European Master of Science of Viticulture and Enology

Master's Thesis

Comparing qualitative and quantitative impacts of different treatments with Horn Silica in grapevine

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STATEMENT

I herewith declare

in lieu of oath that the submitted master's thesis with the title

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has been composed by myself without any inadmissible help

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those given due reference in the text and listed in the list of references.

I further declare that all persons and institutions

that have directly or indirectly helped me

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Volnay, 9th November 2015

PREFACE

This work has been one of the greatest challenges in my life right from the start; dealing with two foreigner languages making easy things complicated, then implementing a long term trial from zero, carrying out all measurements and finally analyzing a huge quantity of data in order to present a written report with useful conclusions.

Fortunately, during this 6 months I have been part of a remarkable group of winegrowers, consultants and researchers, who have been working together as a team for common goals in an excellent example of true cooperation and commitment.

I want to thanks formally to the institutions of Domaine Marquis D'Angerville, Domaine La Pousse D'Or, Domaine Michel Lafarge, Soin de la Terre and Geisenheim University, for get involved in this kind of projects and make it possible.

Particularly I would like to deeply thanks to Frédéric Lafarge, François Duvivier, Vincent Masson, Pierre Masson and Hubert Rossignol, because of their outstanding welcoming, unlimited support, endless motivation, trust and availability to share all their knowledge and experience in order to enrich my work. They were my source of inspiration in most difficult moments.

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Finally, this Thesis is dedicated to those who have given me everything I have, to María Gabriela, Arístides, Fernanda, Martín y Benjamín.

And of course is dedicated to you, L.

Volnay, 9th November 2015

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LIST OF ABBREVIATIONS

AH = after harvest
BD = biodynamic
BDp = biodynamic preparation
BF = before flowering
CHB = Champans Bas
CLL = Les Caillerets
CON = Conventional
FH = between flowering and harvest
HI = Huglin index
INT = integrated
NDVI = Normalized Difference Vegetation Index
OB = oak bark
ORG = organic
SP = silica pure
VL = valerian
VOC = volatile organic compound
WG = winegrower
YN = yarrow and stinging nettle

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I INTRODUCTION

I.1 Overview

In the center of the AOC Cote D'Or of Burgundy, more precisely at Volnay, 5km south of Beaune, a long term research project has been established with the cooperation of three wine producers; Domaine Marquis D'Angerville, Domaine La Pousse D'Or and Domaine Michel Lafarge.

Volnay is settled up in the Chaignot hill and goes downwards from more than 300 down to 230 mt height, following direction Northwest-Southeast which is considered a good orientation to optimize sunlight interception. That slope is higher than the average in the region giving to Volnay a particular identity. Also, Volnay is a traditional *Appellation Village* recognized since centuries as a *terroir* for red wine exclusively, mainly Pinot Noir. From a total surface of 220 ha of vineyards in production, 132 ha are qualified as Volnay *Premier Cru*. Moreover, those high quality vineyards have been grouped in 29 different *Climats*, all *Premier Cru,* located in 2 communes, Volnay and Meursault (BIVB, 2015). Two plots were selected for the research project, both belonging to that category.

D. Marquis D'Angerville, existing since 1906, is working under biodynamic principles since 2006, with a total vineyards surface of 15 ha in Volnay divided in 20 parcels, all certified by Demeter and Ecocert since 2009. 87% of vineyard surface corresponds to Pinot Noir, 10% to Chardonnay, 2% to Aligote and 1% to Gamay. From that, 80 % is qualified as *Premier Cru*. For D'Angerville the biodynamic system of management has become a priority because of the improvement observed at soil and grapevine level, but especially the leap on quality recognized by their customers during wine tastings, consistently through the years. Furthermore, this leap has led them to a better understanding of the typicity of their wines.

D. Michel Lafarge, existing since 1800, is one of the pioneers practicing biodynamic viticulture in Burgundy, since 1997. A total surface of 16 ha of vineyards, 12 of them located at Volnay, 35% qualified as *Premier Cru*. These are divided in 10 parcels planted with: 70% Pinot Noir, 10% Chardonnay, 10% Aligote and 10% Gamay, certified by Demeter since 2009. Based on a large number of trials, Lafarge has developed a solid conviction about the biodynamic system of management, highlighting a careful handling of biodynamic preparations as well as a precise understanding of the effect of complementary herbal infusions used to minimize Sulphur and Copper additions.

D. La Pousse d'Or, even though is the oldest wine property (since 1272) is the newest within the biodynamic system, recently started in 2014. From a total surface of vineyards of 18 ha, 7.56 ha divided in 4 parcels are located at Volnay, all of them converting to biodynamic simultaneously, in process of certification. 100% of these parcels are qualified as Premier Cru, as well as 100% are planted with Pinot Noir.

These three wine producers are good examples of how the biodynamic system has been increasing in popularity last decades; the surface of farmers certified biodynamic has almost doubled last 15 years (Demeter 2015) and for winegrowers the tendency seems to be similar or even higher. The biodynamic system, despite its similarities with the organic system like the absence of fertilizers, pesticides or any chemically synthetized product, as well as the emphasis on soil building and promotion of biodiversity, push further with a strong philosophy behind. Considering the farm as an **individuality**, biodynamics leads to minimize inputs coming from outside arguing that the farm should develop under its natural, economic and social site conditions, a way to become a unique, self-contained and independent organism (Raupp 1999). In addition, it proposes several preparations based on manure, plants and minerals, applied to the soil, the compost and the canopy (Appendix 1), destined to stimulate and regulate life processes and the relationships between cultivated plants and their proximal or distant environment (Koepf 1998). These preparations represent one of the greatest differences from organic agriculture although their exact mode of action remains unexplained (Turinek 2011), in part because they are applied in such small quantities, like homeopathic remedies, that a dose effect is not clearly discernible (Raupp 1999).

According to a survey realized between French winegrowers and farmers practicing biodynamics, the most important subject to research about were the Biodynamic Preparations (BDp) with a 39% of preferences (MABD 2014). These results validates the aim of this Thesis which is to set up two long term comparative trials *on-farm,* meaning on established producing vineyards, focused on the effect of the BDp 501 Horn Silica on vine development. Also, a sort of preliminary scanner of the vines has been done through multiple measurements on vegetative and regenerative aspects, in order to guide and facilitate more specific research during next seasons, as well as to find some quantitative or qualitative impacts already in the first year of treatment.

I.2 Hypothesis

The main hypothesis of the first field trial states that changing the **date of application** of BDp 501 Horn Silica in vineyards managed under biodynamic principles, a qualitative and quantitative impact on vine development can be observed.

The second trial is supported by the hypothesis that **adding different herbal infusions** together with the BDp 501 Horn Silica, at same dates of application, some differences on qualitative and quantitative impacts on vine development can be observed.

For both purposes several variables related to vine physiology and phenology, disease incidence and berry quality were studied.

II LITERATURE REVIEW

II.1 Biodynamic Management Research

Biodynamics (BD) are originated from anthroposophy, a philosophy developed by Rudolf Steiner which goes far away from just an agricultural system of management, easy to apply but hard to understand (Raupp 1999). Looking for the origins of this philosophy, one of the strongest influences of Steiner was Goethe and his particular way of observing nature. He, at the same time, recognized a strong inspiration from Bacchus de Spinoza (Goethe 2009), who already in 17th century described a 3rd level of knowledge or intuitive knowledge (De Espinosa 2011) which could have served as bases for the spiritual science developed by Steiner. Such deep and fundamental roots create sometimes an intimate commitment from farmers and winegrowers to BD, making it a fascinating subject of study, although the application of their principles is what motivates this research.

This short look back to origins can explain why BD has been considered an alternative movement in agriculture for long time and why many institutions based in north-European countries (Switzerland, Germany, Austria, Sweden, Netherlands) have been doing research for decades but mostly supported by private funds.

Several long-term trials in vegetables have described many positive impacts of the BD system, usually focused on soil properties. A **greater biological activity in soil** is quite a common observation which has been described through many different parameters, like enzymatic activity (Carpenter-Boggs et al. 2000a, Mäder et al. 2002, Zaller and Köpke 2004, Granstedt and Kjellenberg 2005), soil organic matter content (Raupp 2001), basal respiration, decomposition rate (Zaller and Köpke 2004), microbial biomass, presence of mycorrhizae on roots and microbial diversity, the last one associated with higher efficiency in the metabolism of organic matter measured as metabolic quotient (Mäder et al. 2002).

Others parameters giving evidence of this positive impact are probably related to that enhanced biological activity. For example, a better buffering capability concerning soil pH changes and more building-up of soil processes in depth (Granstedt and Kjellenberg 2005), higher agronomic or energy efficiency, defined as a ratio of crop yield versus nutrient (N, P, K) input (Mäder et al. 2002, Turinek 2011) and better ecological footprint, a variable which estimate the biologically productive area needed to produce materials and energy used by the population of a certain region (Turinek 2011).

Still in vegetables, Bacchus (2010) has observed that plants of lettuce under BD treatment presented higher fresh weight at 28 and 47 days after transplanting as well as higher N and P uptake. Spraying 2 and 3 times BDp 500 and 501 respectively, even over inorganically fertilized plots, some differences in dry matter content in heads and roots were found. Although these results seem interesting, they were not consistent between them and with other measurements related to N and protein content.

Carpenter-Boggs et al (2000a, 2000b) near Pullman, Washington, found discernible differences in chemical parameters and microbial community structure of **compost** treated with BD preparations. During composting active period the temperature was in average 3.4°C higher than control, at the end of the process 65% more nitrates and a higher ratio of dehydrogenase were measured, suggesting more maturity and more release of CO_2 for the BD treated compost. In a second publication this author applied those composts to some crops, finding higher Nitrate (NO_3^+) content in spring wheat grain, lower Nitrogen (N) and Carbon (C) content in lentil grain and higher Ammonium (NH_4^+) content in soil.

Spaccini et al. (2012) did a **molecular characterization** of 3 samples of BDp 500 horn manure coming from different origins in Italy, through 2 methods: nuclear magnetic resonance spectroscopy and thermochemolysis. Results indicated that BDp 500 tends to preserve more labile or unstable lignocellulosic and alkyl molecules, so that the biochemical recalcitrance could be lower than mature compost having performed full aerobic fermentation. In other words, the BDp 500 can be more biolabile in soil, becoming potentially more bioactive towards plants growth.

One of the most accepted conclusions about the influence of Biodynamics coming from horticulture trials is the **regulation** role of BDp, decreasing or increasing yields depending on the tendency of the season (Raupp 1996, Koepf 1998).

Regarding viticulture, most of the interesting references are coming from long-term trials at academic institutions either at private wine companies. These trials, conducted in many different circumstances (climates, soils, varieties, rootstocks, density of plantation, etc), have compared the BD system as a whole group of practices, to other systems in viticulture. One remarkable difference between the trials is the definition of non-BD systems. In this sense, neither Organic (ORG), nor Integrated (INT) or Conventional (CON) systems had one way of application; each researcher and winegrower has a huge number of choices in terms of products or technical criteria, making the repeatability of this kind of research very low. So, any conclusions coming from each trial has to be considered as local data, where the specific results (values) are less important than the relation between each system concerning the parameter itself, which potentially can give interesting results in other similar trials.

Reeve et al (2005) at Mendocino County, California, have compared Merlot plots planted at 1.83 x 2.44 m., managed under BD and ORG systems for nine years, focused on high quality grapes production. The high fertility of the soil led to a reduction of compost addition in order to control vine vigour. Considering the effect of BD on compost (Carpenter-Boggs 2000a) the lack of it could be one of the reasons why no differences were found in any physical, chemical or biological parameters tested in soil. Also, no results were observed in nutritional content on leaf tissue or productive parameters such as cluster per vine, cluster weight, yield per vine and berry weight. But, the ratio pruning weight/yield was significantly different suggesting a better balance in BD plots and a slight overcropping in ORG plots.

During one particular harvest BD grapes showed significantly higher °Brix, total phenols and total anthocyanins, indicating the existence of a potential influence of BD on grape quality but not consistent through the years.

One of the most fruitful research project related to biodynamic viticulture is a 10 years old trial currently running at Hochschule Geisenheim University, Rheingau, Germany, where the impact of BD, ORG and INT systems on Riesling vines planted at 1,2 x 2 m are compared. Meißner et al (2013) described their results after just 4 years, 2006 to 2009. Already in the first year of conversion differences on **vigour** were observed. The canopy of INT version was denser and more vigorous than the BD-ORG variants, showing higher sucker growth and pruning weight. Is suggested that this difference was due to a greater competition by cover crops, because in BD-ORG variants a legumes emphasized mixture was sown every 2 rows. Concerning regenerative parameters, a statistically significant lower degree of **bunch compactness** was found in BD-ORG systems, despite climatic variations. Every year BD bunches had the lowest degree of compactness and in 2007 it was significantly different from INT and ORG variant as well. It was suggested as a possible effect of BDp. Since these vines tend to be very vigorous plants, both aspects, the reduction of vigor and smaller and loose bunches were considered as something positive, related to healthier and more balanced growth.

Döring et al (2011), at Geisenheim too, studied the impact of the three systems mentioned above (BDY-ORG-INT) over photosynthetic activity measured on old leaves, related to radiation and stomatal conductance. In a rainy year, 2010, BD-ORG systems had a trend towards lower **single leaf net photosynthesis** compared to INT variant, particularly under high radiation conditions or at high stomatal conductance. Between the biological systems, ORG leaves showed lower values than BD leaves. This was complemented by a slight lower chlorophyll content in both, which was significant only at one measurement date.

Simultaneously at Geisenheim, Stöber (2011) observed significant differences on the **lateral leaf area per lateral shoot** measured in each treatment (BD, ORG, INT). The BD system gave lower value, 254.77 cm^2, than ORG and INT systems, 295.00 and 328.98 cm^2 respectively. In terms of **diseases**, the incidence of botrytis and acetic acid bacteria was reduced on BD and ORG systems, compared to the INT.

Later on, Döring et al (2013) assessed the physiological activity and vigour of those Riesling vines, from 2010 to 2012, at Geisenheim. Both, ORG and BD systems showed consistently lower vigour than INT, measured as **lateral shoot growth**; furthermore, in a dryer season like 2011, a reduction of physiological activity was observed two weeks after full-bloom. Parameters like stomatal conductance, assimilation rate and transpiration showed this decline. Similar reduction of physiological activity in ORG and BD systems was observed at *veraison*, this time through a lower **pre-dawn water potential**. All these differences were found even though all treatments received the same level of nutrients and water. In a wetter year, 2012, any difference about these parameters was observed.

Curley (2013) made two separated comparisons, ORG/BD systems and ORG/CON at Pauillac, Bordeaux. In terms of biodiversity it was found a trend on BD inter row spaces to have higher **floristic abundance**, which is the total number of individuals per area. In terms of **vine diseases** it was found that BD vines showed less intensity and frequency of Mildew as well as lower infection by Botrytis, confirming results mentioned before.

Röder (2013) at Margaux, Bordeaux, synthetized the comparison of BD, ORG and Conventional systems in viticulture during 4 seasons, 2008 -2012. Concerning physiological parameters, the **Normalized Difference Vegetation Index (NDVI)**, appeared to be the most significant to study through the years. Looking for **vine diseases**, a weekly observation of them and a precise notation of Oidium, Mildew and Botrytis showed interesting results, but highly dependent on year climatic conditions. Coming to wines produced in each system, a triangular tasting test have led to results too, but still variable through the years.

Johnston et al (2015) worked at McLaren Vale in South Australia since 2008 with a farm-scale trial of Cabernet Sauvignon vineyards planted at 1.8 x 3 m., comparing BD, ORG, CON Low Input and CON High Input systems. Also, the variable of compost application (with or without compost) was added to each treatment. Both biological systems enhanced earthworm population, soil microbial biomass and their activity, confirming previous publications about impact on soil biology, but soil moisture showed lower values at top soil (40 cm), probably because of herbs presence on the row. Regarding the vines during 6 years they showed reduced **bunch number, vine vigour** and **fruit yield** in BD plots, which was highlighted in relation to financial issues.

Lorimer (2014) tested the effect of BDp 500 and BDP 501, isolated and combined, at four different locations in Switzerland (Auvernier, Hauterive, Echandens and Elfingen) planted with Chasselas (3 of them) and a mixture Riesling/Sylvaner, during 6 years. Even if results were not consistent for every site, some of them were remarkable. Concerning physiological parameters the plots treated only with BDp 501 showed a higher amount of **phytoalexins** on leaves (antimicrobial and antioxidative functions, useful facing fungal diseases), higher **wood weight** at pruning (on 3 sites), as well as higher **chlorophyll index,** known as SPAD. Also, it was concluded that the BDp 500 needs a well-nourished soil in order to give interesting results.

From a different perspective Fritz et al (2009, 2014) analyzed grape samples in 2006 taken from the long-term field trial at Geisenheim, Germany. Following 3 different **image forming methods** they compared ORG and INT systems, plus three variants of BD system; without BDp 501, 3 applications of BDp 501 and 4 applications of BDp 501. The picture forming methods are known as biocrystallization, capillary dynamolysis, and circular chromatography (Fritz 2011). Pictures were clearly differentiated and characterized based on the substance and structure, either chaotic or not. In 2010 the author repeated the processes on wine samples coming from the 3 systems (BD, ORG, INT), but without the variants of BDp 501. Using a catalogue of reference the images were grouped by similar form expression and classified as fresh or aged. After that, it was possible rank them according to their freshness. As a result, wine samples coming from BD system showed less structures indicating aging, compared to ORG or INT. These observations allowed to correctly assign each wine sample to its viticulture system of origin.

Laghi et al. (2014) tried to discriminate between ORG and BD wines coming from Sangiovese grapes analyzing their **metabolites** during 3 years. According to their results, quite extensive, the effect of the viticulture system is less significant than vinification protocol, as well as the effect of the year.

Johnston et al (2015) in South Australia also analyzed the effect of BD, ORG, CON Low Input and CON High Input systems on wine chemistry during three years. No consistent differences were found in pH, Total Acidity or Alcohol between the treatments, but sensorial evaluation highlighted BD wines as more complex, rich, textural and vibrant in each season.

Casciano (2013) at Saint-Estèphe, Bordeaux, compared the BD, ORG and CON systems on Cabernet Sauvignon vines, focusing on the incidence of diseases, vine vigour and costs associated to each treatment. Unfortunately, due to a high pressure of Mildew it was not possible to maintain the ORG management as it should, turning plots back to CON. This gave a good example about the preventive character of BD and ORG systems rather than curative, something to consider for any trial of this kind.

Table 1. Summary Literature Review of Biodynamic Management Research

BD VS OTHERS SYSTEMS	HORTICULTURE	Compost	(+)	Biolability of lignocellulosic and alkyl molecules in **BDp 500**, T° of composting, nitrates content, release of CO_2
		Soil	(+)	Enzymatic activity, soil organic matter content, Ammonium (NH4+) content, basal respiration, decomposition rate, microbial biomass, mycorrhizae on roots, microbial diversity (metabolic quotient), buffer ability and soil processes in depth
		Yield	(+)	Agronomic or energy efficiency (yield vs inputs), ecological footprint
		Yield	(+)(-)	Regulation of yields depending on the season trend
		Grain	(+)(-)	Nitrate (NO3+) content in spring wheat, Nitrogen (N) and Carbon (C) content in lentil
		Leaf	(+)	Dry matter content, N and P uptake in lettuce
	VITICULTURE	Plant	(-)	Ratio pruning weight/yield, Sucker growth, pruning weight, vine vigour, single leaf net photosynthesis, chlorophyll content, lateral leaf area per lateral shoot, lateral shoot growth, stomatal conductance, assimilation rate, transpiration and pre-dawn water potential
		Plant	(+)(-)	Normalized Difference Vegetation Index (NDVI)
		Soil	(-)	Soil moisture at top soil (40 cm)
		Yield	(-)	Bunch number and fruit yield
		Grapes	(+)	°Brix, total phenols and total anthocyanins.
		Grapes	(-)	Degree of bunch compactness, incidence of Botrytis, Acetic Acid bacteria, Mildew or Oidium
		Grapes	(+)	Quality according to image forming methods
		Soil	(+)	Floristic abundance per area, Earthworm population, soil microbial biomass
		Wine	(+)	Complexity, richness, texture; freshness according to image forming methods
BD	VITIC	Plant	(+)	**BDp 501**: Pruning weight, phytoalexin content and chlorophyll index
		Soil	(+)(-)	**BDp500**: It needs well nourished soils

II.2 Influence of Silica

II.2.1 Scientific Approach: Silicon

Silicon (Si) is a metalloid element containing four equivalent bonds organized in a tetrahedral structure which gives it a great versatility, usually compared with Carbon in terms of the diversity of Si compounds formed. It is relatively inert, with high melting and boiling point as well as very low solubility in acids. In alkaline media its solubility increases (Royal Society of Chemistry 2015, Wikipedia 2015).

Si has been described as one of the major elements in Earth's crust, found in more than 370 specific rock-forming minerals, usually present as oxide (SiO_2). Despite its abundancy, its concentration in soils is highly variable, from <1 up to 45 wt.-% Si, in part due to the nature of parental rocks and the impact of soil formation processes. In fact, the weathering process of silicated minerals has an important role in regulation of atmospheric CO_2, as well as the flux of Si from mineral sources to the oceans does (Sommer et al, 2006; Struyf et al., 2009). Also, in forests has been observed that 15 to 85 % of Si in soil has biological origin, through litterfall and breakdown of Si contained in plants (Guntzer et al., 2012).

Regarding soil solution and interactions solid/dissolved Si, the presence of aluminum hydroxides, iron oxides and carbonates seems to influence strongly, as well as soil pH, temperature, presence of cations and organic compounds (Sommer et al, 2006). Concentration of Si in soil solution can vary considerably, from 100 to 500 μM (Struyf et al., 2009), but its form is usually the same, the monomeric orthosilicic acid [$Si(OH)_4$] or its ionized form [$Si(OH)_3O^-$], which is able to make Si available for plant uptake (Currie & Perry, 2007), either passively with water flow or actively through specific mechanisms (Struyf et al., 2009).

