leaves. As the berries grow, the content of this acid increases [9]. As the berries soften, the acid already ceases to generate and its content becomes reduced, such that its concentration is diluted [10]. Partial reduction in tartaric acid concentration could by caused when some extent of the substance becomes linked with potassium (K+) contained in the grapes to produce potassium bitartrate, which is difficult to dissolve. For this reason, grapes growing on plants with high stock of K(+) may contain lower quantities of tartaric acid [11]. Malic acid is present in the L(−) form and is less stable than tartaric acid. In the northern hemisphere, the synthesis and storage of malic acid mostly occurs during the first growth stage of the berries [12]. During ripening, malic acid is decarboxylated to produce pyruvate, while a small portion is converted by gluconeogenesis to fructose and glucose. Increased temperature accelerates the process, so grapes in more southerly growing areas are lower in malic acid than northern regions. Grapes from seasons with high temperatures and little rainfall also exhibit a lower quantity of malic acid than those produced in colder seasons with higher precipitation [11,13–15].

Interventions for regulating leaf area form the basis for many vineyard procedures that lead to sourcing grapes with the characteristics required [16]. Reduction of the leaf area can favor nutrient intake for grape ripening. It was previously shown that removal of the leaves can increase growth of transverse shoots and increase photosynthetic activity of other leaves [17,18].

Some organic acids are synthesized in leaves (particularly tartaric), while others are produced in berries [19]. As berries ripen, the tartaric acid ceases to flow from the leaves into the grapes along with assimilates, while malic acid is decarboxylated [8,12]. Carboxylation of phosphoenolpyruvate to oxaloacetic acid, followed by reduction in the berries, is responsible for malic acid synthesis [20,21]. In unripe berries at beginning of veraison, the titratable acidity (TA) is very high and malic acid is higher than tartaric acid. During maturation, TA decreases, especially malic acid [14–16]. Decreasing the leaf area by shoot thinning may result in a delay in the ripening of the grapes [22]. Shoot thinning after blooming shifts fruit ripening by more than 1–2 months [23]. Too dense foliage can cause excessively dense shoots or growth of sublateral shoots [24–27]. Thus, the aim of this study was to observe the effect of the leaf area size on the content of soluble solids (TSS) and acids in grapes during ripening.

## 2. Materials and Methods

### *2.1. Location of the Experiment*

The experiment was conducted in 2017, 2018, and 2019 in an organic vineyard in the municipality of Popice, Mikulov viticultural subregion, the Czech Republic (48°55′47.2″ N, 16°42′04.1″ E). The vineyard was planted in 2004, vines were cultivated using a semi-high training method with a trunk height of 80 cm and the spacing of the plants was 2.4 × 0.9 m. All varieties were pruned by cutting to 1 cane without a reserve spur with five to eight buds. The inter-rows were treated alternately as grass zones and black fallow land zones. The slope was oriented south to southwest at an altitude of 210–260 m, the soil type was chernozem on loess, and, depending on the size of the particles, it was clay soil.

### *2.2. Weather Conditions*

During the experiment, 2017 was the coldest period, showing the average temperature of 15.5 °C. In 2019, the average temperature was 15.8 °C, whereas 2018 was the warmest season, with an average temperature of 17.4 °C. Average temperatures of all three seasons were slightly above the long-term average from April to October (Table 1). Average growing season temperatures (TGS), based on the classification by Jones [28], were intermediate (2017, 2019) or hot (2018). For the 3 seasons, the average Huglin Index [29] was 2066, and the Winkler Index [30] was 1425.

**Table 1.** Average monthly temperatures in °C for the experiment area, Popice, Czech Republic 2017–2019.

| | April | May | June | July | August | September | October | Average Value (April—October) |
|---|---|---|---|---|---|---|---|---|
| 2017 | 8.3 | 15.2 | 20.2 | 20.4 | 20.9 | 13.3 | 10.2 | 15.5 |
| 2018 | 14.4 | 17.6 | 19.2 | 21 | 22.6 | 15.9 | 11.4 | 17.4 |
| 2019 | 10.9 | 11.9 | 22 | 20.1 | 20.7 | 14.7 | 10.4 | 15.8 |
| long-term average | 9.3 | 14.4 | 17.2 | 19.3 | 18.8 | 14.1 | 9 | 14.6 |

In 2018, disproportionately high temperatures were recorded in August and September; in the remaining two seasons of the experiment, September temperatures were below the multi-annual average threshold (14.1 °C). The values of rainfall (mm) and sunshine (hours) per month are shown in Figure 1.

