Effects of different types of leaf removal and shoot trimming performed in different periods on cv. Vermentino

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Dissertation to obtain a Master's Degree in

Engineering of Viticulture and Oenology – Double Degree

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2019

Acknowledgement

I would like to thank the University of Turin for the great opportunity and Professor Vittorino Novello for the support. Thanks to the Higher Institute of Agronomy and to Professor Carlos M.A. Lopes for the shown availability.

I would like to thank my family and my girlfriend, Beatrice, for always being with me.

Abstract

Vermentino is a cultivar traditionally cultivated in the west Mediterranean region; in Italy there are about 5000 hectares with this variety, mainly in Sardinia, Tuscany and Liguria. Each clone has specific characteristics concerning production, disease resistance, agronomic performance. To identify the best techniques to achieve the desire grape composition and quality, a series of treatments were performed in a company located in Sarzana, eastern Liguria: early defoliation (ED), post-flowering defoliation (PFD) for north (PFD-N) and south canopy side (PFD-S), apical late defoliation (ALD) and late shoot trimming (LT) compared with a control (CNTR), trimmed when the shoots exceed the wires placed at the top of the canopy to facilitate the tractors passage. ED reduced powdery mildew damages but leading to a lower TSS at harvest and a greater acidity degradation. PFD-S increased significantly TSS with a strong decrement in acidity. PFD-N led to significant differences for bunch weight, berries number and berries weight resulted lower than control. ALD did not confirmed what reported in literature, showing results similar to the control. LT showed a significant difference delaying sugar accumulation and maintaining higher acidity than control. For future studies, it will be necessary to understand if LT can compromise the future plant reserves for the following seasons; if ALD, tested again, will give significative results and if basal defoliation done without removing all the basal leaves but leaving some of them will achieve all the goals without modifying acidity or pH. In general, considering the results obtained with these treatments and the insufficient information founded in literature, further experiments will be necessary on Vermentino variety to achieve a greater awareness in the management of grapes and wine production.

Keywords: Vermentino – Green pruning – Canopy management – Leaf removal – Trimming – Bunch compactness

Resumo

O Vermentino é uma casta de uva de vinho tradicionalmente cultivada na região mediterrânica ocidental. Cada clone tem uma dimensão particular, forma, morfologia do cacho, produtividade variável, sabores típicos, diferente resistência às doenças. Para identificar as melhores técnicas para obter a composição e qualidade da uva desejada desta variedade, foram realizadas uma série de tratamentos: desfoliação precoce (ED), desfoliação pós-floração (PFD) orientada a norte (PFD-N) e a sul da copa (PFD-S), desfoliação apical tardia (ALD) e desponta tardia (LT) face a um controle (CNTR). A ED reduziu os danos causados pelas doenças fúngicas, mas levou a um TSS inferior na colheita e uma maior degradação da acidez. O PFD-S aumentou significativamente o TSS com um decréscimo forte na acidez. O PFD-N conduziu às diferenças significativas no peso médio do cacho, no número das bagas e o peso médio das mesmas bagas resultou mais baixo no controle. O ALD não confirmou o indicado na literatura, tendo mostrando resultados semelhantes ao controle. O LT mostrou diferença significativa em atrasar o acúmulo de açúcares e manter maior acidez no controle, permitindo reduzir o teor de TSS e manter uma alta acidez, contudo foi uma boa técnica para obter o objetivo da maior uniformidade na maturação da uva. Nos estudos futuros, será necessário aprender se o LT irá comprometer as futuras reservas da planta para as épocas seguintes e se o ALD, testado novamente, dará resultados significativos e se a desfoliação basal, feita sem remover todas as folhas basais, mas deixando algumas delas, atingirá todos os objetivos sem modificar a acidez ou o pH. Em geral, considerando os resultados obtidos com esses tratamentos e a insuficiente informação encontrada actualmente na literatura, novas experiências serão necessárias na variedade Vermentino para obter uma maior compreensão na gestão das uvas e produção do vinho.

Palavras-chave: Vermentino - Poda verde - Gestão da copa - Desfoliação – Consistência do cacho

Resumo alargado

O Vermentino é uma casta de uva de vinho tradicionalmente cultivada na região mediterrânica ocidental. Cada clone tem uma dimensão particular, forma, morfologia do cacho, produtividade variável, sabores típicos, diferente resistência às doenças. Esta característica das uvas pode permitir o estabelecimento de diversos protocolos de vinificação que podem ser escolhidos para diferenciar os vinhos produzidos e/ou para realçar as peculiaridades das uvas nas diversas áreas produtivas. Os objetivos deste estudo foram: avaliar os efeitos da desfoliação precoce (ED), desfoliação pós-floração (PFD) orientada a norte (PFD-N) e a sul da copa (PFD-S), desfoliação apical tardia (ALD) e desponta tardia (LT) face a um controle (CNTR), no cultivo do Vermentino para identificar as melhores técnicas para obter a composição desejada de uva e vinho em três vinhedos com características diferentes, principalmente em relação às condições climáticas e orientação das fileiras. A empresa "Il Monticello", onde os tratamentos foram testados, está localizada em Sarzana, leste da Ligúria, Itália. Consiste em uma adega e mais de 12 hectares de vinhedos, principalmente com cv. Vermentino: todos os vinhos brancos são produzidos com 100% de uvas Vermentino e a denominação de origem controlada "D.O.C. Colli di Luni". O objetivo desta experimentação é continue este trabalho de anos para encontrar o gerenciamento correto da coroa para obter o melhor características da uva em termos dos principais parâmetros de qualidade (pH, acidez, álcool, polifenóis totais). Comparando os resultados obtidos com o apresentado na literatura, houve algumas semelhanças e diferenças. A ED reduziu os danos causados pelas doenças fúngicas, mas levou a um TSS inferior na colheita e uma maior degradação da acidez. O PFD-S aumentou significativamente o TSS com um decréscimo forte na acidez. O PFD-N conduziu às diferenças significativas no peso médio do cacho, no número das bagas e o peso médio das mesmas bagas resultou mais baixo no controle. A desfoliação basal levou à realização de alguns objetivos, mas modificando alguns parâmetros tal como a acidez ou o pH, valores importantes na produção dos vinhos brancos. Assim sendo, seria ideal realizar uma desfoliação no período de floração sem remover todas as folhas basais, mas deixando algumas delas, preferindo, portanto, uma desfoliação manual muito precisa. O ALD não confirmou o indicado na literatura, mostrando resultados semelhantes ao controle. O LT mostrou diferença significativa em atrasar o acúmulo de açúcar e manter maior acidez no controle, permitindo reduzir o teor de TSS e manter uma alta acidez. Foi uma boa técnica para obter o objetivo de uma maior uniformidade na maturação da uva. Nos estudos futuros, será necessário aprender se o LT irá comprometer as futuras reservas da planta para as épocas seguintes e se o ALD, testado novamente, dará resultados significativos e se a desfoliação basal, feita sem remover todas as folhas basais, mas deixando algumas delas, atingirá todos os objetivos sem modificar a acidez ou o pH. Em geral, considerando os resultados obtidos com esses tratamentos e a insuficiente informação encontrada actualmente na literatura, novas experiências serão necessárias na variedade Vermentino para obter uma maior compreensão na gestão das uvas.

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ALD apical late defoliation

CNTR control

ED early defoliation

LT late trimming

PFD post flowering defoliation

PFD-N post flowering defoliation on canopy north exposed

PFD-S post flowering defoliation on canopy south exposed

TSS total soluble solids

1. Introduction

Summer pruning is a broad term that consists of a set of practices performed on the canopy during the growing season with different purposes, including regulation of size, vigour, reduction of the susceptibility to biotic and abiotic stress and usually aim to remediate deviations of vine development and yield from a well-defined pattern (Palliotti & Poni, 2011). It's important to have a focused application of summer pruning operations in the vineyards allowing the grower to obtain better grape composition; they can be used to mitigate specific features connected with the climate change, it is important to avoid them exclusively to adjust the excessive vegetation. The global warming is leading to a progressive shift toward sub-tropicalization of several viticulture areas causing shorter time intervals between phenological stages, an increased probability for berry sunburn, a significant increase in grape sugar concentration at harvest due to a faster ripening (Jones, 2010). Excessive total soluble solids accumulation has been linked to several other factors: an increase of CO_2 in the atmosphere that leads to a higher canopy photosynthetic potential (Ainsworth & Rogers, 2007); improvements in vineyard management; law-enforced yield limitations in several appellation areas; adoption of grapevine cultivars characterised by low cluster weight and/or grafted on low-vigour rootstocks. In the medium-to-long term these factors will likely affect the geographical distribution of viticulture (Keller, 2010; Caffarra & Eccel, 2011). Whereas, in the short term, new management techniques able to mitigate these negative impacts are needed (Palliotti *et al*., 2014a). The traditional summer pruning operations are cluster and shoot thinning, shoot positioning and hedging, elimination of lateral shoots and late season-basal leaf removal (at berry Total Soluble Solids (TSS) of 12-14 °Brix), and more recent techniques such as pre-flowering leaf removal (Palliotti & Poni, 2011; Diago *et al.,* 2010; Intrieri *et al.*, 2008). Most of these operations are easily mechanized with the exception of shoot and bunch thinning which, as a consequence, have a notable impact on yearly vineyard workload (Intrieri & Poni, 1995). Today, under the pressure of this climate change with its earlier, faster crop ripening and higher incidence of multiple summer stresses and sunburn damage, the economic sustainability is a worldwide essential condition and the philosophy and goals of these operations are also evolving (Gatti *et al.,* 2015). For example, an excessive light and thermal exposure of bunches should be avoided, in warm environments, especially in white cultivars, because it led to excessive loss or degradation of aroma, reducing freshness, promoting overripe flavours while concurrently total acidity was reduced because of excessive malic acid degradation. Under these conditions, the grapes often showed a high pH that required the addition of tartaric acid in order to avoid microbiological instability and improve mouth feel (Keller, 2010). Summer heat stresses may aggravate berry sunburn (Marais *et al.,* 1999). So, traditional leaf removal, typically undertaken between fruit set and veraison, was used in a much more conservative manner avoiding abrupted-exposure of bunches to high light by using some leaves around the bunches to protect them (Dry *et al.*, 2009). It can be done by hand with selective removal of primary and/or lateral leaves or by limiting leaf removal to the least exposed row side. Mechanical leaf removal became generally recommended because its action was non-selective and some leaves or portions always remained and cover the bunches (Intrieri *et al.*, 2008). Another goal of the leaf removal was the aromatic profile; in fact, this technique, when applied at the appropriate time, can affect the volatile composition of grapes and the resultant wines (Alessandrini *et al*., 2018). We must also consider the economic reasons behind this choice: in the past, manual bunch thinning was considered a technique to achieve automatically improvement of sugar and colour accumulation, but wine composition had, in several cases, proven to be quite poorly grounded in physiological terms (Howell, 2001). The amount and quality of leaf-to-fruit ratio was a reliable determinant of final grape composition and, in several instances, increasing a too low crop level was the simplest solution under medium to high vigour sites to achieve more balanced vines. Because of the frequent need in warm areas to prevent the rapid ripening that can cause a lacking in typical flavour, Poni *et al. (*2013) showed that with a correct canopy management it can be delayed. Long-term comparisons of different summer pruning techniques applied to induce changes of the seasonal source–sink balance is still rare in the literature (Gatti *et al.,* 2015) and the insufficiency of information is more pronounced for white cultivars, especially for the white cultivar Vermentino.

