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The comparison of two methods for the reduction of cluster compactness in *Vitis vinifera* cv. Pinot gris

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Summary

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Compact grape clusters, as they naturally grow on Pinot gris clusters, often involve higher susceptibility to bunch rot and therefore lower yield quality. In this study, the two bunch thinning methods of leaf removal and the application gibberellic acid were compared in their efficiency to reduce cluster compactness in two viticultural models: One adapted to less fertile, alluvial soils with Guyot as trellis system, and one adapted to more fertile and vigorous soils with vines trained in Pergola.
Leaf removal and the application of gibberellic acid lowered berry number, mean cluster weight and cluster compactness with statistically significant differences to the untreated controls. Cluster compactness, however, showed no statistically significant difference between leaf removal and the application of gibberellic acid in neither of the two studied viticultural models. Leaf removal represents a valuable alternative to the use of plant growth regulators, such as gibberellic acid, especially when considering the context of organic viticulture.

Riassunto:
Grappoli compatti, come spesso si trovano sul pinot grigio, comportano spesso la formazione di marciumi che compromettono sia la resa che la qualità del raccolto. Questo lavoro di elaborato finale confronta due metodi di diradamento del grappolo: la defogliazione e l'uso di acido gibberellico. L'esperimento è stato applicato su due modelli viticoli: uno adottato su terreni alluvionali non molto fertili, con un impianto a Guyot, e uno adottato su terreni fertili e più produttivi con un impianto a Pergola.
Sia l'applicazione di acido gibberellico che la defogliazione hanno diminuito in maniera statisticamente significativa il numero di acini per grappolo, il peso medio del grappolo e la compattezza del grappolo, se comparati al testimone non trattato. Le differenze in compatezza del grappolo tra defogliazione e acido gibberellico non erano statisticamente significative in entrambi modelli viticoli osservati. La defogliazione quindi rappresenta un valido metodo alternativo all'uso di ormoni vegetali per il diradamento del grappolo, sopratutto nel contesto della viticoltura biologica.

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Abbreviations

ABA Abscisic acid. IV, 14

BRI Bunch rot incidence. 26, 41

BRS Bunch rot severity. 26, 41

GA_3 Gibberellic acid. IV, 1, 8, 13, 16, 17, 21, 22, 31, 37, 38, 40–43, 45

IAA Indole-3-acetic acid. IV, 12

IDI Density index by Ipach et al. (2005). V, VI, 5, 26, 38, 39, 41, 42

LA Leaf area. IV, 25, 32

LAi Individual leaf area. 32

LDI Density index by Lemut et al. (2011). VI, 5, 26, 37, 41

LPI Leaf plastochron index. 23, 28

LR Leaf removal. V, 25, 32, 34, 37, 38, 40–43, 45

PGR Plant growth regulator. 8, 10

PI Plastochron index. 23, 28

SDI Density index by Sabbatini and Howell (2010). 5, 26, 38, 41

T1 Treatment 1: untreated control (pergola). 17, 31, 35–37, 39, 41

T2 Treatment 2: leaf removal (pergola). 17, 31, 35–37, 39, 41

T3 Treatment 3: gibberellic acid application (pergola). 17, 31, 35–37, 39, 41

T4 Treatment 4: leaf removal + gibberellic acid application (pergola). 16, 17, 31, 35–37, 39, 41

T5 Treatment 5: untreated control (guyot). 17, 31, 35–37, 39, 41

T6 Treatment 6: leaf removal (guyot). 17, 31, 35–37, 39, 41

T7 Treatment 7: gibberellic acid application (guyot). 17, 31, 35–37, 39, 41

T8 Treatment 8: leaf removal + gibberellic acid application (guyot). 16, 17, 31, 35–37, 39, 41

TDI Density index by Tello and Ibáñez (2014). VI, 5, 26, 37, 41

TSS Total soluble solids. 41

YPH Yield per hectare. V, 26, 40–42

YPV Yield per vine. V, 26, 40–42

Introduction

1.1 Objective

The goal of this study is to compare two methods, deriving from two totally different approaches, to reduce cluster compactness in Pinot gris. The two methods involve the practice of leaf removal and the application gibberellic acid (GA_3). Both bunch thinning measures will be tested singularly and in combination next to an untreated control in two different viticultural models: One adapted to less fertile soils and one adapted to fertile soils. During the growing seasons multiple parameters such as the plastochron index, leaf area, fruit set and bunch weight, among others, will be assessed.

Figure 1.1: A compact Pinot gris cluster at véraison

Overview

2.1 Pinot gris varietal description

Pinot gris, also known as Pinot grigio in Italy and Grauburgunder or Ruländer in the German speaking regions, is a somatic mutation of the Pinot noir (Vezzulli et al., 2012). It belongs to the species *Vitis vinifera* and with 25.000 ha planted in Italy, makes up to 3,7 % of Italy's total vineyard area (OIV, 2017).

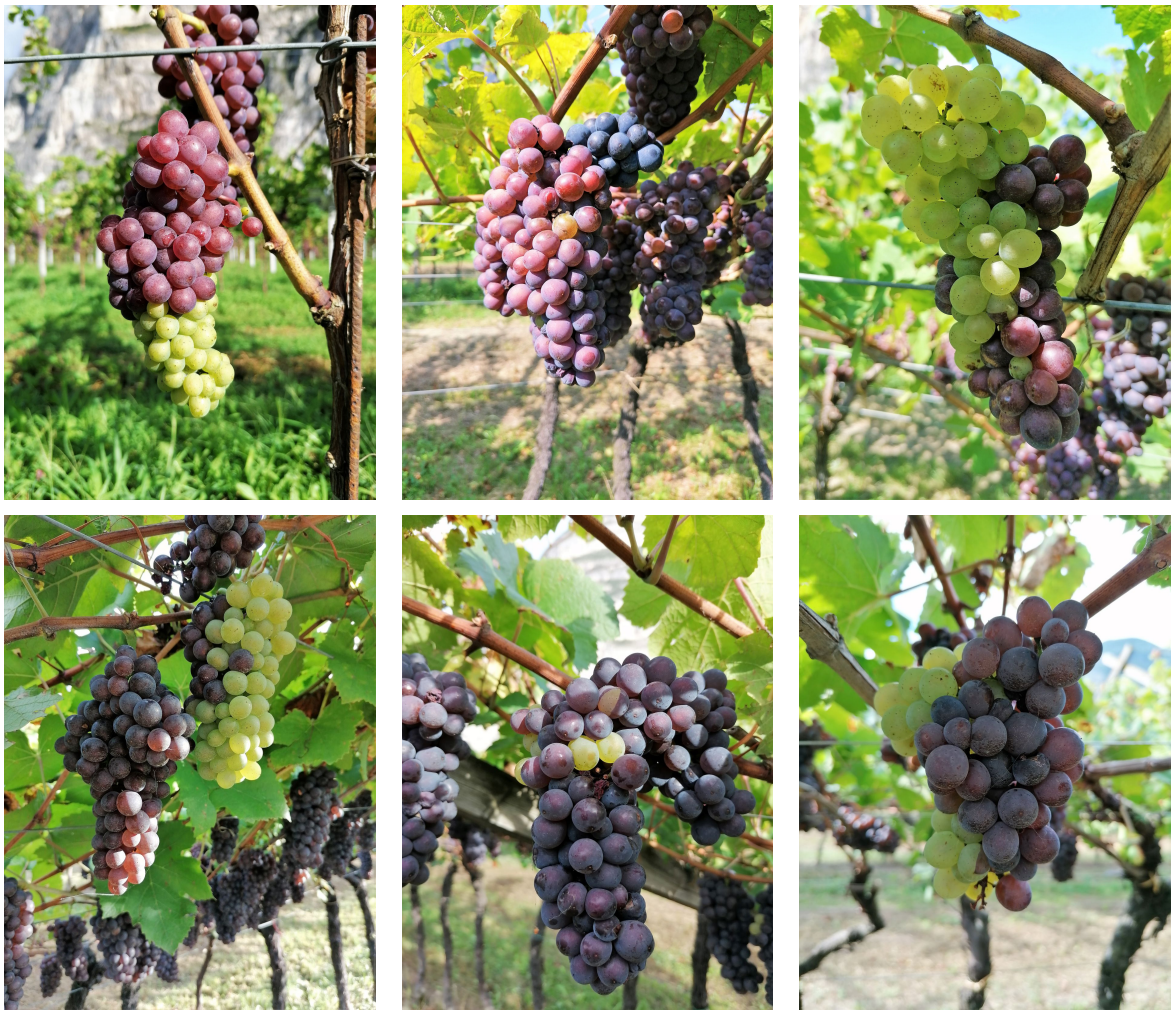


Figure 2.1: Pinot gris clusters presenting somatic mutations

2.1.1 Morphology

From Cosmo and Polsinelli (1960):

Shoots The shoots have an open, pubescent tip of green-gray-ish color, while the main axis is smooth and appears to be slightly twisted. The first fruitful shoots emerge from the second or third bud on. Each shoot carries one or two inflorescences, located on the third and fourth node, respectively. The average internode length ranges from 7 to 10 cm.

Leaves The leaves are small and cordiform with three main lobes. The petiolar sinus is V-shaped and the main leaf veins are bulging. In most cases, the lamina is dark green and shiny. Usually, the apical leaves are hairier than the basal leaves.

Inflorescences The inflorescences have an average length of 13 cm and accordingly, the bunches tend to be stocky. The bunches have a compact, cylindrical shape and usually carry a second, smaller, lateral bunch.

Berries The berries are small and slightly elliptical with gray-violet skin. The pulp is is very juicy and sugar rich and is described to have a neutral aroma. Each berry contains an average of one or two seeds.

Figure 2.2: Illustration of Pinot gris by Jules Troncy (Viala and Vermorel, 1901)

2.1.2 Growth behavior

Pinot gris has an early budburst and is a variety of excellent vigour and very high yield production (Cosmo and Polsinelli, 1960). While these latter mentioned two traits were used in the past to produce high quantities rather than yield of good quality, today's production, on the other hand, has lower production goals (Handels und Landwirtschaftskammer Bozen, 2018).
Simultaneously, also clonal selection went from compact high-yielding clones, such as the Hauser 1, which has an average yield of ca. 4 kg per vine to less compact clones, such as the SMA 505 with an average production of 2,5 kg per vine (Sivilotti et al., 2011).
The difference in size between the Hauser 1 and SMA 505 can be seen in Figure 2.3.

Figure 2.3: San Michele and Hauser clones in comparison

2.2 Cluster compactness and phytopathological aspects

2.2.1 Definition of compactness and measurement

Cluster compactness can be defined as the spacial distribution of the berries on the rachis and the space in between them.

There is a variety of indexes used to classify cluster compactness in different manners (Tello and Ibáñez, 2018). These can range from subjective methods, such as ranking the compactness on a scale from 1 to 9, where 1 implies clearly separated berries and 9 the deformation of berries by compression (OIV, 2007), to objective methods involving mathematical models, 3D scanning technologies and mobile sensing platforms (Cubero et al., 2015; Tello et al., 2016; Palacios et al., 2019).

