Abbreviations

AS =Ammonium Sulphate

C = Control Not Treated

DB = Dried Blood

GC = Gas Chromatography

GDD = Growing Degree Days

HS-SPME = Headspace Solid Phase Microextraction

IBMP = 3-IsoButyl-2-MethoxyPyrazine

IPMP = 3-IsoPropyl-2-MethoxyPyrazine

LR = Leaf Removal

LRCS = Leaf Removal Cluster Shading

MPs = Methoxypyrazines

MS = Mass Spectrometer

NDVI = Normalized Difference Vegetation Index

$N\text{-}NO_3^-$ = Nitric Nitrogen

P = Phosphorous

SOM =Soil Organic Matter

SS = Soluble Solids

TA = Titratable Acidity

TN = Total Nitrogen in juice

WEN = Water Extractable Nitrogen

WEOC = Water Extractable Organic Carbon

Summary

Two field experiment of early post-flowering leaf removal and soil nitrogen nutrition have been set from 2009 to 2011 in a commercial vineyard of Sauvignon blanc, located in the DOC Isonzo, Friuli Venezia Giulia, North East Italy. The experimental field design was complete randomized block with four biological replicates.

The aim of this study was to deepen the knowledge on the effect of these agronomic practices on Sauvignon blanc grape quality, focusing the attention on Methoxypyrazines (MPs): 3-IsoButyl-2-MethoxyPyrazine (IBMP) and 3-IsoPropyl-2-MethoxyPyrazine (IPMP), nitrogen heterocyclic compounds responsible of grassy flavor in the wine.

In 2010 and 2011 leaf removal trial included following treatments:

1) basal leaf removal (LR)
2) basal leaf removal and cluster shading (LRCS) by the shading net
3) control (C), untreated vines;

LRCS was adopted as a treatment for decreasing the effect of light and temperature on grapes. Leaf removal was carried out manually after berry set.

LR did not considerably affect technology maturation parameters of grapes during harvest. Yield was not affected except in 2010 when a severe attack of *botrytis cinerea* reduced mean cluster weight of C, 20% less than LR and LRCS. Concerning MPs, they decrease from veraison to harvest in all varieties where vegetal aromas are synthesized. In our experiment MPs followed the same progression. At Veraison in both 2010 and 2011, LR and LRCS significantly reduced IBMP concentration than C. In 2010, LR and LRCS had lower IBMP concentration than C ($p<0.05$). In 2010, no differences were recorded between LR and LRCS, meaning that cluster shading net had no influence on IBMP content, despite limiting solar radiation and temperature on fruiting zone than LR. In 2011, effects of LR on IBMP confirmed same significance results ($p<0.001$): LR recorded lowest IB concentration, about 50% of C, while LRCS had about 61% of C. As in 2010, also in 2011, IBMP content was similar in LR and LRCS. C and

LRCS At veraison IBMP was much higher in 2011 than in 2010, confirming the strong year effect on these aroma. At harvest, IBMP was below the limit of detection (LD<0.6ng L^{-1}) or below the limit of quantification (LQ<2.0 ng L^{-1}).

In 2009, 2010 and 2011 soil nitrogen nutrition trial was carried out.

Soil nitrogen nutrition treatments included:

1) ammonium sulphate (AS), 40 N units;
2) dried blood (DB), 60 N units;
3) control (C), not fertilized.

Since soil presented a good initial fertility, nitrogen was not applied at very high rates but according to good agronomic practices for the area; so differences between treatments were recorded only for some more sensible variables. IBMP was affected by treatments, increasing differences year by year. Notwithstanding, only at 2009 harvest a detectable amount was recorded.

Considering IBMP at veraison, in 2009 no relevant difference between treatments was registered. On the contrary, results showed highest content in C 25.7 ng L^{-1} than in AS and DB respectively 18.6 and 20.9 ng L^{-1}. Since veraison 2010, treatment effects were going to appear: even if not supported by statistic, C was lower (20.3 ng L^{-1}) than fertilized treatment AS and DB (24.5 and 24.9 ng L^{-1}, respectively). In 2011 C was 30% statistically lower than AS and DB, showing a strengthening of fertilization on IBMP, cumulated through years.

After three years of nitrogen application, fruit composition as soluble solids, titratable acidity and pH was not strongly affected by treatments. Juice total nitrogen (TN) showed differences between treatments in every year but only in 2011 TN was statistically higher in DB and AS than C, according to soil nitrogen rate application. Normalized Difference Vegetation Index (NDVI), correlated to different vigour parameters, showed increasingly significant differences in 2009 and 2010 with highest values corresponding to highest N rate supply.

Concerning the region Friuli Venezia Giulia, *Sauvignon banc* needs to be grown in no limiting conditions of water and nutrient to express its whole complexity. MPs are searched in *Sauvignon blanc* wines by many vinegrowers of the Region. By this study, the year has been recognized to be the most important factor in causing vegetal notes in *Sauvignon blanc*, but finding a strategy to stimulate synthesis and to preserve MPs until harvest could improve the quality of wine obtained, especially in good years, i.e. not too hot summer. LR seemed to be a suitable practice to control pests also in white varieties, characterized by compact cluster, but not to obtain a *Sauvignon blanc* with vegetal notes.

Knowing that N fertilization increases MP concentration and that during ripening MPs fall down below olfactory sensory threshold (2ng L^{-1} in white wines), a high-moderate N fertilization, depending on soil fertility, could be paired with an earlier harvest (e.g. at 14-16°Brix and 9-7,5 TA) than that suggested by usual "technological" maturity (20-22°Brix and 5-6 TA). Normally, Friuli Venezia Giulia wineries are growing different *Sauvignon blanc* clones and often they harvest grapes with high sugar content and low acidity to produce high quality wines, especially during the last hot years.

So, the early harvested grapes could be mixed with full ripe grapes in a correct proportion to obtain balanced *Sauvignon blanc* wine flavours. Future tests in this direction could be useful to improve wine quality of *Sauvignon blanc*.

A world spread variety like *Sauvignon blanc,* that has found a suitable environment for quality productions in Friuli Venezia Giulia, could be a pulling wine for the Region in the international market. High standard Friuli Sauvignon production, being comparable with *Sauvignon blanc* wines coming from most famous winegrowing territories, like France and New Zealand, could lead to the appreciation of excellent local wines of Friuli abroad giving new commercial prospective for wine-makers.

1 Introduction

1.1 Viticulture in Friuli Venezia Giulia

The introduction of grapevines in Friuli Venezia Giulia took place many centuries before Christ by Eneti, people devoted to agriculture and the first inhabitants of the region, which imported the plants and the cultivation techniques from Greece. At that time the trade of agricultural products, and therefore also of wine, was very prosperous. Later during Roman domination, grapevine cultivation and wine production expanded considerably: the Roman senate, as Livy (Titus Livius, 59b.C.-17 A.D.)wrote, sent a colony in Aquileia in 181 b.C., in order to spread viticulture and the friulian city became one of the leading wine stores, the link between markets of the Italian Peninsula and the area of the Danube (http://www.natisone.it).

Nowadays, surface cultivated with vineyard at the regional level is approximately 18,000 hectares, more than 75% in DOC zones. There are about 3,000 wineries in business. Viticultural areas are present in all the plain of Friuli, although the largest concentration is recorded in the hills on the eastern border of the region. There are two organizational forms: the specialized winery (winemakingand direct marketing of their own grapes and wine) and chain with producers of grapes and wine-making / marketing enterprises entrusted into the cooperative system or the agro-industrial system (http:/ / www.ersa.fvg.it).

As a cool climate region Friuli Venezia Giulia is particularly suitable for white wine production, both from autochthonous and international varieties, like Sauvignon blanc. In the last decades Sauvignon blanc became a key variety, with which vinegrowers have expressed the best qualitative potential of Friuli Venezia Giulia viticulture.

1.2 Sauvignon blanc

1.2.1 History and diffusion

Sauvignon blanc is a white wine grape variety internationally widespread (Figure 1). Its origin is uncertain but the main areas where the variety could be originated from are Bordeaux and Loire valley in France (Figure 2) (Vivai Cooperativi Rauscedo, 2008). The name Sauvignon means "wild plant", originated by French *sauvage*.

In France two biotypes are growth: the first characterized by green and large berries and the second, the most cultivated in France and in Italy, characterized by yellow and small berries. Sauvignon was imported in Italy around the half of eighty century and it has adapted in several climate conditions. The first biotype, also called Sauvignonasse, could be connected to Friulano white wine variety, that is growth in Friuli Venezia Giulia as local variety (Cosmo e Polsinelli, 1961).

Figure 1**:** Sauvignon blanc cluster and leaf.

In France four main production regions can be distinguished: Gironde, in the south-west part, where Sauvignon blended with Semillon is used to

produce fortified wines as Sauternes and Barsac; Loire valley where Sauvignon is characterized by strong and persistent aromas; Yonne, Bourgogne, where Sauvignon wines are dry and softener; Provence, important for dry Sauvignon wines (Viva Cooperativi Rauscedo, 2008).

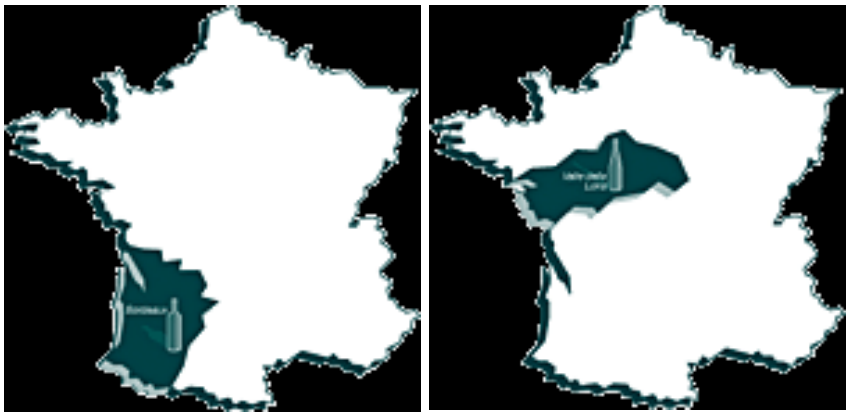


Figure 2:The two main area where Sauvignon blanc possibly was originated: Loire valley (North-West) and .Bordeaux (South-West)

In Italy Sauvignon blanc is cultivated mainly in the Northern, North-eastern part of the Country. Alto Adige and Friuli Venezia Giulia regions (Figure 3) are topic areas for this variety.

In Friuli first references of the variety – initially called Sauterne Sauvignon – date back to 1886 and they concern its tolerance against grapevine downy mildew. Edaphic and climate conditions of Friuli, particularly in the hilly slope areas (GDD – Growing Degree Days about 1750-1850°C; annual rainfall between 1200 and 1400mm; large thermic spread between day and night of 10-11°C during maturation period), confer to Sauvignon typical organoleptic notes and tastes not easy reproducible in other areas (Bigot et al., 2009).

Figure 3: Sauvignon inhilly area of Friuli Venezia Giulia (DOC Colli Orientali del Friuli: Friuli Eastern hills de nomination of origin area).

France and Italy apart, Sauvignon is grown in different East-Europe Countries: Croatia, Serbia, Hungary, Czech Republic, Slovakiaa, Rumania, Moldova and Ukraine. Outside Europe it is cultivated in California, Chile, Argentina, Brasil, Uruguay, South Africa, Australia and New Zealand. This last country made of Sauvignon the top wine production cultivar with l sensory notes of asparagus, gooseberry and green pepper, typical characters of this cultivar in cool climate regions.

1.2.2 Ampelography

Leaf: medium size, curved, trilobate (sometimes pentalobate); petiole sinus open U-shaped when leaf is in a plane situation(and closed in the leaf in its natural position), shallow lateral sinuses; obtuse corner at the top of median lobe , lobes slightly marked, flap folded cup with wavy edges, dark green in the upper surface, light green and pubescent underside, with relevant veins;. red-violet petiole, of medium length. The fall color of the leaves is yellow.

Flower: regular, hermaphrodite, self-fertile.

Inflorescence: 8-10 cm, cylindrical, winged (Figure 4).

Figure 4: Inflorescence of Sauvignon blanc.

Sprouts of 10-20 cm: apex of medium expansion, cottony, white marked with purple-red tinge at the edges; apex leaves (1-3), and expanded the fluffy one, sparse hairs on the upper surface of the other two and yellowish-green color with golden-orange, white below, first with extended shades or purple-red spots, then gray-green because of the tomentum clears, basal leaves (from 4 onwards) opened, glabrous above, pubescent below and light green in colour, with rounded petiole sinus almost always closed axis of the bud a little bent.

Sprouts at bloom: apex expanded, fluffy, whitish green with light shades of pink at the edges; apex leaves (1-3), a little fluffy the first cup, green

with light shades of pink at the edges, basal leaves (from 4 onwards) slightly bent a cup, a few hairs above, light green in colour, axis of the bud a little bent.

Bunch at maturity: less than average, cylindrical, winged, compact, short stalk, thin, woody, short pedicels, verrucous, medium brush, yellowish green, medium berries , sub rounded, with golden green skin, dotted and thick, the average number of seeds is two, medium sized with long beak (Figure 5).

Figure 5: *Sauvignon blanc* bunch at maturity.

Herbaceous shoot: a circular cross section almost smooth external part, hairless, pale green, upward growth habit.

Woody shoot: Medium length, strong, not much branched, with cross section a bit elliptical, streaked surface with darker gray brown on the nodes, internodes 7-8 cm long, nodes evident, protruding buds.

Tendrils: bifid, intermittent, formula 0-1-2-0-1-2.

Trunk: vigorous.

1.2.3 Phenological phases

Bud brake: a little late

Flowering: medium

Veraison: medium

Graperipening: III period (late September)

Leaf fall: early

1.2.4 Variety features

Vigour: good (not too severe pruning is preferred).

Production: good and constant.

Position of the first fruiting shoot: 2nd node.

Average number of inflorescences per shoot: one to two

Fertility of laterals: no fertility.

Resistance to disease and others: normal, a little sensitive to bunch rot (*Botrytis* andsour rot) in clones with compact cluster.

Behavior with respect to multiplication by grafting: normal (Cosmo e Polsinelli, 1961).

1.2.5 Variety agronomical management

Sauvignon blanc gives top quality white wines in cool climate regions, characterized by large temperature differences between night and day. Being a vigorous variety it is recommended for low- medium fertility soils, with a weak rootstock. The canopy can be very vigorous particularly in the first years after planting. Therefore the vine training system adopted should be chosen to contain the vigour, e.g. guyot (Vivai Cooperativi Rauscedo, 2008). Different clones have both different bunch morphology characteristics and qualitative results. Peculiarity of french clones is the compact cluster, that could increase sensitivity to *Botrytis cinerea.*

1.2.6 Sauvignon blanc aroma

The complexity of aromas found in grape and wine varieties is determined by several factors: grape metabolism, clone, rootstock, soil, management and oenological technique. Qualitative profile is characterized by the variety, but the expression is strongly influenced by environment and pedo-climatic conditions.

There are varieties so-called aromatic, like Muscat, that present a berry and a must with flavors similar to resulting wines. In other cases varietal aroma could not be detectable in must. However, inodour must of varieties not categorized as aromatic, while being virtually odorless at the beginning produce aromatic wines with flavors more or less specific to that variety.

The varietal flavor is not identified with a specific compound but by a series of odorous compounds and their precursors which according to their nature and concentrations determine the aromatic characteristic of the variety (Ribéreau-Gayon, 2005).

Sauvignon blanc is belonging to this kind of varieties. It is characterized by a huge variability between clones influenced in different ways by terroir. The great aroma complexity of Sauvignon blanc is connected in a less important part to terpens and mainly to methoxypyrazines (grassy aroma) and thiols (fruity flavors) (Allen et al., 1991).

1.2.6.1 Methoxypyrazines

Methoxypyrazines (MPs) are heterocyclic nitrogen compounds, that are synthesized by aminoacid metabolism. The most important methoxypyrazines that can be found in several different foods are: 3-isopropyl-2-methoxypyrazine, 3-sec-butyl-2-methoxypyrazine and 3-isobutyl-2-methoxypyrazine. Methoxypyrazines are responsible for vegetal flavours (Ribéreau-Gayon, 2005).

Figure 6:Methoxypyrazines molecular structure.

3-Isobutyl-2-methoxypyrazine (IBMP) is the main MP that can characterize the "vegetal" aroma of Sauvignon blanc. The sensory threshold in white wines is 2 ng L^{-1} and its aromatic descriptors in must and wines are green bell pepper (*Capsicum annuum*), green bean (*Phaseolus vulgaris*) and broad bean (*Vicia faba*) (Dubourdieu, 2005).

The IBMP does not vary its concentration with the alcoholic fermentation, as it is not consumed nor produced by yeasts, and increases with the

maceration, because of extraction from the skin is increased. The absence of oxydizable functional groups (-OH,-SH) determines the stability against oxygen, and the group ($-OCH_3$) makes it moderately thermolabile. During the ripening the concentration in must could vary from 100ng/L before veraison to below the threshold limit, 2ng L^{-1}, at harvest.

The content in MPs is strongly influenced by genetic characteristics of the variety (Koch et al., 2010). There are varieties that do not accumulate MPs and varieties such as Cabernet Sauvignon, Cabernet Franc, Merlot and Sauvignon blanc that on the contrary accumulate a quantity of MPs above their olfactive threshold (Koch et al., 2010).

Even the practical experience has shown that MPs are degraded by sunlight: actually , locations with high summer sunshine duration produce Sauvignon blanc with low content in MPs, while locations with less light give higher MPs content (Persello, 2011). Winegrowing areas with high GDD yield grapes with very low levels of IBMP; there is also a positive correlation with malic acid and anegative correlation with degree Brix sugar concentration (Ryona, 2008). So grape ripening has a central role in affecting MPs in must and wine.

1.2.6.2 Thiols

Thiolic sulfur compounds are responsible of some wine olfactory defects but they are also associated with aromas of fruit such as grapefruit and passion fruit (Ribereau-Gayon, 2005). Some of these compounds have been identified in grapes and wines of Sauvignon blanc. The most representative compounds of Sauvignon blanc wine aroma are 4-mercapto-4-methyl-pentan-2-one (4MMP), of 3-mercaptoesan-1-ol acetate (3MHA), 3-mercaptoesan-1-ol (3MH), 4-mercapto-4-methylpentan-2-ol (4MMPOH), 3-mercapto-3-methylbutan-1-ol (3MMB) and benzene-methane-thiol (BMT).

4MMP has an olfactory threshold of 1 ng $L^{-1}$and the concentration in wines can even reach 100 ng L^{-1}. Increasing in concentration, the aromatic

perception vary from white peach to boxwood. The 3MHA has an olfactory threshold of 4 ng $L^{-1}$and in wine it can reach 500 ng L^{-1}. With the increase of concentration it confers character of tropical fruits, such as mango and passion fruit.

3MH has an olfactory threshold of 40 ng L^{-1} and in the wine it can rise up to 3000 ng L^{-1}. Increasing intensity gives notes from fruity to citric like grapefruit. The notes associated with the sensory 4MMPOH are of citrus skin but its concentration in wines rarely reaches the olfactory threshold which is 55 ng L^{-1}. Even the perception threshold of 3MMB(smell of cooked vegetables), 1500 ng L^{-1}, is rarely achieved in wines. BMT has a perception threshold of 0.3 ng $L^{-1}$and in the wine it can be found up to15 ng L^{-1}. It gives aromas of mineral and smoked (Ribereau-Gayon, 2005; Dubourdieu, 2005).

Thiols are oxidizable in disulfides, they can combine easily with copper and are extremely reactive with the quinones formed by oxidation of phenolic compounds of wine, in particular catechins. All of these interactions are negative for the aroma of resultant wine. For this reason, the stability and intensity of the aroma of Sauvignon blanc is greater if the juice contains little copper and phenolic compounds, and it is well protected from oxidation with SO_2, glutathione and lees. Glutathione can bind to and prevent quinones reaction with thiols with consequent loss of aroma (Dubourdieu, 2005).

Sauvignon blanc musts are less odorant. Varietal aroma can be identified during alcoholic fermentation

Peynaud (1980) had sensed the presence of precursors that by fermentation were made free to express their aromatic potential (Dubourdieu, 2005). The thiols are mainly in the conjugated form to cysteine or glutathione where the link provides a sharing of a sulfur atom. They are found in proportion of 50% in the skin and 50% in the pulp. The aromas are released during alcoholic fermentation as a result of the action of the enzyme β-lyase. Some yeast strains (e.g. X5, VL3) were selected according to their ability to liberate aroma (Dubourdieu, 2005).

Some tests have shown that moderate water deficit after veraison and a no limiting nitrogen availability of the plant determines an increase in the potential of aromatic wines; in experiments more precursors linked to cysteine and glutathione were produced in these conditions (Lacroux et al., 2008).

1.2.6.3 Terpenes

The terpenes are isoprene polymers and they are distinguished according to the number of isoprene units. The monoterpenes are formed by two units and are the most important from the point of view of the aromatic potential. The terpenes are easily oxidizable compounds both for the functional group (-OH), as well as for the presence of double bonds between the carbons of the radical R.

The most fragrant terpene compounds belong to the class of monoterpenic alcohols in particular linalool (rose), the α-terpineol (thrush), the nerol (rose), geraniol (rose), the citronellol (citronella) and the trienol (lime). Their perception threshold is rather low, being between a few dozen to a few hundred mgL^{-1} (Ribereau-Gayon, 2005).

The perception threshold is around 50 g L^{-1}. These compounds are essential in the characterization of the aroma of grapes and wines from the Muscat family. Monoterpene concentrations of varieties like Sauvignon blanc are almost always below the olfactory threshold (Ribereau-Gayon, 2005).

In Sauvignon blanc terpenes could influence positively must and wine aroma.

There are some enological products containing fungal β-glucosidase that may be useful to increase the aroma of wine separating in the gucosides the glucose from the volatile part of the aromatic molecule. Some yeast strains are selected for having this activity more pronounced (Persello, 2011).

1.3 Leaf removal management practice

Leaf removal is an agronomic practice that is included within the summer pruning or green pruning. The summer pruning includes all operations of removal of buds, shoots, leaves and bunches performed during the vegetative phase, and just because it is applied on the vine in a phase of active growth, in some cases it assumes an importance even greater than the winter pruning (Poni, 2000).

1.3.1 Technical features

Leaf removal is a widespread practice in the wine world and measurably influences yield quality (Mescalchin et al., 2008). In practice it involves the removal of a part or all the leaves present at the basal level of the shoots, which in spur-pruning trellis system corresponds to the nodes from basal to 9-10 (Figure 7). It can be performed either manually or mechanically and usually in the period between fruit set and veraison, although there is a more and more frequent practice of pre-flowering leaf removal with the aim of disturbing berry set and thus having a control of yield and more loose clusters.

Figure 7: Leaf removal manually applied in the trials further described.

1.3.2 Leaf removal effects

Leaf removal significantly influences fruit development and physiology. The time of application is essential. If the leaf removal determining exposure of grapes to solar radiation if done late (i.e. after veraison),it can cause sunburns (Mescalchin et al., 2008). This can be avoided performing the leaf removal in an earlier stage, such as flowering or fruit set (Poni et al., 2006). Leaf removal in a very early stage (pre-flowering) causes a reduction of berry set because the lack of nutrients going into the cluster impairs physiological processes of setting: the vine sacrifices some flowers being in a stressful situation (Poni et al., 2009).

Some experiments showed that leaf removal on Chardonnay caused higher yields per vine the year after its application (Belvini et al., 2010): this is explained by the fact that normally the leaves are shading basal buds (less light and lower temperature) and this may result in a reduced flower differentiation of fruitful buds in these positions and therefore a lower bud fertility (Buttrose and Hale, 1973). According to some studies, a better exposure of the clusters leads to a reduction of the weight (Poni, 2002). However, there was evidence that the shading of the cluster can lead to an increase of the berry weight, to a decrease or no effect (Bussakorn et al., 2003).

Leaf removal leads to an improvement of cluster microclimate, that normally improves grape quality (Coombe e Dry, 1988). A positive effect in some varieties is the increasing of aroma (Belancic et al., 1997; Zoecklein et al, 1998) and a reduction of vegetal notes but not of fruity character of Sauvignon blanc(Arnold e Bledsoe, 1990).

MPs at harvest are influenced by environmental conditions during berry development and ripening, in particular by temperature and solar radiation in the period from veraison to full maturation (Koch et al., 2012).

Some studies consider solar radiation as directly responsible for the rapid decline of MPs that is observed during ripening (Lacey et al., 1991) but the biological mechanism is not clear yet (Katsumi and Takashi, 1999). The influence of leaf removal is also evaluated on other parameters that determine the quality of the fruit, such as pH, total acidity, the concentration of malic acid, sugars, polyphenols and potassium. In general, exposure of the grapes during ripening allows some increase in sugar content, a reduction of total acidity, a change in the quality of acidic fraction caused by degradation of malic acid (Fregoni, 2005).

Several studies agree with this view, and argue that a better cluster exposure to light with a temperature rise (Smart and Sinclair, 1975) contributes to the demolition of malic acid and to increase of sugar (Ruffner et al., 1975). Even the must obtained from clusters exposed to sun have a higher sugar content, lower acidity and a higher pH (Guidoni et al., 2005), but other studies observed a reduction of pH and of potassium concentration in the must (Kliewer , 1998). However, in some cases an excessive bunch exposure to direct sunlight can lead to a reduction of sugars, malic acid and of total acidity together with an increase of the glycosylated aromatic components (Zoecklein, 1992; Zoecklein et al., 1998).

It is clear that a persistent shading condition is unfavorable to the quality and health of the grapes (Poni, 2002). The reduction of sugar content can occur in clusters excessively shaded due to the low temperature of cluster zone, lower light on the surrounding leaves with a consequent reduction of the photosynthetic activity, or a delay in maturation (Percival et al., 1994); this has also been verified in other tests where a more intense shading caused a slower accumulation of sugars (Valenti et al., 2011). An increase in pH in shaded clusters seems to be due to a greater accumulation of potassium (Bussakorn, 2003). The higher acidity can be correlated to a lower activity of enzymes that degradate malic acid, which, in post-veraison, are stimulated by temperatures greater than 30°C (Lasko, 1975).

It seems clear that the accumulation of sugar in the different exposure conditions is not so much related to the thermal regime of the surroundings of the cluster bright but rather to the amount of leaf area that is still on the vine after defoliation and especially its relationship with the yield load (Poni, 2002). The exposed leaf area in relation with grape production is optimal at levels between 1 and 1.5 m^2/kg (Fregoni, 2005).
Leaf removal performed on vines with balanced vigor did not result in significant changes in sugars and pH(Reynolds et al., 1994; 1996).
Furthermore, the area recovery is proportional to the timing of leaf removal: if applied early in the season the vine is more induced to produce new leaves (Mescalchin et al., 2008; Poni, 2003).
A part of the amount of photosynthesis products that is lost with leaf removal is compensated by the re-exposure to light of other underlying leaves, that increase their photosynthetic efficiency (Poni, 2003).
A part of the amount of photosynthesis products that is lost with leaf removal is compensated by the re-exposure to light of other underlying leaves, that increase their photosynthetic efficiency (Poni, 2003).
Photosynthesis products ensured by the basal portion of the shoot (oldest leaves affected by leaf removal) could not be less than that measured on the medial and apical portions on which are inserted youngest leaves(Poni and Intrieri, 1996). This effect, linked to bigger dimension of basal leaves, suggests not to underestimate the contribution that they could provide when the defoliation is normally carried out (Poni, 2002).
The cluster exposure also leads to an increase of total polyphenols in berry skin (Downey et al., 2006).This phenomenon has been reported in Pinot Noir grapes (Price et al., 1995) and in Shiraz (Hanselgrove et al., 2000) where an increase of grape exposure increased synthesis in the skin of quercetin which protects tissues against ultraviolet radiation (Poni, 2002).
Leaf removal also allows an improving penetration of pesticide spraying on clusters (for example, against moths and *Botrytis*); if applied early in spring, it also improves the efficacy of spraying against *Phomopsis viticola*

and increases the resistance to drought (Fregoni, 2005). So leaf removal applied between flowering and cluster closure can be most effective against *Botrytis* and sunburns (Mescalchin et al., 2008), but even if applied later the effect against *Botrytis* is relevant (Cravero and Rabbi, 2005).

As to sour rot, even if it is assumed a positive effect, it is not yet clear if leaf removal would reduce the severity of this plant disease. The incidence of sunburns caused by late leaf removal is connected directly to row orientation (Mescalchin et al., 2008). A vine grown in East-West row has always the Southern side more exposed to the sun and thus to risk of burns. However, in trellis systems oriented North-South it is not always true that the side less at risk is the one facing East. This variability depends on the time of day when leaf removal is applied, from the orography and row orientations that may differ by only few degrees from the ideal direction with relevant effects on the plant response (Mescalchin et al., 2008).

Vine response to leaf removal and the influence on qualitative and quantitative characters depend on several factors: vigor of the variety, weather conditions, time of application and removal intensity; these factors that must be taken into consideration for implementing the practice with profitable results.

1.4 Fertilization practice

Fertilization is a cultivation technique based on the distribution in the soil of organic or inorganic materials that for their chemical, physical and biological features contribute to the maintenance or improvement of soil characteristics. Fertilizing matters are divided into fertilizers, soil conditioners and correctional with various application purposes (Sequi, 2005).

According to the type, fertilizers can be divided into: mineral fertilizers and organic fertilizers. Mineral fertilizers (inorganic, ready to effect) can

be simple if they contain a single element of fertility or compound if they contain multiple elements of fertility (NP, NK, PK, NPK). Organic fertilizers (slow-acting) are formed by organic compounds of biological origin (Fig. 8), both plant and animal (Sequi, 2005).

The mineral nutrition of grapevine (organic and mineral) is affected by many factors, such as genotype (variety, clone, rootstock), climate, soil and other growing techniques (Bavaresco, 2008).

The purposes of mineral fertilization are:

- restore the level of mineral elements in the soil and plants,
- balance the vegetative-productive development of plants
- increase resistance to disease, drought, frost, etc.

The aims of organic fertilizers are:

- improve the physical, chemical and biological properties of soil,
- improve water availability and mineral nutrients for plants.

Fertilization affects plant physiology and productivity, interacting with photosynthesis and soil water balance.

1.4.1 Grapevine nutrition

Grapevine fertilization did not give much concern to vinegrowers till recent times because until ’70 excessive organic fertilization was performed which guaranteed a resource for growth, development and yield. Often, too many fertilizers were supplied particularly with high nitrogen content, which delayed ripening, decreased sugar content and consequently increased acidity (Eynard and Dalmasso, 2002). This was due to strong vigor and excess of yield that decreased fruit quality. In the past, non-rational nitrogenous fertilization was used to increase yield and to compensate for the low unit price of grapes at the expense of quality (Eynard and Dalmasso, 2002).

Nowadays, grapevine fertilization has changed the approach and it is a very important tool to determine yield and quality of grapes.

1.4.2 Nutritional demands

The fundamental rule of mineral elements' need for plant growth was recognized by J. VonLiebig(1803-1873), who concluded that some elements are essential, because in their absence the plant is unable to complete its life cycle, as their function cannot be replaced by other elements and they are directly involved in the metabolism. A first group consisting of the *macroelements* (N, P, K, S, Mg, Ca) and a second group composed by*trace* elements(Fe, Mn, Zn, Cu, B, Mo, Cl, Ni,Na, Si, Co) are considered essential for plants. The difference between these two categories lies in their different concentration in dry matter of plant tissues: the macroelements have higher values(concentration in plant tissue normally expressed in%) and trace elements have lower values (their concentration is normally expressed in ppm or mg kg^{-1}). The mineral elements are absorbed by plant through the roots but also by other organs, such as the leaves(foliar fertilization) (Bavaresco, 2008).
The vine fertilization is divided into three phases:

1 –Base Fertilization is carried out prior to planting the vineyard: e.g. manure 50-60 t/ha 250-300 units (kg of P_2O_5) of phosphorus/ha, 300-400 units (kg of K_2O) of potassium/ha.

2 –Growing fertilization is made in the first three years after planting:E.g.1st year: 30-50 units (kg of N) of nitrogen/ha; 2nd year: 120 units of nitrogen/ha; 3rd year: 60-120 units of nitrogen/ha, potassium 100-150 units/ha per year.

3 - Production fertilization is carried out in the production period. With reference to a production of 100-150 q/ha of grapes, e.g.50-80 units of

nitrogen/ha, 30-50 units of phosphorus/ha, 100-130 units of potassium/ha, 25-30 units (MgO) of magnesium/ha (Corazzina, 2007).

1.4.3 Nitrogen

Nitrogen has a marked plastic effect on plants: in fact it participates to the formation and reconstruction of tissues. It is an element that is absorbed as nitric (NO_3^-) and ammonium (NH_4^+) forms and it is of dominant importance because it enters into the constitution of many organic compounds such as chlorophyll, proteins, nucleic acids, vitamins, etc.(Corazzina, 2007). The soil nitrogen is present mainly in organic form (amino acids, peptides, amino sugars). The organic nitrogen, including humic acid, is slowly mineralized to ammonia by heterotrophic microorganisms; this conversion is commonly known as mineralization process. The latter could be accomplished at very different times, depending on the stability of the organic material of departure. The organic matrix with a low C/N ratio (<9)has a fast mineralization (Sequi, 2005).