Historicaly, Si has not been considered an essential element for plant life. Excluding some aquatic organisms like algae or diatoms, and terrestrial from Equisetaceae family, the ability of plants to accumulate $Si(OH)_4$ varies from 0.1 to 10% of shoot dry weight (Currie & Perry, 2007). Once $Si(OH)_4$ has penetrated inside the roots and xylem, the main factor regulating transport and deposition of $Si(OH)_4$ on plants shoots is the transpiration rate (Guntzer et al, 2012). Where the accumulation of $Si(OH)_4$ exceeds a concentration of 100–200 mg/kg, a polymerization happens leading to formation and precipitation of more complex silica compounds, said amorphous silica, able to interact with the environment inside cell walls, thanks to its negative charge. Because many factors affect this poly-condensation, a huge diversity of silica solid compounds with different properties and different concentrations can be found, even in organs of the same plant. In all cases silicas formed are built up from the same monomer, $Si(OH)_4$ (Currie & Perry, 2007; Guntzer et al, 2012).

Due to its ability to perform in different plant functions (structural, physiological), Si has an important role alleviating plant stress in general. It may increase resistance to pathogens, insects, wind and rain, alleviate stresses by drought, salinity, mineral toxicity or radiation and it can regulate nutrient uptake (Guntzer et al, 2012). All mechanisms involved are still under discussion, but extensive research, particularly in Si 'acummulator' crops like rice, has documented these benefical properties (further information in the Proceedings of th 6th International Conference of Silicon in Agriculture 2014)

All these benefits coming from Si presence in soils and plants had led to an increasing interest from farmers and researchers in order to promote and protect Si sources. In this sense, the Si recycling has become a crucial subject for agricultural ecosystems, because crop extraction risks to deplete Si in agricultural lands. To face this problem many Si fertilizers has appeared as an alternative, especially those made from crops straw, because of its high content of silica (Guntzer et al, 2012). Also it has been found that faeces of domestic herbivores have 2 to 4 times higher values of readily soluble silica compared with grasses and hay, suggesting an unsuspected impact of herbivore digestion on Si solubility and consequently, on Si cycle (Vandevenne, 2014).

Concerning grapevines, Reynolds et al. (2014) tested the effect of Si fertilization on induced plant defense against insect attacks. The attraction of the predatory beetle *Dicranolaius bellulus* for grapevines infested with larvae of *Epiphyas postvittana* was significantly increased in those plants having the highest silicon tissue content. In a parallel study the authors identified seven volatile compounds released by grapevines infested with *Phalaenoides glycinae*, where *n*-heptadecane production had a significant increase and *Cis*-thio rose oxide production a significant decrease, only on silicon treated vines.

II.2.2 Horn Silica – Biodynamic Preparation (BDp) 501

Well aware of silica abundancy in nature and its properties, Steiner also highlighted the structural role it has in plants, but understood as a balance between silica (Silicon) and lime (Calcium). From his perspective both elements support carbon role as shaper of plants framework, but in opposite ways, lime try to sucks everything from plants pulling downwards while silica wrest away from lime what should be wrested away radiating upwards, into the atmosphere. Furthermore, he associated this lime tendency to earthly formative forces influenced by closer planets, said Mercury, Venus and Moon, instead of silica which brings cosmic formative forces coming from distant planets, said Mars, Jupiter and Saturn. In a similar way of thinking this author stated that lime has a role in the inner ability of plant to growth and reproduction, considered as processes where the only goal is to renew its own specie, meanwhile silica influences fruit development, looked as a phenomena through which the plant can nourish other species (Steiner, 1993).

BDp 501 recommended by Steiner is made from quartz rock crystalline, preferably from the purest possible, which is crushed and grinded until a fine colloidal state. Water is added to form a paste, then is stuffed into a cow horn and left to mature buried in soil during the summer months. It is considered an essential preparation for BD agriculture, complementary to Horn Manure BDp 500 (Masson, 2012).

Cow horns have a role streaming formative forces inwards, into the digestive system, in such a way that digestive processes are fully achieved (Steiner, 1993). Consequently, in the buried horns with silica or manure they work similarly, preserving formative forces already present in manure or silica as well as preserving those formative forces coming from the earth around, during the maturation period (Steiner, 1993).

Considering the physical properties of silicate crystalline minerals like quartz, said low volatility, low solubility, high melting point (1710 °C) and high resistance to mechanical pressure, it has been suggested that quartz crystals have low affinity for air, water, heat or earth, respectively. But, regarding light is totally different because of the remarkable permeability of quartz crystals to light (Julius 2010).

Consequently, Masson (2012) describes Horn Silica as an application of light that helps to improve vitality during the vegetative growth stage. This author states that this preparation brings a luminous, crystalline quality to plants and mitigates tendencies towards disease; moreover, it gives to plants a better relationship with the cosmos as well as reinforces sunlight effect. Because of that it is important in low-sunlight environments and greenhouses, compensating the lack of light, balancing the excess of heat and humidity.

According to Bouchet (2013), BDp 501 stimulates forces of fructification supporting the flower in its ability for searching cosmic influence which will lead it to produce a fruit and Joly (2008) warns about its effect on bare soils reducing the cryptogrammic life into 20 cm depth as well as its potential to increase hydric stress if the season is dry and hot.

Masson (2012) also states that BDp 501 is crucial for internal structures of plants and their development. It reinforces shoots verticality and minimize any tying up of vines, it strengthens plants by increasing flexibility, quality and resistance of leaf and fruit epidermis.

As well, it improves the nutritive quality of grapes, intensifying taste and aromas (Joly 2008, Masson 2012).

Silica has been recognized as curative impact in human health because its close relationship with human senses (Steiner, 1993), involved in the formation of nerves, function of brains, eyes and hair growth (Pfeiffer, 1966). All plants used to complement BDp 501 in this experiment, said Yarrow, Stinging Nettle, Valerian and Oak bark are already include in other BD preparations applied to the compost (Appendix 1) and they as well are known by their beneficial effect in medicine.

II.2.3 Time on Biodynamic Management

Time in BD management is understood as net of rhythms, terrestrial and cosmic, which regulates life processes. Rhythms of the Sun, Moon and planets are in connection with mineral, plant and animal cycles and that is why the BD preparations are conceived according to the four seasons of a solar year (Koepf 1998).

With respect to annual plants, the most remarkable influence is that of the Moon, precisely because of its shorter cycle (Steiner, 1993). Experiments done in wheat for nine years related to the synodic rhythm of the Moon, concluded that plant growth during first 15 days after sowing is better during the waxing Moon phase than during waning Moon phase. Simultaneously, the same author found in maize that Moon influence can be increased if plants are sown 2 days before full Moon, getting better yields than those sown 2 days before new Moon (Kolisko, 1978). For both experiments the water supply has to be adequate either from rain or from irrigation, because there is 'a definite connection between the Moon and the water on the Earth' (Steiner, 1993).

Furthermore, Thun (2008) described the influence of the Moon when passing in front of the twelve zodiac constellations, known as the sidereal rhythm of the Moon, which lasts 27 days. Grouping the constellations in 4 *trigones* associated with the elements earth, water, air/light and heat/fire, this author stated that when the Moon pass through each constellation it promotes the develop of roots, leaves, flowers and fruits, depending on which element is associated to the current constellation. Based on this long term work, but also considering the moments when the Moon is closer or farther to the Earth called Perigee/Apogee, as well as phases of the Moon related to the plane of Earth elliptic orbit called Ascending/Descending; a calendar of seed sowing was created, which has been used and improved by many BD farmers and winegrowers through the years.

The influence of distant planets has been approached with much more prudence, mainly due to the length of their rhythms, i.e. Saturn takes 30 years for one cycle around the Sun, and Jupiter, 12 years. About that Steiner suggests the influence of distant planets (Mars, Jupiter and Saturn) is related to air temperature, only if the air is warm then their formative forces can reach plants. This can be observed in the bark of trees and anything else that make plants perennial (Steiner, 1993).

Nowadays, all these concepts and statements are difficult to grasp and putting together in order to take practical decisions in viticulture management, but as Steiner said, these are only guidelines which will provide bases for long term experiments that will lead to brilliant results if worked into agriculture in an experimental basis (Steiner 1993); that is exactly what this research project has tried to do.

Table 2. Summary Literature Review of Influence of Silica

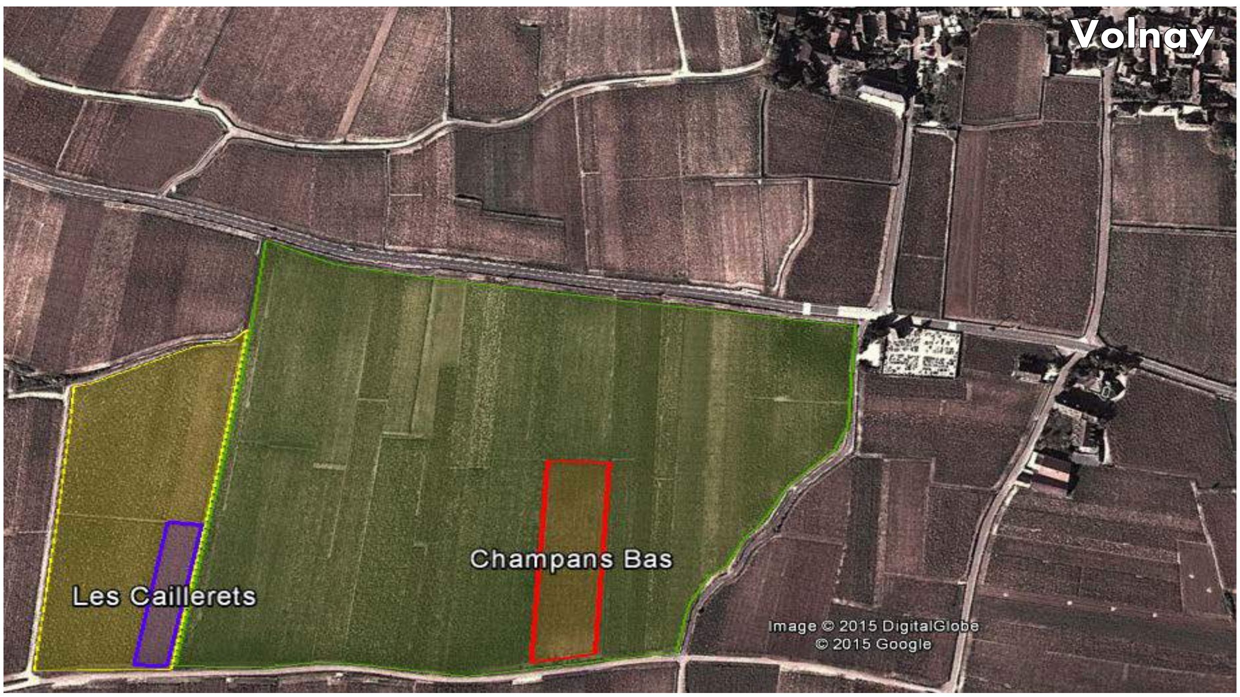
INFLUENCE OF SILICA	SCIENTIFIC APPROACH	Chemistry	Silicon has a tetrahedral structure, is inert, versatile, able to form a huge diversity of compounds
		Ecology	Omnipresent, in parental rocks; <1 up to 45 wt.-% Si, in soil solution 100 to 500 µM, in plants 0.1 to 10% of shoot dry weight, it can vary greatly in different organs of the same plant
		Ecology	Regulate atmospheric CO_2 through weathering of silicated minerals and flux to oceans; in plants is present as Orthosilicic Acid $Si(OH)_4$; in forests plants can provide 15-85% of Si in soil; increases 2 to 4 times solubility through herbivore digestion, has a role within plants cell wall
		Agriculture	Role alleviating plant stress: increase resistance to pathogens, insects, wind and rain, alleviate stresses by drought, salinity, mineral toxicity or radiation
		Agriculture	Role regulating nutrient uptake Si fertilizers mainly made from biological origin, like crops straw
		Viticulture	Increase resistance to insects releasing volatile compounds to attract natural
	BIODYNAMIC APPROACH	Silica	Connected to cosmic formative forces from distant planets (Mars, Jupiter, Saturn) It has to be in balance with Lime, connected to earthly formative forces from closer planets
		Silica	Curative impact in human health, related to human senses, nerves, brains, eyes and hair growth
		Quartz	Raw material for BDp 501, is permeable to light meaning it has affinity for light
		BDp 501	Structural role in plants; reinforces shoots verticality, increases flexibility, quality and resistance of leaf and fruit epidermis, mitigates tendencies towards disease, intensifies grapes taste and aromas
		BDp 501	Complementary to Horn Manure BDp 500, it is an application of light, reinforces sunlight effect, vitality, brings a luminous and crystalline quality to plants, improves relationship with the cosmos, it can reduce cryptogrammic life on bare soils, it can increase hydric stress in hot seasons
		BDp 501	Connection to fruit development in order to nourish other species
		Time	Moon phases are considered to define a root, leaf, flower and fruit day

III SETTING OF THE EXPERIMENT AND METHODOLOGIES

III.1 Location of Experimental Fields

Both experimental fields were placed below Volnay, in the lower part of an area qualified as Burgundian *Premier Cru* (Fig. 1). They belong to neighboring appellations (‘*Climats*’), called En Champans and Les Caillerets, which contain as well many other small properties of different winegrowers but all gathered under the same appellation. Here the priority is the name of the *climat* over the name of the producer. Both parcels are 200 m far from each other.

Figure 1 Experimental Fields at Volnay. Parcel Champans Bas (red) in the AOC Village Premier Cru En Champans (green), parcel Les Caillerets (blue) in the AOC Village Premier Cru En Caillerets (yellow)



III.1.1 Champans Bas

Owned by Domaine Marquis D’Angerville, the parcel Champans Bas is located in the central lower part of the *climat* En Champans, a slightly sloping south-east exposed area. It is a young parcel (10 years old), in transition of trellis system, homogeneous in terms of vigour although some weaker small areas can be distinguished. 2015 was the 9^{th} season under BD management so certain expertise has been developed leading to a well-established vineyards with a defined viticulture strategy. General data are listed below (Table 3).

Table 3. Parcel Champans Bas at Volnay, Burgundy, general data.

LATITUDE	46°59'46'' N
LONGITUDE	04°47'04'' E
HEIGHT	247-258 m
SURFACE (HA)	0.6
YEAR OF PLANTATION	2005
VARIETY	Pinot Noir
CLONES	828, 943, 114, 115, 777
MASAL SELECTION	*ATVB Fin
ROOTSTOCK	161-49
DENSITY OF PLANTATION	1.2 x 0.8 m
VINES/HA	10,000
AVERAGE YIELD 2008-2011 (HL/HA)	40.25
AVERAGE YIELD 2012-2014 (HL/HA)	21.93
N° ROWS:	35
N° BUDS / PLANT (PRUNING CRITERIA)	8-10
EXPECTED YIELD (HL/HA)	35-40
TRELLIS SYSTEMS	Cordon Royat / Guyot Simple

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Source: D. Marquis D'Angerville

MANAGEMENT. The season started in winter 2014 with a 33% of the parcel changed from Cordon Royat into Guyot pruning system. BDp 500P or reinforced Horn Manure (Masson 2012) was applied twice, after harvest on 22nd September 2014 and in spring on 28th April 2015 at dose 130 and 4 g/ha respectively. In both cases BDp 500P was applied together with an infusion of Valerian (*Valeriana officinalis*) at 6 and 5ml/ha. In spring, more precisely on April 1st of 2015 100g/ha of decoction of Horsetail (*Equisetum arvense*) was applied.

Regarding the soil management there was no fertilization during 2014 neither 2015. The last fertilizer applied was 2 ton/ha of compost in 2013. Earthing-over rows was done in October 2014 very softly in a way that earthing-down occurs naturally without any intervention. A superficial tillage was done over the inter-row twice, on March 24th and April 9th; then another one was performed over the row 3 times on April 16th, May 15th and July 7th, at 5-7 cm depth.

To control pests a particular mixture was applied 8 times during the season, at progressively variable dose: 90 lt/ha for the treatments one and two, 150 lt/ha for applications three to five, and 200 lt/ha for treatments six to eight. It was composed by

Bordeaux Mixture at 180 g/ha, an average of 9 kg/ha of wettable Sulphur 80% and 50ml/ha of propolis as a base for every application. Herbal infusions were added as well but also in different combinations and dilutions: Stinging Nettle (*Urtica dioica*), *Osier* (genus *Salix*) and Horsetail at dose of 100 g/ha, Yarrow (*Achillea millefolium*), Dandelion (*Taraxacum officinalis*) and Chamomile (*Chamomilla Officinalis*) at dose of 10g/ha. The specific combination applied changed every time, depending on the wheather and the phenological state of the plants. The first application was on May 7th and included B.Mixture-Sulphur-Propolis-*Osier*-Horsetail-S.Nettle, second and third one were on May 18th and 28th respectively, with B.Mixture-Sulphur-Propolis-*Osier*-Yarrow-Dandelion. From the fourth application until the eighth, which means June 8th, 18th, 30th and July 15th, 30th, the combination was B.Mixture-Sulphur-Propolis-*Osier*-Yarrow-Chamomile. The only exception was the sixth application where B.Mixture was not added. Sulphur powder 96% was added 2 times in dose of 35 and 25 kg/ha on June 5th and 26th.

Regarding canopy management, as it is traditionally in Burgundy vines had a shoot thinning during the first phase of growth, on May 28th, leaving 2 shoots for the spur and 6-8 for the cane. Also shoots were positioned inside the wires of the trellis system twice in the season. After that a hedging was performed 3 times, monthly, on June 16th, July 13th and August 17th focusing only on the upper part of the canopy the first time and the rest including a lateral topping. Harvest was on September 6th.

SOIL. A private association called *Groupement d'Etude et de Suivi des Terroirs* (GEST) studied the soil in Champans Bas using the method BRDA-Hérody. This method qualifies clays and fine silts according to their potential ability to bind minerals [Coefficient of Fixation (CF)] and organic particles in a structure called Mineral-Organic Complex (COM), influenced as well by the presence of Calcium (Ca) and Magnesium (Mg) grouped as bases [Alkaline Earthy (AT)] and Iron (Fe). The potential to form these COM, determined by the nature of parental rocks and soil 'age', is connected with the ability to make organic matter available for plants and consequently, it will support a recommendation for soil management, specifying type, dose and frequency of organic fertilizers. This method has developed a great knowledge based on the geochemistry of calcareous, typical from Burgundy (GEST 2001).

Champans Bas was described in general terms as a quite homogeneous soil, brownie-reddish and calcareous, which all along the slope gradually became thicker, until 60cm depth. It was formed by elements eroded coming from the top of the hill, fine silts, clays, a lot of sands and angular calcareous pebbles.

Looking at the profile (Fig. 2) described by the GEST, the surface around 10 cm depth showed a strong activity of earthworms, high porosity, round aggregates and rootles from adventitious roots. The layer just below was more clayey, with a slightly lower porosity, bigger and more angular aggregates, increasing risk of compaction. Under 35 cm depth porosity improved again thanks to shrinkage cracks presence. At 60 cm depth appeared a calcareous rock of bioclastic nature (composed by corals, shells, etc.) in slow alteration. This rock disintegrates in flat pieces of few cm of thickness, which are able to release sands, shell fragments and iron oxides. Roots were present all along the profile, even under 60 cm, profiting from crack in the rock to go deeper, where humidity is well preserved even during summer.

Figure 2. Soil profile from Champans Bas at Volnay, Burgundy, 2001.

Source: GEST

Interpreting the results of soil analysis (Table 4), CF was medium in surface and rock level (CF=1.6) and calcareous character on surface was low (AT=2.5, CA=6%), creating a COM of biological type which offers a good nutritional regulation to vines. But, not enough to hold more than 5 ton/ha of compost without risk of lixiviation. Also, erosion was not significant when comparing the upper and lower zone of the field.

Total content of organic matter (MTO) was well correlated to those values of CF, but the rate of organic matter easily mineralisable or 'fugitive' (MOF) was weaker on the lower zone so, a slight fertilization was proposed with 10 to 15 units of Nitrogen (N) from organic sources. Soil bases (AT, CA) showed an excess of lime, very saturated (important for rootstock tolerance) but a good mobility of Fe so no problems of structure. Potassium (K) was well supplied but Mg was recommended to add yearly with 200 Kg/Ha of Mg sulphate.

Table 4. Soil analysis for Champans Bas at Volnay, Burgundy.

DATE OF SAMPLING	2001			1996			
PLOT SAMPLED	Champans			Champans Bas			
				Upper Zone		Lower Zone	
DEPTH (ZONE OR CM)	Surface	Mid	Deep	0-5	30-40	0-5	30-40
AT ALKALINE-EARTHY	2.5	2.3	4.0	3.5	3.5	2.0	2.1
CA ACTIVE LIME (%)	6.0	5.0	11.0	20	20.0	10.0	10.0
CF COEFFICIENT OF FIXATION	1.6	2.3	1.7	1.5	1.8	1.5	2.7
MTO TOTAL ORGANIC MATTER (%)	4.1	3.4	3.0	2.5	2.7	4.0	2.7
MOF FUGITIVE ORGANIC MATTER (%)	26.8	18.6	22.3	22	20.0	14.0	22.0
HS STABLE HUMUS (%)	73.2	81.4	77.7	78	80.0	86.0	78.0
K POTASIUM	5+	1	1.5	5	3	5	4.0

***GEST (1996, 2001)**

Source: D. Marquis D'Angerville

III.1.2 Les Caillerets

Belonging to Domaine La Pousse D'Or, Les Caillerets belongs to a *climat* with identic name, as well with south-east exposition and slight slope. Vines are young (12 years old), very homogeneous, being managed under BD principles for first time. Some general data are listed below (Table 5).

MANAGEMENT. Vines in Les Caillerets started the BD management in winter 2014, this included the treatments with BDp 500P applied twice, after harvest on 24th October and beginning of spring on 23rd April 2015 at dose 100 g/ha.

Soil management is very important during transition period from conventional to BD, it started with a fertilization with compost the first week of November 2014, at dose of 5 ton/ha. Then, earthing-over rows in end of November - beginning December, to finally earthing-down at beginning April 2015, as closest as possible to budbreak. Weeding was done mechanically through a superficial tillage 4 times between beginnings of May until beginnings of July 2015, at 4-5 cm depth.