In this paper, cluster compactness will be measured according to following protocols:

bunch weight divided by bunch length, as suggested by Lemut et al. (2011), the formula suggested by Tello and Ibáñez (2014) is similar, but has the intention to give the length of the bunch a greater mathematical weight, the **number of berries per cluster divided by bunch length (cm)**, as suggested by Sabbatini and Howell (2010) and the protocol of Ipach et al. (2005), which consists in categorizing clusters into five classes by not only assessing cluster compactness by a subjective ranking but also links the compactness to the possibility of bending the rachis up to a certain angle, as displayed in Table 2.1. These indexes, when used, will be abbreviated in the following way:

LDI: Density index by Lemut et al. (2011) (Equation 2.1)

TDI: Density index by Tello and Ibáñez (2014) (Equation 2.2)

SDI: Density index by Sabbatini and Howell (2010) (Equation 2.3)

IDI: Density index by Ipach et al. (2005) (Table 2.1)

$$\text{LDI} = \frac{\text{bunch weight (g)}}{\text{bunch length (cm)}} \tag{2.1}$$

$$\text{TDI} = \frac{\text{bunch weight (g)}}{[\text{bunch length (cm)}]^2} \tag{2.2}$$

$$\text{SDI} = \frac{\text{berry number}}{\text{bunch length (cm)}} \tag{2.3}$$

Table 2.1: Cluster density index as described by Ipach et al. (2005)

Index	Description
1	Very loose (no berry contact; bending of the stem to 90° possible)
2	Loose (berry contact; bending of the stem up to 45–90° possible)
3	Dense (berries still flexible; bending of the stem up to 10–45° possible)
4	Compact (berries not flexible; bending of the stem up to 10° possible)
5	Very compact (berries not flexible; bending of the stem not possible)

2.2.2 Current strategies for cluster reduction

Agronomic practices

Throughout the vegetative period, the direction of translocation of photosynthesized carbohydrates coming from the leaves, so called sources, changes destination, also known as sink. Examples of different sinks are for example the growing shoot tips from the moment the leaves become net exporters up until fruitset. Subsequently photosynthates are directed towards other sinks such as the developing bunches, these are a major sink until autumn, where carbohydrates are translocated towards the trunk and roots as reserves (Iland et al., 2011). Caspari et al. (1998) described that fruitset is not only directly linked to the carbohydrate supply, but the availability of carbohydrates is the major determinant of fruitset in grapes. Hence, the loosening of grape clusters can be achieved by adapting agronomic practices that alter the source-sink balance. These include the following:

Leaf removal Defoliation of the cluster-zone has shown to be a powerful method to reduce bunch density at pre-, full-, and even post-bloom (Evers et al., 2010; Gatti et al., 2012; Hed et al., 2014; Poni et al., 2006; Sabbatini and Howell, 2010; Molitor et al., 2011b,a; Smith and Centinari, 2019; Zenoni et al., 2017). Whereby the optimal growth stage for a defoliation treatment to achieve the maximal reduction of cluster density seems to be variety-dependent (Smith and Centinari, 2019; Molitor et al., 2011a).

The reason behind this occurrence is that the carbohydrate supply provided by the basal leaves is crucial to the developing clusters. Fruit set declines exponentially with decreasing carbohydrate supply (Caspari et al., 1998).

Gatti et al. (2012) compared defoliation at pre-flowering, early cluster thinning and late cluster thinning in Sangiovese vines. From the aforementioned methods, defoliation led to the lowest density in grape clusters, making it a considerable alternative method for cluster thinning. However, if the restoration of plant reserves for the initiation and differentiation of inflorescence primordia is affected by too severe leaf removal, the percentage of shootless nodes will increase and the number of clusters per shoot as well as fruitset will decrease the year following the treatment (Sabbatini and Howell, 2010). In Pinot noir, leaf removal during and after bloom (Acimovic et al. (2016) and Evers et al. (2010), respectively) did

not alter the crop yield of the following year negatively.
Molitor et al. (2011a) came to the conclusion that the loosening effect of cluster compactness in Pinot gris was the highest when leaf removal was performed between pre-flowering and early flowering (BBCH 57 and 63 (Lorenz et al., 1994)).

Figure 2.4: A pneumatic leaf removal machine being used on a pergola training system

Canopy shading As Caspari et al. (1998) observed, suboptimal weather conditions, which imply lower levels of temperature and photosynthetically active radiation (PAR), during flowering seem to reduce fruitset. This happens due to lower carbohydrate availability as a consequence of inferior carbon assimilation. Basile et al. (2015) noticed a parallel decline of flower number per bunch and net photosynthesis per shoot by reducing ambient light by 10, 30, 50, 75, and 90% with whole-canopy shading. The objective of the study was to find an efficient alternative method to achieve lower bunch density, as leaf removal, if practiced in dry and warm areas, may lead to sunburn and undesired must compositions (Nuzzo et al., 2009; Basile et al., 2015).
Anti-transpirants Another way to affect carbohydrate supply is to inhibit the transpiration of carbon dioxide by obstructing the stomata, located on the ventral part of the leaves. This can be achieved by applicating substances like paraffin or pinus oils (Tello and Ibáñez, 2018). The application at pre-bloom of the latter mentioned was proven to successfully reduce crop yield and lower bunch compactness by 32% (Palliotti et al., 2010; Intrieri et al., 2013). Among others, Hanni et al. (2013) also tested the application of anti-transpirants on Pinot gris with good, yet not always consistent results. In addition,

the application of transpirants can cause leaf burn and their compatibility with other phytosanitary products has to be further investigated.

Shoot topping Shoot topping during full-bloom is known to increase yield and cluster weight (Vanderlinde et al., 2017), as removing the shoot tip implies removing the main carbohydrate concurrent of the inflorescences (Iland et al., 2011). On the other hand, Molitor et al. (2015), who performed shoot topping at seven different growth stages in Pinot gris and Riesling, found that the loosening effect on the grape clusters increased the later the topping was carried out, since the carbohydrate competition of the shoot tips was maintained for a longer time period.

Plant growth regulators

A common practice in viticulture to achieve cluster compactness reduction is the application of plant growth regulators (PGRs), defined as "plant hormones or their synthetic analogs, inhibitors of hormone biosynthesis or translocation and hormone receptor blockers" (Rademacher, 2015). PGR application is carried out either at pre-bloom or at full bloom with slightly different effects (Tello and Ibáñez, 2018).

Gibberellins The use of gibberellic acid (GA_3) has shown to be an effective tool to reduce the cluster compactness of wine grapes (Hed et al., 2011; Dokoozlian and Peacock, 2001; Evers et al., 2010; Molitor et al., 2016). GA_3 can lead to different results, depending on the phenological growth stage at the moment of application. While an application at pre-bloom (BBCH < 60) or full bloom (BBCH 68) both lead to a reduction of the cluster compactness, the application of GA_3 after flowering (BBCH > 70) led to increased cluster compactness and berry size (Tello and Ibáñez, 2018). Even tough the pre-bloom and full-bloom application of GA_3 achieve a similar effect, their mechanism of action is different. Pre-bloom application of GA_3, as it was examined by Molitor et al. (2016), causes an elongation of the main axe of the grape. They treated Sauvignon blanc grapevines with GA_3 at various phenological stages before flowering, including stages from 3 unfolded leaves (BBCH 13) to 7 unfolded leaves (BBCH 17), which resulted in a 28% longer main axe at fruitset (BBCH 71).

On the other hand, the application of GA_3 at full bloom reduces cluster compactness by lowering the rate of fruitset and berry number (Hed et al., 2011; Evers et al., 2010; Dokoozlian and Peacock, 2001). This could be because gibberellic acid applied at that stage can act as a pollenicide and therefore limits the fertilization, as suggested by Weaver and McCune (1960). As an application of GA_3 also stimulates vegetative growth, this inevitably increments the carbohydrate competition between the flowers and other organs of the vine, leading to an increased number of flower and fruitlet abortions (Caspari et al., 1998).

However, the application of gibberellic acid at bloom may result in prolonged bud dormancy or necroses of the main bud, leaving some buds unable to burst the following year (Eriş and Çelik, 1981; Iwasaki, 1980; Lavee et al., 1981; Weaver, 1959; Ziv et al., 1981) as it can be seen in Figure 2.5. Gibberellins also may, depending on the stage of bud development during the application, inhibit inflorescence primordia differentiation (Li-Mallet et al., 2016).

Figure 2.5: A cane showing symptoms similar to the ones described in Lavee et al. (1981), probably due to the use of gibberellic acid in the previous year

Alternatively to gibberellins, reduction of cluster compactness through application of other plant growth regulators have also been reported (Tello and Ibáñez, 2018), but with less promising results.

Prohexadione-calcium An application at full-bloom, especially in combination with leaf removal has shown to be effective on Pinot gris and Pinot blanc. Prohexadione-calcium disturbs the balance between biologically active and inactive gibberellins, consequently promoting flower or berry abortion (Molitor et al., 2011b). However, the application of the same commercial product led to no significant difference in cluster compactness when applied to Sauvignon blanc (Roschatt and Innerebner, 2017)

Ethephon Ethephon (2-Chloroethylphosphonic acid) is an ethylene releasing molecule, which promotes berry abscission (Ferrara et al., 2016). El-Zeftawi (1982) applied ethephon on various *Vitis vinifera* cultivars and achieved cluster reductions ranging from 70 to 94%.

Even though this may be a valid method to facilitate the harvest of table grapes (Uzquiza et al., 2013), it is not to be considered a favorable method for the control of cluster compactness and yield.

The effectiveness of PGRs seems to depend on a variety of factors, including cultivar, environmental conditions and annual weather fluctuations.

2.2.3 Bunch rot

Bunch rot can be divided into *Botrytis* bunch rot and sour rot. They may both appear due to wet pre-harvest conditions and, in fact, their occurrence is often coincident, even though they are caused by different etiological agents (Wilcox et al., 2015).

***Botrytis* bunch rot**

Grey mould or *Botrytis* bunch rot is caused by a plant pathogen called *Botrytis cinerea* and is responsible for serious economic damage in vineyards all around the world (Elmer and Reglinski, 2006), especially in vineyards dominated by cool and wet conditions during the ripening period (Steel et al., 2013). It is known to infect over 200 different crop species (Williamson et al., 2007) and can lead to moldy or fungal off-flavors in wines (Steel et al., 2013). An infection of *Botrytis cinerea* is facilitated by any factor which may cause damage to the tissue, such as berry compression or lesions caused by other pathogens (Elmer and Michailides, 2007). In fact, many studies show that bunch rot is tightly correlated with cluster compactness (Evers et al., 2010; Hanni et al., 2013; Hed et al., 2014; Molitor et al., 2011b; Zabadal and Dittmer, 1998), as compact clusters have a longer wetness duration than loose clusters (Evers et al., 2010), which promotes successful conidia germination and subsequently an infection through the cuticular membrane (Savage and Sall, 1984). Another reason for the major susceptibility of compact clusters to *Botrytis cinerea* is, that compact clusters have more berry-to-berry contact sites (Vail and Marois, 1991) where the epicuticular wax is deformed, making the contact-surface more vulnerable to *Botrytis cinerea* infections (Marois et al., 1986).

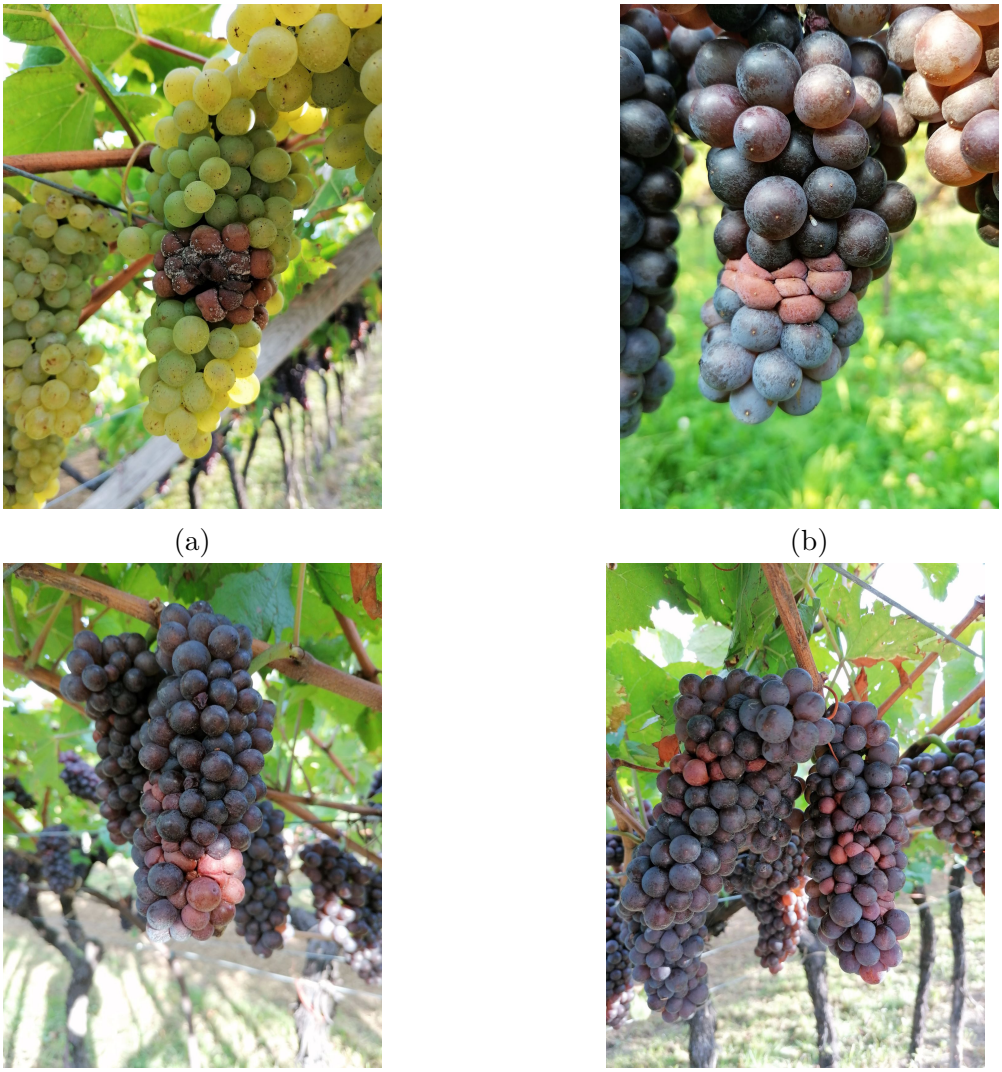
Sour bunch rot

Grape bunches affected by sour rot generally show symptoms of decay, such as brown berry skin, deriving from oxidation, and a strong vinegar-like odor (Wilcox et al., 2015). Sour rot does also involve the presence of fruit or vinegar flies (*Drosophila* ssp.), *Drosophila suzukii* and *Drosophila melanogaster* in particular (Entling and Hoffmann, 2019).
According to Wilcox et al. (2015), a precondition for sour rot are injured berries, by either abiotic factors, such as compression in compact bunches or cracking due to rain, or

biotic factors, like damage caused by pathogens, birds or insects. The latter mentioned include *Drosophila suzukii*, which lacerate the berry skin to lay their eggs, inducing rotting processes and hence cause also financial losses (Walsh et al., 2011). Ioriatti et al. (2017) proved that *Drosophila suzukii*, through feeding and contact on rotting berries, are in fact vectors for spoilage bacteria.

