POLYTECHNIC INSTITUTE OF BEJA

Agrarian School

Master in Food Engineering

Utilization of a banker plant to potentiate *Ampelomyces quisqualis*' efficiency as a biocontrol agent of grapevine powdery mildew (*Eryshiphe necator*) and,

Effects of powdery mildew in quality and yield of grapes (*Vitis vinifera*) at harvest

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Master Thesis

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Abstract

To study the hypothesis of building up a system were *Ampelomyces quisqualis* could develop in time, on a plot, to parasitize grapevine powdery mildew (*Erysiphe necator*) when the first contamination occurs, a banker plant was installed in a trial plot.

The chosen banker plant was purple clover, and its phenological stages were observed during 2016. When purple clover reached its early flower stage, it was infected with *Erysiphe trifolii* and, after visual symptoms of the fungus were spotted, an inoculation with *Ampelomyces* was made.

This season, no visual symptoms of powdery mildew were observed on leaves of the grapevine, there was only infection on clusters with a frequency of attack of 10% and a severity of 5%, in average. A series of samples were collected for further investigation with Polymerase Chain Reaction technique.

A second trial was conducted to evaluate the impact of powdery mildew disease on quality and yield of clusters and musts, of Chardonnay and Cabernet Franc, at harvest period. It was concluded that, powdery mildew has negative effects on quality and yield of grapes at harvest period, even in lower levels of infection (11%), by decreasing the size and number of berries. An increase in total acidity was spotted in both cultivars. In Cabernet Franc the composition in anthocyanins decreased.

Regarding the triangular tests, no statistical differences were found between samples however, the sensory panel noted some of the most aromatic attributes given by the fungal disease, according to literature (viscosity/oil, mould/dust).

Keywords: *Ampelomyces quisqualis,* Banker plant; Crop damage; *Erysiphe necator*; Organoleptic test.

Resumo

Para estudar a eficácia de um sistema onde *Amplemomyces quisqualis* se pudesse desenvolver, numa vinha, de forma a parasitar o oídio (*Erysiphe necator*) aquando da primeira contaminação, uma planta de suporte foi instalada numa parcela.

A planta escolhida foi o trevo violeta e, os seus estados fenológicos foram observados durante 2016. Quando o trevo atingiu o estado de início de flor foi infetado com *Erysiphe trifolii* e, após observação de sintomas do fungo procedeu-se com a inoculação com *Ampelomyces*.

Este ano, não foram detetados sintomas de oídio nas folhas da videira, apenas se verificou a infeção nos cachos com uma frequência de ataque de 10% e severidade de 5%, em média. Uma série de amostras foram recolhidas para futura investigação com recurso ao *Polymerase Chain Reaction*.

Um segundo ensaio foi conduzido para avaliar o impacto do oídio na qualidade e rendimento de cachos e mostos, de Chardonnay e Cabernet Franc, no momento da vindima. Concluiu-se que, o fungo tem efeitos negativos nas uvas, mesmo para níveis baixos de infeção (11%), através de uma redução significativa do tamanho e número de uvas. A acidez total é mais elevada em ambas as cultivares. Para Cabernet Franc a composição em antocianinas é menor devido à presença da doença.

Relativamente às provas triangulares, não se estabeleceram diferenças significativas entre as amostras contudo, o painel de provadores registou alguns dos atributos aromáticos mais comuns dados pela presença de oídio, referidos pela literatura (viscosidade/óleo, bolor/terra).

Palavras-chave: *Ampelomyces quisqualis, Erysiphe necator*; planta de suporte; danos; teste sensorial.

Introduction

This essay was developed in the ambit of the Master in Food Engineering of the Polytechnic Institute, Agrarian School of Beja. The main goal is to study the hypothesis of building up a system, using a banker plant, to increase the efficiency of the bio control agent *Ampelomyces quisqualis* of grapevine powdery mildew (*Erysiphe necator)* and assess the effects of this fungal disease on quality and yield of grapes at harvest period, through measure of clusters and, chemical and sensorial analysis of musts produced with several levels of powdery mildew's infection.

The practical experiment was conducted in France, region of Angers, at *the Institut Français de la Vigne et du Vin* (IFV) through an ERASMUS + program.

In 2008, France initiated a plan called *Ecophyto 1*8 with the goal of diminish in 50% the utilization of pesticides in 10 years, if possible. This plan is englobed on the European directive 2009/128, regarding the use of chemical products compatibles with sustainable development and, having as minimal, as possible, negative effects on environment and human health (Ministère de l'Agriculture, de l'Agroalimentaire et de la Forêt, 2015).

Powdery mildew caused by *Erysiphe necator* is one of the most important grapevine diseases in several viticulture areas, and high fungicide input is required to control it, which is under scrutiny due to concerns about its impact on human health and the environment (Nesler, et al., 2015).

This study was conceived having this concerns in thought and, trying to create a sustainable solution, compatible with organic farming that could fight powdery mildew's disease without using fungicides but a biocontrol agent instead.

The use of *Ampelomyces* as hyperparasite of powdery mildew has been studied before and, a commercial form of the fungus is already available in some countries, for example, Italy. However, its efficiency is controversial and, for this reason, this study is being developed.

The present work is divided in four chapters concerning two trials: a field trial and a tasting trial, being the first chapter dedicated to a bibliographical review about all the subjects approached. These subjects are, firstly, dedicated to grapevine powdery mildew

(*Erysiphe necator*), its morphology and biological cycle, a description of the conditions that favour its appearance on the grapevine and, the most common symptoms associated to an infection of this fungal disease in several organs of the vine. Secondly, an approach on previous studies about the impact of powdery mildew in the quality of grapes, musts and wines. Thirdly, a resume on using pesticides to control powdery mildew's development, their impact to economics, environment and health and, also alternatives to their use, like, bio control agents, for example. Fourthly, an explanation of the cycle of development of *Ampelomyces quisqualis* and its mode of action against powdery mildew. A description about how can it be used as bio control agent and why its use has been appointed with some limitations to fight the grapevine disease. Lastly, it is appointed a hypothesis to overcome the efficiency issues related with this bio control agent and a description of an analysis to detect the presence or absence of *Ampelomyces's* parasitism on powdery mildew.

Second chapter starts by describing the *Institut de la Vigne et du Vin*, where its located, which are its goals and its facilities in Angers. After, this project, named AQ10 Diaplasce, is explained, its main goals are appointed and, the objectives for this essay are summarized. Finishing this chapter are the materials and the necessary methodology to reach the objectives mentioned before.

Third chapter is dedicated firstly, to describe and discuss the obtained results regarding the installation of the tri-trophic system (banker plant – powdery mildew/ *Ampelomyces quisqualis* - grapevine) and, secondly, to demonstrate the obtained results of a tasting trial, where is assessed the effects of grapevine powdery mildew on quality and yield of grapes at harvest period.

The fourth and last chapter approaches a reflexion about everything done in this study, what were the limitations, which improvements can be done to obtain better results and, further possibilities of investigation.

1. Bibliographical Context

1.1. Grapevine Powdery mildew

Grapevine powdery mildew is a fungal disease caused by *Erysiphe necator*. This fungus belongs to the genus *Ascomycota* (table 1) and, it is an obligatory parasite on genera within the *Vitaceae*, including *Vitis*, *Cissus*, *Parthenocissus* and *Ampelopsis* (Pearson & Gadoury, 1992), however, the most economically important host is grapevine (*Vitis*), particularly the European, *Vitis vinifera*, which is highly susceptible to powdery mildew (Gadoury, D., et al., 2012). It attacks the new branches, leaves, clusters and shoots of the vine although, it doesn't destroy these structures, because it only needs to extract nutrients, but harms the vegetal development and production of the fruits.

Table 1 - Scientific classification of the fungus *Erysiphe necator*

Kingdom:	***Fungi***
Division:	*Ascomycota*
Order:	*Erysiphales*
Family:	*Eryphaceae*
Genus:	*Erysiphe*
Specie:	*necator*
Binominal nom:	*Erysiphe necator (syn. Uncinula necator)*

This parasite is native of United States of America and it was introduced, in 1845, on Europe through the introduction of the American grapevine plants into the European vines.

Powdery mildew is one of the most important fungal diseases for the vineyard along with downy mildew and botrytis. Longtime ago, this disease was known as "the disease of the vine" because of the big impact it has on the grapevine and, it represents a real threat for farmers. In fact, if the epidemic is not controlled, the damages caused can be very serious and cause important losses of yield and quality on vines and on final product, meaning wine.

1.1.1. Morphology and reproductive biology

Hyphae of *Erysiphe necator* are 4–5mm in diameter, hyaline and superficial on epidermal cells, with multilobed appressoria at regular intervals. A penetration hypha from the lower surface of the appressorium pierces the cuticle and epidermal cell wall and is subtended by globose haustorium, which envaginates the epidermal cell membrane (figure 1).

Multiseptate conidiophores form themselves perpendicular to the epidermis, densely in rapidly growing colonies on susceptible tissue, less so on more resistant tissues, each producing a single hyaline, cylindro-ovoid conidia. Chains of conidia may accumulate in still air, the oldest conidium at the distal end. Long conidial chains are rarely seen under more turbulent field conditions. Conidia germinate via a single germ tube, which terminates in a lobed appressorium (Gadoury D. , Seem, Ficke, & Wilcox, 2011).

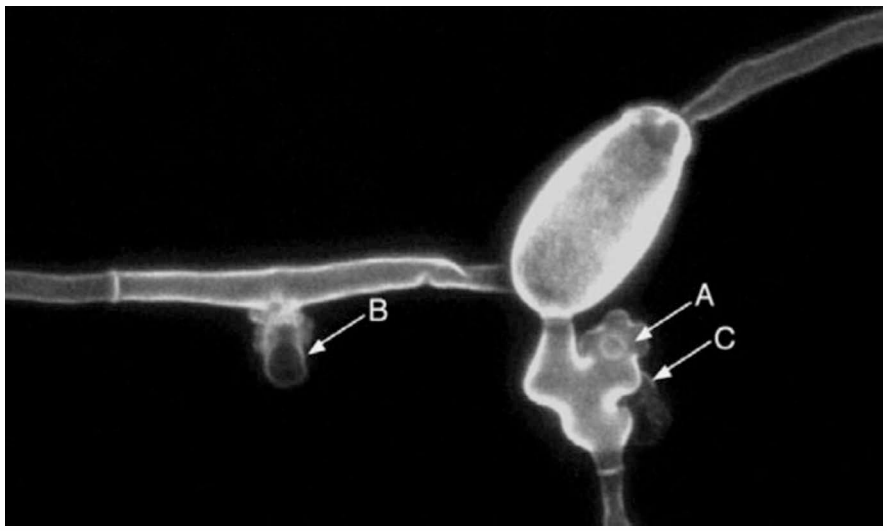


Figure 1 - Laser scanning confocal micrograph of *Erysiphe necator* on an ontogenically susceptible leaf of *Vitis vinifera* at 72h post-inoculation, showing the multilobed primary apressorium and penetration pore (A), and secondary germ tube with appressorium (B). The globose haustorium is faintly and partially visible at the lower left, beneath the primary appressorium (C) (Gadoury, D., et al., 2012).

➢ Asexual reproduction (conidial phase)

Powdery mildew survives in dormant infected buds, early in the previous season. In the following spring, a wind-dispersed spore called a conidium is produced. These spores land on surrounding shoots and vines and, infect the green tissue. Conidia germinate within 24 hours in the absence of free water when relative humidity is greater than 40%. At each of these new infection sites, the fungus multiplies, and around 5-12 days later these new 'colonies' produce another generation of conidia. Regardless of whether initial infection originates from overwinter spores or cleistothecia, unless the disease is controlled, the infection cycle continues many times throughout the growing season resulting in a rapid increase in disease incidence. After about 40 days from budburst, spore numbers increase dramatically and disease severity escalates if controls are not applied, or are ineffective (figure 2).

➢ Sexual reproduction (sporophyte phase)

Cleistothecia are the sexual fruiting bodies produced by the powdery mildew organism. They only form on the surface of heavily diseased vine tissue and, take about 90 days to fully mature. Immature cleistothecia are yellow, and gradually turn brown, then black. When mature they are just visible to the naked eye and look like tiny black specks the size of a pinpoint on the surface of heavily diseased tissues. They form from mid-summer to autumn and survive over winter on the bark around the vine crown and cordon, and on mummified bunches.

When cleistothecia are wetted by 2.5 mm or more through rain or irrigation and temperature is greater than 10°C, they eject ascospores which are spread by wind and water to infect the lower leaves near where the cleistothecia have overwintered. Similarly to infection by conidia, these also produce characteristic yellow leaf splotches as the colony develops. These colonies go on to produce asexual conidia that can then start new infections (figure 2) (Wicks, 2010).

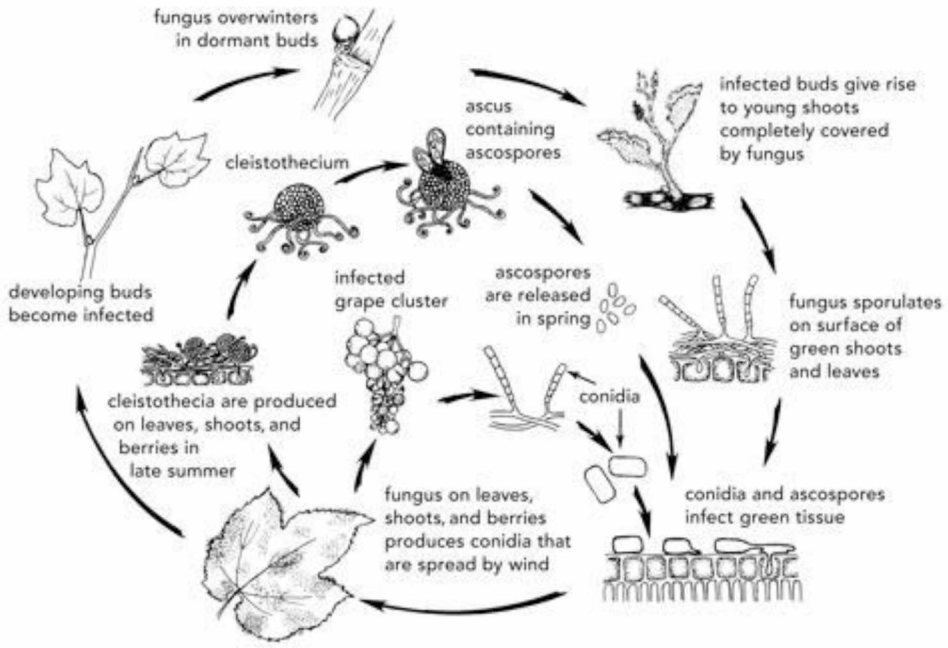


Figure 2- Biological cycle of *E. necator* and its morphological representation (Wicks, 2010).

1.1.2. Conditions favoring development of infection

Seasonal differences of powdery mildew severity varies considerably depending on a number of factors. The levels of the disease in the previous season in conjunction with the effectiveness of controls applied influence the amount of diseased buds and cleistothecia carried over to the next season. The occurrence of conditions favorable to the disease in the current season also has considerable impact (Wicks, 2010).

In general, this pathogenic agent prefers environmental conditions with high humidity, accompanied with vigorous and compact vines where the sun light will have difficult to penetrate and the circulation of air is limited (Arnaud & Arnaud , 1931). The factors of temperature, humidity and sun light are interdepend and their conjugation favors or not the development of powdery mildew in the grapevine.

➢ Temperature

Temperature is an important factor for the growth of *Erysiphe necator*, but the optimal conditions differ in function of the state of the development of the fungus.

Relatively to the germination of conidias, temperatures between 15 – 27ºC allow to an increase of speed of germination. At its optimum, this phase of development starts 1h30 after the deposition of the spores and riches a maximum of germination after 12h. However, the duration of this phase is dependent of the temperature so, for very high or very low temperatures, the start of germination is 4 to 8h after the deposition of the spores and riches one maximum after 14 – 28h. Equally, at 5ºC the conidias are unable to form the appressoria (Cantin, 2015).

Furthermore, temperature influence also the next germination cycle (Fessler & Kassemeyer, 1995):

- If germination occurs at temperatures inferior to 20ºC, the production of conidias will not have any problem;
- If germination occurs at temperatures superior to 24ºC, the production of conidias will not germinate more than until 13ºC.

The first sporulation, in optimal conditions (at 22ºC), are visible after 5 days and they stop 35 days later. Temperatures between 26 -30ºC are the optimal ones for this phase.

➢ Relative Humidity and free water

Relative humidity (RH) is a favorable factor for the germination and sporulation of *Erysiphe necator* however, the sensitivity to this factor is more important for sporulation than for the other phase.

The optimal spectrum of Relative Humidity is between 60% and 76%.

Nevertheless, free water is unfavorable to the development of *Erysiphe necator*, for instance, it conduces to a low germination, inhibit the formation of apressoria and leads to the bursting of conidias.

Rain is one source of relative humidity for the development of the fungus, for example, a drizzle is a favorable factor to the development of the disease because, it will increase the level of relative humidity of the air however, a downpour is unfavorable since it will wash the conidias and the mycelium present on the organs of the vine, especially, on the leaves (Corio - Costet, 2007).

- **Wind**

Another environmental element, which can promote the dissemination of conidias and allow the propagation of the fungus in space, is the wind (more than 3m/s). It can also diminish the high summer temperatures (45°C) that harm the development of the fungus.

- **Sun light exposition**

The duration of illumination, combined with temperature and relative humidity, is favorable for the germination of conidias. In condition of darkness, 42% of conidias germinate but, with a 24h exposition to artificial light, 75.1% the conidias germinate. Yet, the rows exposed to shadow, during the hot hours, are the most affected by the disease. So, the germination of *Erysiphe necator* is easier in conditions when the light is diffuse, and not when the light is direct and strong.

UVB intensity (between 1.3 and 0.7 W/m^2) is considered to be even more important than the duration of illumination. According to Zahavie, Reuveni , Scheglov, & Lavee (2001), strong intensity of illumination is a limitant factor for development of the fungus. The effect of radition is higher when the temperature is also high and, it affects the germination just after the inoculum (Willocquet, Colombet, Rougier, Fargues, & Clerjeau, 1996).

The UV radiation favors the formation of cuticular waxes, creating one mechanic barrier that limits the penetration of *Erysiphe necator* and, so the development of powdery mildew. However, low UV radiation combined with the input of 3g of nitrogen per plant favors development of the disease (Keller, Rogiers, & Schultz, 2003).

➢ Host plants

As told before, *Vitis vinifera* is especially sensitive to powdery mildew infection. The susceptibility to the disease varies according to the variety of the host plant.

Some examples of varieties **less sensitive** to powdery mildew are (Barber, 2014):

- Petit Verdot;
- Syrah;
- Alicante Bouschet;
- Ugni Blanc;
- Graciano and,
- Gewürztraminer.

Known varieties that are **sensitive** to powdery mildew's infection are the following (Barber, 2014):

- Alvarinho;
- Merlot;
- Sauvignon Blanc and,
- Monastrell.

Finally, examples of varieties that are **very sensitive** to powdery mildew are (Barber, 2014):

- Chardonnay;
- Chenin Blanc;
- Verdelho;
- Pinot Noir;
- Tempranillo and,
- Cabernet Sauvignon.

➢ Mineral nutrition

Mineral nutrition affects the plant physiology and can also influence the tolerance or sensibility to diseases.

The input of 40 Kg/ha of nitrogen allows to limit the expansion of the pathogen and to provide a good quality of the berries. However, the larger is the amount of nitrogen, the bigger will be the growth of the plant but, also, the rate of infection will increase (Eynard, Morando, Lembo, & Lovisolo, 2002; Bonomelli C., 2006).

In sum, the effects of nitrogen on the intensity of powdery mildew infection are dependent of the following elements: variety of the host, form of nitrogen, age of the plant, period of the vegetative cycle and the environmental interactions (Huber & Watson, 1974).

Next table resumes the principal conditions that favours development of powdery mildew, exhibiting the limits and the optimal valour's for each one.