Concerning treatments against pests, a specific combination of product was applied 9 times during the season, at dose 150 lt/ha. It was composed by Hydroxide of Copper (Champlo™) at 252 g /ha, Sulphur 10 kg/ha together, herbal infusions of Stinging Nettle

Table 5. Parcel Les Caillerets at Volnay, Burgundy, general data.

LATITUDE	46°59'38'' N
LONGITUDE	04°46'59'' E
HEIGHT	246 – 252 m
SURFACE (HA)	0.25 Ha
YEAR OF PLANTATION:	2003
VARIETY:	Pinot Noir
CLONES:	777, 828
MASAL SELECTION:	*ATVB Fin, ATVB *Moyen*
ROOTSTOCK:	161-49
DENSITY OF PLANTATION:	1 x 1
VINES/HA:	10,000
AVERAGE YIELD 2006-2011 (HL/HA)	37.1
AVERAGE YIELD 2012-2014 (HL/HA)	18.8
N° ROWS:	20
N° BUDS / PLANT (PRUNING CRITERIA)	9-10
EXPECTED YIELD (HL/HA)	35-40
TRELLIS SYSTEM:	Guyot Simple

***ASSOCIATION TECHNIQUE VITICOLE DE BOURGOGNE**

Source: D. La Pousse D'Or

(*Urtica dioica*) and *Osier* (genus *Salix*) at dose of 10 lt/ha from the second to the ninth application, also concentrated maceration Stinging Nettle at 10 lt/ha only the first application and decoction of horsetail for the second and third applications. Sulphur was added as powder 2 times as dose 25 kg/ha on 29th May and 8th June.

Regarding canopy management, as it is traditionally in Burgundy, vines were manage with a shoot thinning on 28th to 30th of April leaving 2 shoots for the spur and 4-5 for the cane. Then on 15th of May shoots were positioned inside the wires of the trellis system and again on 2nd -3rd of June because of the natural difference in growth rate. After that a hedging was performed 4 times, the first one manually on 12th June and the rest mechanically once per month, 24th June, 20th July and 19th August. To finish the season grapes were harvested on September 5th.

SOIL. Soil analysis (Table 6) gives an idea of the character of the soil, for example the high content of fine particles, more than 50% adding clays plus fine silts, would suggest that water and nutrients are naturally well regulated. Lime content and carbonates is typical from the region and even though the organic matter content is medium, it can be enough to promote the structure of the soil and enhanced vines uptake of nutrients.

Table 6. Soil analysis for Les Caillerets at Volnay, Burgundy.

SOIL	Silty – Clayey	
GRANULOMETRY (%)	Clay	36.7
	Fine Silt	26.0
	Coarse Silt	14.2
	Fine Sand	6.2
	Coarse Sand	17.0
ORGANIC MATTER (%)	2.2	Medium
TOTAL CARBONATES (%)	30.3	Medium High
ACTIVE LIME (%)	8.1	Medium High
PH WATER	7.9	High
PH KCL	7.5	Excessive
RESISTIVITY MOHM	8065	Low
C/N RATIO CARBON/NITROGEN	12.4	High
ANHYDRIDE PHOSPHORIC JH (MG/KG)	126	Low
K POTASIUM (G K2O/KG)	0.39	Med High
CA CALCIUM (G CAO/KG)	11.76	High
MG MAGNESIUM (G MGO/KG)	0.18	Medium
K/MG (%)	0.9	Medium
CA/MG (%)	45.9	Medium

*LABORATOIRE DEVELOPPEMENT MEDITERRANEE (2002)

Source: D. La Pousse D'Or

III.2 Treatments Description

In both parcels each application of BDp 501 Horn Silica was done following rigorously the advice of Pierre Masson, expert consultant in BD Agriculture, as well as the choice of date of application was based on the 'Agenda Biodynamique Lunaire et Planetaire' of BioDynamie Services (Masson 2015). The product itself, BDp 501 powdered, was supplied by BioDynamie Services as well, stored outside in a hermetic glass-made container, in a dry place, exposed to daylight and isolated from wi-fi networks.

The procedure of application started early in the morning, 4 g of BDp 501 were diluted in 35 lts of rain water (0.11 g/lt), just after started the dynamization process. This process begins stirring the solution in one direction, vigourously, until a vortex is created at the bottom of the container; then, through a sequence of stop / wait for 1-2 seconds / restart stirring in the opposite direction, a small turbulent chaos is created in the water, which is the main goal of the dynamization. To insure repeatability and a good rhythm, the process was done automatically with a comercial copper-made dinamizer Eco-dyn™. After 1 hour of continuing dynamization, the BDp 501 was applied to the vineyards, through manual spraying. The whole process never finished later than 8:30 am to avoid high temperatures.

III.2.1 Champans Bas

In this field 5 treatments were defined following 4 different scheme of application date for BDp 501:

- 3 Before Flowering (BF)

  13th May at 6 to 8 leaves extended, ascendant Moon, Flower/Leaf day

  18th May at 7 to 9 leaves extended, new Moon, opposition Moon-Saturn, Root day

  26th May at > 9 leaves extended, descendant Moon, Fruit day
- 3 Between Flowering and Harvest (FH)

  17th June at Pea-size, descendent Moon, Flower day

  07th August at Veraison, ascendant Moon, opposition Moon-Saturn, Fruit/Root day

  26th August at Ripening, ascendant Moon, Fruit day
- 3 After Harvest (AH)

  30th September at Leaf discoloration, ascendant Moon, Fruit day

  3rd October Leaf discoloration, ascendant Moon, Root day

  16th October at Leaf discoloration, descendent Moon, Flower/Leaf day
- 2 to 5 according to winegrower criteria (WG)

  18th May at 7 to 9 leaves extended, new Moon, opposition Moon-Saturn, Root day

  26th May at > 9 leaves extended, descendant Moon, Fruit day

  03rd June at mid blooming, ascendant Moon, Leaf/Fruit day

  26th August at ripening, ascendant Moon, Fruit day
- Control (CO)

III.2.2 Les Caillerets

5 treatments were defined for this field, applying BDp501 Horn Silica following the 'Winegrower' modality at Champans Bas for date and number of applications (18th May, 26th

May, 03rd June, 26th August), but changing the composition of the product adding different infusions, as follows:

- 501 pure (SP)

  Dosage Horn Silica: 4 g/ha
- 501 + Infusion of Yarrow (*Achillea millefolium*)/Stinging Nettle (*Urtica dioica*) (YN)

  Dosages: Horn Silica: 4 g/ha   Yarrow and S.Nettle: 20 g/ha
- 501 + Infusion of Valerian (*Valeriana officinalis*) (VL)

  Dosages: Horn Silica: 4 g/ha   Valerian: 5 ml/ha
- 501 + Infusion of Oak Bark (*Quercus robur*) (OB)

  Dosages: Horn Silica: 4 g/ha   Oak bark: 20 g/ha
- Control

Infusions were done early in the morning, before Silica dynamization. 5g of dried Yarrow flowers and 5g of dried Stinging Nettle leaves were put together into 300ml of water. 5g of Oak bark were put into 150 ml of water. Both pots were heated until boil, then immediately stopped to led them macerating for 10 minutes and finally filtrated with a common kitchen strainer. The Valerian infusion was supplied by BioDynamie Services.

Once at the vineyards, the first treatment to apply was always the Horn Silica pure, then each infusion was added to the sprayer just before application, with a short manual homogenization inside the sprayer.

III.3. Reducing Experimental Error

Once the main hypothesis of the experiment was defined with its corresponding treatments or *variable factors* (date of application and additives) it was necessary to control some *nuisance factors* which could possibly affect the results (Montgomery 2001). This process, known in statistics as *Local Control* improves the estimation of the experimental error, helps keeping this experimental error low and increases the general precision of the experiment. It can be made removing the undesirable effect through blocking techniques, keeping the factor constant or measuring the variability of the factor and then adjusting it through data analysis techniques (Gomez and Gomez 1984, Jayaraman 1999, Montgomery 2001, Hoshmand 2006).

In this sense, two sources of variability, age and trellis systems, were identified in Champans Bas counting all plants conducted on Cordon Royat and Guyot (Table 7). At the same time younger or replanted vines were counted in both parcels and then avoided during measurements, especially those related to vegetative development.

Fortunately, the ratio Cordon Royat/Guyot was enough homogeneous to ignore its variability, except for some measurements where the difference could influence significantly the results. This mixed trellis system is part of a process of conversion from Cordon to Guyot, so every year the proportion will change and eventually it will disappear.

Table 7. Percentage of different trellis systems, missing and replanted plants for each treatment in both experimental fields at Volnay, Burgundy, 2015.

Champans Bas						
Treatment Plots	WG	YN	FH	AH	CO	Mean
Cordon Royat	57.1 %	58.0 %	56.0 %	57.4 %	56.8 %	**57.06 %**
Guyot	36.0 %	34.6 %	34.0 %	36.0 %	33.4 %	**34.80 %**
Replanted / Absent	6.9 %	7.4 %	10.0 %	6.6 %	9.8 %	**8.14 %**
Les Caillerets						
Treatment Plots	SP	YN	VL	OB	C	Mean
Guyot	95.3 %	98.4 %	97.9%	95.8 %	95.8 %	**96.64 %**
Replanted / Absent	4.7 %	1.6 %	2.1 %	4.2 %	4.2 %	**3.36 %**

Soil heterogeneity intra-field was addressed reviewing soil studies described before. Also, a minimum of homogeneity should be accepted given that the AOC system gave their names to Les Caillerets and Champans precisely because each of these areas has its own particular features, similar intra-parcel but different inter-parcel, good enough to consider them as unique *Terroir* or *Climat* (BIBV, 2015).

Moreover, this information was compared to updated photos from the same source (GEST) and a soil profile observation made through 9 samples done manually in different sectors of the parcels (Fig. 3 and Fig. 4). The sampling should follow a strategy in order to be representative, in fact, sample size can be calculated using the margin of error from previous experiences (Gomez and Gomez 1984, Jaggi and Varghese 2007) (Appendix 2). That kind of information was not available in this case so, assuming the experimental design as randomized blocks, the minimum size for a sample is equal to the number of blocks, then 4 samples were taken in Champans Bas, 5 in Les Caillerets. No soil analysis was performed.

Figure 3. Soil samples in parcel Champans Bas at Volnay, Burgundy, 2015.

Figure 4. Soil samples in parcel Les Caillerets at Volnay, Burgundy, 2015.

Concerning viticulture variability, each field has to be managed as a whole plot, identical in time, frequency and intensity of practices, as it was described in point III.1. Remaining sources of variability, like macro and mesoclimate factors, sunlight exposition or water availability were assumed as equal for each plot and only two factors, rootstock and clone, were deliberately ignored because they were randomly mixed and planted several years ago.

Finally, to overcome the variability coming from those sources ignored or impossible to control, each treatment was *replicated* 4 times and *randomized,* distributing them independently over the fields. This two processes reduce the experimental error and increase the precision on estimating the effect of BDp 501. Although that precision will be enhanced as much number of replicates are set up, 4 was considered enough to provide valid results with proper accuracy, despite the fact that more than 4 would have been too difficult to manage. (Jayaraman 1999, Montgomery 2001, Hoshmand 2006)

III.4 Experimental Design

Most of the experimental designs used in agriculture are assorted depending on the number of factors under study, in this sense, higher is the numbers of factors higher will be the complexity because of increased number of variables and interaction effects (Gomez and Gomez 1984, Montgomery 2001, Hoshmand 2006). In this research, only one factor was deliberately manipulated in each field, date of application of BDp 501 for Champans Bas and additives to BDp 501 for Les Caillerets, so that a *Randomized Complete Block Design* (RCBD) was chosen, well fitted for experiments with 5 treatments (4 treatments + control) and replicated 4 times.

The slope of the land created a potential gradient in soil heterogeneity especially on surface, so each block containing all 5 treatments was disposed along the slope and each treatment was assigned randomly to each replicate (Fig. 5 and 6). In this way the variability within each block was **minimized** and the variability among blocks was **maximized**, allowing to set homogeneous experimental units of equal size. (Gomez and Gomez 1984, Jayaraman 1999).

Concerning the size of the experimental units (replicates), plots with 6 rows-width and with 45 plants-length were set up in Champans Bas while in Les Caillerets were units of 4 rows-width and 24 plants-length. Taking into account the density of plantation of each field, the surface destined for each replicate was 259 m^2 and 96 m^2 respectively. In order to insure independency on the treatments and minimize contamination risks all measurements were done in the central rows of each replicate.

Finally, the selection of the *response variables* giving useful information about the questions addressed (Montgomery 2001), were focused on vegetative and re-generative aspects. Lack of scientific references about BDp 501 in viticulture led this research to scan multiple variables through a systematic monitoring all along the season, as a kind of initial period of general characterization. Results will define more specific approaches in the future.

Figure 5. Experimental design in parcel Champans Bas, at Volnay, Burgundy, 2015.

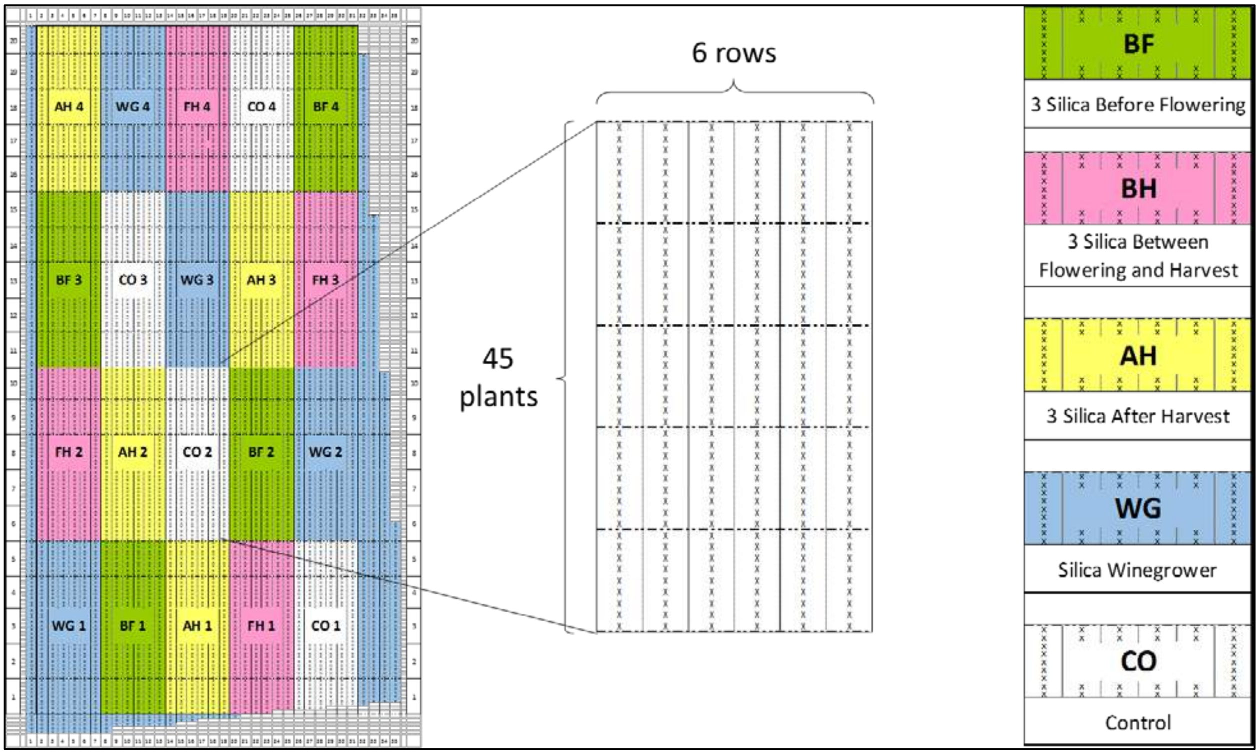
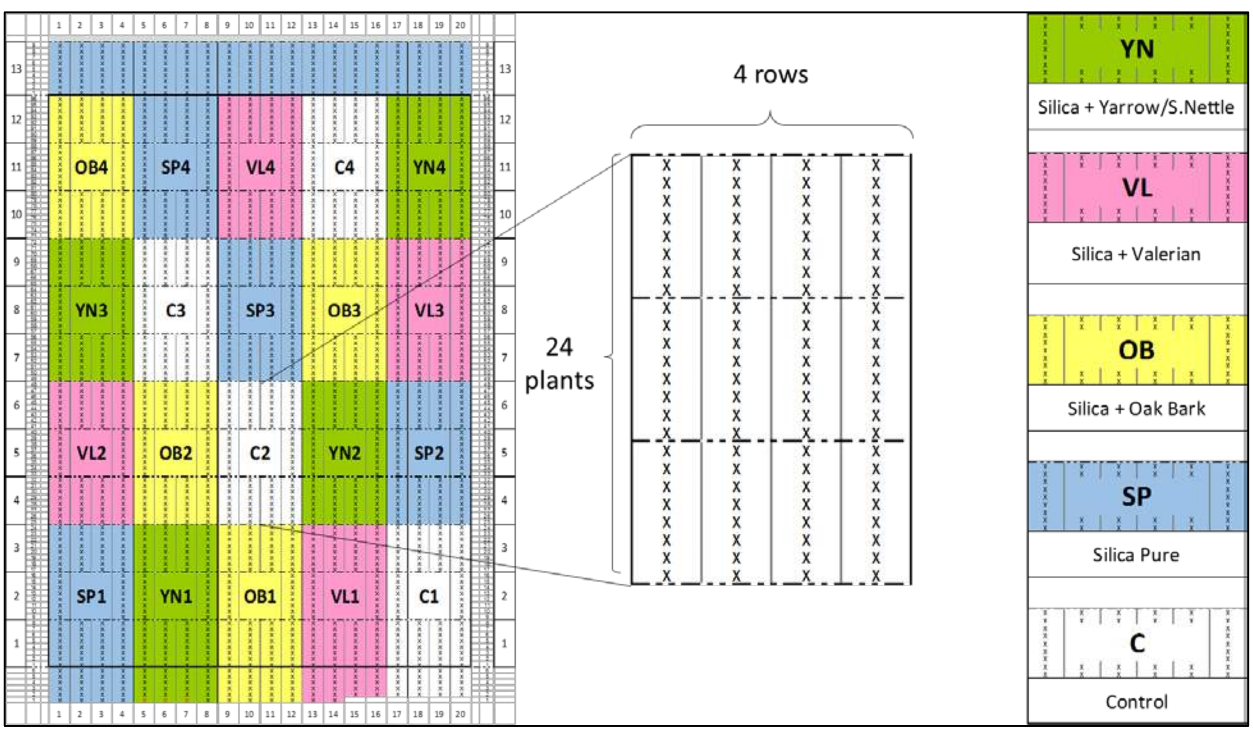


Figure 6. Experimental design in parcel Les Caillerets at Volnay, Burgundy, 2015.

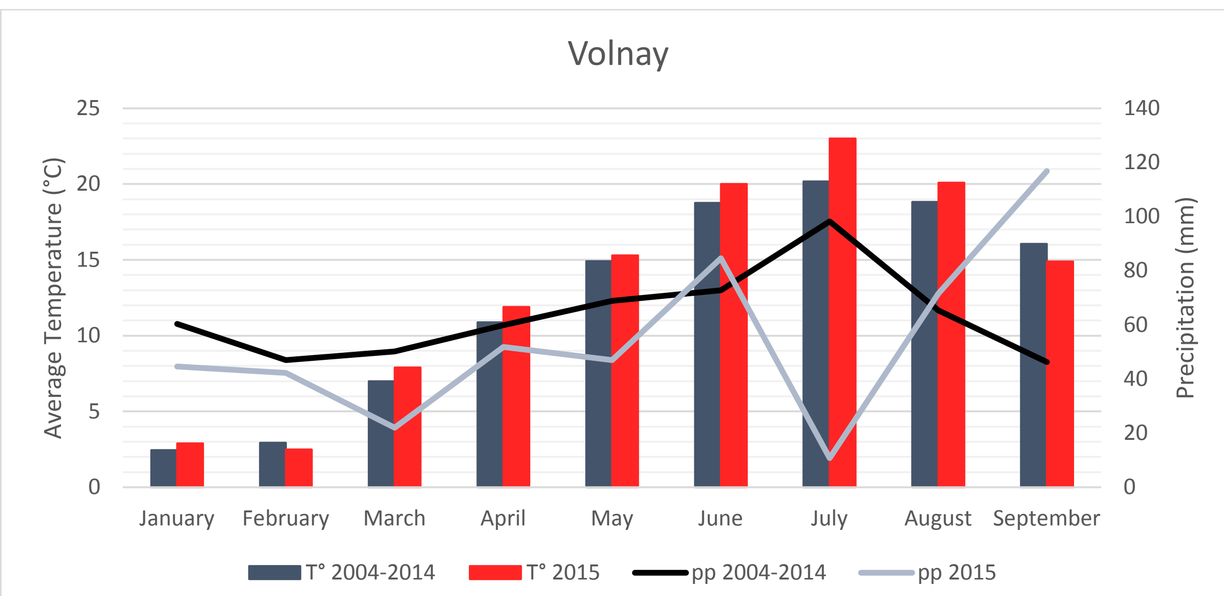


III.5 Climate Conditions

Burgundy has a privileged location where converge three different climatic influences: meridional, oceanic and continental. Globally tempered, it has an average temperature in summer around 20°C, rainfall close to 700 mm/year usually concentrated over May and June and approx. 1300 sunshine hours between April and September which are the key to avoid frost in winter and to reach full maturity in summer. Western winds are carriers of humidity but northern winds regulate it. Volnay is located in Côte de Beaune, one of the typical *coteaux* placed between 200-500m height, with south-east exposition which allows vines to profit from the best sunshine hours. Consequently, is described as a region with hot summers and dry autumns coming from continental influence (BIVB 2015).

Hail is nowadays the greatest concern about weather for winegrowers in Volnay, last 3 years it has been damaging vines and reducing yields dramatically. This phenomenon occurs mainly during thunderstorms and has been monitored in France by the National Association of Study and Fight against Atmospheric Catastrophes (ANELFA) for decades. This 2015 was free of hail, nevertheless vines production was low (~25 HL/Ha), suggesting that the damage from three precedent years is still affecting them.

Figure 7. Ombrothermic diagram of Volnay during season 2015.