These bacteria, in this case acetic acid bacteria belonging to various *Gluconobacter*, *Gluconoacetobacter* and *Acetobacter* subspecies are considered the etiological agents of sour rot (Barata et al., 2012). However, Hall et al. (2018) concluded that sour rot is the product of the interaction between *Drosophila* flies, yeast and acetic acid bacteria and thus, all three components must be present for symptoms to develop.

Sour rot can cause considerable damage, as the infections are able to spread rapidly through adjacent berries (Wilcox et al., 2015), which can be fatal for compact clusters.



(a) (b) (c) (d)

Figure 2.6: Compact clusters affected by *Botrytis* (a) and sour (b, c, d) bunch rot

2.3 Phytohormones

The terms phytohormone, plant hormone or plant growth regulator are interchangeable and are used to characterize organic compounds of either natural or synthetic origin, capable of modifying certain physiological processes inside a plant. Their presence in plant tissues is limited to very low concentrations and each hormone can trigger a myriad of different processes, depending on the tissue, the hormone concentration and the phenological growth stage of a plant. (Urry et al., 2016)
The main hormones can be divided into 5 families:

2.3.1 Auxins

Auxin may be the overall most important plant hormone. It plays a crucial role in phototropism, stem elongation, fruit development and promotes apical dominance (Urry et al., 2016). Even though the term auxin is often abbreviated to IAA (indole-3-acetic acid), a compound that makes up approximately 25% of the total auxin amount, the hormone family, among others, also includes IBA (indole-3-butyric acid), IPA (indole-3-propionic acid) and 4-Cl-IAA (Ludwig-Müller, 2011).
Auxins are produced in the shoot tip as well as in the roots (Zhao, 2010). The hormone level inside the plant tissue can be regulated by either producing new IAA or by freeing IAA from it's conjugated inactive form (Bartel, 1997). During the initial stages of berry development in the grapevine, IAA enacts a vital role, as it delays fruitlet abscission by reducing the ethylene sensitivity of the tissue (Kuhn et al., 2016).

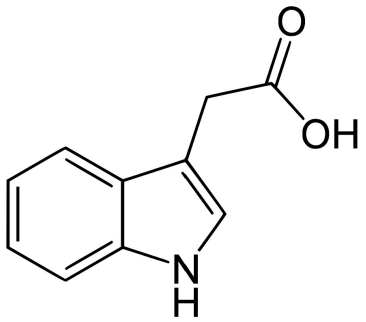


Figure 2.7: The chemical structure of indole 3-acetic acid (IAA)

2.3.2 Gibberellins

Gibberellins (GAs), first discovered towards the end of the 19th century as secretion of a fungus named *Gibberella fujikuroi*, are involved in in several growth processes of the plant (Hedden and Sponsel, 2015). These include germination, stem elongation and fruit development (Urry et al., 2016). Gibberellins have been isolated from bacteria, fungi and vascular plants. To date, 126 gibberellic acids have been identified (MacMillan, 2001). Different developmental processes implicate specific active GAs. Even though numerous bioactive GAs can be detected different plant tissues, in many cases developmental processes are regulated by one prevailing GA type (Giacomelli et al., 2013). In grapevine inflorescences, berries and seeds the gibberellins GA_1, GA_3, GA_4, GA_{17} and GA_{20}, have been detected (Pérez et al., 2000; Giacomelli et al., 2013).

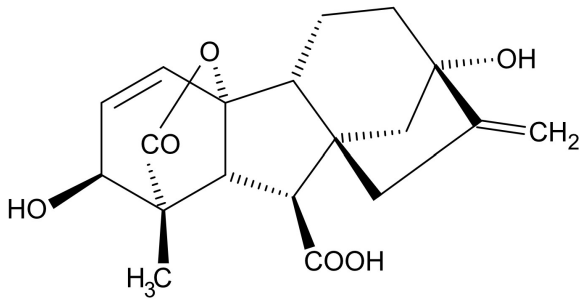


Figure 2.8: The chemical structure of gibberellic acid (GA_3)

2.3.3 Cytokinins

Cytokinins are mostly produced in the roots and are mainly involved in the regulation of cell division in the root apical meristem (RAM) and in the shoot apical meristem (SAM). Their function is often connected with the function of auxins (Schaller et al., 2015). In cell division, for instance, auxins and cytokinins seem to influence different parts of the cell cycle. Whereas auxins influence DNA replication, cytokinins are responsible for cell division (Gaspar et al., 1996).

Another example of the interaction between auxins and cytokinins is the regulation of apical dominance. In contrast to the before mentioned example, in this case the two hormones work as antagonists. Apical dominance is a phenomenon where the growing shoot tip suppresses the growth of axillary buds by producing auxins. Cytokinins, however, counter the effect of the auxins (Tanaka et al., 2006). Because of this occurrence, in many plants the cytokinin-rich axillary buds, closer to the roots, tend to be longer than the auxin-rich axillary buds, located closer to the shoot tip (Urry et al., 2016).

2.3.4 Ethylene

Ethylene is a gaseous hormone that is produced in response to stresses like high temperatures, infections, injuries or mechanical pressure. It's main functions include leaf abscission, the triple response in seedlings and the promotion of fruit ripening in climacteric fruit (Urry et al., 2016). Grapes are not climacteric fruit, as no ethylene or respiration bursts occur during the maturation process (Abeles et al., 1992a). In ethylene action, two types of responses can be distinguished: the concentration response and the sensitivity response. While the latter mentioned is a change in the ethylene-sensitivity of the tissue where the hormone is already present (for example in some senescence or abscission phenomenons), the concentration response is caused by an actual change in the concentration of cellular ethylene. As stated before, many environmental stresses increase the ethylene production. Thus, almost all stress-induced physiological changes belong to the category of concentration responses (Abeles et al., 1992b).

2.3.5 Abscisic Acid

Abscisic acid (ABA) is involved in the regulation of seed and bud dormancy, stomata closure and adaptive responses to biotic and abiotic stresses (Nambara and Kuchitsu, 2011). ABA is essentially a breakdown product of carotenoids such as violaxanthin and neoxanthin (Kende and Zeevaart, 1997).

The synthesis of ABA primarily happens in vascular tissues, where it is transported to target tissues. It is mobile in phloem and xylem, therefore allowing transport from the roots to the shoots and vice versa (Finkelstein, 2013).

Coombe and Hale (1973) suggested that the onset and rate of ripening is directly linked to the accumulation of ABA in the grape berry. In fact, they noticed a gradual raise in ABA before verasion, while before the beginning of ripening it increased rapidly. Pilati et al. (2017) proved that ABA signaling regulates an extensive gene modulation which finally is an essential trigger for the beginning of berry skin ripening.

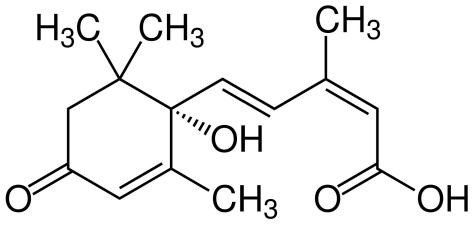


Figure 2.9: The chemical structure of abscissic acid (ABA)

Materials and methods

3.1 Vineyard description

The trial was conducted in two commercial vineyards, both located at Salorno, South Tyrol, Italy. The vineyards are called "Fra gli adigi" and "Puncli". Both vineyards lie at an average altitude of 230 m and have a north-to-south row orientation.

Fra gli adigi

The vines are 8 years old Pinot gris, SMA 505 and SMA 514 clones, grafted on SO4 rootstock. These vines are pruned in Double Guyot, with approximately 11 buds per m^2 with a vine spacing of 0,8 m and a row spacing of 2 m.
The proximity to the river "Adige", whose riverbed is roughly 180 m away from the vineyard clearly has a influence on the soil composition. This alluvial soil can be described as sandy silt of mediocre fertility. Groundwater can be found at a few meters of soil depth.

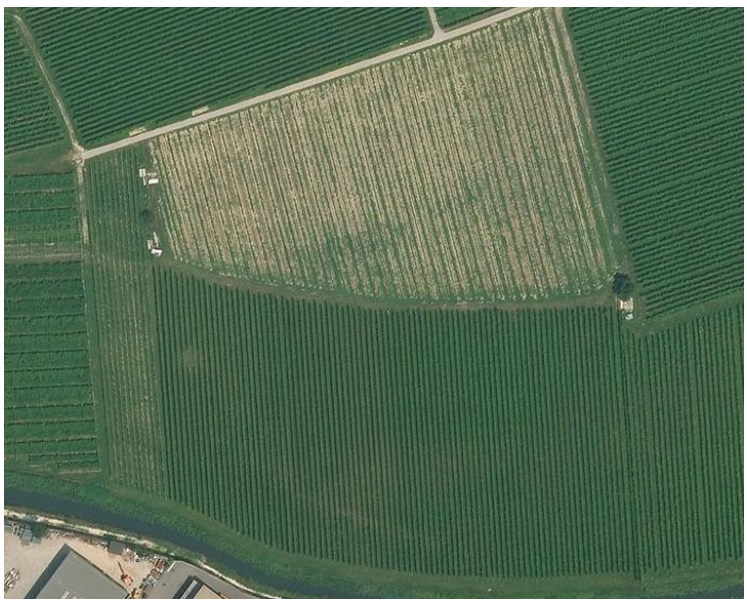


Figure 3.1: "Fra gli adigi" vineyard. Aerial photo from Geobrowser 3

Puncli

In this vineyard, 12-year old Pinot gris vines, SMA 505 and SMA 514 clone grafted on SO4 rootstock, are planted. These vines are trained on a pergola system with approximately 12 buds per m^2 with a vine spacing of 0,8 m and a row spacing of 3 m. The total area of this vineyard is roughly 1 ha.
The soil of this vineyard is influenced by the greater distance to the river "Adige" (800 m from the riverbed) and the proximity of the calcareous mountain "Favogna". The soil consists of very fertile sandy clay loam.

Figure 3.2: "Puncli" vineyard. Aerial photo from Geobrowser 3

3.2 Experimental design

One of the main goals of this study is to evaluate and compare the efficiency of leaf removal, executed at early flowering, and the application of gibberellic acid (GA_3), carried out at full bloom, in their ability to reduce the cluster compactness.
This study implements four treatments: an untreated control, defoliation, GA_3 application and a combination of both. These measures were applied on Pergola and Guyot, making up a total of eight treatments, as summarized in Table 3.1.
The following paragraphs will elucidate how the different treatments were divided into experimental blocks and how these were arranged in each vineyard. It is worthwhile to mention that, regarding the order of the various treatment blocks, the placement of the combined treatments (T4 and T8) in between the treatments in comparison was intentional to allow a more ergonomic treatment procedure.