The aims of this study were: to assess the effects of pre-flowering basal leaf removal, post-flowering basal leaf removal, late-apical leaf removal, late shoot trimming and no-trimming techniques on bunch microclimate, vine performance and physiology in the white cultivar Vermentino to identify the best techniques to achieve the desired grape and wine composition in three vineyards with different characteristics mainly regarding climatic conditions and orientation of rows.

2. Literature review

2.1. Vermentino variety

Vermentino is a wine grape variety traditionally cultivated in the west Mediterranean region and recently introduced in new countries such as Australia, USA (California, North Carolina, Virginia, Texas), South Africa and Argentina where viticulture is developing fast. The most important geographical regions for the diffusion of this cultivar were Sardinia and Corsica, covering 3000 ha (Deidda *et al.,* 2003) and 1110 ha respectively. Vermentino was also found in Piedmont and Tuscany, and DNA analysis established its genetic identity with the traditional varieties Pigato and Favorita. This variety has discordant origins because someone thought that it was born in Spain and spread later in France and Corsica (Nieddu *et al.,* 2016). Fregoni (1999) hypothesizes a Middle Eastern origin, confirmed by the consistent size of the bunch and the grape that would include it within the Pontic Proles: starting from Anatolia, the vine first reached the Turkish coasts and then arrived in the Aegean islands; from there, the Greeks took it to Marseilles where, thanks to the Ligurian navigators, went to the southern coasts of France and subsequently spread to the coasts of Liguria, advancing inland to Piedmont and often changing its names. In fact, nowadays, it has a large number of synonyms (Nieddu *et al.,* 2016). In Italy it is cultivated also in Liguria up to the Livorno area and on Elba island, where it has distinguishable characteristics different from others. The high quality attained by the monovarietal wines, which allowed the growers to achieve a D.O.C. (Controlled Designation of Origin), is one of the reasons of the growing market achievements of this variety. In Italy there are about 5000 hectares with this variety (Nieddu *et al.,* 2016), including 220 hectares of Favorita (in Piedmont) and 264 of Pigato (in Liguria) which allowed the production of 50 D.O.P. (Protected Denomination of Origin) and I.G.P. (Protected Geographical Indication) wines and 21 D.O.C. and 1 D.O.C.G. (Controlled and Guaranteed Designation of Origin) in several regions such as Sardinia, Tuscany, Liguria, Piedmont, Puglia, Sicily, Abruzzo, Lazio, Marche and Umbria. About 75% of the total area is cultivated in Sardinia, 14.5% in Tuscany, 6.2% in Liguria, 2.6% is located in Piedmont and 1.7% in other regions. Vermentino is a relevant product of Italian enology, as it represented the fifth most sold wine in 2008 in Italian large retail stores and showed a continuous increase in the average selling price. Clonal selection of Vermentino was carried out in France and Italy, and several clones were selected (Bagard, 1997; Deidda *et al.,* 1997). Each clone was characterized by having a particular size, shape, morphology of the bunch and grape, variable productivity, typical flavours, different disease resistance and, in relation to the different geographic area selection, dissimilar agronomic and technological performance. The metabolic state of the plant changes rapidly in response to environmental stimuli such as ground exposure to light and drought conditions during ripening: the metabolites levels can be considered as the final response of biological systems to environmental and genetic changes due to complex cellular regulatory processes (Mulas *et al*., 2011). For this reason, the term terroir was coined in enology to describe and connect all the complex effects of environmental factors and agronomical practices that led to grape quality and, after the wine-making process, to wine uniqueness. Climate, soil, rootstock, cultivar, and human practices were the main parameters which define terroir (Van Leeuwen & Seguin, 2006).

In general, the cultivar Vermentino has these characteristics:

- Inflorescence: medium size, length 10-15 cm, cylindrical, tight;
- Leaf: medium-large, pentagonal, five-lobed, with a wide U-shaped petiole sinus and a closed lyre; bristly or arachnoid inferior page of light green color, protruding and green veins; glabrous upper page, dark green and with green veins; very pronounced irregular teeth, with convex margins;

- Bunch (at maturity): medium or medium-large size (15-20 cm long), mostly cylindrical but also pyramidal, average sparse; visible, herbaceous, medium-large peduncle;
- Berry: medium-large, regular in shape, spheroid with a circular cross-section, pruinose peel, averagely consistent, amber-yellow in color in years with favourable climatic conditions during maturation, otherwise the color is greenish-yellow; regular color distribution; juicy pulp with colourless juice, neutral flavour; medium pedicels that easily separate from the green grape, with an evident green leaf;
- Position of the first fruiting shoot: 1st-2nd node;
- Average number of inflorescences for each shoot: 2;
- Lateral shoots fertility: poor, however negligible;
- Resistance to adversity: rather sensitive to frost; it rather fears to the downy mildew and *Lobesia botrana*, while it is fairly resistant to powdery mildew;
- Rootstock: several plants in typical areas of production are without rootstocks; *Rupestris du Lot*, "420A" and "Kober 5BB" have proved to be excellent rootstocks.

(Italian Vitis Database)

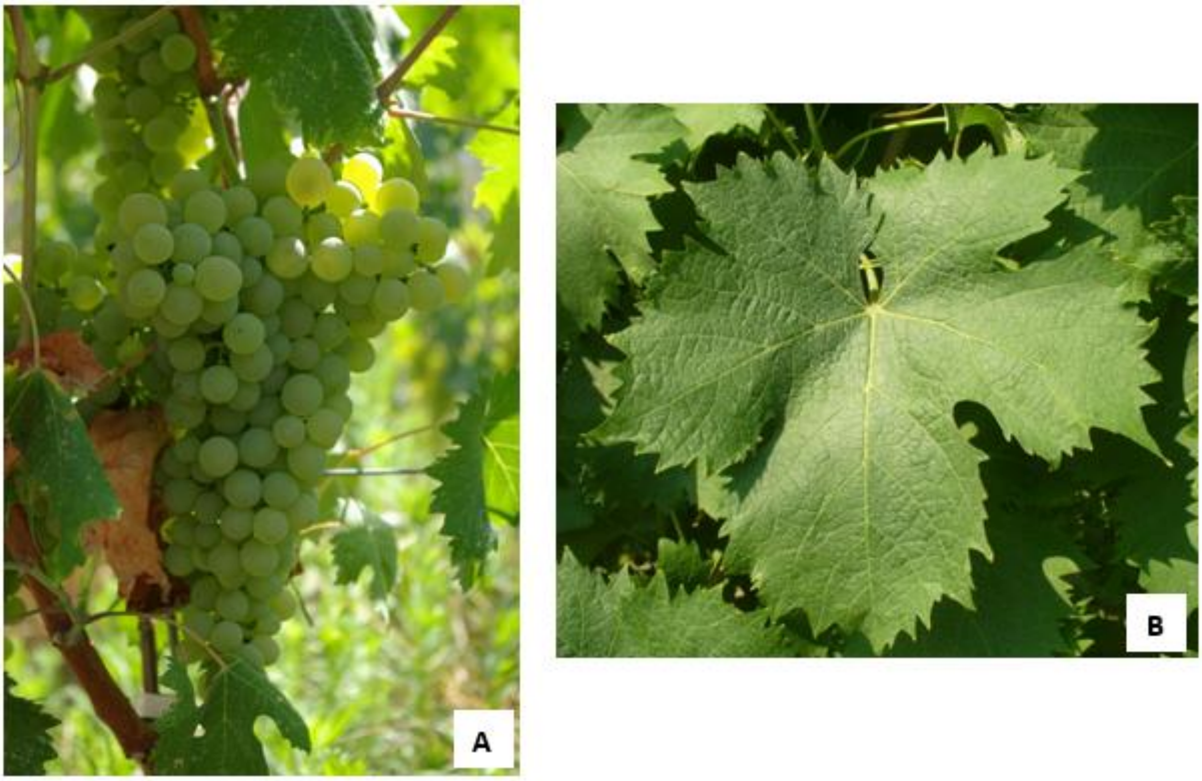

Figure 1: Typical bunch (A) and leaf (B) of Vermentino variety (Italian Vitis Database)

For the updating and qualification of the ampelographic national platform, the cultivar Vermentino was recommended for new plantations as it had interesting wine making characteristics. In fact, several works in the literature on the phenolic component of grapes (Corino *et al*., 1999) showed a good amount of total flavonoids and proanthocyanidins compared to other typical vines of the area such as Bosco and Albarola (in Liguria Region) and showed a sensitive production of secondary metabolites, aroma precursors such as vitispirani, Riesling acetal and 1,1,6-trimethyl-1,2-dihydro-naphthalene. It was also reported a reduced sensitivity to oxidation (Iannini *et al.,* 1985). These properties of the grapes can therefore allow a strong characterization of the wines produced (Bucelli & Storchi, 2007). Giordano *et al.* (2012) showed the unexpressed richness of the grape Vermentino, but also its oenological flexibility. This characteristic of the grapes can allow the setting of different wine-making protocols that can be chosen to differentiate the wines produced and/or to enhance the peculiarities of the grapes in the various production areas.