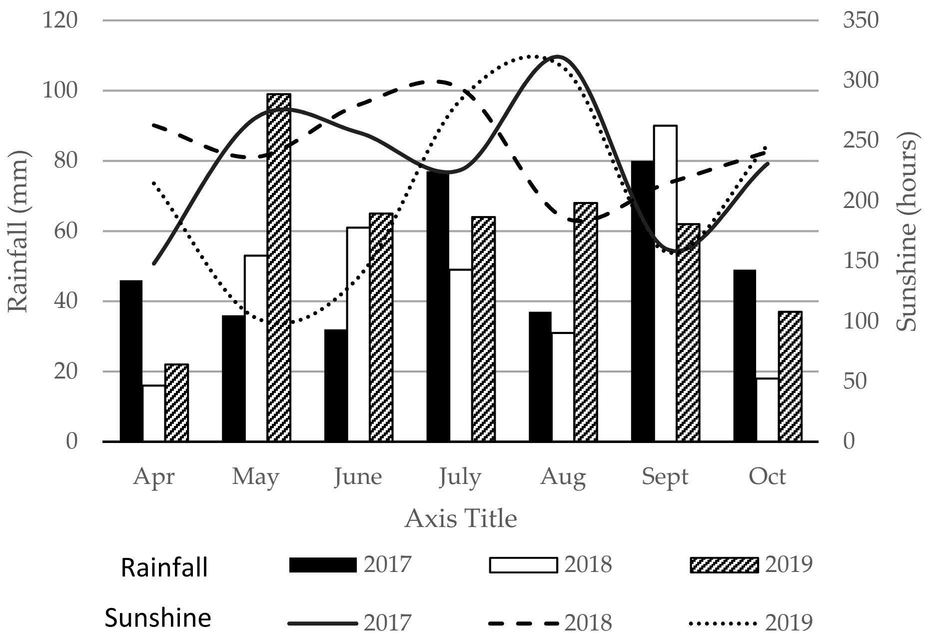


**Figure 1.** Sunshine (hours) and rainfall (mm) for the experiment area, Popice, Czech Republic 2017–2019.

### 2.3. Grape Varieties and Treatments of Experiment

Grapes of three *Vitis vinifera* L. varieties were investigated: 'Rhine Riesling' (RR), clone R 2, rootstock Kober 5 BB, origin: VCR Rauscedo Italy. 'Pinot Gris' (PG), clone R 6, rootstock SO 4, origin: VCR Rauscedo, Italy. 'Sauvignon Blanc' (SB), clone 107, rootstock Teleki 5C, origin: Germany.

Each variety was split into three treatments, depending on the intensity of the agrotechnical interventions aimed primarily at reducing the leaf area. Tipping away excess foliage on shoots took place two to three times during the growing season, with the aim of removing 40% (variant B) and 70% (variant C) of the total leaf area (Table 2). For variant A (control), no regulation was carried out on the leaf area. The removal of shoots was carried out by mechanization at about 30-day intervals. The treatment was performed depending on the growth rate of the shoots. The first reducing was always after blooming. Every treatment consisted of three replicates, with fifty vines per replicate.

**Table 2.** The leaf area of each of three grape varieties (A—without leaf area reduction, B—40% leaf area reduction, C—70% leaf area reduction).

| | | Leaf Area ($m^2$/vine) | | |
|---|---|---|---|---|
| **Variant of Leaf Area Treatment** | **Season** | **'Pinot Gris'** | **'Sauvignon Blanc'** | **'Rhine Riesling'** |
| A | | 3.03 [c] | 2.67 [d] | 4.25 [c] |
| B | 2017 | 1.97 [b] | 1.54 [b] | 2.61 [b] |
| C | | 1.02 [a] | 0.99 [a] | 1.17 [a] |
| A | | 3.38 [c] | 2.90 [d] | 4.82 [d] |
| B | 2018 | 1.95 [b] | 1.71 [b,c] | 2.99 [b] |
| C | | 1.14 [a] | 1.12 [a] | 1.40 [a] |
| A | | x | 3.29 [e] | 4.63 [c,d] |
| B | 2019 | 2.20 [b] | 1.95 [c] | 2.72 [b] |
| C | | 1.17 [a] | 1.14 [a] | 1.39 [a] |

ANOVA was used to compare data. Means within columns followed by different letters ([a,b,c,d,e]) are significantly different from each other at $p < 0.05$ based on Fisher's unprotected least significant differences test (LSD).