Table 3.1: Overview of the treatments applied in this study

Treatment name	Vineyard	Trellis form	Measures	Developmental stage (Lorenz et al., 1994)
T1	Puncli	Pergola	Untreated control	
T2	Puncli	Pergola	Leaf removal	BBCH 62
T3	Puncli	Pergola	GA_3	BBCH 65
T4	Puncli	Pergola	Leaf removal + GA_3	BBCH 62 and BBCH 65
T5	Fra gli Adigi	Guyot	Untreated control	
T6	Fra gli Adigi	Guyot	Leaf removal	BBCH 62
T7	Fra gli Adigi	Guyot	GA_3	BBCH 65
T8	Fra gli Adigi	Guyot	Leaf removal + GA_3	BBCH 62 and BBCH 65

Puncli

The experiment-specific treatments were applied in the first six rows of the vineyard. Each row has a length of 70 m and contains more or less 100 vines. Every two rows correspond to a cluster reduction measure, with an exception in row six, where one half of the row represents the untreated control.

Fra gli adigi

The trials T5 to T6 were conducted in the first 11 rows of the 4 ha large vineyard. The approximately 120 m long rows in "Fra gli adigi", were divided in three equal sections of 40 m, each containing 32 vines. The growth in this vineyard is very homogeneous, hence the use of a randomized block design was waived in order to avoid cross-contamination from spray drift, coming from GA_3 application. Thus, the whole testing parcel consists of three sections, corresponding to one treatment each, exclusive of row one, where the first two sections constitute the untreated control.

Analog to the colors assigned to the different treatments (Table 3.2 and 3.3), plastic panels were cut out and affixed to delimit the beginning and the end of each treatment block (Figure 3.3 and 3.4).

Table 3.2: Experimental design in “Puncli”

Row number	
1	
2	
3	
4	
5	
6	

T1 T2 T3 T4

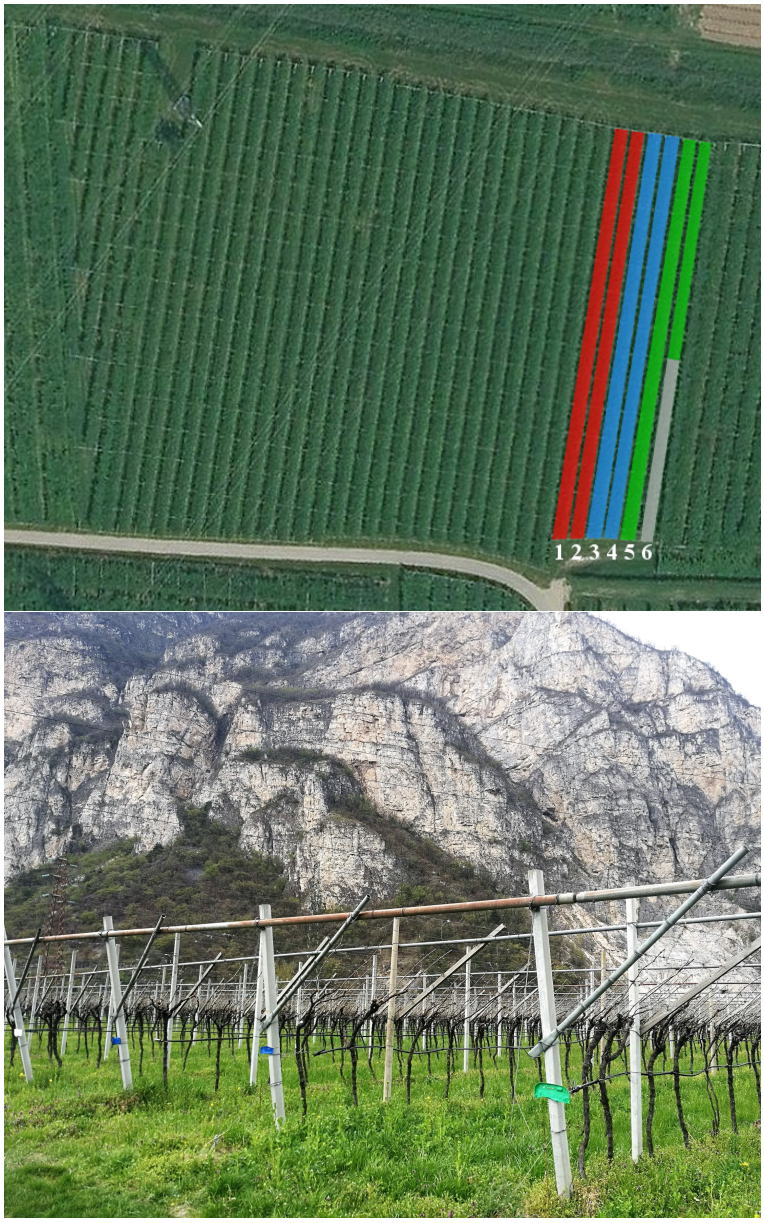


Figure 3.3: Experimental design in Puncli. Picture above from Geobrowser 3, edited

Table 3.3: Experimental design in “Fra gli adigi”

Row number	Section 1	Section 2	Section 3
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			

T5 T6 T7 T8

Figure 3.4: Experimental design in Fra gli Adigi. Picture above from Geobrowser 3, edited

3.3 Cluster reduction measures

3.3.1 Leaf removal

Leaf removal was performed at early flowering (code 62 on the BBCH developmental stage scale) with a treatment intensity of removing approximately 5 basal leaves. Leaf removal was carried out mechanically using an OLMI (Olmi s.n.c., Costigliole d'Asti, Italy) pneumatic leaf removal machine on Pergola and a Binger EVB 2000 (Binger France, Niederhergheim, France) de-leafer on Guyot.
Apart from the fact that the two machines are being used on a different training system, they also have a distinct operating principle: while the OLMI creates air pulses that mangle the leaves, the Binger attracts the leaves with a suction fan and cuts them off with a rotating blade.

Figure 3.5: Pictures of the Machines used for defoliation

3.3.2 Application of gibberellic acid

GA_3 was applied at full flowering, which corresponds to stage 65 on the BBCH scale. The Treatment was carried out with Lochmann (Lochmann Plantatec, Nalles, Italy) RPS series sprayers and a GA_3 concentration of 2 g/hl with 10 hl per hectare.

Figure 3.6: The sprayer used for GA_3 application in Fra gli Adigi

3.4 Timelines

The timing of the key phenological growth stages and the application of measures are represented in Table 3.4.

Table 3.4: Timeline of the growth stages and treatment application

Fra gli Adigi		
Date	BBCH stage	Applied Measure
09.04.2020	09 - Bud burst	
14.04.2020	13 - 3 leaves unfolded	
19.04.2020	15 - 5 leaves unfolded	
24.04.2020	53 - Inflorescences clearly visible	
09.05.2020	55 - Inflorescences swelling	
16.05.2020	57 - Inflorescences fully developed	
18.05.2020	60 - First flowerhoods detach	
19.05.2020	61 - Beginning of flowering	
20.05.2020	62 - 20% of flowerhoods fallen	Leaf removal
20.05.2020	63 - Early flowering	
21.05.2020	65 - Full flowering	GA_3 application
28.05.2020	71 - Fruit set	
27.07.2020	81 - Véraison	
31.08.2020	89 - Harvest	
Puncli		
Date	BBCH stage	Applied Measure
11.04.2020	09 - Bud burst	
17.04.2020	13 - 3 leaves unfolded	
21.04.2020	15 - 5 leaves unfolded	
27.04.2020	53 - Inflorescences clearly visible	
09.05.2020	55 - Inflorescences swelling	
16.05.2020	57 - Inflorescences fully developed	
18.05.2020	60 - First flowerhoods detach	
19.05.2020	61 - Beginning of flowering	
20.05.2020	62 - 20% of flowerhoods fallen	Leaf removal
20.05.2020	63 - Early flowering	
21.06.2020	65 - Full flowering	GA_3 application
29.05.2020	71 - Fruit set	
24.07.2020	81 - Véraison	
31.08.2020	89 - Harvest	

3.5 Assessment of canopy and yield parameters

3.5.1 Canopy measurements

Determination of the plastochron index

During the growing season, each vineyard's vigour was monitored by measuring the plastochron index (PI). A plastochron is the timespan in-between the initiation of two successive leaf primordia and the plastochron age can be calculated using the formula

$$\mathrm{PI} = n + \frac{\ln L_n - \ln 30}{\ln L_n - \ln L_{n+1}} \tag{3.1}$$

(Erickson and Michelini, 1957), where n is the node position of the youngest leaf with a lamina length above 30mm (Freeman and Kliewer, 1984; Schultz, 1993), L_n is the length of the aforementioned leaf and L_{n+1} is the length of the leaf on the subsequent node.
The developmental age of a specific leaf can be determined by calculating the leaf plastochron index (LPI.), which can be achieved by subtracting the node position from the leaf of interest, i, from the plastochron index (Erickson and Michelini, 1957).

$$\mathrm{LPI.} = \mathrm{PI} - i \tag{3.2}$$

The shoots of 10 vines in the testing parcel of each vineyard were randomly selected and kept track of. The measurements were carried out in regular intervals of approximately seven days in length, starting from five unfolded leaves (BBCH 09) until 10 days after leaf removal.

Assessment of flowering and fruit set

Fruit set was determined by using the method of Poni et al. (2006). The fruit set ratio was calculated by dividing the number of berries, counted at BBCH 75, by the number of flowers, quantified at BBCH 57 (Equation 3.3).

$$\text{Fruit set} = \frac{\text{Number of berries}}{\text{Number of flowers}} \times 100 \tag{3.3}$$

For the quantification of the number of flowers, 20 clusters in the testing parcels were photographed against a dark background and the number of flowers on each inflorescence was manually counted. Later, the linear regression between the number of visible flowers on the photograph and the actual number of flowers present on each cluster was calculated. The resulting function was used to estimate the mean flower number of each treatment.

Figure 3.7: Pinot gris inflorescences photographed against a dark background

Berry number on the other hand was determined by weighting 500 berries in each vineyard at pea-size (BBCH 75) and dividing the total weight by the number of collected berries, obtaining the mean berry weight. Berry number per cluster was then calculated by dividing the total weight of the berries present on a cluster by the mean berry weight (Equation 3.4). In order to exclude the influence of the rachis on the bunch weight, the berries were peeled off of the cluster for weighing (Figure 3.8).

$$\text{Number of berries} = \frac{\text{Total weight of berries on cluster}}{\text{Mean berry weight}} \tag{3.4}$$

Figure 3.8: Berries at pea size being weighed

Defoliation intensity

Defoliation intensity was calculated by measuring the leaf area (LA) before and after defoliation (Equation 3.5). Leaf area values were obtained by analysing pictures of the canopies with *ImageJ*, an open source program for scientific image analysis (Schneider et al., 2012).

$$\text{Defoliation intensity} = (1 - \frac{\text{LA after LR}}{\text{LA before LR}}) \times 100 \tag{3.5}$$

A total of 74 leaves, of which 30 were basal leaves, 22 were leaves from the middle section of the shoot and 22 were apical leaves, have been collected and scanned in order to create a regression between the length of the central vein and the area of the leaf blade according to the equation

$$\text{LA}_i = a\text{L}^b \tag{3.6}$$

by Montero et al. (2000), where LA_i is the area of an individual leaf and L is the length of leaf lamina. Afterwards, LA was calculated by measuring the length of the central vein off of every leaf on 20 randomly chosen shoots on digital pictures before and after defoliation took place. The pictures of the canopies were taken with a digital camera after tensioning white fabric behind the canopy as background (Figure 3.9).
Furthermore, defoliation intensity was also measured by calculating the percentage of removed or damaged leaves in proportion to the leaf number before defoliation. The damage of pneumatic defoliation was additionally estimated by fitting the leaf length into the regression curve and comparing the potential LA_i to the actual LA_i computed by image analysis.

Figure 3.9: Digital pictures of the canopy were used for LA estimation

3.5.2 Yield, Fruit quality and cluster compactness

Bunch rot

Bunch rot incidence (BRI) and severity (BRS) was determined at harvest. The mean severity was calculated by assigning clusters, based on the severity into one of seven classes (0%; 1–5%; 6–10%; 11–25%; 26–50%; 51–75%; 76–100%), according to the EPPO guideline PP1/17, and dividing the total severity by the number of analysed clusters (Molitor et al., 2015).