Table 2 -Limits of conditions favouring development of powdery mildew at vineyard

Factor	Minimum	Optimal	Maximum
Temperature	4°C	20 – 27°C	40°C
Relative Humidity	10%	30 – 85%	99%
Wind	-	>3 m/s	-
Sun light exposition	-	diffuse light	-

1.1.3. Symptoms of the disease on the grapevine

The fungus causes ash-grey to white powdery growth on green tissue of the grapevine (it is an ectoparasite). In particular, the upper and lower surfaces of young leaves, shoots or clusters are highly susceptible. The chains of conidia that develop from powdery mildew hyphae give the infected vine tissue the characteristic grey-white powdery or dusty appearance. Severely infected vines emit a musty aroma mid to late season.

Main symptoms on leaves are irregular yellow blotches (figure 3) best seen on the upper surfaces.

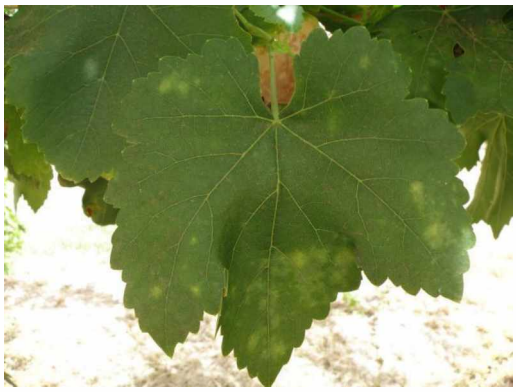


Figure 3 - Yellow blotches on the upper surface of the leaf (Taylor , Fisher, & Gordon, 2016).

The blotches soon show the typical white mildew growth, as spots expand and merge across upper and lower leaf surfaces. Eventually, entire leaf surfaces can be covered (figure 4). The earliest infected leaves become distorted and discolored, sometimes giving the vines a flaccid appearance (New Zealand Winegrowers Fact Sheet, 2014).

Figure 4 - Several powdery mildew infection on the upper surface of grapevine leaf (Taylor , Fisher, & Gordon, 2016).

Clusters of most cultivars are susceptible between flowering and up to five weeks later. Although berries (figure 5) develop resistance with age the bunch stalk and stems remain susceptible. Severely infected berries may develop irregular shapes, crack or split and site for *Botrytis cinerea* infection (Taylor , Fisher, & Gordon, 2016).

Figure 5 - Example of infected berries by powdery mildew (Taylor , Fisher, & Gordon, 2016).

1.2. Grapes, must and wine quality affected by powdery mildew disease

Powdery mildew not only reduces the yield and marketability of grapes, but also the quality of the wine. Levels of disease on grapes as low 3% can taint wines (Pool, Pearson, Welser, Lakso, & Seem, 1984). Low levels of disease are difficult to assess visually in the vineyard and, to quantify in large consignments of grapes. Currently, winery staff visually assess powdery mildew on grapes at the field and/or when consignments arrive at the winery. Grapes are rejected if the level of powdery mildew is too high.

Calonnec, Poupot, Dubourdieu, & Darriet (2004) determinaded that powdery mildew affects the composition of diseaded grapes, being these effects more pronounced when the disease was severe. Bunches, of cultivars Cabernet Sauvignon and Sauvignon blanc, infected with the fungus had grapes with reduced size, less number of berries per cluster and delayed growth.

On analyses of grape juice, it was evident that there was an increase of sugar concentration with the increase of the level of infection. However, Rusjan, Jug, & Bavcon Kralj (2012) observed that content in sugars and tartaric acids decreased with the increasing degree of infection.

Stummer, et al. (2004) conducted a study during 2000, 2001, 2002 and 2003, in Australia, using the composition of musts and wines from Chardonnay, Syrah and Cabernet Sauvignon grapes with increasing levels of powdery mildew infection. It was concluded that the infection on Chardonnay, Shiraz and Cabernet Sauvignon grapes substantially affected the chemical composition of both grapes and wine, notably the concentration of phenolics and total acidity.

On Chardonnay grapes, even low levels of powdery mildew infection (1-5% infection of bunches) increased the concentration of total phenolics (which in white wines is considered, normally, as a negative effect on quality), hydroxycinnamates, flavonoids and brown pigments on grape juice but, Chardonnay bunches with more than 30% powdery mildew had lower total soluble solids (TSS) than bunches with lower categories of disease and healthy grapes.

Powdery mildew infection of Cabernet Sauvignon bunches (1-20%) also resulted in lower total soluble solids, and in lower wine phenolic concentration and spectral colour values compared with healthy grapes.

Regarding sensorial properties, the development of powdery mildew on berries' surface leads to organoleptic defects (mushroom-like, mouldy, earthy and geranium leaf odours) on musts (Darriet, et al., 2002).

According to Stummer, et al. (2004), in Chardonnay, there was a clear impact of the powdery mildew in enhancing a viscous and oily mouth-feel character, even at the lowest level of infection, which may be considered an undesirable attribute in a white table wine.

1.3. Use of pesticides to control powdery mildew

Powdery mildew fungi (*Erysiphaceae*) are one of the most conspicuous groups of plant pathogens, compromising more than 500 species that attack more than 1500 plant genera (Braun, 1987). Important crops, including wheat, barley, grapevines, apple and a number of vegetables and ornamentals, grown in the field and greenhouses are among the major targets of powdery mildew fungi (Kiss L. , 2003).

It is one of the major reasons of fungicide use in viticulture, along with *Plasmopara viticola* (grapevine downy mildew). According to a report on the use of fungicides in the European Union, over the period 2001–2003, indicated that while viticulture only accounted for 3.3% of the agricultural area, a staggering 81,000 tonnes of active substance were applied annually to grapevines in European vineyards, which represented 67% of all fungicides applied to crops in the European Union (EUROSTAT EC, 2007). Not only does this translate into increased production costs for growers, but there is also the potential impact of these chemicals on the health of beneficial organisms in the vineyard (Gadino, Walton, & Dreves, 2011) and vineyard workers (Le Moal, et al., 2014), as well as increased carbon emissions generated from their frequent application and, have leaded to the development of powdery mildew strains resistant to different fungicides wildly used decreasing their efficiency against this fungal disease (Qiu, Feechan, & Dry, 2015).

Cultivars resistant or tolerant to powdery mildew infections have been developed in a number of crops, but their use is limited, especially in fruit and vegetable crops (Bélanger & Benyagoub, 1997). Furthermore, for questions of wine marketing, the plant variety used is very important because, usually, wines are recognized, named and sold according to the grapevine variety by which they were produced so, it seems complicated to use new resistant grapevine cultivars to powdery mildew, without compromising the marketability of the final product (wine).

All these constraints associated with the use of fungicides and resistant cultivars have led to the search of alternative methods to control powdery mildews. Non-fungicide products, such as soluble silicon, oils, salts and plant extracts, inducing resistance in plants infected with powdery mildews or acting as prophylactic and/or curative factors are in focus, especially in greenhouse production (Kiss, et al., 2001). Biological control, a phenomenon based on the antagonism between micro-organisms (Andrews, 1992), is also considered as an alternative way to prevent or suppress powdery mildews in some crops. Attempts have been made to use mycolytic bacteria (Highland, 2000), mycophagous arthropods (Norton, English - Loeb, Gadoury, & Seem , 2000) and other possible non-fungal biological control agents (Yarwood, 1957) against powdery mildews but, these studies have provided no promise of practical control to date.

The most promising biological control trials have involved a number of fungi antagonistic to powdery mildews and, have resulted in the development of two biofungicide products - AQ10 Biofungicide® and Sporodex® - which have been registered and commercialized in some countries (Kiss L. , 2003).

1.4.*Ampelomyces quisqualis*, a hyperparasite of Powdery Mildew

Ampelomyces is a mycoparasite and it is usually considered to be a mono-species genus (the unique species being *A. quisqualis*) (Kiss, Russell, Szentiványi, Xu, & Jeffries, 2004). It was discovered in 1852 on colonies of powdery mildew in grapevine leaves and, it was De Bary (1870) who identified *Ampelomyces* as an intracellular parasite of *Erysiphales.*

1.4.1. Cycle of development and infection

One interesting characteristic of *Ampelomyces* is that it is very polyphagous and can parasitize a very broad spectrum of powdery mildews, on a variety of host plants. Even though it has mainly been described on cultivated species (Kiss, Russell, Szentiványi, Xu, & Jeffries, 2004).

- **Mode of action**

Ampelomyces kills the parasitized powdery mildew cells by causing a rapid degeneration of the cytoplasm (Hashioka & Nakay, 1980). They infect and form pycnidia (fruiting bodies) within powdery mildew hyphae, conidiophores (specialized spore-producing hyphae) and cleistothecia (closed fruiting bodies of powdery mildews). These mycoparasites suppress both asexual and sexual sporulation of the attacked powdery mildew mycelia by colonizing and destroying the conidiophores, and the immature ascocarps, respectively. The early stage of mycoparasitism is apparently biotrophic, but the invaded cytoplasm then begins to die and a necrotrophic interaction results (Sundheim & Krekling, 1982).

The pycnidia are receptacles containing conidia. The conidia are unicellular, hyaline, mostly filled with a fine guttulate and embedded in a mucilaginous matrix inside the pycnidia.

In presence of water, these matrices swell and conidia are released from intracellular pycnidia by the rupture of the pycnidial wall. After 10 -20 h, under conditions of high humidity, conidia germinate and the hyphae of the mycoparasites can then penetrate the hyphae of powdery mildews, by the mobilization of the mechanical process through the formation of apressoria at the level of prenetration (De Bary, 1870).

After penetration, the hyphae of the mycoparasite continue their growth internally and produce their intracellular pycnidia after 5 -8 days in the mycelia of their host fungi. The life cycle starts again when pycnidia are mature (Kiss, Russell, Szentiványi, Xu, & Jeffries, 2004).

The following figure resumes the life cycle of the parasitism of *Erysiphe necator* by *Ampelomyces*.

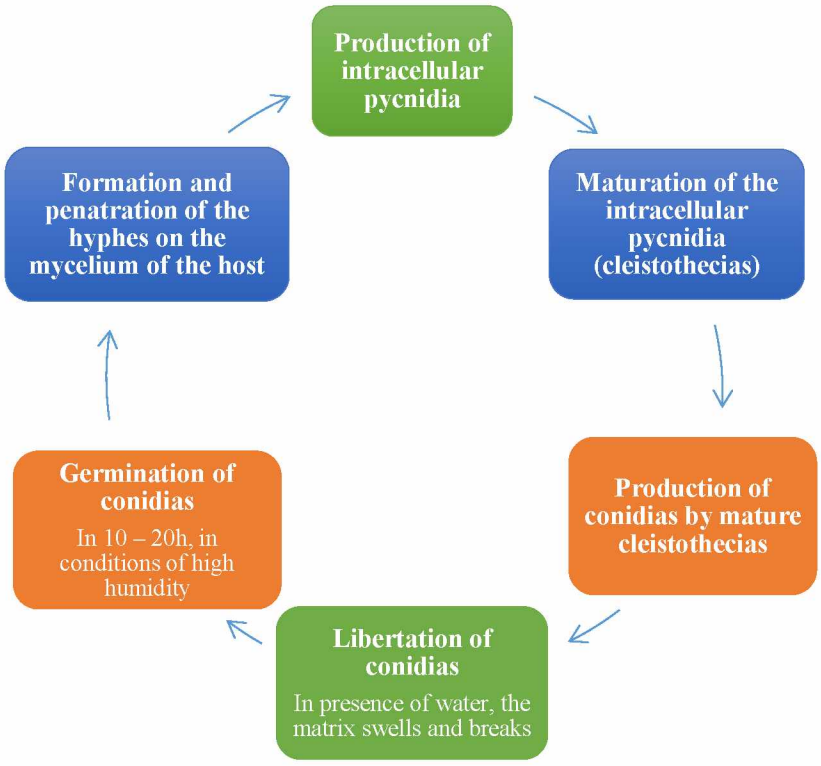


Figure 6 – Simplified scheme of the life cycle of the hyperparasite of *Erysiphe necator* (*Ampelomyces quisqualis)*.

➢ Propagation

Conidia can be dispersed within the plant canopy by rain-splash or water runoff from plant surfaces. *Ampelomyces* can also spread to long distances as hyphal fragments in parasitized and detached powdery mildew conidia (Jarvis & Slingsby, 1977; Speer, 1978 and Sundheim & Krekling, 1982). Furthermore, *Ampelomyces* can be transported far from its original fungal host and can parasitize other powdery mildew species. After penetration, the hyphae of *Ampelomyces* invade the host mycelia internally, and produce their pycnidia mostly in the conidiophores and young, immature ascocarps of powdery mildews.

➢ Development conditions

A good development of the hyperparasite is dependent of the temperature, relative humidity and other abiotic factors such as, the presence of anti-fungi molecules on the chemical products, like Sulphur (annexes 1 and 2).

The presence of water is indispensable for the development of *Ampelomyces,* as mentioned above, this factor allows the rupture of pycnidia and the liberation of the conidia.

According to Gu & Ku (1997), the concentration of Ampelomyces conidia on the leaves is also important because, germination rapidly decreases above a concentration of 10^6 CFU/ml due to the production of a self-inhibitor.

1.4.2. Use of AQ10 biofungicide and its limitations

Toxin production has not been detected in *Ampelomyces* (Beuther, Philipp, & Grossmann, 1981) in contrast to other pycnidial mycoparasites, such as *Coniothyrium minitans Campbell* (Machida, et al., 2001). However, some obstacles have been found by Kiss et al. (2004) to the use of *Ampelomyces* as a biocontrol agent:

- It needs high RH (Relative Humidity) level (>90%).
- As an obligatory parasite, its host needs to be present. Therefore, there is often a gap between the powdery mildew contamination and its infection by *Ampelomyces*, leading to an ineffective control of the disease.
- It is affected by some plant protection products, including sulphur, which is an issue concerning the potential use of *Ampelomyces* in organic farming. However, it is compatible with several others, so it can nevertheless be used in an IPM (Integrated Pest Management) program.

The first point makes *Ampelomyces* useful in greenhouses, where relative humidity can be under control, but harder to use in vineyards, and very climate-dependent. The second point would not seem to be an issue, as spraying conidial solution can be done as soon as the powdery mildew infection starts, but the conjunction with the first point makes it tricky, has the solution would be to spray immediately before or during a rain event – which causes technical issues (Lafond & Cantin, 2015).

1.4.3. Hypothesis of increase *Ampelomyces quisqualis's* efficience

According to Lafond and Cantin (2015), could be possible to overcome the problems with the begging and velocity of the development of *Ampelomyces* by increasing their level on the plot at the time of the first contamination, through the use of banker plants. These plants would be infected by another powdery mildew before the vine, allowing the population of *Ampelomyces* to build up. This way, when the first ascosporic infections of *Erysiphe necator* will be triggered by rain, *Ampelomyces* conidia will be already available in the vineyard and could get in touch with the new, fresh, powdery mildew colony and begin the hyper parasitism process.

The use of intercrops to build up a population of biocontrol agent is not totally new in viticulture. For instance, the green leafhopper (*Empoasca vitis*) has a very strong interaction with the vineyard environment since it hibernates as adults on evergreen trees and shrubs (mainly conifers) outside the vineyard; it migrates to intermediate hosts (*Rosaceae*) during spring migrations (before entering the vineyard) and it also uses intermediate hosts during autumn emigrations before returning to overwintering plants. Its parasitoid, *Anagrus atomus*, can't realize whole cycle on this host, as it overwinter as a parasitized egg, while *Empoasca vitis* overwinters as an adult. This parasitoid overwinters on other leafhoppers species that lives on plants in the neighbourhood, and reaches the vineyards while *Empoasca vitis* lay its eggs (Helden, Decante, & Papura, 2003). So, if a system is built where another crop within the plot can be a support of leafhoppers that could allow *Anagrus atomus* to perform its cycle. Although the same approach has never been tried concerning fungal diseases (Lafond & Cantin, 2015).

1.4.4. PCR technique for detection of *Ampelomyces quisqualis*

The Polymerase Chain Reaction (PCR) is a method of replicating DNA. It is capable of taking a small amount of DNA or even a single molecule and amplifying a specific region exponentially such that once the reaction is finished, there may exist up to 230 copies of each starting DNA molecule. PCR reactions can complete many rounds of replication and produce billions of copies of a DNA fragment only in few 2-3 hours (Flanagan, Kulakov, Kulakova, Spence, & Allen, 2014; Alfonso , et al., 2015 and Alibi, 2015).

This method has been successfully used to study the hyperparistism relation between powdery mildew and *Ampelomyces quisqualis* and, to detect differences among various strains of *Ampelomyces* used in these biocontrol experiments (Liang, et al., 2007; Kiss, et al., 2011; Pintye, et al., 2012; Tollenaere, et al., 2014).

To confirm the presence of *Ampelomyces*, sequency of the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA gene (nrDNA) has been the procedure reffered on these studies about *Ampelomyces*. DNA of the sample is extracted and the ITS region is amplified several times by the utilization of specific primers then, the products from PCR are purifyed (Ito & Takamatsu, 2010). The amplification of the ITS region correspondig to the the reference bank of DNA allows to identify the fungus and its strain (Schoch, et al., 2012).

For example, in the study of Tollenaere, et al., (2014), about *Podosphaera plantaginis* (powdery mildew's specie that naturally infects its wild host plant *Plantago lanceolata*), allowed to detected 6 strains of *Ampelomyces* using the sequence primer AQF264/AQ-R462. However, for *Erysiphe necator* (grapevine powdery mildew) Pintye, et al., (2012) demonstrated that exists a huge diversity of Ampelomyces' strains and, Kiss, et al., (2011) concluded that there are several sequencies of *Ampelomyces*' ITS for the same host plants infected by the same powdery mildew specie.

Previous studies revealed that straines of Ampelomyces coming from the same specie of powdery mildew (or not), can point a divergence of sequency for the same genetic marker until 10-15% (Kiss & Nakasone, 1998; Sullivan & White, 2000; Szentiványi, et al., 2005; Liang, et al., 2007). Thus, for detection of *Ampelomyces* in *Eryshipe necator,* a preliminary investigation is necessary to determine which primers can be used.

2. Practical Context

2.1. IFV Pays de Loire

Institut Français de la Vigne et du Vin (IFV) (figure 7), is the technical organization who serves all the wine producers in France, being is general mission "to conduct general studies for the entire wine industry in the areas of plants selection, viticulture, winemaking and marketing". It comes from the merge of ENTAV (*Etablissement National Technique pour l'Amélioration de la Viticulture*) and ITV (*Institut Technique du Vin*) in France and, it benefits the double qualification of Agricultural Technical Institute and the Institute of Food Technology.

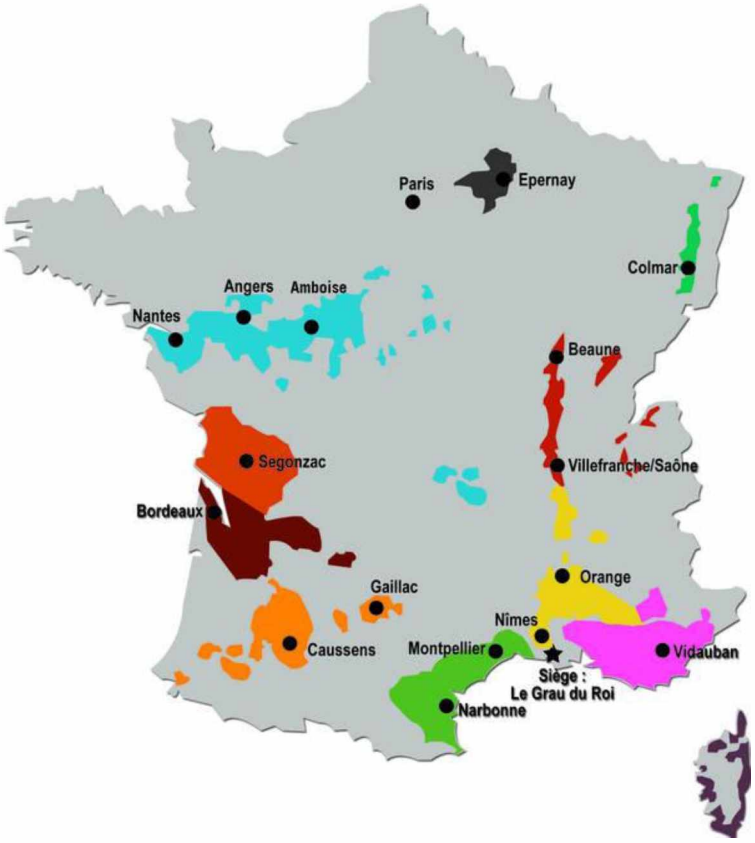


Figure 7 - Distribution of the all Research Units of IFV and their production areas (IFV, 2016a).