Source: Cooperative Vitivinicole de Beaune

Season 2015 at Volnay was hot and early, but considered as a very good one from climatic point of view (Fig. 7). Right from March the weather was drier than historic and temperatures were warmer but still under 20°C as maximum. April warmed up overpassing 20°C maximum and only a couple of days were below zero. Rainfall increased as well concentrated on the beginning and the end of the month but still not enough to reach the historic average. These conditions allowed buds to break without much risk of early pests.

In May precipitations remained quite stable but again concentrated on the first three days of the month. Temperature continued to increase with minimum never below 3°C and maximum for first time surpassing the barrier of 30°C in one day, 13th, promoting shoot growth. When the weather became hotter in June, 70 mm of precipitation on two days, 14th and 15th, prepared vines to tolerate July's 19 days with maximum temperature over 30°C. The hottest day, July 4th, reached a maximum of 37°C. These consecutive hot days were the most difficult for plants, where the incidence of sunburn on the south-exposed side of vines was evident Rainfall during this month was almost null. In August precipitation came back but temperatures kept very high with 11 days over 30°C and the record of 37.7°C on 7th. This led to a full maturity of grapes and early harvest at beginning of September (Data source: Cooperative Vitivinicole de Beaune, Volnay meteorological station).

III.6 Methodologies

During the period when observations and measurements were done, the treatment '3 BDp 501 after Harvest' in Champans Bas was never performed, so never was assessed. The same for the treatment '3 BDp 501 between Flowering and Harvest' concerning the earliest measurement.

III.6.1 Phenology

Phenology was assessed through an individual periodic observation, which means that 1 single plant per treatment was studied weekly. A notation of phenological changes was done every time, between 06:30 and 09:30 AM, from 19th May until harvest. Each week, the same plant was observed, described and photographed from the same angle, one selected bunch as well.

First weeks observations were focused on vegetative growth, the density and regularity of the canopy, colour of leaves and inflorescences. Then fruit set, development of lateral shoots and leaf turgency. Always diseases were noted as well as a brief description of the weather for each morning.

Final observations were compared to a group discussion in order to contrast different perspectives and obtain a more precise qualitative results. Comments and photos were summarized showing as best as possible phenological differences.

III.6.2 Physiology

III.6.2.1 Primary Shoot Growth

Primary Shoot Growth was calculated as the difference between 2 consecutive measures of shoot length. This shoot length was measured from the base of the shoot until the last leaf with a blade diameter larger than 2 cm. The sample was 3 shoots of 3 vines per repetition (36 shoots per treatment), which were marked with tape (Fig. 8) and measured weekly before the first topping. In Champans Bas only 3 treatments were applied at those dates so that in total 108 shoots were measured 4 times on May 20th, 26th, June 3th and 9th. In Les Caillerets 180 shoots were measured on May 20th, 27th, June 3th and 8th.

In Champans Bas, before the first measurement of shoot growth all three applications of the treatment '3 BDp 501 before Flowering' and 2 applications of 'Winegrower' treatment were performed. Before the second measurement of shoot growth the 'Winegrower' replicates received a 3rd application of BDp 501.

Figure 8. Tape identyfing shoots measured for Primary Shoot Growth at Volnay, Burgundy, 2015

In Les Caillerets, the timing was exactly the same than 'Winegrower' treatment of Champans Bas, which means 2 applications of BDp 501 before the first measurement of shoot growth and a 3rd application before the second.

III.6.2.2 Rate of Primary Shoot Growth

Rate of Primary Shoot Growth was calculated relating measurements of shoot length with the °Huglin Index (HI), in order to include climatic features at Volnay (Huglin 1978). The day length at highest latitudes is considered together with the daily temperature in order to estimate potential maturity on grapes based in shoot length (Eq.1, Eq.2).

Equation 1. RSG = Rate of shoot growth, δ length= difference in shoot length, δ HI= difference in Huglin Index

$$R_{SG} = \frac{\delta \text{ length}}{\delta \text{ HI}}$$

Equation 2. Tx= max.temperature (°C), Tm= average temperature (°C), k= coefficient of day length dependent on latitude (Volnay = 1.05)

$$HI = \sum_{01.04}^{30.09} \frac{[(Tx-10)+(Tm-10)]*k}{2}$$

III.6.2.3 Number of Internodes

The number of Internodes was measured in the same shoots and plants marked for the Primary Shoot Growth. This was counted from the base of the shoots until the last leaf with a blade diameter larger than 2cm. The remaining upper part of the shoot, usually very short, was counted as 1 internode. This measurement was done on May 20th in both parcels, meaning that 2 BDp 501 applications of '3 before Flowering' treatment and 1 application of 'Winegrower' treatment were already performed in Champans Bas, as well as 1 application of all treatments in Les Caillerets. The difference δ between trellis systems was calculated as well. Within 36 shoots evaluated per treatment, 24 were in Cordon and 12 in Guyot.

III.6.2.4 Lateral Shoot Area

Lateral Shoot Area is well correlated to the length of lateral shoots (Mabrouk and Carbonneau 1996). The length was measured once after Veraison using the same shoots of the same three vines chosen for Primary Shoot Growth. The measurement was done from the base, taking the first clearly differentiated node from the crown until the last node with lateral shoot. Nodes without lateral shoot were also counted. In Champans Bas was done on August 6th and in Les Caillerets on August 14th.

To facilitate the work the lengths were classified in 7 different groups, adopted considering the most observed values after 4 repetitions measured (Table 8).

Table 8. Lateral Shoot Length Classification

Class	Lateral Shoot Length
0	No lateral shoot
1	< 5 cm
2	5 – 10 cm
3	10 – 15 cm
4	15 – 20 cm
5	20 – 30 cm
6	> 30 cm

Using the correlation found by Mabrouk and Carbonneau (1996) at Veraison an estimation of the foliar area covered by lateral shoots was done (Eq. 3).

Equation 3. Y_i = foliar area (cm^2), X_i = Lateral Shoot length at Veraison (cm)

$$Y_i = (18.884 * X_i) + 9.4956$$

III.6.2.5 Canopy Density

Canopy Density was addressed through the Point Quadrat analysis developed by Smart (Smart and Robinson 1991). Using a wood stave of 1,8m long hanging from the wires as horizontal base, a copper bar of 10mm diameter was inserted into the canopy perpendicularly (Fig. 9), each 10 cm, 100 times per repetition (50 per trellis system) in Champans Bas and 50 times per repetition in Les Caillerets. Every leaf and bunch touched by the tip of the bar was recorded, as well as gaps. In Champans Bas measurements were done only where two vines with identical trellis system were placed. For vines under Guyot system several times individual plants were measured because of lack of plants.

Figure 9. Point Quadrat technique to approach canopy density at Volnay, Burgundy, 2015.

III.6.3 Vine Diseases

III.6.3.1 Downy & Powdery Mildew

Downy and Powdery Mildew were monitored permanently since their occurrence determined if they have to be estimated quantitatively or not. No significant presence was observed for both diseases, nevertheless one measurement was done for Powdery Mildew at Bunch Closure on the 30th of June, following this classification (Table 9) (EPPO 2009):

Table 9. Classification of vine diseases intensity of incidence in bunches.

Class	Incidence Description
1	No disease
2	1 - 5 %
3	5 - 25 %
4	25 - 50 %
5	> 50 %

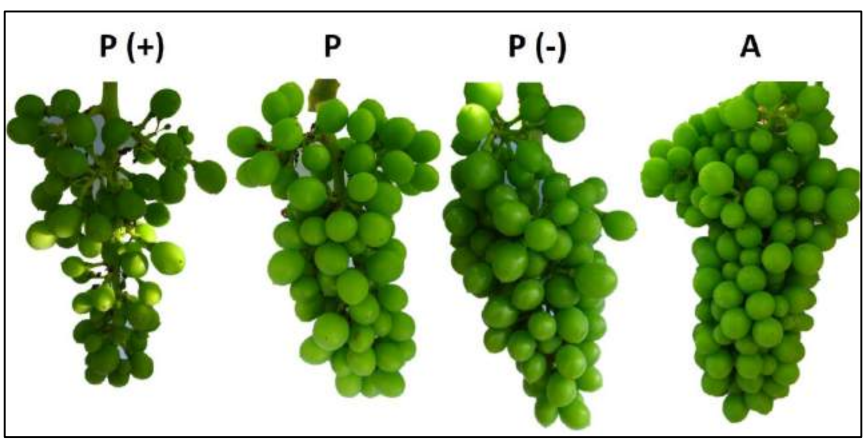
III.6.3.2 Coulure

Coulure was studied setting a scale of intensity of incidence based on Absence/Presence criteria. According to the impact of Coulure presence on bunch structure it was qualified as lower 'P(-)', higher 'P(+)' or in between, 'P' (Table 10 and Fig. 10). A first

Table 10. Coulure classification for intensity of incidence in bunches.

Class	Description
A	Absence
P(-)	Presence is visible but without impact on bunch structure
P	Presence is high associated to a looser bunch structure
P(+)	Presence is very high associated to a very loose bunch structure

Figure 10. Coulure classification for intensity of incidence in classification at Volnay, Burgundy, 2015.



measurement was done at Fruit Set on June 9th and 10th. To improve precision it was repeated in Champans Bas on June 16th (still Fruit Set). Both measurements were performed on 15 shoots per replicate (60 shoots per treatment), including all inflorescences present in each shoot, in both fields.

At Pea-size (turning to Bunch Closure) a second measurement was performed only in Champans Bas, on June 23th. This included 12 marked bunches per replicate (48 bunches per treatment).

III.6.3.3 Sunburn and Sulphur burn

Sunburn and Sulphur burn were measured simultaneously but estimated separately, using the classification of EPPO guidelines for diseases in grapevine bunches, but modified (Table 11). When dehydrated berries appeared were qualified as an effect of Sunburn exclusively, excluding the fact that in many cases both phenomena were observed in the same berries, therefore, Sulphur burn was noted only in non-dehydrated berries (Fig. 7).

Table 11. Sunburn and Sulphur burn classification for intensity of incidence in bunches.

Class	Incidence Description
1	No disease
2	> 1 < 5 %
3	> 5 < 25 %
4	> 25 < 50 %
5	>= 50 < 100 %
6	100 %

Observations were done at beginning of Veraison (around 1 red berry per bunch) on July 15th, after several days of high temperatures, only in the south-east exposed bunches which is known as the afternoon sun-exposed side of the canopy. According to climatic data, the average maximum temperature until July 15th was 32.1°C.

40 bunches per replicate (160 per treatment) were assessed in Champans Bas, 10 bunches per replicate (40 per treatment) in Les Caillerets. At the moment of the measurement only 1 BDp 501 application of '3 between Flowering and Harvest' treatment

in Champans Bas was performed, while '3 before Flowering' and 'Winegrower' had 3 applications already done.

Figure 7. Sunburn (red) and Sulphur burn (yellow) identification criteria at Volnay, Burgundy, 2015.

III.6.3.4 Botrytis and Acetic Rot

Since the occurrence of Botrytis and Acetic Rot determined if they should be estimated quantitatively or not, measurements were delayed until last week before harvest because no significant presence was observed. Botrytis and Acetic rot were estimated simultaneously four days before harvest, on August 31st. 50 bunches per replicate (200 per treatment) were observed in Champans Bas, 36 bunches per replicate (144 per treatment) in Les Caillerets. They were qualified according to the same table of vine disease incidence in bunches (Table 9) used for other diseases, proposed by EPPO guidelines.

III.6.4 Regenerative Development

III.6.4.1 Inflorescences/Shoot

Inflorescences were counted at Fruit Set on 9th and 10th of June in Champans Bas and Les Caillerets respectively, including only plots which have been treated with BDp 501 at that date, 'Before Flowering' and 'Winegrower'. Champans Bas was repeated on 16th June, still Fruit Set, in order to include differences in trellis systems and it All measurements were performed on 15 shoots per replicate (60 shoots per treatment), including all inflorescences present in each shoot and taking into account bunch size when it was too small.

III.6.4.2 Bunches/Shoot & Bunches/Vine

Bunches were counted very accurately at Pea-size turning to Bunch Closure, on June 23rd in Champans Bas, while in Les Caillerets it was done one week later at Bunch Closure, on July 01th. The characterization included bunch size when small, trellis system, double shoots, suckers, shoot size when smaller than average and not developed buds. A number of 6 vines per replicate (24 vines per treatment) were chosen for this case.

III.6.4.3 Berries/Bunch

Berries were counted once only in Champans Bas, at Pea-size on June 17th. To that effect 6 bunches per replicate were selected and marked, 24 bunches per treatment as total.

III.6.4.4 Berry and Bunch Weight

Berry Weight was calculated as average of 50 berries taken randomly from each replicate before harvest on 2nd September, a total of 200 berries per treatment, in both fields.

Bunch Weight was measured on samples of 10 bunches per replicate, 40 per treatment, harvested and weighted on 4th September in Champans Bas, one day before harvest. In Les Caillerets samples were composed by 6 bunches per replicate, 24 per treatment, weighted on 2nd September, two days before harvest.

In Champans Bas, previously, 6 bunches per replicate were marked for Coulure and berry counting so, the same 6 bunches plus 4 randomly chosen complete the 10 bunches sample needed to measure weight.

III.6.4.5 Berry Maturation Sampling

As a complement for previous measurements, each sample of 200 berries per treatment picked on September 2nd was pressed to extract the juice, which was analyzed by Infrared spectrophotometry in a Wine Scan™ FT 120 from FOSS brand.

III.6.4.6 Bunch Compactness (OIV descriptor n°204)

Bunch Compactness was performed visually classifying each bunch according to the angle of torsion tolerated by the vertical axe, from 1 to 5 following the protocol of Ipach et al. (2005)(Table 12). With one hand the upper half of the bunch is held and with the other hand the lower half is turned until its maximum. Every measurement was done by the same person in order to avoid changes on qualitative terms (criteria, force used, holding technique, hand size).

A total of 80 bunches per treatment, 20 bunches per replication, 50 from each side of the row, were assessed at beginning of Veraison, on July 21st for both fields.

Table 12. Bunch Compactness Classification (Ipach 2005)

Class	Description
1	Very Loose Bunch, berries do not touch each other Torsion angle of rachis higher than 90°
2	Loose Bunch, berries touch each other Torsion angle of rachis between 45° and 90°
3	Strong Bunch Structure, berries still can be moved Torsion angle of rachis between 10° and 45°
4	Compact Bunch, berries not movable but not deformed Torsion angle of rachis until 10°
5	Very Compact Bunch, berries deformed by pressure of neighboring berries Torsion of the rachis is not possible

III.6.5 N-tester (Chlorophyll Index)

N-tester is an optical device measuring the intensity of green colour in leaves. This depends on Chlorophyll content which at the same time is related with the nitrogen content of vines (Bavaresco 1995, Van Leeuwen and Friant 2011). To estimate this nutritional state of vines, 4 measurements were done at Full Bloom, Bunch Closure, 50% Veraison and Harvest, on June 9th and 25th, July 25th and September 2nd respectively. Each replicate was measured once, which means the mean of 30 different leaves. Leaves had to be placed at the main shoots otherwise results change clearly, as well as healthy and free of pest treatment residues. In Champans Bas, because of excess of residues over the canopy (powder sulphur), the second measurement was performed on upper leaves (above the upper wire) rather than lower leaves at fruit zone. Consequently, third and fourth observations considered these differences, although in Les Caillerets only upper leaves were measured in every case, due to similar reasons.

III.6.6 Statistical Analysis

Results were analyzed with the statistical software R version 3.2.2 Copyright (C) 2015 created by the R Foundation for Statistical Computing. ANOVA one way was done to each result related to the treatments, or two ways when the influence of other factors was determinant. Results obtaining a p-value < 0.05 were considered significant and results with p-value < 0.1 as a trend.

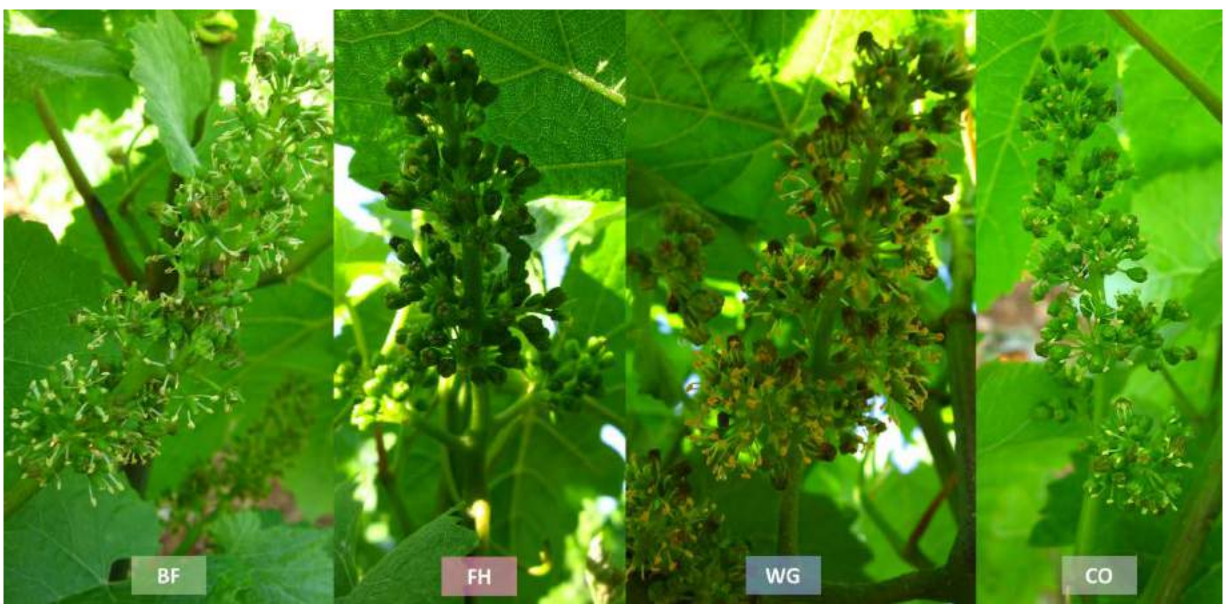
IV RESULTS

IV.A CHAMPANS BAS

IV.A.1 Phenology

Flowering occurred between last days of May and first week of June. During these days weather was temperate, no rain, with average temperatures around 17°C until June 2nd, then it rose through several consecutive days with maximum over 30°C until June 7th, which accelerated the process. At Flowering vines could be classify from more advanced to less advanced in phenological development like this: BF > WG - CO > FH. The vine under BF treatment showed 80-90% of floral caps fallen down while FH only 30-40% (Fig. 11). Fertility was assessed counting number of inflorescences per vine, obtaining: BF >> WG >> FH > CO.

Figure 11. Flowering in each treatment on June 4th in Champans Bas, Volnay, Burgundy, 2015.



Canopy development was described according to the gaps observed which gave the following classification from higher to lower: FH >> BF – CO >> WG; consequently the regularity of shoot growth almost invert that sequence: WG >> CO > FH > BF. Color of the leaves was assessed from darker green to more pale green: WG >> CO > BF - FH.

Two weeks after Flowering the Fruit Set pass to Pea-Size very fast, then the incidence of coulure on bunch structure was already noticeable leading to classify them from less to more intensity of coulure: CO > FH >> BF > WG (Fig. 12). During this period the growth and abundancy of lateral shoots was assessed obtaining: WG > BF – CO > FH, as well as petiole length of leaves opposed to bunches: WG > BF – CO > FH.

Figure 12. Fruit-set to Pea-size in each treatment on June 18th in Champans Bas, Volnay, Burgundy, 2015.

Veraison started on July 15th in the middle of a very hot wave of heat of nine over ten consecutive days trespassing 30°C as maximum temperature. It begun with 1 coloured berry per bunch, until August 5th when more than 95% of berries were coloured in all bunches.

Figure 13. Veraison in each treatment on July 31st in Champans Bas, Volnay, Burgundy, 2015.

At Veraison the order by phenological state was FH > CO > BF > WG (Fig. 13), damage by sunburn and sulphur burn appeared affecting all treatments in a similar intensity, re-growth of shoots observed after topping was WG – BF > CO – FH. In general, the vitality of leaves was usually WG > BF - CO > FH during this hot period.

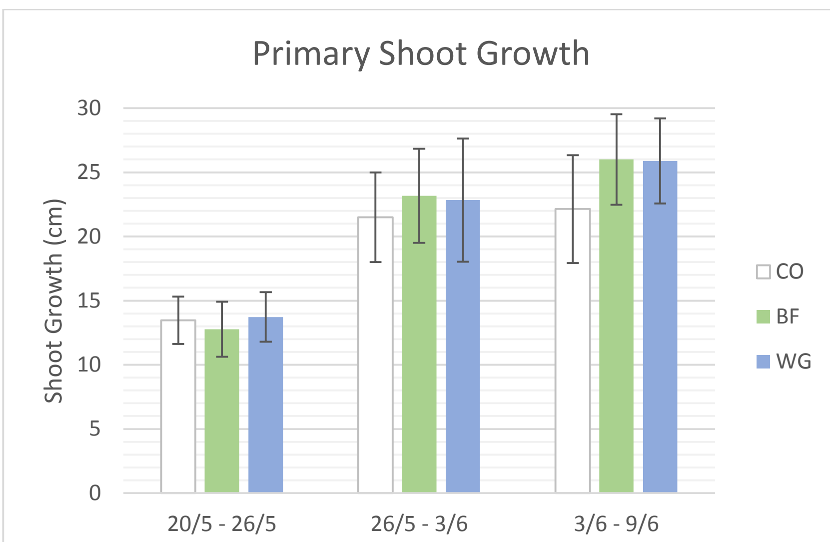
From Veraison until Maturity the only difference observed was on intensity of dehydrated berries on bunches, obtaining: BF > WG > CO > FH.

IV.A.2 Physiology

IV.A.2.1 Primary Shoot Growth

As a global result the impact of treatments BF and WG was not significant, except at the third measurement on June 9th when both increased the primary shoot growth (p-value < 0.05). The WG treatment showed higher growth than CO in every date, while BF treatment only in last two periods. A clear difference in growth between the first measurement and the other two was observed (Fig.14).