2.2. Defoliation

This operation was historically defined as *"the removal of some leaves from the fruiting area between fruit set and veraison"* (Smart, 1973) with the prevailing aim to obtain a better bunch microclimate and reduce rot incidence in canopies that were too dense (Gubler *et al.,* 1991). Leaf removal is a viticultural practice that is known to influence grape quality and production. With this technique, grape growers are able to manipulate and to reduce canopy density in order to improve fruit exposure and, in general, to induce changes of the seasonal source–sink balance using the right technique at the right time (Alessandrini *et al.*, 2018)**.** Leaf removal is one of the most interesting canopy management practices due to its simplicity and suitability to mechanisation (Palliotti *et al.,* 2014a).

2.3. Typologies of defoliation

On-going research has provided knowledge to distinguish different types of leaf removal aimed at quite distinct goals (Palliotti & Poni, 2011). This practice could be applied from pre-flowering to full veraison (Alessandrini *et al.*, 2018)**.**

2.3.1. Traditional method

Usually, leaf removal is carried out from fruit-set to veraison on high-density canopies to improve the exposure to light and the circulation of air around the clusters, with benefits in terms of tolerance to rot (Smart, 1985; Gubler *et al.,* 1991; Percival *et al.,* 1994). This operation can be done manually, requiring about 60 hr/ha, but with the increasing of labour costs and considering the working time it's possible to use a mechanical approach (2 hr/ha). The best timing for machine use, to make defoliation more effective, is about one-two weeks before veraison when berries are still hard while bunch weight is already much higher than that of leaves. Yield may not change (Hunter *et al.,* 1995) or might even increase as compared with non-defoliated vines (Zoecklein *et al.,* 1992). The variability of the impact that leaf removal has on yield and its components is strictly dependent on the negative effects on fruit-set and berry growth in the current year and the positive effects on budburst for the next year due to an improvement in canopy microclimate. This type of defoliation usually led to improvements in grape composition, with, more frequently, a slight increase in sugars and a decrease in malic acid content, attenuating also the herbaceous and grassy characters in the final wines (Smart, 1985; Zoecklein *et al.,* 1992; Scheiner *et al.,* 2010). Moreover, Petrie *et al.* (2003) found that leaf removal from the lower quarter of the canopy during the lag phase of berry growth caused a significant decrease of whole-vine photosynthesis suggesting that the lower portion of the canopy contributed more than the upper portion to the whole-vine carbon amount. A possible explanation is that the basal and older leaves are removed by defoliation, but they are also the largest leaves along the shoot and their size can compensate lower photosynthetic rates (Poni *et al.,* 1994). Therefore, lowering the photosynthesis of the shoots cannot be insignificant especially for leaf removals performed after fruit-set. Additionally, the removal of all the leaves from the fruiting area exposes the clusters to full sun and might lead, in warm climates, to compromise fruit composition due to excessive berry temperatures, which can compromise the colour formation and cause a reduction in malic acid concentrations (Tarara *et al.,* 2008). For these reasons and considering the increasing preoccupation about berry sunburn, the criteria to apply leaf removal have become more restrictive with differentiation in the actual need and/or severity of this removal depending up to specific planting choices. For example, no or very light defoliation is usually applied on the south facing side in an east-west oriented row, while more severe leaf removal can be necessary on the north facing row side. The popularity of this traditional method is decreased over the last two decades probably due to the scientific progress in leaf and whole-canopy physiology and to new pressure from global warming (Palliotti & Poni, 2011).

2.3.2. Early leaf removal

Early leaf removal carried out at pre-flowering is a viticultural practice to regulate yield components and improve grape quality (Reynolds, 1989). At this stage, the defoliation reduces fruit-set and berry weight, leading to smaller and looser clusters, increasing the skin-to-berry and seed-to-berry ratio (Tardaguila *et al.,* 2010). Moreover, the temporary source limitation induced by removing an average of six main basal leaves before flowering has led, in a series of genotypes and growing conditions, to a significant reduction of cluster compactness resulting in a reduction of bunch rot incidence maybe due to an improve in ventilation in the canopy (Intrieri *et al.,* 2008; Diago *et al.,* 2010; Gatti *et al.,* 2015). This feature may be important for cultivars grown in humid climates (Poni *et al.*, 2006). Furthermore, defoliated shoots generally have a higher final leaf-to-fruit ratio than control (Poni *et al*., 2006). This effect on crop regulation due to early leaf removal is constant and repeats, mainly because carbohydrate supply at the pre-flowering stage is the primary regulator of subsequent fruit-set (Coombe, 1962) and a temporary foliar stress can reduce cell division rates during the green stage of berry growth; this has negative effects on final berry size (Palliotti *et al.,* 2009). Moreover, this technique showed significant improvement in final grape composition and greater wine appreciation, due to the fact that a precocious source limitation carried out in the form of defoliation hastens translocation of assimilates towards the cluster (Quinlan & Weaver, 1970), improving grape composition in the defoliated shoots that is also related to the quality of the source. For example, removing the main six basal leaves at pre-bloom causes an abrupt and severe decrease in vine photosynthesis: 75% less than not-defoliated (Poni *et al.,* 2008). However, removing source leaves around bloom also triggers a series of dynamic changes in canopy growth: defoliated vines have a younger canopy at veraison because leaves in a median and apical shoot position at this time are mature and more lateral leaves may be present as a compensating reaction to early main leaf removal. Palliotti *et al.* (2011 b) shown that early defoliation applied before flowering with the elimination of 80% of the leaf area as compared with a non-defoliated control was quite effective in limiting yield per vine, cluster weight, cluster compactness, rot incidence, berry set and mass in two of three seasons. New leaves on lateral shoots of defoliated plants have higher assimilation rate, intrinsic water use efficiency, tolerance to photoinhibition than non-defoliated ones (Palliotti *et al.,* 2011 a). The accumulation of sugar in berries was accelerated in defoliated vines, leading, at harvest, at higher must TSS and phenolic and anthocyanin concentrations than non-defoliated vines (Palliotti *et al.,* 2011 b). Essentially, the most important outcome is that, irrespective of genotype, the early leaf removal considerably improves grape composition and wine sensory properties as compared to non-defoliated shoots (Diago *et al.,* 2010; Palliotti *et al.,* 2011 b). This technique showed to be reliable, especially where legal limiting of yield was associated with traits of high vegetative vigour, high node fruitfulness and compact clusters. Seasonal changes in the crop load determined by early leaf removal increased canopy efficiency, especially during the veraison/ripening period; such characteristics, together with the ability to control vigour without compromising the vine capacity and wood carbohydrate replenishment, were the physiological bases for improved grape and wine composition regardless of year-to-year variability (Palliotti *et al.,* 2011 b). Furthermore, Mescalchin *et al.* (2008) have shown on the variety Pinot Gris that an earlier defoliation leads to lesser incidence of skin burning on VSP and pergola-trained varieties due to the formation of a thicker skin. Removing all the leaves from the fruiting area, there is an exposure of the clusters to full sun and it might lead in warm climates to compromised fruit composition due to excessive berry temperatures, which can hinder colour formation and cause a clear drop in malic acid concentrations (Bergqvist *et al.,* 2001). The quantity of tartaric acid was studied by Kliewer & Schultz (1964) who reported higher amounts of $^{14}CO_2$ incorporated into tartaric acid for berries held in full sun as compared with shaded ones. The increasing of total soluble solids shown no effects on titratable acidity, corresponding to a better quality of the resulting must (Alessandrini *et al.*, 2018)**.** In addition, berry sensibility to temperature and light varies among ripening stages and it is influenced by cultural practices (Downey *et al*., 2006). At the third stage of berry growth (veraison), low night temperatures seem to stimulate anthocyanin accumulation while extremely high temperatures are known to inhibit polyphenols synthesis and to promote anthocyanin degradation (Mori *et al*., 2007). The higher anthocyanin and phenol content in berries on defoliated vines agree with the increase of skin-to-berry ratio and phenolic and colour substances, mainly present in skin tissues (Roby *et al.,* 2004). Leaf removal was shown to affect grape volatile composition and the resultant wines (Alessandrini *et al.*, 2018); indeed, it increases the monoterpene content in Sauvignon blanc grapes (Hunter *et al.,* 2004), enhances potentially volatile terpene content in berries and in the musts of Gewürztraminer and Chardonnay Musqué varieties. The resulting wines had higher citrus, tropical and floral aromas and in an amount higher than those of untreated vines (Reynolds *et al.,* 2007). For instance, Zoecklein *et al.* (1998) found that the concentration of total glycosidic precursors in Riesling and Chardonnay grapes was higher when leaves had been pulled. Generally, defoliation increases terpenes and norisoprenoids but reduces methoxypyrazines in grapes and wines. It is associated as well with an increased on the perception of fruity/tropical fruits and floral, at the contrary of untreated vines with an increase of vegetal nuance and acidity (Suklje *et al.,* 2014). This technique can be carried out with machines, but mechanization is feasible by preferably using at pre-flowering (closed-flower stage) an air pressure blowing machine which can run two passages per row in about 5-7 hr/ha (Intrieri *et al.,* 2008). The best performance is obtained on canopies characterized by vertical and well positioned shoots, on cultivars with mostly erect inflorescences. It has to be kept in mind that early leaf removal is specifically recommended in highly productive vineyards which often present heavy, thick bunches very susceptible to rot. These data indicated that early mechanical defoliation is a viable crop adjustment tool in a Mediterranean growing area. Based on the constancy of the results obtained under the above circumstances, this practice is nowadays an interesting alternative to the other traditional methods of crop control, such as bunch thinning. Indeed, Palliotti *et al.* (2011 b) showed advantages like feasibility of mechanization, cost saving and different mechanisms to adjust the final yield. Post-flowering leaf removal appears to be more effective than an earlier removal when the goal is also to obtain some control on berry size, and even post-bloom defoliations are quite effective in causing abortion or growth arrest of set berries (Petrie *et al.,* 2003). Although many studies have focused the attention on the effects of leaf removal on grapevine yield and quality, there are still many agronomic and economical aspects that need further investigation. For instance, the definition of the best period and methodology of defoliation for a given terroir and their implication on berry colour development need to be analysed and addressed (Oliveira *et al.*, 2011). Another mechanism acting in favour of non-limiting final leaf-to-fruit ratio in the defoliated plots is the tendency to offset the loss of removed leaf area by promoting a stronger lateral shoot growth, which was more pronounced under earlier treatments according to the Trebbiano data (Poni *et al.*, 2006). Leaf removal influences light interception and plants architecture, which must be adapted to local climate and soil conditions as well as varietal behaviour (Chorti *et al*., 2010).