### 2.4. Measurement of Leaf Area and Sampling

At each sampling (from 50 days before harvest to harvest), the number of mature leaves per shoot was recorded on shoots from which berries were sampled. At the end of the experiment, 50 leaves per treatment (variant) were sampled and their surfaces measured using the CI-202L Area Meter (CID, Inc., Camas, WA, USA) equipment, expressed in $m^2$. On the basis of these values, an average size of the leaf was calculated for each variety and treatment and multiplied by the number of leaves on the shoot bearing the examined grape.

Each sample was represented by 100 berries. During the transportation period, the berries were kept in open plastic bags with detailed labelling and stored in a plastic crate. The berries were crushed and the loosely drained must was analyzed.

### 2.5. Titratable Acidity (TA) and pH

A digital pH meter OP 122/1 (WTW, Waltham, Germany) was used to determine pH. The titratable acidity (TA) was determined using alkalimetry. Exactly 20 mL of must was titrated using 0.1 M NaOH to reach pH 7, as is recommended by OIV methods [31]. The quantity of NaOH consumed was the basis for calculating the content of TA expressed as tartaric acid (g/L). For each sample, the measurement was made in three runs.

### 2.6. Total Soluble Solids (TSS)

Soluble solids were established in the samples at a temperature of 20 °C using digital refractometer (DR201-95, A. Krüss Optronic, Hamburg, Germany) and expressed in °Brix degrees (°Bx).

### 2.7. Tartaric and Malic Acid (TtA, MA)

Each sample was diluted by demineralized water (1:10 proportion) and subsequently filtered through a microfilter, grain size 0.2 µm. Concentrations of tartaric acid and malic acid were determined using an HPLC system with the Chrom SDS 150 pump, a thermostat (at 60 °C), and the Thermo-Spectra System UV 6000 LP DAD Detector (Thermo, Alaucha, FL, USA). The column used was a Watrex Polymer IEX H 10 µm, 250 × 8 mm, with a mobile phase of 2 mM sulfuric acid, flow: 0.7 mL/min. The absorbance of the acids was set at a wavelength of 210 nm. The concentration was determined using a 10-point calibration system using tartaric acid and malic acid standards. The $r^2$ value for tartaric acid was 0.9918 and for malic acid, it was 0.9965. The sample volume was 20 µL.

### 2.8. Statistical Analysis

The statistical assessment was carried out using STATISTICA 12. Statistical operations were used to obtain means and standard deviations from three determinations made in parallel. Cochran, Hartley, and Bartlett tests were used to confirm the variance homogeneity. The method of multivariate analyses was selected to confirm a conclusive difference between values of leaf area reduction and TtA/MA, TtA, Ma, and pH, with subsequent use of Fisher's unprotected least significant differences test (LSD) test at a significance level of $p < 0.05$.

## 3. Results and Discussion

Reduction of the leaf area was carried out in variant B (40%) and C (70%) for all varieties. The control treatment (A) had no leaf area reduction during grape ripening. The individual leaf area values for the variants are given in Table 2. The analysis of results showed that the variant of leaf area treatment had a significant influence of the leaf area, but not the season.

Leaf area size was positively correlated with TSS (Figure 2) (Pinot Gris, r = 0.587; Sauvignon Blanc, r = 0.435; Rhine Riesling, r = 0.438), consistent with other studies [32–35]. This trend was particularly apparent for the 'Rhine Riesling' and 'Sauvignon Blanc' varieties (Figure 2c,e). The difference in cluster weight for these two varieties or different weather conditions of each season could be the possible reason. The 3-year average cluster weight of 'Pinot Gris' was 98 g, while for both 'Sauvignon Blanc' and 'Rhine Riesling', it was 110 g. Schiefer and Thin [36] mentioned that for grape ripeness to be optimized, 16 to 22 $cm^2$ of leaf area per gram of grape weight is the necessary leaf to fruit ratio. The size of the leaf area has a significant effect on the TSS, while Ollat and Gaudillere [16] reported that the grapes had a lower TSS on the vine with a lower leaf area. The greatest differences were recorded in 2017 for the 'Sauvignon Blanc' and 'Pinot Gris' varieties (Figure 2). The value of TSS in grapes measured for plants with the highest leaf area reduction (variant C) was up to 3° Bx less than for plants with greater leaf areas (Sauvignon Blanc and Pinot Gris). The sensitivity of 'Sauvignon Blanc' to reduced leaf area in relation to TSS accumulation was confirmed by Petrie et al. [18] as well. The smallest difference in terms of TSS values was found in 2019 between the variants, when the figure ranged between 0.5 and 1.1° Bx across all three varieties.