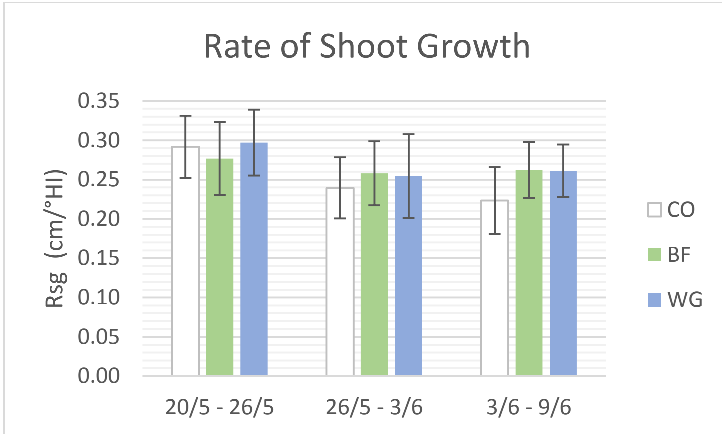
Figure 14. Primary Shoot Growth before first hedging in Champans Bas at Volnay, Burgundy, 2015.



IV.A.2.2 Rate of Primary Shoot Growth

Results of rate of shoot growth associated with the Huglin Index (cm/°HI) were not significant (Fig.15). Temperatures rose dramatically at the end of May beginning of June, increasing differences in HI and therefore reducing values for rate of shoot growth.

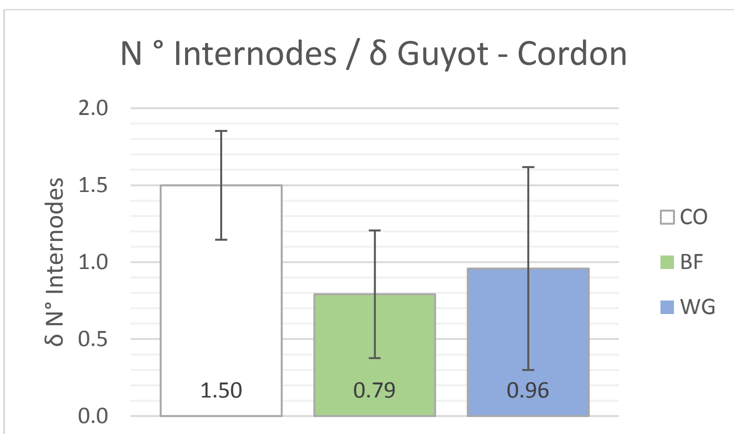
Figure 15. Rate of Shoot Growth (Rsg) before first hedging in Champans Bas at Volnay, Burgundy, 2015.



IV.A.2.3 Number of Internodes

Number of Internodes did not showed significant difference between the treatments (Fig. 54 in Annex 3), but the effect of trellis systems influenced significantly (p-value < 0.001). Regarding closer this phenomenon it was found that the difference between Cordon Royat and Guyot was more extreme in the control than BF or WG treatments (Fig. 16).

Figure 16. N° of Internodes difference between trellis systems in Champans Bas at Volnay, Burgundy, 2015.



IV.A.2.4 Lateral Shoot Area

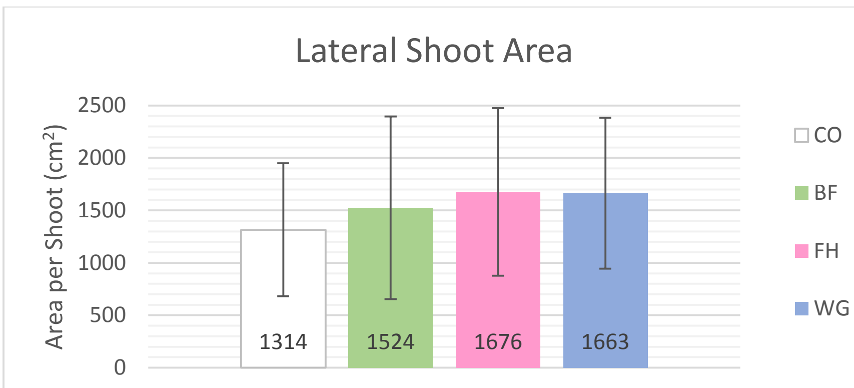
For any lateral shoot length the influence of the treatments was not significant. Considering the correlation between area and length of lateral shoots topped or not topped (Mabrouk and Carbonneau 1996), it was found that over 65.1 % of the observed lateral shoots have lengths of 1 to 10 cm (Table 13).

Table 13. Lateral Shoot Length (L) and Area (A) distribution in Champans Bas at Volnay, Burgundy, 2015.

LENGTH	CONTROL			BEFORE FLOWERING			FLOWERING HARVEST			WINEGROWER		
	%L	%A	cm^2	%L	%A	cm^2	%L	%A	cm^2	%L	%A	cm^2
0-5	47.4	20.5	8733	39.8	13.7	7542	36.5	11.8	7088	36.7	11.7	7031
5-10	30.5	24.1	10288	33.5	30.8	16926	33.3	28.6	17228	28.4	24.2	14508
10-15	11.4	21.3	9085	14.1	21.0	11541	14.9	20.8	12523	19.2	26.7	15960
15-20	7.1	18.3	7819	5.7	11.8	6459	6.7	13.0	7819	9.8	18.7	11219
20-30	2.2	7.9	3371	4.8	14.0	7706	5.8	16.0	9632	3.6	9.7	5779
>30	1.5	7.9	3352	2.1	8.6	4693	2.6	10.0	6034	2.4	9.0	5363

The abundancy of lateral shoots shorter than 5 cm made CO treatment get the lowest total foliar area, but still not significant (Fig. 17).

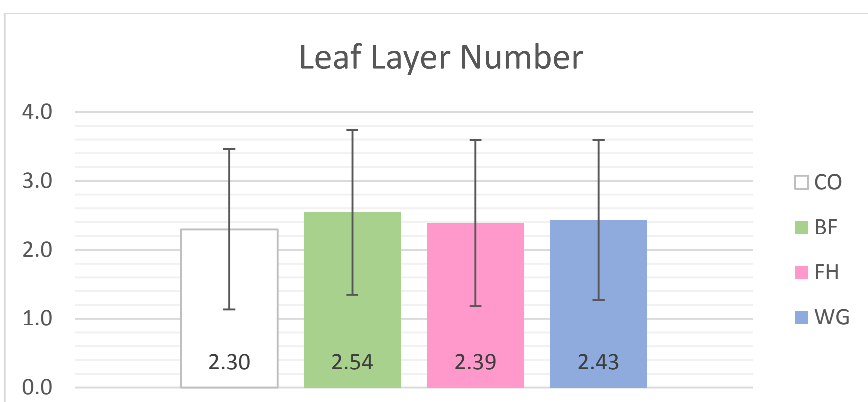
Figure 17. Lateral Shoot Area per shoot in Champans Bas at Volnay, Burgundy, 2015



IV.A.2.5 Canopy Density

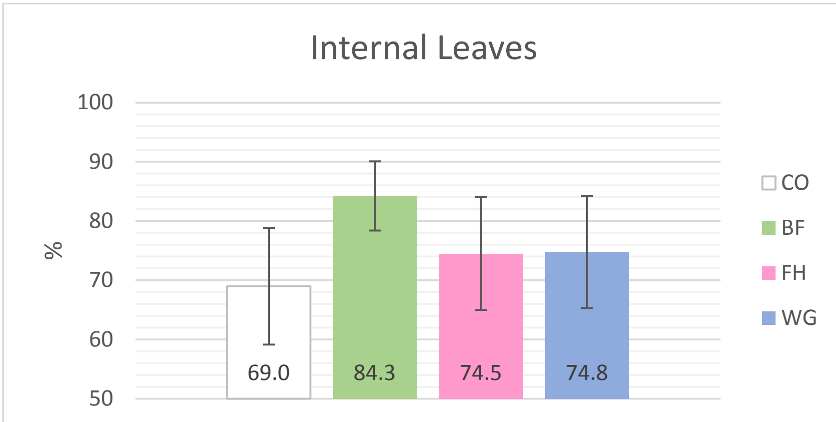
Leaf Layer Number was the parameter of the Point Quadrat analysis most significantly affected by the treatments (p-value < 0.05), all their vines had more than 2.39 layers of leaves, while control only 2.30. BF treatment got the highest value, 2.54 (Fig. 18).

Figure 18. Leaf Layer Number of canopy in Champans Bas at Volnay, Burgundy, 2015.



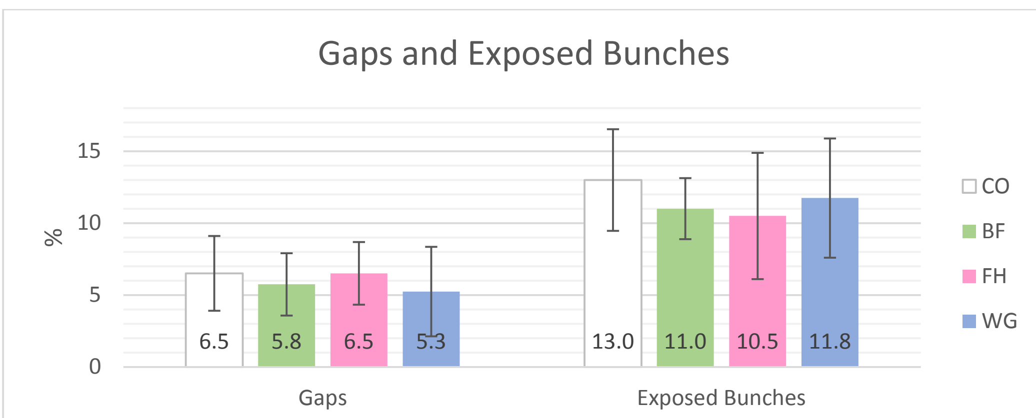
Internal Leaves were in a similar situation related to the impact of treatments but with less significance (p-value < 0.1), the control vines were the only ones under 70% of internal leaves. Again the BF treatment had the highest value, 84.25 % (Fig. 19).

Figure 19. Internal Leaves percentage in canopy in Champans Bas at Volnay, Burgundy, 2015.



When it comes to gaps or exposed bunches, only in the second character control showed some differences but these were not significant (Fig. 20).

Figure 20. Gaps and Exposed Bunches percentages on canopy in Champans Bas at Volnay, Burgundy, 2015.



A summary of the impact of Trellis System is presented (Table 14) because it was significant particularly in exposed bunches (p-value < 0.001), internal leaves (p-value < 0.01) and gaps percentage (p-value < 0.1). Guyot system increased internal leaves and exposed bunches but decreased gaps presence.

Table 14. Point Quadrat parameters for canopy density comparing Guyot (G) and Cordon Royat (CR) systems in Champans Bas at Volnay, Burgundy, 2015.

	CONTROL		BEFORE FLOWER		FLOWER HARVEST		WINEGROWER	
	CR	G	CR	G	CR	G	CR	G
GAPS (%)	7.50	5.50	8.00	3.50	7.50	5.50	5.50	5.00
LEAF LAYER NUMBER	2.28	2.32	2.45	2.64	2.30	2.47	2.47	2.39
INTERNAL LEAVES (%)	64.0	74.0	74.0	94.5	66.5	82.5	75.0	74.5
EXPOSED BUNCHES (%)	6.0	20.0	6.0	16.0	6.5	14.5	9.5	14.0

IV.A.3 Vine Diseases

IV.A.3.1 Downy & Powdery Mildew, Botrytis & Acetic Rot

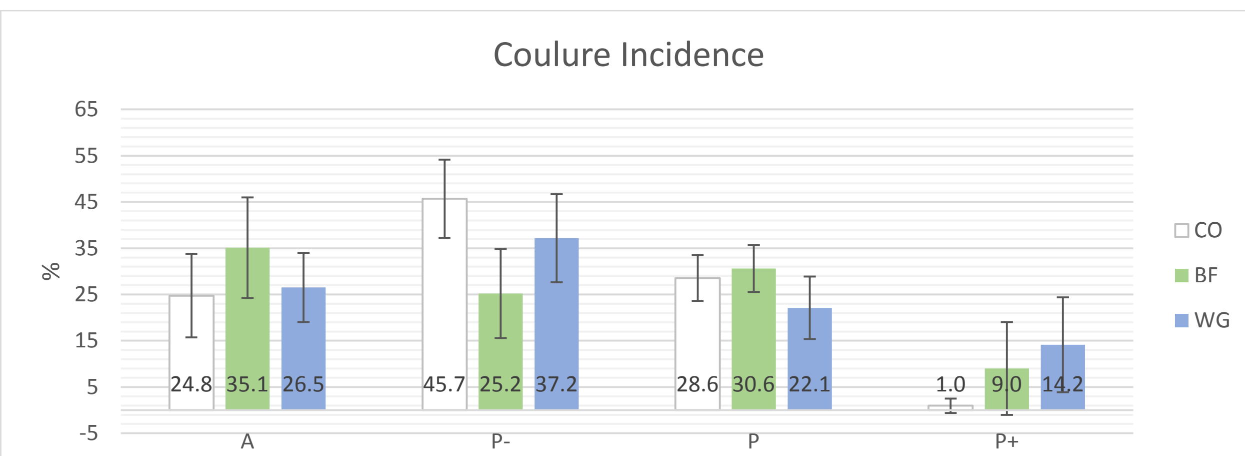
2015 was a great year for winegrowers in terms of grape health. Downy and Powdery Mildew were monitored permanently without success, one exhaustive observation done for Powdery Mildew in bunches gave less than 1 % of incidence.

Similarly, Botrytis and Acetic Rot were measured once before harvest and results were ignored because infected bunches did not surpass the 1% of the cases.

IV.A.3.2 Coulure

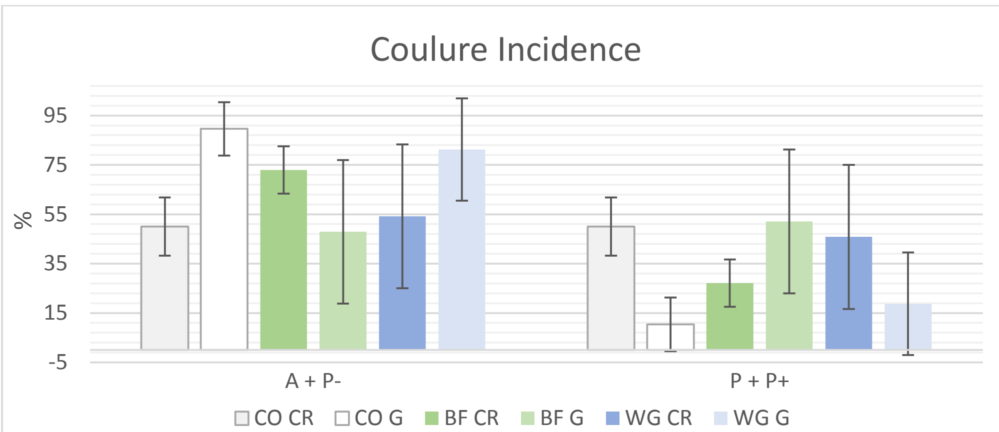
Merging measurements done at Fruit Set (June 16th) and Bunch Closure (June 23rd) in Champans Bas no significant results were found, nevertheless when regarding bunches highly impacted by coulure (P+) both treatments with BDp 501 were higher than control in both dates (Fig. 21).

Figure 21. Coulure incidence on bunches in Champans Bas at Volnay, Burgundy, 2015.



When merging those categories where the incidence of coulure did not impact the structure of the bunch ("A" + "P-") as well as those where coulure clearly changed bunch structure ("P" + "P+"), the interaction between the trellis systems and the treatments became significant on June 23rd (p-value < 0.01) as well as the effect of the trellis alone (p-value < 0.05). While WG treatment and control showed stronger incidence of Coulure on Guyot vines, BF treatment had higher impact but in Cordon Royat vines (Fig. 22).

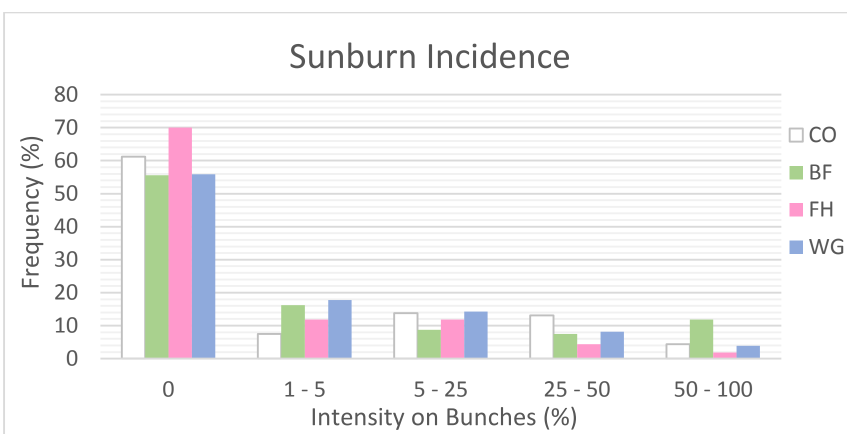
Figure 22. Coulure incidence on bunches grouping categories and distinguishing between Guyot (G) and Cordon Royat (CR) in Champans Bas at Volnay, Burgundy, 2015.



IV.A.3.3 Sunburn & Sulphur Burn

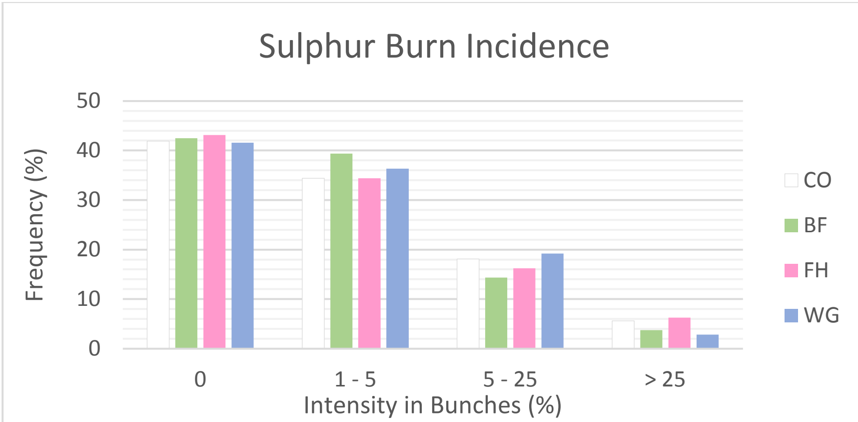
Sunburn incidence was affected significantly by treatments (p-value < 0.01). Mean of Sunburn frequency was consistently lower for FH treatment (30%) than BF and WG (45%) or control (39%). In terms of intensity on bunches the treatment BF showed a specific high level on strongly impacted bunches (50-100%) (Fig. 23).

Figure 23. Sunburn distribution of incidence on bunches in Champans Bas at Volnay, Burgundy, 2015.



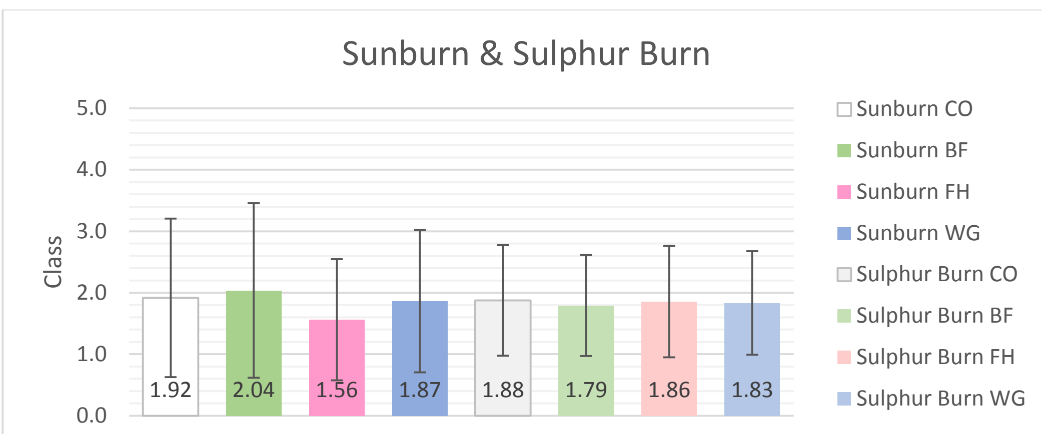
Sulphur Burn impact was smaller and differences between treatments not significant. Means showed an incidence between 56.9 and 58.4 % for any treatment (Fig. 24).

Figure 24. Sulphur burn distribution of incidence on bunches in Champans Bas at Volnay, Burgundy, 2015.



Means of intensity on bunches either for Sunburn or Sulphur Burn were closer to class 2 (1-5 %), disregarding treatments. Higher variability of Sunburn is noticeable as well in this figure, clearly associated to treatments (Fig. 25).

Figure 25. Sunburn and sulphur burn intensity on bunches in Champans Bas at Volnay, Burgundy, 2015.

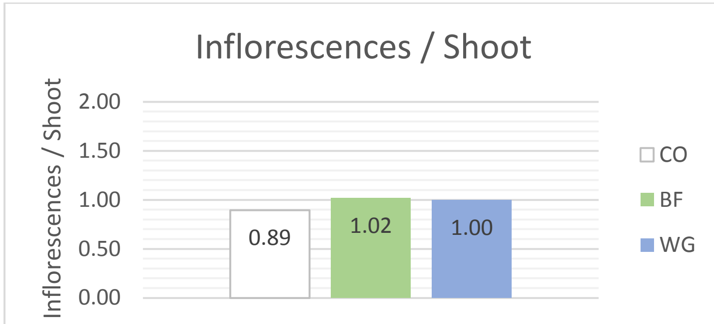


IV.A.4 Regenerative Development

IV.A.4.1 Inflorescences/Shoot

Treatments BF and WG had over 1 inflorescence per shoot which was higher than control, but not significant (Fig. 26).