The head office of IFV is on the Espiguette's domain, at the Le Grau du Roi, which operates a vineyard of about 40 hectares, where is the National Conservatory of varieties and clones and pre-multiplication of plots required for the dissemination of the French plants' selection.

This Institute has 140 collaborators and operates with an annual budget of 11 million euros (IFV, 2016b).

Unit of Angers:

The IFV Angers Unit is situated in the old INRA's (*Institut National de la Recherche Agronomique*) office at Beaucouzé (figure 8). The Angers department dedicated to viticulture researches is based, with 10 hectares, in the experimental area at Montreuil –Bellay.

This unit collaborates with the Viticulture Lyceum of Montreuil-Bellay and, also features some researches for private wine producers.

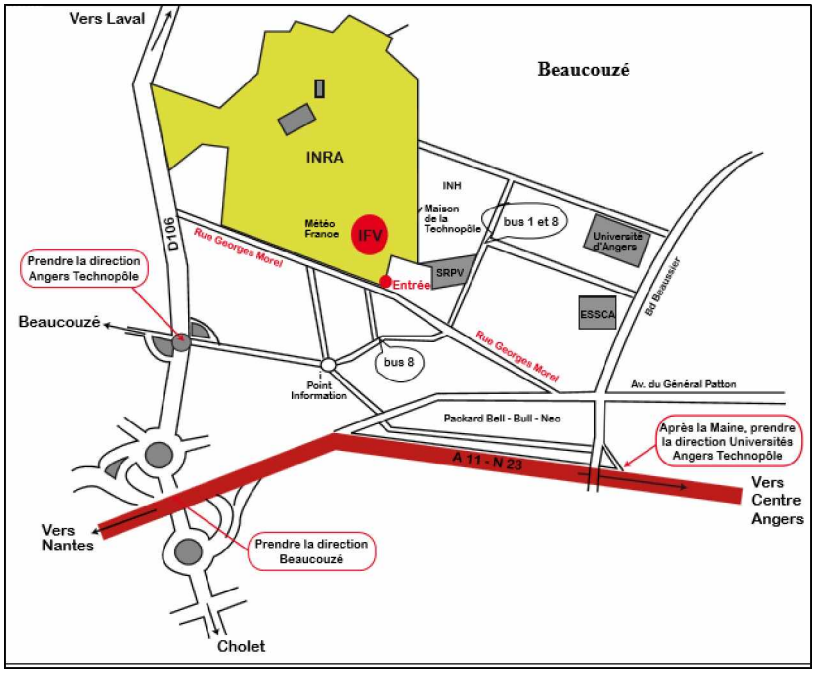


Figure 8 – Unit of IFV in Angers (France) (IFV, 2013a).

The cellar, where the IFV Engineers work, has 4 rooms with temperature control. Two rooms are equipped with mass loss continuous sensors and Carbon dioxide release.

The micro – vinifications are conducted on wine laboratories with 500ml to 50 litters capacity. The laboratories are equipped with colorimetry, enzyme dosage, HPLC chromatography, spectrophotometry, pH measure technique, centrifugation system and freezers who reach -80ºC of temperature.

Finally, there is also a wine store room (of $50m^2$) with controlled temperature (IFV, 2013b).

2.2. Project AQ10 Diaplasce

AQ10 Diaplasce is an on-going national project being developed on *Institut Français de la Vigne et du Vin* (IFV - Angers), since 2014, under supervision of the Engineer David Lafond – chef of the plant protection department of the Institute. It consists on a creation of a tri-trophic system, with the aim of fight powdery mildew infection, in the vineyard, through biological control.

Ampelomyces quisqualis (commercialized under the name AQ10® biofungicide) is a hyperparasite of *Erysiphe necator* (vulgar known as grapevine powdery mildew) and it is the most promising biocontrol agent against this fungal disease. However, previous studies showed that the efficacy of AQ10 is controversial because of the limitations mentioned on sub-chapter 1.4.2.

In order to overcome such limitations, the research team of IFV (Angers) is studying the hypothesis of the introduction of a banker plant, in the vineyard, that would help to build up the population of *Ampelomyces,* on the environment, in time to parasitize *Erysiphe necator*, soon as it contaminates the green tissues of the grapevine.

The first part of this project consisted on selection of an appropriate intercrop to be used as banker plant. After, considering a series of criteria [geographical distribution of the plant in France, temperature, relative humidity of the air, soil quality, dimension of the plant, sensibility to powdery mildew, biological cycle, and necessity of maintenance (Rolland & Guérin, 2014)] purple clover (*Trifolium pratense*) was selected. Three reasons were appointed for the choice of purple clover as a banker plant (Cantin, 2015):

3. Purple clovers are easy to find (they provide easily seeds);
4. They are sensitive to powdery mildew infection;
5. It is easy to implant purple clovers at a vineyard (on the inter-row), and since they are legumes, they are not too competitive relatively to water and nitrogen, which is a big issue in vineyards managed with green cover.

The Second part of the project resided on an in vitro study of the dynamic of the parasitism by AQ10 on purple clover.

It was concluded that 89% of the samples of purple clover, used during the trial, were contaminaded by powdery mildew (*Erysiphe trifolii*), this result is very favorable for the use of purple clover as banker plant. Furthermore, *Ampelomyces* parasitize powdery mildew after **10 to 15 days** of its development cycle and it was observed that Ampelomyces needs, at least, 30% of intensity of atack by powdery mildew (on purple clover) to start his development.

The next phase consists, based on the in vitro trial, to construct a trophic system, at a vineyard, in order to study the interaction, under natural environmental conditions, between the banker plant, the *Ampelomyces'* spores, powdery mildew infection and the grapevine.

The goals of this field project are:

- Test the efficiency of application AQ10® wg at the field;
- Determinate the natural presence/absence of *Ampelomyces* spores on the environment;
- Ascertain if there is transference of *Ampelomyces* spores from the banker plant to the vineyard and,
- If *Erysiphe necator* attacks the vineyard, assess if the parasitism occurs or not.

Figure 9 summarizes the schematization of the tri-trophic system created by the research team of IFV.

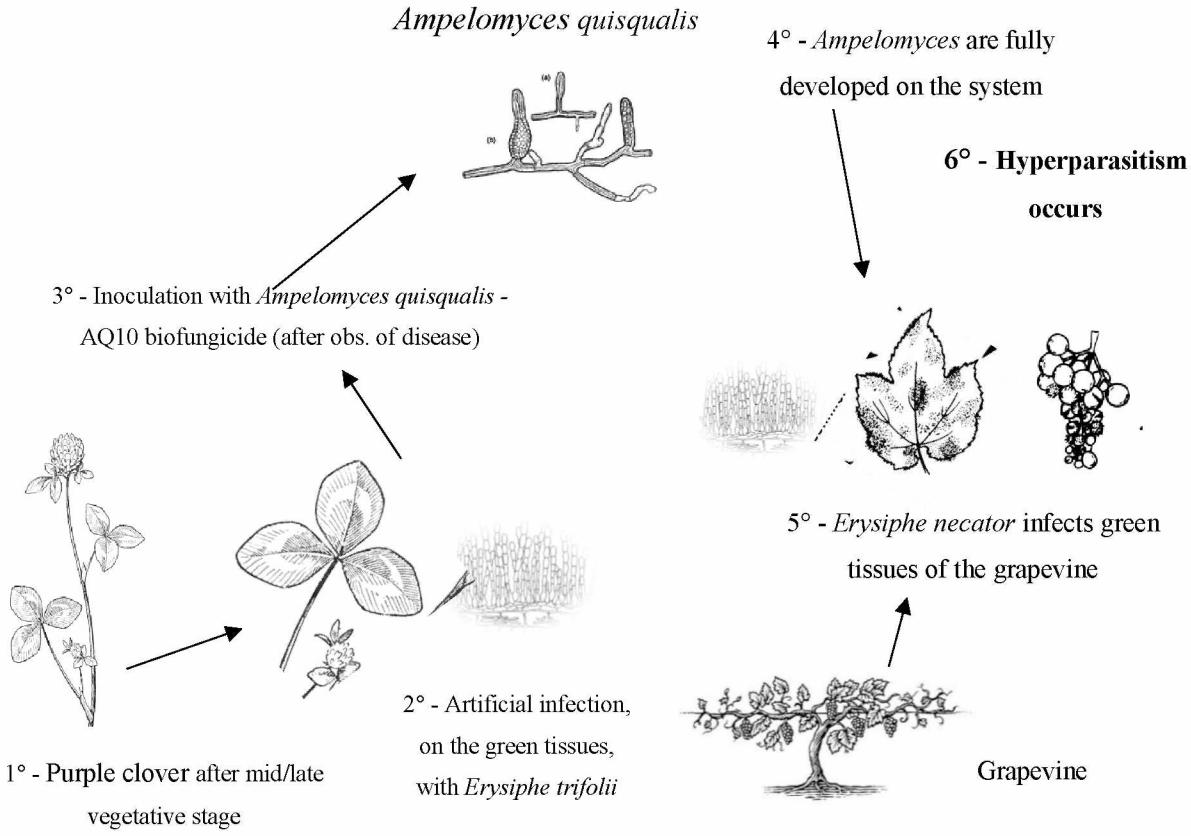


Figure 9 - Scheme of a trophic system where a banker plant (purple clover) helps building up a biocontrol agent population.

2.3. Objectives

The aims of this study are the following ones:

- Accompany the development of the banker plant already installed last year;
- Infect the banker plant with powdery mildew (*Erysiphe trifolii*);
- Inoculate the banker plant with AQ10® WG, when visual symptoms of powdery mildew are visible;
- Install removable cover plastics in specific zones of the vineyard in study, to protect those zones from chemical treatments against powdery mildew;
- Weekly observation of the non – treated zones, against powdery mildew, of the vineyard to spot visual symptoms of powdery mildew's disease;
- Collection of a series of samples (from the environment, vineyard and banker plant) at the plot to be analysed for presence/absence of *Ampelomyces quisqualis* and, finally,
- evaluate the impact of several levels of powdery mildew infection on the yield of clusters and quality of the obtained musts at the harvest period of the plot in study and, other plot with a red variety, since this subject is not well studied for red varieties of grapes as it has been for white varieties, specially, Chardonnay.

2.4. Materials and methods

All the operations to perform during this trial are described in the following sub-chapters.

2.4.1. Trial design

This study is being conducted in one plot (annex 3), with 0,26ha, composed by the cultivar Chenin Blanc (*Vitis vinifera*) of the Lycée Viticole at Montreuil Bellay (Edgard PISANI, 49).

It is composed by 16 rows, with 960 grapevine plants in average (29 missed or death plants were counted), distributed in 12 zones per row. The interval between plants is 1.10 metre and between rows is 1 metre. Two rootstocks, SO4 and 101.14, are present at the plot in equal proportion since, within each row the rootstock exchange one zone to two.

Regarding the inter-row management, a superficial tillage is made although, on the inter-row 9-10, in the central part corresponding to the 5^{th}, 6^{th}, 7^{th} and 8^{th} zones, is reserved to install the banker plant, which is the variety JUSTIN of purple clover.

2.4.1.1. Installation of purple clover on the inter-row of the plot

Purple clover was installed on March of 2015, with high humidity conditions, making difficult to prepare a good seedbed since the soil was very humid. For this reason the seeds were added to the soil manually and, there was not a passage with a roller after, which difficult the contact seeds/soils and the future implantation of plants. This installation was not well succeeded and, a manually re-installation was conducted in April because of, the high humidity conditions, it was, again, impossible to perform a soil work.

Since purple clover was installed lately (during spring instead of autumn), without a seedbed previously prepared with a superficial tillage and, the impossibility of make a passage with a roller after, the addiction of the seeds, leaded to a bad development of purple clover, at the plot.

The purple clover used on this study results from some plants that, even though the difficulties, grew in 2015 and developed during 2016's spring.

2.4.1.2. Inoculation of powdery mildew (*Erysiphe trifolii*) on purple clover

When purple clover is well developed, which means from mid/late vegetative stage, the inoculation of powdery mildew is conducted.

Using powdery mildew infected leaves of purple clover – obtained in the laboratory - (figure 10), delicately rub and leave them on the banker plants.

Figure 10 - samples of diseased purple clovers to be used to infect the banker plants at the field.

If there is no observation of development of the disease on the banker plant, this procedure must be repeated.

2.4.1.3. Inoculation of *Ampelomyces quisqualis*

Two to five days after the observation of the symptoms' disease on leaves of the banker plant (Cantin, 2015), proceed with the inoculation of *Ampelomyces quisqualis*.

The inoculation is made with the biofungicide AQ10® WG, in granules. The product contains vital spores of the fungus and, the recommended dose is 50-70g/ha, in water suspension (annex 4).

This operation is made with manual sprayer. For the preparation of the product, it is necessary to mix the adequate dose of AQ10® with water and left it 30 to 60 minutes at environmental temperature (figure 11). To increase the germination and installation of *Ampelomyces quisqualis* on the vegetation, it is recommended that this operation occurs in conditions of high relative humidity (>70% in optimal conditions).

Figure 11 - Preparation of the solution with AQ10® WG biofungicide.

According to the area to be treated (22m^2 / 0,0022ha), the dose of AQ10® to be used should be 0,154g however, to increase the chances of having the installation of the spore on the vegetation, the dose to be applied is 5g of AQ10® in 1L of water (sprayer capacity).

Seven to ten days after the first inoculation with *Ampelomyces*, it is recommend a second application with the same dose at the same environmental conditions.

The following figure exemplifies the application of AQ10® in the banker plant.

Figure 12 - Inoculation with *Ampelomyces'* spores in the banker plant.

2.4.1.4. Inoculation of Powdery mildew on grapevines

If no visual symptoms of powdery mildew are detected during these season, at the grapevines, an artificial inoculation with *Eryshiphe necator* must be made because, it is impossible to determinate if *Ampelomyces* is present or not at the plot (after being spread on infected purple clover) if the fungus is not present (since *Ampelomyces* is an obligatory parasite).

For this propose, leaves of a grapevine that show early visual symptom of the fungus' mycelium, must be collected and putted in plastic bags with humid papers. Next, they must be, delicately, rubbed on young leaves of selected grapevines at the trial plot (grapevines that belong to the non-treated zones for powdery mildew) and left there so, the fungus can start spreading and infect those grapevines.

2.4.1.5. Treatment programme of the plot

The treatments are made with a grapevine sprayer using OPTIDOSE (tool to reduce the quantity of chemical products applied).

The application of chemical products is performed when symptoms of diseases are observed and, according to the weather conditions (the treatments are inefficient when made in raining days and/or wind is present).

Giving data from previous years on the plot, it is known that, it is sensible to powdery and downy mildew infections, which means, the treatments that have to be made are against these two diseases. However, the type of chemical products to be used has to be compatible with the utilization of AQ10® otherwise, it will compromise the experimentation (annexes 1 and 2).

2.4.1.6. Removable cover plastics to protect vineyard against powdery mildew treatments

Eight zones of the plot (annex 3) are protected, by a removable cover plastic, every time an anti-powdery mildew treatment occurs (exclusively), with the finality of having non-treated zones for this disease where the study can be effectuated.

For each protection dispositive, it is necessary to have a cover plastic with 5 meters of length per 2 meters of height plus, 10 staples, 18 eyelets and rope (<6mm of diameter) that will fix the plastic to the non-treatment zone.

To install the cover plastic, it must be opened over the vineyard zone, be stapled in the beginning of the zone, then place the eyelets in function of the plants on the below area of the plastic and, also, at the level of the end of the zone to finish the fixation (figure 13).

Figure 13 – Example of an installed cover plastic to protect a trial zone against powdery mildew treatment.

Once the powdery mildew treatment is finished, the cover plastics are rolled and fixed at the beginning of the zone (figure 14).

Figure 14 - Example of the cover plastic after a powdery mildew treatment occurs.

2.4.2. Visual assessment

The visual assessments to perform during this trial are:

- Observation of purple clover's phenological stages;
- Scout of diseases at the vine (specially grapevine powdery mildew) and,
- Collection of samples for detection of *Ampelomyces*.

The procedures for all these visual assessments are described below.

2.4.2.1. Observation of phenological stages of purple clover

The development of purple clover has to be followed, in order to accompany its phenological evolution and, program when to, artificially, proceed with the inoculation of powdery mildew (*Erysiphe trifolii*) on it.

These observations are visually made and the following parameters are used:

- Spreading rate of the plants;
- Height of the plants;
- Development stage (Vignau-Loustan & Luyghe, 2008);
- Natural presence of powdery mildew (*Erysiphe trifolii*);
- Presence of another plants

Two scales of phenological stages are used to accompany the purple clover development and, they can be visualized on the following table.

Table 3 - Phenological stages of purple clover

Vignau-Loustan & Luyghe (2008)		Adapted from Kalu & Fick (1981)		
Stage name	**Stage definition**	**Stage level**	**Stage name**	**definition**
Vegetative	Total absence of floral buds	0	Early vegetative	Stem length < 15 cm; no buds, flowers or seed pods
		1	Mid–vegetative	Stem length 16 – 30 cm; no buds, flowers or seed pods
		2	Late vegetative	Stem length > 31 cm; no buds, flowers or seed pods
Beginning of bud	5% of the plants have buds	3	Early bud	1 to 2 nodes with buds; no flowers or seed pods
Bud	50% of the plants have buds	4	Late bud	3 or more nodes with buds; no flowers or seed pods
Beginning of flower	5% of the plants have blooming flowers	5	Early flower	One node with one open flower (standard open); no seed pods
Flower	50% of plants have blooming flowers	6	Late flower	Two or more nodes with open flowers; no seed pods

2.4.2.2. Observation of powdery mildew at the plot

This operation consists in observe and register the presence/absence of powdery mildew (exclusively) on the plot however, if other diseases are spotted, for example, downy mildew, black rot and/or botrytis, they are noted as a comment.

Powdery mildew on leaves

Period of observation: starting on the stage of 6 adult leaves until the formation of bunches, with a frequency of one time per week.

The first observation is performed all over the plot to have a notion of its sanitary state but, after the installation of the cover plastics, it will only be effectuated on the 8 non-treated zones of the plot.

For detecting the symptoms of the disease, it is necessary to observe very carefully the two faces of each leaf (especially the inferior face). The intensity of the attack is esteemed by the percentage of the attack on the surface of the leaf.

The frequency and intensity, of the attack per plant, is evaluated according to the total appearance of the symptoms per plant.

Powdery mildew on clusters

Period of observation: since the formation of clusters until veraison, with a frequency of one time per week.

For each plant, the number of clusters is counted in order to determine the frequency of attack. For the intensity, it is esteemed by percentage of surface of the attack at the totality of bunches per plant.

2.4.2.3. Detection of Ampelomyces in the plot

On the environment

Install a series of collector devices in several spots of the plot (annex 3) where AQ10® was sprayed, with the aim of detect the presence/absence of *Ampelomyces* after 10 days of the inoculation. Also, select other spots where there was not inoculation to determine if *Ampelomyces* appears naturally on the plot.

The collector device is composed by a funnel, a graduated plastic tube of 65ml (where the samples will be deposed), and plastic tape (which fixes the tube to the wood), as exemplified on the figure 15.

Figure 15 - Collector device for spores of *Ampelomyces*.