Regarding TA, a declining trend was observed in the course of berry ripening. The highest TA of the clusters was contained in the 'Rhine Riesling' variety at harvest time, regardless of the % of leaf reduction; this was particularly true in 2019, when TA values were up to 2 g/L higher than seen in the preceding seasons (values ranging between 12.36 and 17.15 g/Lat harvest). The effect of the leaf area size was shown most considerably for 'Sauvignon Blanc' (Figure 2c). In 2018, the value of 7.02 g/L was found for grapes growing on plants from the variant without leaf area reduction, for plants from variant B it was 7.63 g/L, and, finally, plants without reduced leaf area (variant A) achieved 8.63 g/L. While the other varieties exhibited the same trend as 'Sauvignon Blanc' in the course of ripening, the differences were not as significant as for this variety. Similar conclusions were reached by Candolfi-Vasconcelos and Koblet [37] and Kozina et al. [38]. The results confirmed that while regulating the leaf area after blooming can quite effectively influence the content of TA in grapes, the development of climatic conditions in that season remains a very considerable factor, as shown on Figure 2. Above all, the most rainy season, 2019, significantly affected the production of acids, which were above average in all variants. Gutiérrez et al. [39] also studied the effect of leaf area on TSS content in grapes. They found that in some varieties (Sauvignon Blanc, Cabernet Sauvignon, Syrah) the declining leaf area reduced the TSS content in grapes, while in the Carmenére variety, the leaf area did not affect the TSS in grapes.