Cluster compactness

Cluster compactness was assessed through the density indexes described in Section 2.2.1. Bunch weight (used for LDI and TDI) was determined at harvest (BBCH 89) by using a precision scale. The estimation of IDI and the measurement of bunch length (used for LDI, TDI and SDI) also took place at harvest. Berry number, however, was calculated at pea-size by using the method previously described for the determination of fruit set. The mean berry weight was calculated again at harvest for the comparison between the treatments.

Total soluble solids

The sugar content of each treatment was determined at harvest by using a portable refractometer.

Yield

Yield was estimated in terms of yield per vine (YPV), as well as yield per hectare (YPH). Yield per vine, expressed in kg/vine, was calculated by multiplying the mean bunch weight by the average number of clusters per vine. On the other hand, Yield per hectare, expressed in dt/ha, was determined by multiplying YPV by the number of vines per hectare which, again, was calculated by dividing 10.000 m^2 by row spacing per vine spacing (both in metres).

3.5.3 Statistical analysis

Measured parameters, where possible, were compared by performing a one-way ANOVA with post-hoc Tukey HSD Test.

Results and Discussion

4.1 Results

4.1.1 Meteorological aspects of the vegetative period

During the growing season 2020, the data collected at the weather station of Salorno were used to describe the course of the weather during the vegetative period, as summarized in Figure 4.1 (Weather and avalanche service, 2020).

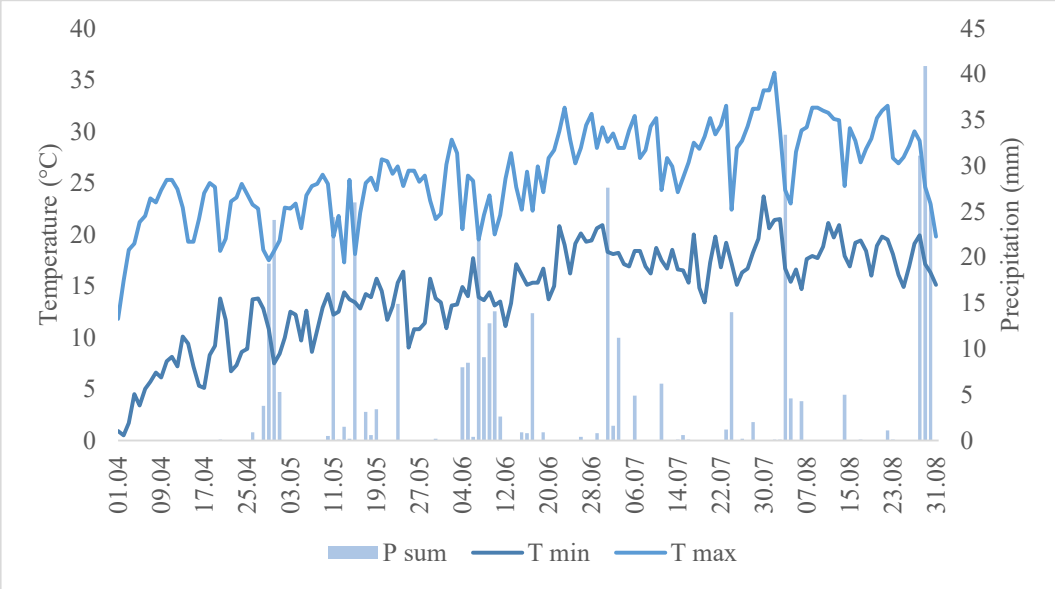


Figure 4.1: Precipitation, min and max temperatures (April through August)

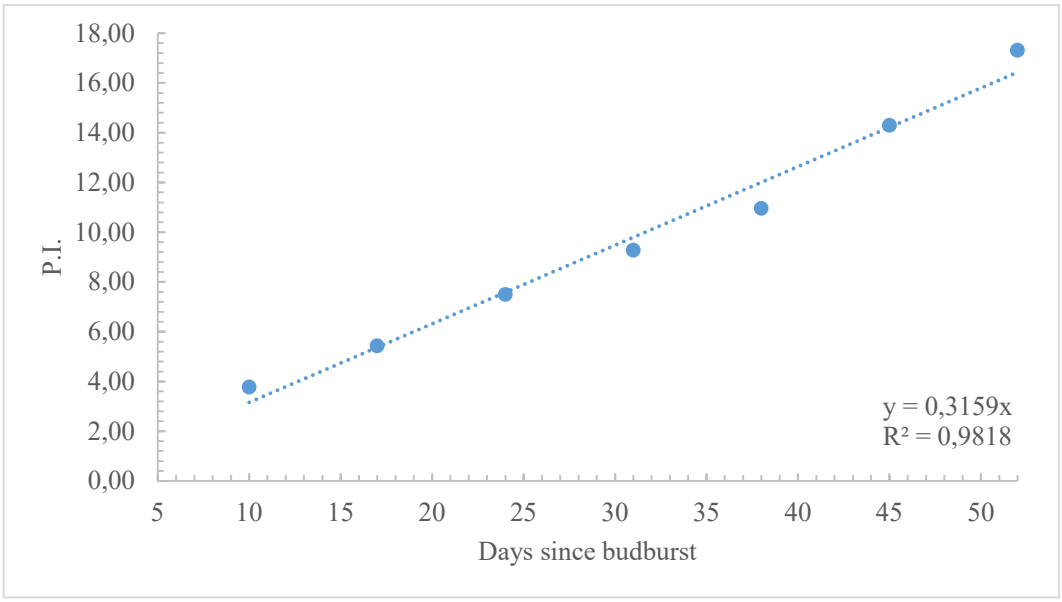
April had relatively high average temperatures, which resulted in an earlier budburst than in previous years and a quick development of the shoots (Südtiroler Beratungsring für Obst- und Weinbau, 2020c). In fact, the phenology was anticipated by approximately three weeks compared to the previous year (Südtiroler Beratungsring für Obst- und Weinbau, 2020a). The week before flowering was characterized by multiple precipitation events, therefore not allowing any of the defoliation treatments, which took place at the early

stages of flowering instead. The following high temperatures and the presence of a high pressure system contributed on a quick progression of the flowering process (Südtiroler Beratungsring für Obst- und Weinbau, 2020b). Multiple precipitation events also occurred during the period after flowering, which led to a higher amount of *Plasmopara viticola* infections when compared to previous years (Südtiroler Beratungsring für Obst- und Weinbau, 2020d).

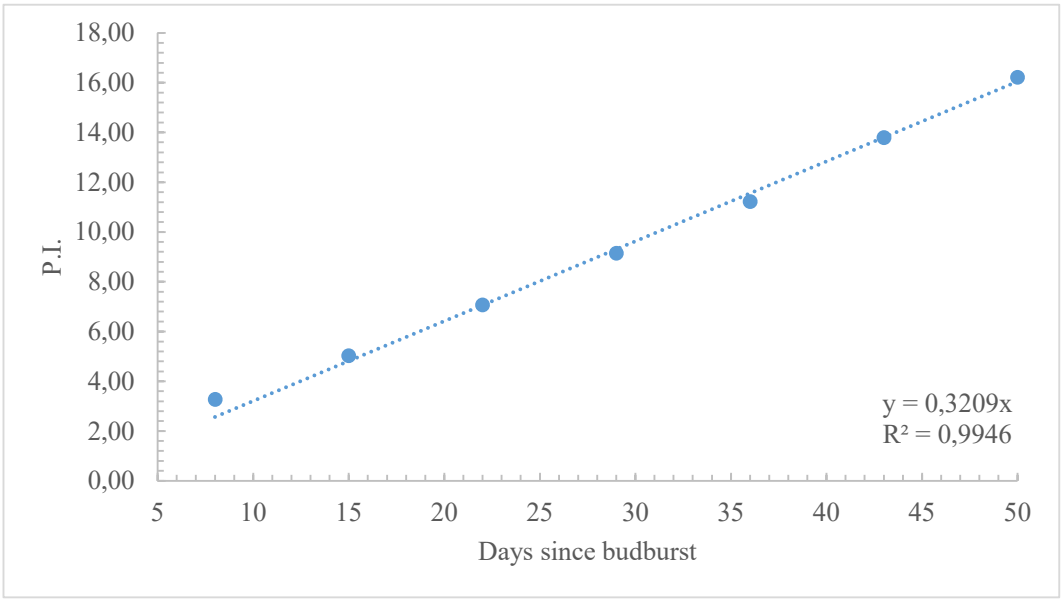
4.1.2 Canopy measurements

Determination of the plastochron index

The plastochron index was monitored for 50 days after budburst on a total of 20 tagged shoots. The data of the mean PI in relation to the days after budburst, inserted into a scatter plot, could be approximated to the linear functions $y = 0,3159x$ for Fra gli Adigi and $y = 0,3209x$ for Puncli with R^2 values of 0,98 and 0,99 respectively (Figure 4.2). It is noteworthy to mention that, during the active growth phase, the plastochron curve can be simplified as a linear function (Freeman and Kliewer, 1984; Poni and Giachino, 2000), however, if plotted over a longer period of time (until growth cessation), the PI would follow a logistic growth curve (Schultz, 1992).
Comparing the plastochron curves to the meteorological events of the first part of the growing season (Figure 4.1), the deviation from the linearity at around 40 days after budburst can be justified by precipitation events and therefore lower temperatures and a lower availability of photosynthetically active radiation.
The foliar age can be calculated by multiplying the LPI of the leaf of interest at a given date by the reciprocal value of the slope of the linear regression (Poni and Giachino, 2000). The reciprocal value of the slope of the linear function describing the PI indicates the number of days that need to pass to increment the PI by one unit. The multiplication of the LPI by the days per plastochron therefore results in the leaf age expressed in days. At the date of leaf removal, the average age of the three basal leaves was 39,83 days, 36,66 d and 33,50 d in Fra gli Adigi and 37,69 d, 34,57 d and 31,45 d for node positions one, two and three, respectively.



(a)



(b)

Figure 4.2: The evolution of the plastochron index in Fra gli Adigi (a) and Puncli (b) in function to the days since budburst

Comparison of fruit set in the different treatments

Fruit set was determined for each treatment by dividing the number of berries, calculated at pea-size, by the number of flowers, estimated inserting the number of flowers, counted on a picture of the inflorescence against a dark background, into the function $y = 1,6233x + 3,986$ obtained by calculating the linear relationship between the numbers on the photograph and the actual number of flowers present on the cluster (Figure 4.3).

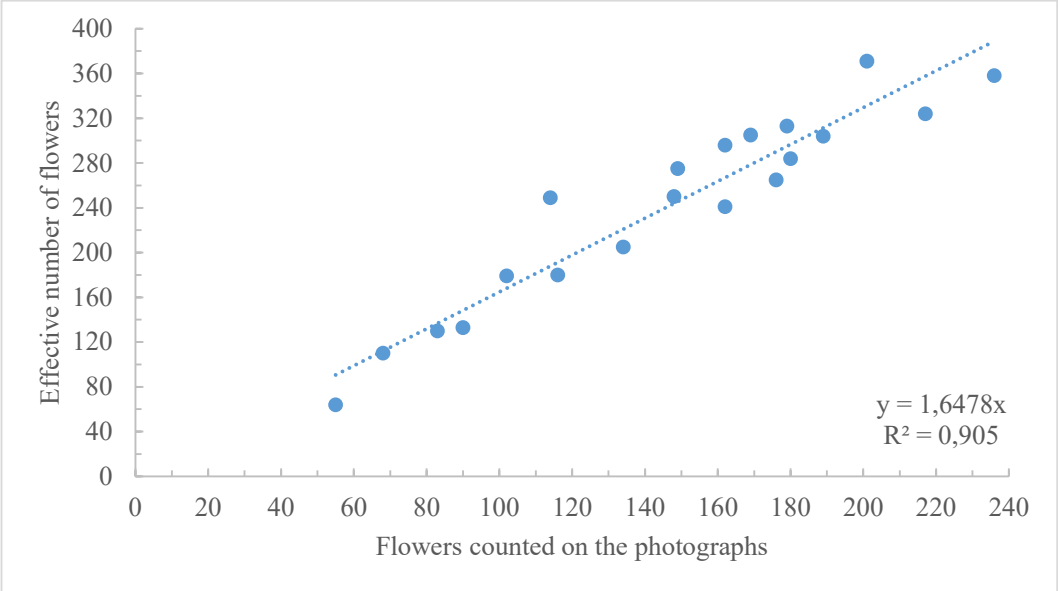


Figure 4.3: Linear regression between the flowers visible on the pictures and the actual number of flowers determined by manual counting

Berry number was estimated at pea-size (BBCH 75) by dividing the mean weight of 500 berries by the mean weight of the berries on the clusters. 500 berries amounted to a weight of 154 g in Fra gli Adigi and 187 g in Puncli, making the mean berry weight 0,308 and 0,374 g per berry, respectively.
For statistical treatment, a one-way ANOVA with post-hoc Tukey HSD Test was performed. While there was no statistically significant difference between flower number in the various treatments, all of the cluster reduction measures showed statistically significant ($p < 0,01$) differences in berry number when compared to the untreated controls. There was no significant difference between the berry number of the two untreated controls. The various methods for the reduction of cluster compactness were unable to show statistically significant differences in berry number when compared to each other.
Since fruit set was calculated by using the means of representative samples from each treatment and not by estimating the fruit set of marked clusters, a post-hoc test was not performed.