2.3.3. Late leaf removal

The global warming is implicated in causing earlier vintages with the policy guidelines in Italy that strictly limit yield per hectare in appellation areas and the vineyard efficiency relative to berry sugar-storage capacity that has increased. This increment can be due to the progressive rise in atmospheric CO_2 concentration leading to a higher leaf assimilation rate, often result in a crop that, at a notably early stage (mid-August), shows a high potential alcohol content and low acidity, a fruit composition would suggest immediate harvesting (Poni *et al.,* 2013). At the same time, however, pH is usually high, thereby favouring microbial instability of wines and constraining the expression of full grape aroma potential (Jackson, 2008). Based on some pioneer work done on Riesling, a severe leaf removal apical to the bunch zone prior to veraison causes a ripening delay of about 2 weeks as compared to non-defoliated vines (Stoll *et al.,* 2010). This quite revolutionary approach has recently undergone to a more rigorous evaluation (Palliotti *et al.,* 2013a), starting from its physiological background: around veraison, the leaves located on the apical two-third of the canopy are the most functional having reached full expansion and are still far from senescence (Poni *et al.*, 1994). A two-year study by Palliotti *et al.* (2014a) on mechanical post-veraison, apical to the bunch zone, leaf removal on cv. Sangiovese showed a reduced leaf-to-fruit ratio (from 1.77 to 1.13 m^2/kg) and demonstrated its potential to delay optimal total soluble solid accumulation in the berry by about 2 weeks as compared to non-defoliated vines. At the same harvest date, defoliated vines had 1.2 °Brix lower in the must and such difference was evident in the wine alcohol contents (−0.6% vol.). Similar results have been obtained on cv. Montepulciano by Lanari *et al.* (2012) and Poni *et al.* (2013) shown a straightforward relationship between physiological adaptation to late-season leaf removal applied above the bunch area and final grape composition. A temporary delay of technological ripeness without affecting colour and phenolics was in fact driven by defoliation above the bunch zone applied at post-veraison (12 °Brix). This canopy management technique is easily mechanized, and the target is a canopy area spatially distant from the fruiting area, therefore the problem of cluster damage is solved, allowing a speed machine higher than traditional one (Palliotti *et al*., 2014a). Additionally, while being able to significantly delay berry TSS accumulation, this technique did not affect the phenolic compounds in grapes and wines or the replenishment of reserves storage in canes and roots (Palliotti *et al*., 2013a); to be effective at significantly delaying TSS accumulation in the berries it is advised to remove leaves at around 14–15 °Brix while ensuring that at least 30–35% of the leaf area is removed (Palliotti *et al*., 2014a). Thus, it appears there is a possibility for a decoupling between the two ripeness types that would result in a wine style that is much in demand by the market nowadays: one that is less alcoholic to meet the trend for 'light drinking' while at the same time largely retaining its phenolic substances, which have a well-documented positive action on human health. More work is now needed in the field to verify over a longer term the consistent reproducibility tasting different genotypes and environments (Poni *et al.,* 2013).

2.4. Shoot trimming

Practices aimed at manipulating vegetative growth during late-spring and summer, particularly in vigorous vineyards, are used substantially to influence yield and grape composition. Hedging is a common management practice used to maintain canopy shape, reduce vine vigour, improve the microclimate in the fruiting zone, increase the efficiency of disease treatments, facilitate harvest and the access of machines to the vineyard rows. Compared with other summer management practices used for similar goals, such as leaf removal and pulling of lateral shoots, hedging is commonly used because it can be done completely mechanically and is fast, easy and cheap. The effects of hedging on yield and fruit quality, considering the variables of timing and severity of application, are strictly associated to the ability of the cultivar to develop lateral shoots and their photosynthetic capacity from veraison to harvest (Cartechini *et al.,* 1998). The impact of hedging severity on vine performance is well known; severe hedging, less than six main leaves retained per shoot, generally reduces grape quality (Palliotti, 1992), whereas the time of application is rather controversial because other factors may also influence these effects such as bud load, shoot orientation, training system, environmental conditions, soil characteristics, water availability. Vertical shoot positioned (VSP) training systems are normally trimmed when their shoots exceed the wires placed at the top of the canopy and, usually, the timing is poorly dependent on grower's decisions and it is instead a function of intrinsic shoot vigour and vine balance. A balanced vineyard would reach the height suitable for trimming around fruit set, whereas an excessively vigorous one would get to the same growth stage much earlier (Palliotti & Poni, 2011). The positive outcomes of the early hedging are dependent upon a cultivar's ability to develop lateral shoots after trimming: all the cultivars with a good capacity to produce laterals, such as Cabernet Sauvignon, Verdello, and Sauvignon blanc, responded better to early summer pruning as shown by the increased cluster weight and yield and improved contents of soluble solids, total polyphenols and nitrogen content. Trimming vines increased lateral growth and the total final leaf area was always less than that recorded in control vines in the study of Palliotti & Poni (2011). In this work, at harvest, in all the grapevines tested, early-hedging reduced the leaf/fruit ratio in comparison to the control vines and improved the soluble solids content, whereas late-hedging caused a reduction of both leaf/fruit ratio and soluble solid accumulation in the berries. The rejuvenation of leaf area in the canopy following early-hedging and their high photosynthetic efficiency from veraison to harvest of the newly formed lateral leaves likely reduced the leaf area per gram of fruit required to achieve adequate ripeness. These laterals also translocate assimilates to the subtending clusters very efficiently (Candolfi-Vasconcelos & Koblet, 1990). Negative results found on late-hedged vines, also reported by other authors (Palliotti, 1992), are probably linked to the fact that lateral shoots compete with the developing grapes for carbohydrates, causing delayed berry growth and sugar accumulation. Early trimming reduced titratable acidity as compared to control vines due to greater cluster exposure to sunlight transpiration activity. We can have different physiological responses based on the variety's characteristics: Filippetti *et al.* (2011) applied a shoot trimming on cv. Sangiovese one week after veraison and achieved a significant reduction in the TSS accumulation without changing the pH and the content of organic acids, tannins and anthocyanins in seeds and skins; for other cultivars like Grenache and Tempranillo, a particularly aggressive shoot trimming made at fruit-set immediately above the distal bunches, significantly decreased the leaf area-to-yield ratio and slowed down ripening in terms of lower TSS in the must as well as phenols, anthocyanins and pH in wine (Balda *et al*., 2011). Stoll *et al.* (2010) have reported for a shoot trimming carried out on cv. Riesling at fruit-set a maturity delay of about 20 days, and a significant reduction of the sugar accumulation in the must (4 °Brix less than untrimmed vines). Late shoot trimming (5 weeks after flowering) as compared to an earlier one (1 week after flowering) determined a lag in the accumulation of TSS and in the degradation of organic acids, delaying the optimal time of harvest of several grapevine varieties trained to high wire cordon (Palliotti, 1992; Cartechini *et al.,* 1998). Regardless of the variability linked to variety and harvest, these results are due to two main effects: significant reduction of the leaf area-to-yield ratio; carbon competition between the developing laterals and the accumulation of sugar into grapes (Palliotti *et al*., 2014a). It is obvious that the results expected from these techniques are tightly bound to the timing and severity of the intervention and also to the vine vigor, soil fertility and environmental factors, primarily rainfall, which may promote laterals growth late in the season. Thus, the unpredictable weather course after trimming is really important for the efficacy of this technique. In theory, a relatively late shoot trimming might induce a competitive re-growth of laterals with subsequent reduction in total soluble solid production and their accumulation in berries (Palliotti *et al.,* 2014a). In all the grapevine cultivars that develop many laterals after hedging, the greater transpiration rate estimates in these leaves, compared with primary ones, particularly in August and September, may aggravate susceptibility to vine water stress especially in hot environments and in particularly dry years (Palliotti & Poni, 2011). During the first two weeks of November, the laterals on the vines had net photosynthesis values that ranged from 0.7 to 1.6 µmol CO_2 m^{-2} s^{-1}, in a period when all the carbohydrates fixed are very useful for the reserve accumulation, and therefore for increased cold hardiness (Wample & Bary, 1992) and even for budbreak and initial shoot growth the following season. Thus, at the end of the season the integrity of these leaves must be maintained until total abscission occurs. Early winter pruning, practiced in some viticulture areas, should be avoided (Palliotti & Poni, 2011). It would be very interesting to study other trimming intensities, as well as other times of intervention.

2.5. Other canopy management techniques

2.5.1. Late winter pruning

Generally, winter pruning applied late in the season, when buds have already begun to swell, is done to postpone bud-break by a few days, helping at escaping spring frost (Coombe, 1962) and, moreover, it can be a promising tool to delay ripening (Palliotti *et al.*, 2014a). In New Zealand, Friend & Trought (2007) worked on Merlot and shown that winter pruning applied when the upper part of the canes has shoots around 5 cm long, decreases berry total soluble solid, slow down the degradation of acids and helps to maintain an optimum pH of the must. Palliotti *et al.* (2014b) worked on spur-pruned cv. Sangiovese and shown that postponing winter pruning to beginning of May (inflorescence swelling, and flowers closely pressed together) caused a significant decrease of yield per vine and delay TSS accumulation as compared to vines pruned at dormancy and beginning of bud swelling. This late pruning reduced the number of inflorescences per vine, decreasing fruit-set and berry and bunch weight. These results suggest that a source limitation induced with a very late winter pruning cause that the inflorescence primordia already initiated during late spring/summer of the previous year partially lose their ability to differentiate flower initials or to complete floral formation. Additionally, at the single bud level, the differentiation of flower is highly influenced by the strong nutritional competition exerted by the growing shoot apices (Lebon *et al.,* 2008). However, this promising technique needs further studies to clarify: seasonal changes of the whole canopy and the source-sink balance triggered by late winter pruning; the repeatability and consistency of effects under the variables of different cultivars, environments and pruning dates to find the best compromise (Palliotti *et al.*, 2014a).