After a period of rain (the spores travel through water), identify and collect all the plastic tubes and replace with new ones.

Reserve all the samples on a freezer until analysis.

On purple clover

Several samples of leaves of purple clover are gathered in order to be analysed through PCR technique.

On clusters

Based on observation of symptoms of powdery mildew on clusters at verasion, 19 grapevines are selected to collect samples of infect berries; 1 to 5 berries if the vine is not heavily infected, 10 if there is a lot of powdery mildew.

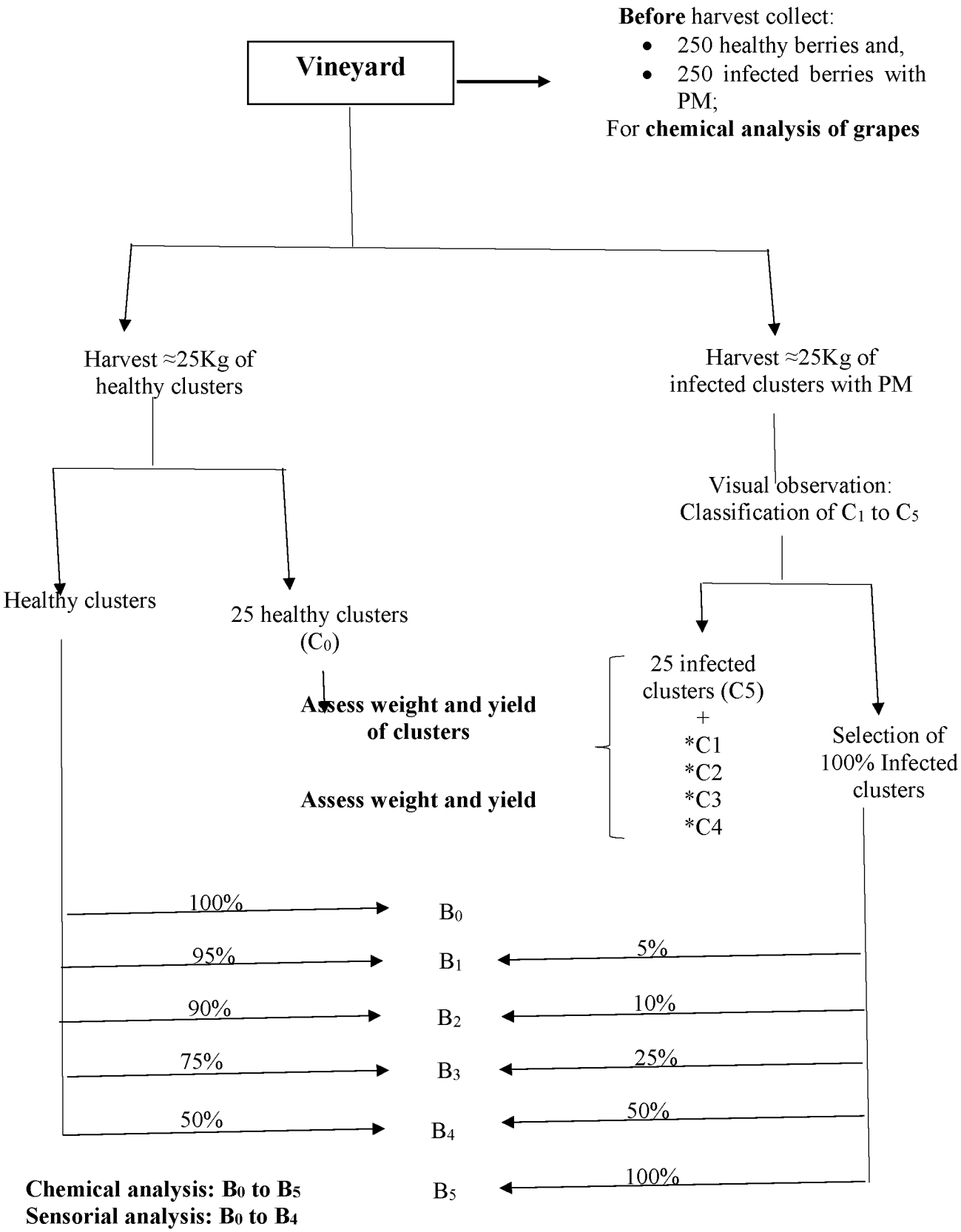
All samples are saved in tubes, previously identified with the local of where the sample was taken and date of collection, and placed at a freezer for conservation.

To detect the presence or absence of spores of *Ampelomyces*, a PCR analysis will be made.

2.4.3. Tasting trial

Samples of white and red grapes were collected from 2 vineyards, which were highly infected with powdery mildew's disease, in the Loire region, France.

Different samples were collected according to the type of analysis to be performed that can be visualized on the experimental design represented in figure 16. All procedures are described on the next sub-chapters.



* Variable number of samples for both cultivars; PM = Powdery mildew

Figure 16 - Trial design for the cultivars in study.

Table 4 synthetizes the calendar of all operations to be performed.

Table 4 - Calendar of operations for the tasting trial

Date	Operation	Description/obs.
20th of September	1st – Collection of 250 healthy berries plus 250 Powdery mildew infected ones	To be deposed in Brissac for chemical analysis; Parameters for analysis: Total Acidity, pH; sugar content and nitrogen.
	2nd- Harvest of white variety	Stored at 4°C until 22nd of September
21st of September	1st - Preparation of all the materials for the must samples	Wash and clean all the vessels.
	2nd – weight and yield of clusters of Chardonnay	
22nd of September	1st - Collection of 250 healthy berries plus 250 Powdery mildew infected ones of Cabernet Franc	To be deposed in Brissac for chemical analysis; Parameters for analysis: Total Acidity, pH; sugar content; nitrogen, anthocyanins and total polyphenols.
	2nd- Harvest and preparation of musts of red variety	1st – destemmed 2nd – preparation of the samples according to the level of infection with powdery mildew; 3rd – putted in cuvees with carbo ice (solid form of carbon dioxide); 4th – maceration (2-3H) at room temperature; 5th – after maceration, press and put the juices in carboys with carbo ice; 6th - Store at 2°C all night.
	3rd – Preparation of all musts of white variety	1st – Press; 2nd – preparation of the samples according to the level of infection with powdery mildew; 3rd – stored in carboys at cold
23rd of September	1st - Preparation of all samples for tasting	Clarification and bottling of all samples to be reserved at room temperature
	2nd - Sensorial Analysis	Triangular test and/or descriptive test
	3rd- Chemical analysis of musts	Assess sugar content, alcohol, total acidity and pH.
26th of September	weight and yield of clusters of red variety	

2.4.3.1. Chemical analysis of berries

- **Samples**

Through visual observation of the clusters in the vineyard, selection of 250 berries infected with powdery mildew and 250 healthy berries before harvest.

- **Methods**

Samples were sent to the Laboratoire Oenologique U.A.P., in Brissac, to test the following parameters:

- Total acidity;
- pH;
- sugar content;
- Potential alcohol;
- nitrogen;
- anthocyanins (only for red variety) and,
- total polyphenols (only for red variety).

2.4.3.2. Quality and yield of clusters, according to different levels of powdery mildew infection

- **Samples**

Approximately 25Kg of healthy clusters were harvested, for each cultivar, and 25 clusters were chosen and separated (C_0). Then, 25Kg of infected clusters were also

harvest, for each cultivar, and through visual observation of the clusters (figure 17), attribution of a score (1-5) reflecting the proportions of diseased berries in each cluster:

- C_1, 1-5% of diseased berries;
- C_2, 6-10% of diseased berries;
- C_3, 11-25% of diseased berries;
- C_4, 26-50% of diseased berries and,
- C_5, 51-100% of diseased berries.

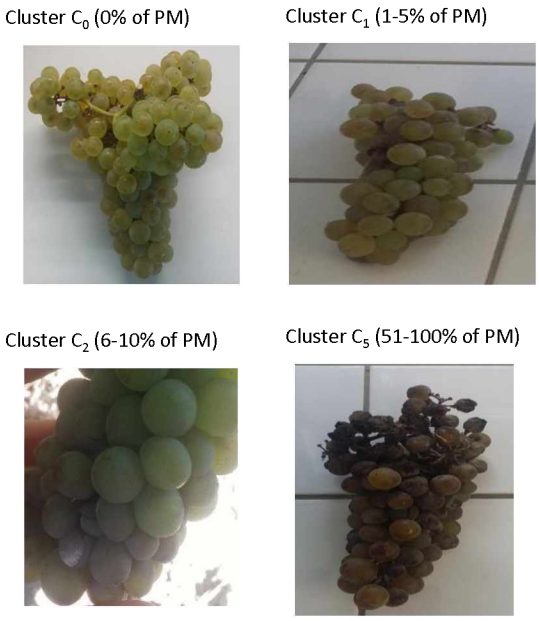


Figure 17 - Examples of clusters being classified

- **Methods**

According to the disease level classification (C_0 – C_5), assess **average** of:

- weight of a cluster, throughout the measure of weight of each selected cluster, for each classification level of disease;
- number of berries per cluster, by counting the number of berries of each selected cluster, for each classification level of disease and,
- weight of a berry per cluster, estimated using the number of berries and total weight of berries per cluster.

2.4.3.3. Chemical and sensorial analysis

- **Samples**

The remaining clusters collect at harvest, which were healthy and totally infected by powdery mildew disease, were mixed in order to obtain the following categories, for chemical and sensorial analysis:

- B_0, absence of powdery mildew;
- B_1, 5% of diseased berries;
- B_2, 10% diseased berries;
- B_3, 25% diseased berries and,
- B_4, = 50% diseased berries for sensorial analysis.

An additional category was added to the previous ones only for chemical analysis of musts: B_5, corresponding to 100% diseased berries.

For the red variety, this mixture was made with weight of clusters although, for the white variety, it was after obtain the must.

- **Methods**

Preparation of musts:

Materials to be prepared and used:

- Balance;
- Press machine, model STOSSIER;
- Gardevin 11;
- Carbo ice (carbon dioxide in solid form);
- 5 cuvees to be used for maceration of the red grapes identified per level of infection;
- 2 x 5 glass carboys identified for cultivar and level of infection;
- 3 x 5 x 2 = 30 bottles identified for cultivar and level of infection;
- Covered black wine glasses.

After harvest, the clusters of the white variety are stored at 4°C. Two days later, direct press during 3 minutes at 2bar plus 2 repressing of 3 minutes at 2bar of healthy clusters and infected ones separately (figure 18). After, according to the volume of musts obtained, proceed with their mixture in order to obtain 6 levels of infection (0%, 5%, 10%, 25%, 50% and 100%) in glass carboys.

Figure 18 - Preparation of clusters for pressing procedure.

In the following figure, the obtained musts, after press, are putted in glass carboys.

Figure 19 - Must, from white variety of grapes, after press.

Before closing the glass carboys, carbo ice (solid form of carbon dioxide) is added to the musts and then, they are stored at 4ºC. Hours before the tasting, all musts are clarified and bottled to be reserved at room temperature.

For the red variety, the healthy and infected clusters are destemmed separately (figure 20).

Figure 20 - Destemming clusters.

After destemming, berries and some juice obtained are kept in a GARDEVIN, where carbo ice is added, as exemplified on the next figure.

Figure 21 – GARDEVIN 11.

Preparation of 6 categories of infection level (0%, 5%, 10%, 25%, 50% and 100%) through different weight proportions and then, put them in separated black cuvees with carbo ice for maceration (figure 22), during 2-3hours (until a rosé colour is obtained) at room temperature.

Figure 22 – Beginning of Maceration's process.

Subsequently, press during 3 minutes at 2bar plus 2 repressing of 3 minutes at 2bar and put the resultant musts in glass carboys with addiction of carbo ice to be stored at 2°C, during all night. Hours before the tasting, all musts are clarified and bottled to be reserved at room temperature.

All must samples (30ml) are served in covered black ISO standard wine glasses (figure 23) at room temperature (≈ 20°C).

Figure 23 - Preparation of wine glasses for tasting.

Chemical analysis of musts

After obtaining the musts from each level of disease, samples (30ml of volume) were collected for analysis of the next parameters:

- Potential alcohol;
- sugar content;
- total acidity and,
- pH.

These samples were identified by variety and category of disease and, stored in a fridge until analysis in the laboratory of *Institut Français de la Vigne et du Vin* - Angers.

Determination of Potential alcohol:

To determinate the potential alcohol (% volume) of each must sample, a digital Wine refractometer model HI 96816 is used (figure 24). This device has an accuracy of ±0.2 % V/V / ±0.3°C (±0.5°F) (Hanna Instruments, n.d).

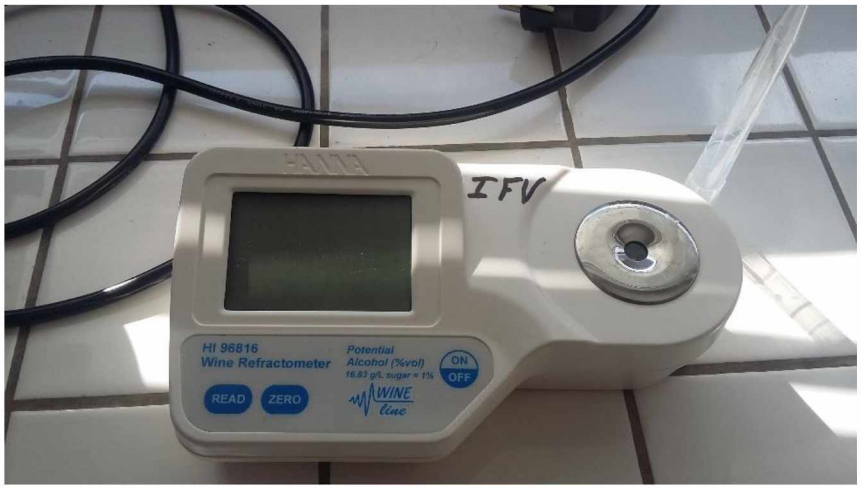


Figure 24 - Digital Wine refractometer model HI 96816.

Before taking measurements, calibrate the instrument with distillate water. Wipe off prism surface located at the bottom of the sample well and make sure the prism and sample well are completely dry. After, using a plastic pipette, the must sample is dripped onto the prism surface (fill it completely) and proceed with the reading. Between each sample, the prism surface must be cleaned with distillate water.

Sugar content:

Sugar content (g/l) is estimated through a mathematical equation using the potential alcohol valour's:

$$Sugar\ content\ \left(\frac{g}{l}\right) = PotentialAlcohol\ (\%V) \times 16.87$$

Where 1% = 16.87 g/l sugar.

Total acidity:

Total acidity (g/l) was determined for each sample using potentiometric titration method. The reagents used were:

- 5 ml of sample (must);
- 0.1N sodium hydroxide (NaOH);
- 5 drops of blue of Bromothymol at 4 g/l;

The materials used were:

- 10 ml pipette
- 50 ml beaker
- 25 ml burette and burette stand

In the beaker 5 ml of the sample and 5 drops of blue of bromothymol were introduced. Sodium hydroxide 0.1N was poured into the burette and its initial volume was registered. The titration was conducted until the change of colour was observed and, the volume of Sodium hydroxide used was registered at that point.

Figure 25 - Titration operation.

pH:

The measurement of pH is made with a meter, model S220 SEVEN COMPACT pH/Ion Mettler Toledo (figure 26). This device has a pH-relative accuracy of ± 0.002.

Calibration of the device must be made after measurements. To proceed with the reading, the sensor is placed into the must sample and, press READ to start measurement. After each measure, the sensor must be cleaned with distillate water.

Figure 26 - pH Meter model S220 SEVEN COMPACT pH/Ion Mettler Toledo.

Sensorial analysis

Panel of tasters

Ten people related with the *Institut Français de La Vigne et du Vin* – Angers – (workers, previous interns and, colleagues from the sensory department of *École Supérieure d'Agricultures* d'Angers) composed the panel of tasters for a difference testing and/or a sensory descriptive analysis. For these tests, descriptors and the testing sheets are in French language (annexes 5, 6, 7 and 8).

The next figure shows a schematization of the tasting room according to the code attributed to each taster.

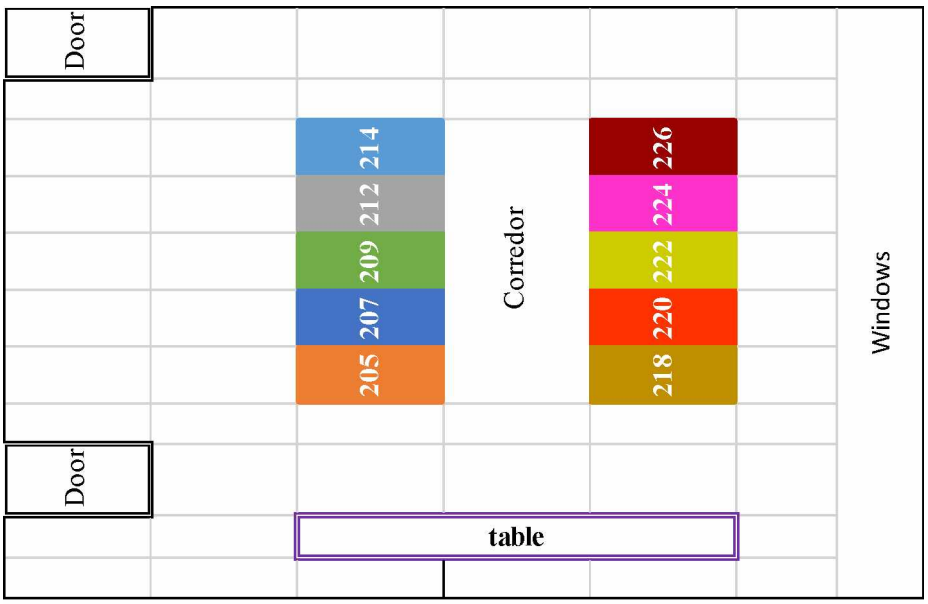


Figure 27 - Scheme of the tasting room.

Difference testing

The four musts, of each of the four infection group category, are first tested out using triangular method (Amerine, Pangborn, & Roessler, 1965). This test is based on the recognition of a different must between three samples, where two of them are identical. Tasters will assess aroma and taste for this purpose. The aim is to test if there is or not sensory differences between musts, made from different categories of infection.

If no statistical differences are found, the descriptive test will not be made.

Descriptive analysis

Six attributes in-mouth (sweetness, acidity, bitter, oily/viscosity, aromatic persistence and taste quality) and 13 aromas (fruit, pineapple, pear, banana, coco, cooked tomato, floral, geranium leaves, mushroom/mould, earth, candied, dust and aromatic quality) are judged for the white variety (annex 9) (Stummer, et al., 2004 ; Rusjan, Jug, & Bavcon Kralj, 2012; Calonnec, Cartolaro, Poupot, Dubourdieu, & Darriet, 2004).

For red variety, six attributes in-mouth (sweetness, acidity, bitter, oily/viscosity, aromatic persistence and taste quality) and 10 aromas (red fruits, spices, geranium leaves, floral, mushroom/mould, earth, cooked tomato, candied, dust and aromatic quality) are evaluated (annex 10).

The intensity of each aroma and palate attribute is rated using a structured 10 point line scale, with endpoints of "absente" and "high", with the tasters instructed to assess and rate aroma attributes for a sample, followed by the in-mouth attributes (Stummer, et al., 2004).

2.4.4. Statistical analysis

Program STATISTICA 7.0 and Excel were used for all data analysis, except, for statistical analysis of chemical composition of musts with different levels of powdery mildew infection that, was assessed with the program Analyse-it for Excel.

Quality and yield of clusters according to different levels of powdery mildew infection was subjected to analysis of variance (one-way ANOVA). Fisher's Least Significant Difference means comparison test (P = 0.05) was performed to determine which means were significantly different.

Sugar content, Potential Alcohol, Total Acidity and pH of musts were compared by a multivariate method: principal components analysis (PCA).

The results of triangular difference tests were analysed using binomial probability tables (annex 11) (Amerine, Pangborn, & Roessler, 1965).