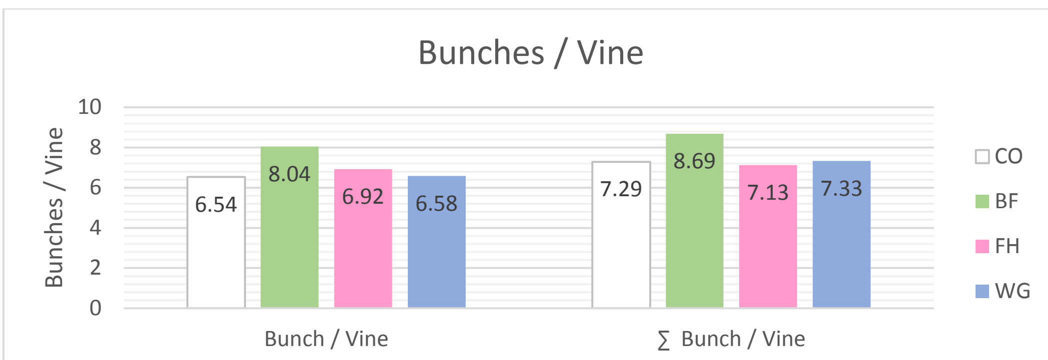
Figure 26. Inflorescences per shoot in Champans Bas at Volnay, Burgundy, 2015.



IV.A.4.2 Bunches/Shoot & Bunches/Vine

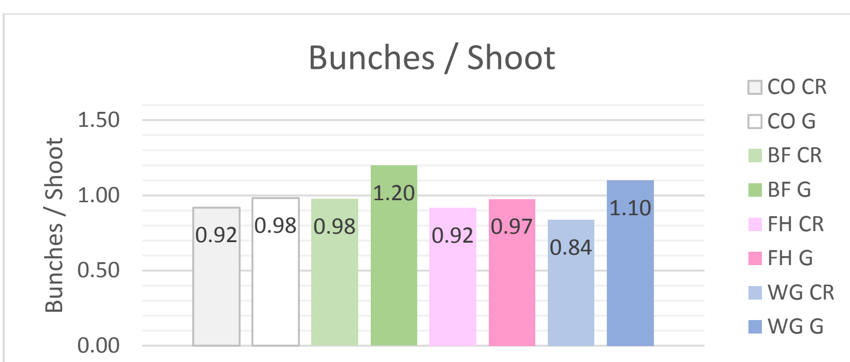
Treatment impact was not significant in yield estimation. BF treatment showed higher bunches per vine when looking at shoots pruned for productive goals as well as when considering all bunches from suckers, double shoots or not well developed shoots (Fig. 27).

Figure 27. Bunches per vine from productive and others shoots in Champans Bas at Volnay, Burgundy, 2015.



The impact of the trellis system was significant (p-value < 0.05), looking at bunches per shoot Guyot vines were higher than Cordon Royat vines (Fig. 28).

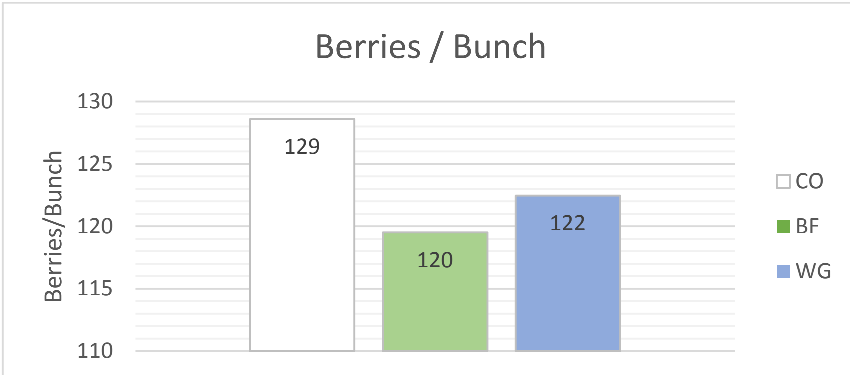
Figure 28. Bunches per shoot comparing Guyot and Cordon Royat in Champans Bas at Volnay, Burgundy, 2015.



IV.A.4.3 Berries/Bunch

Berries counted in Champans Bas were not significantly different between treatments. Mean of control was higher than treatments BF and WG (Fig. 29).

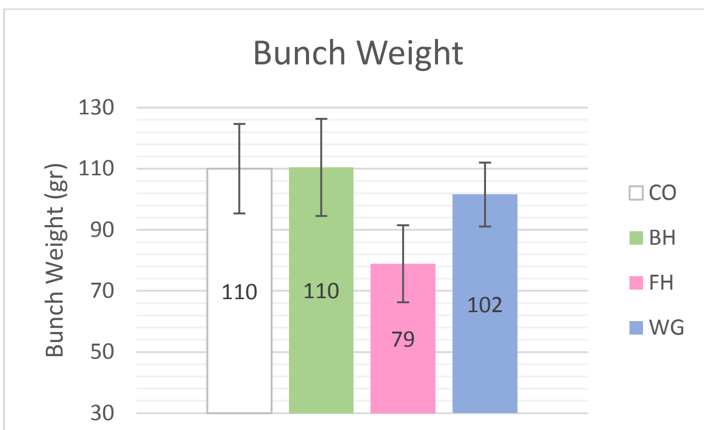
Figure 29. Berries per bunch in Champans Bas at Volnay, Burgundy, 2015



IV.A.4.4 Berry & Bunch Weight

Treatments had a significant effect over the bunch weight (p-value < 0.01). A very low value was observed on bunches of FH treatment compared with BF, WG or control (Fig. 30). Instead, in berry weight no significant difference was observed (Fig. 55 in Annex 4).

Figure 30. Bunch weight in Champans Bas at Volnay, Burgundy, 2015.



IV.A.4.5 Berry Maturation Sampling

Must analysis did not give significant results. Nevertheless, WG treatment showed a lower potential alcohol and sugar than the rest, as well as higher acidity in any parameter of acids and lower pH. Control got higher values in Nitrogen parameters (Table 15).

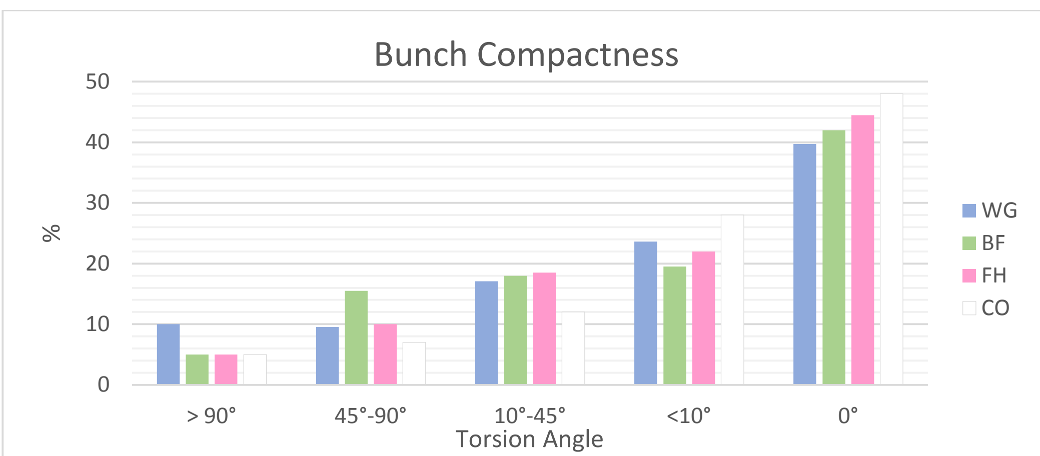
Table 15. Must analysis through spectrophotometry in Champans Bas at Volnay, Burgundy, 2015.

	CONTROL	BEFORE FLOWER	FLOWER HARVEST	WINEGROWERG
POT ALCOH REFRACTO (%VOL)	13.50	13.40	13.50	13.60
POT ALCOH TABLE (%VOL)	14.12	14.12	14.18	13.88
SUGAR (G/L)	240	240	241	236
PH	3.15	3.11	3.13	3.07
TOTAL ACIDITY (G H2SO4/L)	3.20	3.39	3.29	3.52
TARTARIC ACID (G/L)	4.23	4.45	4.54	5.02
MALIC ACID (G/L)	3.12	3.22	3.18	3.26
K	845	819	842	836
AMMONIUM NH3 (MG/L)	82	82	75	78
ALPHA AMINO NITROGEN (MG/L)	106	89	93	87
ASSIMILABLE NITROGEN (MG/L)	186	167	171	172

IV.A.4.6 Bunch Compactness

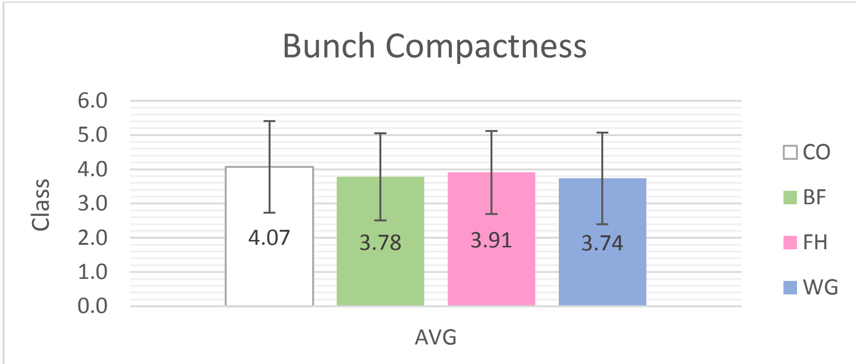
Bunches tolerating over 10° torsion on the vertical axe were higher in treatments with BDp 501 than control, evidencing the significance of treatment effect (p-value < 0.05), consequently the control vines had higher degree of compactness in their bunches (Fig. 31).

Figure 31. Bunch compactness distribution in Champans Bas at Volnay, Burgundy, 2015.



Averages by class confirmed that control treatment with the highest bunch compactness and WG treatment with the loosest bunches. All values were around class 4, meaning the torsion angle allowed by bunches was between 0° and 10° (Fig. 32).

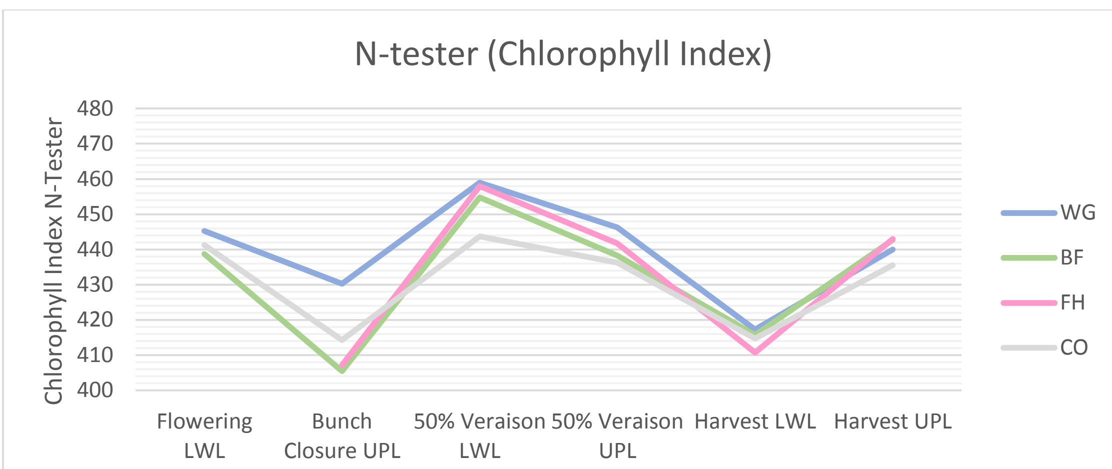
Figure 32. Bunch Compactness intensity by class in Champans Bas at Volnay, Burgundy, 2015.



IV.A.5 N-tester (Chlorophyll Index)

N-tester index did not have significant differences along the season, but a certain predominance of WG leaves over the others was observed (Fig. 33).The effect of measuring at lower (LWL) or upper (UPL) leaves did have statistical significance, particularly at harvest (p-value < 0.01).

Figure 33. N-tester evolution comparing lower (LWL) and upper leaves (UPL) in Champans Bas at Volnay, Burgundy, 2015.



Disregarding leaf type (LWL or UPL) the variability of N-Tester values between phenological states was noticeable. Comparing treatment values between Flowering and Bunch Closure versus Veraison, it was found that the treatment effect became significant (p-value < 0.05), where the BF treatment showed the greatest reduction in N-tester values. FH treatment was affected, but it was assessed only after Bunch Closure (Fig. 56 in Annex 5).

IV.B. LES CAILLERETS

IV.B.1 Phenology

At Flowering vines could be classify from more advanced to less advanced in phenological development like this: YN – OB - C > SP - VL. Vines under YN, OB and C treatments showed around 90% of floral caps fallen down while SP and VL still were around 50% (Fig. 34). Fertility was assessed counting number of inflorescences per vine, obtaining: VL > C > SP > OB > YN.

Figure 34. Flowering in each treatment on June 4th in Les Caillerets at Volnay, Burgundy, 2015.

Canopy development was described according to the gaps observed which gave the following classification from higher to lower: SP – YN – OB > VL >> C; consequently the regularity of shoot growth almost invert that sequence: C – YN > VL > SP > OB. Color of the leaves was assessed from darker green to more pale green: YN > SP – VL – OB > C.

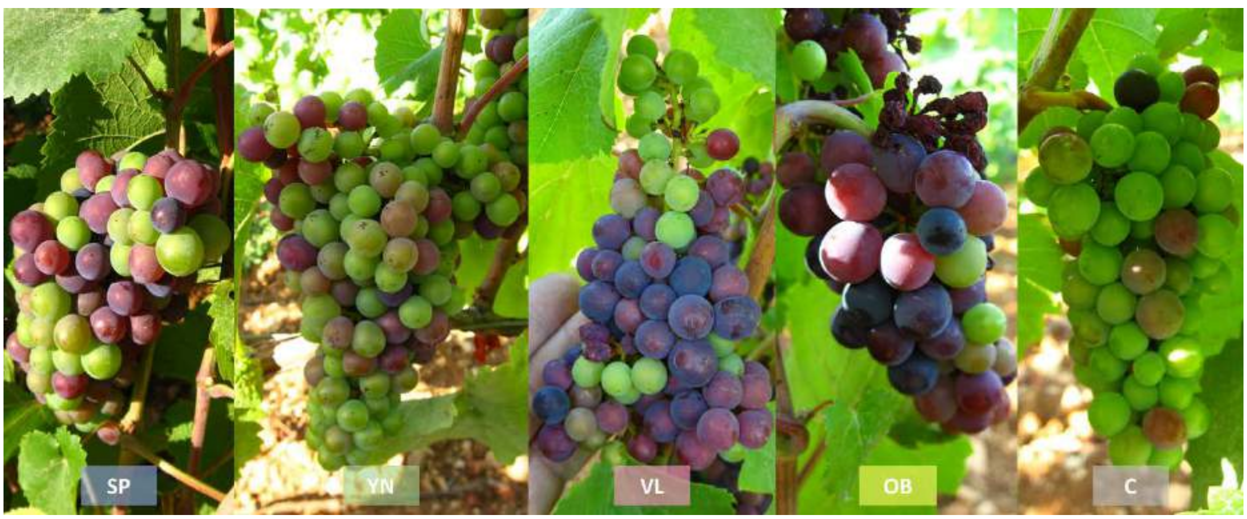
Two weeks after Flowering the Fruit Set pass to Pea-Size very fast, then the impact of coulure on bunch structure was already noticeable, leading to classify them from lower to higher incidence into: YN << SP - OB < VL - C (Fig. 35). During this period the growth and abundancy of lateral shoots was assessed obtaining low results for all treatments. The petiole length of leaves opposed to bunches had only one different: SP < YN – VL – OB - C.

At Veraison the order by phenological state was OB > VL > SP > YN > C (Fig. 36), damage by sunburn and sulphur burn appeared affecting in order of intensity OB > SP > VL

> C - YN, re-growth of shoots observed after topping was very low for all treatments. Through the hot period in July the vitality or firmness observed on leaves was YN – SP > VL > OB - C.

Figure 35. Fruit-set to Pea-size in each treatment on June 18th in Les Caillerets at Volnay, Burgundy, 2015.

Figure 36. Veraison in each treatment on July 31st in Les Caillerets at Volnay, Burgundy, 2015.



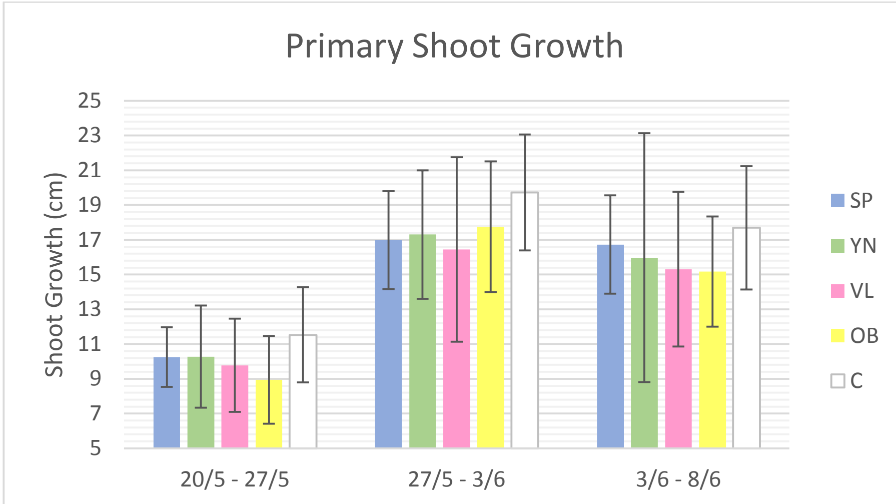
From Veraison until Maturity the only difference observed was on intensity of dehydrated berries on bunches, obtaining: VL > SP - OB > YN - C.

IV.B.2 Physiology

IV.B.2.1 Primary Shoot Growth

Treatments showed no significant impact on primary shoot growth. A certain regular order was observed, SP-YN > VL > OB, during the first and third weeks, although the OB treatment reverted this trend in the second week (Fig. 37).

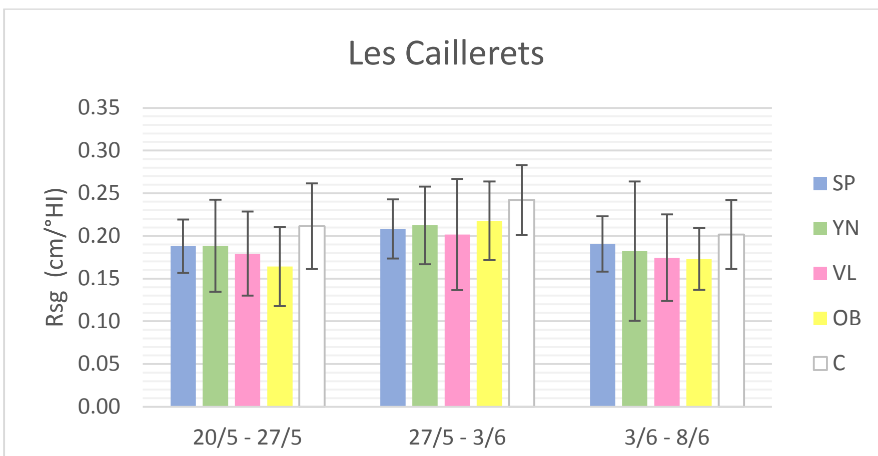
Figure 37. Primary Shoot Growth before first hedging in Les Caillerets at Volnay, Burgundy, 2015.



IV.B.2.2 Rate of Primary Shoot Growth

Results of rate of shoot growth were not significant and quite stables (Fig. 38).

Figure 38. Rate of Shoot Growth (Rsg) before first hedging in Les Caillerets at Volnay, Burgundy, 2015.



IV.B.2.3 Number of Internodes

Number of Internodes did not showed significant difference between the treatments with all ranging from 10 to 10.5 internodes at May 20th (Fig. 53 in Annex 3).

IV.B.2.4 Lateral Shoot Area

The length of lateral shoots in Les Caillerets did not show a significant impact from treatments. All plots gave predominance of short laterals < 10 cm long, having rates over

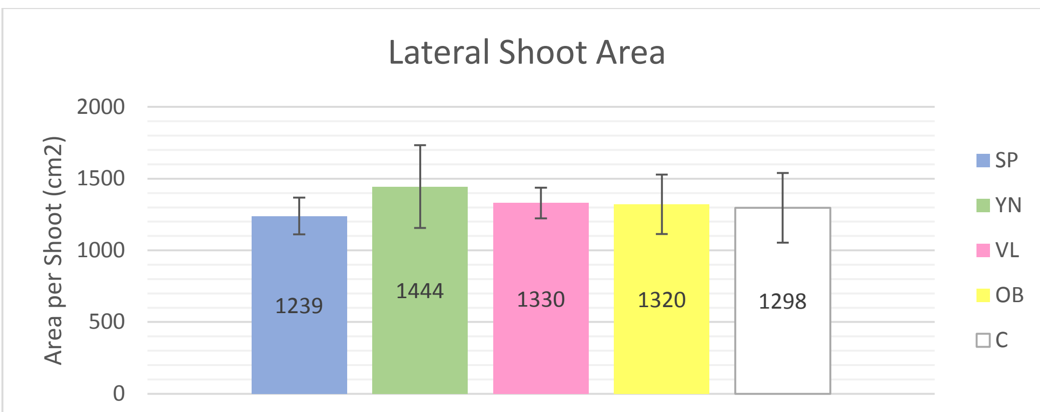
78.6 % (Table 16). When estimating the foliar surface of these lateral shoots using the equation of correlation from Mabrouk and Carbonneau (Mabrouk and Carbonneau 1996), proportions changed reducing short laterals < 10 cm to rates under 62.9 %.

Table 16. Lateral Shoot Length (L) and Area (A) distribution in Les Caillerets at Volnay, Burgundy, 2015.

LENGTH (CM)	SILICA PURE			YARROW S.NETTLE			VALERIAN			OAK BARK			CONTROL		
	%L	%A	cm^2	%L	%A	cm^2	%L	%A	cm^2	%L	%A	cm^2	%L	%A	cm^2
0-5	53.5	24.0	10717	51.2	20.4	10604	54.6	24.5	11738	56.8	25.9	12305	55.0	25.4	11851
5-10	30.6	36.6	16322	27.4	29.1	15113	30.1	36.0	17228	25.7	31.2	14810	30.5	37.5	17531
10-15	9.9	19.3	8594	10.4	17.9	9331	8.7	16.9	8103	11.0	21.7	10313	8.4	16.8	7857
15-20	3.4	9.1	4080	6.3	15.0	7819	3.7	9.9	4760	3.9	10.7	5099	3.7	10.2	4760
20-30	1.7	6.5	2890	3.3	11.1	5779	1.8	7.0	3371	2.4	9.1	4334	1.8	7.2	3371
>30	0.8	4.5	2011	1.4	6.4	3352	1.1	5.6	2682	0.3	1.4	670	0.5	2.9	1341

Looking at the total foliar area calculated from these data, the treatment YN got the highest total foliar surface based on the abundancy of lateral shoots longer than 15 cm, but still not significant (Fig. 39).