Although there was no statistically significant difference in berry number between the various measures for the reduction of cluster compactness, fruit set, however, was generally the lowest in the treatment with combined compactness reduction measures (T4 and T8). Furthermore, despite being very close, the fruit set ratio of the defoliation treatments (T2 and T6), was slightly higher than the treatments with GA_3 application (T3 and T7). The untreated controls had the overall highest fruit set ratio.

Table 4.1: Flower number, Berry number and Fruit set in the different treatments

Treatment	Vineyard	Trellis form	Flower number	Berry number	Fruit set (%)
T1	Puncli	Pergola	264,09 a	129,65 b	49,09
T2	Puncli	Pergola	252,00 a	84,42 a	33,50
T3	Puncli	Pergola	266,61 a	88,06 a	33,03
T4	Puncli	Pergola	245,41 a	73,81 a	30,08
T5	Fra gli Adigi	Guyot	244,42 a	122,94 b	50,30
T6	Fra gli Adigi	Guyot	261,90 a	77,06 a	29,53
T7	Fra gli Adigi	Guyot	286,72 a	82,90 a	28,91
T8	Fra gli Adigi	Guyot	268,15 a	72,29 a	26,96

Results marked with different letters indicate a statistically significant difference according to Tukey's HSD test ($p < 0,01$)

(a) (b)

Figure 4.4: Extreme examples of high (a) and low fruit set (b) in Fra gli Adigi

Defoliation intensity and leaf area

Defoliation was carried out mechanically in both training systems. The two types of leaf removers, besides their operating principle, also differed in their impact on the LA (Table 4.2), which was estimated by calculating the relationship between the individual leaf area (LAi) and the length of the midvein (Figure 4.5).

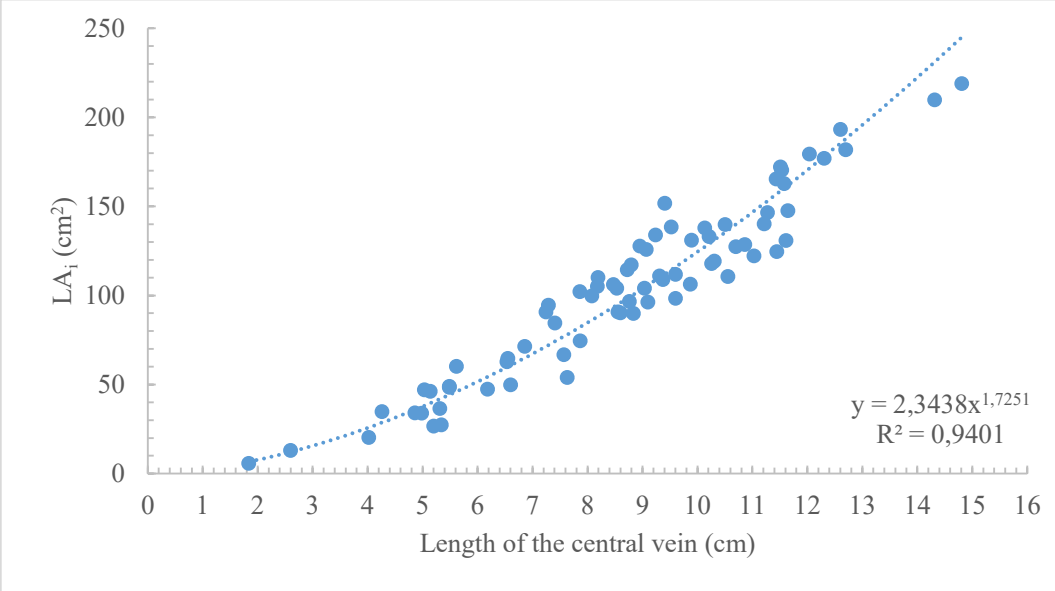
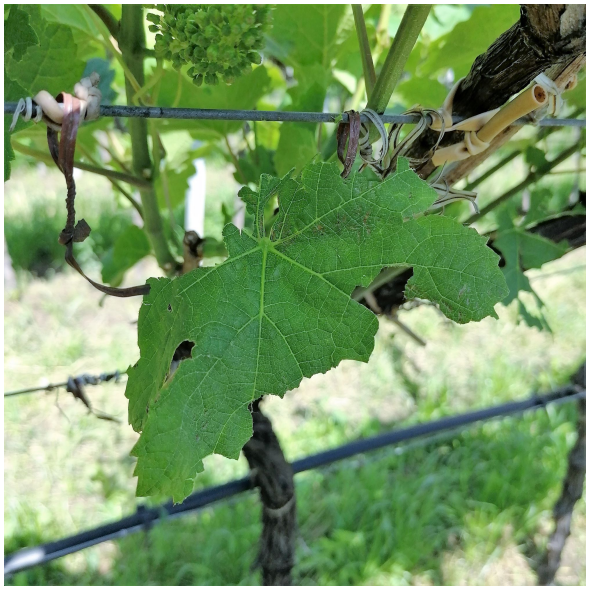


Figure 4.5: The relationship between the area of a single leaf and the length of the midvein

While the leaf remover used in Fra gli Adigi completely cut off the leaves in the cluster zone, the pneumatic leaf remover's air pulses shredded the leaves only partially (Figure 4.6 and 4.7).
Pneumatic leaf removal damaged approximately 45% of the leaves of each shoot and lowered LAi by 32,5% on average and LA per vine by 21%, while the leaf plucker removed 29% of the number of leaves per shoot completely, reducing the total LA per vine by 49%.

Table 4.2: Defoliation intensity

	Fra gli Adigi		Puncli	
	before LR	after LR	before LR	after LR
LA per shoot (cm^2)	656,200	336,172	657,398	522,322
LA per vine (cm^2)	8530,593	4370,238	9203,567	7312,510
Removed LA (cm^2)	-	320,027	-	135,075
Defoliation intensity (%)	n.a.	49	n.a.	21

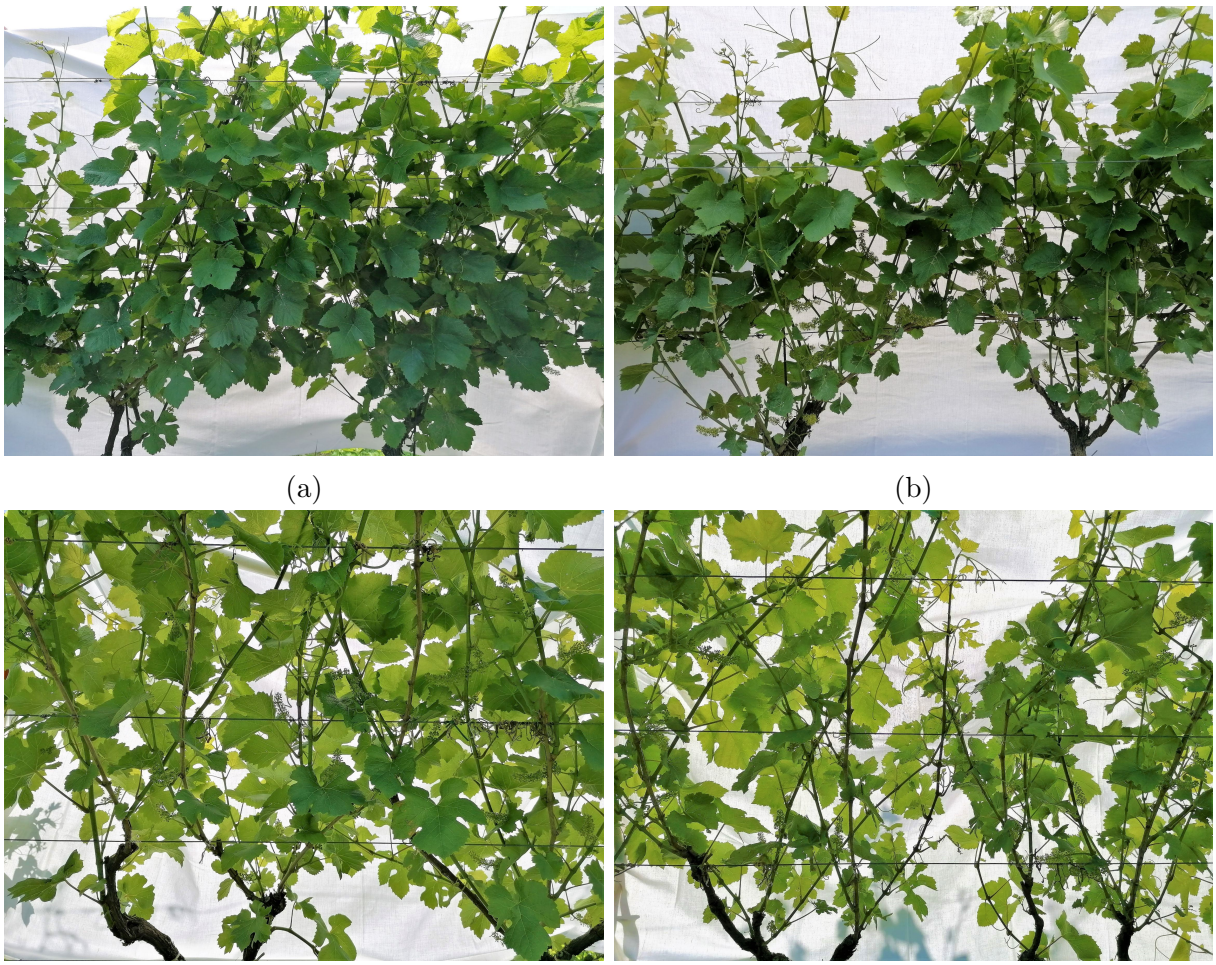


(a)

(b)

(c)

Figure 4.6: Partially mangled leaves (a, b) and a pneumatic leaf remover hurling foliar debris through the air (c)



(a) (b)

(c) (d)

Figure 4.7: Pictures of the canopies before (a, c) and after LR (b, d)

4.1.3 Fruit quality and cluster compactness

Cluster compactness

For calculating the various cluster compactness indexes, multiple parameters had to be assessed, for instance the mean rachis length and mean bunch weight. Table 4.3 gives information on how these parameters were influenced by the various treatments.

Table 4.3: Parameters used for the assessment of cluster compactness

		T1	T2	T3	T4	T5	T6	T7	T8
Berry number		129,65	84,42	88,06	73,81	122,94	77,06	82,90	72,29
	σ	29,77	20,81	30,71	24,97	30,18	29,39	32,85	26,18
	Effect (%)	-	−34,89	−32,08	−43,07	-	−37,32	−32,57	−41,20
	HSD	b	a	a	a	b	a	a	a
Rachis length		12,60	12,53	12,44	12,51	12,80	12,70	12,83	12,79
	σ	1,79	1,46	2,23	1,12	1,41	1,32	1,46	1,89
	Effect (%)	-	−0,57	−1,25	−0,68	-	−0,78	0,22	−0,11
	HSD	a	a	a	a	a	a	a	a
Cluster weight		149,52	114,09	120,09	110,80	138,26	115,93	106,07	82,95
	σ	56,89	32,44	34,82	37,66	34,95	32,53	44,42	35,44
	Effect (%)	-	−23,70	−19,68	−25,89	-	−16,15	−23,29	−40,01
	HSD	d	bc	bc	b	cd	b	b	a

σ indicates the standard deviation, effect shows the difference to the untreated control expressed in %, different letters in HSD indicate a statistically significant difference according to Tukey's honest significant difference test ($p < 0,01$)

As stated in Section 4.1.2, berry number showed statistically significant differences towards the untreated control in all bunch thinning measures. However, there was no statistically significant difference between the cluster compactness reduction measures themselves.