3. Results and Discussion

Based on the procedures mentioned in the previous chapter, the following results were obtained and discussed.

3.1. Observation of phenological stages of purple clover

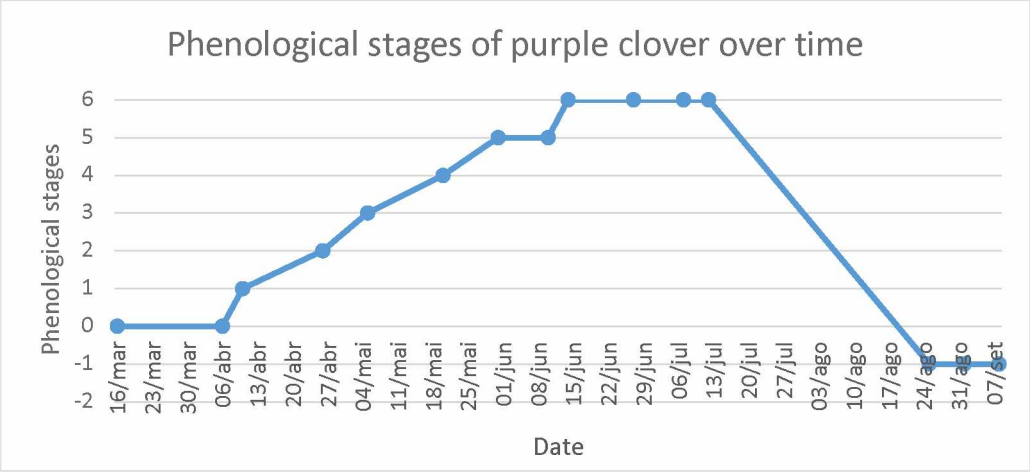
The development of purple clover was accompanied since, March 16th until the beginning of September of the present year. It was observed a spreading rate of, approximately, 80% of purple clover in the trial area (annex 12), co-existing with other flora [dandelion (*Taraxacum officinale);* fescue (*Festuca arundinacea*); Geranium (*Geranium pratens*); Vesce (*Vicia villosa*); grass plot (*Gramineae*); white clover].

In graphic 1 can be visualized the development of the banker plant over time. The period between, mid-vegetative and flower of purple clover matched the development season of powdery mildew (spring), which is favourable for the choice of purple clover as banker plant because, it is important that the banker plant is well developed at the same time that powdery mildew can appears otherwise, it would compromise this study.

According to climate conditions (cold temperatures and rain), purple clover ended its vegetative stage between the 4th and 18th of May. No visual symptoms of powdery mildew were found on clovers until that period, it was necessary to artificially infect the vegetation with *Erysiphe trifolii,* which was operated in May, the 20th.

In contrast with a cold and raining spring, during the month of August, temperatures reached 30 to 35 degrees (annexes 13 and 14), which leaded to dryness of the soil and burnt of the purple clover.

Graphic 1- Development of purple clover over time



Legend: -1 – no development; 0 – Early vegetative; 1- Mid- Vegetative; 2- Late-vegetative; 3- Early Bud; 4- Bud; 5- Early Flower; 6 –Flower; Adapted from Kalu & Fick

On figure 28, it is possible to visualize the growth of purple clover during the observation period of the present year.

Figure 28- Observation of purple clover stages over time.

3.1.1. Observation of powdery mildew infection (*Erysiphe trifolii*)

After artificial contamination of purple clover with the fungus *Erysiphe trifolii* (20th of May), visual symptoms of the disease were observed on 10th of June (figure 29).

Figure 29 - visual symptoms of powdery mildew (*Erysiphe trifolii*) on purple clover (10th of June).

3.1.2. Inoculation of AQ10® bio fungicide

On 14th of June, the first inoculation of *Ampelomyces*' spores was made in, fresh infected purple clovers with powdery mildew. Seven days later, a second inoculation was conducted as advised by the manufacture of the bio fungicide.

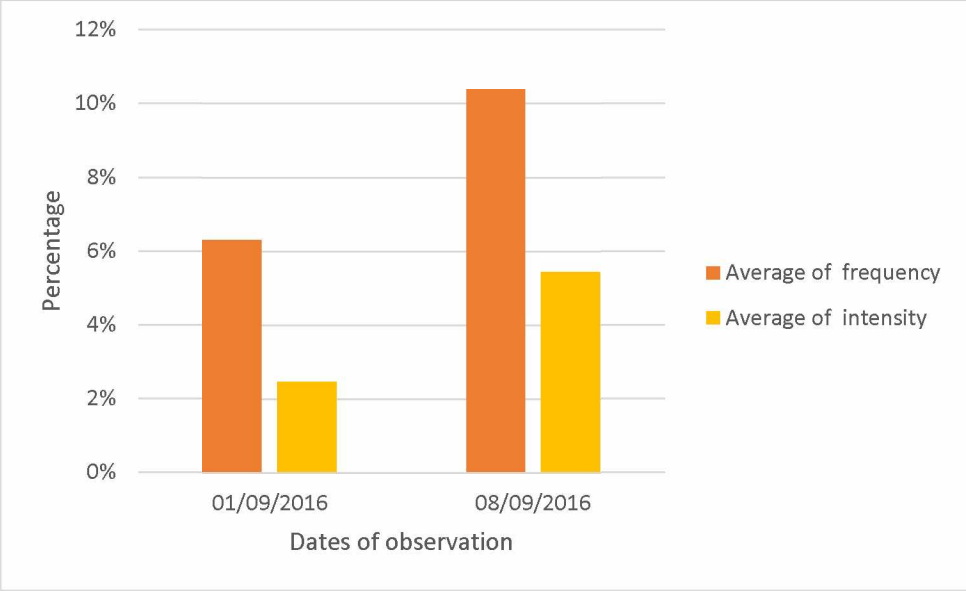
3.2. Observation of powdery mildew at the plot

Observation of powdery mildew started on 27th of July by visual detection of symptoms in leaves and clusters at the non-treated areas of the grapevine (annex…). These scoops finished on 8th of September when *veraison* started because, high levels of sugar concentration in grapes decrease the development of the fungus.

Throughout weekly observation (annexes 15, 16 and 17), no symptoms were found on leaves however, in clusters, powdery mildew infection was spotted on 2nd of August in two clusters and, started to spread in more spots of the grapevine during the summer. It was also observed a slight evolution of frequency and intensity of attack over time. There was not spotted other diseases on the plot.

Graphic 2 shows a frequency of 10% of the global attack with powdery mildew (in clusters) with 5% of intensity, in average. For the purpose of this trial, these low results are not favourable, since the presence of powdery mildew is mandatory for the development of *Ampelomyces* (it is an obligatory parasite).

Graphic 2 – Average of frequency and intensity of Powdery mildew's attack in clusters of AQ10 Diaplasce, at *veraison*



3.2.1. Factors which may affect powdery mildew's development at the plot

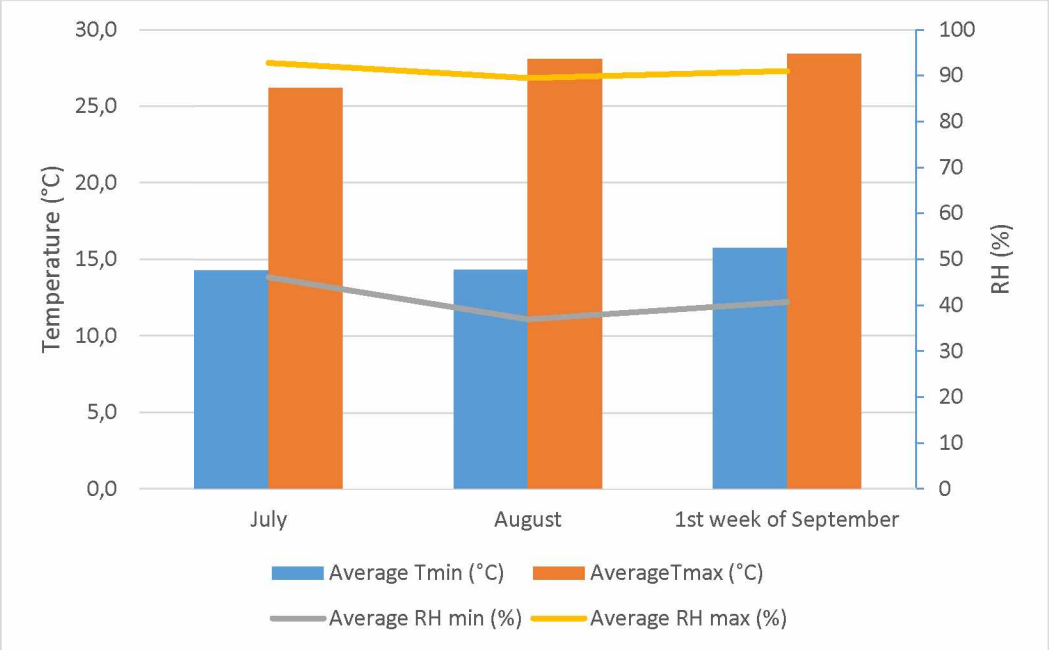
Climate

Appearance and development of powdery mildew are dependent of the climate conditions (temperature, relative humidity, wind and, sun light exposition). A spectrum of temperatures between 15 and 28 degrees associated with high relative humidity and, diffuse exposition to sun light leads to a good development of powdery mildew.

During the period of scouting the fungus at the plot, maximum temperatures were around 26 to 28 degrees and the minimum ones around 14 to 15 degrees, in average. Relative humidity was also favourable for powdery mildew, as it can be visualized in the graphic 3.

In sum, temperature and relative humidity could not be the cause for the low development of this disease in clusters and, total absence on leaves.

Graphic 3 – Averages of temperature and Relative Humidity during the months of observation of powdery infection at the plot



Source: Meteorological Station of Montreuil Bellay (2016) (annexes 13 and 14).

The dominant wind (south west) influenced the distribution of powdery mildew in clusters since, it helped disseminating the fungus according to its orientation. It also may have diminished the high temperatures, which were verified during the month of August (>30°C) (annex 14), not influencing its development in the clusters.

Management of the vineyard

The grapevines in study have an equilibrated vigour, not being too compacted and, with a good sun light exposition which difficult appearance and development of powdery mildew since, it prefers compacted grapevines where there is no air circulation.

No irrigation system is installed at the vineyard or nitrogen fertilization is made, which also can be considered as negative factors for powdery mildew's growth because, according to Valdés-Gómez, Gary, Cartolaro, Lolas-Caneo, & Calonnec (2011), vigorous grapevines, which benefited from a high water and nitrogen supply, develop a larger number of diseased leaves and a higher percentage of diseased berries than vines with balanced/low vigour.

During the scouting period, it was observed that the infected berries belonged to clusters not exposed to sun light, where there were a higher concentration of leaves, which promoted a barrier for air circulation creating a good micro-climate condition for powdery mildew.

Figure 30 provides a scheme of the plot, at *veraison* point, marking the grapevines where, powdery mildew was detected (in clusters) and, the severity of its attack in percentage.

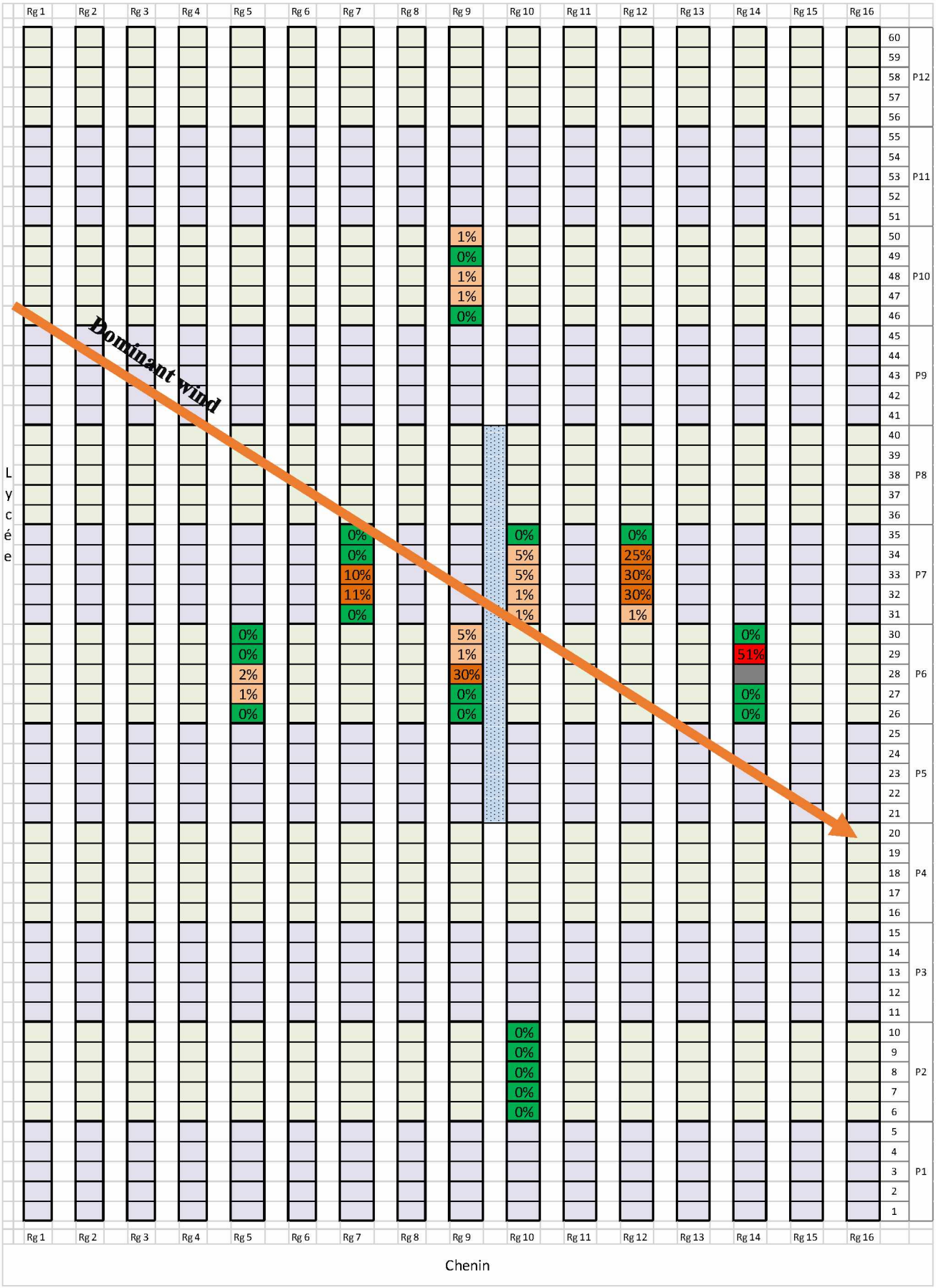


Figure 30 - Distribution and intensity of attack by Powdery mildew at the grapevine on the 8th of September.

Soil management strategy

Soil management can be appointed as a factor for a low development of powdery mildew at the plot because, as mentioned above, this fungus grows in grapevines with high vigour which means, soils that provide high water and nitrogen contents to the vines benefits powdery mildew infection.

In the same study conducted by Valdés-Gómez, Gary, Cartolaro, Lolas-Caneo, & Calonnec (2011), it was evident a relationship between vigour of the vine, powdery mildew development and soil management. More disease is developed on the most vigorous vines mostly, in soils conditions of weed control and higher water and nitrogen supply.

Regarding this knowledge, choosing a legume plant for the inter-row as an important element of this trial (purple clover), could also contribute positively for powdery mildew growth since, it increases nitrogen availability on the soil for the grapevine. Although, the area covered by the banker plant is too small to create a significant impact, as source of nitrogen for all the trial scouting area and, probably, to influence powdery mildew's disease.

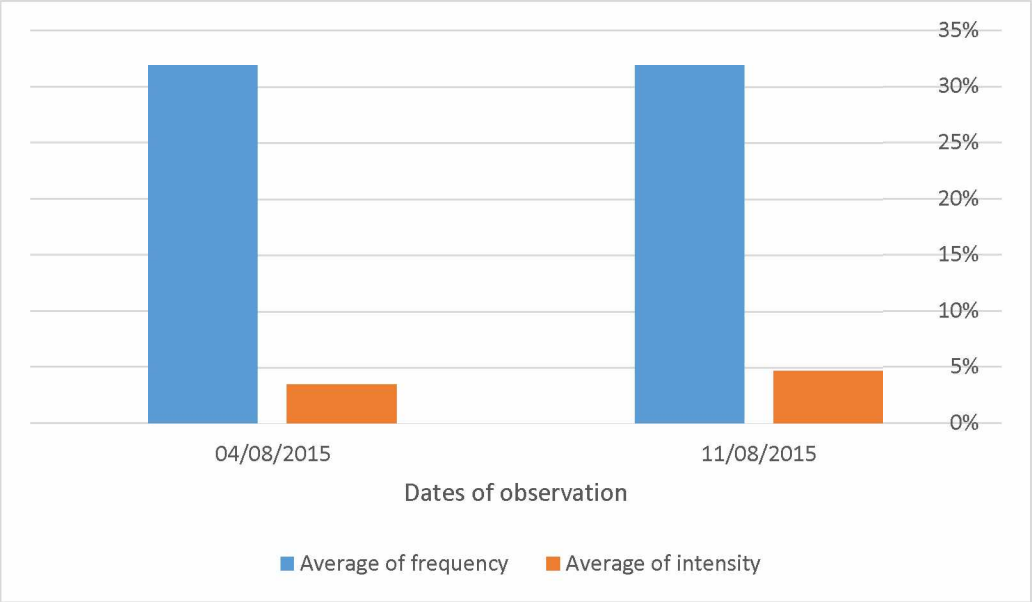
In the future, it would be interesting to cultivate a legume on the inter-row of all plot to promote nitrogen retention in the soil and, evaluate if it has impact on disease appearance since, it is a plot with low incidence of powdery mildew infection.

Legacy/inheritance factor

Powdery mildew's incidence on the vineyard is dependent on previous year legacy because, disease cycle extends itself over two seasons. Which means, if the disease is allowed to develop on season one, some buds will be infected in early season and cleistothecia will develop from mid to late season. (New Zealand Winegrowers Fact Sheet, 2014).

According to last year's data of this plot, no powdery mildew's symptom was found on leaves, only on clusters. At *veraison* point (11/08/2015), 32% of frequency of attack by powdery mildew in clusters was observed, corresponding to an intensity of 5%, in average (graphic 4).

Graphic 4 - Average of frequency and intensity of Powdery mildew's attack in clusters of AQ10 Diaplasce, at *veraison* (year of 2015)



Adapted from (Cantin, 2015)

Thus, legacy factor can be considered as the main cause for absence of powdery mildew's incidence, on leaves, this season since, overwinter inoculum of the fungus did not existed from last season, even though temperature and relative humidity were favouring the infection. However, the artificial inoculation of powdery mildew, made on July 12th, may had contributed for appearance of this disease on clusters.

3.3. Detention of *Ampelomyces* in the plot

A series of samples were collected to detect the presence/absence of *Ampelomyces'* spores at the plot and verify if the parasitism occurred. These samples will be analysed through PCR technique using specific primers for *Ampelomyces.*

This part of the trial is a work in process and the primers that will be used for PCR analysis are confidential.

On the next sub-chapters are presented the number, type and location of all the samples taken for detention of Ampelomyces' spores.

3.3.1. On the environment

After inoculation of Ampelomyces' spores in the banker plant, nine collector devices were placed in different zones of the plot, with the aim of gather, if present, spores' samples of the fungus once a rain period occurs.

The next table displays the designation of each of the nine samples that corresponds to the zone of the plot where the collector was placed, the dates of setting and removal of the samples and, finally, the volumes of collected samples.

Two sets of samples were collect, the first one, in 12/07/2016 that was only removed on 05/08/2016 because, it could only be pick up after a period of rain, which occurred at that time. The second set of samples was placed at the same day the first one was removed, at the exact locations and, it was collected on 15/09/2016 after a rain fall.

All samples were identified by location, date of setting and date of removal and, they were reserved at a freezer.