2.5.2. Shading nets application

The application of shading net within-canopy: shading is a great contributor to unbalanced must and poor-quality wine (Smart, 1985). Both natural and artificial shades delayed berry ripening, reduced TSS, anthocyanin and phenolic compounds and, on the contrary, increased total acidity (Morrison & Noble, 1990). Recently, these results have been verified and showed that they were more due to leaf shading rather than bunch shading: while, referencing to flavonoids, the effects were mainly linked to the expression of their biosynthesis genes (Downey *et al.,* 2004). However, Jeong *et al.* (2004) on Cabernet Sauvignon have found a significant decrease of anthocyanins accumulation following the shading of clusters at veraison (78–80% of reduction of incoming radiation) due to suppression and/or delay of mRNA accumulation of VvmybA1, a putative regulatory gene of anthocyanins biosynthesis. Shahak *et al.* (2006) reported, studying Red Globe table grapes, that the kinetics of berry TSS was affected by net color: red and black nets delayed TSS accumulation, contrary to the white net. Beyond this potential, several issues need clarification and among them: better understanding of the relationship between timing and duration of shading and degree and extent of the maturity delay;

comparison of whole-vine and specific canopy portion shading and, at the end, analysis of technical feasibility of canopy shading (training systems, mechanization of setting and removal of the net, cost-to-benefit ratio, etc.) (Palliotti *et al.*, 2014a).

2.5.3. Antitranspirant sprays

Apply antitranspirant compounds able to induce a partial, although, persistent stomatal closure which can limit water loss and photosynthesis is a third option to induce a controlled source limitation in healthy, mature leaves (Palliotti *et al.,* 2010). Antitranspirants have been widely used to hinder drought events since, once applied to leaves, they significantly reduce water loss and heat stress (Gale & Poljakoff-Mayber, 1967). The following two types of antitranspirant have been classified, depending on the mode of action: film-forming polymers sprayed on leaf surfaces and stomata-closing compounds, alkenyl-succinic acids, phenylmercuric acetate, abscisic acid and chitosan (Zelitch, 1969). The film-forming polymer kaolin, an inert clay mineral, was effective at controlling heat stress, reducing leaf and fruit tissue temperature (Rosati, 2007). Additionally, the effectiveness of a post-veraison application of the film-forming antitranspirant named Vapor Gard was investigated as a technique to delay grape ripening and reduce TSS accumulation in berries (Palliotti *et al.,* 2013b); applied with a concentration of 2% to the upper two-thirds of the canopy, to the most functional leaves, reduced sugar accumulation in berries and alcohol content in the final wine, without compromising storage of carbohydrates and total nitrogen in canes and roots. Moreover, organic acids, pH and phenolic compound of grapes and wines were unaffected, except a lowering in anthocyanin content that is certainly undesirable if the wines are destined for aging, but it would be acceptable for young, rosé or base wines. On the other hand, in cultivar naturally rich in extractable anthocyanins (>1 g/kg) such as Teroldego, Marzemino, Syrah, Merlot, Montepulciano, etc., a loss of 15–20% of anthocyanins are quite sustainable. Finally, to be effective it is advised to perform the spraying at around 14–15 °Brix making sure that the lower epidermis is fully wetted by the product. Generally, using an anti-transpiration to trigger the source limitation needed to achieve a significant ripening delay is quite a solution fully non-invasive and does not require any specific equipment or skill, unlike the practices of leaf removal and shading. Besides, it is flexible as the desired effect can be obtained through adjustment of dosage, timing and number of sprays and target size (whole-canopy or specific portions) (Palliotti *et al.*, 2014a).

3. Materials and methods

3.1. The company structures

The company's name is "Il Monticello" and it is situated in Sarzana, Eastern Liguria (Fig. 2), Italy.

Figure 2: Italy map with in evidence the region Liguria (Tuttitalia website)

It consists of a cellar and more than 12 hectares mainly of Vermentino and Sangiovese varieties which vineyards are dedicated to the production of white, red, rosé wine, raisin and grappa. The company was founded in 1982 and it has a family management. Thanks to the cooperation with a Piedmontese oenologist, "Il Monticello" approached organic farming. Vineyards are grown with huge efforts, implementing natural farming methods, since the respect of the environment and the search for quality. The production is more than 80'000 wine bottles exclusively from local selected vine varieties of Vermentino, Sangiovese, Canaiolo, Ciliegiolo, Pollera Nera and Massaretta. The variety that characterizes this area is Vermentino: all the white wines are produced with Vermentino grapes 100% and it's shown on the label the controlled designation of origin "D.O.C. Colli di Luni Vermentino". For this quality assurance there are rules to be respected in the vineyards and in the cellar, for example:

- maximum yield per hectare 11 tons;
- minimum alcohol content 11% vol.;
- it is allowed only the rescue irrigation;
- yield grapes-wine maximum 70%;
- titratable acidity minimum 4.5 g/L;
- minimum dry extract 15 g/L.

During the past, different techniques have been tested in the vineyards because each parcel has different characteristics regarding soil, planting system, local climate, clone, and rootstock. The goal of this experimentation is to continue this work of years to find the right canopy management and obtain better grapes characteristics in terms of quality main parameters (pH, acidity, alcohol, total polyphenols), resistance to pathogens, especially powdery mildew.

3.2. Experimental site and plant material

The trial was carried out in three non-irrigated vineyards (Fig. 4) located in Liguria, Northern Italy: the first one (A) is situated in Sarzana (Fig. 3) (44°08'01.7"N 9°57'08.0"E, 53 a.s.l.), near the border with Tuscany, with row orientation E-W; the second one (B) is situated in Ponzano (44°09'03.7"N 9°55'38.7"E, 50 a.s.l.), near La Spezia, with row orientation NW-SE; the third one, vineyard C, is situated near Sarzana (44°07'08.2"N 9°58'49.7"E, 40 a.s.l.).

Figure 3: Map of the region Liguria with Sarzana, the city of the study, circled in red (Treccani website)

Vines were 10-year-old Vermentino grapevines (*Vitis vinifera* L.) grafted onto K5BB, spaced 2.30 × 0.90 m in the vineyard A; 15-year-old Vermentino grapevines grafted onto K5BB, spaced 2.00 x 0.70 m in B; 10-year-old Vermentino grapevines grafted onto K5BB, spaced 2.30 x 0.90 m in C (Fig. 4). The soil of these vineyards is similar: sandy, not too much calcareous, slightly alkaline pH. In these vineyards the vines are trained to a VSP with a Guyot pruning type. In fact, Vermentino presents the following characteristics: poor basal fertility, poor ability to regrow from dormant or crown buds, long internode length. So, during winter pruning, the 1-year-old canes bearing 10–11 buds are positioned on a horizontal galvanised steel wire located at 80 cm above ground; during the vegetative season, the growing shoots are positioned vertically using a vertical trellis system composed of three pairs of horizontal movable wires located at 70, 115 and 160 cm above ground. Regarding the soil management, each year after the harvest it is decided, with the agronomist, which vineyards need a green manure or grassing with specific essences or manuring with a manure spreader. The choices are based on deficiencies and vigour problems. The vineyard A, B and C had a natural grassing between the rows during winter, and it was used a mechanical disk-weeder which covered the under-row with soil. Between January and February, a tool coupled to a tractor was used to remove the shoots cut with pruning left between rows and another one was used to break the processing sole formed by the passage of tractors during the year, in alternate rows. During spring months, a mechanical weeder undervine with sensing device to avoid posts and vine trunks was used to control weeds between plants in the rows and equipment for inter-rows management with chain mowers to cut the weed. During May a shoot thinning was carried out to remove non-fruitful and/or less fertile shoots in order to reduce shoot density. It was done manually, and the shoots usually removed were short, non-fruitful, twins. A simultaneous cluster thinning can also be done if fruitful shoots were also removed. In June, the vegetation was positioned inside the wires manually. During the entire vegetative phase numerous visual checks were performed and, based also on the forecast models, the treatments for downy mildew and powdery mildew were decided.

Figure 4: Three vineyards object of study where A has an area of 2730 m^2, B of 6703 m^2 and C of 7060 m^2(Google maps website)

3.3. Climatic characterization

The hills lying immediately beyond the coast together with the sea account for a mild climate year-round. Average winter temperatures were 7 to 10 °C and summer temperatures were 23 to 24 °C. Rainfall can be abundant at times, as mountains very close to the coast create an orographic effect. Genova and La Spezia can see up to 2'000 mm of rain in a year; other areas instead showed the normal Mediterranean rainfall of 500 to 800 mm annually. The area around La Spezia was included in the area of warm temperate climates, in particular of the sub-Mediterranean type, even though the winters were often windy and, thanks to the mitigating action of the Ligurian Sea and the scirocco wind from Africa, it was possible to have days with rather cold temperatures. Two meteorological stations were located in the municipal area of Sarzana: the meteorological station of Sarzana and the meteorological station of Luni. They were officially recognized by the World Meteorological Organization and were the reference point for the study of the climate that characterizes the city and its plain. According to the climatic averages of the period 1971-2000, the average temperature of the coldest month, January, was +7.3 °C, while that of the hottest month, August, was +23.4 °C. On average, there were 17 days of frost per year and 25 days with a maximum temperature equal to or higher than +30 °C. The extreme temperature values recorded in the same thirty years were -9.0 °C in January 1985 and +38.2 °C in August 1985. Average annual rainfall amounts to 1106.4 mm, averagely distributed in 89 rainy days, with relative minimum in summer, maximum peak in autumn and secondary maximum in spring for accumulations. The average annual relative humidity showed a value of 73.4% with a minimum of 70% in July and a maximum of 77% in October and November; on average, there are 6 days of fog a year. The tables (1 and 2) below show the climatic averages and the absolute maximum and minimum values recorded in the thirty years 1971-2000 and published in the Climatological Atlas of Italy for the same thirty years.

Table 1: Climatic averages and the absolute maximum and minimum values recorded from January to July in the thirty years 1971-2000 (Climatological Atlas of Italy, Wikipedia website).