Rachis length was not influenced by any of the treatments, while cluster weight, on the other hand, varied significantly among the treatments. Most importantly, all the cluster reduction measures showed statistically significant differences to the untreated controls. There was no statistically significant difference between the cluster reduction measures, with an exception in the combined treatment in Guyot, where bunch weight was the overall lowest (Figure 4.8).

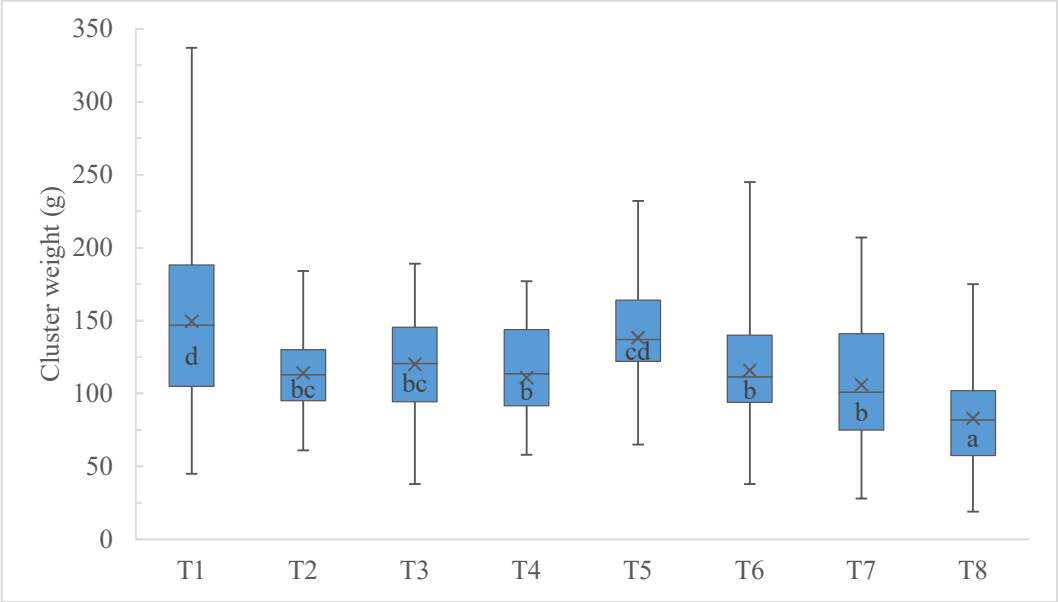


Figure 4.8: Box-plot of measured cluster weights. Different letters indicate a statistically significant difference according to Tukey's honest significant difference test ($p < 0,01$).

Further statistical treatment was made by assigning the collected clusters to six weight classes and calculating the relative frequency of each class for every treatment (Figure 4.9).

The relative frequency of extrema (weight below 49 or above 200 grams) was generally low, with exceptions in treatments T1, T5, T7 and T8. Clusters with total weight below 49 g were the highest in the treatments 7 and 8 with a relative frequency of 9,46 and 18,18%, respectively. In T2, the aforementioned class made up 3,5% of the analysed clusters, whereas it was below 1,5% in treatments 1, 3, 4, 5 and 6. On the other hand, clusters with a total weight above 200 g were the highest in the two untreated controls, T1 and T5, making up 18,88 and 8,33%, respectively, followed by 3,25% in T4, while the other treatments had frequencies below 1,5%. Clusters with weights between 50 and 99 g were predominant in the treatments 8, 2 and 7 with frequencies of 55,84%, 42,66% and 37,84%, respectively. This class made up 20-30% of T1, T3, T4 and T6, as well as 11,11% of T5. The weight class between 100 and 124 g was where the majority of treatments have their mean weight in. The class forms 21-36% of all clusters in treatments 2, 3, 4, 5 and 6. T1, T7 and T8 have a frequency of 14,69%, 18,92% and 11,69%, respectively. Cluster weights between 125 and 149 g were more frequent in the treatments 3, 4, 5 and 6 (20-36%) and less frequent in the treatments 1, 2, 7 and 8 (9-19%). The frequency of clusters between 150 and 199 g was the highest in T1 with 30,07%, ranged from 18% to 21% in T3, T4, T5 and T7 and from 5% to 13% in T2, T6 and T8.

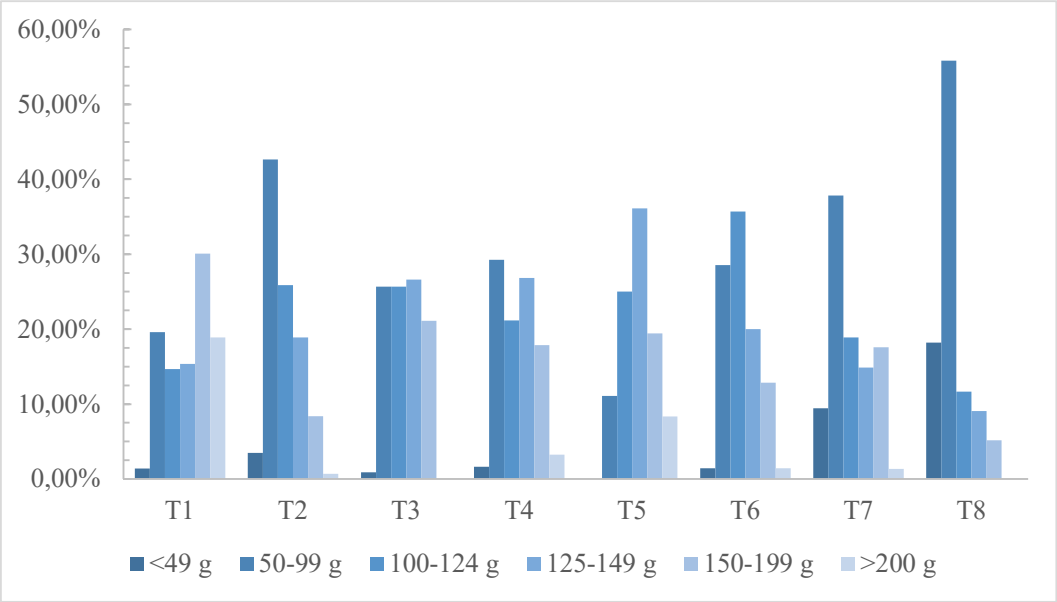


Figure 4.9: Relative frequency of cluster weight classes in each treatment

Cluster weight and rachis length were used to determine LDI and TDI, which expressed cluster density in g/cm and g/cm^2, respectively. Table 4.4 portrays the results of both calculated indexes and how the bunch thinning measures differ from their respective untreated control.

Table 4.4: LDI and TDI in the various treatments

	T1	T2	T3	T4	T5	T6	T7	T8
LDI (g/cm)	11,87	9,11	9,65	8,85	10,80	9,13	8,27	6,49
Effect (%)	-	−23,26	−18,67	−25,39	-	−15,49	−23,46	−39,94
TDI (g/cm^2)	0,94	0,73	0,78	0,71	0,84	0,72	0,64	0,51
Effect (%)	-	−22,82	−17,64	−24,88	-	−14,83	−23,63	−39,87

Effect shows the difference to the untreated control expressed in %

The untreated control in Puncli, T1, had the most compact clusters with 11,87 g/cm or 0,94 g/cm^2. It is closely followed by the untreated control in Fra gli Adigi with a compactness of 10,80 g/cm or 0,84 g/cm^2. Leaf removal led to similar compactness values in both Pergola and Guyot: T2 had an LDI of 9,11 and TDI of 0,73, whereas T6 had an LDI of 9,13 and TDI of 0,72. GA_3 application led to a more severe reduction of cluster compactness on Guyot than on Pergola; T3,had an LDI of 9,65 and TDI of 0,78, where T7, however, had an LDI of 8,27 and TDI of 0,64. The treatments where LR and GA_3 were combined, were the least compact in their respective vineyards. T4 resulted to have an LDI of 8,85 and TDI of 0,71 and T8 had an LDI of 6,49 and TDI of 0,51, making it the overall least compact.

Accordingly to the compactness values, also the relative change to the untreated control altered. The intensity of the effect of the cluster compactness reduction measures was not consistent through the measures on the two trellis systems. For instance, while the effect of GA_3 was not as intense as LR on Pergola, on Guyot the effect of GA_3 application was stronger than LR. When compared to the untreated control, clusters in Puncli were 18 to 25% less compact. In Fra gli Adigi, single cluster compactness reduction measures lowered the compactness by 15 to 24% in relation to the untreated control, whereas the combination of LR and GA_3 lowered cluster compactness by approximately 40%.

The density index proposed by Sabbatini and Howell (2010), which expresses cluster density as the proportion of the berry number to the cluster length, had the following results: While both untreated controls had approximately 10 berries per cm of the rachis main axis (10,29 in Pergola and 9,60 in Guyot), the bunch thinning measures had values around 6 and 7. More precisely, in Pergola LR had an SDI of 6,74 berries per cm, GA_3 had an average of 7,08 and the combined treatment had 5,90. While maintaining the same proportion among the treatments, SDI values in Guyot were lower with 6,07 berries per cm in LR, 6,46 in GA_3 and 5,56 in the combined treatment.
In contrast to the previously mentioned density indexes, the density index by Ipach et al. (2005), beyond the mean cluster compactness, also gave insight on how the different compactness classes were represented numerically in the different treatments (Figure 4.11). Overall, the control treatments were predominantly represented by a high presence of compact clusters with a IDI of five (81 out of 100 clusters in Pergola and 75 in Guyot). All the cluster reduction measures, except the combined treatment in Guyot, were dominated by clusters with a rating of four. It is noteworthy to mention that, even though GA_3 and LR had means with no statistically significant difference, in field, there was a visible difference in the distribution of clusters per compactness class. Defoliated treatment parcels had a relatively high numbers of clusters in the classes 3 and 4 (78 out of 100 clusters in Pergola and 65 in Guyot) with lower numbers with rating 1, 2 or 5, whereas the application of gibberellic acid had a more uneven effect, thus covering a broader spectrum of classes all while maintaining it's peak at clusters belonging to class 4. In some cases, the cluster reduction treatments resulted in excessive coulure, classified with a rating of 1 on the IDI scale. These latter mentioned were more frequent in Guyot rather than Pergola, with a maximum of 19 out of 100 clusters in the combined treatment in Fra gli Adigi. Furthermore, excessive coulure was recurrent in all treatments that involved the application of GA_3, especially when combined with leaf removal.

Table 4.5: IDI with standard deviation and relative difference to the untreated control

	T1	T2	T3	T4	T5	T6	T7	T8
IDI	4,77	3,53	3,58	3,31	4,73	3,53	3,45	2,61
σ	0,53	0,85	1,07	1,05	0,49	1,05	1,31	1,16
Effect (%)	-	−26,00	−24,95	−30,61	-	−25,37	−27,06	−44,82
HSD	c	b	b	b	c	b	b	a

σ indicates the standard deviation, effect shows the difference to the untreated control expressed in %, different letters in HSD indicate a statistically significant difference according to Tukey's honest significant difference test ($p < 0,01$)

Figure 4.10: Cluster compactness classes from 1 to 5, according to IDI

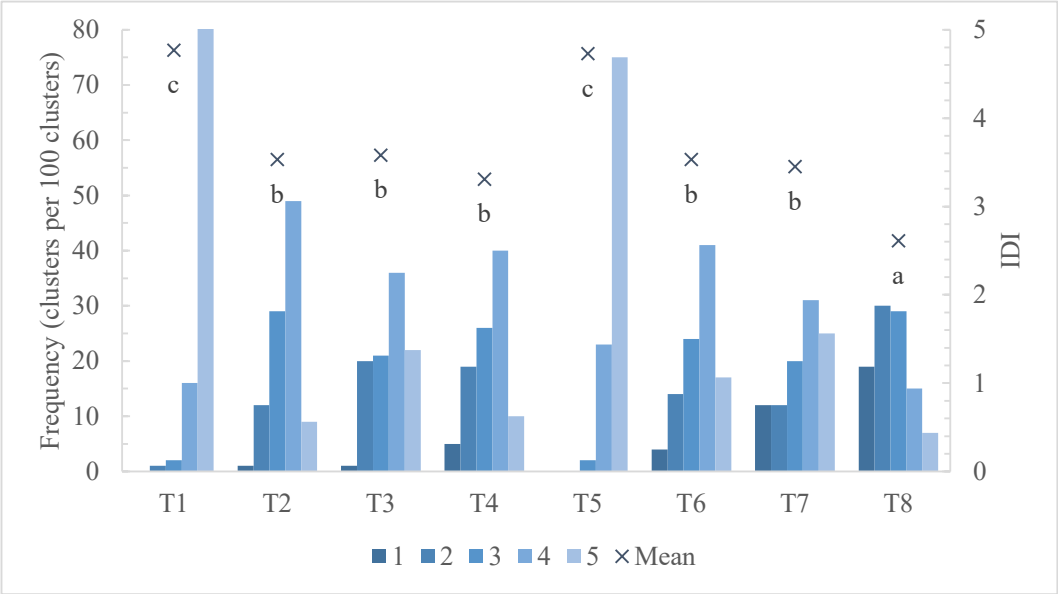


Figure 4.11: Distribution of the IDI classes in each treatment, expressed in frequency per 100 clusters, with mean IDI and HSD ($p < 0,01$)

Yield

If each trellis system is observed separately, yield per vine, in compliance with bunch weight, was the highest in the untreated control and the lowest in the combined treatment, whereas the effect of single bunch thinning measures was not consistent through the trellis systems. Figure 4.12 presents YPV and YPH sorted by the nature of the applied treatment.