Table 5 – Collected Samples of *Ampelomyces*

Sample	Dates of setting		Dates of removal		Volumes of samples (ml)	
	1st	2nd	1st	2nd	1st	2nd
R 1. Z 1-2	12/07/2016	5/08/2016	5/08/2016	15/09/2016	65ml	65ml
R 7. Z 6-7	12/07/2016	5/08/2016	5/08/2016	15/09/2016	65ml	65ml
R 9. Z 4-5	12/07/2016	5/08/2016	5/08/2016	15/09/2016	65ml	65ml
R 9. Z 11-12	12/07/2016	5/08/2016	5/08/2016	15/09/2016	65ml	65ml
R 10. Z 1-2	12/07/2016	5/08/2016	5/08/2016	15/09/2016	65ml	65ml
R 10. Z 8-9	12/07/2016	5/08/2016	5/08/2016	15/09/2016	65ml	65ml
R 16. Z 3-4	12/07/2016	5/08/2016	5/08/2016	15/09/2016	65ml	65ml
R 16. Z 6-7	12/07/2016	5/08/2016	5/08/2016	15/09/2016	65ml	65ml
R 16. Z 9-10	12/07/2016	5/08/2016	5/08/2016	15/09/2016	65ml	65ml

Legend: R= Row; Z=Zone

3.3.2. On purple clover

Five leaves of purple clover were collected, on 15/09/2016, and placed in plastic tubes, which were reserved on a freezer until analysis.

The aim of analysing purple clovers' leaves is to determine if the AQ10® solution, sprayed on the banker plant after visual symptoms of powdery mildew, build up a population and parasitize (or not) the powdery mildew present on clovers' leaves.

3.3.3. On clusters

In the last scouting made at the plot (08/09/2016), corresponding with the *veraison* point, 19 grapevines had infected clusters by powdery mildew. According to the severity of the infection, a series of samples of infected berries were collected and putted in plastic tubes, identified by localization of collection (row, zone and grapevine) and date of sampling, and they were placed on a freezer until PCR analysis.

The idea is to verify if, *Ampelomyces* build up a population in time to parasitize powdery mildew's fungus present on the clusters.

The tables below demonstrate the number of berries collected per infected grapevine by powdery mildew, on 15/09/2016, according with the scouting data at *veraison*.

Table 6 - Collection of samples of infected berries by powdery mildew (rows 14, 12 and 10)

Localization			***Veraison***				**Sampling**
Row	Zone	Grapevine	08/09/2016				15/09/2016
			Frequency			Intensity	
			NB Clusters	Infected Clusters	%	%	NB berries
14	6	30	6	0	0%	0%	0
		29	17	2	12%	51%	5
		28					
		27	14	0	0%	0%	0
		26	4	0	0%	0%	0
12	7	35	9	0	0%	0%	0
		34	17	8	47%	25%	5
		33	17	4	24%	30%	5
		32	19	2	11%	30%	3
		31	12	1	8%	1%	1
10	7	35	11	0	0%	0%	0
		34	10	5	50%	5%	3
		33	14	1	7%	5%	1
		32	14	2	14%	1%	1
		31	10	1	10%	1%	1

NB = Number

Table 7 - Collection of samples of infected berries by powdery mildew (rows 10, 9, 7 and 5)

Localization			***Veraison***				**Sampling**
Row	Zone	Grapevine	08/09/2016				15/09/2016
			Frequency			Intensity %	
			NB Clusters	Infected Clusters	%		NB berries
10	2	10	4	0	0%	0%	0
		9	5	0	0%	0%	0
		8	15	0	0%	0%	0
		7	5	0	0%	0%	0
		6	9	0	0%	0%	0
9	10	50	16	3	19%	1%	1
		49	4	0	0%	0%	0
		48	13	1	8%	1%	1
		47	10	1	10%	1%	3
		46	17	0	0%	0%	0
9	6	30	10	2	20%	5%	3
		29	11	2	18%	1%	1
		28	10	8	80%	30%	5
		27	5	0	0%	0%	0
		26	6	0	0%	0%	0
7	7	35	11	0	0%	0%	0
		34	12	0	0%	0%	0
		33	17	3	18%	10%	3
		32	5	1	20%	11%	3
		31	11	0	0%	0%	0
5	6	30	17	0	0%	0%	0
		29	9	0	0%	0%	0
		28	10	1	10%	2%	1
		27	5	1	20%	1%	1
		26	8	0	0%	0%	0

NB = Number

3.4. Tasting trial

It was initially previewed to collect a series of samples of healthy and infected clusters by powdery mildew, from the plot in study and, evaluate the impact of several levels of powdery mildew infection on the yield of clusters and quality of the obtained musts at the harvest period. Although, at *veraison* point, the frequency of infection by the fungus was 10% with a severity of 5%, in average (see chapter 3.2), meaning, there was a very low level of infection on clusters of this plot, with which would be impossible to obtain enough samples to perform this tasting trial.

Since, it also previewed to obtain samples clusters from a red variety of grapes that would be known to be highly infected by this disease, it was decided to choose another vineyard, from a white variety of grapes, which presented high levels of infection by powdery mildew on clusters.

Thus, samples of clusters were collected from 2 vineyards in the Loire region, France, from Saint Cyr en Bourg (Chardonnay) and Parnay (Cabernet Franc).

The next sub-chapters demonstrate the obtained and discussed results on this trial.

3.4.1. Chemical analysis of berries

250 healthy berries and 250 diseased berries of Chardonnay's clusters were sent to the Laboratoire Oenologique U.A.P., in Brissac, for analysis of sugar content (g/l), potential alcohol (% volume), total acidity (g H_2SO_4/l), pH and nitrogen (mg/l). Table 8 displays the obtained results for each of the chemical parameters, according to presence and absence of infected berries by powdery mildew.

The grape juice resultant from 250 berries free of visual symptoms of powdery mildew, from Chardonnay's clusters, showed a content in sugar of 203.30g/l corresponding to a volume of potential alcohol of 12.08%. In terms of total acidity, its valour was 3.82g H_2SO_4/l with a measured pH of 3.30 and, finally, 175mg/l of nitrogen (annex 18).

For the resultant grape juice of 250 diseased berries of Chardonnay's clusters, the result of sugar concentration was 231.10g/l corresponding to a volume of potential

alcohol of 13.73%. Total acidity was 5.19g H_2SO_4/l with a pH of 3.43 and, finally, concentration of nitrogen was 248mg/l (annex 19).

Thus, these results demonstrate that, powdery mildew may have increased concentration of sugar, volume of potential alcohol, total acidity and nitrogen compared with grape juice obtained from powdery mildew free berries. An increase in sugar concentration in juice made from diseased berries was previously reported, more recently, by Stummer, et al., (2004) and Calonnec, Cartolaro, Poupot, Dubourdieu, & Darriet (2004) and can be a consequence of a reduction in the volume of, and an increased transpirational water loss by, diseased berries, enhanced by cracked skin (symptom associeted to powdery mildew infection in berries mention on sub - chapter 1.1.3). An increase of total acidity in juice from diseased berries was also reported by Gadoury D. , Seem , Pearson, Wilcox, & Dunst (2001) and, Stummer, et al. (2004) in samples of Chardonnay's grapes. Regarding composition in nitrogen, Valdés-Gómez, Gary, Cartolaro, Lolas-Caneo, & Calonnec (2011) described a relationship between powdery mildew's growth and content of nitrogen for the grapevine, meaning, powdery mildew develops in more vigours grapevines (more vegetative growth and more nitrogen concentration). Vines with high amounts of nitrogen will also have highest concentration of this parameter in all organs, inclunding berries (Hilbert, et al., 2003).

Table 8 - Results of chemical analysis of berries from **Chardonnay**

Category	Sugar (g/l)	P. Alcohol (% vol.)	Total Acidity (g H_2SO_4/l)	pH	Nitrogen (mg/l)
Healthy	203.30	12.08	3.82	3.30	175
Diseased	231.10	13.73	5.19	3.43	248

Table 9 shows the obtained results for chemical parameters of sugar content (g/l), potential alcohol (% volume), total acidity (g H_2SO_4/l), pH, nitrogen (mg/l), anthocyanins (mg/kg) and total polyphenols from 250 healthy and 250 infected berries of Cabernet Franc. Regarding the analysis of grape juice produced from 250 diseased berries of Cabernet Franc's clusters, the volume of the obtained juice was not sufficient for analysing the concentration of sugar, potential alcohol, total acidity, pH and nitrogen; it

was only possible to determinate anthocyanins (mg/kg) and total polyphenols. These analysis were carried by the Laboratoire Oenologique U.A.P., in Brissac (annexes 20 and 21).

Grape juice resultant from 250 berries with no visual symptoms of powdery mildew infection displayed a concentration of sugar of 167g/l, corresponding to a volume of potential alcohol of 9.92%. Total acidity was 6.86g H_2SO_4/l with a valour of pH of 3.18 and nitrogen concentration was 104mg/l. Anthocyanins component was 741mg/kg and total polyphenols 36.40.

Regarding the concentration of anthocyanins and total polyphenols of grape juice obtained from diseased berries, of Cabernet Franc, they were, respectively, 649mg/kg and 51.10. Valour of anthocyanins (pigments responsible for the red and purple colour of the grapes and wines) was higher in the resultant grape juice of healthy berries than, the one from diseased grape berries, other studies had also reported a decrease in anthocyanins of diseased berries (Amati, Piva, Castellari, & Arfelli, 1996; Piermattei, Piva, Castellari, Arfelli, & Amati, 1999; Calonnec, Cartolaro, Poupot, Dubourdieu, & Darriet, 2004). The opposite occurred with the valour of total polyphenols.

Table 9 - Results from chemical analysis of berries from **Cabernet Franc**

Category	Sugar (g/l)	P. Alcohol (% vol.)	Total Acidity (g H_2SO_4/l)	pH	Nitrogen (mg/l)	Anthocyanins (mg/kg)	Total Polyphenols
Healthy	167	9.92	6.86	3.18	104	741	36.40
Diseased	-	-	-	-	-	649	51.10

3.4.2. Quality and yield of clusters according to different levels of powdery mildew infection

Clusters, of Chardonnay, from categories C_3, C_4 and C_5 were significantly lighter than healthy ones (C_0), averaging, respectively, 140.84g, 114.65g and 76.21g compared with 177.07g, corresponding to weight decreases of, respectively, 20%, 35% and 57%.

Number of berries from Chardonnay's clusters C_4 and C_5 were statistically less than healthy ones, meaning, correspondingly, 99.40 and 93.36 comparing to 140.16. Chardonnay's clusters C_4 had 29% less number of berries, per cluster, than healthy ones and, clusters C_5 had 33% less number of berries, per cluster, compared with clusters without diseased berries.

Diseased berries from clusters C_3, C_4 and C_5, of Chardonnay, were statistically lighter than healthy ones, corresponding, in average, to 1.05g, 1.04g and 0.75g, respectively. This translates in weight losses of 14%, 15% and 39%, in the same order.

Table 10 displays the effects of the different levels of powdery mildew in clusters of Chardonnay, in terms of weight and numbers of grapes (annexes 22, 23and 24).

Table 10- Effects of powdery mildew on weight and numbers of Chardonnay's grapes, $P<0.05$. Mean (Standard deviation)

Category	N	Mean weight of a cluster (g)	Mean number of berries	Mean weight of a berry per cluster (g)
C_0	25	177.07 (69.96)a	140.16 (52.33)a	1.22 (0.26)a
C_1	14	161.76 (51.46)ab	151.57 (47.70)a	1.08 (0.26)ab
C_2	14	156.99 (46.90)ab	140.43 (37.09)a	1.09 (0.29)ab
C_3	15	140.84 (44.11)bc	137.86 (53.11)a	1.05 (0.30)b
C_4	15	114.65 (29.34)c	99.40 (24.65)b	1.04 (0.11)b
C_5	25	76.21 (41.11)d	93.36 (34.98)b	0.75 (0.21)c

Category of cluster: C_0 = no diseased berries, C_1 = 1-5% of diseased berries, C_2 =6-10% of diseased berries, C_3 = 11-25%, C_4 = 26-50% and C_5 =51-100% of diseased berries on visual scale. N= number of clusters used for analysis. Note: variable number of clusters assessed because of number of clusters found to fit these categories of infection

Clusters of Cabernet Franc, from categories C_1, C_2, C_3, C_4 and C_5 were significantly lighter than healthy ones, having, in average, 94.79g, 93.91g, 90.40g, 83.98g and 28.65g, in that order, compared with 125.00g from the healthy clusters. These weights correspond to decreases of 24%, 25%, 28%, 33% and 77%, respectively, in relation with weight of healthy ones.

Number of berries from Cabernet Franc's clusters C_4 and C_5 were, statistically, less than healthy ones. In average, C_4 and C_5 had 88.58 and 87.96 berries, in that order, compared with 113.84 berries from C_0, C_4 and C_5 had, 22% and 23%, respectively, less number of berries in comparison with clusters with no diseased berries.

Cabernet Franc's diseased berries, from clusters C_3, C_4 and C_5 were statistically lighter than healthy ones, corresponding, in average, to 0.93g, 0.87g and 0.29, in the same order, comparing to 1.07g from clusters with no diseased berries. These averages leaded to weight decreases of, respectively, 13%, 18% and 73%.

Next table shows the effects of different levels of powdery mildew on weight and numbers of grapes from Cabernet Franc (annex 25, 26 and 27).

Table 11 - Effects of powdery mildew on weight and numbers of Cabernet Franc's grapes, $P<0.05$. Mean (Standard deviation)

Category	N	Mean weight of a cluster (g)	Mean number of berries	Mean weight of a berry per cluster (g)
C_0	25	125.00 (37.92)a	113.84 (30.11)a	1.07 (0.32)a
C_1	12	94.79 (30.04)b	91.18 (28.86)ab	0.96 (0.08)ab
C_2	12	93.91 (19.19)b	93.08 (25.32)ab	0.95 (0.14)ab
C_3	12	90.40 (33.25)b	91.58 (35.76)ab	0.93 (0.10)b
C_4	12	83.98 (25.56)b	88.58 (25.93)b	0.87 (0.15)b
C_5	25	28.65 (20,91)c	87.96 (41.19)b	0.29 (0.12)c

Category of cluster: C_0 = no diseased berries, C_1 = 1-5% of diseased berries, C_2 =6-10% of diseased berries, C_3 = 11-25%, C_4 = 26-50% and C_5 =51-100% of diseased berries on visual scale. N= number of clusters used for analysis. Note: variable number of clusters assessed because of number of clusters found to fit these categories of infection

Thus, powdery mildew had a clear effect on grape yield since, an increase in the percentage of diseased berries of reduced size, and a reduction in the number of berries per cluster. More than 10% of infection caused a delay of growth of berries (decrease in berry size). Berry set and size are mainly limited by carbon availability during early stages of development, and most of the sugar imported into the berry is metabolized, with little storage. The growth reduction observed in diseased berries could be the consequence of modification of the source–sink relationships for carbohydrates in the developing berry, with powdery mildew competing for the glucose and fructose (Calonnec, Cartolaro, Poupot, Dubourdieu, & Darriet, 2004).

The volume of extractable juice from diseased berries can be considered lower than from healthy berries due to the fact of diseased berries are significantly smaller.

3.4.3. Weight of clusters and their proportion levels to obtain musts

Chardonnay's clusters were harvest on 20/09/2016 in two different containers, with, approximately, 25kg capacity, one container was used to put healthy clusters of this cultivar and, the other one was used for clusters that showed visual symptoms of powdery mildew. The two containers were reserved at 4°C until 22nd of September.

The weight of the two containers with the clusters was measured and noted, after proceeding with tare of the containers, it was obtained 20.14kg of healthy clusters of Chardonnay and 16.83kg of the infected ones. Then, the healthy and infected clusters were pressed, separately, during 3 minutes at 2bar plus two repressing under the same conditions, using a press model STOSSIER. It was obtained 12.81kg of healthy pressed clusters and 9.68kg of infected ones.

Table 12 displays the weight of healthy and infected clusters of Chardonnay at harvest and, after press.

Table 12 - Weight of healthy and infected clusters of **Chardonnay** at harvest and after being pressed

Category	Harvest	Press during 3min at 2 bar + 2 repressing			
	Weight net (Kg)	Weight of cloths used for press (Kg)	Weight brut after press (Kg)	Tare of container (Kg)	Weight net (kg)
Healthy clusters	20.14	3.59	16.55	3.74	12.81
Infected clusters	16.83	3.41	13.42	3.74	9.68

Based on the weight net obtained after press, six modalities were esteemed in order to have musts with 0% (B_0), 5% (B_1), 10% (B_2), 25% (B_3), 50% (B_4) and 100% (B_5) of infection with powdery mildew.

Next table, exhibit the quantities, in litres, of chardonnay that were mixed according to respective their categories, where 0% corresponds to 2.50L of healthy must

of chardonnay; B_1 is combination of 2.38L of healthy must with 0.13L of infected one (matching a proportion of 5% of level of disease present in the final must); B_2 relates 2.25L of healthy chardonnay with 0.25L of powdery mildew's must (corresponding to a proportion of 10% of level of disease present in the final must), B_3 is a mixture of 1,88L of healthy must with 0,63L of infected must (regarding to a proportion of 25% of level of disease present in the final must); B_4 that corresponds to half quantity of healthy must with half infected in order to obtain 2.50L in total (meaning a proportion of 50% of level of disease present in the final must) and, finally, B_5 that corresponds to 2.50L of infected must with powdery mildew (corresponding to a proportion of 100% of level of disease present in the final must).

After obtaining the 6 modalities of chardonnay, they were reserved in carboys, in a cold chamber, for clarification and bottling on the next day.

Table 13 – Proportions of healthy and infected musts used to obtain the different categories of musts of Chardonnay

Chardonnay (L)	**B_0**	**B_1**	**B_2**	**B_3**	**B_4**	**B_5**	**Total**
Healthy must	2.50	2.38	2.25	1.88	1.25	0.00	10.25
Powdery mildew's must	0.00	0.13	0.25	0.63	1.25	2.50	2.25
Total	2.50	2.50	2.50	2.50	2.50	2.50	15.00

B_0, absence of powdery mildew; B_1, 5% of diseased berries; B_2, 10% diseased berries; B_3, 25% diseased berries; B_4, = 50% diseased berries for sensorial analysis and, B_5 = 100% diseased berries.

For cabernet franc, harvest occurred on 22/09/2016, where healthy and infected clusters were, separately, putted in two containers with a capacity of 25Kg, approximately (figure 31).

Figure 31 – Infected clusters on the left, healthy clusters of Cabernet Franc on the right, after harvest.

Table 14 displays the obtained weights of Cabernet Franc at harvest and after the process of stalk, where the berries were separated from the stems. It is possible to observe that it was collected 25.11Kg of healthy clusters and 20.89Kg of infected ones. Next, these clusters were destemmed, separately, and it was attained 17.71Kg of healthy clusters and 14.12Kg of the infected ones.

Table 14 - Weight of healthy and infected clusters of **Cabernet Franc** at harvest and after stalk

Operations	**Weights**	**Category**	
		Healthy	Infected
Harvest	Weight brut (Kg)	27.52	23.30
	Tare of the box (Kg)	2.41	2.41
	Weight net (Kg)	**25.11**	**20.89**
Stalk	(1) Stem + bucket (Kg)	2.59	5.98
	(2) Tare of bucket (Kg)	0.76	2.46
	(1)-(2) Stems (Kg)	1.83	3.52
	Clusters with PM (Kg)	0.55	-
	Rests of grains + stems (Kg)	1.23	-
	Gardevin 11 + destemmed clusters (kg)	21.96	18.37
	Tare of Gardevin 11 (kg)	4.25	4.25
	Destemmed clusters	**17.71**	**14.12**

With the weight net obtained after stalk, six modalities were esteemed (table 15) and mixed in order to have cuvees with 0% (B_0), 5% (B_1), 10% (B_2), 25% (B_3), 50% (B_4) and 100% (B_5) of infection with powdery mildew, macerating during 3 hours.