Figure 39. Lateral Shoot Area per shoot in Les Caillerets at Volnay, Burgundy, 2015.



IV.B.2.5 Canopy Density

In Les Caillerets differences between the treatments were not significant, only there is some trend related to the gaps percentage (p-value < 0.1). In this sense, while SP and OB tended to have more gaps, YN and VL vines showed less (Fig. 40.).

VL treatment showed certain consistency towards higher density of the canopy, said more internal leaves and leaf layer number as well as less gaps and exposed bunches, but without statistical significance (Table 17).

Figure 40. Gaps percentage on canopy in Les Caillerets at Volnay, Burgundy, 2015.

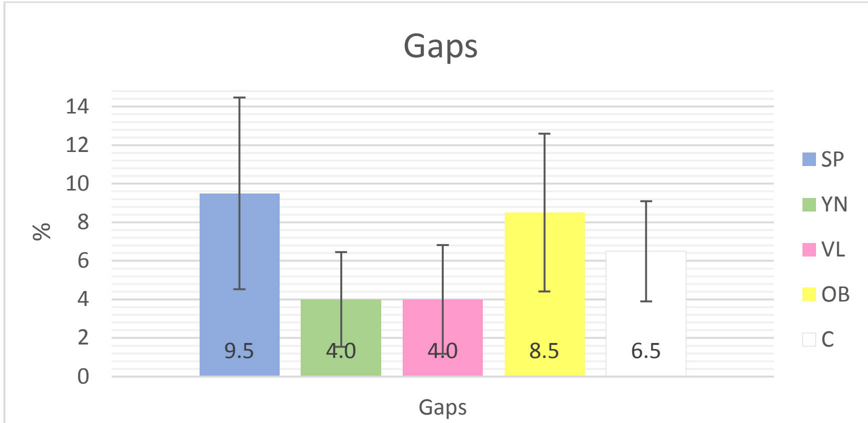


Table 17. Point Quadrat parameters for canopy density in Les Caillerets at Volnay, Burgundy, 2015.

	SILICA PURE	YARROW S.NETTLE	VALERIAN	OAK BARK	CONTROL
GAPS (%)	9.50	4.00	4.00	8.50	6.50
LEAF LAYER NUMBER	2.13	2.27	2.36	2.09	2.17
INTERNAL LEAVES (%)	65.50	63.50	69.50	58.50	61.00
EXPOSED BUNCHES (%)	15.00	17.00	13.50	14.50	17.50

IV.B.3 Vine Diseases

IV.B.3.1 Downy & Powdery Mildew, Botrytis & Acetic Rot

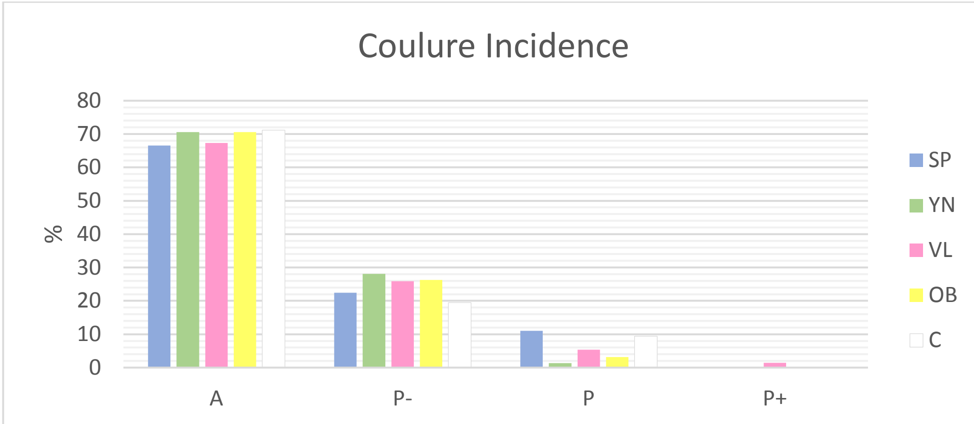
2015 was a great year for winegrowers in terms of grape health. Downy and Powdery Mildew were monitored permanently without success, one exhaustive observation done for Powdery Mildew in bunches gave less than 1 % of incidence.

Similarly, Botrytis and Acetic Rot were measured once before harvest and results were ignored because infected bunches did not surpass the 1% of the cases.

IV.B.3.2 Coulure

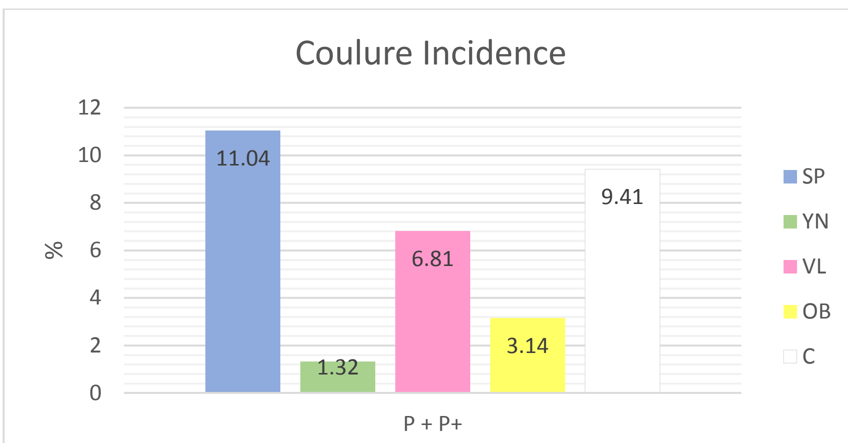
Observations registered at Fruit Set (June 9th) showed a weak incidence of Coulure in Les Caillerets as a general overview. Almost no bunches with severe impact were found (Fig. 41) and treatments showed no effect over coulure.

Figure 41. Coulure incidence on bunches in Les Caillerets at Volnay, Burgundy, 2015.



When merging categories of absence and low coulure incidence (“A” + “P-”) as well as those with high coulure incidence (“P” + “P+”) the impact of treatments increased but it was not enough to consider it as a trend (Fig. 42).

Figure 42. Coulure incidence on bunches grouping most affected categories in Les Caillerets at Volnay, Burgundy, 2015.



IV.B.3.3 Sunburn & Sulphur Burn

Sunburn and Sulphur Burn impact in Les Caillerets were noticeable but significant differences between treatments were not observed. Sunburn was quite homogenous in terms of intensity on bunches having means around class three, 5-25 % (except VL treatment), instead, Sulphur Burn had means of intensity close to class two, 1-5% (Fig. 43 and 44).

Figure 43. Sunburn distribution of incidence on bunches in Les Caillerets at Volnay, Burgundy, 2015.

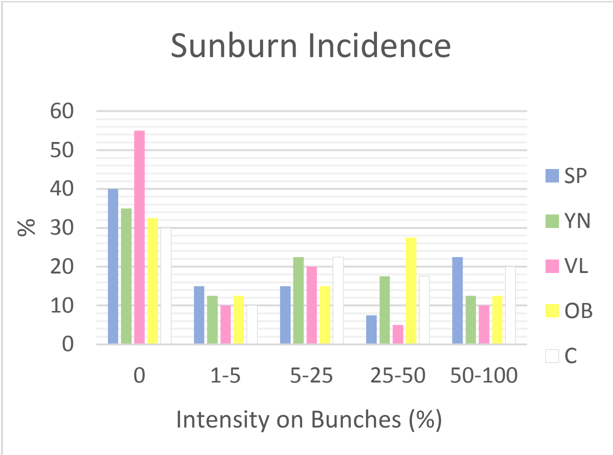
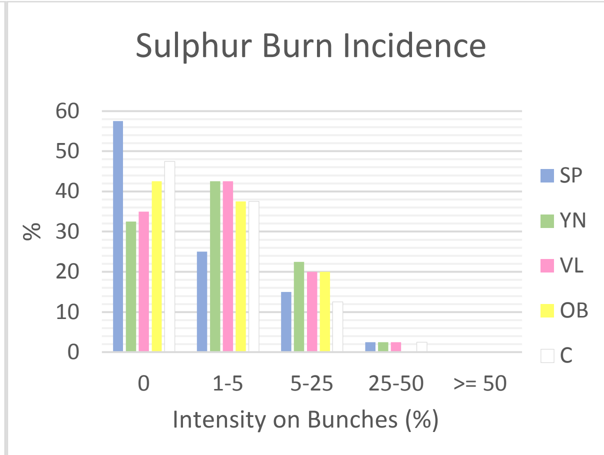


Figure 44. Sulphur burn distribution of incidence on bunches in Les Caillerets at Volnay, Burgundy, 2015.

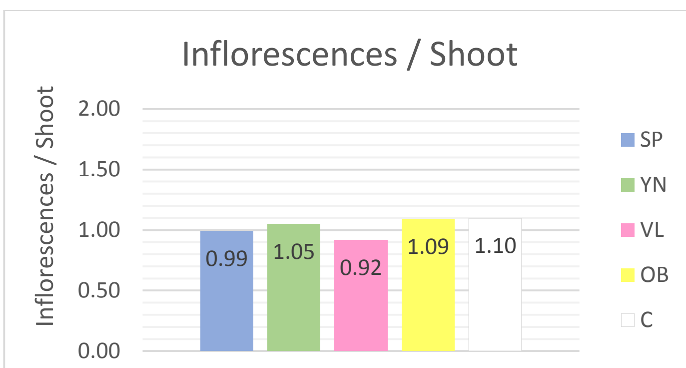


IV.B.4 Regenerative Development

IV.B.4.1 Inflorescences/Shoot

Inflorescences counted had no significant differences between treatments and all of them were lower than control (Fig. 45).

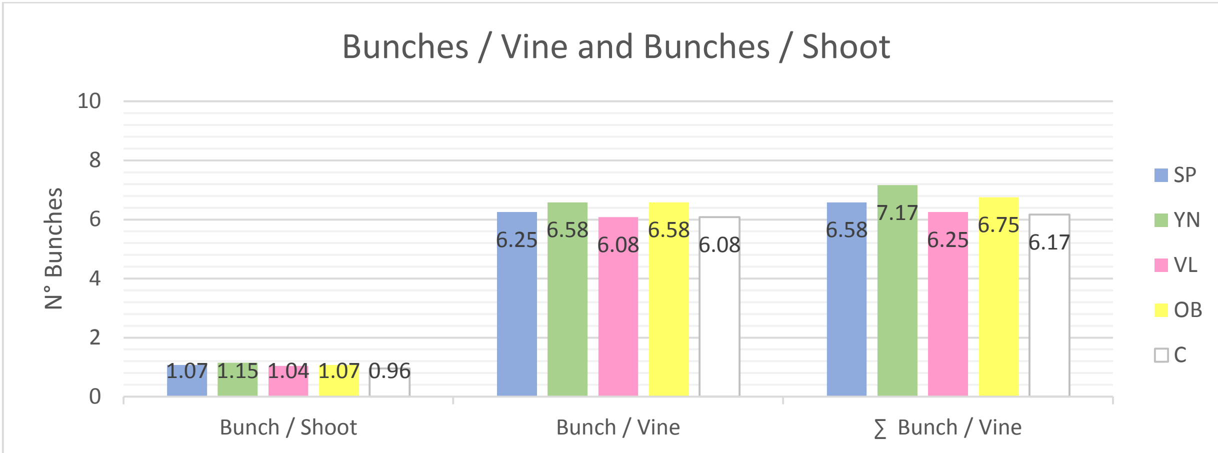
Figure 45. Inflorescences per shoot in Les Caillerets at Volnay, Burgundy, 2015.



IV.B.4.2 Bunches/Shoot & Bunches/Vine

Results of yield estimation counting bunches did not have significant differences analyzing treatment means (Fig. 46).

Figure 46. Bunches per vine from productive and others shoots and bunches per shoot in Les Caillerets at Volnay, Burgundy, 2015.

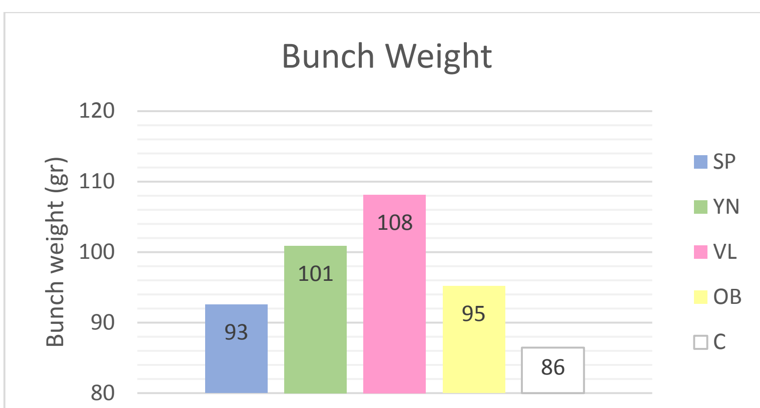


The increase of bunches per vine when counting all bunches coming from suckers, doubles or not well developed shoots, is the parameter which was closer to some significance but still not enough. In this sense, YN treatment showed the greatest increase because of these bunches (8.86%).

IV.B.4.3 Berry & Bunch Weight

No significant results were found on bunch weight, nevertheless it was observed that all treatments had heavier bunches than control, particularly the VL treatment (Fig. 47). Similar non-significant results were found in berry weight (Fig. 54 in Annex 4).

Figure 47. Bunch weight in Les Caillerets at Volnay, Burgundy, 2015.



IV.B.4.4 Berry Maturation Sampling

Must analysis before harvest gave no significant differences between treatments in any of the parameter (Table. 18).

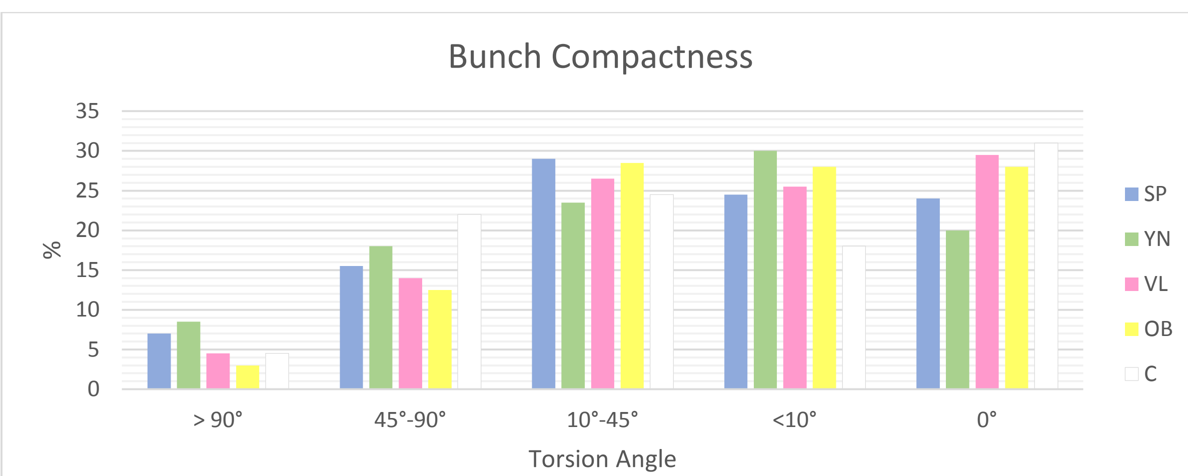
Table 18. Must analysis through spectrophotometry in Les Cailleret at Volnay, Burgundy, 2015.

	SILICA PURE	YARROW S.NETTLE	VALERIAN	OAK BARK	CONTROL
POT ALCOH REFRACTO (%VOL)	13.30	13.30	13.40	13.40	13.30
POT ALCOH TABLE (%VOL)	13.82	14.00	13.94	14.06	14.00
SUGAR (G/L)	235	238	237	239	238
PH	3.14	3.13	3.12	3.12	3.16
TOTAL ACIDITY (G H2SO4/L)	3.09	3.27	3.24	3.23	3.18
TARTARIC ACID (G/L)	4.65	4.71	4.66	4.47	4.36
MALIC ACID (G/L)	3.07	3.23	3.14	3.12	3.23
K	820	851	853	824	851
AMMONIUM NH3 (MG/L)	67	74	59	70	65
ALPHA AMINO NITROGEN (MG/L)	89	90	79	85	87
ASSIMILABLE NITROGEN (MG/L)	146	144	133	145	138

IV.B.4.5 Bunch Compactness

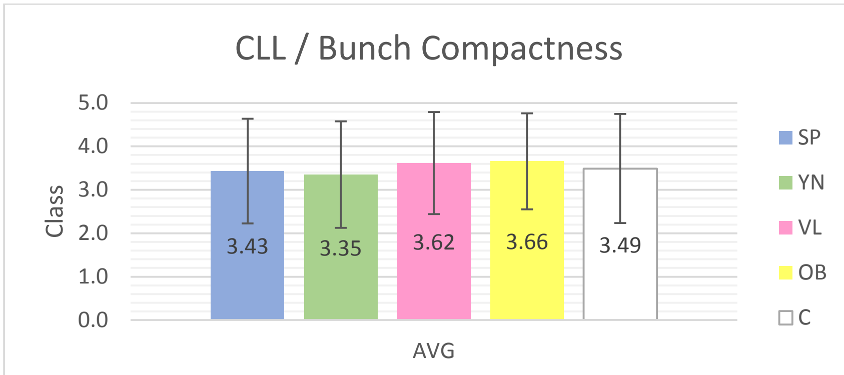
The impact of treatments on the angle of torsion tolerated by the bunch vertical axe can be considered as a trend (p-value < 0.1), in this sense, SP and YN treatments tended to reduce compactness, VL and OB treatments tended to enhance compactness and control stayed in between (Fig. 48).

Figure 48. Bunch compactness distribution in Les Caillerets at Volnay, Burgundy, 2015.



Averages by class confirmed that OB and VL treatments had higher bunch compactness with values around class 4, meaning a torsion angle between 0° and 10°, while YN and SP were closer to class 3 meaning looser bunches with a torsion angle between 10° and 45° (Fig. 49).

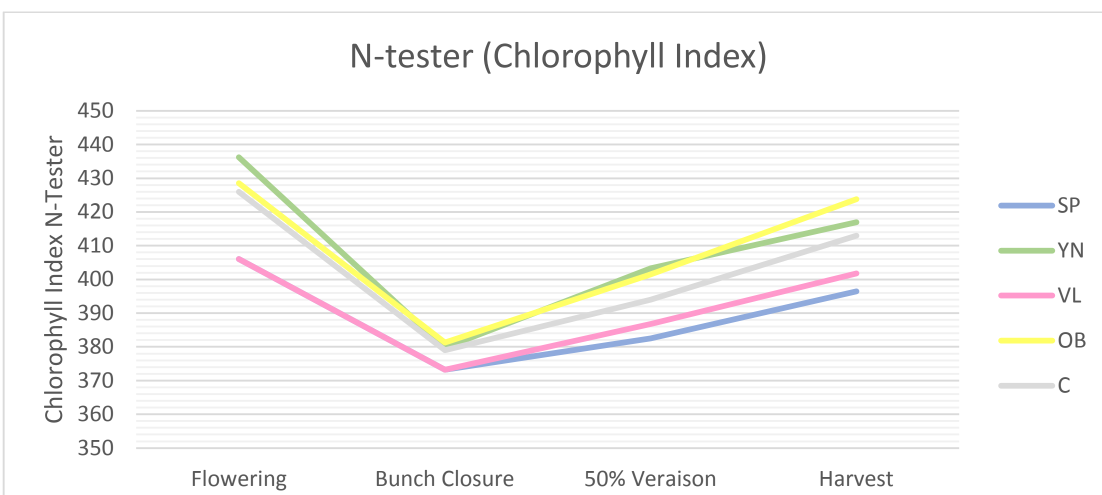
Figure 49. Bunch compactness intensity by class in Les Caillerets at Volnay, Burgundy, 2015



IV.B.5 N-tester (Chlorophyll Index)

Results indicated certain trend related to treatment effect (p-value <0.1) on N-tester values, allowing to group some treatments according to their behavior along the season. Leaves from treatments YN and OB showed consistently higher values than treatments VL and SP (Fig. 50). When analyzing each phenological state separately no significant influence of the treatments was found.

Figure 50. N-tester evolution in Les Caillerets at Volnay, Burgundy, 2015..



VI DISCUSSION

Results in Les Caillerets (CLL) were much less significant than in Champans Bas (CHB), suggesting that the date of application influences more the function of BDp 501 than additions of complementing herbal infusions. But, due to practical restrictions, many measurements were done on smaller samples in Les Caillerets, which could have affected reducing the degree of freedom and limiting the possibility of significant results.

Phenology

Due to the lack of statistical significance of one individual in a population, conclusions coming from this methodology were summarized at the end of this work (Annex. 4)

Physiology

Analyzing both fields together, physiological parameters showed contradictory results. In CHB treatments with BDp 501 applied before flowering (BF) and winegrower (WG) consistently **increased vegetative development**. Primary shoot growth at third measurement (June 9^{th}), canopy density measured as leaf layer number and internal leaves were all significantly higher in BF and WG than control (CO), particularly the treatment BF showed values slightly higher. Individual and group observations confirmed this firmness and strength of the canopy on plots belonging to earlier applications of BDp 501 (Abarzúa et al. 2015). This is the opposite to what have been observed in previous trials, where vegetative parameters showed lower vigour and more balanced growth, either comparing only biodynamic plots (Webber-Witt 2013, Lorimer 2014) or comparing biodynamic with other systems of viticulture (Reeve et al. 2005, Stöber 2011, Gonfrier 2012, Döring et al 2013, Meißner et al. 2013, Johnston et al. 2015).