Mineralization is followed by nitrification operated by aerobic autotrophic bacteria (Nitrosomonas, Nitrobacter) which consists in the biological oxidation of ammonia to nitrate via nitrite (toxic to plants) (Sequi, 2005).

The loss of nitrate nitrogen may occur by denitrification (formation of molecular nitrogen) by anaerobic microorganisms in a reducing environment or by leaching.

1.4.4 Vine Nitrogen Needs

The plant has a significant need of nitrogen in both the time of initial vegetative growth, when it exploits previous year's stored reserves , and immediately after fruit set, when it will need to find the element readily absorbable in the form of nitrate to meet the increased requirements. Annually the nitrogen requirements for grapevine is around 40-60 kg/ha.

In the leaves and shoots the nitrogen has a high turnover, so that these parts can act as a temporary nitrogen reserves for the roots and berries (Conradie, 1986). According to Wermerlinger, 1991, the nitrogen content in the shoots and leaves is mobilized towards the berries during ripening. During the summer the leaves become the primary storage organ of nitrogen and so in the phase of foliar senescence a certain amount of foliar nitrogen is translocated in order to be stored in woody tissues during fall and winter (Millard, 1993).

Normally, as reported by Titus and Kan (1982) for Apple tree and by (Conradie, 1991), nitrogen is stored in the bark of branches and trunk during the winter in the form of aminoacids according to the availability of nitrogen present in the plant in relation to fertilizations, and in the roots in the form of proteins rich in arginine (Millar and Proe, 1991).

Thus, it is possible to understand how the application of nitrogen fertilizer immediately before or after grape harvest may influence the rate of nitrogen that will be stored. It is more probable that nitrogen spread later in the season will be mobilized from leaves to root and trunks that nitrogen spread earlier (Porro et al., 2001).

This nitrogen will be mobilized from trunk and roots in spring to contribute to canopy development. The mobilization occurs before root activity of the new season; actually before bud break there is low nitrogen uptake and most of N uptake from the soil occurs after bloom (Keller, 2005).

In the four weeks after flowering nitrogen uptake and need of vine reach the greatest level. Nitrogen uptake in this period could be 1.5-1.6 kg/ha/day with peaks of 1.8 kg/ha/day. A second uptake peak occurs after harvest when daily a vineyard can uptake 1kg/ha /day of nitrogen (Porro et al., 2001).

The nitrogen partitioning in the different parts of a balanced vine is about 26% in permanent structures (roots, trunk, branches), 41% in the leaves and shoots and 33% in clusters during ripening (Conradie, 1991).

1.4.5 Nitrogen uptake

Mineral element uptake by plant takes place in a passive or active modality. In passive uptake nutrients are absorbed by the plant using solute concentration difference exploiting the transpiration flow both as mass and as diffusion. The active transport requires energy to absorbe elements and transport them across the membranes of rooting system cells (Keller, 2005; Porro et al., 2001).

The passive mechanism requires a soil in good conditions, with appropriate element availability and sufficient water supply to dilute nutrients . A strong and active transpiration flow is necessary to move the soil nutrient solution into the plant ("the thirsty plant is also hungry"). Undoubtedly, weather conditions greatly influence this process. For the other type of mechanism, the active one, the root activity will be favoured by good respiration with optimum values of temperature and available oxygen as well as sufficient quantities of carbohydrates for the best performance (Porro et al., 2001; Rom, 1996). The largest transport of carbohydrates in leaves and shoots, that occurs during rapid active growth through intense photosynthetic activity, can limit the rate of root respiration and hence nutrient absorption through the active mechanism (Porro et al., 2001).

The nitrogen is absorbed by the plant from the soil as nitrate and ammonium forms and subsequently it is converted into aminoacids for the formation of proteins and enzymes; nitrate, however, is considered to be the main form of nitrogen uptaken by the roots, also because of its greater concentration in the soil in comparison with ammonium, which is rapidly converted to nitrate by soil micro-organisms through the process of nitrification (Keller, 2005; Porro et al., 2001).

The input of nitrate radicals within the cells, which occurs against electrochemical gradient, requires simultaneous input(co-transport) of protons with consequent increase in pH of the medium extra-radical. In addition, the reductive assimilation of the anion involves a consumption of protons that exceeds the quantity of those necessary for the transport;

this leads to an accumulation of hydroxyl ions that are released in the external medium (Sequi, 2005). Also the absorption and the subsequent assimilation of ammonium determines the release of protons outside the root with consequent acidification of the rhizosphere (Sequi, 2005).

The assimilation process of nitrate in the plant occurs in leaf chloroplasts, and it is strictly connected to photosynthesis. In several vegetal species nitrate reduction occurs in the roots, when nitrate availability is low (Raven et al., 2005).

Ammonium is the main form of inorganic nitrogen involved in the synthesis and catabolism of organic nitrogen, but it is potentially highly toxic and difficult to partition and therefore needs to be detoxified. Both the ammonium formed by the reduction of nitrate, as that absorbed from the soil in the cell are immediately inserted at radical level in organic compounds such as amino acids and amides in order to then be transported through the vascular system (xylem and phloem).In contrast, the nitrate is harmless and its assimilation is regulated by the carbohydrate oxidation and it is associated with the production of organic acids.

The nitrogen uptake is maximum at neutral pH and when the roots are in the soil conditions of sufficient water supplied. All environmental conditions of the soil (temperature, available oxygen, etc..)that allow a better metabolism of the root, facilitate the absorption of mineral elements; roots are active between 5 and 30°C (Porro et al., 2001).

1.4.6 Nitrogen fertilizers

The management of nitrogen fertilization requires a good knowledge of the soil, climate and agronomic conditions, and their interactions with the type of fertilizer used (Sequi, 2005). The chemical form in which nitrogen is used and its behaviour in the soil, are crucial in the development of the plant. Nitrate is not retained by the electronegative soil colloids, otherwise occurs for the ammonium ion.

The main types of mineral nitrogen fertilizers are:

- ammonia
- urea
- ammonium sulphate
- ammonium nitrate
- ammonium chloride
- calcium cyanamide

Urea and calcium cyanamide are defined organic nitrogenous fertilizers. Ammonium sulphate- which is the fertilizer used in this study - has been the most used fertilizer in the past, now replaced by fertilizers with a higher nitrogen concentration and lower production cost, such as urea and ammonium nitrate (Sequi, 2005). Ammonium sulphate (granular or liquid) is a nitrogenous ammonia, its title (% of nitrogen) is 20-21% (Giardini, 1994).The main advantages of this fertilizer are the low hygroscopicity and good efficiency; it is also a good source of sulphur.

Commercial fertilizers are prepared in compounds formed by more than one element (N, P, K) and also slow release nitrogen fertilizers (formurea) (Sequi, 2005).

A second very important group are organic fertilizers, organic compounds formed by the carbon of vegetal or animal origin. Organic fertilizers must contain only nitrogen and organic nitrogen expressly originated by animal or plant materials, but they can also contain micro-nutrients. The value of these fertilizers is more related to the content and the kind of organic matter than to the nutrients they contain; they contribute to improve physical, chemical and microbiological fertility.

Organic fertilizers are divided as:

- of mixed origin (manure, composts)
- of animal origin (solid manure, urine, blood, bones, residues of leather, etc.)
- ofplant origin (crop residues, green plants-green manure.)
- commercial organic fertilizers (Giardini, 1994).

Their effectiveness depends on the content of nutrients but especially by the C/N ratio of the organic matrix.

Dried blood was the manure used in this study. The dried blood (granular) has a high amount of organic nitrogen (14%), presents a very rapid mineralization, and also makes available a high amount of iron (> 200mg kg^{-1}) which is resulting from the hemoglobin: it is in the form biologically more active and it is therefore useful to solve phenomena of iron chlorosis.

1.4.7 Nitrogen fertilization effects

From recent studies it has been described as vine nutritional status affects the organoleptic quality of the wine. Nitrogen pushes vine development, changing substantially both the distribution and the concentration of nitrogen in berries and deeply influencing the quality of wine. The nitrogen fertilization changes the microclimatic conditions of the clusters, acting in particular on the rate of degradation and synthesis of acidic phenolic and aromatic compounds (Linsemeier et a., 2008; Keller, 2005; Hilbert et al., 2003; Porro et al., 2001).

Some authors indicate that moderate deficit of nitrogen increase the synthesis of phenolic compounds (Peyrot des Gachons C. et al., 2005). Often, wines from vineyards subject to heavy nitrogen fertilization have greater aromatic intensity (Porro et al., 2001).

The nitrogenous components of must influence the fermentation activity of yeast with the possibility of producing fermentation aromas (higher alcohols, acetals of higher alcohols, etc..) responsible for fruity olfactory notes (Porro et al., 2001). The quality of white grapes depends on its aromatic potential. As to Sauvignon blanc, the most important aromatic component derives from thiols, but these are present in the form of its grapes precursors, S-conjugates to cysteine. Some studies have linked the influence of water and nitrogen on thiolic precursors of Sauvignon blanc with the result that a moderate water deficit and unlimited nitrogen

availability maximize the expression of the aroma (Peyrot des Gachons et al., 2005).

Studies carried out in Friuli Eastern hills have not revealed a clear link between nitrogen and sulfur in must although some experiments carried out in France in the Bordeaux area had shown a positive relationship between nitrogen and thiol precursors in musts. However, the sensory analysis confirmed that foliar fertilization "nitrogen + sulfur" fostered the best aromatic properties (notes of peach, grapefruit, black currant, plant and boxwood) in Sauvignon blanc, thus confirming the role of fertilization on the development of aromatic notes (Bigot et al., 2009).

Another category of compounds that significantly contributes to the aroma of Sauvignon blanc is represented by MPs (Hashizume and Samuta, 1999; Roujou de Boubée et al, 2002). Nowadays, there are no studies showing that nitrogen fertilization affects the concentration of MPs in grapes and wine. However, since Mps are nitrogen-containing compounds, it can be supposed that nitrogen fertilization can influence the accumulation of MPsin fruits (Hanschke Bell, 2005). Nitrogen fertilization may also affect indirectly the concentration of MPs acting on the leaf microclimate (vigor).

Another effect of the nitrogen fertilization regards leaf growth: the increase of nitrogen availability in the soil corresponds to an increase of the foliar density (Bell and Robson, 1999), and it is known that a reduction of the nitrate reductase activity in leaves is occurring in poor light conditions (Perez and Kliewer, 1982).

Many studies indicate that the application of nitrogen increases must total acidity, especially in long terms trials when huge amounts of organic fertilizers were applied (Morlat R. and Chaussod R, 2008; Morlat, 2008; Morlat R. and Symoneaux, 2008). Furthermore, wine quality reduction was described in the same trials.

The influence of nitrogen fertilization on pH and potassium showed mixed results when studied, but most researches have found that it is not significant. However, it is evident the increase of nitrogen compounds in berries (Bell and Hanschke, 2005).

The nitrogen fertilization shifts the balance between vegetation and production towards the production of vegetative organs and that, if the nitrogen supplyis adequate to the situation of the vineyard, may cause only a slight delay of grape ripening. Furthermore an excess of vigor due to an excessive use of fertilizer can also lead to decrease degradation of malic acid (Fontana and Castellari, 2004).

A balanced nitrogen fertilization helps natural vegetative state and leads to an improvement of grape quality. On the other hand, if nitrogen fertilization is carried out on a soil already rich in nitrogen, this does not lead to a qualitative improvement but may cause a decline. This is due to an excessive increase of the density of the foliar and production to strain with consequent ripening delay, greater susceptibility to adverse environmental conditions and to fungal diseases (*Plasmopara viticola, Oidium tukeri, Botrytis cinerea*).

In leaves, nitrogen is a key component of chlorophyll and promotes the photosynthetic capacity. In addition, 70-85% leaf nitrogen is used by Rubisco, an enzyme that operates organication of CO_2via incorporation of the carbon in precursors of sugars during the photosynthetic process to form new sugar (Rom, 1994).

The ratio nitrogen-photosynthesis is decisive: at low nitrogen levels, leaves reduce their size, in consequence of a lower net photosynthesis intensity (Pn). When leaf nitrogen is limiting for vegetative growth of vines (<2% of ss) plants respond to the light stimulus by reducing the photosynthetic activity (Porro et al., 2001).

A well balanced organic fertilization allows optimal vegetative-productive ratio. The mineral fertilization is able to satisfy the nutritional needs of the vineyards also when very productive. The efficiency of the use of mineral nutrients by the roots, however, is much influenced by physical and chemical characteristics of the soil itself and on the performance forecast and expectations (Bavaresco, 2008).

The mineral fertilization often is carried out in spring at the time of initial vegetative growth of the plant, but this contribution may not be sufficient

for the entire production cycle of the plant. On the contrary, an organic fertilizer is more efficient because it is able to release gradually the nutrients in the soil, and its use is more suitable for unproductive vineyards (Bavaresco, 2008). The organic fertilizer is also able to improve the physical and microbiological characteristics of soil.

Effects of not balanced nitrogen fertilization could be:

- nitrogen deficiency: sometimes temporary as a result of low temperatures, excess of water in the soil, etc.. it induces chlorosis (yellowing) basal older leaves, poor vegetative growth and low percentage of fruit set and reduced production, imperfect and not simultaneous ripening of the grapes, reduction of sugar and total acidity (Figure 8).

Figure 8: nitrogen deficiency in vine leaf (photo Pastorelli s.p.a.).

- Nitrogen excess: more frequent than deficiency, occurs with an intense vegetative development (large and dark leaves), with an increase of respiration (from which increase water consumption and and reduce sugar in berry), mutual shading of the leaves, increased sensitivity to downy mildew and *Botrytis*, strong and prolonged vegetative growth (low resistance to drought and cold winter),

casting (early fall) of flowers, delayed ripening of grapes with poor sugar in the must, high titratable acidity and herbaceous aromas.

2 Materials and methods

2.1 Vineyard description and farm management

The vineyard is located in Romans d'Isonzo, North-East Italy, in Isonzo river plain, location 45°89'72''N, 13°45'16''W. It is part of "DOC Isonzo", one of the winegrowing areas in which Friuli Venezia Giulia Region is divided. Soil type is connected directly with Isonzo river depositions. The area is located in the right part of Isonzo river.

The Sauvignon blanc plants were planted in 1991, grafted onto Kober 5bb rootstock. Distance between rows is 2.70m and between vines is 1.0m, with a vineyard density of approximately 3700 vines per ha. Row orientation is North-South. The field has a regular shape and It's about 300m according to row length and 85m width wise.

Vines are pruned as single guyot system with 8-10 buds per branch. Cluster is closed with medium size berries.

Farm fertilization management includes the use of mineral fertilizers only, applied normally 2 times for ammonium sulphate in pre-anthesis, rate 40 Nkg ha^{-1}, and after harvest, rate 15 Nkg ha^{-1}. Farm uses a travelling sprinkler irrigation system. Irrigation is applied only when water stress is to severe. Irrigation water is purchased from Irrigation Consortium of Isonzo plain by pressurized distribution net. Pest control is conventional, applying a schedule strategy. Space between the rows is maintained covered by spontaneous grass cover, cut 2 or 3 times per year, according to its growth rate.

Weed control in the row is performed with chemical herbicide locally spread at the first weed manifestation.

Topping is performed 2 times during the growing seasons to regulate canopy shape.

Farm chooses mechanical harvest executed by external services.

Yield destination use from this vineyard is blending with other masses to obtain a fresher and more aromatic Sauvignon wine.

Between 2009 and 2011, years of experimentation, Sauvignon blanc as other sensitive varieties like Traminer, Chardonnay and Pinot noir, was affected by esca disease. Symptoms were particularly widespread in 2011.

Symptoms appeared in all the typical forms since pre-anthesis to post-harvest. This aspect increased the large variability normally present in the field.

2.2 Soil description

Soil type is connected directly with Isonzo river depositions of coarse carbonate material. Soil type can be classified by following the WRB (World Reference Base, 2006) as Rhodi Cambisol Endoskeletic. Soil profile is constituted by a shallow Ap horizon until a depth of 35cm and a deeper diagnostic Bw horizon until 70cm on a 2C horizon with coarser river depositions. Gravel is frequent in topsoil. Texture class is clay-loam with brown-red wet soil colour.

Parameter	Unit	Value	Analysis method
Texture			
Clay	% w/w	26	
Silt	% w/w	45	
Sand	% w/w	29	
Gravel	%v/v	28	
Bulk density	Mg/m3	1.42	
pH	in H2O	6.99	
Organic Carbon	%w/w	2.6	
Available P	mg/kg	41	
Exchangeable Ca	mg/kg	3195	
Exchangeable K	mg/kg	408	
Exchangeable Mg	mg/kg	250	
Exchangeable Na	mg/kg	232	
Cation Exchange Capacity (C.E.C.)	meq/100g	20.05	

Source: Photo provided by ERSA-FVG, that described soil profile; soil analysis were performed by us at the starting of the filed

Root depth is confined among the first 70cm by gravel in the sub-soil. The available water content is estimated low or medium because of texture and the constricted soil depth that root can explore. Therefore irrigation is required for almost any crop to reach adequate qualitative and quantitative yield.

Soil water permeability is moderate to high and It doesn't entail soil water drainage.

2.3 Weather 2009-2011

Data collected by the weather station of Gradisca d'Isonzo were used for this study. The weather station belongs to Friuli Venezia Giulia Meteorological Regional Observatory (ARPA-OSMER).

Friuli Venezia Giulia climate is characterised on the average by rainy spring and autumn with the summer months July and August generally warm (mean temperature 23.3°C, means 1990-2011) and not much rainy (103mm, spread in 8 rainy days, means 1965-2000), forcing farmers to exploit irrigation water for supplying almost any crop. Huge differences in mean annual rainfall are present in the restricted territory of the Region, due mainly to its orography (Figure 9).

The three vintages differ widely both for rainfall and temperature trends, especially during the growing season. Generally, 2009 and 2011 were warmer and less rainy than 2010.

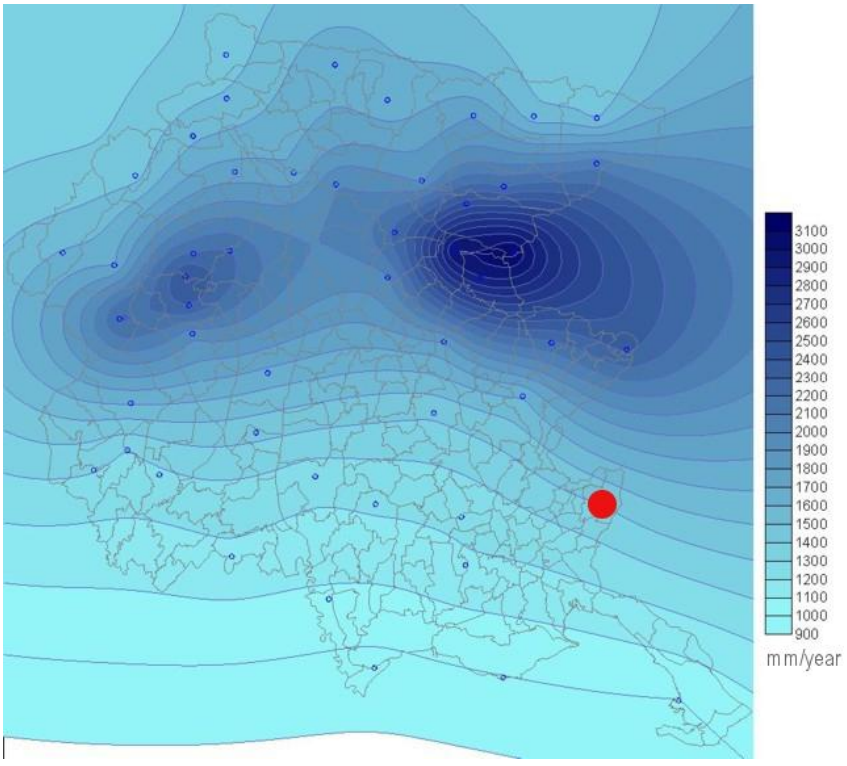


Figure 9: mean annual interpolated rainfall in Friuli Venezia Giulia, data 1965-2000; maximum rainfall occurs going closer to the mountain chains; the red spot indicates Gradisca d'Isonzo weather station 2km as the crow flies far from experimental vineyard; the station recorded a mean annual rainfall of 1431mm from 1965 to 2000 (source: ARPA-OSMER FVG);

2009 temperatures during July and August were respectively 23.9 and 25.1°C, above the average, especially in August during grape ripening. 2010 was characterized by lower mean temperatures during growing season than 2009 particularly in august 22.4°C, but except for July 24.4 °C. Instead, 2011 showed temperatures more similar to 2009 with a particularly hot August, mean 25.1°C with several consecutive days of peak max air temperature between 36 and 38°C during grape ripening. Besides, 2011 bud break was characterized by relative high temperatures for the period and a lack of precipitation (Figure 10). Indeed, few days before harvest, youngest vines showed severe water stress symptoms (data not shown).

Daily rainfalls were different in the three years of experimentation (Figure 10): 2009 and 2011 were quite similar, even then 2011 showed less rainfall event number and less severe and consequently higher temperature.

Cumulated rainfall (Figure 11)was higher in 2010 than 2009 and 2011,inboth a whole year basis (1845mm vs 1405mm and 839mm) and a growing season (April-October) basis (924mm vs 719mm and 625mm).

Growing Degree Days (GDD) calculated with 10°C-base since April 1st to October 1stwere 2003, 1697 and 2078°C, in 2009, 2010 and 2011 respectively (Figure 11).

Global Solar radiation was affected by year: consistently with other climatic variables, solar radiation was higher in 2011 and 2009 than in 2010. Focusing on the fruit development and ripening period, the 2010 season had a solar radiation expressed on a monthly basis (Figure 12) higher in June and lower in August than 2009 and 2011.

Cooler and more rainy weather in 2010 extended growing season, conditioning grape composition, yield and disease incidence and diffusion, if compared with 2009 and even more with 2011.

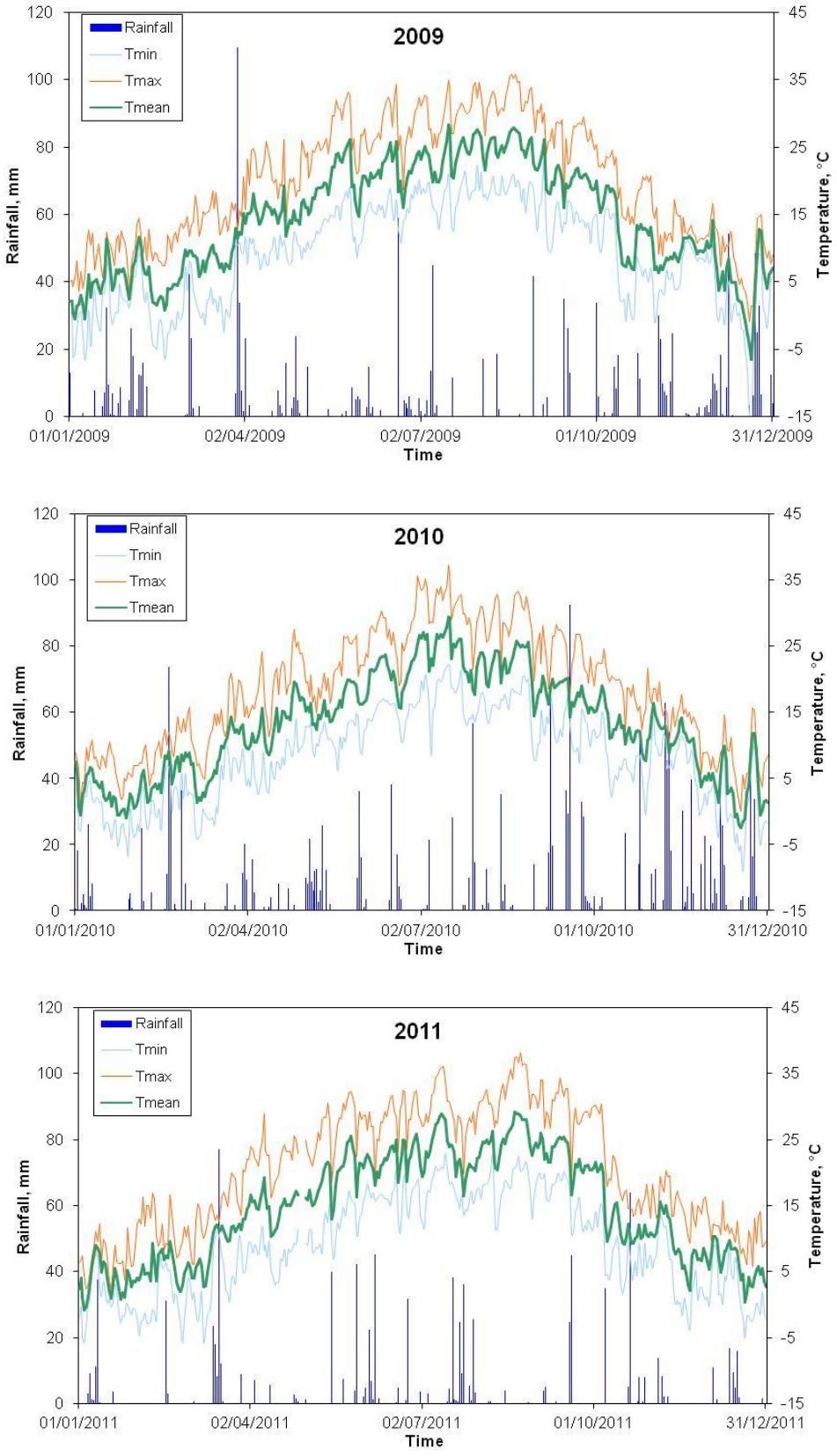


Figure 10: Minimum, mean and maximum daily temperatures and daily rainfall in 2009, 2010 and 2011; weather station managed by OSMER, Friuli Venezia Giulia Region installed in Gradisca d'Isonzo

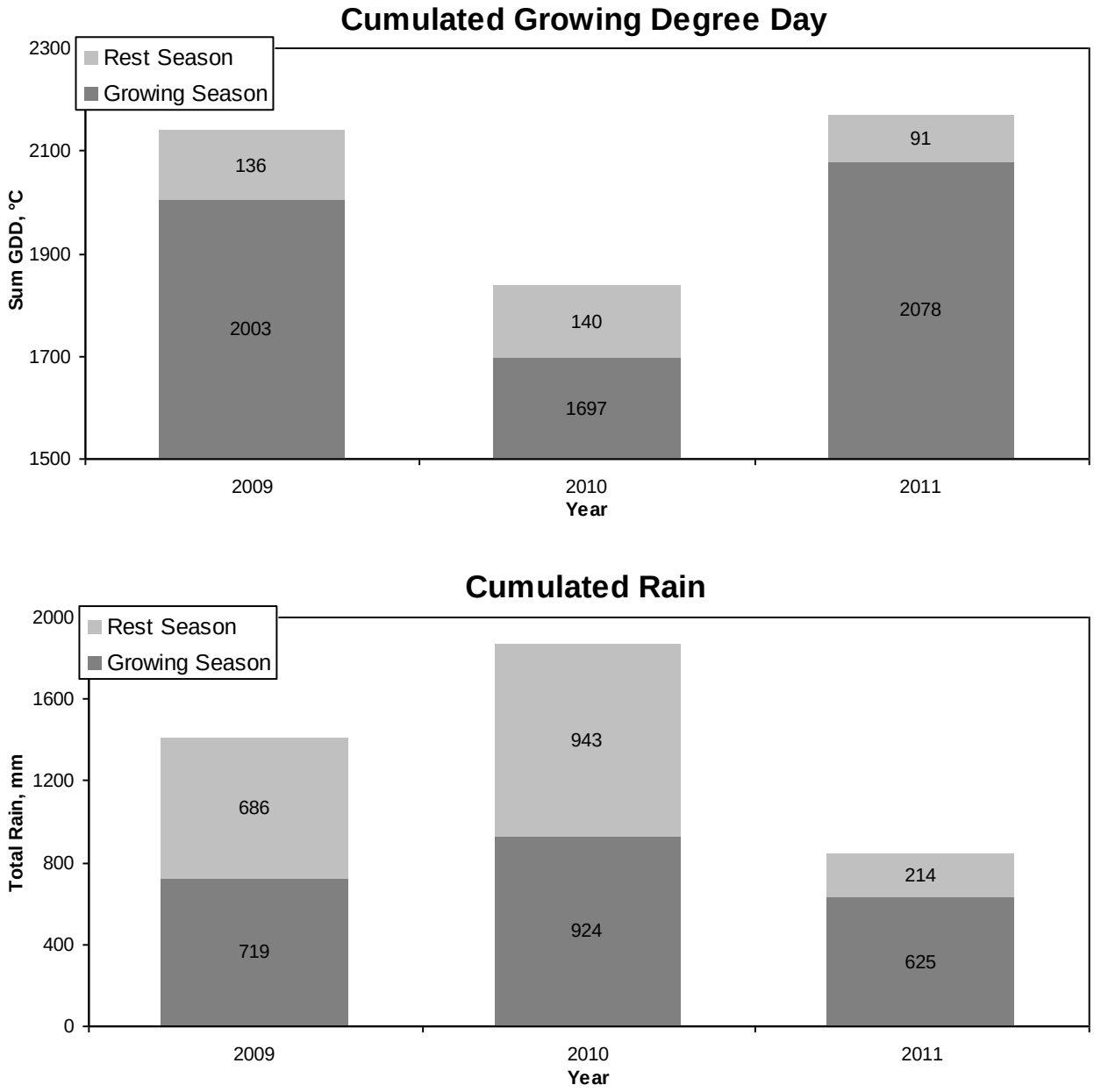


Figure 11: Cumulated rainfall in mm and GDD during the three years of experimentation divided in rainfalls amount in the growing season (since April to October) and occurred during the rest part of the season.

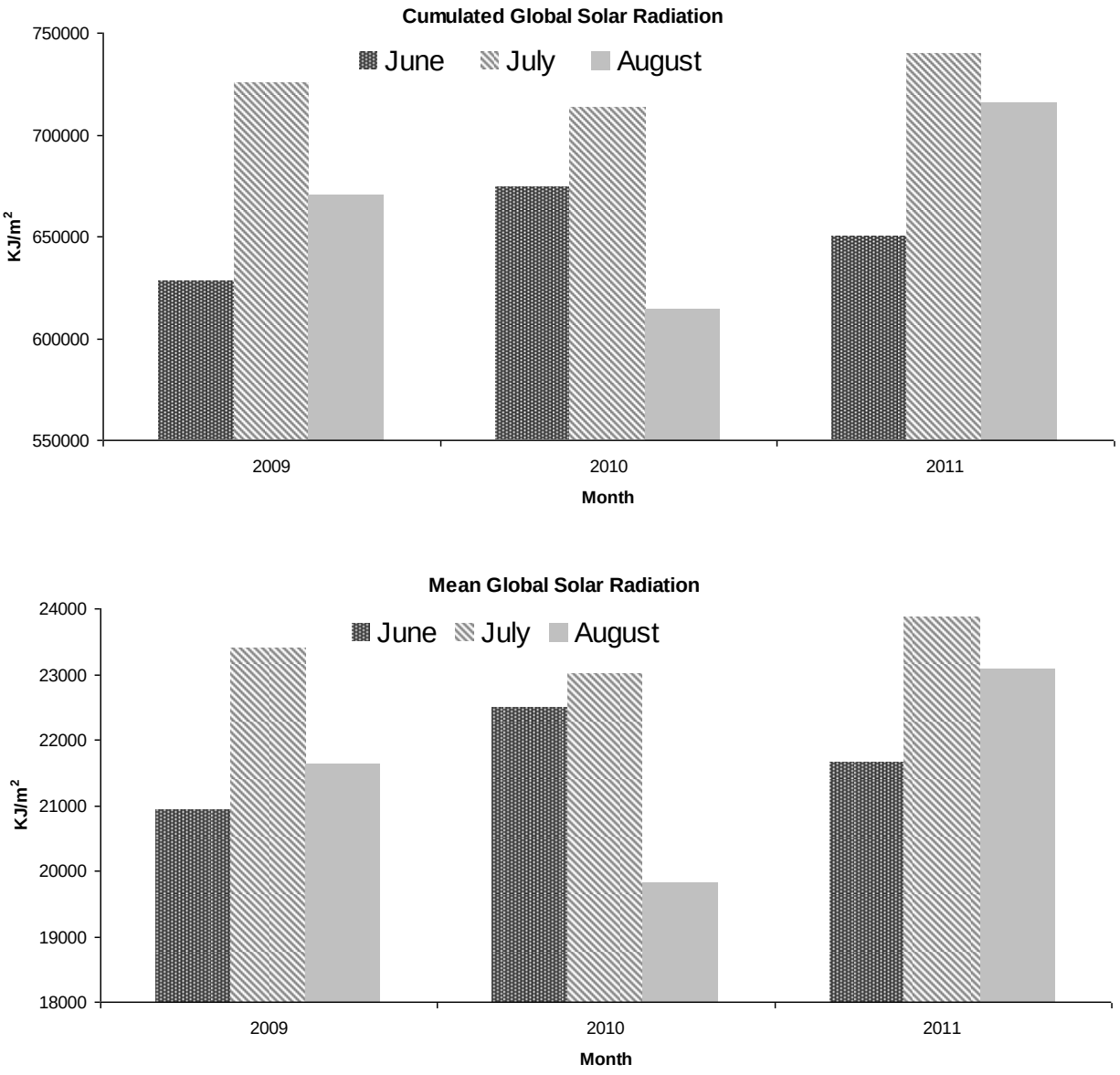


Figure 12: Cumulated and Mean Global Solar Radiation during the three years of experimentation on a monthly basis (since June to August), the time of berry development

2.4 Experimental field design

Different experimental field designs were chosen for 2009 than 2010 and 2011 field trials.