Table 15 - Proportions of healthy and infected clusters of **Cabernet Franc**, to be used to obtain the different levels of infection in musts

Cabernet Franc (Kg)	**B_0**	**B_1**	**B_2**	**B_3**	**B_4**	**B_5**	**Total**
Healthy clusters	4.00	3.80	3.60	3.00	2.00	0.00	16.40
Powdery mildew's clusters	0.00	0.20	0.40	1.00	2.00	4.00	3.60
Total	4.00	4.00	4.00	4.00	4.00	4.00	20.00

B_0, absence of powdery mildew; B_1, 5% of diseased berries; B_2, 10% diseased berries; B_3, 25% diseased berries; B_4, = 50% diseased berries for sensorial analysis and, B_5 = 100% diseased berries.

When maceration was finished, the 6 modalities of Cabernet Franc were pressed during 3 minutes at 2 bar plus, two more repressing under the same conditions, using a press model STOSSIER. On the table below, it is possible to visualize the weights, in Kg, acquired after press for each modality of Cabernet Franc.

The obtained musts were stored in glass carboys, in cold chamber, for bottling on the next day.

Table 16 - Weights of musts of **Cabernet Franc**, per level of infection, after press

Press during 3min at 2 bar + 2 repressing 3min at 2 bar				
Modality	Weight of cloths used for press (Kg)	Weight of container + plug + must (kg)	Tare of container (kg)	Weight of must (Kg)
B_0	0.99	2.74	1.75	0.99
B_1	1.21	2.59	1.75	0.84
B_2	1.05	2.87	1.75	1.12
B_3	1.23	2.47	1.75	0.72
B_4	1.19	2.94	1.75	1.19
B_5	1.45	2.65	1.75	0.90

B_0, no presence of powdery mildew; B_1, 5% of diseased berries; B_2, 10% diseased berries; B_3, 25% diseased berries; B_4, = 50% diseased berries for sensorial analysis and, B_5 = 100% diseased berries.

3.4.4. Chemical composition of musts with different levels of powdery mildew infection

The data gathered from the chemical analysis of musts with different levels of powdery mildew (annexes 28 and 29) was evaluated using the Principal Component Analysis method, which allowed to note possible correlations on quality components of musts and the infection levels of powdery mildew disease. Four variables were accessed: sugar (g/l), potential alcohol (% volume), total acidity (g/l) and pH.

For Chardonnay's musts, the first (PC1) and second (PC2) principal components were used which account for 79.6 % and 16.8%, respectively, of the variance in the four variables, with a cumulative proportion of 96.5% of the variance represented.

The colour maps in table 17 shows to what degree each variable is represented by the first and second principal component (PC1, PC2). The main variables represented by PC1 are in receding order sugar/potential alcohol and total acidity. With respect to PC2, pH is the main variable represented.

Table 17 - Coefficients of variables, for **Chardonnay**'s musts, in each the first (PC1) and second (PC2) principal component

Variables	PC1	PC2
Sugar (g/l)	0.515	-0.481
Total Acidity (g/l)	0.498	0.442
pH	0.472	0.584
Potential Alcohol (% vol.)	0.515	-0.481

The monoplots represents the variables as vectors. Those which are close to one another are linked by a positive correlation, while those pointing to opposing direction are negatively correlated, and those closing a right angle with another present no correlation. In the monoplot, for Chardonnay's musts (annex 30) in correspondence with previous observations, the vectors of the following variables are located close to one

another, thus are positively correlated: sugar (g/l) and potential alcohol (R = 1.00) and pH with total acidity (R = 0.85).

When data is represented on a biplot (annex 31), each dot represents the level of infection by powdery mildew (each marked with a different coloured symbol). When observing the position of musts, according to the level of infection with powdery mildew, musts from 0%, 10% and 25% categories of disease are located in the same part of the plot, exhibiting similar valour's for sugar (g/l), potential alcohol (% vol.), total acidity (g/l) and pH. Must with 5% of level of infection displayed similar valour's with the musts previously mentioned, although with, a slight difference for potential alcohol (% vol.) compared with 0%, 10% and 25%, which explains its position in the plot. Chardonnay's musts from 50% and 100% are both located in other part of the plot displaying different valour's in comparison with the others' musts. In addition, comparing the position between the dot that represents 50% with the 100% one, there is a clear long distance that corresponds to a significant difference for pH and total acidity concentrations between them.

Therefore, the different levels of powdery mildew infection had a strong impact on pH and total acidity composition of Chardonnay's musts, representing a strongly positive correlation with these two components (R = 0.994 and R = 0.792). For sugar and potential alcohol, the different proportions of powdery mildew's infection on musts were approximately correlated (R = 0.576).

Regarding Cabernet Franc's musts, the first (PC1) and second (PC2) principal components were used which account for 78.4 % and 20.4%, respectively, of the variance in the four variables, with a cumulative proportion of 98.8% of the variance represented.

The colour maps in table 18 demonstrates to what degree each variable is represented by the first and second principal component (PC1, PC2). The main variables represented by PC1 are in receding order sugar/potential alcohol and total acidity. With respect to PC2, pH is the main variable represented.

In the monoplot Cabernet Franc's musts (annex 32), the vectors of the following variables are located close to one another, thus are positively correlated: sugar (g/l) and potential alcohol (R = 1.00) and pH with total acidity (R = 0.92).

Table 18 - Coefficients of variables, for **Cabernet Franc**'s musts, in each the first (PC1) and second (PC2) principal component

Variables	PC1	PC2
Sugar (g/l)	0.509	-0.477
Potential Alcohol (% vol.)	0.509	-0.477
Total Acidity (g/l)	0.522	0.378
pH	0.457	0.634

When data is represented on a biplot (annex 33), each dot represents the level of infection by powdery mildew (each marked with a different coloured symbol). Through observation of the position of musts, according to the level of infection with powdery mildew, musts from 0% and 5% categories of disease are located in the same part of the plot, exhibiting similar but slightly increasing valour's for sugar (g/l), potential alcohol (% vol.), total acidity (g/l) and pH, respectively. Musts with 10% and 25% level of infection are closely located on the plot, which means, their chemical composition is similar even though, valour's for musts with 25% level of infection was a little higher than the other one. Musts from 50% and 100% levels of infection are located in distinct parts of the plot, being isolated from each other and from the rest of the musts.

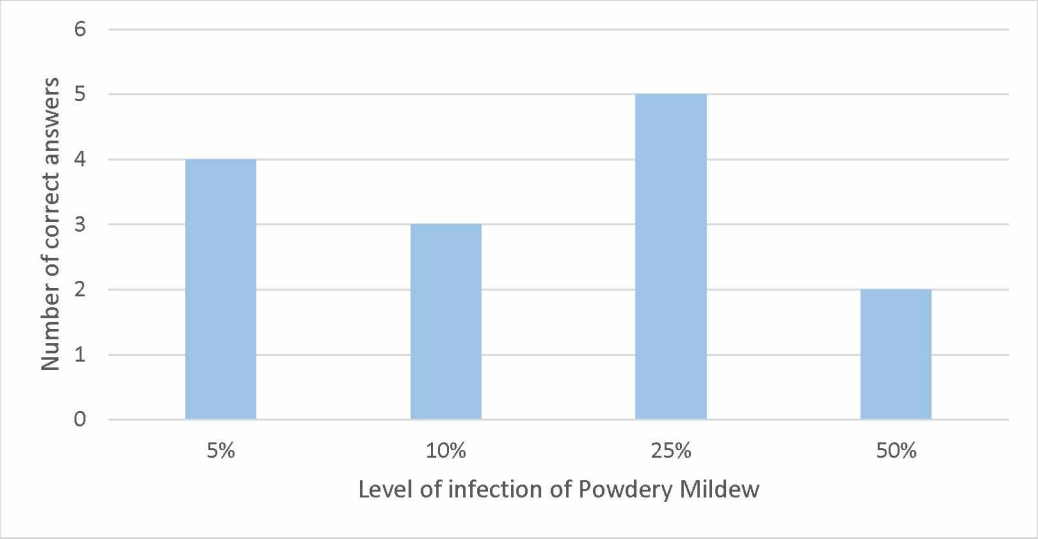
In sum, the different levels of powdery mildew infection had a strong impact on pH and total acidity composition of Cabernet Franc's musts, representing a strongly positive correlation with these two components ($R = 0.986$ and $R = 0.941$, in the same order). For sugar and potential alcohol, the different proportions of powdery mildew's infection on musts were approximately correlated ($R = 0.509$).

3.4.5. Sensorial analysis of musts with different levels of infection by powdery mildew

A triangular test was prepared on 23/09/2016 with the propose of verify if a panel of 10 tasters were able to note sensorial differences between musts made, of Chardonnay and Cabernet Franc, with different levels of infection by powdery mildew (5%, 10%, 25% and 50% in comparison with 0%).

Graphic 5 demonstrates the number of correct answers, given by the tasting panel, on the triangular tests for Chardonnay's musts. There were no statistical differences between Chardonnay's musts with 5%, 10%, 25% and 50% in comparison with 0%, even at a 5% level of significance (annex 11).The panel had difficulties in distinguish differences between samples, which translates the low number of correct answers on this test, because of the high composition of sugar present in the musts, since no fermentation occurred.

Graphic 5 – Total number of correct answers for triangular tests of **Chardonnay**'s musts

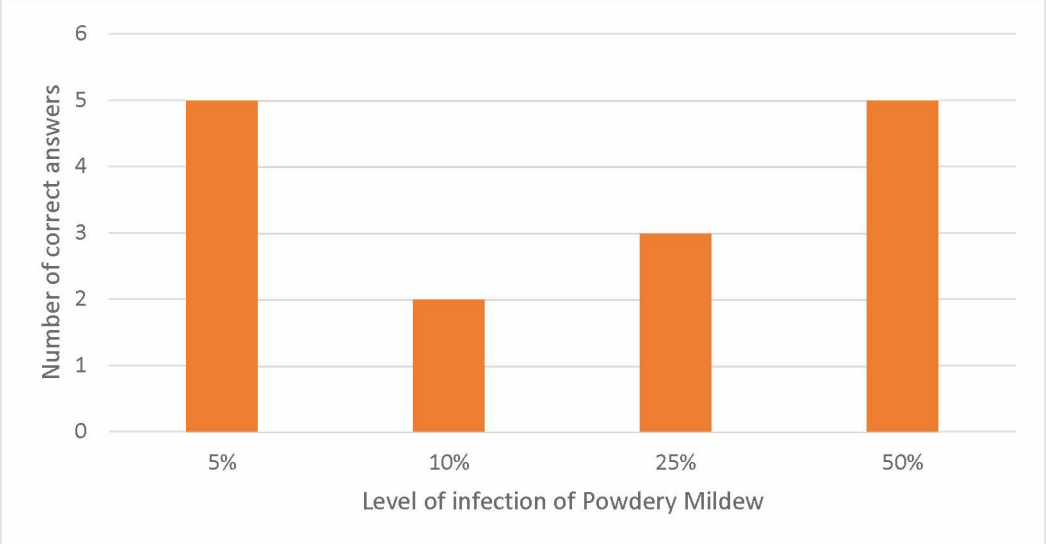


Regarding the triangular tests of Cabernet Franc's musts, no statistical differences were found on samples with 5%, 10%, 25% and 50% in comparison with 0% of level of

powdery mildew. Next graphic displays the number of correct answers, given by the tasting panel, in the triangular test for Cabernet Franc's musts.

Tasters had difficulty in assess sensorial differences between samples because of, high concentration of sugar in the musts, as occurred with Chardonnay's triangular tests.

Graphic 6 - Total number of correct answers on triangular tests of **Cabernet Franc**'s musts

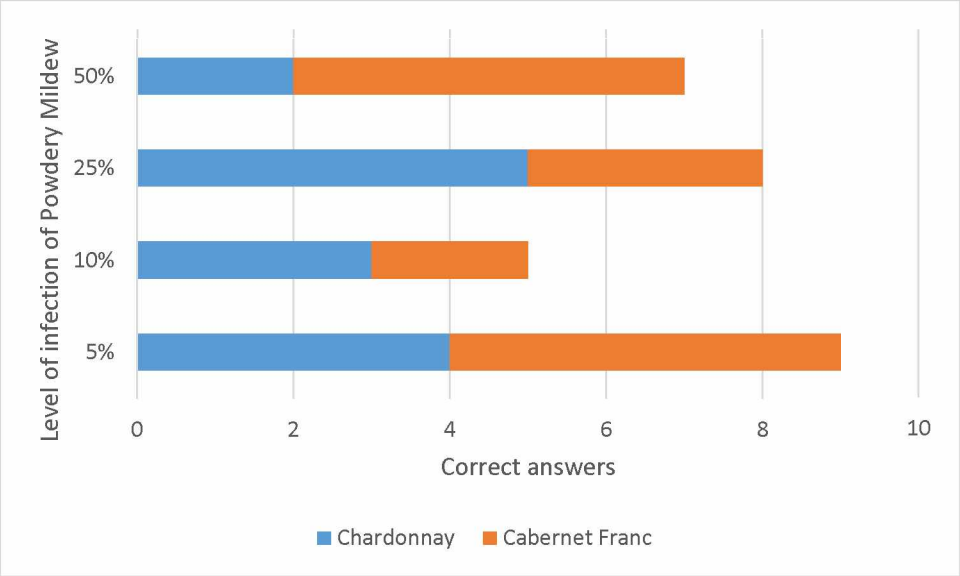


The high sugar content present disguised other aromas present in the musts and confused tasters when asked to appoint sample that is different from the other two. In wines, literature referrers a strong impact, in sensorial attributes, by powdery mildew even at the lowest level of infection but, there are not references about non fermented musts. It is clear that, it was hard to taste grape juices and try to find differences between them, specially, when the panel of tasters was not trained for these tests.

The methodology used to obtain musts is also a factor to have in count, since tasters had more difficulty in distinguish samples from Chardonnay than from Cabernet Franc (graphic 7). Clarification of Chardonnay's musts before bottling may have contributed negatively for perception of sensorial differences between musts, since some aromas were lost after separation of the must from the deposit, which associated with the fact, of a high concentration of sugar clearly confused the sensory panel.

Maceration before press of each modality separately, in Cabernet Franc, enhanced the liberation of aromas from the skin of berries to the musts and leaded to a better perception of sensorial characteristics in all samples.

Graphic 7 - Comparison between number of correct answers, by tasters, in Chardonnay's and Cabernet Franc's triangular tests

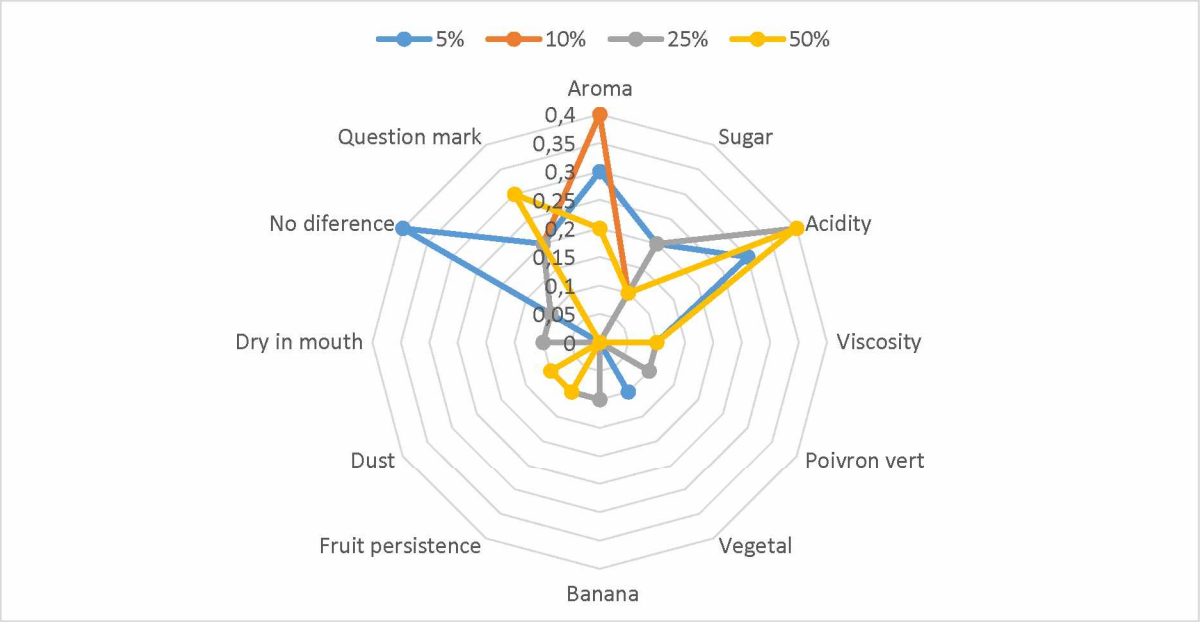


Although no statistical differences were found on the triangular tests, which means descriptive tests were not conducted, tasters were asked, during the triangular assessments, to write what were the sensorial attributes that, they thought were distinct in the sample they were appointing as different from the others.

Graphic 8 demonstrates the sensorial attributes answered as criteria of distinction, in average by the panel of tasters, for each of Chardonnay's triangular test. The sensorial distinctive attributes were aroma, sugar content, acidity, viscosity, *banana*, *poivron vert* (green pepper), vegetal, fruit persistence, dust, dry in mouth, no difference on, at least one sensorial characteristic (for example, no perceptive difference in aroma and/or others) and, question mark when tasters were not able to appoint a sensorial attribute. Three and four out of 10 tasters attributed aroma as a distinction criteria for the triangular test, where samples with 5% and 10%, respectively, of level of infection with powdery mildew were compared with 0% but, only two appointed this criteria for the triangular test of 50%. In sugar content, two out of ten judges attributed this sensorial criteria as distinct in the

triangular tests that compare 5% and 10% of level of infection with 0%. However, one out of ten mentioned sugar as a sensorial distinctive criteria for the triangular tests that compare 25% and 50% of level of infection with 0%. Four out of ten tasters, appointed acidity as a distinctive criteria for the triangular tests that compare 10%, 25% and 50% of level of infection with 0% though, three out of ten judges related acidity as distinctive parameter for the triangular test that compares 5% of infection with 0%. Lastly, some specific attributes were noticed by few tasters as aromas of: poivron vert (green pepper), banana, vegetal, dust, and viscosity, differences in fruit persistence and dry in mouth feeling.

Graphic 8 – Mean of answers for sensorial criteria of distinction between samples for each of the **Chardonnay**'s triangular tests (5%, 10%, 25% and 50%)



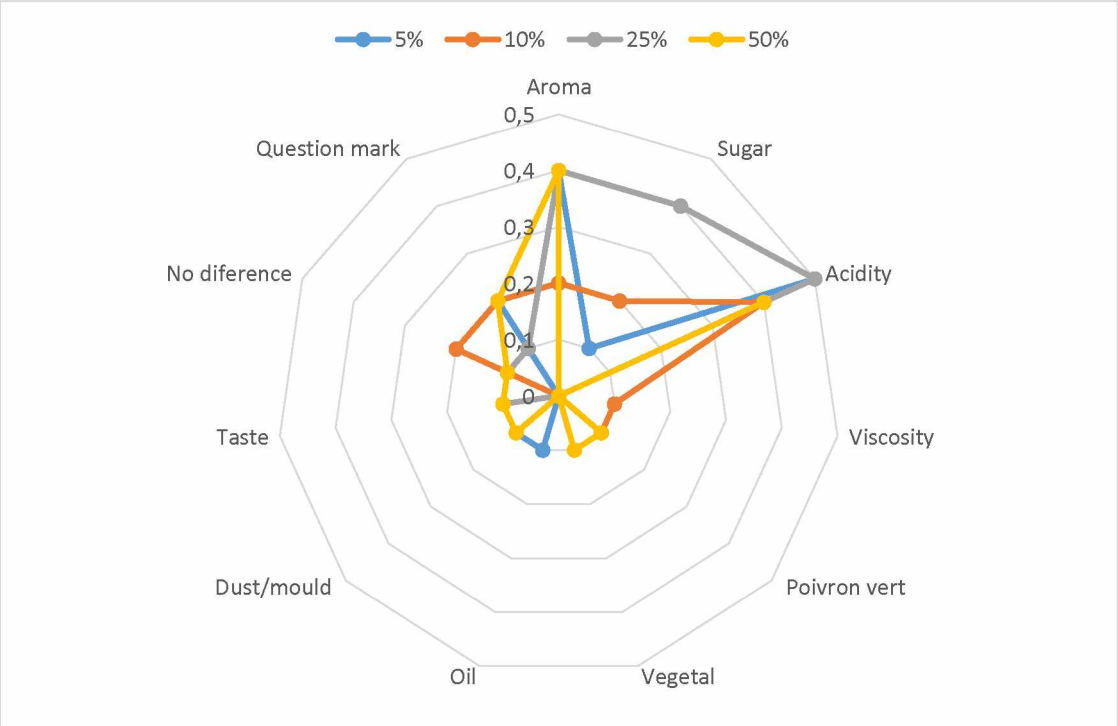
According to Emmett, et al., (2004), in sensory attributes of Chardonnay's wines, made from different levels of powdery mildew's infection, there was a clear impact of the fungus in enhancing a viscous mouth-feel character, even at the lowest level of infection, sensorial parameter detected among tasters during the triangular tests.