Month	Jan	Feb	Mar	Apr	May	Jun	Jul
Record high (°C)	18.4	20.0	24.0	26.2	32.4	34.8	36.4
Average high (°C)	11.3	12.3	14.6	17.1	21.7	25.2	28.7
Daily mean (°C)	7.3	8.0	10.1	12.6	16.8	20.2	23.2
Average low (°C)	3.3	3.7	5.6	8.1	11.9	15.2	17.8
Record low (°C)	-9.0	-5.0	-4.9	-3.6	3.8	7.8	10.4
Average precipitation low (mm)	116.0	91.5	79.4	100.8	74.1	62.5	25.4
Average precipitation days (≥1.0mm)	9.4	7.5	7.5	9.2	7.6	5.7	2.9

Average relative humidity (%)	73	69	68	72	72	72	69

Table 2: Climatic averages and the absolute maximum and minimum values recorded from August to September in the thirty years 1971-2000 (Climatological Atlas of Italy, Wikipedia website).

Month	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Year**
Record high (°C)	38.2	34.2	29.0	24.4	19.2	38.2
Average high (°C)	28.8	24.9	20.2	15.0	12.1	19.3
Daily mean (°C)	23.4	19.8	15.7	11.0	8.2	14.7
Average low (°C)	17.9	14.8	11.3	7.0	4.4	10.1
Record low (°C)	7.6	6.0	0.0	-3.6	-6.6	-9.0
Average precipitation low (mm)	50.2	101.9	157.9	134.0	112.7	1106.4
Average precipitation days (≥1.0mm)	4.2	6.4	10.0	8.8	8.7	87.9
Average relative humidity (%)	70	71	74	74	73	71

Rainfall and air temperature were measured hourly throughout the experiment in two weather station located at the experimental sites. In Figure 5 the temperatures have been reported; humidity and precipitations (Fig. 6) were recorded during the 2019 year by two weather stations located near vineyard A and B. Total rainfall was 578.8 mm until August.

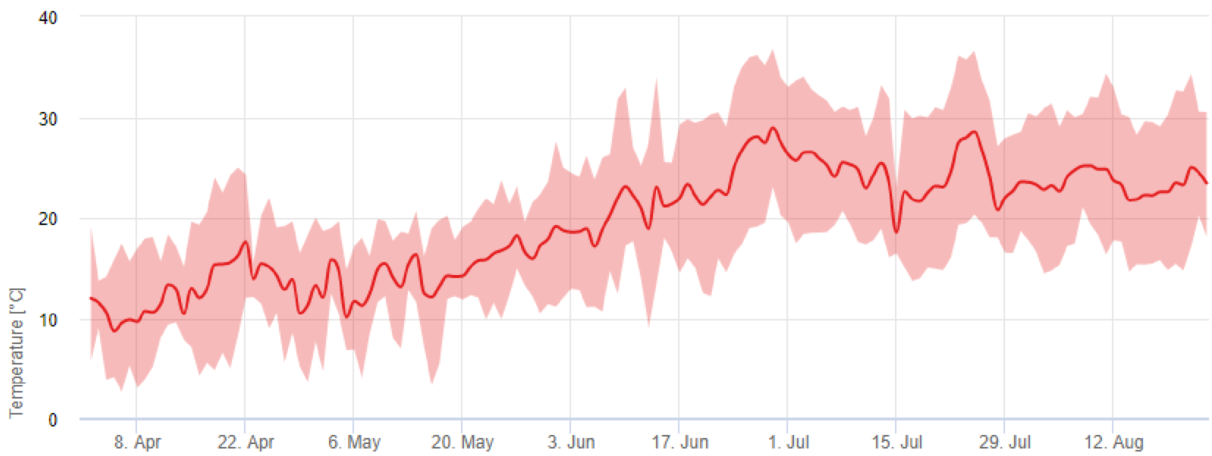

Figure 5: Air temperature of the year 2019 (Field climate website).

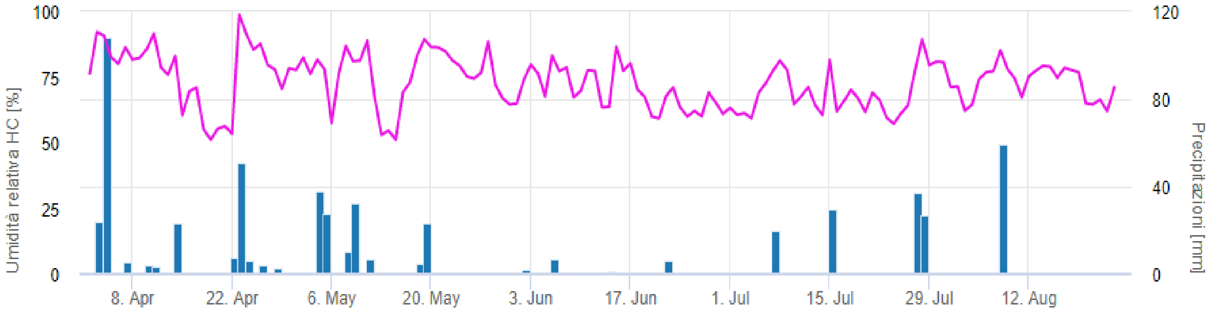

Figure 6: Humidity and precipitation for the year 2019 (Fieldclimate website).

3.4. Phenological stages and method of detection

To describe the annual cycle of the vine and in particular the vegetative sub-cycle, the BBCH code was used, doing the observation once a week from before budburst to harvest. This code followed a guide that expected the collection of data on a minimum of three representative plants or at least five buds per plant (on a VSP training system is considered the central part of the cane); the assigned pheno-phase should cover at least 50% of observed organs (leaves, shoots, bunches) and the data should be collected weekly or at least each 14 days (Lorenz *et al*., 1995). The range of values went from:

- 000-009: from dormancy to bud burst (Fig. 7);
- 101-114: from the first unfolded leaf to the fourteenth (Fig. 7);

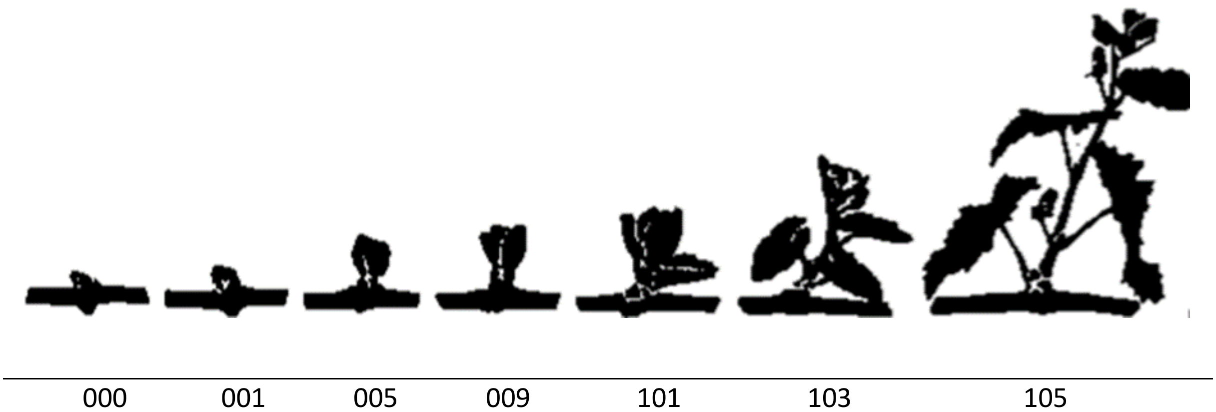

Figure 7: Code BBCH examples from 000 to 105, five unfolded leaves (Lorenz et al., 1995).

- 503-507: from inflorescences clearly visible to inflorescence full developed; flower separating;
- 601-609: from 10% to 100% of flowering (Fig. 8)

601 603 605 608

Figure 8: Code BBCH examples, from 601 to 608 (Lorenz et al., 1995).

- 701-709: from fruit set to the majority of berries touching (Fig. 9);

701 703 705 707

Figure 9: Code BBCH examples, from 701 to 707 (Lorenz et al., 1995).

- 801-809: from 5% to 100% of veraison, with °Brix detection until harvest.

To have a more detailed code was followed the Annex 1 and Annex 2.

3.5. Treatments and experimental design

For the experimental design, thirty vines were chosen per each treatment (ALD-apical defoliation; ED-early defoliation; PFD-post-flowering defoliation; LT-late trimming): three blocks in three different rows were selected with ten labelled vines per block. Each vineyard had blocks called control (CNTR) used to understand the treatments effect. The control was subjected to the normal canopy management: trimmed when the shoots exceed the wires placed at the top of the canopy to facilitate the tractors passage, about two weeks after fruit set and later in the season if necessary.

Between treatments: a post-flowering defoliation (PFD) in the vineyard A was performed, in particular a post flowering defoliation on canopy north exposed (PFD-N) and a post flowering defoliation on canopy south exposed (PFD-S). The defoliation treatment was carried out after flowering in the middle of June (BBCH 701-706) and it was compared with the control. The goal was to improve sugar accumulation without changing the acid content.

An early defoliation (ED) in bunch zone was performed in the vineyard B. This type of defoliation was performed at the phenological stage of pre-flowering during the first week of June (BBCH 600-603) and compared with the control (CNTR). At harvest, bunch rot was determined as incidence (percentage of infected clusters per vine) and as severity (percentage of infected berries per cluster) as explained by Sabbatini & Howell (2010). It was done only in this vineyard because in previous seasons had problems with powdery mildew; in fact, the goal was to improve powdery mildew tolerance.

For these two treatments (ED, PFD) six basal leaves for all the main shoots, not laterals, per vine were removed manually.

In the vineyard C, an apical defoliation (ALD) and a late vigorous trimming (LT) were performed: the defoliation treatment removed 25% of the leaf surface in the apical part of the canopy, while the trimming treatment removed all the shoots portions above the last bunches, except for the Guyot shoot renewal. These different treatments were compared to the control (CNTR). They were performed manually and were applied when the TSS were around 12-14 °Brix (Poni *et al*., 2006).

The normal canopy management practices were applied for all the treatments except the LT: trimming when the shoots exceed the wires placed at the top of the canopy to facilitate the tractors passage.

3.5.1. Yield components and berry ripening

From beginning of ripening until harvest, the TSS (total soluble solid) content measurement for a sample of 50 berries was performed using a temperature-compensating refractometer [HHTEC] for six times (once a week) per each treatment.