In 2009 a complete randomised block split-plot experimental design was adopted to evaluate the interaction between nitrogen fertilization and leaf removal. Four biological replicates per treatment were imposed. The nutrition treatments included:

4) ammonium sulphate (AS), 40 N units;
5) dried blood (DB), 60 N units;
6) control (C), not fertilized.

Nitrogen rates were chosen according to the agronomical practises normally adopted for conventional and organic farming in Isonzo DOC area.

Blocks were divided according to vine rows, considering soil variability and soil features. Soil organic matter content, determined before planning the study, revealed statistical differences between row fertility, as reported in Figure 13.

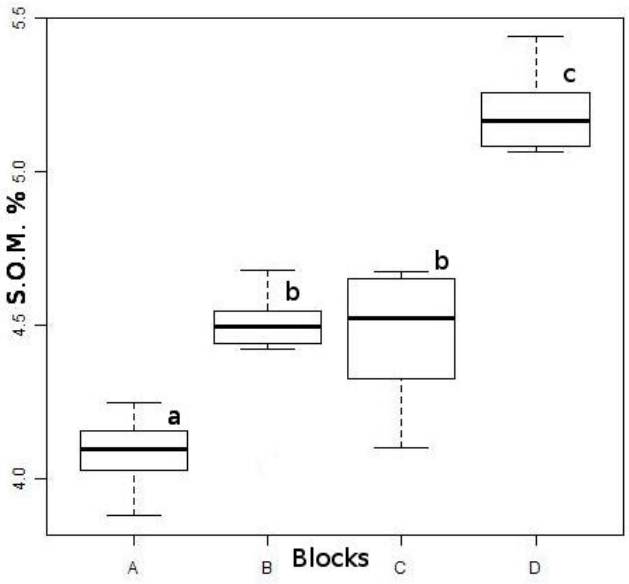


Figure 13: Soil Organic matter determined in each plot before starting the trial in spring 2009

Each plot included 20 vines, divided afterwards into 2 sub-plots of 10 vines. One of the two sub-plots was chosen for leaf removal. Leaf removal

was carried out after anthesis, removing manually five leaves from the proximal cluster node towards distal nodes. Every week, foliage was set up in the trellis to control undesired shading.

In 2010 and 2011 a complete randomised block experimental design separated for nutrition and leaf removal trials was chosen. Nutrition plot remained the same of 2009 for the three years. Instead, leaf removal trials were transferred on the same rows/blocks but outside the fertilization trials in the same vineyard. Dividing the two trials, a further treatment in leaf removal study was applied. The established treatment was the same basal leaf removal with the cluster shading by a 50% solar radiation shading netting. The net was mounted immediately after leaf removal with clips connected directly to the first metallic wire for supporting the canopy. The net was 30cm large to shade only cluster layer.

The resultant treatments in 2010 and 2011 were:

4) basal leaf removal (LR)
5) basal leaf removal and cluster shading (LRCS) by the shading net
6) control (C), untreated vines;

LRCS was adopted as a treatment for decreasing the effect of light and temperature on grapes.

Treatments were applied on days reported in Table 1

The location field experiments is reported in

Figure 14.

	year		
Trial	**2009**	**2010**	**2011**
Nutrition	19/05	24/05	11/05
Leaf Removal	18/06	22/06	29/06

Table 1: Time of Nitrogen nutrition and Leaf Removal treatment application

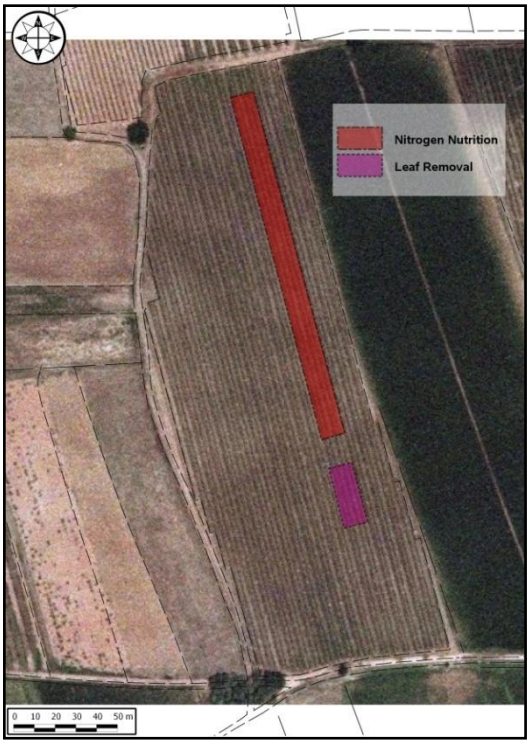


Figure 14: experimental plot location in the vineyard;

2.5 Field measurements

2.5.1 Normalized Difference Vegetation Index (NDVI)

NDVI (Rouse et al., 1974) was chosen as the index to describe the vine condition, since It is high correlated with biomass. NDVI is calculated as:

$$NDVI = (R\ NIR - R\ VIS) / (R\ NIR + R\ VIS)$$

Where:

R NIR = Near Infrared Reflectance

R VIS = Visible (Red) Reflectance

NDVI was measured by proximal sensing sensors, instead of remote sensing approach, more suitable for large-scale surveying. Advantages of proximal sensors are:

- no destructive measurement: measures can be replicated on the same plant;
- the distance between the sample and the sensors can be set;
- rapid measurements: a lot of data can be recorded from moving vehicles;

- measurements are not affected by natural light, relief slope and aspect, soil cover and cloudiness, so you can choose the acquisition time;
- high spatial resolution: few centimetres.

The sensors used in this study were Crop Circle ACS-210, Holland Scientific. This Sensors have a light source (PolySource®), that emits simultaneously VIS and NIR light from a single led. Reflected light portion, coming from the canopy, is recorded by a couple of sensors, sensitive to the different wavelengths (Figure 15).

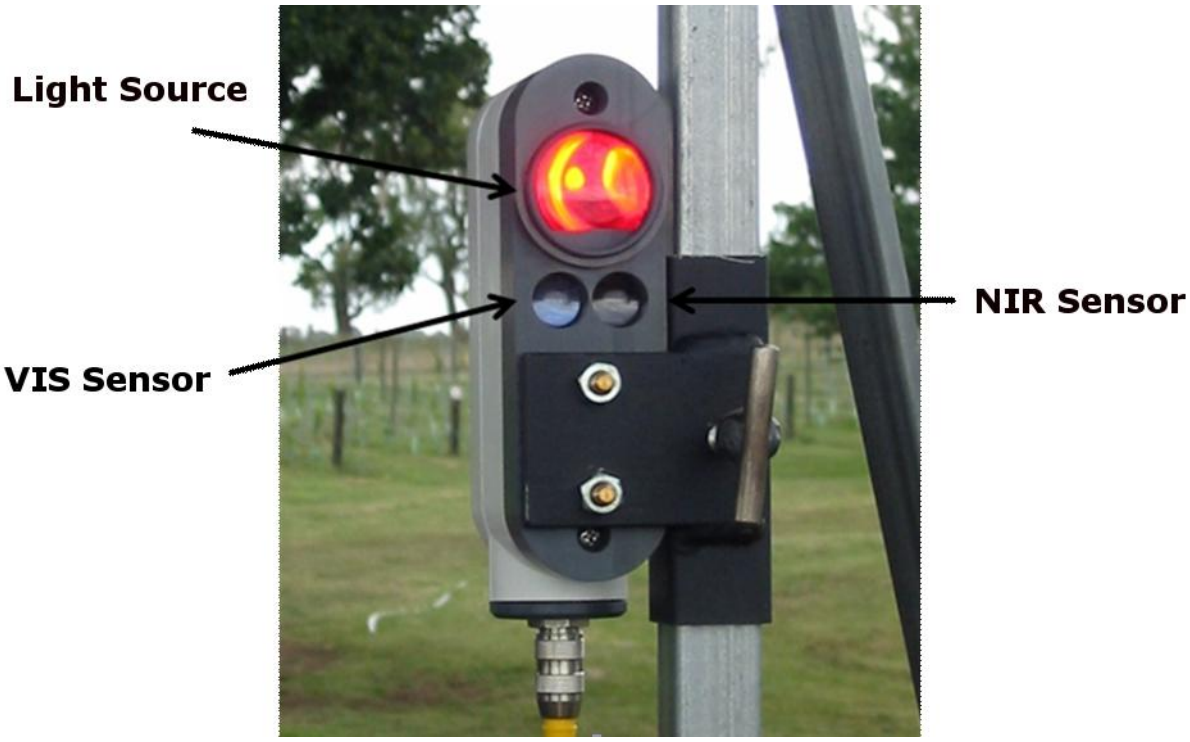


Figure 15: detail of the light source VIS and NIR Sensors.

They were mounted on a Quad-bike and connected with a datalogger, model ACS-210, Holland Scientific, equipped with a GPS aerial with WAAS-EGNOS frequency (Figure 16). Two sensors were directed at right angles to the canopy to record data in the internal side of two next vine rows. The height was set up according to the mean canopy height. Software Arvagreen 1.6, Arvatec S.r.l., was used for data collection. The vehicle speed was 10km/h and records were registered every 30cm of progress. For each of the three growing season data acquisition were carried out few days before harvest.

Figure 16: QUAD equipped with NDVI sensors and PC-datalogger;

2.5.2 Berry sampling

In 2009, berries were collected 4 times, in 2010 7 times and in 2011 5 times (Table 2). For each plot a 60-Berry sample was taken by cutting the whole pedicel with a manicure scissor for MPs analysis. A second sample of 60 berries was carried out for must parameters analysis; in this case, berries were blown off manually without the pedicel. For both the sample kinds, a randomized sampling approach was followed since each single berry was collected casually from clusters and from the location in the cluster.

Samples were closed carefully in a small plastic bag, tagged and placed in a cool box. In the laboratory samples were conserved in the freezer at -80°C until analysis.

Year			**Date**				
2009				27/7	10/7	18/8	22/8
2010	24/6	1/7	13/7	22/7	5/8	18/8	31/8
2011			29/6	12/7	21/7	3/8	19/8
Time				*Veraison*			*Harvest*

Table 2: Time of berry sampling

2.5.3 Leaf area estimation

In 2011 growing season two surveys for vine area leaf estimation were performed on June, 29th, when the leaf removal treatment was established, and on August, 10th, before harvest.

Hundred leaves were collected following a complete random approach from main shoots and another hundred from lateral shoots. Every leaf was measured for the length of the main vein and the blade area was measured in every leaf with a meter and a LI-3100C Area Meter (LI-COR Lincoln, Nebraska USA).

Two typical regressions of the experimental vineyard were calculated between the length of the main vein and the area of the single leaves.

One vine per C and LR plot was then chosen for measuring the main vein of all the leaves. Then the leaf area was calculated according to the relation between the main vein and the measured area.
A second equation calculated according to the relation between the number of leaves per shoot and the respective area was identified. Two different curve regressions were calculated for C and LR plots. Furthermore two vines per plot were counted for the main and lateral leaf number separated per each single shoot. Hence, the leaf area of the four biological replicates of C and LR were estimated.
Because of LRCS plot was tested for being not different than LR, area leaf estimation was achieved in C and LR plots.

2.5.4 Microclimate in 2010 and 2011

Canopy and cluster microclimate were monitored during ripening by using a data acquisition system connected to a datalogger (Campbell Scientific, CR200X-series, UK) (Figure 17). Thermocouples for temperature acquisition were mounted i) inside six representative clusters per treatment, three on the East and three on the West side of the row ii) outside the clusters and iii) in the canopy, three on the East and three on the West side of the row. Tube solarimeters were placed in the cluster zone of one vine per each treatment, eventually removing clusters that could shade sensors. Every 30 min mean temperature inside and outside the clusters and mean solar global radiation on clusters and above the canopy were registered. The instrumentation was powered by a high amperage battery.

Figure 17: Datalogger Campbell scientific equipped with multiplexer for probe connections

In two days during the 2010 and 2011 season, surface berry temperature was measured by an infra-red thermometer (Spectrum Technologies, Inc., Raytec Raynger ST, USA) at 9 am, 12 am, 3 pm (solar time), collecting about 100 measurements per plot.

2.5.5 Harvest parameters

At harvest seven plants per plot were chosen to record yield parameters and cluster disease. Cluster were harvested and the amount of cluster per vine was scored. Yield per vine, number of cluster per vine and mean cluster weight were calculated. For the former hundred clusters counted in each plot, the severity of botrytis, sun burn and sour rot damage per

cluster was recorded to know the effect of the imposed treatments on disease intensity and diffusion.

2.6 Laboratory analysis

2.6.1 Soil samplings and analysis

Soil samples from each plot of nutrition trial were collected 7 times during the growing season, from bud break to post maturation. At any sampling time, 6 sub-samples were collected from each plot from 5-25 cm depth and merged to obtain a composite soil sample. Samples were sieved moist (2 mm) and stored at 4 °C. Soil samples were analysed for water soluble organic C and N (WEOC, WEN), N-NO_3^-, available P, organic matter (SOM), soil microbial biomass C and N.

WEOC and WEN were determined using the analyzer TOC-Vch with the TN unit TNM-1.

DTC – DTN – equipment and technique - carrier gas (purified air) is passed at a controlled flow rate of 150 ml/min through an oxidation catalyst-filled, heated to 680°C for the TC determination and to 720°C for the TN determination. When the sample injection system injects the sample into the combustion tube, the TC in the sample is oxidized or decomposes to create carbon dioxide, TN thermally decomposes to create nitrogen monoxide. The carrier gas carrying the combustion products from the combustion tube is cooled and dehumidified in the dehumidifier before passing via the halogen scrubber into the sample cell of the non-dispersive infrared detector (NDIR), where the carbon dioxide is detected. The NDIR analog signal forms a peak, and the data processor calculates the peak area.

N-NO_3^- content was measured by means of UV-vis spectrofotometry set at 220 nm.

2.6.2 Berry juice determinations

On berry juice classical quality parameters were determined for each sampling date. Samples were put out of the fridge and melt in a heated water bath at 65°C for 1 hour to dissolve tartrates (Scheiner et al., 2010). Afterwards berries were squeezed manually into the plastic bag and the juice was collected for the further analysis. Soluble solid were measure with a digital refractometer; titratable acidity (TA) was measured by titration using 10ml of juice and 0.1N NaOH solution to reach the pH 8.2; pH was measured with the same electrode used for TA determination.
At harvest a larger sample of about 2kg was collected. The same analyses were done on the obtained juice.

2.6.3 Total Nitrogen in juice (TN)

Total nitrogen (TN) was measured by using the automatic analyser for fluids Shimadzu TOC-VCS/CP with TNM-1 equipped with the ASI-V autosampler, as explained for Soil analysis (paragraph 2.6.1) with suitable dilutions.

2.6.4 Malic and Tartaric Acids

Organic acids were measured by using enzymatic kit of Megazime, Assay Procedure. Sample preparation and analysis protocol has been followed by Megazyme manuals. The detection of L-malic acid requires two enzyme reactions. In the first reaction catalysed by L-malate dehydrogenase (L-MDH), L-malic acid is oxidised to oxaloacetate by nicotinamide-adenine dinucleotide (NAD+). However, since the equilibrium of the first reaction lies firmly in the favour of L-malic acid and NAD+, a further reaction is required to "trap" the NADH product, and this is achieved by the conversion of oxaloacetate to L-aspartate and 2-oxoglutarate, in the presence of a large excess of L-glutamate, by glutamate-oxaloacetate transaminase (GOT). The amount of NADH formed in the above coupled

reaction is stoichiometric with the amount of L-malic acid. It is the NADH which is measured by the increase in absorbance at 340 nm.
Similar analysis was carried out for tartaric acid according to a specified double enzymatic reaction.
Sample were prepared in 96-well plystyrene clear flat bottom microplates (Matrix technologies Corp.) and red by SPECTROstar omega bmg labtech device.

2.6.5 MPs analysis

For determination of Methoxypyrazines, analysis were performed at theCentral Laboratories, Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia.
Preparation of Standards and Solvents. IBMP (Sigma-Aldrich, St. Louis, MO, USA) with apurity of 99%, 2-isobutyl-3-methoxy-d3-pyrazine ([2H3]-IBMP) (C/D/N/Isotopes, Quebec, Canada) with a purity of 99%, and IPMP(Sigma-Aldrich) with a purity of 99% were used for the preparation ofstandards in solvent. Stock solutions of IBMP (250 mg/L), [2H3]-IBMP (500 mg/L), and IPMP (280 mg/L) were prepared in methanol(Sigma-Aldrich). Intermediate solutions (IBMP = 2.5 mg/L, [2H3]-IBMP = 5.0 mg/L, and IPMP =2.8 mg/L) and working solutions(IBMP = 2.5 µg/L, [2H3]-IBMP = 5.0 µg/L, and IPMP = 2.8 µg/L)were prepared in methanol as well.
Preparation of Sugar Solution. Five hundred milliliters of water purified by a Milli-Q system (Bedford, MA, USA) was placed in a 1000mL volumetric flask. Ninety grams of fructose (Sigma-Aldrich), 90 g of glucose (Sigma-Aldrich), and 1 g of tartaric acid (Merck, Darmstadt,Germany) were added and dissolved. The volumetric flask was made up to volume with purified water, and the pH was adjusted to 3.2 with NaOH. Dearomatization of Grape Juice. Forty-five milliliters of Sauvignon blanc juice was placed in the 50 mL tube and centrifuged for 5 minutes at 5000 min−1. The liquid was then transferred to a 5 L flask; 3 L of previously centrifuged Sauvignon blanc juice was evaporated under reduced pressure to

approximately 90% of the initial volume. The evaporated liquid was replaced by purified water. Afterward, the juice was transferred to a beaker and heated until it reached 80 °C to evaporate or decompose the MPs still present in the juice. Preparation of Alcoholic Solution. Five hundred milliliters of purified water, 120 mL of absolute ethanol (Sigma-Aldrich), and 1 g of tartaric acid were added to a 1000 mL volumetric flask. The volumetric flask was then made up to volume with purified water, and the pH was adjusted to 3.2 with NaOH.

Preparation of Calibration Standards. Calibration standards were prepared in a sugar solution, an alcoholic solution, and a dearomatized must using working solutions of IBMP, [2H3]-IBMP, and IPMP. Some sugar solution, alcoholic solution, or dearomatized must was transferred to a 25 mL volumetric flask, [2H3]-IBMP, IBMP, and IPMP were added, and then the flask was made up to the volume to reach the final concentration of 25 ng L-1 of [2H3]-IBMP and IBMP and 28 ng L-1 of IPMP. NaCl was placed into a 20 mL SPME vial along with a stir bar, followed by 1.6 mL of the prepared solution, 6.4 mL of purified water, and 2 mL of 4 M NaOH. The vial was closed and placed onto a magnetic stir plate to dissolve the NaCl.

Preparation of Sample. The grape juice sample was prepared byhand-crushing undamaged berries in a plastic bag for 2 min. Some strained grape juice was transferred to a 25 mL volumetric flask, 125μL of [2H3]-IBMP (internal standard) was added with a concentration of 5 μg/L, to reach the final concentration 25 ng L-1 of [2H3]-IBMP, and the flask was made up to the volume with grape juice. NaCl was placed into a 20 mL SPME vial along with a stir bar, followed by 1.6mL of the prepared sample, 6.4 mL of purified water, and 2 mL of 4 MNaOH. The vial was closed and placed onto a magnetic stir plate todissolve the NaCl.

Apparatus and Determination Procedure. The samples were analyzed using a gas chromatograph (Agilent Technologies 7890A,Shanghai, China) equipped with a Gerstel MPS2 multi purpose sampler (Gerstel, Mu̇lheim an der Ruhr, Germany) and two successively connected columns, an HP 1 MS (Agilent Technologies,30 m, 0.32 mm i.d., 0.25 μm film thickness) and an

HP INNOWAX(Agilent Technologies, 30 m, 0.32 mm i.d., 0.25 μm film thickness),with a constant flow of helium at 1.5 mL/min. The vial was incubated for 5 min at 40 °C. The extraction on fiber DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) was performed for 40 min at 40 °C with constant stirring at 250 min−1. The injector was held at 250 °C for 3 min for the analytes to desorb from the fiber. The GC oven was programmed as follows: 60 °C for 10 min, from60 to 100 °C at 7 °C/min, held at 100 °C for 10 min, from 100 to 170°C at 7 °C/min, from 170 to 230 °C at 40 °C/min, held at 230 °C for20 min, from 230 to 60 °C at 40 °C/min, and held at 60 °C for 3 min. For the determination of analytes, a mass spectrometer (AgilentTechnologies 5975C, upgraded with a triple-axis detector, Palo Alto,CA, USA) was used. The temperature of the ion source was 230 °C, the auxiliary temperature was 250 °C, and the quadrupole temperature was 150 °C. For qualitative determination, retention time and mass spectrum in selective ion monitoring mode (SIM) were used. The mass channel was m/z 137 and 152 for IPMP, m/z 124 and151 for IBMP, and m/z 127 and 154 for [2H3]-IBMP. Ions 137, 124,and 127 were the target ions used for quantification, whereas 152, 151,and 154 were used as qualifier ions. Calibration was performed with calibration standards in sugar solution for must and in alcoholic solution for wine. Linearity was verified by using spiked samples of dearomatized must and alcoholic solutions for wine (four repetitions for one concentration level, nine concentration levels for the calibration curve). Linearity and range were determined by multiple linear regressions, using the F test.

Calibration curves were derived using increasing amounts of IBMP (1−196 ng L-1) and IPMP (1−200 ng L-1) spiked in a dearomatized must, a sugar solution, and an alcohol solution. Good linearity was obtained for both analytes: IBMP (R2 for dearomatized must was 0.9996; for sugar solution, 0.9991; and for alcohol solution, 0.9986) and IPMP (R2 for dearomatized must was 0.9992; for sugar solution,0.9981; and for alcohol solution, 0.9985). The limit of detection (LD) and the limit of quantification (LQ) were calculated from the calibration curve. For IBMP,

the LD of the dearomatized must was 0.6 ng L-1 , and for the alcohol solution it was0.4 ng L^{-1}. The LQ for IBMP was 2.0 ng L^{-1} for the dearomatized must and 1.2 ng L^{-1} for the alcohol solution. For IPMP the LD of the dearomatized must was 0.6 ng L-1 , and for the alcohol solution it was0.5 ng L^{-1}. The LQ for IPMP was 2.1 ng L^{-1} for the dearomatized must and 1.6 ng L^{-1} for the alcohol solution.

2.7 Statistical analysis

Data were analysis for statistics with SPSS statistical package ver.17 (SPSS inc., Chicago, Illinois).

In 2009, a split-plot experimental field design Leaf removal x Nitrogen nutrition was adopted, so one-way ANOVA was performed evaluating the interaction between factors. For multiple year trials in complete randomized block design, General Linear Mixed Model were used to evaluate treatment effect (fix factor) and year effect (random factor). Year x Treatment interaction was assessed.

Means were compared by choosing among different post-hoc tests: for vine variables Duncan's test was adopted and for soil variables LSD test was used as suggested by Webster, 2007.

3 Effect of Leaf Removal and cluster shading on Methoxypyrazines Sauvignon blanc expression

3.1 Results

3.1.1 Temperature and solar radiation

3.1.1.1 2010 microclimate

In 2010, cluster temperature and solar radiation during ripening were influenced by the imposed treatments. Cluster temperatures were higher ($p<0.01$) for LR and LRCS than C during sunny days, but not in heavy cloudy days, when no significant differences were recorded (Figure 22).

LRCS created intermediate conditions between C and LR of temperature and solar radiation in the fruiting zone. Although there were no sensor replicates of solar radiation and we were not allowed to assess statistics, LR was always higher then LRCS and C during sunny days.

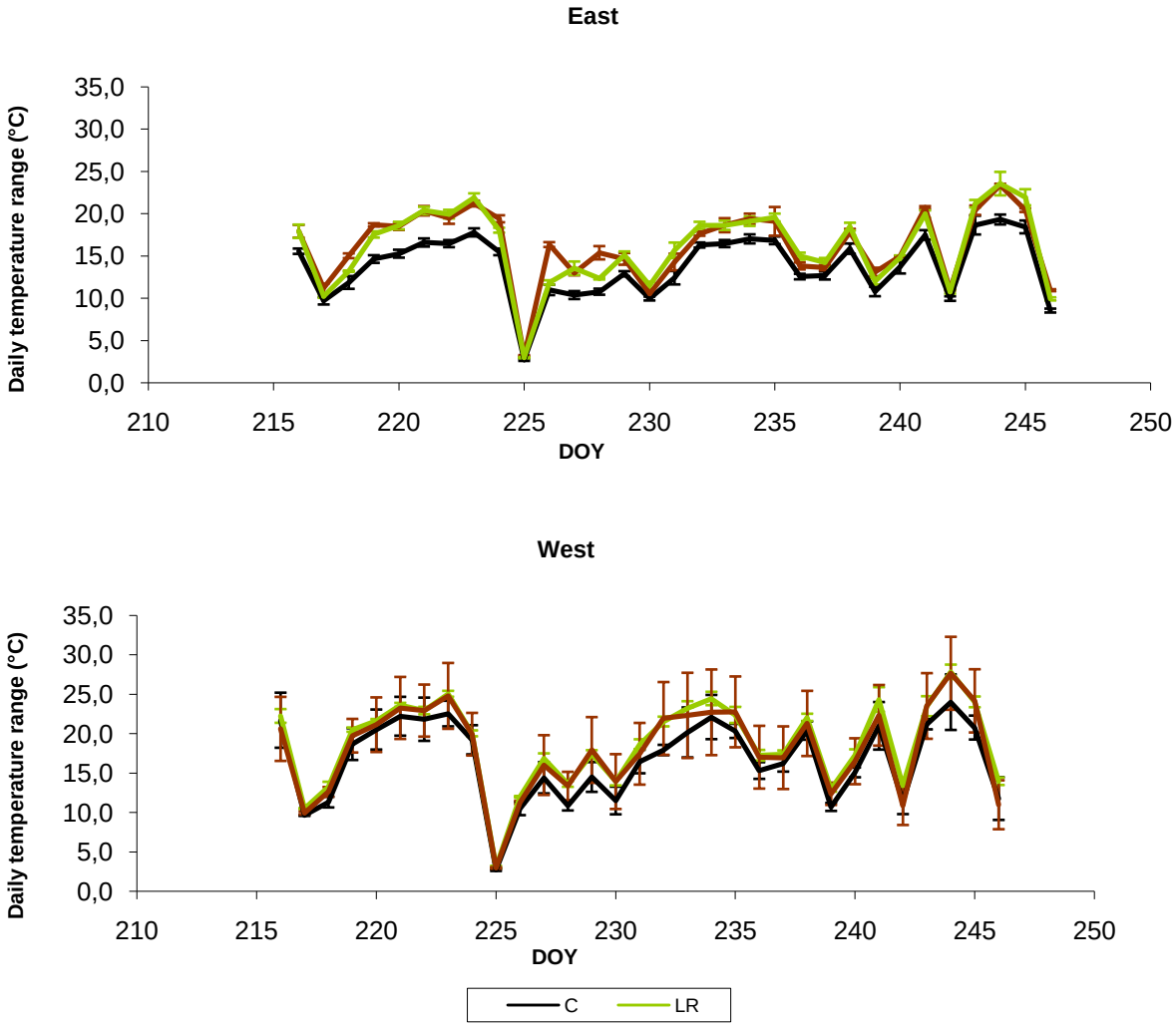


Figure 18:Daily temperature range recorded by east and west thermocouples inserted in the clusters of the three treatments.

Mean daily temperature in sunny days was 3 or 4°C higher in LR and LRCS than in C in east sunlight exposed clusters, while in west side mean daily temperature differences were about 2°C in the same days (Figure 18).

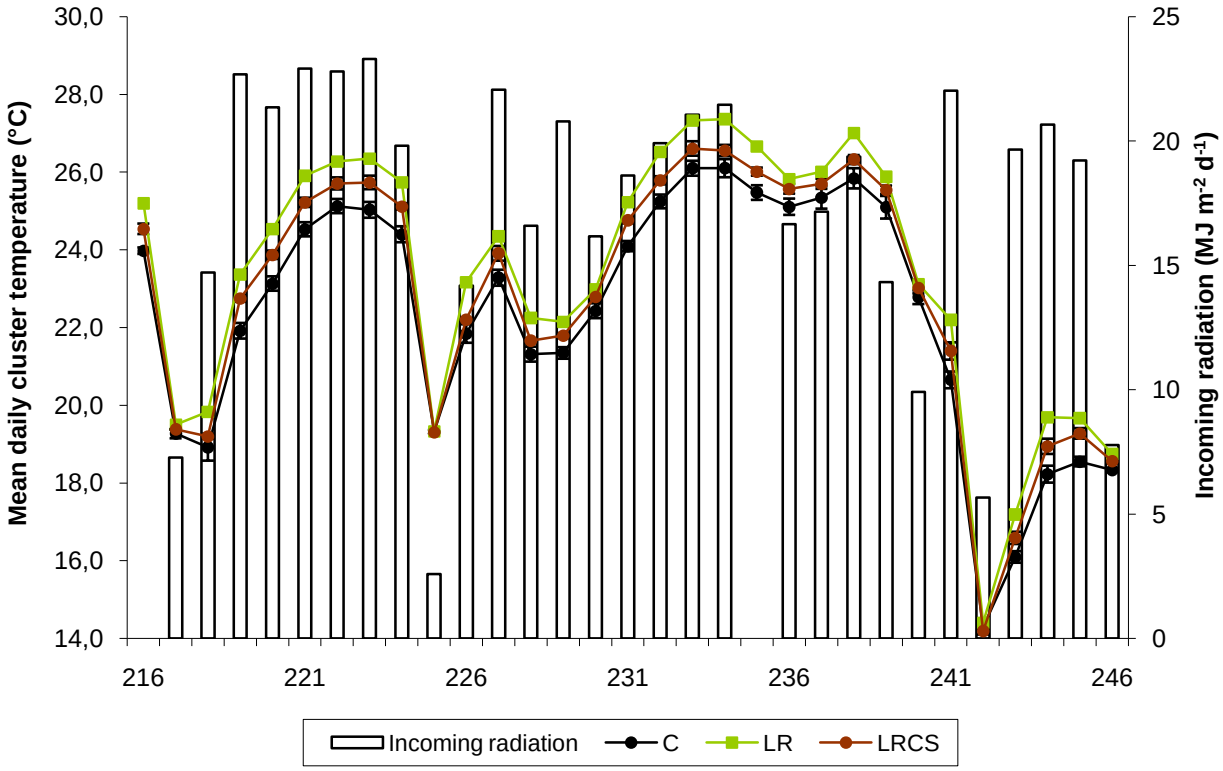


Figure 19: mean daily cluster temperature and incoming radiation above the canopy.

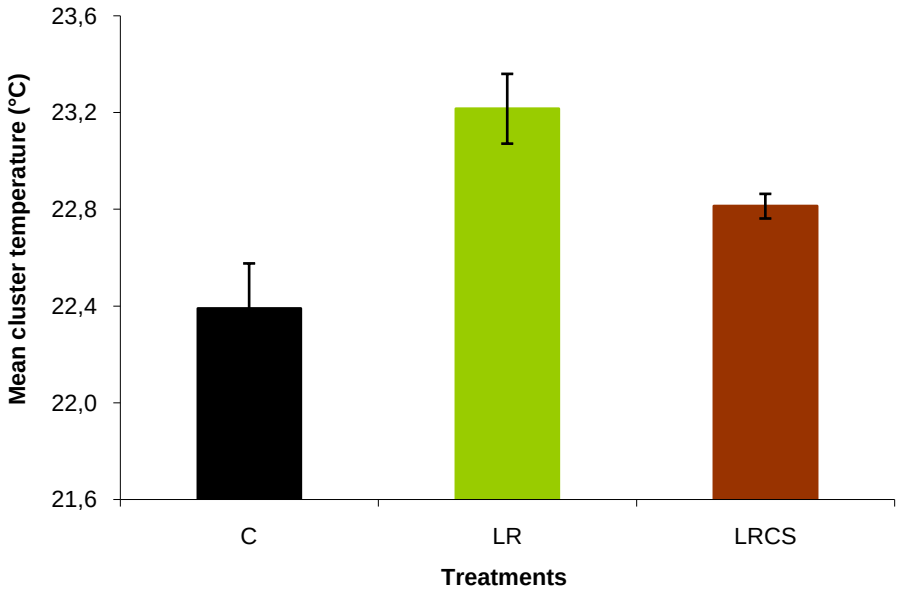


Figure 20: mean cluster temperature of the imposed treatments during the recording period.

Figure 20 shows mean cluster temperatures inside clusters during the recording period: LR increased on average cluster temperature of 0.8°C if compared to C and of 0.4°C if compared to LRCS, with high statistical difference between treatments (p<0.001). Thus, the shading net assessed intermediate grape microclimate conditions, between C control not defoliated and simple LR leaf removal (Table 3).