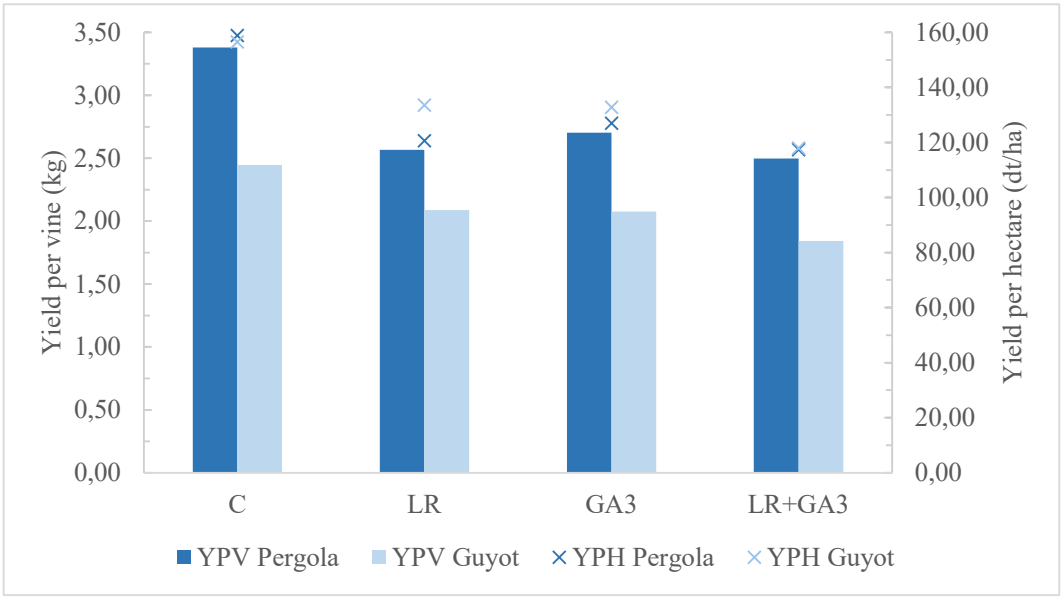


Figure 4.12: YPV and YPH per type of treatment

YPV in Pergola was higher than Guyot in all the treatments; with values ranging from 3,38 kg per vine in the untreated control to 2,50 kg in the combined treatment. The untreated control in Guyot had a mean weight of 2,45 kg per vine and the combined treatment 1,84kg with marginal differences between LR and GA_3 (2,09 and 2,08 kg per vine, respectively). YPH values in were similar in both trellis systems, especially the untreated control and the combined treatment. Yield per hectare in the controls was 158,85 dt/ha in Pergola and 156,51 dt/ha in Guyot. The combined treatment beared 117,95 dt/ha in Guyot and 117,43 dt/ha in Pergola. Yield per hectare in single bunch thinning measures was higher in Guyot with 133,60 and 132,80 dt/ha for LR and GA_3 than 120,65 and 127 dt/ha for LR and GA_3, respectively.

Bunch rot

Bunch rot incidence (BRI) and severity (BRS) was assessed at harvest. Low disease levels were present and, according to the EPPO guideline PP1/017(3) (EPPO, 2000), that case requires that only the % of infected bunches, also known as incidence, shall be assessed. 13 out of a total of 1500 inspected clusters showed bunch rot symptoms. None of these infected clusters had more than 25% of the cluster affected by bunch rot. BRI was lower than 3% in every treatment.

Total soluble solids

Differences in the average berry sugar content between the treatments were evident in Pergola but non-existent in Guyot, where all treatments roughly contained the same amount of soluble solids. The untreated control in Puncli had an average of 20,5 °Brix, LR and GA_3 both had an average of 21,5 °Brix and the combined treatment had the overall highest sugar content with 22 °Brix.

Table 4.6 summarizes the results obtained in this section of the study.

Table 4.6: Yield, fruit quality and cluster compactness

	T1 (C)	T2 (LR)	T3 (GA_3)	T4 (LR+GA_3)	T5 (C)	T6 (LR)	T7 (GA_3)	T8 (LR+GA_3)
Fruit set(%)	49,09	33,50	33,03	30,08	50,30	29,53	28,91	26,96
Berries/cluster	129,65 b	84,42 a	88,06 a	73,81 a	122,94 b	77,06 a	82,90 a	72,29 a
Berry weight (g)	1,40	1,46	1,44	1,43	1,42	1,42	1,43	1,42
Cluster weight (g)	149,52 d	114,09 bc	120,09 bc	110,80 b	138,26 cd	115,93 b	106,07 b	82,95a
Rachis length (cm)	12,60 a	12,53 a	12,44 a	12,51 a	12,80 a	12,70 a	12,83 a	12,79 a
LDI (g/cm)	11,87	9,11	9,65	8,85	10,80	9,13	8,27	6,49
TDI (g/cm^2)	0,94	0,73	0,78	0,71	0,84	0,72	0,64	0,51
SDI (berries/cm)	10,29	6,74	7,08	5,90	9,60	6,07	6,46	5,65
IDI	4,77 c	3,53 b	3,58 b	3,31 b	4,73 c	3,53 b	3,45 b	2,61 a
TSS (°Brix)	20,5	21,5	21,5	22	21	21	20,8	21
YPV (kg)	3,38	2,57	2,70	2,50	2,45	2,09	2,08	1,84
YPH (dt/ha)	158,82	120,65	127,00	117,43	156,51	133,60	132,80	117,95
BRI (%)	0,95	0,00	0,00	0,00	1,67	1,10	2,66	0,56

Results marked with different letters indicate a statistically significant difference according to Tukey's HSD test ($p < 0,01$)

4.2 Discussion

The goal of this study was to compare two different methods to reduce cluster compactness in two different growing conditions, previously referred to as viticultural models. These viticultural models, one adapted to less fertile, alluvial soils with Guyot as trellis system, and one adapted to more fertile and vigorous soils with vines trained in Pergola were selected on purpose, as any approach of rational viticulture adapts an appropriate system based on the prevalent environmental and pedological conditions. In this context, the vines in the Pergola vineyard were able to display higher productivity and vigour, which was visible especially through a larger leaf area, higher fruit set and bigger bunch weight. The fact that the same clone was planted in both vineyards and every treatment parcel, which is, among others, confirmed by the absence of statistical differences in terms initial flower number and mean rachis length, highlighted not only the differences between the various treatments, but also those between the two trellis systems.

These last mentioned differences were mainly apparent in cluster weight, the density indexes depending on this parameter and yield per vine. Higher YPV in Pergola, which has fewer vines per hectare than Guyot, can be explained as the direct result of the agronomic aim of producing a given yield per hectare: when considering the equation where YPH equals to YPV times the number of vines, in order to maintain a constant YPH, a reduction of vines per hectare has to be compensated by a higher YPV. Considering the fact that not only yield, but also vine balance and fruit composition are strongly related to the trellis system, the choice of an appropriate trellis system can be used to optimize yield and vine balance without having a negative impact on the quality of the grapes (Reynolds and Heuvel, 2009).

Also fruit set and berry number were tendentially higher in Pergola than in Guyot, however, with no statistically significant differences between the trellis systems. Regarding berry number, the two untreated controls were statistically different from all the bunch thinning measures. Cluster compactness was the highest in the untreated controls. Leaf removal and the application of GA_3 were statistically insignificant from one another, whereas both were significantly different from the untreated control.

Another remarkable aspect to consider is the difference between the distribution of the IDI cluster compactness classes: Leaf removal seemed to have lower frequencies of clusters belonging to the extrema (class 1 and 5) than GA_3. This would suggest that the response of the vine is more homogeneous and implements a more balanced and consistent ripening of the different clusters.

A few things have to be noted regarding the different methods of execution of the cluster compactness reduction measures in the viticultural models. The spatial orientation of

the canopy directly influences the mechanical accessibility and therefore the choice of machine to use and therefore directly how the treatment is applied. For instance, in Guyot the cluster reduction measures (leaf removal and GA_3) can be aimed directly and exclusively towards the cluster zone. In Pergola however, this is not possible because of the way the canes are oriented (as the "cluster zone" extends itself to the whole canopy) and cluster reduction measures have to be implemented by incorporating the canopy in it's entirety. This, in fact, could explain why the bunch thinning effect (especially GA_3) seemed to be more severe on Guyot. Supposedly, the circumstance that in Pergola the cluster reduction measures are distributed on the whole canopy instead of being able to be specifically aimed towards a cluster zone like in Guyot, might mitigate the treatment's effect. This mitigating effect could be responsible for the differences in the combined treatments. While the combination of LR and GA_3 in Pergola showed no statistically significant difference to the singularly used cluster compactness reduction methods, in Guyot, however, the combination had economically fatal consequences and led to the overall least compact clusters of the whole experiment. An explanation for this peculiar difference in the trellis systems could be obtained by investigating the exact mechanism of action of, or the reaction (for example by studying the consequences for the phytohormonal balance) to, the treatments.

The obtained results are in conformity with previously cited literature that investigate defoliation and/or the application of gibberellic acid as bunch thinning measure (Dokoozlian and Peacock, 2001; Evers et al., 2010; Hanni et al., 2013; Hed et al., 2011, 2014; Intrieri et al., 2008; Lemut et al., 2011; Molitor et al., 2011a; Poni et al., 2006; Roschatt and Innerebner, 2017). For instance, Hanni et al. (2013) obtained similar percentages of cluster weight and berry number reduction when defoliating pneumatically in Pinot blanc and Pinot noir. Comparable changes between untreated control, LR and GA_3 in cluster compactness, expressed in berries per cm, were achieved on Chardonnay by Hed et al. (2014).

The suggested mechanisms of action are, for LR, to cause a carbohydrate shortage, which results in lower fruit set (Caspari et al., 1998) and, for GA_3, to either act as pollenicide (Weaver and McCune, 1960) or by stimulating vegetative growth and therefore increasing the carbohydrate competition between the flowers and other organs of the vine (Caspari and Lang, 1996).

Due to the low bunch rot presence in both untreated control and treatments it is not possible to make statements or comparisons about the bunch rot susceptibility of the different agricultural practices conducted in this study. Significant data regarding the differences in bunch rot susceptibility could be obtained by repeating the experiment over multiple years. However, when favorable climatic conditions exist, many works have shown

the existence of a close correlation between the bunch compactness and the incidence of bunch rot (Hed et al., 2009; Tello and Ibáñez, 2014; Kocsis et al., 2018).

Conclusion

This work highlights that in both viticultural models defoliation and the application of Gibberellic acid (GA_3) both constitute valid methods for the reduction of cluster compactness by lowering berry number (as a consequence of fruit set), mean cluster weight and cluster compactness with statistically significant differences to the untreated controls. The results in cluster compactness, however, showed no statistically significant difference between LR and GA_3 in neither of the two studied trellis systems.
However, in an vine growers context, it does not make much sense to associate both techniques, as leaf removal should be carried out anyway shortly after fruit set and it would not be much of a financial burden to anticipate said operation by a few weeks. Thus, especially when considering the context of organic viticulture, leaf removal represents an efficient alternative to the use of plant growth regulators, such as GA_3, to avoid eventual bud necroses or reduced bud fruitfulness in the years following the treatment.

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