Nine clusters per treatment were harvested at maturation; the type of cluster sampled were the ones from the first order. They were tagged for detailed measurements: number of berries, average cluster weight, average berries weight, bunch compactness, for all the three vineyards and incidence and severity of powdery mildew only for vineyard B. A microanalytical precision scale was used to determine the berry weight of berries without pedicel and then the ratio between berries weight and the number of total berries allowed to estimate the average berries weight. Cluster compactness was visually estimated using code O.I.V. code 204 that classifies bunches into five categories, based on the mobility of the berries and the visibility of the pedicels: very loose (notation 1); loose (3); medium (5); compact (7) and very compact (9) (O.I.V., 1983).

At harvest, 50 berries per block were collected, and the analyses performed were: pH, total acidity, TSS (g/L), potential alcohol (% vol.), total phenolic content. To ensure a representative sample, this was composed of berries picked from the top to the bottom and from the exterior to the interior of the bunch. These berries were then crushed, and the juice was used to determine grape composition. Total soluble solids (TSS) expressed in °Brix were measured using a temperature-compensating refractometer [HHTEC]; pH was analysed with the O.I.V. method MA-AS313-15 R2011 using a pH meter [HANNA instruments] and a glass electrode, and TA was determined following the O.I.V. method MA-AS313-01 R2015 with a titrator [HANNA instruments] using 0.25 N of sodium hydroxide (NaOH) to a pH 7.00 endpoint, expressed as g/L of tartaric acid equivalent. The sugar content was determined using the O.I.V. method MA-AS2-02 R2012 which used the refractive index at 20 °C, expressed either as an absolute value or as a percentage by mass of sucrose, and the appropriate table to provide the sugar concentration in grams per litre and in grams per kilogram for grape musts. To determine total polyphenols, it was used the O.I.V. method MA-F-AS2-10 R2009; the extract was diluted with water and 1-mL portion was transferred into a 20-mL calibrated flask, and 2 mL of methanol, 5 mL of water, and 1 mL of Folin–Ciocalteu reagent was added. After 3 min, 4 mL of sodium carbonate (10%) was added and the solution was left to stand for 90 min. Then, absorbance was registered at 700 nm on the spectrophotometer [PCE instruments] using 10-mm cuvettes. Concentrations were determined using a calibration curve and expressed as (+)–catechin, mg/kg of grape.

3.6. Data Analysis

The data collected were organized using the Excel program. In particular, an Excel file was used to record the values for the BBCH code from budburst to beginning of ripening. A second Excel file was intended to collect the data at harvest (TSS, acidity, pH, potential alcohol, phenolic content, yield and yield components). The statistical analysis has been done, separately per each experiment, with the program Statistix 9.0 and Excel. The ANOVA model (two-way ANOVA) was done for the vineyard A and C with two treatment and the control. The statistical differences were indicated with difference letters (Bonferroni's test). For the vineyard B, with one treatment and the control, a Student's t test between unpaired samples was applied to understand if the differences were significantly or not.

4. Results and discussion

This section showed the data collected during the season related to phenological stages, vegetation and the main parameters to evaluate the grapes ripening.

4.1. From budburst to veraison: the BBCH code

Table 3 shows the BBCH values which were the same for all the these under examination until veraison.

Table 3: Data collected from bud burst to full bunch closure.

Observation day	BBCH code	Comment
20/03/2019	001	Buds enlargement
04/04/2019	007	Buds opening
11/04/2019	009	Budburst (Fig. 10)
18/04/2019	101	Figure 10
02/05/2019	503-505	Due to lower average temperatures the development wasn't high (Fig. 10)
16/05/2019	504-505	The development of floral clusters was not evident
23/05/2019	507-509	The delay can be estimated at 7-10 days compared to the previous year
30/05/2019	600-601	Close to flowering
06/06/2019	601-603	Flowering (Fig. 11)
17/06/2019	609-701	Significant millerandage caused by pre-flowering temperature changes
24/06/2019	705-706	Pea size (Fig. 11)
08/07/2019	709	Cluster closure
15/07/2019	800	10-15 days later than the previous year
01/08/2019	801	Evidence of veraison

Figure 10: From left to right: BBCH 09, BBCH 101, BBCH 505.

Figure 11: BBCH 61, BBCH 73, BBCH 75.

From the beginning of ripening until harvest, the sugar content (Brix) for each vineyard and for each treatment were measured with a refractometer on 50 berries. The data, expressed in °Brix, were presented in Tables 4 for each vineyard.

Table 4: Sugar content (Brix) from beginning of ripening to harvest for the vineyards A, B and C.

Vineyard	Treatment	05/08	19/08	26/08	02/09	09/09	16/09
A	***PFD-N***	7.5	12.6	15.8	18	18.8	19
	PFD-S	8.6	13	16.5	19.7	20.5	21
	CNTR	8	13	16.3	18.2	19	19.4
B	***ED***	8.7	12.7	15.8	16.4	18	19.5
	CNTR	9	12.5	16	18.5	19	20.7
C	***ALD***	9	12.8	16.1	18	21	22.8
	LT	8	13.1	16	17.1	19.2	21.8
	CNTR	7.5	13.3	15.9	18	20.6	23

ALD – apical late defoliation; CNTR – control; ED – early defoliation; LT – late trimming; PFD-N – post flowering defoliation on canopy north exposed; PFD-S – post flowering defoliation on canopy south exposed. n = 50

The trend of values was quite indicative. Defoliation (PFD-S) in vineyard A, late trimming (LT) in vineyard C confirmed what reported by other authors. In particular, treatment PFD-S showed results close to the ones reported by Palliotti & Poni (2011): the accumulation of sugar in berries was accelerated in defoliated vines. Treatment LT resulted in agreement with the study by Palliotti & Poni (2011) confirming the delay of ripening. On the contrary, early defoliation (ED) in the vineyard B and apical late defoliation (ALD) gave different results regarding sugars. In fact, treatment ALD showed the opposite results if compared with the study by Stoll *et al.* (2010) where was reported that a severe leaf removal apical to the bunch zone prior to veraison causes a ripening delay of about two weeks as compared to non-defoliated vines. In general, these differences were less evident during the first half of August than in the second half.

In the Table 5 are reported the data relating to August 19th sampling in comparison with those of the same week of the 2018 to understand the different maturation trends mainly due to the climatic conditions. Even though they are clearly lower than the previous year, the sugars have undergone a good progress in that week, with an increase of 6 °Brix and a decreased in acidity. In the middle of August 2018, 14 of the 20 vineyards monitored by the region were already harvested, this year only 3

Table 5: Sarzana, Vermentino variety at maturation in two different years.

Chemical Analysis	**°Brix**	**% vol**	**Acidity**	**pH**
2018	19.0	10.7	5.9	3.19
2019	13.9	7.4	12.6	2.84

4.2. Effects of the treatments on yield and grape composition

Table 6 shows the results obtained for each treatment and the control at harvest. In particular, bunch weight (g), berries number, berries weight (g) and bunch compactness were obtained on at least nine bunches per treatment (3 vines per block). The values for the control were the average of the control of each vineyard.

Table 6: Bunch weight, berries number, berries weight and bunch compactness for the treatments.

Vineyard	Treatment	Bunch weight (g)	N. berries/bunch	Berries weight (g)	Bunch compactness
A	PFD-N	264.6 b	98 b	2.70 b	5
	PFD-S	217.5 c	87 c	2.50 c	3
	CNTR	284.0 a	100 a	2.80 a	7
B	ED	234.0 b	90 b	2.60 b	5
	CNTR	286.2 a	102 a	2.83 a	7
C	ALD	282.8 a	101 a	2.80 a	7
	LT	272.7 b	101 a	2.70 b	7
	CNTR	284.4 a	103 a	2.80 a	7

CNTR – control; LT – late trimming; ALD – apical late defoliation; PFD-N – post flowering defoliation on canopy north exposed; PFD-S – post flowering defoliation on canopy south exposed; ED – early defoliation. n = 9

For A and C: means per column followed by the same letters are not significantly different at $\rho \leq 0.05$ by the Bonferroni significant difference test.

For B: different letters across a column show significant differences between values according to Student's t-test ($P = 0.05$)

These results confirmed what reported by other author. As reported by Tardaguila *et al*. (2010), the defoliation reduced fruit-set and berry weight, leading to smaller and looser clusters, increasing the skin-to-berry and seed-to-berry ratio. ED and PFD-S treatments were significantly different from the control; also, PFD-N treatment but in a lesser way. In agreement with Poni *et al.* (2006) this study indicated that post flowering leaf removal appears to be quite effective as an earlier removal when the goal is also to achieve some control over berry size. The differences were visually noted by the panel of people who judged the compactness. Regarding trimming results there weren't precise information about the parameters considered but LT treatment was significantly different from the control for the berries weight.

In Table 7 was reported the percentage of incidence and severity of bunch rot in the vineyard B, for both treatment (ED) and control (CNTR) to understand if defoliation was able to reduce the number of infected bunches.

Table 7: Percentage of incidence and severity of infected bunches in vineyard B.

Treatments	**% of incidence**	**% of severity**
ED	5 b	4 b
CNTR	15 a	20 a

CNTR – control; ED – early defoliation. n=9
For B: different letters across a column show significant differences between values according to Student's t-test (P = 0.05)

As shown by previous authors (Intrieri *et al.,* 2008; Diago *et al.,* 2010; Gatti *et al.,* 2015) this type of defoliation led to a significant reduction of bunch compactness resulting in a reduction of bunch rot incidence maybe due to an improve in ventilation in the canopy. As reported by Poni *et al.* (2006), the effect achieved by defoliation is the decrease in cluster compactness, which in turn had a positive impact on the incidence of powdery mildew on grapevines and this feature may be important in humid climates. There was significantly difference between treatments due to the previous explanation and to the improved efficiency of the treatments. Another mechanism that could occur is the thickening of the skin.

At maturation the grape composition was determined in each vineyard and the results were reported in Table 8. The parameters analysed were: total soluble solids (g/L); pH; titratable acidity (g/L of tartaric acid), potential alcohol (% vol) and total phenol index (mg/L of gallic acid).

Table 8: Grape composition at maturation for the three vineyards (A, B and C).