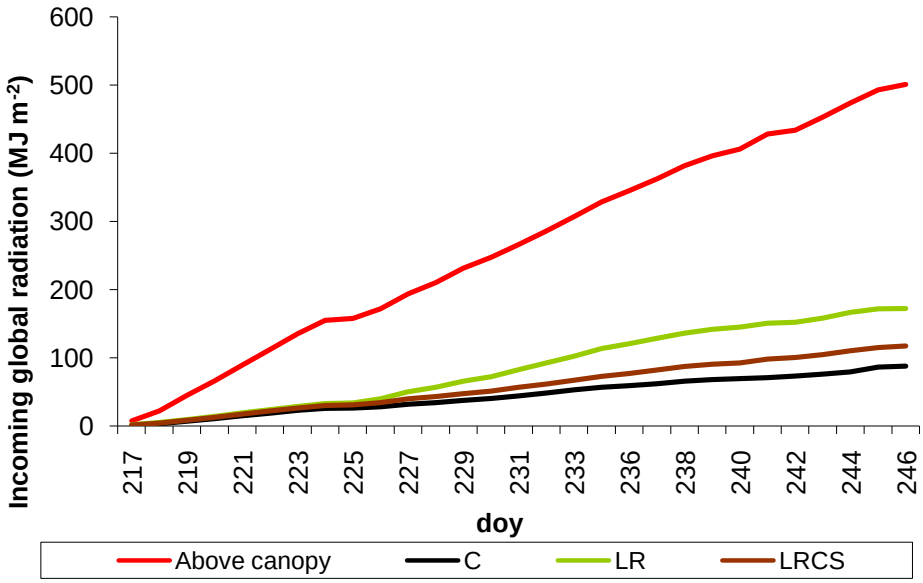


Figure 21: Incoming global radiation of the imposed treatments during the recording period.

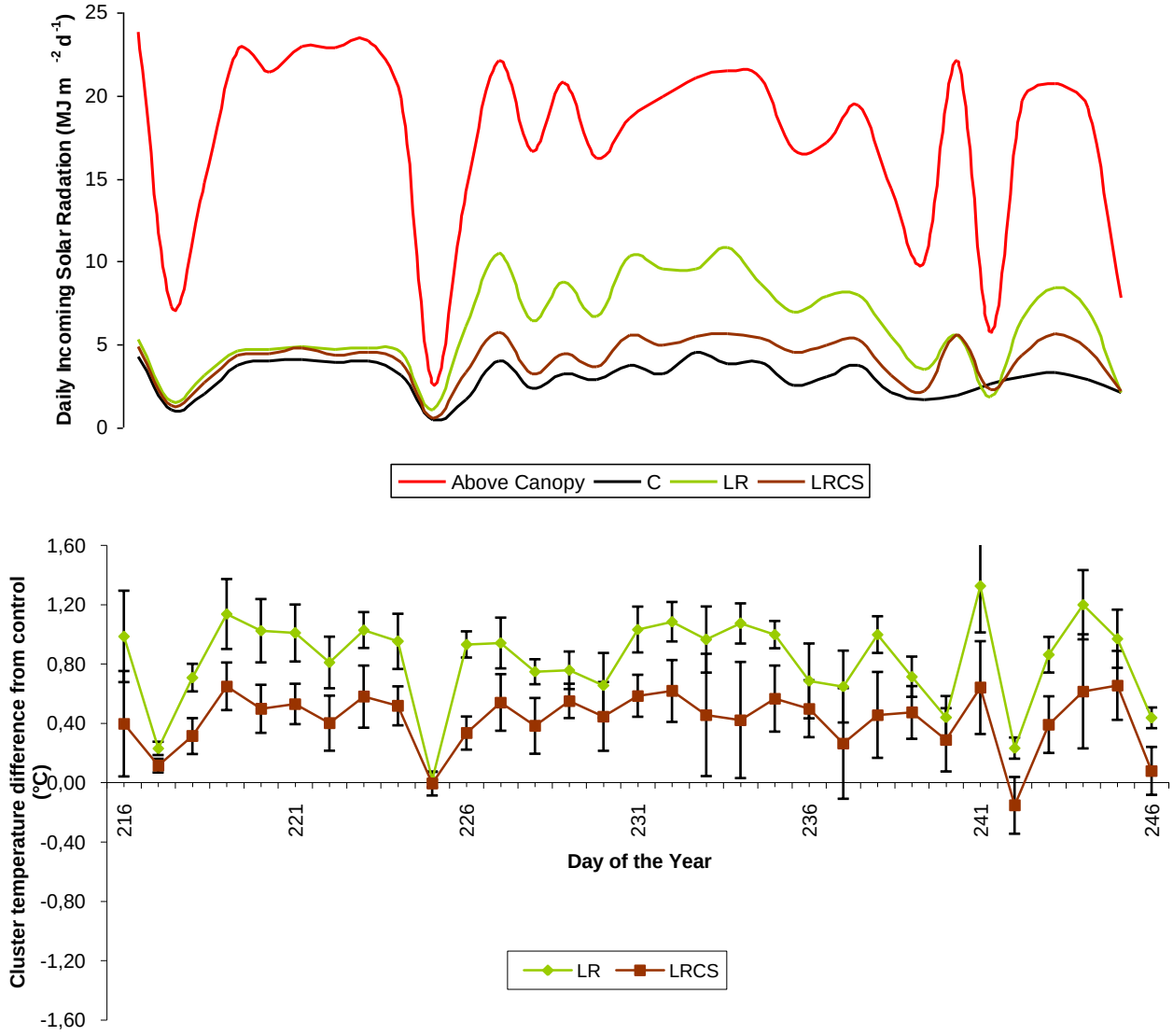


Figure 22: daily incoming solar radiation (MJ m-² d-¹) and daily cluster temperature (°C) difference from control during 2010 ripening.

Cumulated incoming solar radiation on clusters during the monitored period was highest for LR, lowest for C and intermediate between LR and C for LRCS (Figure 21 and Table 3).

During the day, cluster temperatures were strongly related to solar radiation, and this was true for all imposed treatments (Table 3). Among treatments, LR recorded the highest radiation followed by LRCS and C.

Treatments	Incoming solar radiation (MJ m^{-2})	Mean air temperature (°C)	Mean cluster temperature (°C)
Above Canopy	546	21.89	-
C	92	-	22.39+-0.19
LR	189	-	23.21+-0.14
LRCS	127	-	22.81+-0.05
Significance	-		**

Table 3: summary of incoming solar radiation (MJ m^2) and mean cluster temperature (°C) monitoring during ripening 2010.

In a full sunny day in the experiment vineyard temperature and solar radiation varied as showed in Figure 23. The cluster temperature and solar radiation measured in the fruiting zone increased rapidly in the morning (once the sun was east of the north/sud row axis), decreased rapidly as the sun moved to the zenith and increased again afterward (as the sun was west of the row axis).

Solar radiation on LR had similar intensity as radiation above canopy until 9.00-9.30 in the morning and from 3.00 p.m, until sunlight rays reach directly clusters. In the central hours of the day solar radiation on clusters fell down to similar C and LRCS intensity.

Grape temperature increased after solar radiation picks in the morning and in the afternoon, when solar radiation reached directly clusters. Maximum daily temperature were recorded in the afternoon. In the central hours of the days cluster temperatures slightly increased following air temperature increasing.

Small variations of solar radiation recorded above the canopy could be due to light temporary cloudiness during the central hours of the day (Figure 23).

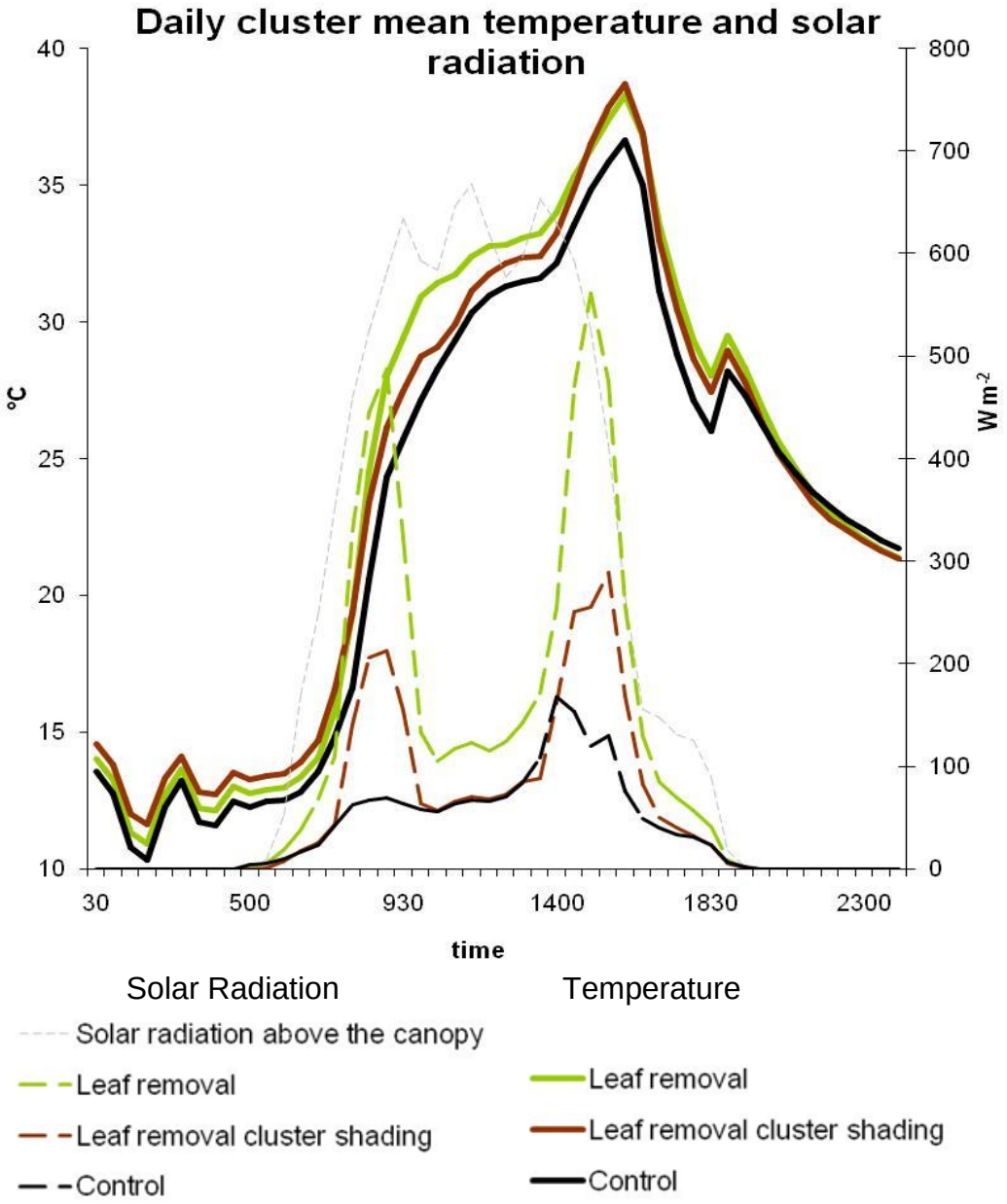


Figure 23: data collected in an example pre-harvest 2010 day: dotted lines indicate incoming solar radiation and full lines indicate temperature.

3.1.1.2 Cluster surface temperature

The measurement of cluster surface temperature described the thermal status of external berries of clusters subjected to different treatments. Measurements were taken one day before harvest in 2010 and 2011. Temperature varied depending on the day time. Since experiment vineyard is planted in North/south orientation, light affected directly grape temperature from sunshine to sunset.

In both seasons, LR and LRCS significantly affected surface cluster temperature in the morning on the east side and in the afternoon on the west side.

In 2010, morning east side cluster temperature was recorded 10°C and 4° C higher in LR than in C and LRCS, respectively (Table 4); on the contrary on west side there were no differences in the temperature of the west side clusters, and the temperature was for all the treatments similar to the one recorded for east side clusters in C. At noon temperatures were not statistical different between east and west clusters and among treatments. Conversely, in the afternoon west side recorded highest temperatures in LR and LRCS.

Year	Row side	Treatment	10.00am	sd	12.30pm	sd	3.30pm	sd
2010	E	C	30.8a	0.9	32.8a	0.9	34.0a	1.5
		LR	40.6c	1.5	34.6a	0.7	35.1a	1.5
		LRCS	36.6b	1.7	35.0a	0.5	34.7a	1.3
	W	C	30.5a	1.1	33.0a	0.4	36.2a	0.2
		LR	31.2a	1.0	33.8a	0.3	41.6b	0.5
		LRCS	31.0a	1.0	33.7a	0.4	42.9b	1.4
significance			**		*ns*		**	
2011	E	C	36.6a	1.4	37.3a	0.7	37.2a	1.0
		LR	43.0b	1.3	37.5a	0.7	37.8a	1.0
		LRCS	38.9ab	1.9	36.8a	0.5	37.5a	1.0
	W	C	34.6a	1.4	36.9a	0.3	38.9a	0.6
		LR	35.1a	1.3	36.8a	0.5	43.3b	3.5
		LRCS	34.8a	2.0	36.2a	0.5	40.7ab	0.9
significance			**		*ns*		**	

Table 4: surface cluster temperature collected at 2010 and 2011 pre harvest sunny and cloud clear day, with a IR thermometer at morning, noon and afternoon, when sunlight reached directly grapes.

In 2011, temperature patterns were similar to the one observed in 2010. Generally, 2011 cluster temperatures were higher than 2010 ones. This is consistent with the fact that harvest was carried out ten days earlier in 2011 than 2010 and that average air temperatures recorded the day when the measurements were performed were significantly higher in 2011 than 2010. This is probably at the basis of the lower differences observed in 2011 among clusters in sun light exposed clusters.

Temperatures registered on cluster surface were high correlated (r^2=0.75) to temperature inside the cluster (Table 5). Clearly, surface temperature was always higher than inside cluster one. This points out that some berries were more influenced by treatments than others also within the same cluster.

		10.00am		12.30am		3.30pm	
Row side	Treatment	Inside	Surface	Inside	Surface	Inside	Surface
E	C	29.8	34.7	32.4	34.4	32.3	35.3
	LR	36.4	40.9	34.1	36.8	37.1	33.9
	LRCS	31.3	37.1	33.1	35.3	35.2	35.4
W	C	25.8	29.2	31.5	32.7	36.2	36.2
	LR	27.7	30.7	32.8	33.5	38.4	43.3
	LRCS	27.8	31.4	32.6	33.6	40.2	42.2

Table 5: surface cluster temperature and inside cluster temperature collected on 2010 pre-harvest time.

3.1.2 Grape ripening variables

3.1.2.1 Fruit composition

Must ripening parameters soluble solids (SS) (°Brix), pH, titratable acidity (TA) (tartaric acid g/L) showed statistical differences only for some sampling dates.

The effect of the year was predominant if soluble solids were compared (Table 7). As showed in the climatic introduction section, 2011 season was appreciable warmer during the ripening time, so that °Brix were higher both at veraison and harvest in comparison to 2010. The ripening curve didn't show statistical differences except in 2010 sampling before harvest, when LR and LRCS increased SS concentration (Table 6).

At 2010 harvest SS were statistically lower (18.0°Brix) than in 2011 (20.5°Brix). In both years treatments did not affect consistently SS at harvest: comparing histograms in Figure 24 a trend of delaying SS accumulation could be noticed for LRCS both in 2010 and in 2011, although no statistical differences were determined.

TA showed statistically differences between treatments both in 2010 and 2011 especially at the sampling date before veraison. Contrary to SS, TA was higher in 2010 than in 2011 and LRCS increased TA at harvest especially in 2011, when LRCS (5.5g/L) was statistically higher than C (4.4g/L) and LR (4.5g/L) (Figure 24).

TA was affected by high variability and did not show a clear trend due to treatments during grape ripening.

Year effect was significant ($p<0.067$) at harvest supporting the year effect on SS.

pH in the must showed statistical differences only in 2010 when LR treatment reduced pH both at veraison and at harvest (Table 6). Interaction between year and treatment effects was found both at veraison and at harvest (Table 7). Year effect was not significance.

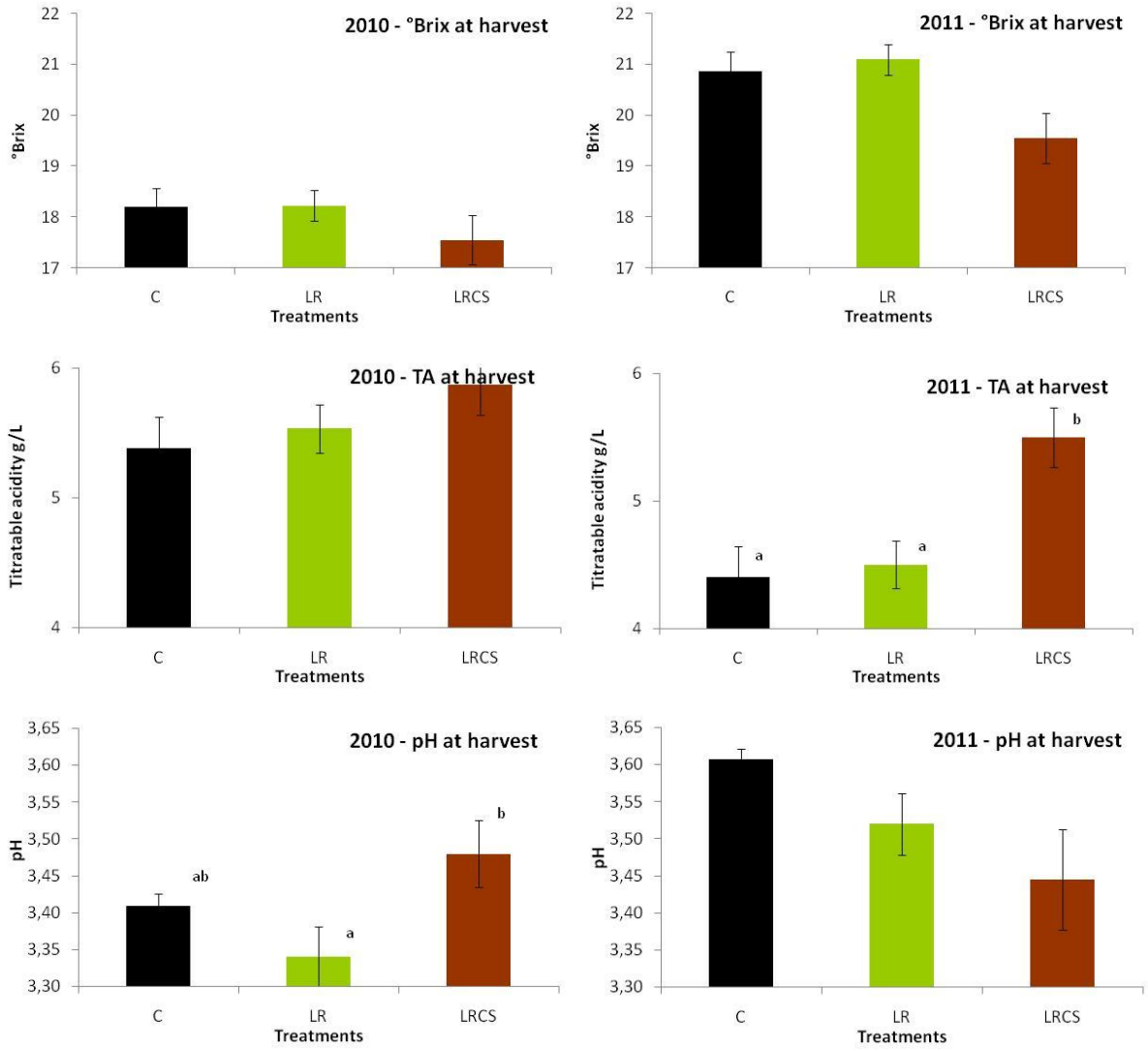


Figure 24: Soluble solids (°Brix), Titratable acidity (g/L) and pH as affected by treatments C (control not defoliated, LR (Leaf Removal) and LRCS (Leaf Removal Cluster Shading) at harvest in 2010 and in 2011 seasons.

			°Brix						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p value***
	174	19	3.6	0.1	3.6	0.1	3.6	0.1	-
	181	26	3.5	0.1	3.7	0.2	3.9	0.2	0.056
	193	38	4.0	0.1	4.2	0.1	4.1	0.1	0.096
2010	202	47	4.3	0.3	4.2	0.2	4.2	0.1	0.744
	216	61	10.9	0.5	9.7	0.4	9.8	0.9	**0.042**
	229	74	13.8a	0.8	15.7b	0.9	14.1a	0.4	**0.002**
	242	87	18.2	0.7	18.2	0.6	17.6	1.0	0.260
	181	44	3.8	0.1	3.8	0.0	3.8	0.0	0.919
	192	55	5.4	0.3	5.7	0.4	5.2	0.0	0.193
2011	201	64	9.9	0.9	10.9	0.8	9.9	1.3	0.346
	214	77	15.7	1.3	15.8	1.1	14.7	0.9	0.172
	230	93	20.9	0.6	21.1	1.7	19.6	2.0	0.479

			Titratable acidity (g/L)						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p value***
	174	19	18.9	0.1	18.9	0.1	18.9	0.1	-
	181	26	23.4a	0.3	25.3b	0.3	24.8c	0.2	**0.001**
	193	38	28.2a	0.9	30.9b	1.1	30.4b	0.5	**0.022**
2010	202	47	29.7a	0.8	33.3b	1.0	31.0a	0.5	**0.002**
	216	61	20.5	0.8	20.3	1.0	21.5	2.1	0.372
	229	74	6.6	4.2	9.6	1.8	8.0	0.5	0.311
	242	87	5.4	0.5	5.5	0.4	5.9	0.5	0.332
	181	44	31.5b	1.3	29.4a	0.3	30.1ab	0.9	**0.042**
	192	55	35.8	2.5	34.4	0.8	35.6	0.7	0.412
2011	201	64	22.0	4.9	20.5	1.7	22.9	3.0	0.678
	214	77	9.9	0.7	10.2	1.0	11.0	1.6	0.532
	230	93	4.4a	0.1	4.5a	0.2	5.5b	0.7	**0.024**

			pH						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p value***
	174	19	2.57	0.01	2.57	0.01	2.57	0.0	-
	181	26	2.60	0.01	2.59	0.06	2.54	0.0	0.307
	193	38	2.64	0.03	2.54	0.12	2.59	0.1	0.255
2010	202	47	2.59b	0.03	2.39a	0.06	2.52b	0.0	**0.002**
	216	61	2.80	0.01	2.70	0.07	2.73	0.1	0.069
	229	74	3.11	0.05	3.06	0.11	3.15	0.0	0.348
	242	87	3.41ab	0.03	3.34a	0.08	3.48b	0.1	**0.046**
	181	44	2.43	0.05	2.48	0.05	2.49	0.0	0.117
	192	55	2.55	0.05	2.50	0.01	2.51	0.0	0.226
2011	201	64	2.74	0.03	2.71	0.02	2.69	0.0	0.151
	214	77	3.07	0.05	2.93	0.08	2.89	0.1	0.061
	230	93	3.61	0.03	3.52	0.08	3.45	0.1	0.147

Table 6: Grape ripening soluble solid content (°Brix), pH and titratable acidity (g tartaric acid/L) in 2010 and 2011 seasons determined in samples subjected to different treatments. P-value indicates significance level for the different treatments; different letters indicate different means.

					p value		
Variable	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Veirason							
Brix°	4.2	0.2	5.4	0.3	**0.014**	0.598	0.192
pH	2.50	0.10	2.52	0.07	0.681	0.290	**0.003**
Titratable acidity	31.3	1.7	35.3	1.6	0.116	0.831	**0.003**
Harvest							
Brix°	18.0	0.8	20.5	1.6	**0.011**	0.124	0.781
pH	3.41	0.09	3.52	0.11	0.280	0.742	**0.016**
Titratable acidity	5.6	0.5	4.8	0.7	**0.066**	0.161	0.308

Table 7:Grape ripening variables soluble solid content (°Brix), pH and titratable acidity (g tartaric acid/L) analyzed by year; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments.

3.1.2.2 Organic acids: Malic and tartaric

Malic and tartaric acids were strongly affected by high variability. Malic acid (MA) showed strong statistical differences between treatments in 2010 at veraison when C (30.4 g L^{-1}) was higher than LR (23.4 g L^{-1}) and LRCS (23.3 g L^{-1}). Shading net (LRCS) did not affect MA content if compared to simple leaf removal application (LR), and at harvest no differences were observed among treatments (Table 8). First sample after veraison showed statistical differences with LR and LRCS, 22.4 and 22.3 g L^{-1} respectively, stationary if compared to veraison, and C, 16.2 g L^{-1}two times decreased than veraison.

In 2011, MA was not different among treatments, even though the mean amount at harvest was higher in C than in LR treatment: C had 2.76 g L^{-1}, LR 2.11 g $L^{-1}$and LRCS 2.38 g L^{-1}.

A significant year x treatment interaction was observed at veraison, when the LR treatment had a significant effect in 2010 and not in 2011 (Table 21). As regards the MA content at harvest, a significant effect of the year was observed; when MA concentration was higher in 2011 than 2010, despite the fact that harvest was carried out ten days before 2010 (Table 9).

Tartaric acid (TA) did not show statistical differences between treatments apart at veraison both in 2010 and in 2011. In fact TA was higher at veraison in 2010 in LR (15.3 g L^{-1}) and LRCS (17.0 g L^{-1}) than in C (13.3 g L^{-1}) (Table 8). 2011 results confirmed the strong effect of leaf removal treatments (C 13.6 g L^{-1}, LR 15.8 g L^{-1} and LRCS 16.8 g L^{-1}) on this parameter (Table 9). Differences were observed at harvest only in 2011 when TA was lower in C (3.76 g L^{-1}) and LR (4.83 g L^{-1}) than LRCS (7.15 g L^{-1}).

At harvest, a significant Year x Treatment interaction was observed for TA concentration (*p value* 0.018).

			Malic Acid						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p value***
2010	181	26	9.98b	1.06	8.02a	0.97	6.95a	0.02	**0.002**
	193	38	18.92	1.77	20.97	4.55	23.75	5.12	0.342
	202	47	30.42b	4.24	23.41a	0.75	23.27a	1.91	**0.011**
	216	61	16.12	2.27	22.41	4.39	22.31	3.05	**0.041**
	229	74	6.46	0.77	6.58	1.23	6.21	0.53	0.853
	242	87	3.96	0.79	3.11	0.30	4.25	0.67	0.150
2011	181	44	18.91	1.18	21.47	3.21	21.74	1.18	0.182
	192	55	24.07	0.97	26.06	0.64	28.52	3.56	0.065
	201	64	18.48	4.52	16.57	2.41	15.38	1.92	0.499
	214	77	7.23	0.62	6.31	0.52	7.09	0.69	0.233
	230	93	2.76	0.37	2.11	0.08	2.38	0.28	**0.079**

			Tartaric Acid						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p-value***
2010	181	26	17.23	1.34	18.01	1.17	17.25	0.24	0.124
	193	38	16.31	2.59	18.25	3.14	19.41	3.01	0.255
	202	47	13.13a	2.13	15.27b	1.84	17.05b	0.68	**0.007**
	216	61	10.08	1.81	8.91	0.78	10.21	1.40	0.476
	229	74	5.71	1.04	5.82	1.00	5.79	2.03	0.994
	242	87	5.98	1.15	7.76	1.12	6.95	0.71	0.166
2011	181	44	18.13a	1.12	22.05b	2.51	22.31b	2.30	**0.014**
	192	55	13.57a	1.25	15.81ab	1.23	16.80b	2.37	**0.039**
	201	64	12.61	2.49	12.23	0.23	13.79	1.42	0.453
	214	77	6.42	0.63	7.59	1.41	8.71	1.75	0.074
	230	93	3.76a	0.42	4.83a	1.33	7.15b	0.52	**0.005**

Table 8: grape organic acid content malic and tartaric (g L^{-1}) in 2010 and 2011 seasons determined in samples subjected to different treatments. P-value indicates significance level for the different treatments; different letters indicate different means.

					p value		
Variable	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Veirason							
Malic	25.7	4.3	26.2	2.7	0.898	0.854	**0.001**
Tartaric	15.0	2.3	15.4	2.1	0.420	**0.014**	0.779
Harvest							
Malic	3.8	0.8	2.4	0.4	**0.036**	0.226	0.244
Tartaric	6.9	1.2	5.25	1.67	0.225	0.335	**0.018**

Table 9: grape organic acid content, malic and tartaric (g L^{-1}) analyzed by year; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.

3.1.3 Yield variables

Yield variables were not affected by imposed treatments but for mean cluster weight in 2010.

As reported in Table 11 year effect was clearly decisive to control number of clusters per vine and yield. Yield per vine was 2.3 kg vine in 2011, lower than in 2010, 3.3 kg vine. Cluster per vine were also consistently lower in 2011 than in 2010, 18.7 and 22.5 respectively.

In 2010, mean cluster weight of C was 18g and 28g statistically lower than LR and LRCS. In the same year yield per vine was not significant different among treatments, although yield per vine of C was 0.2 kg and 0.5 kg lower than LR and LRCS respectively (Table 10).

Berry weight recorded for each berry sampling (Table 12) showed statistical differences in 2010 at the 5^{th} sampling (61DAA) in which LRCS was the lowest (1.07g/berry). In 2011, berry weight at the sampling before harvest was lower in LRCS (1.46g/berry) than in C (1.64g/berry), while in LR (1.57) It was not statistically different from C and LRCS. At harvest in both years berry weight was not different among treatments. In 2010 berry weight was higher at harvest (1,77g/berry) than in 2011 (1,57 g/berry). At veraison there was no difference between years.

		treatments						
Year	**Variable**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p value***
2010	Cluster weight (g)	132.9a	15.6	150.7b	7.5	160.0b	4.8	**0.023**
	Clusters per vine (n)	23.3	2.3	22.0	2.3	22.3	1.6	0.750
	Yield per vine (kg)	3.1	0.3	3.3	0.2	3.6	0.2	0.131
2011	Cluster weight (g)	121.9	4.5	121.3	24.7	146.3	28.4	0.369
	Clusters per vine (n)	20.2	1.6	17.3	1.5	16.9	2.7	0.142
	Yield per vine (kg)	2.5	0.2	2.1	0.4	2.4	0.2	0.127

Table 10: mean cluster weight (g), cluster per vine and yield per vine (kg)collected on 2010 and 2011 harvest. P-value indicates significance level for the different treatments; different letters indicate different means.

					p value		
Variable	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Cluster weight (g)	147.9	15.1	129.8	23.3	0.088	0.126	0.575
Clusters per vine (n)	22.5	2.0	18.1	2.4	**0.024**	0.189	0.568
Yield per vine (kg)	3.3	0.3	2.3	0.3	**0.034**	0.524	0.088

Table 11: mean cluster weight (g), cluster per vine and yield per vine (kg) analyzed by year; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.

			Treatments						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p value***
2010	174	19	0.27	0.02	0.29	0.02	0.26	0.02	0.345
	181	26	0.53	0.04	0.52	0.02	0.48	0.04	0.311
	193	38	0.82	0.05	0.78	0.06	0.76	0.06	0.474
	202	47	0.89	0.05	0.89	0.03	0.85	0.05	0.496
	216	61	1.19b	0.08	1.16b	0.07	1.07a	0.09	**0.039**
	229	74	1.55	0.12	1.51	0.07	1.47	0.13	0.631
	242	87	1.81	0.12	1.75	0.04	1.74	0.08	0.559
2011	181	44	0.78	0.05	0.84	0.04	0.83	0.03	0.063
	192	55	0.88	0.01	0.89	0.03	0.87	0.06	0.791
	201	64	1.07	0.09	1.17	0.07	1.13	0.13	0.363
	214	77	1.64b	0.12	1.57ab	0.10	1.46a	0.05	**0.026**
	230	93	1.56	0.05	1.62	0.20	1.51	0.11	0.666

Table 12: mean berry weight recorded for each berry sampling during the trial; P-value indicates significance level for the different treatments; different letters indicate different means.

					p value		
Berry weight	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Veraison	0.88	0.04	0.88	0.04	0.852	0.089	0.894
Harvest	1.77	0.08	1.57	0.13	**0.030**	0.459	0.587

Table 13: mean berry weight at veraison and harvest analyzed by year; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.

3.1.4 Bunch diseases

Botrytis and sour rot were affected by the imposed treatments, when diseases were promoted by climate. Indeed, in 2010, a season characterized by several consecutive rainfalls during fruit ripening and harvest period, LR and LRCS reduced significantly the incidence and severity of rots (Table 24). The interaction year x treatment was highly significance ($p<0.001$) for *Botrytis* severity in particular, because of seasonal meteorological events are a decisive factor for grape rot development and leaf removal create unfavourable environment for rots. In 2010 *Botrytis* incidence was 74% in C and 44% and 51% in LR and LRCS respectively, significantly lower than C (Figure 25).