On the other hand, in CLL vegetative parameters described a general lower vigour or more depressed canopy than CHB, but when looking for differences between treatments these were neither significant nor consistent. Nevertheless, in primary shoot growth it was interesting that all treatments with BDp 501 grew less than control in every date. This is supported by previous trials mentioned above, but mechanical and wind damaged on shoot apexes could have influenced as well. While treatments with BDp 501 had an average of 7.5 apexes damaged or broken, only 1 was found in control replicates. This shoot weakness is coherent with the group conclusion that 4 applications of BDp 501 for a first year in biodynamics was excessive for CLL (Abarzúa et al. 2015), considering that in CHB a total of 3 damaged apexes were observed in the whole field.

These different results lead to the question about the real effect of early applications of BDp 501 over the vegetative parameters, does it promote the vegetative development or does it regulate? More research has to be done to answer this question.

Regarding treatments with BDp 501 plus herbal infusions, only isolated results were observed, like the trend on silica pure (SP) and silica oak bark (OB) treatments to have more gaps in the canopy than silica yarrow/stinging nettle (YN) and silica valerian (VL) treatments, but this lacks of relevance without any other result supporting it. Another example was certain inclination showed by VL vines towards higher density of the canopy, which is opposed to that stated by Masson (2012) that valerian reduces formation of lateral shoots between other functions. Similarly, differences are very subtle and more research has to be done in this sense as well.

Vine Diseases

In a year almost without disease problems, any aspect related to vines health was secondary and with a minimal effect. In both fields results about **coulure** were not significant although in CLL the general incidence was lower. The influence of the trellis system, in CLL Guyot versus the mixture Cordon Royat/Guyot in CHB, could have an impact on that.

In CHB significant differences in **sunburn** frequency were seen: FH (30%), CO (39%), BF - WG (45%). In CLL the frequency of **sunburn** was higher, 45 to 70%, maybe due to its more depressed canopy, with control (C) bunches showing clearly the highest frequency and VL the lowest, but, without statistically significant differences. The risk of BDp 501 related to hot or too exposed environments has been supported (Joly 2008, Masson 2012), but CLL showed an exactly opposite behavior with BDp 501 reducing that impact. These opposite effects has not been reported before, so following years can confirm or refuse these results. In any case, is unlikely that 3 applications of BDp 501 before hot temperatures (BF-WG) had increased sunburn frequency and then 1 application in the beginning of hot period (FH) had reduced it that much. This suggested the influence of uncontrolled factors, and it can be interesting to consider UV rays in the analysis, because of their impact in bunch quality (Song et al. 2015) and the global warming context.

When it comes to **sulphur burn**, in CHB a general higher incidence compared to sunburn (56.9 to 58.4 %) has to be remarked because it could be improved by management. In CLL was the opposite, the incidence of Sulphur burn was lower than sunburn. Regarding treatments, no significant influence was found, in any field.

Regenerative Development

Previous references about impact of BD system in **yield** described a negative effect (Johnston et al. 2015). Results tended to be the opposite in both parcels, but these cannot be related with treatments because floral induction is determined by conditions on the previous season. For 'secondary' bunches coming from double shoots, suckers or not well developed shoots is the same; the exception would be pimples, which could have been influenced by the treatments, but they were not counted. So, to infer a conclusion about these parameters is not possible this year.

In CHB, the significantly lower **bunch weight** on FH (79 g) treatment compared to the others, CO, BF and WG (> 102 g) was interesting because FH was the only treatment done exactly during maturation period. A lower berry weight has been seen in BD plots compared to ORG (Gonfrier 2012) but this is not enough to support this result. In CLL for example, although not significant, treatments with BDp501 got higher bunch weight but lighter berry weight compared to control, which evidence the variability in this aspect.

A parameter aspect well documented is related to **bunch compactness**. Meißner et al. (2013) already found significant difference between BD vineyards compared to INT and ORG management. In CHB the three treatments with BDp 501 reduced bunch compactness compared to control, especially both with application before flowering (BF – WG). In CLL a trend observed describes SP and YN as more loose bunches than C, VL and OB respectively. This is a trend to confirm during next years, but what is noticeable is that this parameter has been consistently significant in both experimental fields so that, is worthy to study.

N-Tester

Although no significant results were found in CHB, it was noticeable that an early BDp 501 application like WG treatment had consistently higher and more stable values for this index of chlorophyll, usually related to nitrogen balance in plants and vigour. Some trials using this technology had similar results (Webber-Witt 2013, Lorimer 2014). In CLL the N-Tester showed a trend of YN and OB treatments having higher values than treatments VL and SP, which could be connected to group and individual observations on the field but only concerning the YN treatment that was seen with more alive canopy (Abarzúa et al. 2015).

Looking at differences between leaf position, it seemed that lower leaves tend to reduce their chlorophyll content at the end of the season while upper leaves rise up. At the beginning is exactly the opposite, upper leaves showed low values and lower leaves high values. Veraison is the moment when upper and lower leaves had similar values and should be the moment to measure.

To complement the discussion a summary with group observations on the field about each treatment in both parcels is presented below (Table 19).

Table 19. Summary of group observations made on the field in Champans bas and Les Caillerets, Volnay, Burgundy (Abarzúa et al. 2015).

<table>
<tr><td rowspan="13">GROUP OBSERVATIONS</td><td rowspan="6">Champans Bas</td><td>General</td><td>Problem of homogeneity coming from the different trellis systems. Guyot seemed to have better productivity than Cordon Royat vines</td></tr>
<tr><td>General</td><td>Observations in the morning are useless because leaves are in a situation of comfort, they all look similar, turgent and hydrated; but in the evening is when they could show if treatments with BDp 501 help them or not to tolerate weather conditions during the day</td></tr>
<tr><td>WG</td><td>good canopy structure and grape taste</td></tr>
<tr><td>BF</td><td>BF a canopy a bit more depressed and lacking some tension on taste</td></tr>
<tr><td>FH</td><td>FH thicker leaves, more balanced canopy and tension on taste</td></tr>
<tr><td>CO</td><td>CO the most depressed canopy, with grape skin tasting differently</td></tr>
<tr><td rowspan="7">Les Caillerets</td><td>General</td><td>Upper area of the parcel looked weaker than lower area</td></tr>
<tr><td>General</td><td>4 applications of BDp 501 was a bit excessive considering it was its first season under biodynamic management, explaining with this a certain general weakness perceived on vines</td></tr>
<tr><td>YN</td><td>One of the best resisting the hot weather of 2015</td></tr>
<tr><td>SP</td><td>One of the best resisting the hot weather of 2015</td></tr>
<tr><td>VL</td><td>More vigorous than control</td></tr>
<tr><td>OB</td><td>Weak, affected by the sun</td></tr>
<tr><td>C</td><td>Less organized canopy</td></tr>
</table>

About the implementation of long term trials

One of the main goals of this research was to understand what is needed to implement long term trials of this kind in established vineyards, working with winegrowers. In this sense, a short summary of the main conclusions are listed as following:

1.- Requirements. Two concrete conditions emerged from this experience as primordial for scientific purposes:

- **4 replications**
- **200 measurements per treatment per parameter**

These two conditions help to translate accurately observations on the field into statistical terms and distinguish an impact of BDp, which is usually subtle and not so discernible because they work in low dosages (Raupp 1999). Smaller samples tended to give not significant results even when clear differences were observed. These conditions demand a physical effort that has to be considered. An exception could be samples repeated on time, in those cases less than 200 measurements per treatment can be an option.

2.- Experimental Design. Considering the previous point an experimental design should consider **no more than 3 modalities**, especially if there are more than one research field. If it is just one parcel then 5 modalities are possible to manage, but this is the maximum. All difficulties coming from the randomization of the replicates has to be assumed by the student, meaning that the application of the treatments has to be done by him/her. In a randomized trial is not possible to trust the application to the winegrowers.

3.- Methodologies. Any **method used should be easy to implement and practical**, unless the university take responsibility for more complex methods with more sophisticated technology and prepare everything in advance. In this sense, the student in charge of the trial has to be fully aware about the methodologies and materials to use because winegrowers usually don't have scientific resources, sometimes not even internet.

4.- Student schedule. In a practical context is absolutely necessary that winegrowers get in contact with the student, to share perspectives, to appreciate the work each one does, and connect the research with the reality around. In this sense, to dedicate **2 or 3 days per week working as a common intern** with the winegrowers is necessary. Also, the student has to **stay in the place**, so in any case of difficulty or mistake he can go easily to fix it or to re-measure or anything that could be needed. To overcome the lack of the experience of the student a **weekly description and photography of one plant** per treatment was essential. It allows him/her to accompany the evolution of the vines and be aware about the timing of each measure. It is a deep tool of learning as well.

5.- Winery role. **One person of the winery should take responsibility** about the trial, knowing what kind of measurements should be done and having regular communication with the student about the trial.

6.- University role. The university should **provide the student and train him/her before its departure to the winery**. Every protocol, methodology and structure of the work has to be defined in advance in order to ensure a proper scientific work. This is priority because the student will be in isolated conditions, without the support of an academic body. Moreover, for the first year **the university should be present on the field** as starting point, particularly to define the experimental design which is the most important issue for a long term trial. Winegrowers demand this presence, otherwise any cooperative work will not be feasible.

Projection of the trial

Taking in consideration the methodologies used, practical limitations observed and the results obtained, several proposals will be done in order to improve the performance of this trial and push it further. In this sense, one of the first challenges is to keep the same level of accuracy in both parcels, rising the sample size in Les Caillerets to equal Champans Bas. To do that the ideal solution is to have one student per field; they can work separately or as a team when most difficult measurements have to be carried out.

A second point is that parameters of vegetative development should continue being studied, to confirm or reject results obtained this season. In this line, new variables could give new interesting information. Starting from the simplest ones, **shoot verticality** is a variable to study from budbreak to the first lift up of wires useful to compare earlier applications of BDp 501 (Masson 2012). The **blade angle** of leaves related to the shoots is closely related to canopy density, because in more dense canopies leaves tend to compress themselves rising up their blade angles, while in less dense canopies leaves tend to appear extended and fully open, so that blade angles descend apparently. Then, is very important to compare plants in equal conditions of vigor and canopy density, which means the trellis system has to be the same. Another variable to try could be **petiole length**, which is influenced by shoot vigour and the variability along the shoot. For instance, in Guyot vines it was observed that both shoots located at the spur tend to be more vigorous and showed wider leaf blades and longer petioles. Moreover, basal leaves tend to have shorter petioles than middle leaves, usually correlated with **leaf blade diameter**. In order to measure petiole length both source of variability should be considered. Looking at more sophisticated alternatives, **NDVI,** an aerial vigour description has proved its significance in previous experiences (Röder 2013) and it can be assumed by external companies. Considering the global warming context, **thermographic** measurements on the canopy with Multiplex ™ or any others equipment can approach the temperature regulation of each treatment. This could be performed by Geisenheim University.

Regarding regenerative parameters, it seems that **coulure** is connected with **bunch compactness**. If this is verified, results should be analyzed considering **disease incidence on bunches** and **bunch weight** as well. In other words, if any year coulure has a strong impact and this is correlated with reduced bunch compactness, it cannot be judged as negative if diseases on bunches and bunch weight are not assessed as well. The natural 'compensation effect' balance the coulure impact in a way that the final yield is not affected. Studying this group of parameters together, it can occurs that BDp 501 before flowering could have unexpected influence. **Must analysis** is very interesting for producers so is a priority that should be done once or twice, but always analyzing each replicate separately, for statistical requirements. **Sunburn and Sulphur burn** have increasing interest because of global warming, but their impact depends on climatic conditions more than applications of BDp 501 so they should be taken as alternatives more than priorities to measure.

Parameters of yield estimation should be discarded because they are more interesting when comparing BD to others systems than in a study between two BD plots.

Finally, from a very personal point of view, the effect of BDp 501 should be addressed always from different and innovative approaches, it is not something that traditional visual measurements will describe in its real dimension. In this sense, scientific research done on analysis of **Volatile Organic Compounds** (VOC) released by plants (Zhang and Li 2010, Reynolds et al. 2014) could be interesting if they are described when BDp 501 is applied, although it implies a great challenge to implement on-farm.

VII CONCLUSIONS

Two research projects were implemented and carried out in two different established Pinot Noir vineyards, in order to find out the impact of the BDp 501 – Horn Silica – on vegetative and regenerative development of vines.

Regarding results about different dates of application it was found that BDp 501 applied before flowering (BF), between flowering and harvest (FH), or according to winegrower criteria (WG) has no negative effect on vines related to canopy development or yield. In a hot and dry year like 2015 where sunburn and sulphur burn became important, this observation gain in value because reject the usual believe that 501 increase risk of vine stress coming from excess of light and heat. Moreover, results indicated a trend of both treatments with applications at the beginning of the season (BF and WG) to strength canopy development and shoot growth, but this has to be confirmed during next years.

From a methodological point of view the measurement of bunch compactness appeared as the most significant one. All treatments with BDp 501 applied in different dates showed looser bunches than control, which in a year of such low pressure of diseases does not look having practical importance, but it could have in the future of this research.

Looking at the impact of BDp 501 with infusion of Yarrow/Stinging Nettle (YN), Valerian (VL), Oak bark (OB) or pure (SP), results are much less significant and difficult to extrapolate to practical consequences, at least in its first year of research. In the same line than the first parcel, here were not seen negative effect from BDp 501 applications, confirming the previous statement.

As a projection of this research, several new variables were proposed to study in order to improve precision and representativity, because when it comes to biodynamic research is clear that we deal with subtle impacts that should be approached from several perspectives.

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ANNEXES

1. Biodynamic Preparations (Webber-Witt 2013).

BD Prep	Origin	Use and influence
500	Cow manure	Soil health used as field spray
501	Silica powder	Photosynthesis and balance used as field spray
502	Yarrow blossoms (*Achillea millefolium*)	Allows for reduction in dose of sulfur; Influence in K and S. Used as compost
503	Chamomile blossoms (*Chamomilla Officinalis*)	High in calcium, potash and sulfur. Promotes general heath. Influence in Ca and K. Used as compost
504	Stinging nettle (*Urtica dioica*)	Regulate and stimulates vegetative growth. Prophylactic for mildew. Control aphids and allows reduction in dose of copper. Influence in N, Fe, K, Ca and Mg, S. Used as compost
505	Oak bark (*Quercus robur*)	Anti-fungal. Influence in Ca. Used as compost
506	Dandelion flowers (*Taraxacum officinalis*)	Reinforces the silica process, improving the quality of plant tissues and resistance to fungal attacks. Influence Si, S and Mg. Used as compost
507	Valerian flowers (*Valeriana officinalis*)	Helps to give plants strength after hail or frost. Stimulant during flowering stage. Influence in P. Used as compost
508	Horsetail (*Equisetum arvense*)	Anti-fungal agent rich in silica. Helps to strengthen the resistance of vines. Used as field spray.

2. Sampling Strategy

$$n = \frac{(Z_\alpha^{\,2})(v_s)}{r(D^2)(\overline{X}^2) - (Z_\alpha^{\,2})(v_p)}$$

where n is the required sample size, Z_α is the standardized normal value at α level of significance, v_s is the sample variance, $\overline{X}$ is the mean value, d is the margin of error expressed as a fraction of the plot mean, r is the number of replications, v_p is the variance between plots of the same treatment and D is the margin of error expressed as a fraction of the treatment mean (Gomez and Gomez 1984, Jaggi and Varghese 2007).

3. Number of Internodes

Figure 51. Number of internodes and trellis system in Champans Bas at Volnay, Burgundy, 2015.

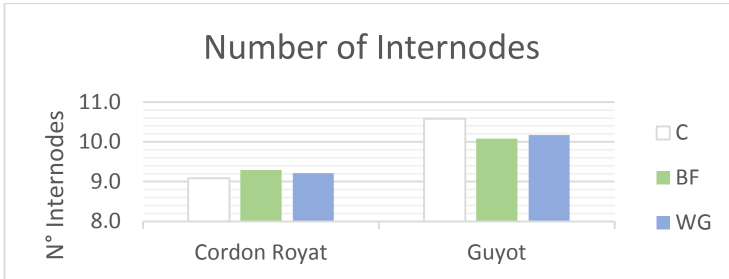
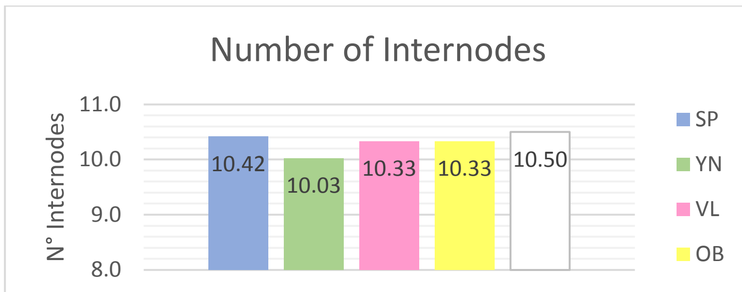


Figure 52. Number of internodes in Les Caillerets at Volnay, Burgundy, 2015.



4. Berry Weigth

Figure 53. Berry weight in Champans Bas at Volnay, Burgundy, 2015

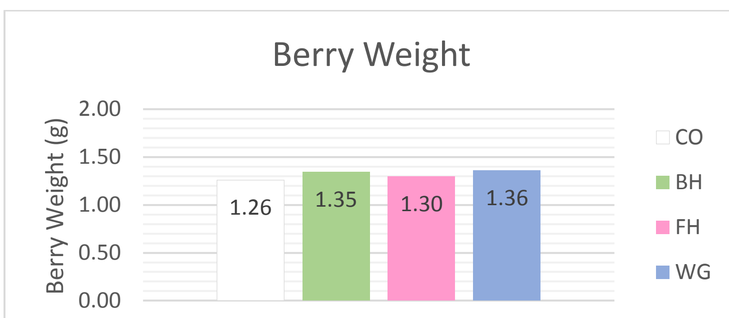
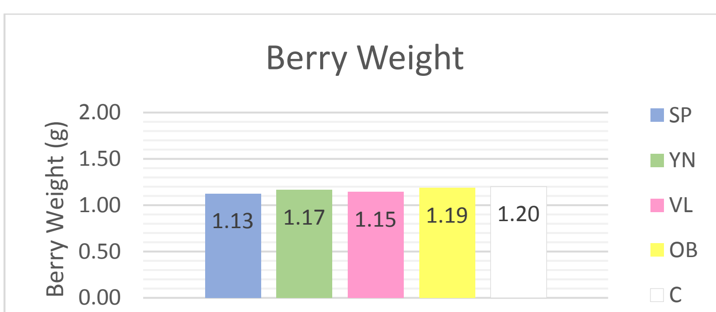
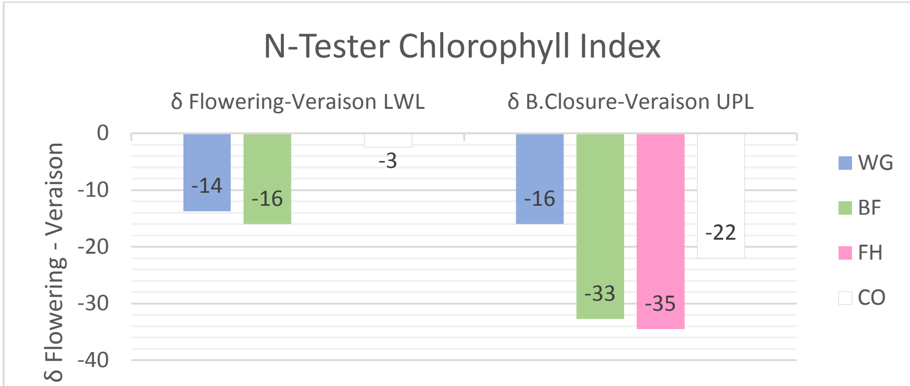


Figure 54. Berry weight in Les Caillerets at Volnay, Burgundy, 2015



5.

Figure 55. Differences in N-Tester values between phenological states in Champans Bas at Volnay, Burgundy, 2015



6. Individual Observations on Phenology

Champans Bas: During Flowering temperature changed suddenly getting days with maximum over 30°C, which accelerated the end of the process. Differences observed (BF > WG - CO > FH) on 3-4th June suggested some impact of the BDp 501 applied before Flowering on BF and WG treatments, but the almost inversion of this order at Veraison (FH > CO > BF > WG) limited this argument. BF and WG treatment had higher number of bunches per plant as well, which influence delaying bunch development (Jackson and Lombard 1993). Also, it was noticeable that the plant under WG treatment expressed consistently higher values for parameters related to vegetative development, increasing the leaf/fruit ratio and suggesting that it was able to resist the hot and dry environment during maturation period, regulating better its growth. Instead, the FH plant was often associated with poorest canopy development, so that it was more affected by the weather, leading it to a more accelerated development, which can explain differences observed at Veraison. If this development was influenced by treatments of BDp 501 is still unclear, more observations are needed to get any conclusion from that in this research. In any case, a stronger canopy development usually is not considered as something positive for quality vineyard, especially if it is excessive, but in a hot year like 2015 this can be interesting as a natural regulation, with better adaptability. Another point was that both plants, WG and FH, were conducted in Cordon Royat, suggesting that the impact from treatments with BDp 501 can be stronger than the effect of the trellis system. Finally, at Maturity, both treatments treated before Flowering showed higher dehydration of berries than FH and CO, but it did not correspond with observations of sunburn or sulphur burn.

Les Caillerets: Individual observation showed that vines under treatments with more developed state at Flowering were YN – OB - C > SP – VL. These difference seemed to disappear at Veraison (OB

> VL > SP > YN > C) where SP and VL tended to a more advanced state while YN and C tended to a more delayed one. The plant from OB treatment was the exception to this observation, without a clear explanation coming from oak bark properties. The fertility observed (VL > C > SP > OB > YN) seemed to be inversely related to coulure incidence (YN << OB - SP < C - VL) which is not consistent with that observed at Champans Bas. Dehydrated berries (VL > SP - OB > YN – C) observed has followed a similar order observed at Veraison, except again OB vines. These observation did not allow to take any conclusions from them. In terms of canopy seemed that YN treatment emerged with better vegetative parameters, higher regularity, color, petiole length and leaf vitality, as well as less sunburn damage, but simultaneously YN had high proportion of gaps and low lateral shoots.