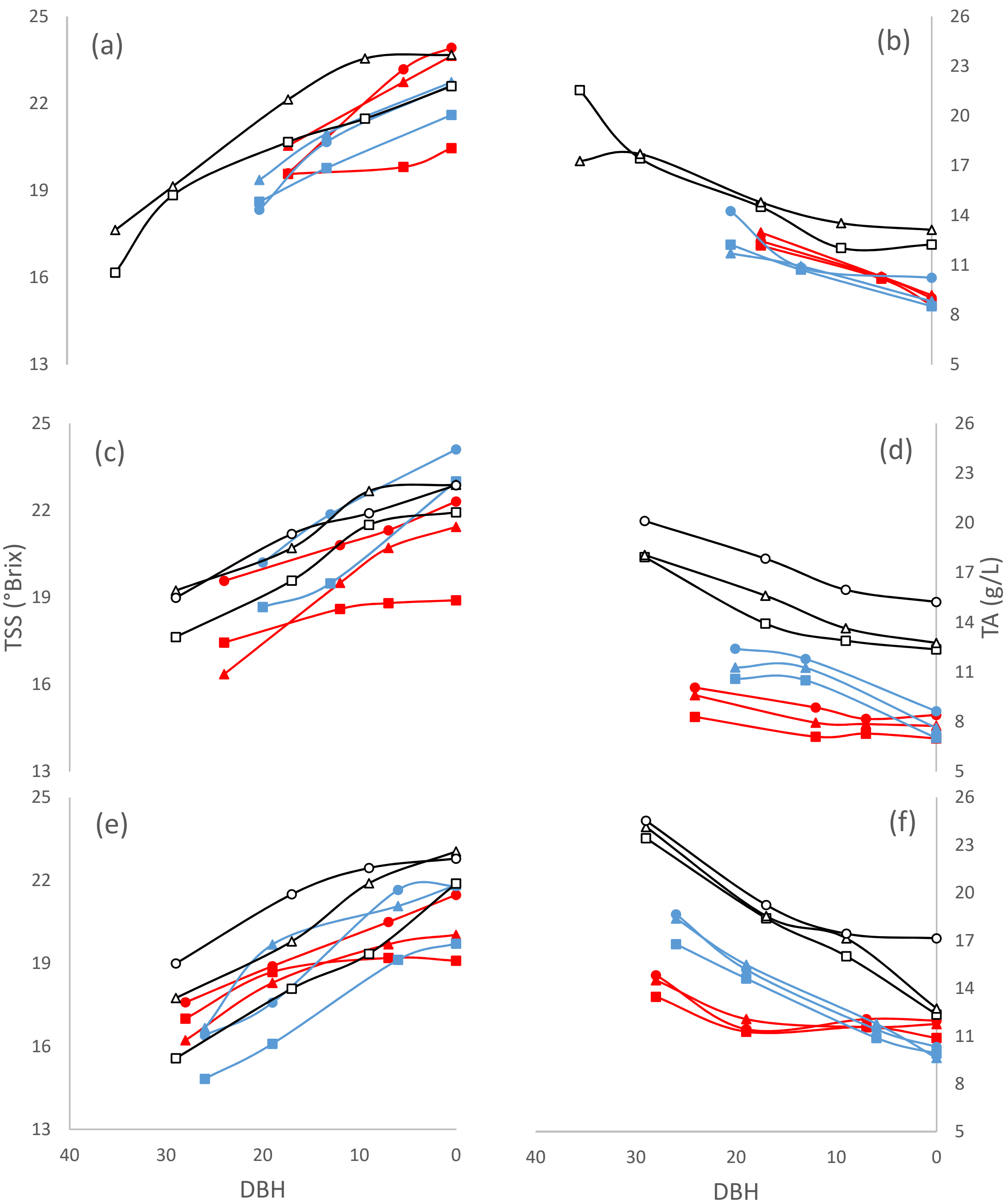


**Figure 2.** The effect of leaf area reduction on the grape content of total soluble solids (TSS) and total titratable acidity (TA) during ripening (days before harvest (DBH)) in grapes of varieties 'Pinot Gris' (**a**,**b**), 'Sauvignon Blanc' (**c**,**d**), and 'Rhine Riesling' (**e**,**f**) in 2017 (—red), 2018 (—blue), and 2019 (—black) when reducing 0% (●), 40% (▲), and 70% (■) the leaf area.

The tartaric acid content remains more stable during ripening than malic acid content [40]. Malic acid begins to break down in the grapes as sugar begins to accumulate in them. This is most pronounced at higher temperatures. In cool conditions, at temperatures of 12–22 degrees, however, the synthesis of malic acid still persists in the grapes even after the accumulation of sugar [41]. This experiment brought significant attention to changes in the tartaric acid to malic acid proportion depending on the leaf area (Table 3). This proportion showed considerable differences depending on the variety and, in particular, climatic conditions. For the surveyed varieties, the proportions ranged between 1.28 and 4.90. 'Sauvignon Blanc' grapes growing on plants without leaf area reduction showed the acid ratio to be 0.17–2.64 less than for grapes from plants with the highest reduction of leaf area (Table 3). In almost all cases, the lower leaf area caused a lower content of malic acid in the grapes. Similar results were found by Ollat and Gaudillére [16], who studied the effect of leaf area ratio on content of substances in the grapes, 51 days after bloom. In the Cabernet Sauvignon variety, they observed higher concentrations of malic acid in grapes on vines with a higher leaf area. Van Leeuwen et al. [42] suggested that the content of malic acid in grapes is mostly dependent on the season, which was confirmed through the results of the present paper. In particular, 2019 was the season to show significantly higher levels of malic acid than in previous seasons.

**Table 3.** The effect of variety and leaf area on content of tartaric acid, malic acid, and pH in grapes at harvest time 2017–2019.