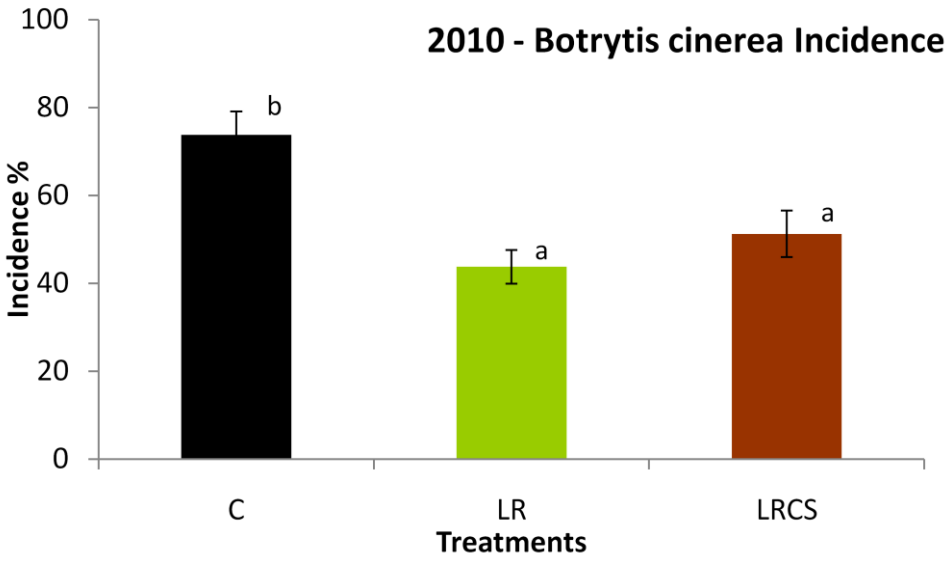


Figure 25: *Botrytis cinerea* Incidence (%) at harvest in 2010: LR (44%) and LRCS (51%) reduced consistently rot incidence than C (74%)

Although high incidence was recorded also in LR and LRCS, severity showed the high power of LR to reduce disease attacks. In fact, *Botrytis* severity showed very high statistical differences between treatments with nonessential bunch damages in LR and LRCS treatments: C was 25%, LR 5% and LRCS 6% (Figure 26).

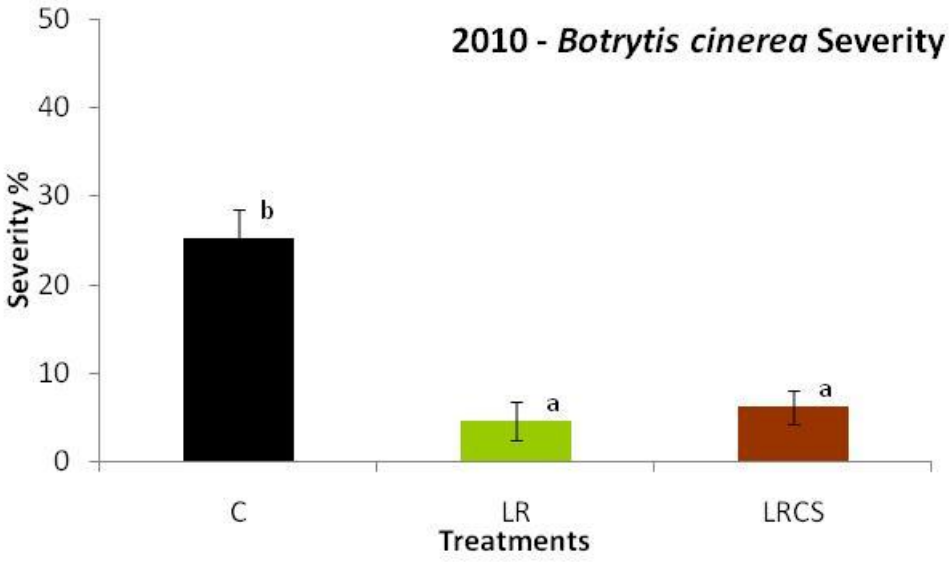


Figure 26: *Botrytis cinerea* severity (%) at harvest in 2010: LR (5%) and LRCS (6%) reduced consistently rot severity than C (25%)

In C 75% of counted bunches with an overall damage of 25%was affected by *Botrytis*, while in LR and LRCS, respectively 44% and 51% of counted bunches with an overall damage of 5% and 6% was affected by *Botrytis*.

In 2011 no effects of LR and LRCS on *Botrytis* were found: incidence was similar in C than in LR and LRCS, 39%, 28% and 33% respectively and no statistical differences were achieved. In 2011 *Botrytis* severity was very low (around 4%) in all treatments, so no important yield loss was recorded.

Bunch sour rot reflected *Botrytis cinerea* development in both 2010 and 2011, but with very narrow incidence and severity: in 2010 very low incidence (5%) was recorded in C while in LRC and LRCS the disease was not present. Severity was so weak in all theses that no consistent treatment effects could be assessed.

In 2010 no sunburn occurred, on the contrary it was recorded in 2011,very hot season during grape ripening (Table 24). Although burn severity was very low, incidence was more extended in LR (48%) than C (9.3%). LRCS maintained the incidence below 17% (Table 24), affecting positively cluster protection (Figure 27).

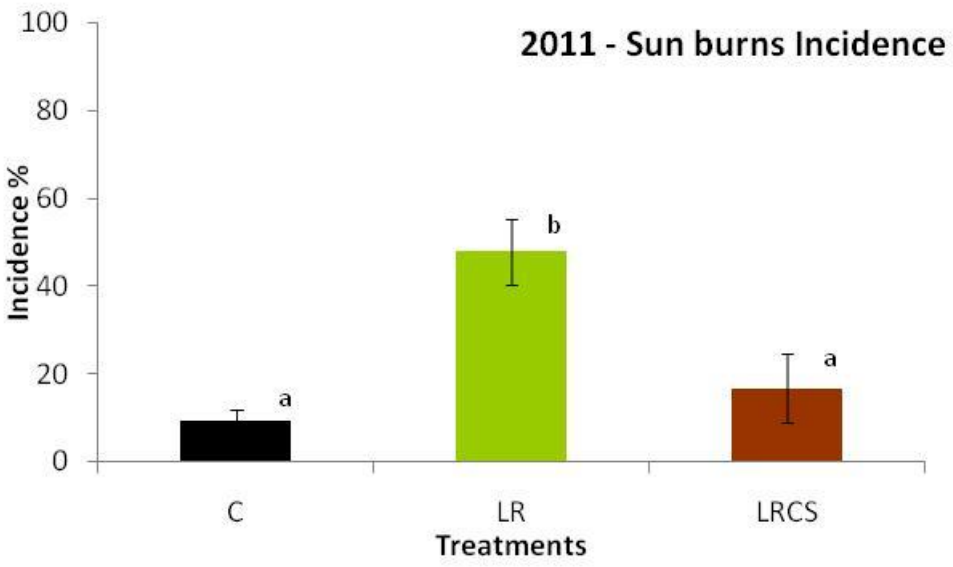


Figure 27: *Sun burns* Incidence (%) at harvest in 2011: LR (48%) and LRCS (17%) reduced consistently rot incidence than C (9%)

As sunburns did not develop in 2010 and treatments induced sunburns only in 2011, the interaction year x treatment was significant.

		Incidence						
Year	**Variable**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p value***
2010	Botrytis bunch rot	73.7a	10.8	43.7b	7.7	51.2b	10.6	**0.029**
	Sour rot	5.5a	2.9	0.0b	0.0	0.3b	0.5	**0.005**
	Sunburn	0.0	0.0	0.0	0.0	0.0	0.0	-
2011	Botrytis bunch rot	38.8	17.6	27.7	15.5	32.6	21.3	0.755
	Sour rot	8.3	9.2	5.6	4.1	2.0	4.0	0.483
	Sunburn	9.3a	5.6	47.9b	14.7	16.8a	15.8	**0.028**

		Severity						
Year	**Variable**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p value***
2010	Botrytis bunch rot	25.2b	3.2	4.6a	2.2	6.1a	1.9	**0.000**
	Sour rot	0.7	0.6	0.0	0.0	0.0	0.0	**0.051**
	Sunburn	0.0	0.0	0.0	0.0	0.0	0.0	-
2011	Botrytis bunch rot	6.3	4.1	2.2	1.2	1.4	0.7	0.088
	Sour rot	0.7	0.8	0.1	0.1	0.2	0.3	0.348
	Sunburn	0.4	0.2	3.6	2.8	0.8	1.0	0.109

Table 14: main grape disease incidence and severity collected on 2010 and 2011 harvest. P-value indicates significance level for the different treatments; different letters indicate different means.

	Incidence						
Variable	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Botrytis bunch rot	56.3	16.0	33.0	17.2	**0.059**	0.192	0.436
Sour rot	1.9	3.1	5.3	6.3	0.099	0.100	0.722
Sunburn	0.0	0.0	24.7	21.0	0.173	0.500	**0.003**

	Severity						
Variable	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Botrytis bunch rot	12.0	10.0	3.3	3.2	0.234	0.288	**0.000**
Sour rot	0.2	0.5	0.3	0.5	0.184	**0.014**	0.936
Sunburn	0.0	0.0	1.58	2.15	0.254	0.500	**0.051**

Table 15: main grape disease incidence and severity analyzed by year; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.

3.1.5 Leaf area estimation

Leaf area showed clear differences in the determination on July, 5th, but lower area and not different between C and LR on August, 10th (Figure 28).

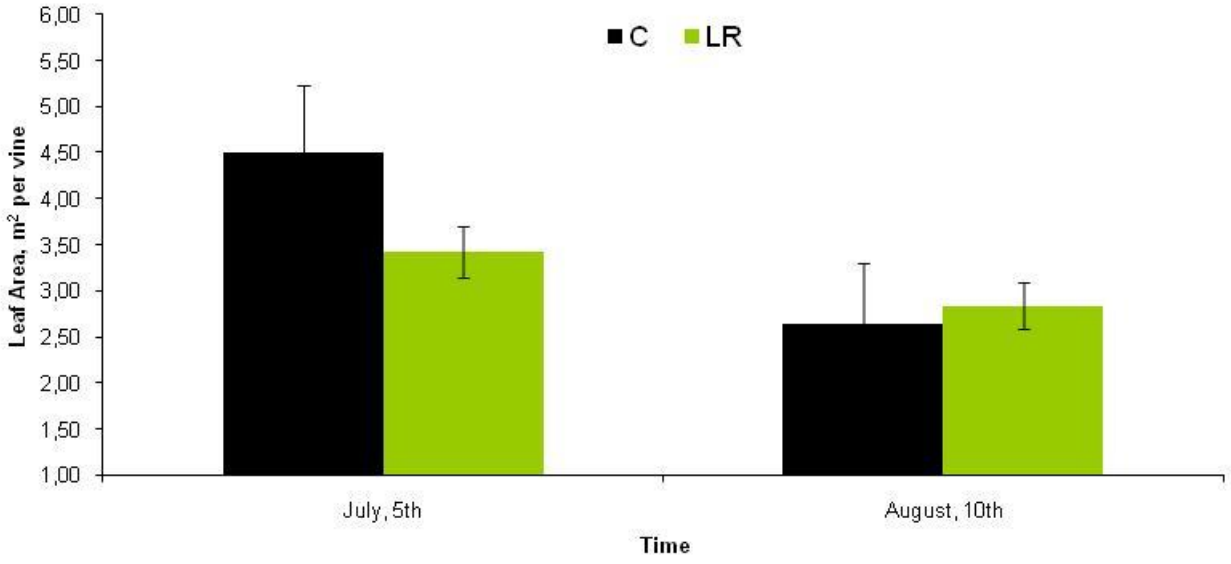


Figure 28: leaf area per vine estimated in July and August 2011; on July, 5th leaf area of LR is statistically lower then C, ($p<0.05$); on August, 10th leaf area doesn't different depending on the two treatment and it is lower then in July.

The effect of the LR treatment is put in evidence by the leaf area estimated in July. Since the regression calculated between the main leaf number and the respective area of LR was not plenty satisfactory (R^2 0.41), differences between treatments were not high at $p<0.01$.

Probably LR leaf area of main leaves was slightly overestimated, due to pour R^2 with a slope curve higher than C. This increase of variability was introduced probably by the treatment: removing leaves from basal to the 5th leaf above the grapes, leaves different for number and dimensions could be caught specially on sprouts growth on vine head trunk.

Nevertheless this measure distinguished leaf areas between C and LR, close the treatment time.

As reported in Figure 28 leaf area was not different between C and LR in august, 45 days after the leaf removal treatment. Vines could have

produced more laterals as consequence of the treatment, growing the leaf area to the untreated vines. Therefore both the areas were lower in the second date survey. It might be due to the topping practice performed by the farm in the early August.

3.1.6 Effect of LR treatments on Methoxypyrazines

MPs were affected by treatments only at veraison in both years. At harvest MPs were below limit of detection (LD=0.6ng L-1). IPMP did not show any difference between treatments in any year. However, the results were well correlated to IBMP concentration (Pearson's r, 0.644 $p<0.01$ in 2010 and 0.698 $p<0.01$ in 2011), highest IPMP in C than in LR and LRCS.

IBMP concentration was affected only at veraison (Table 16). In 2010, LR and LRCS had a remarkably lower IBMP concentration than C. Differences were statistically significant at $p<0.05$. In 2010, no differences were revealed between LR and LRCS, meaning that cluster shading net had no influence on IBMP content, despite affecting light and temperature on the fruiting zone (Figure 28). In 2011, effects of LR on IBMP confirmed significance results ($p<0.001$): LR recorded lowest IB concentration, about 50% of C, while LRCS had about 61% of C, same relationship obtained in 2010(Figure 28). As in 2010, also in 2011, IBMP content was similar in LR and LRCS. C and LRCS IBMP contents were much higher in 2011 than in 2010, while LR showed the same concentration in both years, interaction between year and treatments was stressed by generalized mixed model statistical analysis ($p<0.07$).

Comparing treatment effect in sampling time during ripening in 2010 (DAA = 61), differences were put in evidence by ANOVA ($p=0.058$) but not by mean comparing by Duncan's test. This was due to the high standard deviation in C results were not statistically confirmed. Therefore C hadthe highest IBMP concentration (7.6ng L-1) if compared with LR (2.4ng L-1) and LRCS (3.6ng L-1). In 2011, IBMP at a similar ripening stage was lower and the comparison among treatments did not show statistical differences. At harvest, C, LR and LRSC IBMP concentrations were always lower than the limit of quantification (LQ<2.0ng L-1). Comparing 2010 and 2011, a more rapid degradation process of MP in 2011 than in 2010 could be observed (Table 16)(Figure 28).

			IBMP						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p value***
2010	174	19	3.5	0.9	4.0	1.1	3.8	0.5	0.469
	181	26	37.0	2.4	28.0	6.2	22.7	1.9	0.185
	202	47	24.5b	3.6	15.6a	2.4	14.6a	4.0	**0.015**
	216	61	7.6	4.4	2.4	0.5	3.6	0.7	**0.058**
	229	74	2.8	0.8	3.2	0.5	3.4	1.5	0.347
	242	87	<LD	-	<LD	-	<LD	-	-
2011	181	44	57.3	8.8	48.9	3.1	55.9	2.2	0.288
	192	55	36.7b	4.8	18.5a	2.3	22.8a	3.1	**0.000**
	201	64	5.1	2.3	3.5	0.7	3.6	0.1	0.120
	214	77	3.0	1.7	1.1	0.4	1.1	0.4	**0.085**
	230	93	<LD	-	<LD	-	<LD	-	-

			IPMP						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**LR**	**s.d.**	**LRCS**	**s.d.**	***p value***
2010	174	19	<LD	-	3.6	0.6	<LD	-	
	181	26	27.3b	1.6	15.6a	4.9	12.6a	0.6	**0.032**
	202	47	3.1	0.6	2.7	0.3	2.7	0.5	0.166
	216	61	2.1	0.2	1.6	0.2	2.0	0.4	0.139
	229	74	<LD	-	<LD	-	<LD	-	-
	242	87	<LD	-	<LD	-	<LD	-	-
2011	181	44	28.3	1.1	23.4	1.3	29.8	0.1	**0.063**
	192	55	4.4	0.7	3.6	0.3	3.8	0.3	0.114
	201	64	<LD	-	<LD	-	<LD	-	-
	214	77	<LD	-	<LD	-	<LD	-	-
	230	93	<LD	-	<LD	-	<LD	-	-

Table 16: IBMP and IPMP in 2010 and 2011 different samplings. P-value indicates significance level for the different treatments; different letters indicate different means;<LD means that all samples were analysed but MPs values were below the Limit of Detection.

					p value		
Variable	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
IBMP	18.3	5.6	26.0	8.7	0.101	0.090	**0.062**
IPMP	2.9	0.5	4.0	0.5	**0.011**	0.083	0.689

Table 17: IBMP and IPMP analyzed by year at veraison; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.

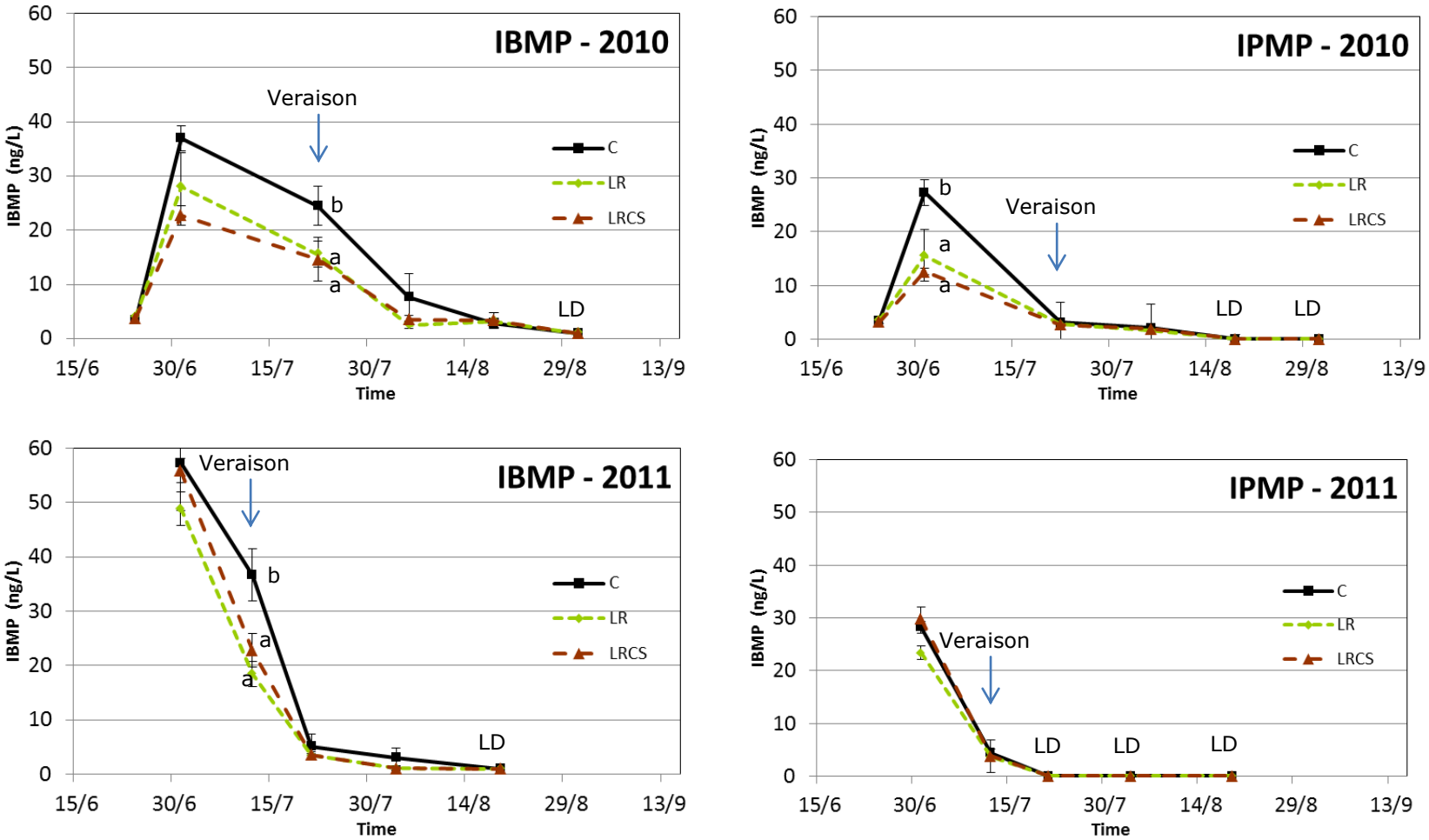


Figure 29: IBMP and IPMP concentrations during 2010 and 2011 growing seasons; the error bars reflect standard deviation of four biological replicates; different letters indicate different means according to Duncan's test; LD means that all samples were analysed but MPs values were below the Limit of Detection of the method.

3.2 Discussion and Conclusions

According to the most recent work on LR related to MP in red varieties (Koch et al, 2012; Scheiner et al. 2010, Ryona et al. 2008, Ryona et al., 2009), the same progression of MPs during ripening also in *Sauvignon blanc was* described. MPs concentration both IBMP and IPMP decreased from veraison to harvest. Unfortunately, all sampling concentration at harvest were below the limit of detection (LD<0.6ng L-1) or below the limit of quantification (LQ<2.0ng L-1), put in evidence the difficulty to obtain *Sauvignon blanc* with vegetal notes in Friuli Venezia Giulia Region. Other authors, (Šuklje et al. 2012) working on the same variety in South Africa, found the same lack of MPs in *Sauvignon blanc* at harvest. Šuklje et al 2012 described that *Sauvignon blanc* berries with more than 20.2 °Brix showed MPs below LD. In our study in 2011, harvest was performed when SS were over 20.2°Brix and no MPs were detected. In 2010 at harvest SS were lower than 18.5°Brix in all treatments and also MPs were no detected. As reported by Koch et al., 2012 IBMP concentration at harvest is affected by light and temperature environment before and not during ripening. So that, year and bunch light exposure play a crucial role in determining MPs concentration at harvest only during a limited part of the season.

IPMP was weakly affected by treatments, being in very low concentration already at veraison (2.7-4.4 ng L^{-1}). Differently, Šuklje et al. 2012 at veraison IPMP concentration was already below the LD (LD<0.6 ng L^{-1}) in LR application, while in C results were similar to ours.

At veraison, strong differences were found among treatments for IBMP: LR affected IBMP concentration at veraison in both years, reducing MP of about 50 and 60% than C, control not defoliated. In 2010 LRCS, leaf removal with cluster 50% solar radiation shading net application, showed the same results of LR. No statistical differences were obtained in 2011 at veraison comparing LR and LRCS, even if the latter treatment had 4.2 ng L^{-1} more IBMP than the former. On the other hand, shading net strongly

moderated temperature and solar radiation than grape exposed direct to sunlight in LR. Nonetheless these implications, shading net did not preserve MP from degradation. In order to emphasize the shading effect 100% solar radiation shading net could be used, so that only diffused light could reach clusters.

According to Koch et al., 2012 that demonstrated that light intensity before ripening affects IBMP, our study showed that leaf removal treatment carried out between berry set and earlier than veraison reduced largely IBMP concentration at veraison and until IBMP were detectable with applied analytical method. Similar results were obtained by Scheiner et al. 2010, Ryona et al. 2008 that recorded the same outcomes for red varieties Cabernet Sauvignon and Merlot, even if in this works a consistent amount of MP were determined also at harvest because of variety expression.

In the presented case of study, 2010 and 2011 have been two years deeply dissimilar in North East Italy and Friuli Venezia Giulia Region. 2010 was characterized by low mean temperatures during ripening but high between the end of June and the beginning of July during berry set and berry development. Moreover, rainfalls have been importnat in the same period and in the rest part of the season. On the contrary, 2011 showed temperatures with a particularly hot in August mean 25.1°C, with several consecutive days of peak max air temperature between 36°C and 38°C during grape ripening. Rainfalls were much lower in 2011 than 2010 during ripening. Nonetheless, incoming solar radiation was higher in June2010 than in June 2011, 675 MJ/m^2 and 650MJ/m^2 respectively. On the contrary, in August during ripening incoming solar radiation was 614 MJ/m^2 in 2010 and 715 MJ/m^2 in 2011.

This huge season difference induced different berry ripening: SS analysed at 2010 harvest were 18.0°Brix compared to 20.5°Brix in2011. Also TA was affected by year: conversely of SS, 2010 was higher than 2011. At veraison the same ripening differences were not found, indeed both SS and TA were slightly higher in 2011 than in 2010. Concerning IBMP, It’s

interesting to notice that there was a consistent difference between 2010 and 2011 at veraison: 2011, the hottest and driest year during ripening, recorded higher IBMP concentration, 26.0ng L^{-1} against 18.3ng L^{-1} in 2010. As demonstrated by Koch et al., 2012, also in this study, light and temperature environment before ripening seemed affect IBMP in the final stages. Indeed, 2010 showed higher solar radiation in June and part of July than 2011. This aspect could have affected the IBMP concentration at veraison, even though there was a delaying veraison set in 2010 than in 2011.

Actually, in 2011 MPs degradation was faster than in 2010, since comparing the same ripening stage between veraison and harvest, 2011 had less IBMP than 2010.

No correlation between Malic acid and MPs were found in this study, comparing data at veraison, even if in 2010 LR and LRCS decreased consistently Malic acid concentration. Tartaric acid was found higher in LR and LRCS treatments both at 2010 and 2011 veraison. At harvest only LRCS was consistently higher than C.

In 2010 *Botrytis* bunch rot affected heavily C if compared to LR and LRCS and mean cluster weight was consistently reduced in C than in LR and in LRCS.

As reported by several authors (Duncan et al. 1995, Tardaguila et al. 2010) LR affected positively grape healthy: rots incidence and severity in 2010 were consistent reduced in the same way by LR and LRCS treatments than the C. The loss of production in C could be attributed to *Botritys* incidence that was recorded in the same plots, since inverse correlation was found between Botrytis incidence and mean cluster weight. LRCS had the same power of LR to contrast bunch rot infections. Positive effects on rot limitation in LR treatments were confirmed by other studies like Duncan et al. 1995, Tardaguila et al. 2010, Cravero e Rabino, 2005 on several varieties and different environmental condition. Conversely LR in 2011 enhanced sunburn incidence, while LRCS didn't

show statistical differences if compared to C. LRCS was useful to reduce rots and to protect grapes against negative effects of sunlight.

Other studies (Bledsoe et al., 1998) confirmed that LR imposed after berry set doesn't significantly affect yield, cluster per vine, cluster weight and berry weight independently by LR intensity. Instead, It's clarified that LR imposed before anthesis affects yield variables like cluster weight (Poni et al, 2008).

Sauvignon blanc is a very important international variety, widely cultivated in cool climate regions. From the time when climate change is bringing to increase temperature and decrease rainfalls, aromatic or semi-aromatic grape varieties could be penalized. Aroma complexity of Sauvignon is well accepted by markets and vegetal notes in wines coming from Friuli Venezia Giulia are difficult to be obtained particularly during the last years. So, conservative strategies to protect this kind aroma could be a goal to produce complete and high quality Sauvignon wines. So that, shading net could be used as action strategy to reduce rots, to protect clusters against sunburn and hail but not at 50% solar radiation filtering as a conservative tool for vegetal grape aromas.

4 Effect of Nitrogen Nutrition on Methoxypyrazines Sauvignon blanc expression

4.1Results

4.1.1 WEOC, WEN and $N\text{-}NO_3^-$ as affected by treatments in soil

4.1.1.1 WEOC -Water extractable organic carbon

During the three-year experiment, WEOC in the soil increased after application of both AS and DB (Figure 30). In particular in 2010 and 2011 DB plots had higher WEOC than AS and C, in samples collected after fertilizer application. Highest WEOC were determined in samples collected in early summer when temperature and water disposal accelerate soil biological cycles. In the end of the season, after harvest, there was a residual effect of treatment on WEOC especially in 2010 and 2011, when WEOC in fertilized plots was 10-20 mg/Kg higher than in C ($p<0.05$).
Seasonal effect on WEOC showed also consistent differences ($p<0.01$). 2010 reported highest values, in particular between June and August.

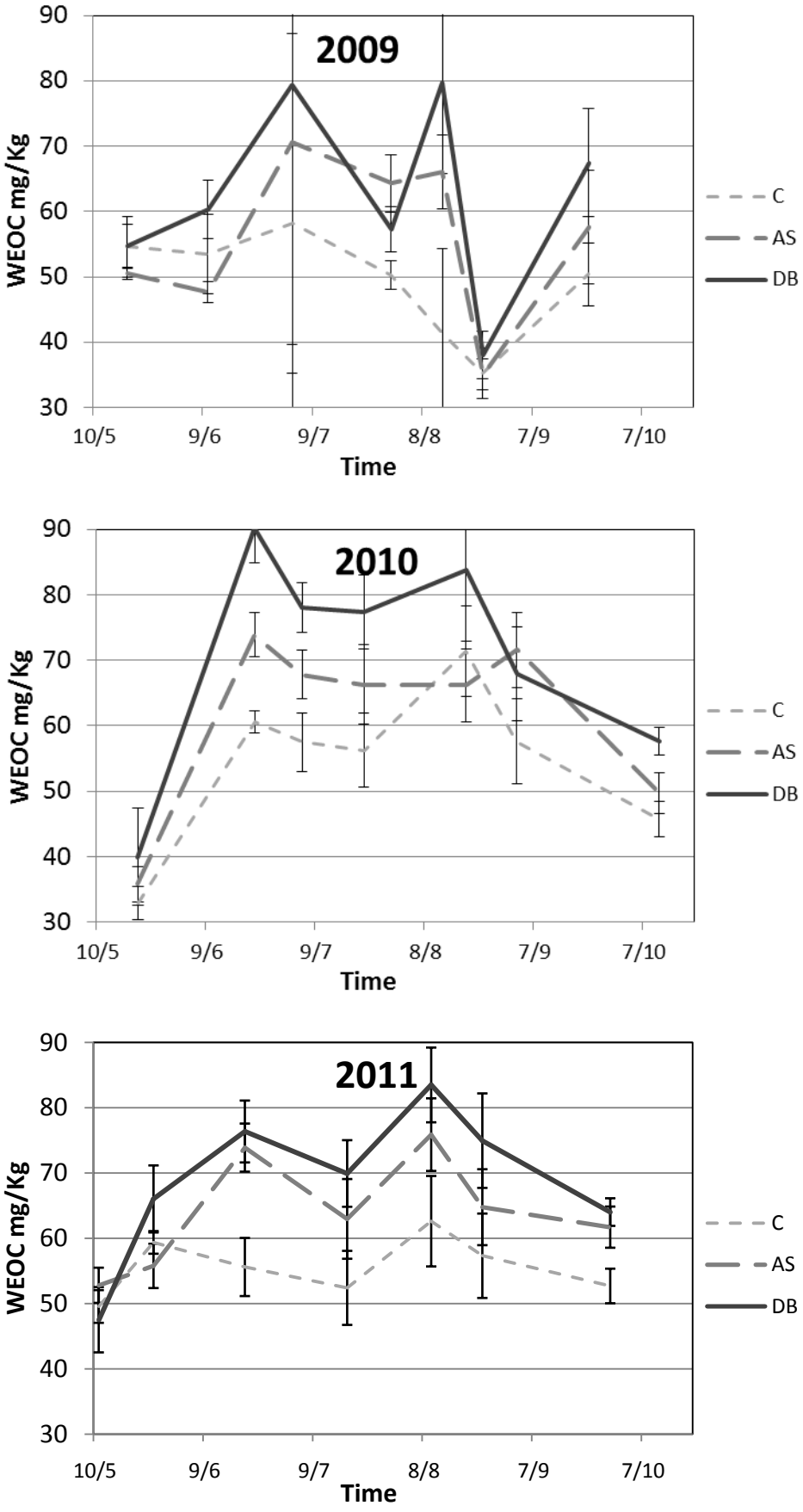


Figure 30: WEOC, mg/kg measured in the soil of the trial.

4.1.1.2 WEN - Water extractable nitrogen

Over three-year field experiment WEN was strongly affected by treatments in the two sample time after fertilization (Figure 31). In 2009 AS had the highest content with 200 and 180 mg/Kg, followed by DB that was stable on 140 mg/Kg. On the contrary in 2010 DB showed the highest peak after the spread, 106 mg/Kg. Finally, in 2011 AS reached 159 mg/Kg and after that It fell to C level. In contrast, DB maintained the same WEN both in the first and second sampling date after application (Figure 31).

Since N in its mineral form is the most soluble and movable element in the soil, It is strongly affected by flows of the soil water system. So that, a certain portion of soluble nitrogen could be lost (Reid et al 1969). Furthermore nitrogen cycle is affected also by soil physical and chemical characteristics (Zhang Rui and Wienhold, 2002). Because of high rainfall variability, the effect of treatments on soil, WEN was quite different between years.

Nevertheless, It is important to stress that vine N-uptake was highest from two-weeks before bloom until veraison and form harvest until leaf fall (Peackok et al. 1989; Schreiner et al., 2006).

In this way, by WEN analyses we described that N was at maximum in vine root zone between bloom and berry growing.