Vineyard	Treatment	TSS (g/L)	pH	Acidity (g/L)	% vol	TPI (mg/L)
A	**PFD-N**	180 b	3.35 b	6.00 a	10.4 b	130 b
	PFD-S	202 a	3.40 a	4.69 b	12.0 a	194 a
	CNTR	181 b	3.34 b	6.05 a	10.7 b	123 b
B	**ED**	186 b	3.29 a	5.97 b	11.1 b	170 a
	CNTR	200 a	3.28 a	6.53 a	11.9 a	160 a
C	**ALD**	203 a	3.36 a	4.95 b	12.1 b	184 a
	LT	185 b	3.26 b	5.81 a	11.0 a	175 a
	CNTR	198 a	3.28 b	5.24 b	11.8 b	180 a

ALD – apical late defoliation; CNTR – control; ED – early defoliation; LT – late trimming; PFD-N – post flowering defoliation on canopy north exposed; PFD-S – post flowering defoliation on canopy south exposed; TSS – total soluble solids; TPI – total phenol index. n = 150
For A and C: per column means followed by the same letters are not significantly different at ρ ≤ 0.05 by the Bonferroni significant difference test.
For B: different letters across a row show significant differences between values according to Student's t-test (P = 0.05)

These results confirmed what reported by other authors. Removing all the leaves from the fruiting area, as reported by Bergqvist *et al.* (2001), there is an exposure of the clusters to full sun and it might lead in warm climates to compromised fruit composition due to excessive berry temperatures, which can cause a clear drop in malic acid concentrations. This mechanism occurred in treatment PFD-S where the values of TSS and pH were significantly different. In fact, as explained by Poni *et al.* (2006), the response of lower malic acid can derive from increased cluster exposure; for this reason, very light defoliation is usually applied on the south facing side in an east-west oriented row, as reported by Palliotti & Poni (2011). Roby *et al.* (2004) explained that the higher phenol content in berries on defoliated vines (PFD-S, ED) agree with the increase of skin-to-berry ratio. Regarding the treatments PFD-S, PDF-N and ED, Petrie *et al.* (2003) showed that post-flowering leaf removal appeared to be more effective than an earlier removal when the goal was to obtain some control on berry size, post-bloom defoliations were quite effective in causing abortion or growth arrest of set berries.

As described by Palliotti *et al.* (2013a), the apical late defoliation (ALD) did not affect the phenolic compounds in grapes. Palliotti (1992) showed that the late trimming treatment led to a delayed berry growth and sugar accumulation, probably linked to the fact that lateral shoots compete with the developing grapes for carbohydrates. A two-year study by Palliotti *et al.* (2014a) on mechanical post-veraison, apical to the bunch zone, leaf removal showed a reduced leaf-to-fruit ratio and demonstrated its potential to delay optimal total soluble solid accumulation in the berry as compared to non-defoliated vines. Additionally, Palliotti *et al.* (2013a) reported that, while being able to significantly delay berry TSS accumulation, this technique did not affect the phenolic compounds in grapes and wines or the replenishment of reserves storage in canes and roots. ALD treatment wasn't significantly different from the control for TSS value while it was for pH value which resulted higher.

As reported by Palliotti & Poni (2011), at harvest, late-hedging caused a reduction of both leaf/fruit ratio and soluble solid accumulation in the berries and these effects are probably linked, as explained by Palliotti (1992) to the fact that lateral shoots compete with the developing grapes for carbohydrates, causing delayed berry growth and sugar accumulation and to a significant reduction of the leaf area-to-yield ratio. Filippetti *et al.* (2011) applied a shoot trimming one week after veraison and achieved a significant reduction in the TSS accumulation without changing the pH and the content of organic acids and tannins in the skins. In theory, as shown by Palliotti *et al.* (2014a), a relatively late shoot trimming might induce a competitive re-growth of laterals with subsequent reduction in total soluble solid production and their accumulation in berries. The results confirmed these hypotheses because the TSS value resulted significantly different from the control while the pH value wasn't.

5. Conclusions

The aims of this study were: to assess the effects of pre-flowering basal leaf removal, post-flowering basal leaf removal, late-apical leaf removal, late shoot trimming technique on bunch microclimate, vine performance and physiology in the white cultivar Vermentino to identify the best techniques to achieve the desired grape and wine composition in three vineyards with different characteristics mainly regarding climatic conditions and orientation of rows.

The ED treatment was a valid technique to reduce the powdery mildew damages due to less bunch compactness and a more efficient product spraying in the bunch zone. The infection rate resulted significant lower compared to the control, but it led to a lower TSS value at harvest and a greater acidity degradation.

The PFD-S treatment increased significantly the TSS content, as expected from the literature, leading to a total potential alcohol of 12% instead of 10.7% of the control. The goal of increasing the sugar content had been achieved but the treatment had led to a strong decrease in acidity (-1.36 g/L): the early exposure and the greater skin to pulp ratio had not maintained the same acid content, or a higher one, in comparison with the control as reported by some authors. Contrarily, the total phenol index resulted higher than the control. In addition, the lower yield must be considered in relation to the legislation and the company's needs.

The PFD-N was not so effective; this treatment led to significant differences for the average bunch weight, berries number and average berries weight that resulted lower than the control.

The basal defoliation led to the achievement of some objectives but by modifying some parameters like acidity or pH, important values in the production of white wines. It would be better to carry out a defoliation around the flowering period without removing all the basal leaves but leaving some of them; thus, preferring a very precise manual defoliation.

ALD treatment showed results similar to the control. The apical defoliation applied late in the season (12-14 °Brix) will be tried again to better understand its potential.

In the same vineyard the LD treatment was quite effective, with a significant difference, in delaying sugar accumulation and maintaining higher acidity than control (+0.57 g/L). For this late vigorous trimming there were interesting results because it permitted to reduce the TSS content at harvest maintaining a high acidity value. It was a good technique to achieve the goal obtaining greater uniformity in the total maturation of that vineyard. It will be necessary to investigate if this treatment can compromise the future reserves in the plant if repeated for several consecutive years, limiting the accumulation of reserves in the plant during autumn.

Considering the results obtained with these treatments and the insufficient studies presented in literature, many experiments will be necessary to test these techniques for this cultivar.

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Appendix

Annex 1: BBCH code, from 001 to 114 (Lorenz *et al.,* 1995).

Principal growth stage	Mesostage	Secondary stages	Phenophase description	BBCH CODE
0	0	1	Beginning of bud swelling: buds begin to expand inside the bud scales	0-0-1
0	0	2		0-0-2
0	0	3	End of bud swelling: buds swollen, but not green	0-0-3
0	0	4		0-0-4
0	0	5	"Wool stage": brown wool clearly visible	0-0-5
0	0	6		0-0-6
0	0	7	Beginning of bud burst: green shoot tips just visible	0-0-7
0	0	8	Bud burst: green shoot tips clearly visible	0-0-8
0	0	9		0-0-9
1	0	0		1-0-0
1	0	1	First leaf unfolded and spread away from shoot	1-0-1
1	0	2	2 leaves unfolded and spread away from shoot	1-0-2
1	0	3	3 leaves unfolded and spread away from shoot	1-0-3
1	0	4	4 leaves unfolded and spread away from shoot	1-0-4
1	0	5	5 leaves unfolded and spread away from shoot	1-0-5
1	0	6	6 leaves unfolded and spread away from shoot	1-0-6
1	0	7	7 leaves unfolded and spread away from shoot	1-0-7
1	0	8	8 leaves unfolded and spread away from shoot	1-0-8
1	0	9	9 leaves unfolded and spread away from shoot	1-0-9
1	1	0	10 leaves unfolded and spread away from shoot	1-1-0
1	1	1	11 leaves unfolded and spread away from shoot	1-1-1
1	1	2	12 leaves unfolded and spread away from shoot	1-1-2
1	1	3	13 leaves unfolded and spread away from shoot	1-1-3
1	1	4	14 leaves unfolded and spread away from shoot	1-1-4

Annex 2: BBCH code, from 601 to 907 (Lorenz *et al.,* 1995).

6	0	1	Beginning of flowering: 10% of flowerhoods fallen	6-0-1
6	0	2		6-0-2
6	0	3	Early flowering: 30% of flowerhoods fallen	6-0-3
6	0	6		6-0-6
6	0	7	70% of flowerhoods fallen	6-0-7
6	0	8		6-0-8
6	0	9	End of flowering	6-0-9
7	0	0		7-0-0
7	0	1	Fruit set: young fruits begin to swell, remains of flowers lost	7-0-1
7	0	2		7-0-2
7	0	3	Berries groat-sized, bunches begin to hang (4 mm in diameter)	7-0-3
7	0	4		7-0-4
7	0	5	Berries pea-sized, bunches hang (7 mm in diameter)	7-0-5
7	0	6		7-0-6
7	0	7	Berries beginning to touch (if bunch are tight)	7-0-7
7	0	8		7-0-8
7	0	9	Majority of berries touching (lag phase)	7-0-9
8	0	0		8-0-0
8	0	1	Beginning of ripening: berries begin to soften	8-0-1
8	0	2	Berries change color	8-0-2
8	0	3	12 °Brix	8-0-3
8	0	4		8-0-4
8	0	5	15 °Brix	8-0-5
8	0	6		8-0-6
8	0	7		8-0-7
8	0	8		8-0-8
8	0	9	Berries ripe for harvest (Brix according to the cultivar)	8-0-9
9	0	0		9-0-0
9	0	1	After harvest; end of wood maturation	9-0-1
9	0	2	Beginning of leaf discolouration	9-0-2
9	0	3	Beginning of leaf-fall	9-0-3
9	0	4		9-0-4
9	0	5	50% of leaves fallen	9-0-5
9	0	6		9-0-6
9	0	7	End of leaf-fall	9-0-7

INSTITUTO SUPERIOR DE AGRONOMIA
Universidade de Lisboa

Departamento de Ciências e Engenharia de Biossistemas

PARECER

Na qualidade de orientador da dissertação de mestrado do aluno **Fillipo Conti** entitulada "**Effects of leaf removal and shoot trimming performed in diferent period on CV. Vermentino",** confirmo que o aluno procedeu às alterações sugeridas pelos membros do júri.

Instituto Superior de Agronomia, 21 de Janeiro de 2020

Orientador

(Carlos M. A. Lopes; Prof. Associado)