| Variety | Season | Variant of Leaf Area Treatment | TtA/MA | TtA (g/L) | MA (g/L) | pH |
|---|---|---|---|---|---|---|
| 'Pinot Gris' | 2017 | A | 2.41 [a] | 8.14 [b] | 3.38 [c] | 3.11 [a] |
| | | B | 2.38 [a] | 8.05 [b] | 3.38 [c] | 3.11 [a] |
| | | C | 2.33 [a] | 7.37 [a] | 3.16 [c] | 3.09 [a] |
| | 2018 | A | 2.92 [b] | 9.18 [c] | 3.14 [c] | 3.37 [b] |
| | | B | 3.57 [c] | 8.52 [b] | 2.39 [b] | 3.42 [c] |
| | | C | 4.12 [d] | 8.07 [b] | 1.96 [a] | 3.39 [b,c] |
| | 2019 | A | × | × | × | × |
| | | B | 2.45 [a] | 10.50 [d] | 4.90 [d] | 3.62 [d] |
| | | C | 2.14 [a] | 11.27 [e] | 4.60 [d] | 3.65 [d] |
| 'Sauvignon Blanc' | 2017 | A | 2.89 [b] | 8.81 [c,d] | 3.04 [d] | 3.03 [a] |
| | | B | 3.75 [c,d] | 7.14 [a] | 1.90 [b] | 3.01 [a] |
| | | C | 4.90 [e] | 7.11 [a] | 1.45 [a] | 3.10 [a] |
| | 2018 | A | 3.18 [b,c] | 8.08 [b] | 2.54 [c] | 3.49 [b] |
| | | B | 4.00 [d] | 7.82 [b] | 1.97 [b] | 3.56 [c] |
| | | C | 4.42 [d,e] | 8.28 [b,c] | 1.87 [b] | 3.36 [c] |
| | 2019 | A | 1.52 [a] | 9.90 [f] | 6.50 [e] | 3.67 [c] |
| | | B | 2.69 [a,b] | 9.18 [e] | 3.41 [d] | 3.69 [c] |
| | | C | 2.62 [a,b] | 9.25 [d] | 3.52 [d] | 3.69 [c] |
| 'Rhine Riesling' | 2017 | A | 3.84 [e] | 11.35 [e] | 2.96 [b] | 2.78 [a] |
| | | B | 3.29 [d] | 9.71 [d] | 2.95 [b] | 2.87 [b] |
| | | C | 4.10 [e] | 9.05 [b,c] | 2.21 [a] | 2.89 [b] |
| | 2018 | A | 2.69 [c] | 8.45 [a] | 3.14 [b] | 3.18 [c] |
| | | B | 3.02 [c,d] | 8.75 [a,b] | 2.89 [b] | 3.19 [c] |
| | | C | 3.18 [d] | 9.24 [c] | 2.91 [b] | 3.14 [c] |
| | 2019 | A | 1.28 [a] | 10.92 [e] | 8.54 [d] | 3.67 [d] |
| | | B | 1.85 [b] | 10.08 [d] | 5.46 [c] | 3.68 [d] |
| | | C | 1.68 [a,b] | 9.30 [c] | 5.55 [c] | 3.66 [d] |

ANOVA was used to compare data. Means within each variety column followed by different letters ([a,b,c,d,e,f]) are significantly different from each other at $p < 0.05$ for Fisher's unprotected least significant differences test (LSD). X—the A variant was not monitored in 2019.

pH of grapes is related to the proportion of malic acid, tartaric acid, and $K^+$ concentration [43]. Samples with a higher amount of malic acid were shown to have higher pH values. There was no consistent treatment effect on pH. Despite the high TA in 2019, musts of all varieties had relatively high pH values. Such levels could be caused by the development of weather in that season, when there was significantly higher precipitation than in 2017 and 2018 (Figure 1). Under such conditions, there is a more pronounced accumulation of $K^+$ in leaves, which could have led to an increase in pH [38].

## 4. Conclusions

For each the of three varieties, the removal of 70% of leaf area led to the accumulation of lesser quantities of sugar in the grapes; therefore, these grapes contained the lowest values of TSS. Similarly to TSS, TA increased with the leaf area. 'Sauvignon Blanc' was particularly high in its sensitivity towards reducing the leaf area and acidity production. On the contrary, a minimum response was observed on this intervention in 'Pinot Gris'.

The tartaric acid to malic acid ratio changed depending on the season and the extent of the leaf area. The relationship between the proportion of these acids and the extent of the leaf area was confirmed for 'Sauvignon Blanc' and 'Pinot Gris' in more warm seasons (2018 and 2019), when the leaf area without reduction resulted in higher concentrations of malic acid. This study showed the complexity of the relationship between leaves and production of sugars and acidity of the grapes. As a result, any handling and regulation related to leaf area should be subject to careful considerations and adapted to not only the site-specific circumstances and climatic conditions, but also to intrinsic varietal features.

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