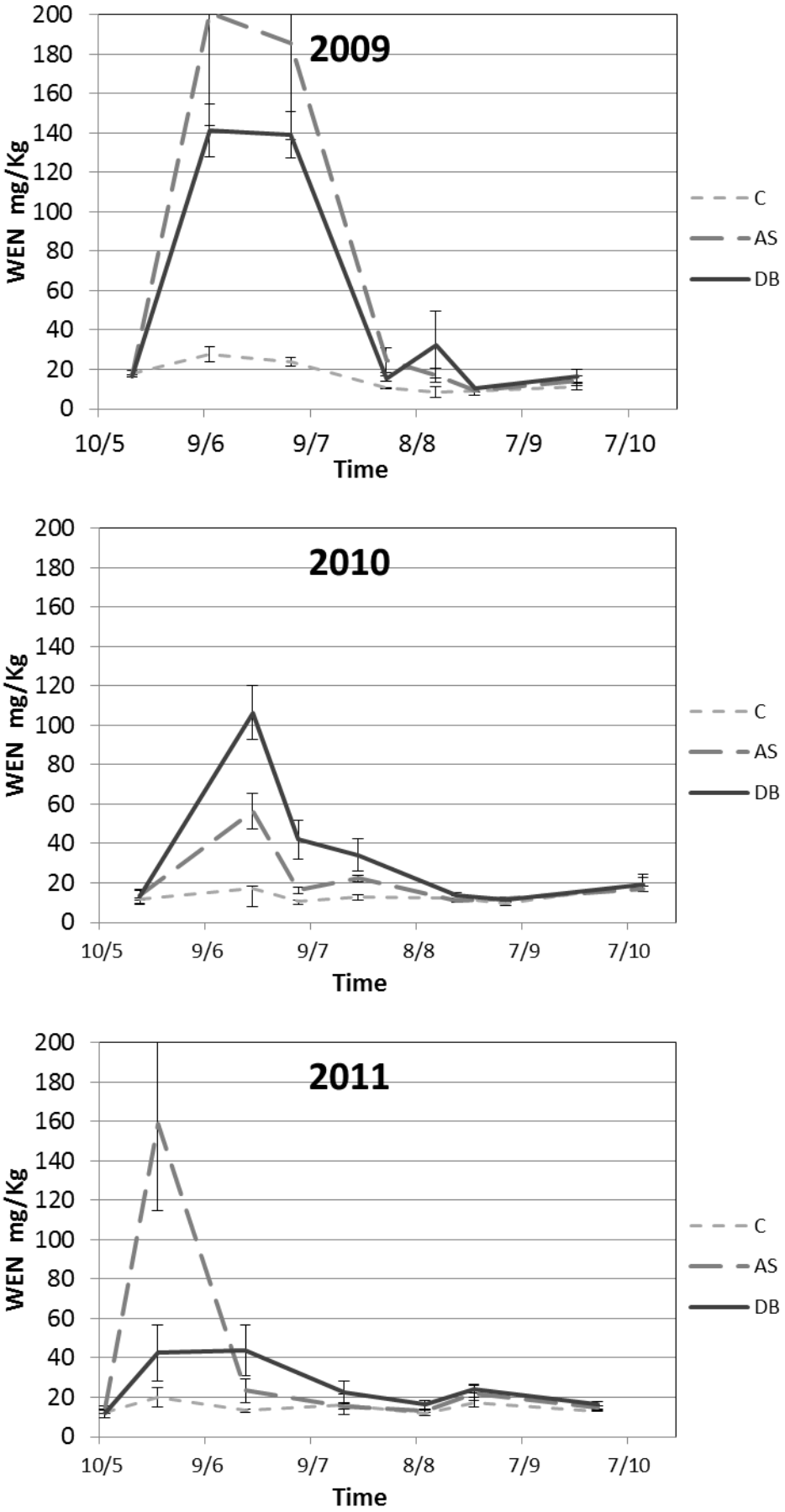


Figure 31: WEN, mg/kg measured in the soil of the trial.

4.1.1.3 N-NO_3^- Nitric Nitrogen

N-NO_3^- followed the same trend of WEN: rapid increase immediately after application and then depletion with N-NO_3^- concentration like C (Figure 32). After harvest of all years, supplementary ammonium sulphate has been spread with lower rate than spring fertilization, about 15 kgN/ha. The effect in soil was put in evidence by soft peaks in the last part of the season of each year defined by Figure 32.

In 2009 N-NO_3^- of AS was half time lower than WEN during mineralization process. On the contrary DB showed the same values of WEN. In 2010, N-NO_3^- was similar to WEN, in both fertilization treatments stressing the fact that in this year N mineralization occurred mainly in the first month after fertilization. In 2011 N-NO_3^- was an the whole lower than the former years, but as in 2009 N-$NO_3^-$of AS was half time lower than WEN and DB had the same values of WEN, not over 40mgN/kg during the mineralization process.

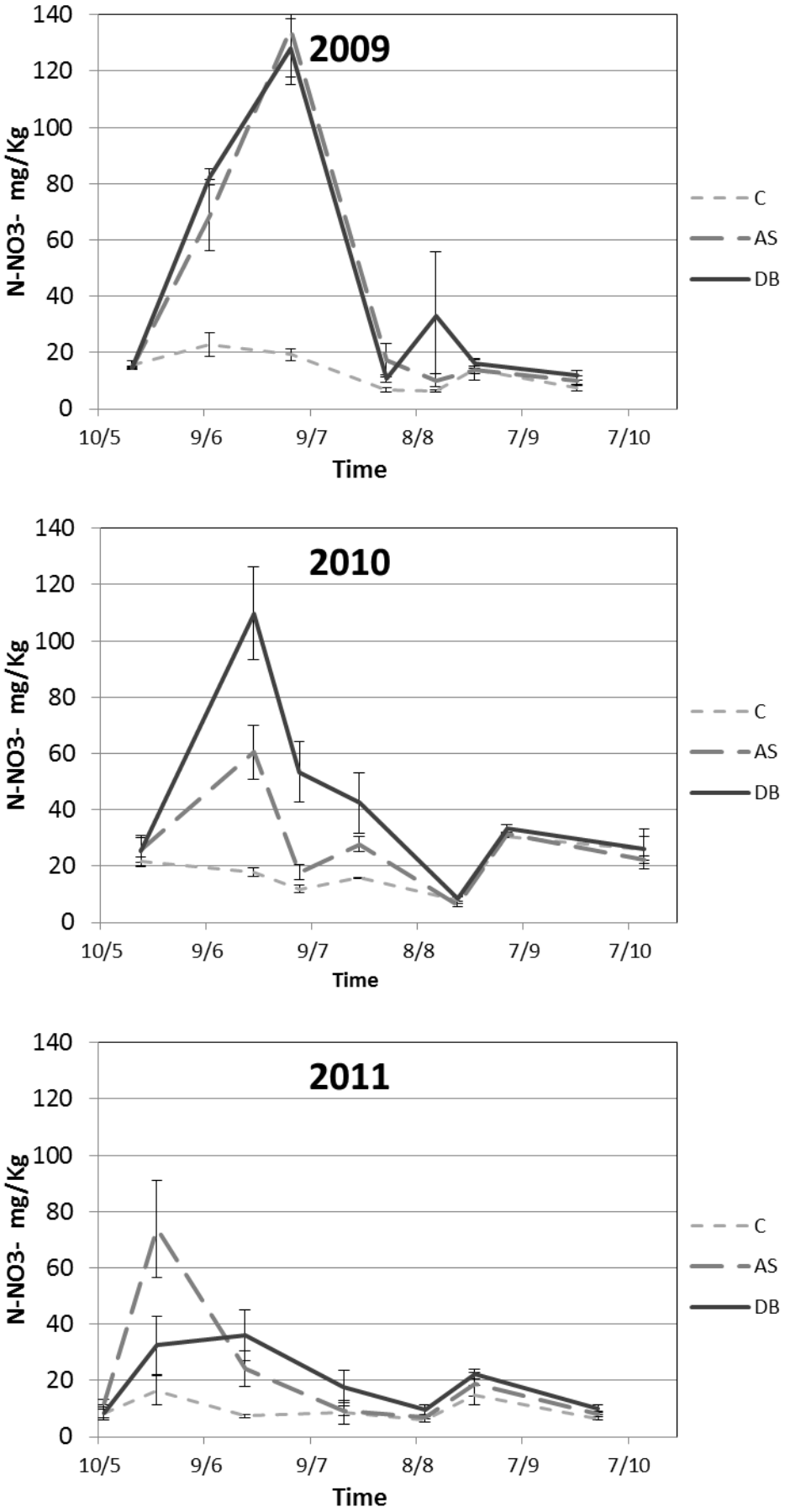


Figure 32: N-NO3-, mg/kg measured in the soil of the trial.

4.1.2 Grape ripening variables

4.1.2.1 Fruit composition

Must ripening parameters soluble solids (SS) (°Brix), pH, titratable acidity (TA) (tartaric acid g/L) showed statistical differences only for some sampling dates.

In 2009, SS were higher in samples collected after veraison in C plots if compared to AS and DB. Sampling points after veraison showed already different maturation level between treatments, AS and DB reduced ripening. At harvest, AS and DB had respectively 0.7 °Brix and 1.8 °Brix less than C (Table 18).

In 2010, differences between treatments were not as clear as in 2009. At DAA 61 on the contrary AS had more SS than DB and C. After this sampling, even though not significant, SS were lower in DB and AS than in C (Table 18).

Also in 2011, only in two sampling dates statistical differences were determined when treatments means were compared. In 2011, although differences were not statistically supported, C showed higher SS (21.3 °Brix) than AS (19.6°Brix) and DB (21.0°Brix). At sampling point after veraison (DAA 64), differences were statistically significant, showing C with higher SS (16.1°Brix) than AS and DB (14°Brix).

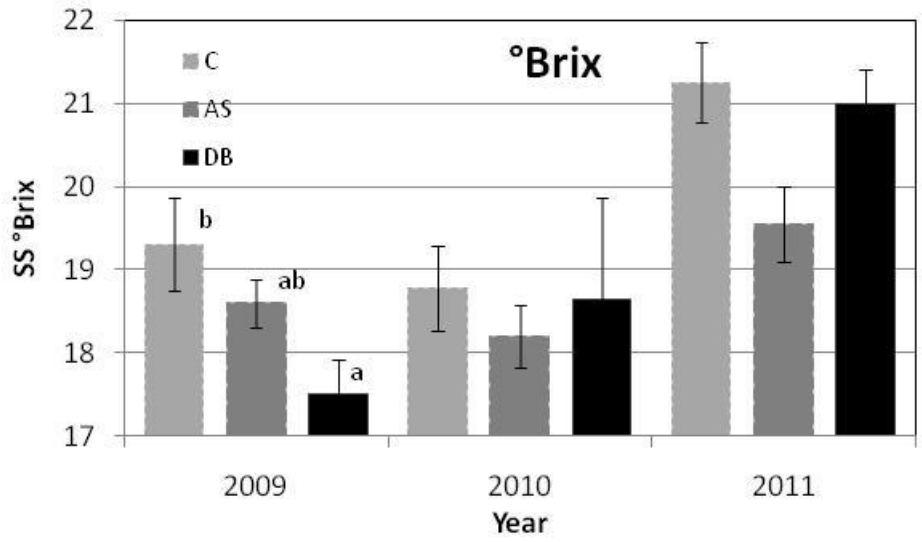


Figure 33: SS (°Brix) at harvest in the trial years: only at harvest 2009 differences were consistent between treatments, DB recorded the lowest concentration.

pH was not affected by imposed treatments (Figure 34). During the three years of investigation no difference evidences were found. It is interesting to notice that pH at harvest 2009 was significative higher than in 2010 and in 2011 (Table 19).

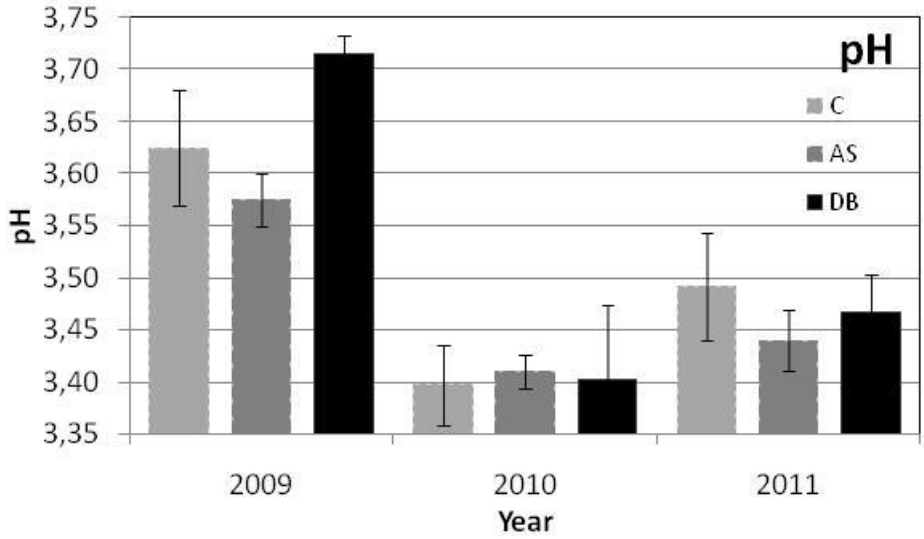


Figure 34: pH at harvest in the trial years: no consistent effects of treatments were determined at harvest on pH.

Also TA did not show clear differences between treatments in any trial year. Notwithstanding, apart 2010 harvest, when TA was lower in AS and DB than in C, in 2009 and 2011 AS and DB had higher TA than C, according to treatments effects on SS (Figure 35).

All the three analysed ripening parameters were clearly affected by year. Year effect was high significance: 2011 favoured grape ripening increasing SS and cutting TA at harvest time (Table 19).

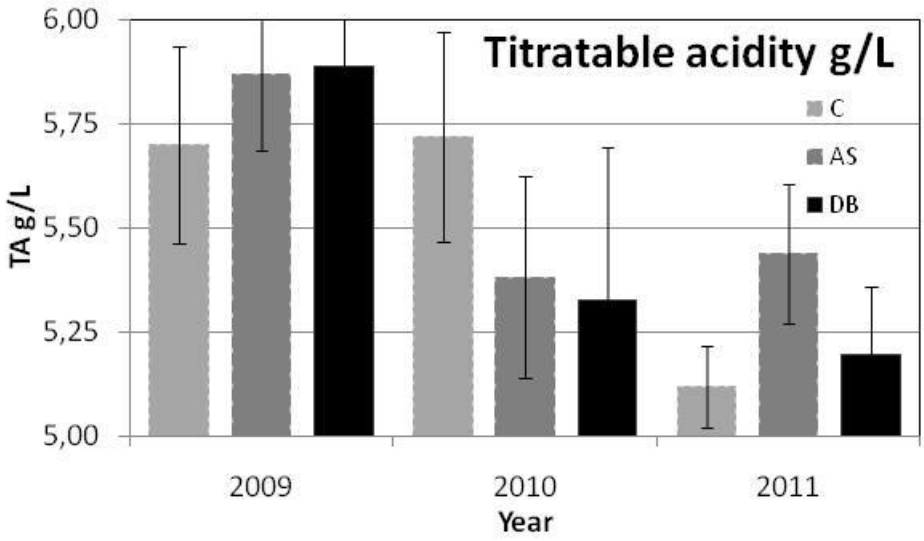


Figure 35: Titratable acidity at harvest in the trial years: no consistent effects of treatments were determined at harvest on TA

In 2009 and 2010 same SS (18.5°Brix) were recorded at harvest, while in 2011 highest SS were determined (20.6°Brix). PH was different among years: in 2009, particular high pH (3.60) was measured, in 2010 the lowest (3.40) and in 2011 (3.47). TA was inversely correlated to SS among years at harvest. In 2009, highest TA were recorded (5.8 g/L) despite pH were very high. in 2010 TA was not very different (5.6 g/L) and in 2011, conversely to SS, TA was the lowest (5.3 g/L).

			°Brix						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***
2009	207	64	10,0	0,8	10,5	0,0	10,3	0,9	0,657
	221	78	15.9b	0,8	15.7b	0,9	13.5a	0,9	**0,024**
	229	86	18.3b	0,7	18.2b	0,1	17.0a	0,7	**0,011**
	233	90	19.3b	1,1	18.6ab	0,6	17.5a	0,8	**0,082**
2010	174	19	3,5	0,2	3,6	0,1	3,6	0,1	**0,443**
	181	26	3.4b	0,1	3.4b	0,1	3.2a	0,0	**0,060**
	193	38	3,8	0,0	4,0	0,1	3,9	0,1	**0,143**
	202	47	4,1	0,1	4,3	0,3	4,2	0,2	0,411
	216	61	10.2ab	0,2	10.8b	0,5	10.0a	0,4	**0,058**
	229	74	14,5	0,6	13,8	0,8	13,5	0,9	0,157
	242	87	18,8	1,0	18,2	0,7	18,7	2,4	0,816
2011	181	44	3.8b	0,1	3.6a	0,1	3.7ab	0,2	**0,011**
	192	55	5,7	0,3	5,7	0,2	5,6	0,3	0,406
	201	64	11.9b	0,6	10.5a	0,5	10.3a	0,5	**0,023**
	214	77	16,1	1,2	14,0	1,5	14,0	2,1	0,244
	230	93	21,3	1,0	19,6	0,9	21,0	0,8	0,114

			Titratable acidity (g/L)						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***
2009	207	64	23,2	2,4	23,2	1,6	22,0	1,6	0,362
	221	78	9,8	0,7	9,4	0,4	9,7	0,8	0,744
	229	86	6.41a	0,2	7.42b	0,5	7.21b	0,2	**0,005**
	233	90	5,7	0,5	5,9	0,4	5,9	0,3	0,684
2010	174	19	18,5	0,4	18,9	0,1	18,9	0,3	0,244
	181	26	23,6	0,1	23,4	0,3	23,6	0,6	0,787
	193	38	28,7	0,2	28,2	0,9	28,1	0,6	0,103
	202	47	30,5	0,4	29,7	0,8	30,2	0,7	0,380
	216	61	20,1	0,6	20,5	0,8	20,9	1,8	0,551
	229	74	8,7	0,2	6,6	4,2	8,3	0,4	0,474
	242	87	5,7	0,5	5,4	0,5	5,3	0,7	0,639
2011	181	44	29.9b	0,6	27.2a	0,9	29.8b	0,5	**0,004**
	192	55	36.4b	1,3	34.3a	0,5	33.3a	0,3	**0,009**
	201	64	18,8	0,5	19,1	0,9	19,7	2,2	0,612
	214	77	10,8	0,3	10,3	0,7	10,1	1,5	0,659
	230	93	5,1	0,2	5,4	0,3	5,2	0,3	0,441

			pH						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***
2009	207	64	2,78	0,02	2,80	0,02	2,75	0,04	0,096
	221	78	3,15	0,08	3,21	0,02	3,21	0,04	0,354
	229	86	3,41	0,07	3,46	0,02	3,49	0,05	0,133
	233	90	3,63	0,11	3,58	0,05	3,72	0,04	0,096
2010	174	19	2.52a	0,01	2.56b	0,01	2.56b	0,00	**0,004**
	181	26	2.57a	0,02	2.60ab	0,01	2.64b	0,04	**0,030**
	193	38	2,65	0,04	2,64	0,03	2,66	0,04	0,184
	202	47	2,57	0,04	2,59	0,03	2,59	0,02	0,422
	216	61	2,77	0,04	2,80	0,01	2,78	0,07	0,805
	229	74	3,10	0,05	3,11	0,05	3,14	0,03	0,389
	242	87	3,40	0,08	3,41	0,03	3,40	0,14	0,982
2011	181	44	2,48	0,03	2,54	0,03	2,53	0,03	0,108
	192	55	2,52	0,01	2,56	0,02	2,51	0,04	0,087
	201	64	2,78	0,01	2,76	0,03	2,76	0,02	0,534
	214	77	2,91	0,03	2,93	0,05	2,96	0,08	0,564
	230	93	3,49	0,10	3,44	0,06	3,47	0,07	0,500

Table 18: grape ripening variables soluble solid content (°Brix), pH and titratable acidity (g tartaric acid/L) in 2009, 2010 and 2011 seasons determined in samples subjected to different treatments. P-value indicates significance level for the different treatments; different letters indicate different means.

							p value		
Variable	**2009**	**s.d**	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Veirason									
Brix°	10.3	0.7	4.2	0.2	5.7	0.3	**0.000**	0.352	0.731
pH	2.78	0.00	2.59	0.07	2.53	0.04	**0.000**	0.172	0.169
Titratable acidity	22.8	1.8	30.1	0.7	34.7	1.5	**0.000**	0.187	0.116
Harvest									
Brix°	18.5	1.1	18.5	1.4	20.6	1.1	**0.033**	0.306	0.196
pH	3.64	0.22	3.40	0.09	3.47	0.08	**0.006**	0.394	0.449
Titratable acidity	5.8	0.4	5.5	0.6	5.3	0.3	**0.057**	0.849	0.505

Table 19: grape ripening variables soluble solid content (°Brix), pH and Titratable acidity (g tartaric acid/L) analyzed by year; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.

4.1.2.2 Organic acids: Malic and tartaric

As reported for TA, also malic and tartaric acids didn't show clear differences between treatments (Table 20). Although not significatively different, in 2011 both malic acid and tartaric were higher in AS and DB than in C.

Year affected malic acid at harvest. Malic acid in grapes was affected by temperatures during ripening (Table 21). In fact, 2011 had lowest malic acid concentration at harvest (2.4 g/L). 2009 was the year that recorded the highest Malic acid concentration at harvest (4.2 g/L) while in 2010 malic acid was 3.6 g/L.

Tartaric acid was not affected by treatments: its concentration decreased from veraison to harvest in the same way for all the imposed treatments. Year also did not affected tartaric acid concentration at harvest: its concentration was 7.6, 6.7 and 7.7 g/L, in 2009, 2010 and 2011 respectively.

			Malic Acid							Tartaric Acid						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***
2009	207	64	18.0	2.0	19.4	1.4	20.7	2.1	0.217	9.67b	1.2	9.47b	0.4	5.75a	1.5	**0.009**
	221	78	7.1	0.6	6.8	0.4	6.7	0.5	0.575	7.6	0.6	7.8	0.2	8.0	0.8	0.662
	229	86	4.8	0.5	5.0	0.7	5.3	0.6	0.353	6.6	1.8	9.0	1.0	8.1	0.5	0.123
	233	90	4.1	0.7	4.1	0.3	4.4	0.6	0.669	7.3	0.6	7.9	0.5	7.8	0.3	0.329
2010	174	19	8.1b	0.5	-	-	6.5a	0.4	**0.013**	22.6b	1.3	-	-	18.6a	0.4	**0.004**
	181	26	9.0a	1.2	10.0ab	1.1	10.9b	0.6	**0.027**	17.0	0.6	17.2	1.3	18.9	1.5	0.095
	193	38	17.1	6.9	18.9	1.8	19.0	2.4	0.804	14.5	3.8	16.3	2.6	16.3	4.1	0.583
	202	47	26.8	1.4	30.4	4.2	26.7	2.4	0.211	15.6	3.5	13.1	2.1	13.4	1.5	0.327
	216	61	18.9	2.5	16.1	2.3	19.4	2.2	0.237	9.6	1.5	10.1	1.8	9.2	1.2	0.709
	229	74	6.5	0.2	6.5	0.8	6.7	1.0	0.848	5.1	1.2	5.7	1.0	6.1	1.1	0.406
	242	87	3.4	0.4	4.0	0.8	3.4	0.5	0.430	7.2	1.1	6.0	1.2	7.0	0.9	0.371
2011	181	44	21.3	1.2	20.0	2.5	19.6	0.5	0.485	20.8b	1.3	18.2a	1.5	19.3ab	1.0	**0.096**
	192	55	26.1	0.7	26.6	1.8	28.0	3.4	0.277	16.2	2.4	17.9	1.9	14.8	1.6	0.129
	201	64	12.6	1.3	13.9	0.9	15.7	3.3	0.141	13.8	1.9	14.4	0.2	13.4	0.6	0.458
	214	77	6.6	0.6	5.7	0.8	6.2	1.2	0.472	10.5	1.1	8.4	1.7	10.0	1.6	0.226
	230	93	2.2	0.3	2.4	0.6	2.4	0.3	0.488	7.4	0.7	8.0	0.9	7.7	0.5	0.358

Table 20: grape organic acid content malic and tartaric (g/L) in 2009, 2010 and 2011 seasons determined in samples subjected to different treatments. P-value indicates significance level for the different treatments; different letters indicate different means.

							p value		
Variable	**2009**	**s.d**	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Veirason									
Malic	19.4	2.0	28.0	3.2	26.9	2.2	**0.004**	0.376	0.164
Tartaric	8.3	2.1	14.0	2.5	16.3	2.2	**0.004**	0.149	0.109
Harvest									
Malic	4.2	0.55	3.6	0.60	2.4	0.39	**0.002**	0.410	0.515
Tartaric	7.6	0.5	6.7	1.1	7.7	0.7	0.168	0.878	0.168

Table 21: grape organic acid content content malic and tartaric (g/L) analyzed by year; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.

4.1.3 Yield

Yield variables were slight affected by imposed treatments (Table 22). Cluster weight and cluster number per vine were not consistently different between treatments in any trial year. Only in 2010, yield per vine was statistically higher in DB (3.54 kg) than C (2.98 kg) and AS (3.04 kg). Indeed, in 2010 there were no limiting conditions for vine growing. Conversely, in 2011 yield was constrained by water scarcity, even if sprinkler irrigation was performed as needed. Cluster weight was consistently different between years: highest in 2010 with 138g and lowest in 2011 with 127g. Cluster weight was also affected by treatments, according to the means of the three seasons (Table 23).

Significance interaction was found between year and treatment according to yield per vine, because of in 2009 differences between treatments were casual, in 2010 there was a consistent increasing of yield with nitrogen application and in 2011, even if differences were not statistical based, nitrogen nutrition trials had ever higher yield than C.

Year effect was clearly decisive to control number of clusters per vine and yield. 2011 showed fewer yields than 2010 both for fertility and mean cluster weight.

		treatments						
Year	**Variable**	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***
2009	Cluster weight (g)	132.7	13.3	128.2	3.5	139.2	11.2	0.347
	Clusters per vine (n)	20.7	1.0	19.5	2.5	22.7	3.4	0.245
	Yield per vine (kg)	2.75	0.36	2.50	0.35	3.14	0.42	0.110
2010	Cluster weight (g)	137.0	4.5	132.9	15.6	142.9	15.8	0.619
	Clusters per vine (n)	21.3	1.7	23.3	2.3	24.9	1.5	0.142
	Yield per vine (kg)	2.92a	0.21	3.08a	0.33	3.54b	0.22	**0.014**
2011	Cluster weight (g)	125.3	18.3	127.5	11.0	129.1	6.4	0.910
	Clusters per vine (n)	18.2	0.5	21.1	3.8	18.9	1.3	0.264
	Yield per vine (kg)	2.28	0.33	2.66	0.27	2.43	0.06	0.244

Table 22: yield parameters collected in 2009, 2010 and 2011. P-value indicates significance level for the different treatments; different letters indicate different means.

							p value		
Variable	**2009**	**s.d**	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Cluster weight (g)	133	10	138	13	127	12	**0.023**	**0.058**	0.920
Clusters per vine (n)	21.0	2.6	23.2	2.3	19.4	2.5	0.094	0.348	0.141
Yield per vine (kg)	2.8	0.4	3.2	0.4	2.5	0.3	0.246	0.246	**0.048**

Table 23: yield parameters analysed by year; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.

4.1.4 Bunch diseases

2009 and 2011 seasons have not led to rot development during grape ripening.

Sunburns were not affected by the imposed treatments.

Year was the predominant effect affecting disease development (Table 25). In 2009 climate conditions did not lead to *Botrytis cinerea* bunch rot development: incidence was lower than 15% in all treatments, while severity was about 2% with no differences between treatments.

Sour rot showed the same incidence and severity of *Botritys cinerea* with no differences between imposed treatments.

Sunburns were negligible in 2009 both for incidence and severity (Table 24).

In 2010, rots were widespread in the trial vineyard, but only weak statistical differences between treatments were determined for sour rot. Sour rot incidence was higher in AS (5.5%) and DB (2.3%) than C (0.8%), however with nonessential severity (Table 24). *Botrytis cinerea* incidence and severity (respectively about 71% and 23%)were very high in all plots but with no differences between not fertilized and nitrogen applied plots. On the contrary, sunburns were not occurred in 2010 in all plots.

In 2011 *Botrytis cinerea* bunch rot was recorded with average incidence (about 40% in the vineyards) expanded only a couple of days before harvest. Severity was not important, about 6%. No differences between treatments were assessed. Concerning sun burn in 2011 they were more spread (incidence 10%) than other trial years but with negligible severity (1%) and no differences between treatments.

All bunch diseases, like bunch stem necrosis, berry shrivel and Black rot were checked for at harvest but no presence was recorded.

		Incidence							Severity						
Year	**Variable**	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***
	Botrytis bunch rot	15	9	9	4	10	4	0.550	3	2	2	1	1	1	0.372
2009	Sour rot	13	6	18	8	17	4	0.815	3	2	4	2	4	2	0.960
	Sun burn	0	0	4	7	4	5	0.732	0	0	1	1	1	2	0.777
	Botrytis bunch rot	68	9	74	11	68	6	0.602	20	7	25	3	21	5	0.519
2010	Sour rot	0.8a	2	5.5b	3	2.3ab	2	**0.043**	0.065	0	0.685	1	0.24	0	**0.095**
	Sun burn	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Botrytis bunch rot	38	21	51	12	29	2	0.263	5	5	7	2	5	2	0.606
2011	Sour rot	12	9	12	7	3	4	0.112	1	2	1	1	0	0	0.154
	Sun burn	11	4	9	6	9	8	0.882	1	0	1	1	0	0	0.639

Table 24: main grape disease incidence and severity collected in 2009, 2010 and 2011. P-value indicates significance level for the different treatments; different letters indicate different means.

	Incidence									Severity								
Variable	**2009**	**s.d**	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**	**2009**	**s.d**	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Botrytis bunch rot	11	6	70	8	39	16	**0.001**	0.344	0.251	2	2	22	5	6	3	**0.000**	0.280	0.635
Sour rot	16.1	5.8	2.8	2.8	9.0	7.7	**0.014**	0.268	0.265	3.6	2.1	0.3	0.4	0.9	1.1	**0.002**	0.467	0.680
Sun burn	2.73	4.84	0	0	9.25	5.55	**0.047**	0.951	0.656	0.67	1.18	0	0	0.50	0.38	0.588	0.771	0.446

Table 25: main grape disease incidence and severity analyzed by year; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.

4.1.5 Vigour variables

TN, analysed at harvest of each year (Table 26), showed differences between treatments in every year but only in 2011 it was statistically higher in DB (674 mg/L) and AS (557 mg/L) than C (462 mg/L) (Figure 36), according to soil nitrogen rate application. Nevertheless, there is a consistent interaction between TN in grape and nitrogen fertilization, demonstrated by grand mean high statistical differences (p= *0.011*) if per year and per treatment data were compared: AS and DB had 18% and 34% more TN concentration than C (Table 27). The year effect was also put in evidence (p = *0.018*): 2010 was particularly low, 25% smaller than 2009 and 2011. This difference coincided with highest yield recorded in the same year.

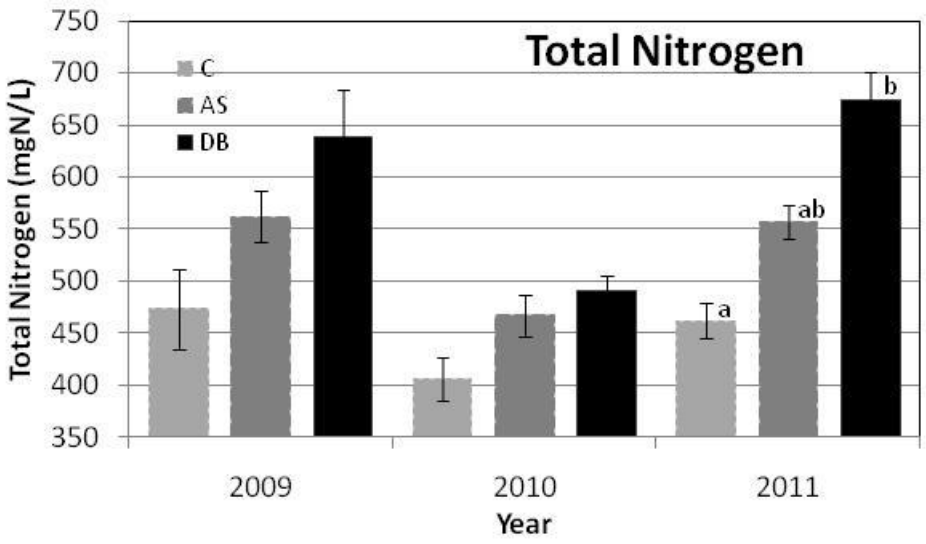


Figure 36: TN (Must Total Nitrogen) measured at harvest in each trial year. Statistical differences between treatments were achieved only in 2011, when DB was higher than C and AS; while in 2009 and 2010 no consistent differences were found

Normalized difference vegetation index (NDVI) at harvest showed statistical differences between treatments already in 2009 and in 2010 (Figure 37), when DB was higher than C in both years and AS only in 2010 (Table 26).

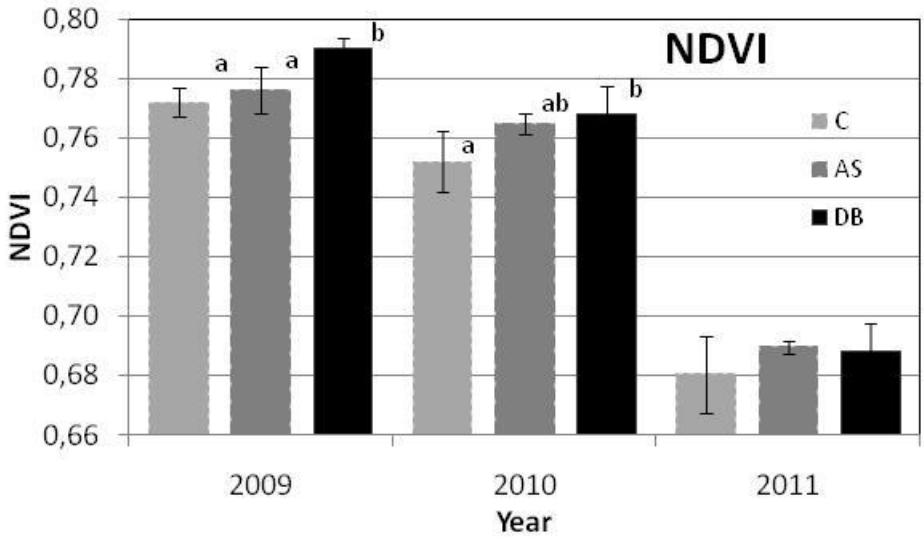


Figure 37: NDVI (Normalized Difference Vegetation Index) collected at harvest in each trial year. Statistical differences between treatments were achieved in 2009 and in 2010, when DB was higher than C and AS; while in 2011no consistent differences were found.

In 2011, NDVI was statistically smaller than the former trial years (Table 27). NDVI less than 0.70 appeared very low for Sauvignon blanc NDVI means of the area (data not showed), a variety gifted with medium/high vigour. Lack of treatment effect and low NDVI in 2011 were probably connected to severe water stress, affecting only youngest vines in the plots at harvest. We were not able to split within-vine variability to put in evidence treatment effect also in 2011.

		treatments						
Year	**Variable**	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***
2009	TN	473	129	562	82	639	151	0.112
	NDVI	0.772a	0.010	0.776a	0.016	0.790b	0.007	**0.025**
2010	TN	406	69	467	68	490	50	0.250
	NDVI	0.752a	0.020	0.765ab	0.007	0.768b	0.019	**0.059**
2011	TN	462a	56	557ab	55	674b	90	**0.024**
	NDVI	0.681	0.026	0.690	0.005	0.688	0.019	0.699

Table 26: Juice total nitrogen (TN) and NDVI collected at 2009, 2010 and 2011 harvest. P-value indicates significance level for the different treatments; different letters indicate different means.

							p value		
Variable	**2009**	**s.d**	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
Harvest									
TN	558	133	454	68	574	109	**0.018**	**0.011**	0.759
NDVI	0.780	0.000	0.762	0.000	0.686	0.000	**0.000**	**0.046**	0.550

Table 27: Juice total nitrogen (TN) and NDVI collected at 2009, 2010 and 2011 harvest analysed by year; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.

4.1.6 Effects of nitrogen nutrition on Methoxypyrazines concentration

IPMP didn't show any difference and was below detection limit (DL<0.6ng L^{-1}) already in early ripening stages.

IBMP were affected by treatments with a consistent explanation year by year (Table 28). Notwithstanding only at 2009 harvest a detectable amount was recorded (Figure 38).

MPs decrease from veraison to harvest in all varieties where vegetal aromas are synthesized. In our experiment MPs followed the same progression. We determined highest concentration at veraison and lowest or not detachable (<2 ng L^{-1}) at harvest.

In 2009, IBMP were still present in quietly high concentration 12 days before harvest, 8.5, 9.0 and 8.4 ng L^{-1}, in C, AS and DB respectively.

In 2010, the sampling time 25 days before harvest showed low MPs content (Figure 38), for IBMP 5.8, 7.6 and 6.9 ng L^{-1}, in C, AS and DB respectively. In 2011, samples collected at the intermediate ripening time (12-15 days before harvest) had already very low IBMP content, 1-3 ng L^{-1}, often above the nose detection limit in white wines (2 ng L^{-1}).

Considering IBMP at veraison, in 2009 any consistent difference between treatments was achieved. On the contrary, results showed highest content in C, 25.7 ng L^{-1}, than in AS and DB, 18.6 and 20.9 ng L^{-1} respectively. Since veraison 2010, treatment effects were going to appear: even if not supported by statistic, C was lower (20.3 ng L^{-1}) than fertilized thesis AS and DB (24.5 and 24.9 ng L^{-1}, respectively). In this sampling DB samples were affected by very high variability (s.d.=11.2), decreasing significance level.

In 2011, C (21.9 ng L^{-1}) was 30% statistically lower than AS (29.0 ng L^{-1}) and DB (29.2 ng L^{-1}) (Table 28), showing a strengthening of fertilization application cumulated through years (Figure 38).

At veraison, year effect was not put in evidence by statistics (Table 29): IBMP was not different among years. Anyway, IBMP increased from 2009

to 2011 because of the increased concentration year by year unfertilized plots. Furthermore 2011 showed the highest TN at harvest and it was the only year when statistical difference between treatments was found for this variable, following nitrogen application rate.

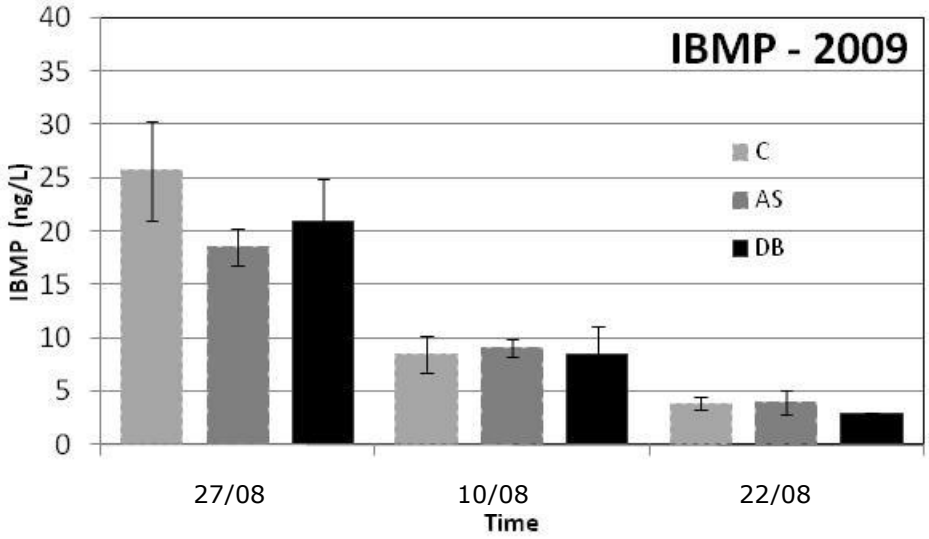
In addition year-treatment interaction effect was stressed by statistics (Table 29), demonstrating an accumulation of treatment effect year by year.

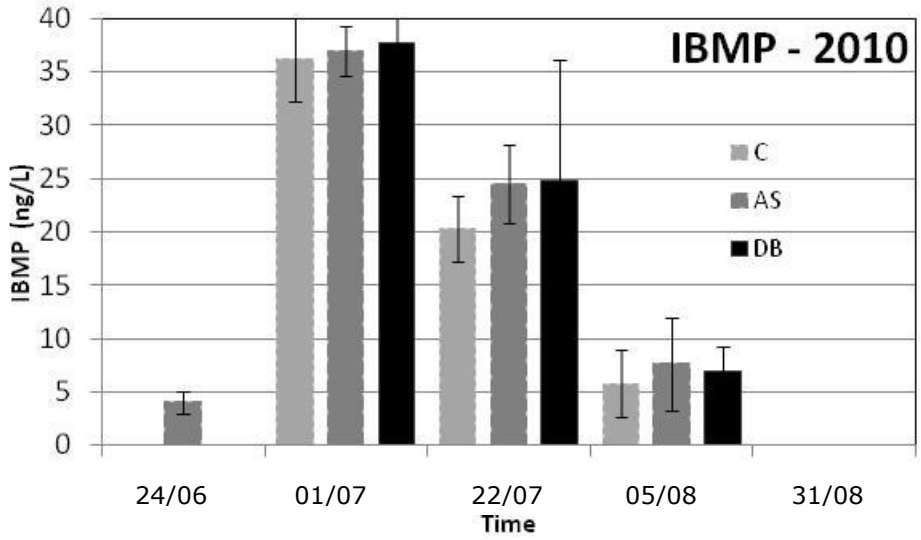
			IBMP							IPMP						
Year	**DOY**	**D.A.A.**	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***	**C**	**s.d.**	**AS**	**s.d.**	**DB**	**s.d.**	***p value***
	207	64	25.7	4.7	18.6	1.7	20.9	4.1	0.11	3.2	0.8	3.0	1.3	3.2	0.6	0.904
2009	221	78	8.5	1.7	9.0	0.8	8.4	2.7	0.233	<LD	-	<LD	-	<LD	-	-
	233	90	3.8	0.6	4.0	1.2	2.9	0.1	0.365	<LD	-	<LD	-	<LD	-	-
	174	19			4.0	1.0						3.6	0.6			
	181	26	36.3	3.9	37.0	2.4	37.7	2.6	0.298	27.3	2.5	27.3	1.6	26.5	1.4	0.748
2010	202	47	20.3	3.1	24.5	3.6	24.9	11.2	0.567	2.8	0.4	3.1	0.6	2.9	0.2	0.588
	216	61	5.8	3.2	7.6	4.4	6.9	2.3	0.201	1.9	0.3	2.1	0.2	1.9	0.4	0.726
	242	87	<LD	-	<LD	-	<LD	-	-	<LD	-	<LD	-	<LD	-	-
	192	55	21.9a	4.0	29.0b	1.2	29.2b	4.9	**0.073**	4.0	0.6	4.1	0.2	3.7	0.2	0.485
2011	201	64	3.1	1.1	4.0	0.8	5.2	2.3	0.238	0.7	0.2	0.7	0.2	0.7	0.2	0.419
	230	93	<LD	-	5.7	-	<LD	-	-	<LD	-	<LD	-	<LD	-	-

Table 28: IBMP and IPMP in 2009, 2010 and 2011. P-value indicates significance level for the different treatments; different letters indicate different means. <LD indicates that the concentration is below the limit of detection 0.6 ng/L

							p value		
Variable	**2009**	**s.d**	**2010**	**s.d**	**2011**	**s.d**	**Year**	**Treat**	**Year*Treat**
IBMP	21.7	4.6	23.2	6.7	26.4	4.8	0.438	0.817	**0.073**
IPMP	3.1	2.8	2.9	0.4	4.0	0.4	**0.004**	0.745	0.853

Table 29: IBMP and IPMP analyzed by year at veraison; year, treatment and year*treatment interaction effects are evaluated. P-value indicates significance level for the different treatments; different letters indicate different means.





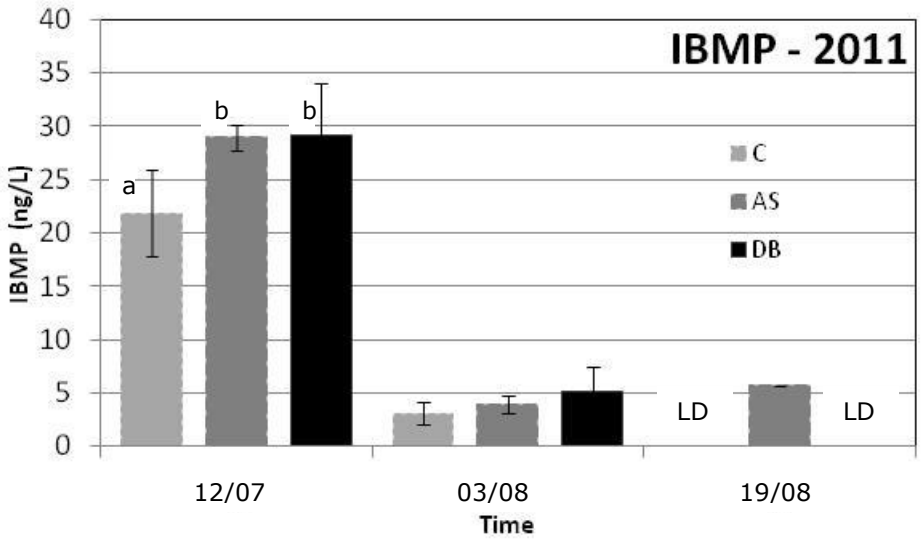


Figure 38: IBMP and IPMP concentrations during 2009, 2010 and 2011 growing seasons; the error bars reflect standard deviation of four biological replicates; different letters indicate different means according to Duncan's test; LD means that all samples were analysed but MPs values were below the Limit of Detection of the method (0.6 ng/L).

4.2 Discussion and Conclusion

Hilbert et al. 2003 demonstrated that in controlled experiments on Merlot high N supply delayed fruit ripening in the same year of application. In long term nitrogen supply trials on Cabernet Franc (Morlat R. and Chaussod R, 2008; Morlat, 2008; Morlat R. and Symoneaux, 2008) found ripening braking when organic fertilizers were used with high rate for several years.

In our study so clear outcomes cannot be put in evidence since not very high N application has been used, basal soil fertility and vine vigour were high and trials were limited to three year applications. Maybe longer time is needed to spread difference between no N supplied plots and agronomical rate nitrogen applications. Even though, evidences of ripening delay in nitrogen nutrition plots than in C not fertilized were slightly distinguished. In this case of study SS were statistical higher in C than in AS and DB in some samplings during ripening. Already at harvest 2009 C had 19.3°Brix, AS 18.6 and DB 17.5. Although in 2010 differences were not so clear, in 2011, fruit ripening slowed slightly down especially after veraison in fertilized plots. As attended TA was inversely correlated to SS in almost samples. In 2011 at harvest, grape ripening was consistently higher than 2010 and 2009. Always in 2011 at harvest, berry weight was consistently lower (1.67g) than in 2009 (1,91g) and in 2010 (1,80g). Evaluating berry enlargement during ripening among the three years, in 2011 from the sampling before harvest (DAA 77) berry of AS (1.65g) and DB (1.50g) did not get weight at harvest (1.67g and 1,61g respectively), while C increased berry weight from 1,54g to 1,73g. On the contrary in 2009 and in 2010 between in the same time, berry get 0,18g on average. So that in 2011, a concentration effect could have weakened SS differences between AS, DB and C at harvest. In 2011, in the last part of the season, few days before harvest water stress symptoms were recorded in the experimental plots, despite irrigation was normally applied. Since no stem leaf water potential measures were not performed

in each plots, we could not certainly correlate berry growth stop to water deficit in DB and AS. Indeed in 2011, NDVI also was not different among treatments at harvest, probably because of water stress. On the basis of this considerations, nitrogen nutrition effect on slight vigour enforcement could have led to higher water consumption in AS and DB plots than in C. AS and DB could have suffered before C of water stress and could have blocked before C berry weight increase and sugar loading.

Yield variables were slight affected by imposed treatments. Cluster weight and cluster per vine were not consistently different between treatments in any trial year. Only in 2010 per vine yield was statistically higher in DB than C and AS. Results could be consistent with Neilsen et al., 2010 that evidenced yield increase in merlot vine plots managed with mineral fertilizers spread at bloom if compared with budbreak spreading. Since vine N-uptake is at the most during bloom (Peackok et al. 1989; Schreiner et al., 2006), no fertilization at bloom (C treatment) could be compared with a budbreak spreading.

Three years of field trial evidenced the effect of soil nitrogen nutrition on IBMP secondary metabolite expression in Sauvignon blanc. As assessed by our LR trials, high MPs concentration was determined at veraison, but no MPs were found at harvest, except for 2009.

Šuklje et al. 2012 found that at Sauvignon veraison berry of the same diameter having SS difference of less 1°Brix showed huge differences in IBMP concentration of about 3 times more in less mature berries. Since in our study no differences were found between treatments at veraison 2010 and 2011 for SS, but strongly differences were determined in IBMP concentration particularly at the third year of application 2011, starting slightly since the second year 2010, a different process beyond the ripening delay effect of N supply could be occurred. In fact at the third year of application IBMP at veraison were 30% lower in C than in AS and DB. Also in 2010 same differences were evidenced but not so high to produce statistical significance. Supporting the idea of accumulation effect of nitrogen on IBMP after year, year*treatment effect (p=0.073) was

assessed by statistics. Only in 2011 TN was statistically higher in DB (674 mg/L) and AS (557 mg/L) than C (462 mg/L). AS was intermediate between C and DB, following N rate application. NDVI, correlated to different vigour parameters, showed increasing significant differences since 2009 to 2010 with highest values corresponding to highest N rate supply. In 2011, because of the water stress influence on young vine shortly before harvest, no differences were revealed.

Unfortunately, at harvest 2010 and 2011, where treatments were effective on aroma at veraison, IBMP concentration was below the limit of detection (LD<0.6 ng L^{-1}). So, certain results on the effects of nitrogen nutrition on MPs at harvest can not be described for *Sauvignon blanc*. Only in 2009 4 ng L-1 of IBMP were found at harvest, confirming that year played a crucial role to asses MPs in grape.

In 2011, causes of IBMP increasing in N supply plots than in not fertilized one could be associated with different reasons that have worked together: since MPs are heterocyclic nitrogen compounds, the increase of vine nitrogen availability in the vine encouraged MP biosynthesis, involved in amminoacid degradation pathways, and the delay of grape ripening due to N supply, according to MPs rapid degradation from veraison to harvest. The influence on vigour and subsequently to a more developed aerial part that increased cluster shading would be a remote hypothesis, considering that in our leaf removal trial, LR treatment until the 5^{th} leaf above clusters reduced of about 50% IBMP concentration at veraison and a small increased grape shading should not be so decisive.

Vine nitrogen status was affected by N supply in all years independently by nitrogen mineralization in soil, but only in 2011 TN was consistently higher in fertilized plots than in C, that could depend on accumulation effect of treatments through years.

Monitoring N forms in soil after fertilization in each plots showed different behaviour in each year concerning to mineralization processes. Although DB had an organic origin fast mineralization rate in soil were recorded. So, even if in 2011 seemed to be a lower effect on N soil availability of DB

than AS, no limitation have occurred in N uptake according to TN in juice. Furthermore, DB affected positively WEOC in soil, variable connected microbial biomass in soil (Gregorich et al. 1998), an important factor for soil biodiversity enrichment and soil cycles and soil fertility. Mineralization rate of DB was slightly lower than mineral fertilizer, buffering N leaching risk particularly in coarse soils. For this reasons, organic fertilizers, even if with rapid nitrogen mineralization rate, are to be preferred for agricultural purposes when soils are pour in physical characteristics. Since WEOC is connected to several soil processes such as denitrification (Burford and Bremner 1975), microbial respiration (Gregorich et al. 1998;) and N cycling (Murphy et al. 2000), significance soil WEOC enrichment could be due to the kind of applied fertilizer, organic DB and mineral AS, and the N-rate application that both influence the above soil processes. Similar results were obtained by other studies about nitrogen nutrition on other varieties like Riesling in cool climate, Cabernet Sauvignon and Merlot, where nitrogen nutrition increased must nitrogen according to N rate and time and affected consistently nitrogen mineralization in soil (Neilsen et al., 2010; Linsenmeier et al., 2008).

Long term nitrogen supply trials (Morlat R. and Chaussod R, 2008; Morlat, 2008; Morlat R. and Symoneaux, 2008) described negative effects of huge organic material vineyard fertilization on soil, grape and wine quality. In particular, the increasing of vigour through the years increased herbaceous odours of Cabernet franc. In our study in 2011, a moderate negative effect of nitrogen application has been associated to the increase of sensibility to water stress. Even though, high nitrogen fertilization is reputed to increase grape susceptibility to *Botrytis cinerea* and Rouma et al, 1998 demonstrated that high nitrogen fertilization predisposed grapevines to infection by *Botrytis cinerea* bunch rot and increased disease severity, in this study *Botrytis bunch* rot was not affected by nitrogen application. Only in 2010 slight negative effect was recorded for sour rot in AS and DB, but incidence (2-5% for supplied nitrogen plots) and severity (<1%) were not important from practical point of view.

As general result, a moderate multi strategy adapted to soil condition and environment should be adopted. A mean time fertilization strategy to implement vegetal note in Sauvignon planted in loamy soils in Friuli Venezia Giulia plain, could be use both organic matter not well decomposed and organic nitrogen provided by high N mineralization rate, increasing rate in spring applications.

Concerning to Friuli Venezia Giulia and recalling conclusions of leaf removal trail, *Sauvignon banc* needs to be growth in no limiting conditions of water and nutrient to express its whole complexity. MPs are researched in *Sauvignon banc* wines with effort by many winegrowers of the Region. Year effect has been recognized to be the most important in imparting vegetal notes to *Sauvignon banc*, but finding a strategy to stimulate synthesis and to conserve MPs until harvest could improve resultant wine quality. Knowing that N fertilization rate increases MP concentration and that during ripening MPs fall down below nose sensory threshold (2ng L^{-1} in white wines), N fertilization could be paired with a harvest in advance than that suggests by technological maturity parameter (14-16°Brix and 9-7,5 TA).

Wine-making could be difficult to be managed with must not well matured, especially for very low pH that reduce inoculated yeast activity.

Normally, Friuli Venezia Giulia wineries cultivate different Sauvignon blanc clones and often harvest grapes with high sugar content and low acidity to produce high quality wines.

So that, the mass harvested in advanced could be matched with a high mature one in right proportion to obtain complete *Sauvignon banc* wine flavour and balance. Future tests in this way could be useful to improve wine quality.

5 Methodological activity to ascertain an analytical method to quantify 3-Isobutyl-2-MethoxyPyrazine in whole grape berries

5.1 Introduction

In this section a further method to analyse IBMP in whole grape berries is described and tested. For sample analysis in the main part of the research the method developed by AIS, Slovenia, was used to perform more analysis and get also IPMP concentration in juice. In the last part of the work we wanted to set up a method for IBMP analysis with the devices available in our laboratory. To test and set up the method samples collected in 2009 from the trial field were used.

Since ’70, several methods for MPs determinations in grapes and wine has been set up. A review on different method to analyse MPs in grape, must and wine has been purposed by Sala et al., 2004.

The first problem is aroma extraction from samples. First methods used liquid-liquid extraction (LLE), where solvent were used to obtain concentrated matrix of the interested compounds to be submittied to analysis. Solvents usually were toxic or dangerous compounds. Besides, it required big concentration factors and other compounds present in the sample appeared as interferences during analysis. Thus, It is now recommended to use complementary separation techniques to clean the extract, together with very sensitive and specific detector systems (Sala et al., 2004).

Head-space solid-phase microextraction (HS–SPME) is simpler and more suitable for MPs extraction. The most used fiber for these purposes is divinylbenzenecarboxen-polydimethylsiloxane (DVB/CAR/PDMS), which has best performance with alcolic solution if compared to others.

Several authors (Koch et al. 2012; Suklje et al 2012; Sheiner et al. 2010; Ryona et al 2009; Godelmann et al. 2008), used head-space solid-phase microextraction (HS–SPME) extraction method both for wines and musts. Well checked innovative methods use last technologies to perform analysis. For quantification gas chromatography time-of-flight mass spectrometry (GCxGC–TOF–MS) is used as more sensitive than gas chromatography mass spectrometry (GCxMS).

In our work HS-SPME coupled with GCxMS was used to perform analysis. All steps for sample preparation until injections into GC system were carried out manually. For berry sampling preparation Ryona et al., 2009 method was followed, modifying some operations according to laboratory material availability.

5.2Sample preparation

Samples were let out of the freezer until they get room temperature.30g-Berry sample for each biological replicate was weighted and the number of berries was counted into a 100ml-centrifuge tube. Following the sample preparation suggested by Ryona et al 2009, an aliquot of 50%w/w of 0.1M EDTA solution adjusted at pH 7.8 with NaOH was added to the sample. To avoid enzymatic reactions a CaCl2 5%w/w of the mixture weight was added.

Then the mixture was grinded carefully with an IKA Ultra Turrax Homogeniser. 10g of the homogenate were weighted into 40ml-Brown vial for SPME extraction, previously prepared with stir bar, 3g of NaCl and 10g of small glass marbles (diameter 2mm). Glass marbles were not used by Ryona et al., 2009 but they were tested to show the improved MP extraction from the whole berry sample.

After that 50ul of Deuterated standard of IBMP (d-IBMP) solution at 4000ng L-1 were spiked into the vial.

Vial was closed with its screw cap and silicone septum and vortexed for 2 minutes until salt was dissolved. The prepared vials were finally put in ultrasonic bath at 80°C at max ultrasonic intensity for 10 minutes.

Vials were conserved in the freezer at –22°C until Headspace Solid Phase Microextraction (HS-SPME).

5.3 Headspace Solid Phase Microextraction (HS-SPME)

A 2 cm, 50/30 µm divinylbenzene-carboxen-polydimethylsiloxane (DVB/CARB/PDMS) SPME fiber was used.Vials were put out of the freezer, conditioned at 40°C for 10 minutes in a water heated bath to homogenously defrost them, vortexed 1 minute and then reconditioned at 50°C for 10 minutes before fiber insertion.

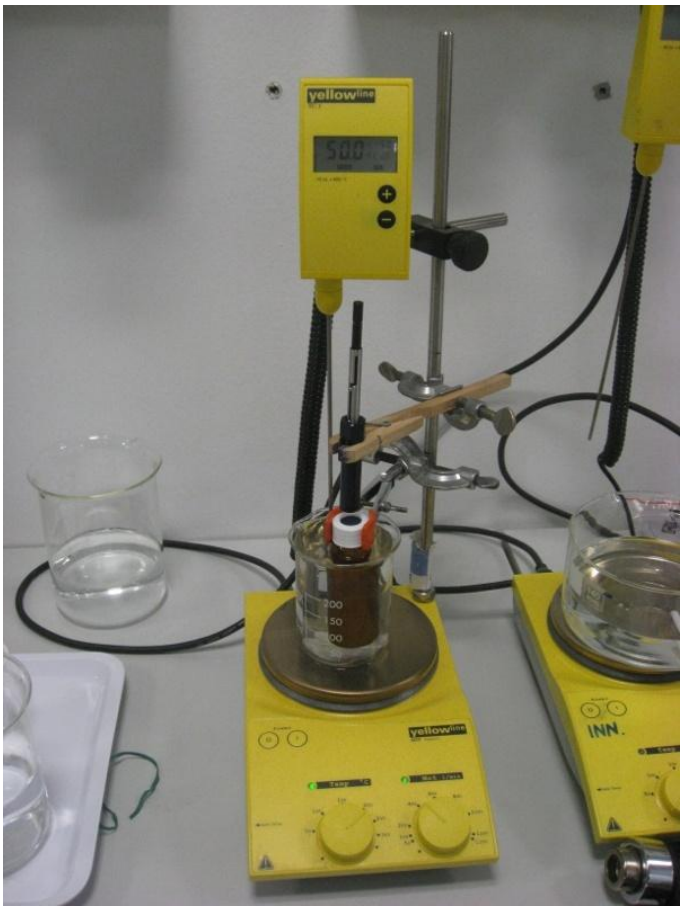


Figure 39: heated bath with brown vial and inserted fiber for SPME extraction

Fiber was exposed for 30 minutes at 50°C before the manual injection into GC (Figure 39).

After fiber injection into GC injector, the fiber was cleaned into a GC device at 250°C for 5 minutes eluted with helium.

5.4 MPs standard solution

IBMP (Sigma-Aldrich, St. Louis, MO, USA) with apurity of 99%, 2-isobutyl-3-methoxy-d3-pyrazine ([2H3]-IBMP) (C/D/N/Isotopes, Quebec, Canada) with a purity of 99%, and IPMP(Sigma-Aldrich) with a purity of 99% were used for the preparation of standards in solvent.

Sugar Solution added with standards were prepared to test the suitability of GCxMS to separate and detect MPs. Five hundred milliliters of waterpurified by a Milli-Q system (Bedford, MA, USA) was placed in a 1000mL volumetric flask. Ninety grams of fructose (Sigma-Aldrich), 90 g ofglucose (Sigma-Aldrich), and 1 g of tartaric acid (Merck, Darmstadt,Germany) were added and dissolved. The volumetric flask was madeup to volume with purified water, and the pH was adjusted to 3.2 with NaOH.

5.5Calibration curve

A calibration curve (CC) prepared as the samples was set up to check the linearity and the repeatability of the method. The CC was prepared with Muscat grape variety known as MP-free, collected at harvest 2008 and with Sauvignon blanc grape collected at harvest 2011, presumably being without MPs or with very low concentration. Seven different standard concentration of 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) were prepared. Concentration range varied from 0 to 91.2ng L-1 (197pg/g fresh berry weight) for IBMP and 0 to 48.8 ng L-1 (97pg/g fresh berry weight) for IPMP. Tri-Deuterated standard was spiked into the vial with the sample to reach a final concentration of 32 ng L-1 (69.2pg/g fresh berry weight). Table 30 reports the concentrations of calibration curve points.

STD Solution	IBMP pg/g	d-IBMP pg/g
1	0.0	69.2
2	9.9	69.2
3	19.6	69.2
4	39.3	69.2
5	98.5	69.2
6	157.7	69.2
7	197.0	69.2

Table 30: Standard concentration used for calibration curve

Tri-Deuterated standard solution was provided by C/D/N ISOTOPES INC., Point-Clare, Quebec, Canada (Figure 40).

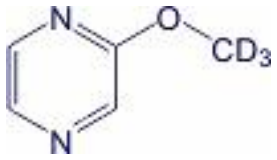


Figure 40: 2-Methoxy-d3-pyrazine

Since sample preparation was on weight-based dilution using the whole ground berries, results were expressed in pg of 2-Alkyl-3-Pyrazine per g of fresh weight berry.

5.6 Instruments tested and used

Originally, a GC model 17A Shimadzu equipped with a Shimadzu GCMS-QP5050 detector was used. The device was equipped with a chemical ionisation chamber, using methane as ionisation gas. The CI-50 gas controller was used to control gas flow into the ionisation chamber (Figure 41).

Figure 41: equipment used for MP analysis during the first part method development: GCxMS and CI-50 gas controller

Figure 42: common MPs you can find in food; isobutyl and isopropyl are the most abundant

Since the device equipped with CI did not work properly, analyze continued with the GC17A MS-QP5000 Shimadzu, provided with the Electrospray Ionization (EI) device, as suggested in several works (Koch et al., 2012). The column was the DB-5MS, J&W Scientific, Agilent Technologies, length 30m, I.D. 0.25mm, Film 0.25um.

SPME injection was splitless with a desorption temperature of 250°C. Helium was used as carrier gas at the flow rate of 1mL/min. The temperature program was: initial hold for 5min at 40°C, followed by 4°C/min ramp to 140°C, then 15°C/min ramp to 300°C and final hold for 10 min.For qualitative determination, retention time and mass spectrum in selective ion monitoring mode (SIM) were used. The mass channel was m/z 137 and 152 for IPMP, m/z 124 and151 for IBMP, and m/z 127 and 154 for [2H3]-IBMP. Ions 137, 124,and 127 were the target ions used for quantification, whereas 152, 151,and 154 were used as qualifier ions. To quantify IBMP and IPMP concentration, pick area ratio between IBMP or IPMP and d-IBMP was used.

5.7 Calibration curve results and tests

Unfortunately, IPMP resulted unpredictable with the used method at very low concentration (<20 pg/g) and it was not suitable for Sauvignon blanc sample analysis. Thus, we concentrated efforts on IBMP, because of they are the most important within Sauvignon blanc MPs.

In order to evaluate method linearity and to exclude the matrix effect on IBMP extraction two replicates of calibration curve at 5 point concentration has been prepared. First one was prepared by adding STD solutions in increasing concentration to a well homogenised Sauvignon sample collected at harvest 2009 (with very low IBMP content) and the same Sauvignon sampled at veraison (with expectably high IBMP content) (Figure 43).

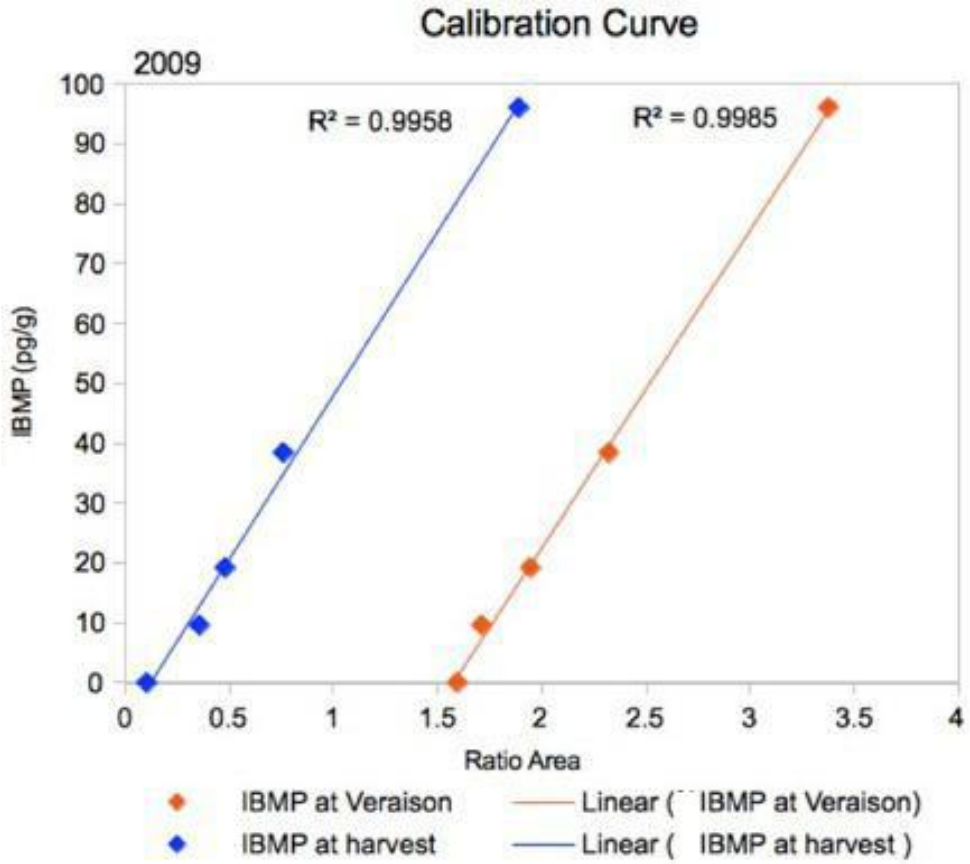


Figure 43: Calibration curves prepared with Sauvignon grape collected in trial field at harvest and veraison 2009

Linearity of the method was assessed by very high linear regression in both cases. Indeed same regression curve slope factor demonstrated that IBMP was extracted without differences even if grape matrix was completely different.

5.8 Sample analysis

The method was finally used to analyse IBMP in whole berries of samples collected in 2009 split-plot trial Leaf Removal per Nitrogen nutrition as explained in materials and method section.

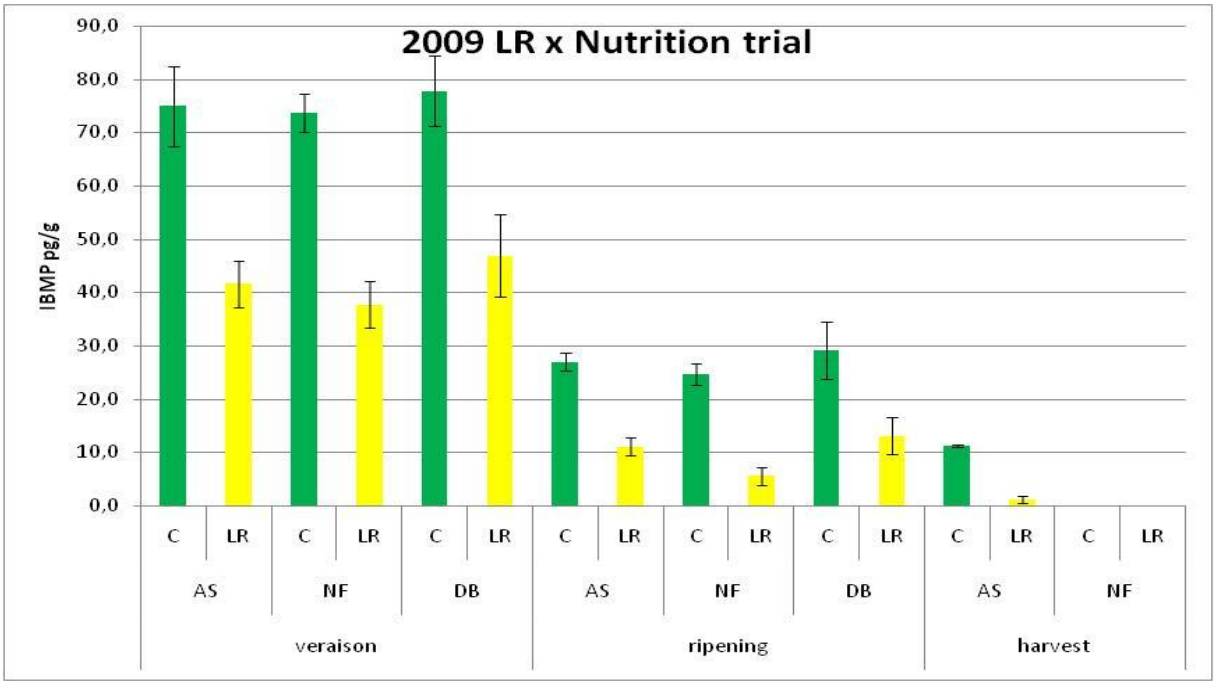


Figure 44: Results on IBMP concentration in Sauvignon grape collected in trial field in 2009; green bars indicate not defoliated plots (C) and yellow leaf removal treatments (LR); NF indicates control not fertilized, AS ammonium sulphate application and DB dried blood;

According to results obtain in the other years and with method applied for the complete analysis, we found high statistical differences between LR and C in all samplings included at harvest. Concentration of IBMP decreased from veraison to harvest and also at harvest a little amount of IBMP were detected.

No differences were found between nitrogen nutrition trial, since it was the first application year.

By mixed model analysis not statistical evidence was revealed for nitrogen nutrition and Leaf removal interaction.

From this preliminary results the method works properly to determine IBMP concentration in whole berries.

5.9 Advantages of the method

Method advantages could be summarized in:

- small sample is needed: at least 30g of fresh grape berries are needed to perform analysis and handle variability within the same sample;
- sample before veraison can be analysed: small green berries can also crushed and prepared for analysis without problems, It's enough to have more than 30g;
- results are expressed directly into weight ratio IBMPweight/BerryWeight: this is helpful to determine absolute IBMP potential and not only a relative concentration in juice;
- simple and safe sample preparation: no solvent or hazardous materials are used;
- analysis without new and expensive instruments: analysis can be carried out completely manually since sampling preparation to injection into GC with a classic GCxMS EI.

6 Final Conclusion

It is proved that agronomic management, both on soil and canopy, affects grape quality production. Oenological aims have to be considered and appointed before choosing solutions to be applied in the vineyard. A better knowledge of variables and factors that determine wine grape quality is important for vinegrowers to improve agronomic management and reach the followed goal.

While some wine quality parameters could be corrected and improved by oenological techniques, others are strictly connected to vine performance. Concerning *Sauvignon blanc* grape aroma complexity, the two major aromatic groups that characterize the wine are Thiols and Methoxypyrazines (MPs). Thiols are present in grape pulp and skin as precursors: their concentration depends on several environmental parameters and on vine clone features, but aroma in resultant wines is largely determined by yeast power to release volatile thiols during the alcoholic fermentation due to the transformation of the corresponding S-cysteine conjugate (Masneuf-Pomarède et al., 2006). Some yeast strains were selected according to their ability to liberate aroma (Dubourdieu, 2005). On the contrary MPs are present in grapes as volatile forms: their concentration in resultant wines is directly connected to their concentration in grapes at harvest (Ryona et al., 2009). MPs are not easy to be decomposed and, for better or for worse, the amount present in grapes is found in resultant wine.

It has been demonstrated that agronomical practices connected to bunch exposure to solar radiation influence considerably MPs concentration during ripening and at harvest (Koch et al., 2012; Scheiner et al., 2010; Ryona et al., 2009) especially in red variety that synthesizes relevant amounts of MPs.

In our study we applied i) severe basal leaf removal (LR),ii) the same leaf removal with 50% solar radiation shading net application (LRCS) and a iii) not defoliated control treatment (C)at the beginning of berry growt hin 2010 and in 2011; in a separate trial we applied soil nitrogen nutrition

with i) Ammonium Sulphate (40 kg ha^{-1} of nitrogen) and ii) Dried Blood, an organic fertilizer (60 kg ha^{-1} of nitrogen), to test their effect on *Sauvignon blanc* grape, focusing on MPs, 3-IsoButyl-2-MethoxyPyrazine (IBMP) and 3-IsoPropyl-2-MethoxyPyrazine (IPMP).

Microclimate monitoring in 2010 showed strong differences between treatments. On a daily basis, cluster temperatures were higher ($p<0.01$) for LR and LRCS than C during sunny days, but not in heavy cloudy days, when no significant difference was recorded.

Concerning the whole ripening period, LR increased on the average cluster temperature of 0.8°C if compared to C and of 0.4°C if compared to LRCS, with high statistical difference between treatments ($p<0.001$).

On a daily basis, as for temperatures, in cloudy days no difference was recorded between treatments for incoming solar radiation.

Notwithstanding, cumulated incoming solar radiation on clusters during the monitored period was highest for LR, lowest for C and intermediate for LRCS, because of strong differences in sunny days.

Measurements of surface cluster temperature were taken one day before harvest in 2010 and 2011. Temperature varied depending on the day time as reported for temperature inside clusters, but differences between LR and C were up to 10°C, in the morning when solar radiation was hitting directly the clusters.

LRCS created intermediate conditions between C and LR of temperature and solar radiation in the fruiting zone.

These differences recorded for cluster microclimate affected in different ways grape quality, yield and disease susceptibility.

Concerning MPs, they decrease from veraison to harvest in all varieties where vegetal aromas are synthesized (Koch et al., 2012; Scheiner et al., 2010;Ryona et al., 2009). In our experiment MPs followed the same progression. At veraison, consistent differences were found among treatments for IBMP: LR affected IBMP concentration at veraison in both years, reducing MP of about 50 and 60% less than C, control not defoliated. In 2010 LRCS, leaf removal with cluster 50% solar radiation

shading net application, showed the same results of LR. No statistical differences were obtained in 2011 at veraison comparing LR and LRCS, even if the latter treatment had 4.2 ng L^{-1} more IBMP than the former. On the other hand, shading net strongly moderated temperature and solar radiation with respect to grape exposed direct to sunlight in LR. Nonetheless, shading net did not preserve MPs from degradation at veraison. Differently from varieties studied in other works, Merlot, Cabernet Sauvignon and Cabernet franc (Koch et al., 2010; Scheiner et al., 2010;Ryona et al., 2009), all sampling concentration at harvest were below the limit of detection (LD<0.6ng L^{-1}) or below the limit of quantification (LQ<2.0 ng L^{-1}). This fact put in evidence the difficulty to obtain *Sauvignon blanc* with vegetal notes in Friuli Venezia Giulia Region.

No clear and wide differences were determined for fruit composition at harvest both in 2010 and 2011, indicating that classical ripening parameters for studied *Sauvignon blanc* are not consistently influenced by sunlight exposure. Also Scheirer et al, 2010 did not find statistical differences for Cabernet franc and Merlot grape composition in 2007 and 2008.

Really strong and interesting effects were recorded for LR and LRCS treatments to control bunch rots in 2010, a very rainy year particularly during ripening .

In 2010 *Botrytis* incidence was 74% in C and 44% and 51% in LR and LRCS respectively, significantly lower than C

Although high incidence was recorded also in LR and LRCS, severity showed the high power of LR to reduce disease attacks. In fact, *Botrytis* severity showed very high statistical differences between treatments with nonessential bunch damages in LR and LRCS treatments: C was 25%, LR 5% and LRCS 6%. Probably due to *Botrytis* bunch rot attack, yield was about 20% lower in C than in LR and LRCS.

In 2011 bunch rots did not develop but bunch sunburns were recorded: LR in 2011 enhanced sunburn incidence, while LRCS didn't show statistical differences if compared to C. Although burn severity was very low,

incidence was more present in LR (48%) than C (9.3%). LRCS maintained the incidence below 17%, affecting positively cluster protection. LRCS was useful to reduce rots and to protect grapes against negative effects of sunlight.

The application of the shading net at 50% of solar radiation on leaf removal cluster conferred positively effects of simple leaf removal for rots risk development and protected bunches against sunburns. Grape composition was not affected by LR and LRCS as compared to C. Although no MPs were detected at harvest because of year effect (warm season), in the previous sampling moments LR and LRCS cut down MPs in must. According to oenological aim, LR and LRCS appear to be useful agronomical practices to reduce rot incidence and probably also vegetal aromas (in years when MPs synthesis is promoted) without modifying grape composition.

Thiol precursors were not taken into account in this study but from other studies has been demonstrated that this aroma could be more connected to a combined nitrogen and sulphur foliar nutrition, while soil nitrogen nutrition did not affect their expression (Lacroux et al., 2008). That being so, LR and LRCS are agronomical practices that meet needs of recent European legislation (Directive 2009/128/EU, Sustainable Use of Pesticides in Agriculture) to reduce pesticides and that could be applied also in white grape varieties with compact clusters. In order to reinforce the shading effect, 100% solar radiation shading net could be used, so that only diffused light could reach clusters.

In the same vineyard soil nitrogen nutrition trial was set up from 2009 to 2011. Applied thesis were C (not fertilized), AS (Ammonium Sulphate, 40 N units) and DB (Dried Blood, 60 N units).

Since soil presented a good initial fertility, nitrogen was not applied at very high rates but according to good agronomic practices for the area; so differences between treatments were recorded only for some more sensible variables. IBMP was affected by treatments, increasing

differences year by year. Notwithstanding only at 2009 harvest a detectable amount was recorded.

We determined the highest content at veraison and not detectable (<2ng L^{-1}) at harvest, apart from 2009 harvest. Samples collected at the intermediate ripening time (12-15 days before harvest) had very low IBMP content, 1-3 ng L^{-1} just above the olfactory detection limit in white wines (2 ng L^{-1}). In 2010 the same sampling moment had already no detectable MPs concentration. In 2010 the sampling time 25 days before harvest showed very low MPs content.

Considering IBMP at veraison, in 2009 no relevant difference between treatments was registered. On the contrary, results showed highest content in C 25.7 ng L^{-1} than in AS and DB respectively 18.6 and 20.9 ng L^{-1}. Since veraison 2010, treatment effects were going to appear: even if not supported by statistic, C was lower (20.3 ng L^{-1}) than fertilized treatment AS and DB (24.5 and 24.9 ng L^{-1}, respectively). In 2011 C was 30% statistically lower than AS and DB, showing a strengthening of fertilization on IBMP cumulated through years.

After three years of nitrogen application, fruit composition as soluble solids, titratable acidity and pH was not strongly affected by treatments. Juice total nitrogen (TN) showed differences between treatments in every year but only in 2011 TN was statistically higher in DB and AS than C, according to soil nitrogen rate application. In 2010 TN was the lowest than other years, when yield was highest.

Normalized Difference Vegetation Index (NDVI), correlated to different vigour parameters, showed increasingly significant differences in 2009 and 2010 with highest values corresponding to highest N rate supply. In 2011, because of the water stress influence on young vine shortly before harvest, no difference was revealed.

Concerning the region Friuli Venezia Giulia, *Sauvignon banc* needs to be grown in no limiting conditions of water and nutrient to express its whole complexity. MPs are searched in *Sauvignon blanc* wines by many vinegrowers of the Region. By this study, the year has been recognized to

be the most important factor in causing vegetal notes in *Sauvignon blanc*, but finding a strategy to stimulate synthesis and to preserve MPs until harvest could improve the quality of wine obtained, especially in good years, i.e. not too hot summer. LR seemed to be a suitable practice to control pests also in white varieties, characterized by compact cluster,. Since LR has been demonstrated to reduce MPs concentration at veraison, during ripening and presumably also at harvest (in years when present), it is not recommended to use LR to obtain a *Sauvignon blanc* with vegetal notes.

Knowing that N fertilization increases MP concentration and that during ripening MPs fall down below olfactory sensory threshold (2ng L^{-1} in white wines), a high-moderate N fertilization, depending on soil fertility, could be paired with an earlier harvest (e.g. at 14-16°Brix and 9-7,5 TA) than that suggested by usual "technological" maturity (20-22°Brix and 5-6 TA). However, wine-making could be difficult to be managed starting from grapes not well mature, especially because of very low pH reducing inoculated yeast activity.

Normally, Friuli Venezia Giulia wineries are growing different *Sauvignon blanc* clones and often they harvest grapes with high sugar content and low acidity to produce high quality wines, especially during the last hot years.

So, during the vinification process the early harvested grapes could be mixed with full ripe grapes in a correct proportion to obtain balanced *Sauvignon blanc* wine flavours. Future tests in this direction could be useful to improve wine quality of *Sauvignon blanc*.

A world spread variety like *Sauvignon blanc,* that has found a suitable environment for quality productions in Friuli Venezia Giulia, could be a pulling wine for the Region in the international market. High standard Friuli Sauvignon production, being comparable with *Sauvignon blanc* wines coming from most famous winegrowing territories, like France and New Zealand, could lead to the appreciation of excellent local wines of Friuli abroad giving new commercial prospective for wine-makers.

7 References

Allen M. S., Lacey M. J. 1993. Methoxypyrazine grapeflavour: influence of climate, cultivar and viticulture. Wein-Wiss. 48: 211–213

Allen M. S., Lacey M. J., Hars, R. L. N., Brown, W. V. 1991. Contribution of Methoxypyrazines to Sauvignon Blanc wine aroma. Am. J. Enol. Vitic., 42, 109−112.

Arnold R.A., Bledsoe A.M. 1990. The effect of various leaf removal treatments on the aroma and flavor of Sauvignon Blanc wine. American Journal of Enology and Viticulture, 41: 74-76

Bavaresco L. 2008. Concimi organici e fogliari per vigneti poco produttivi. Informatore Agrario, 7: 50-53

Belancic A.E., Agostini I.A., Bordeu E., Baumes R., Razungles A., Bayonove C. 1997.Influence of sun exposure on the aromatic composition of Chilean muscat grape cultivars Moscatel de Alejandria and Moscatel Rosada. American Journal of Enology and Viticulture, 42: 181-186

Bell S.J. and Robson A. 1999.Effect of Nitrogen Fertilization on Growth, Canopy Density, and Yield of Vitis vinifera L. cv. Cabernet Sauvignon. Am. J. Enol. Vitic 50 (3): 351-358

Bell S-J. and Hanschke P.A. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. Australian Journal of Grape and Wine Research, 11: 242-295

Belvini P., Pascarella G., Dalla Costa L., Sivilotti P. 2010. Con interventi al verde su vite aumenta l'efficienza delle foglie. informatore agrario, 17 : 46-53

Bergqvist J., Dokoozlian N. and Ebisuda N..Sunlight Exposure and Temperature Effects on Berry Growth and Composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California.Am. J. Enol. Vitic 2001 vol. 52 no. 1 1-7

Bigot G., Degano F., Chiavoni A., Palladin M., Visentini A., 2009. Zolfo e Azoto per aromi al top. VQ, 7:20-26

Bledsoe A. M., Kliewe. W.M., MaroisJ.J. 1988.Effects of Timing and Severity of Leaf Removal on Yield and Fruit composition of Sauvignon blanc Grapevines. Am.J. Enol. Vitic. 39 (1): 49:54

Burford J. R. and Bremner J. M. 1975.Relationships between denitrification capacities of soils and total water soluble and readily decomposable soil organic matter. Soil Biol. Biochem. 7: 389–394.

Buttrose M.S., Hale C.R. 1973. Effect of temperature on development of the grapevine inflorescence after bud burst. Am.J. Enol. Vitic., 24: 14-16

Cargnello G. 1992. Recherchers sur l'influence de l'envirronement sur la physiologie, sur la -histologie et sur l'etat phytosanitarie des differents grains de la meme grappe de la vigne (Vitis Vinifera L.). Atti IV Simp. Int. Fisiol. Vite, Torino, 631-634

Conradie W.J. 1986.Utilization of nitrogen by the grapevine as affected by time of application and soil type. S. Afr. J. Enol. Vitic, 7: 76-83

Conradie W.J. 1991. Translocation and storage of nitrogen by grapevines as affected by time of application. Proc. of int. Symp. on Nitrogen in Grapes and Wine, Seattle (Usa), 32-42

Coombe B.G., Dry P.R. 1998. Viticulture volume 2: Practices. Aldelaide, Australia: Winetitles.

Corazzina E. 2007. La concimazione. La coltivazione della vite. Edizioni L'Informatore agrario. IV edizione, 133-155

Cosmo I., Polsinelli M. 1961. Sauvignon. Principali vitigni da vino coltivati in Italia. Volume I, tomo IV. Ministero dell'agricoltura e Foreste. Roma.

Cravero S., Rabino M. 2005. Sfogliatura e botrite, marciume acido ed alter alterazioni del grappolo. Informatore Agrario, 21: 43-46

Denis A. A., Martin H. C., Philippe R. and Bernard G. 2006. Dynamics of soil water-extractable organic C following application of dairy cattle manures. Canadian Journal of Soil Science, 86(5): 851-858

Duncan R. A., Stapleton J. J., Leavitt G. M. 1995. Population dynamics of epiphytic mycoflora and occurrence of bunch rots of wine grapes as

influenced by leaf removal. Plant PathologyVolume 44, Issue 6, pages 956–965Fregoni M., 1996. Nutrizione e fertilizzazione della vite. Ed. Agricole, Bologna.ISBN 88-206-1996-2;

Godelmann R., Limmert S., Kuballa T. 2008. Implementation of headspace solid-phase-microextraction–GC–MS/MS methodology for determination of 3-alkyl-2-methoxypyrazines in wine. Eur. Food Res. Technol. 227:449–461

Greer D. H., Rogiers S. Y. And SteelC. C. 2006. Susceptibility of Chardonnay grapes to sunburn. Vitis 45 (3), 147–148

Gregorich E. G., Rochette P., McGuire S., Liang B. C. and Lessard R. 1998.Soluble organic carbon and CO2 fluxes in maize fields receiving spring-applied manure. J. Environ. Qual. 27: 209–214.

Hilbert G., Soyer J. P., Molot C., Giraudon J., Milin S. And Gaudillere J. P. 2003.Effects of nitrogen supply on must quality and anthocyanin accumulationin berries of cv. Merlot. Vitis 42 (2): 69–76

Keller M. 2005.Deficit Irrigation and Vine Mineral Nutrition. Am. J. Enol. Vitic. 56:3

Koch A., Doyle C.L., Matthews M.A., Williams L.E., Ebeler S.E. 2010. 2-Methoxy-3-isobutylpyrazine in grape berries and its dependence on genotype. Phytochemistry 71: 17-18

Koch A., Ebeler S.E., Williams L.E. and Matthews M.A. 2012. Fruit ripening in *Vitis vinifera*: light intensity before and not during ripening determines the concentration of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon berries. Physiologia Plantarum 145: 275–285

Lacey M.S., Allen M.S., et al. 1991. Methoxypyrazines in Sauvignon Blanc grapes and wines. American Journal of Enology and Viticulture, 42: 103-108

Lacroux F., Trgoat O., Van Leeuwen C., Pons A., Tominaga T., Lavigne-Cruege V., Dubordieu D. 2008. Effect of foliage nitrogen and sulphur application on aromatic expression of Vitis vinifera L. Sauvignon blanc. J. Int. Sci. VigneVin, 42 (3): 125-132

Lakso A.N., Kliewer W.M. 1975. The influence of temperature on malic acid metabolism in grape berries. Plant Physiol., 56: 370-372

Linsenmeier Albert W., Loos U. and Löhnertz O. 2008. Must Composition and Nitrogen Uptake in a Long-term Trial as Affected by Timing of Nitrogen Fertilization in a Cool-Climate Riesling Vineyard. Am. J. Enol. Vitic.59 (3):255-264

Lisjak K., Baša Česnik H., Š uklje K. 2011. Determination of methoxipyrazines content in Slovenian must and wine by SPME-GC-MS. In Proceedings of the 2nd ms food day.Forum Editrice Universitaria Udinese srl.

Masneuf-Pomarède I., Mansour C., Murat M.L., Tominaga T., Dubourdieu D. 2006. Influence of fermentation temperature on volatile thiols concentrations in *Sauvignon blanc* wines. Int. J. F. Microb. 108: 385–390

Mescalchin E., Bottura M., Cainelli R., Fellin F., Gobber M., Lucin R., Margoni M., Mattedi F., Michelotti F., Patton A., Penner F, Ribolli F. 2008. Sfogliare precocemente la vite per evitare scottature e botrite.Informatore Agrario, 17: 39-43

Millard P. 1993. Internal cycling of nitrogen in trees. Proc. 2nd int. Symp. on Diagnosis of nutrition al status of deciduous fruit orchards, Acta Horticolturae, 383: 3-14

Morlat R. and Chaussod R. 2008.Long-term Additions of Organic Amendments in a Loire Valley Vineyard. I. Effects on Properties of a Calcareous Sandy Soil.Am. J. Enol. Vitic. 59:4

Morlat R. and Symoneaux R. 2008. Long-Term Additions of Organic Amendments in a Loire Valley Vineyard on a Calcareous Sandy Soil. III. Effects on Fruit Composition and Chemical and Sensory Characteristics of Cabernet franc Wine Am. J. Enol. Vitic. 59:4

Morlat R. 2008 Long-Term Additions of Organic Amendments in a Loire Valley Vineyard on a Calcareous Sandy Soil. II. Effects on Root System, Growth, Grape Yield, and Foliar Nutrient Status of a Cabernet franc Vine. Am. J. Enol. Vitic 59:4

Murphy D. V., Macdonald A. J., Stockdale E. A., Goulding K. W. T., Fortune S., Gaunt J. L., Poulton P. R., Wakefield J. A., Webster C. P. and Wilmer W. S. 2000. Soluble organic nitrogen in agricultural soils. Biol. Fertil. Soils 30: 374–387.

Neilsen Gerry H., Neilsen Denise, Bowen Pat, Bogdanoff Carl and Usher Kevin. 2010. Effect of Timing, Rate, and Form of N Fertilization on Nutrition, Vigor, Yield, and Berry Yeast-Assimilable N of Grape. Am. J. Enol. Vitic 61 (3) 327-336

Nicolini G., Versini G., Corradin L., Larcher R., Beretta C., Olivari A., Eccli E. 2004. Misura dell'azoto prontamente assimilabile dal lievito nei mosti d'uva ed esempi di applicazione. Riv. Vitic. Enol. 1/2, 13-27;

Persello R. 2011. Gli aromi del Sauvignon dalle uve al vino. Progetto tipicità Sauvignon dei Colli Orientali del Friuli. Consorzio Colli Orientali del Friuli.

Peyrot des Gachons C., Van Leeuwen C., Tominaga T., Soyer J.P., Gaudillère J.P., Dubourdieu D. 2005.Influence of water and nitrogen deficit on fruit ripening and aroma potential of Vitis Vinifera L. cv *Sauvignon blanc* in field conditions. J. Sci. Food Agric, 5: 73-85

Poni S. 2000. La potatura verde del vigneto. Il Corriere Vinicolo., 17: 9-13

Poni S. 2002. Microclima della chioma e parametri qualitative delle uve. Informatore Agrario, 40 : 73-79

Poni S. 2003. La potatura verde del vigneto aspetti fisiologici e colturali. Informatore Agrario, 26: 37-49

Poni S. and Intrieri C. 1996. Physiology of grape leaf ageing as related to improved canopy management and grape quality. Proceedings of 9th Australian Wine Industry Technical Conference. Adelaide, Australia, 113-122

Poni S., Bernizzoni F. and Civardi S. 2008. The effect of early leaf removal on whole-canopy gas exchange and vine performance of *Vitis vinifera* L. 'Sangiovese'. Vitis 47 (1), 1–6

Poni S., Bernizzoni F., Civardi S., Libelli N. 2009 Effect of pre-bloom leaf removal on growth of berry tissues and must composition in two red Vitis Vinifera L. Cultivars. Australian Journal of Grape and Wine Research, 185-193

Poni S., Casalini S., Bernizzoni F, Civardi F., Intrieri C. 2006.Effect of early defoliation on shoot photosynthesis, yeld components, and grape composition. American Journal of Enology and Viticulture, 57(4):397-407

Porro D., Colugnati G., Stefanini M. 2001. Approccio nutrizionale alla viticoltura di qualità. Informatore agrario, 13: 63-76

R'Houma A., Chérif M., Boubaker A. 1998.Effect of nitrogen fertilization, green pruning and fungicide treatments on botrytis bunch rot of grapes. Journal of Plant Pathology 80 2

Raven P.H., Evert R.F., Eichhorn S.E. 2005. Assimilazione dell'azoto. Biologia delle piante. VI edizione Zanichelli.

Reid A. S. J., Webster G. R., Krouse H. R. 1969. Nitrogen movement and transformation in soils. Plant and Soil 31 (2) 224-237

Reynolds A.G., Price S.F., Wardle D.A., Watson B.T. 1994. Fruit environment and the crop level effects on Pinot Noir. I. Vine performance and fruit composition in British Columbia. Am. J. Enol. Vitic., 4: 452-459

Reynolds A.G., Wardle D.A., Naylor A.P. 1996. Impact of training system, wine spacing and basal leaf removal on Riesling. Vine performance , berry composition, canopy microclimate, and vineyard labor requirements. Am. J. Enol. Vitic., 47: 63-76

Ribéreau-Gayon P., Glories Y., Maujean A., Dubourdieu D. 2005.Aromi varietali. Trattato di enologia II. Edagricole.

Ryona Imelda, Pan Bruce S. and Sacks Gavin L. 2009. Rapid Measurement of 3-Alkyl-2-methoxypyrazine Content of Winegrapes To Predict Levels in Resultant Wines. J. Agric. Food Chem. 57, 8250–8257

Ryona I., Pan B. S., Intrigliolo D. S., Lakso Alan N. 2008. Effects of Cluster Light Exposure on3-Isobutyl-2-methoxypyrazine Accumulation and Degradation Patterns in Red Wine Grapes (*Vitis vinifera* L. Cv. Cabernet Franc) J. Agric. Food Chem, 56: 10838-10846

Sala C.; Busto O. and Guasch J. 2004. Determination of 3-alkyl-2-methoxypyrazines ingrapes, musts and wines: a review.

Scheiner J.J, Sacks G.L., Pan B., Ennahli S., Tarlton L., Wise A., Lerch S.D., Vanden Heuvel J.E. 2010. Impact of Severity and Timing of Basal Leaf Removal on 3-Isobutyl-2-Methoxypyrazine Concentrations in Red Winegrapes. Am. J. Enol. Vitic.61(3) 358-364

Schreiner P.R., Carolyn F.S. and BahamJ. 2006.Nutrient Uptake and Distribution in a Mature `Pinot noir' Vineyard. Hort Science 41(2): 336-345

Sequi P.. 2005. Fondamenti di chimica del suolo. II edizione. Patron editore

Smart R.E., Sinclair T.R.. 1976. Solar heating of grape berries and other spherical fruits. Agricultural Meteorology, 17: 241-259

Šuklje K., Lisjak K., Baša Česnik H., Janeš L., Du Toit W., Coetzee Z., Vanzo A. and Deloire A. 2012. Classification of Grape Berries According to Diameter and Total Soluble Solids To Study the Effect of Light and Temperature on Methoxypyrazine, Glutathione, and Hydroxycinnamate Evolution during Ripening of Sauvignon blanc (Vitis vinifera L.). J. Agric. Food Chem., 60: 9454−9461

Tardaguila J., de Toda F. M., Poni S., Diago M..2010.Impact of Early Leaf Removal on Yield and Fruit and Wine Composition of *Vitis vinifera* L. Graciano and Carignan. Am. J. Enol. Vitic 61 (3): 372-381.

Titus J.S., Kan S.M..1982. Nitrogen metabolism translocation and recycling in Apple trees. Hort. Rev, 4: 204-246

Valenti L., Mattivi F., Bravi M., Dell'Orto M., Ghiglieno I. 2011. Potatura ed ombreggiamento controllano il grado zuccherino. Informatore Agrario, 13:22-25

Vercesi A. 2000. Concimi organici a terreno e foglie in viticoltura. Informatore Agrario, 6: 83-90

Vivai Cooperativi Rauscedo. 2008. I cloni originali di VCR di Sauvignon. Quaderni tecnici,

Webster R. 2007. Analysis of variance, inference, multiple comparisons and sampling effects in soil research. Europ. J. of Soil Science, 58: 74–82

Zhang R. and Wienhold B. J. 2002.The effect of soil moisture on mineral nitrogen, soil electrical conductivity and pH. Nutrient Cycling in Agroecosystems 63: 251–254

Figure Index

Ringraziamenti

Questo lavoro è stato realizzato grazie al contributo in misura diversa di molti tra colleghi e amici, vecchi e nuovi.

Ringrazio il prof. Enrico Peterlunger per avermi dato la possibilità di seguire il corso di dottorato e completarlo, anche se tra qualche difficoltà dovuta ai miei troppi impegni contemporanei e ai miei ripetuti ritardi. È stato peggio di un parto ma è fatta.

Il dott. Simone Castellarin per l'assistenza, le indicazioni, i suggerimenti nel corso delle varie prove: tra i mille lavori in piedi è riuscito a trovare un po' di tempo da dedicarmi.

La dottoressa Lara Tat per l'aiuto in laboratorio e per avermi trattenuto dal rompere a martellate il gas- cromatografo quando puntualmente non funzionava.

Il dott. agr. Giovanni Bigot e staff (di cui per altro attualmente faccio parte con entusiasmo) per le prove di campo, i consigli, le interpretazioni, i rilievi NDVI e alla raccolta con spettacolari vendemmie delle parcelle sperimentali fino alle 22 con torcia alla mano.

Il gruppo di ricerca CRA-RPS di Gorizia per avermi dato lavoro, possibilità di imparare e di seguire le mie attività di dottorato senza problemi e anche un aiuto nei lavori: Gilberto, Claudio, Adriano, Flavio, Philippe, Chiara e Tania.

Klemen Lisjak e il suo gruppo di lavoro del Kmetijski inštitut Slovenije di Lubiana per l'aiuto nelle analisi delle famigerate pirazine nell'uva.

Il dott. Giorgio Alberti per i rilievi microclimatici della chioma, la messa a disposizione dei materiali e l'aiuto nella gestione dei dati.

Il buon tesista Marco Pinat per la condivisioni dei campionamenti di uva, alle 6 del mattino e per l'aiuto nelle analisi di laboratorio. Direi che ci siamo divertiti.

L'altro buon tesista Laith Kokali, per l'aiuto con le analisi di laboratorio, anche se non l'ho sfruttato abbastanza. Ho ancora il rosario regalatomi per la sua laurea.