For Cabernet Franc's triangular tests, the sensorial distinctive attributes were aroma, sugar content, acidity, viscosity, *poivron vert* (green pepper), vegetal, oil, fruit persistence, dust/mould, taste, no difference on, at least one sensorial characteristic (for

example, no difference in aromas or others) and, question mark when tasters were not able to appoint a sensorial difference.

Graphic 9 displays the sensorial distinctive attributes answered by the judges, in average, for each of the triangular tests made for Cabernet Franc's musts. Four out of ten judges considered that existed differences in aroma between samples of Cabernet Franc with 0% of infection and 5%, 25% and 50% but, only two out of ten considered that aroma was different between a sample with 0% of infection and 10%. Regarding sugar content, four out of ten tasters appointed a high concentration of sugar on the triangular test performed for the modality of 25% of infection with powdery mildew. Acidity was a criteria of distinction very highlighted by judges in all performed tests. Finally, some specific attributes were noticed by few tasters as aromas of: *poivron vert* and dust/mould for tests of 5%, 10% and 50% of infection level in comparison with 0%, viscosity on the triangular test of 10% of infection, oil for the test of 5% of infection and vegetal for the triangular tests of 25% and 50% of infection level with powdery mildew in comparison with 0%.

Graphic 9 - Mean of answers for sensorial criteria of distinction between samples for each of the **Cabernet Franc**'s triangular tests (5%, 10%, 25% and 50%)



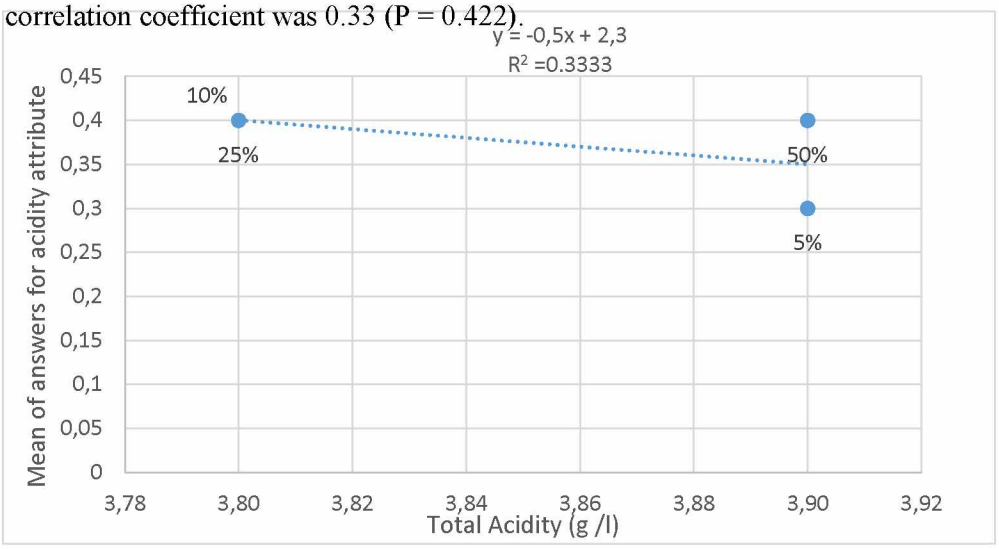
Viscosity/oil and dust/mould are sensorial attributes associated to the presence of powdery mildew infection on wines, according to literature, that were perceived by the sensory panel on the triangular tests for Cabernet Franc's musts.

Green pepper was an attribute appointed for Chardonnay's and Cabernet Franc's triangular tests although, there is not bibliographical references associating this parameter with the effects of powdery mildew in wines.

Although, it was very difficult for tasters to perceive differences between samples of non-fermented musts, especially on Chardonnay's, the sensory panel was very perceptive of some of the most common sensorial attributes given by the presence of the fungus in wines, according to bibliography. This means, even in grape juice powdery mildew may have a clear impact on quality, although further investigation is required and, methodology must be reviewed in order to be able to achieve better conclusions on this subject.

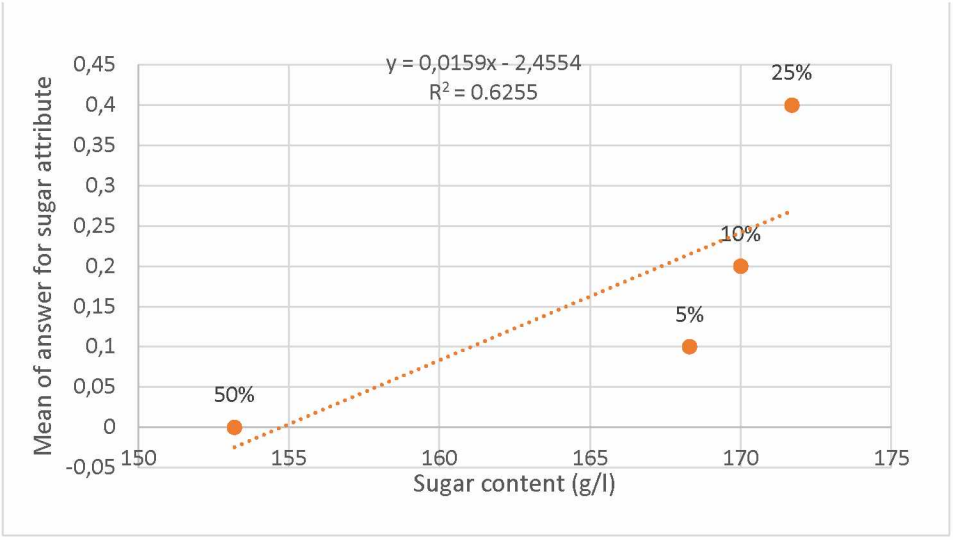
Another interesting point is the sensorial criteria of distinction most answered was, by the sensory panel, acidity for all the triangular tests of Chardonnay. Correlating the mean of answers, given by the judges, appoint acidity as a different parameter between samples of each triangular test and Total acidity (g/l) analysed in laboratory, a very low correlation was found ($R^2 = 0.33$, $P = 0.422$, $n = 4$), as it can be visualized on graphic 10.

Graphic 10 - Correlation between mean of answers for Acidity attribute as distinctive attribute and Total Acidity (g /l) for **Chardonnay's** triangular tests. The values next to the symbols indicate the level of powdery infection of each of the samples. The correlation coefficient was 0.33 (P = 0.422).



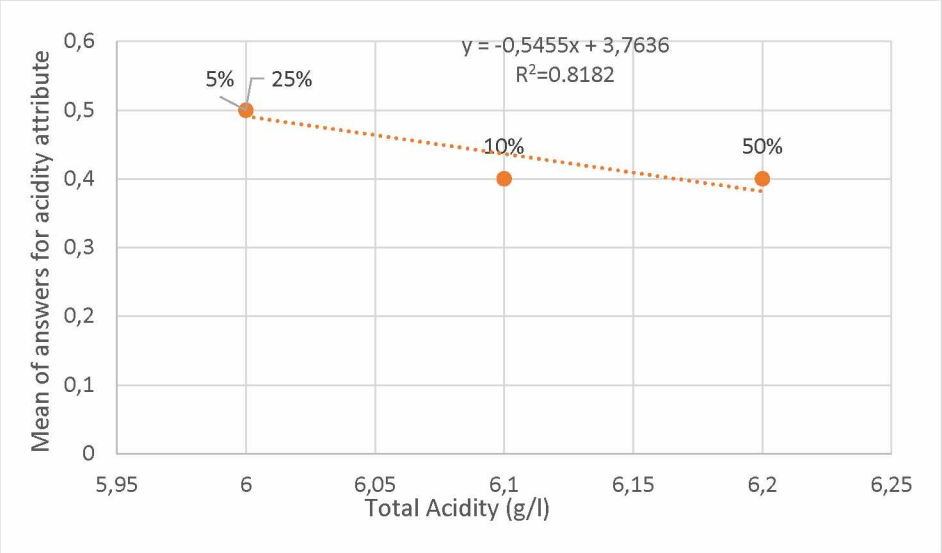
In Cabernet Franc's triangular tests, the sensory panel perceived sugar and acidity as the most common criteria of distinction between samples. Mean of answers for sugar parameter was correlated approximately equally (R^2= 0.63, P = 0.209, n = 4) with Sugar Content (g/l), as it can be visualized on graphic 11.

Graphic 11 - Correlation between mean of answers for sugar as a distinctive attribute and Sugar content (g/l) for **Cabernet Franc**'s triangular tests. The values next to the symbols indicate the level of powdery infection of each of the samples. The correlation coefficient was 0.63 (P = 0.209).



Mean of answers for acidity parameter was positively strongly correlated (R^2 = 0.83, P = 0.095, n = 4) with Total Acidity (g/l), as it can be visualized on graphic 12.

Graphic 12 - Correlation between mean of answers for Acidity attribute as distinctive attribute and Total Acidity (g /l) for **Cabernet Franc's** triangular tests. The values next to the symbols indicate the level of powdery infection of each of the samples. The correlation coefficient was 0.83 (P = 0.09).



Thus, statistical differences were not found on the triangular tests for Chardonnay's and Cabernet Franc's musts however, the sensory panel was able to perceived some characteristics refereed on literature as, an effect of presence of powdery mildew in young wines (viscosity/oil, mould/dust). These results are not sufficient but, they mean there are further investigation to be done.

The methodology to obtain the musts needs to be reviewed, since clarification of Chardonnay's musts leaded to loss of aromas, which associated with high level of sugar concentration confused the sensory panel. However, the maceration process in Cabernet Franc's musts contributed for the better perception of sensorial attributes during the triangular tests.

The tasting panel was not prepared to perceive differences in non-fermented musts, where sugar concentration was very high, although, this can be overcome if the panel has previous training sessions before a triangular test then, it will be easier to found differences between infected samples in comparison with a non-infected one since, throughout the distinction criteria, some tasters were able to detect some of the most common attributes given by the disease in wines.

4. Limits, improvements and further perspectives

➢ Limits

For studying the mode of action of *Ampelomyces quisqualis* on a plot, the first limitation is related with the presence of powdery mildew (since it is an obligatory parasite). Banker plant and the grapevines need to be infected with the disease in order to *Ampelomyces* starts developing. For the banker plant, this was easily overcame by an artificially inoculation with powdery mildew however, for the grapevine it is more complex, as it was observed in 2015 and 2016, since grapevine powdery mildew is dependent of a series of factors to appear on a plot (see sub-chapter 1.1.2).

Ampelomyces and *Erysiphe necator* are both dependent of the environment, the first fungus needs high levels of relative humidity and, the second on requires temperatures around 25°C associated with high levels of relative humidity and light diffuse but, clime can differ from one year to the next.

Management of the grapevines can also be limiting for powdery mildew's growth, as it prefers vines with more vigour.

Regarding the tasting trial, the first limit was the low attack by powdery mildew on the trial plot, which leaded to the choice of another plot for collection of clusters, known to be highly infected by the disease.

The second limit was related with the choice of procedures to obtain musts for tasting. Maceration before press helped the sensory panel to perceive some differences in Cabernet Franc's musts, which did not occurred in Chardonnay's samples due to the absence of this procedure and after, the clarification before bottling. Opting to not ferment the musts, due to lake of time, also leaded to the inconclusive results on the triangular tests.

Lastly, tasting panel was not prepared for perceive differences in samples with high levels of sugar content and, this was also a limit as the judges were confused and had difficulties on the tests.

- **Improvements and further perspectives**

Regarding the study of the use of a banker plant as a source of development of *Ampelomyces* to fight grapevine powdery mildew, management of the vines should be re –thought, meaning, any operation that increases plant vigour, leading to more compacted vines, where air circulation of the air is poor and indirect sun light helps the development of powdery mildew (obligatory element for this study). Although, clime will be, always, a factor favouring or limiting the interaction between the variables in study at the field.

The realization of a PCR analysis is mandatory to understand if this system can or not be functional. Since, its use provides a rapid and inexpensive method for screening for genetic diversity across a large number of isolates of pycnidial fungi isolated from colonies of powdery mildew fungi (Szentiványi, et al., 2005). Also, it will be important to do further investigation about the practical functionality of this tri – trophic system, meaning, how to adequate these procedures to lead farmers to choose this solution instead of applying fungicides.

Concerning the tasting trial, methodology to obtain samples should be reviewed and, perform a maceration before press of the samples separately.

Sensory panel must be trained before performing triangular tests so, the limitation related with sugar concentration could be, probably, overcome.

Sensorial trial has been assessed in wines but there is no evidence about free run grape juice. It seems interesting to study the impact of powdery mildew in sensory attributes in non-fermented musts and, try to understand if fermentation has or not influence in perception and on type of aromas attributed to the presence of contamination by this fungus.

Conclusion

The study about the use of a banker plant, meaning purple clover, to potentiate the efficiency of *Ampelomyces* as a biocontrol agent of grapevine powdery mildew will is still on-going work in process.

At this point, the tri - trophic system was implemented by using purple clover as a banker plant. This plant was observed since its early vegetative stage until flower and, it was successfully infected with powdery mildew (*Erysiphe trifolii*). The period of development of the banker plant matched with the season of development of powdery mildew in the vineyard, which is a very important aspect for this trial. After, being infected, purple clover was inoculated with AQ10® WG. Collector devices for *Ampelomyces'* spores were placed and several samples were collected and conserved.

Regarding the visual control of symptoms of grapevine powdery mildew (*Erysiphe necator*), during this year there was not infection by this fungus on leaves and, only a 10% frequency with 5% of severity, in average, of attack by the fungus on clusters of the vineyard in study. These low results compromised further investigation concerning *Ampelomyces'* efficiency as a bio control agent, using a banker plant since, it is an obligatory parasite and it needs its host present to develop itself. Although, few samples of infected berries with powdery mildew were also collected and conserved. These collected samples will be analysed through PCR technique to detect the presence/absence of the biocontrol agent.

Thus, for the moment was not possible to concluded if this system is functional or not, and further investigation is required in the area of PCR assessment, meaning, the choice of adequate primers for detention of strains of *Ameplomyces.*

The second phase of this study was related with quality and yield of grapes, from Chardonnay and Cabernet Franc infected by grapevine powdery mildew, at harvest period.

A series of assessments were conducted, being the first one chemical analysis of infected berries in comparison with healthy ones and, it was concluded that powdery mildew increased the concentration in sugar content and total acidity of berries of Chardonnay. The presence of the fungus is also related with higher levels of nitrogen in the grapevine and, so in the berries. For Cabernet Franc, the presence of powdery mildew

decreased the concentration in anthocyanins in berries and, it leaded to a slight increase in total polyphenols.

The second assessment was evaluate the quality and yield of clusters with different levels of powdery mildew infection, which were visually observed. Powdery mildew had a clear effect on grape yield since, an increase in the percentage of diseased berries of reduced size, and a reduction in the number of berries per cluster was determinate. More than 10% of infection caused a delay in growth of berries, which means a decrease in berry size thus, the volume of extractable juice from diseased berries can be considered lower than from healthy berries due to this fact.

The third assessment was on chemical analysis of musts with different levels of powdery mildew infection. The presence of different categories of contamination had a strong impact on pH and total acidity composition of Chardonnay and Cabernet Franc's musts, being the most infected musts more acid than the non-infected ones.

Finally, a triangular test was performed but no statistical differences were found in musts with several levels of disease compared with the healthy ones. The judges had difficulty in assess aromas due to high levels of sugar content (since the musts were not fermented) and, these difficulties were more intense in Chardonnay's musts, which can be explained by the fact that a maceration, before press, was not made as it was for Cabernet Franc's musts. With these assessments, it was possible to conclued that, in fact powdery mildew has negative effects on quality and yield of grapes at harvest period, even in lower levels of infection (11%).

Therefore, regarding the field trial, if this system proves to be efficient, it will be important to do further investigation about its practical functionality, meaning, how to adequate these procedures to lead farmers to choose this solution instead of applying fungicides. Concerning the tasting trial, the procedure method of obtain the musts should be improved to lead to better sensory perceptions: maceration of different categories of infection must be done before pressing in all varieties of grapes so, the aromas can be more pronounced in the obtained samples. Plus, the chosen tasters must have preparation sessions before doing triangular tests, to overcome the high sugar concentration limit because, even though it is hard to taste non- fermented musts, it seems very promising to, try to understand if fermentation has or not influence in perception and, on type of aromas attributed to the presence of contamination by this fungus.

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Annexes

Annex 1 - Active substances compatible with the utilization of AQ10

Active substance	Commercial name	Compatibility with AQ10	Downy mildew	Powdery mildew	Black-rot	Excoriose	Botrytis	Insecticide
DIFENOCONAZOLE	SCORE	-		Yes	Yes			
FENBUCONAZOLE	INDAR	-		Yes	Yes			
TETRACONAZOLE	DOMARK	-		Yes	Yes			
POLYSULPHUR OF CALCIUM	POLICALCIO	-		Yes				
BUPIRIMATE	NIMROD	-		Yes				
IMAZALIL	DECCOZIL	-		Yes				
PROCHLORAZ	divers	-						
PROPAMOCARBE	PREVICUR	-						
THIABENDAZOLE	divers	-						
TOLCLOFOS METHIL	divers	-						
BENALAXIL	divers	2	Yes		Yes	Yes		
CYMOXANIL	CURZATE 50%	2	Yes			Yes		
FOSETYL-ALL	ALIETTE 80%	2	Yes			Yes		
IPROVALICARB	MELODY COMPACT	2	Yes			Yes		
CYAZOFAMID	MILDICUT	2	Yes					
FENAMIDONE	ELICIO	2	Yes					
FLUAZINAM	OHAYO	2	Yes					
ZOXAMIDE	ELECTIS R	2	Yes					
MYCLOBUTANIL	THIOCUR	2		Yes	Yes			
TEBUCONAZOLE	FOLICUR	2		Yes	Yes			
TRIADIMENOL	BAYFIDAN	2		Yes	Yes			
THIPHANATE-METHYL	divers	2		Yes			Yes	
CYPROCONAZOLE	ATEMI/CADDY	2		Yes				
PENCONAZOLE	TOPAS	2		Yes				
QUINOXYFEN	ARIUS	2		Yes				
SPIROXAMINE	PROSPER	2		Yes				
Bacillus amyloliquefaciens	AMYLO-X	2					Yes	
Bacillus subtilis	SERENADE MAX	2					Yes	
BOSCALID	CANTUS	2					Yes	
IPRODIONE	ROVRAL	2					Yes	
MEPANIPYRIM	FRUPICA	2					Yes	
METALAXIL	RIDOMIL	2					Yes	
PYRIMETANIL	SCALA	2					Yes	
EMAMECTINE	PROCLAIM	2						Yes
PROPICONAZOLE	TILT	2						
PROPIZAQUID	TALENDO	2						

Source: adapted from (Cantin, 2015)

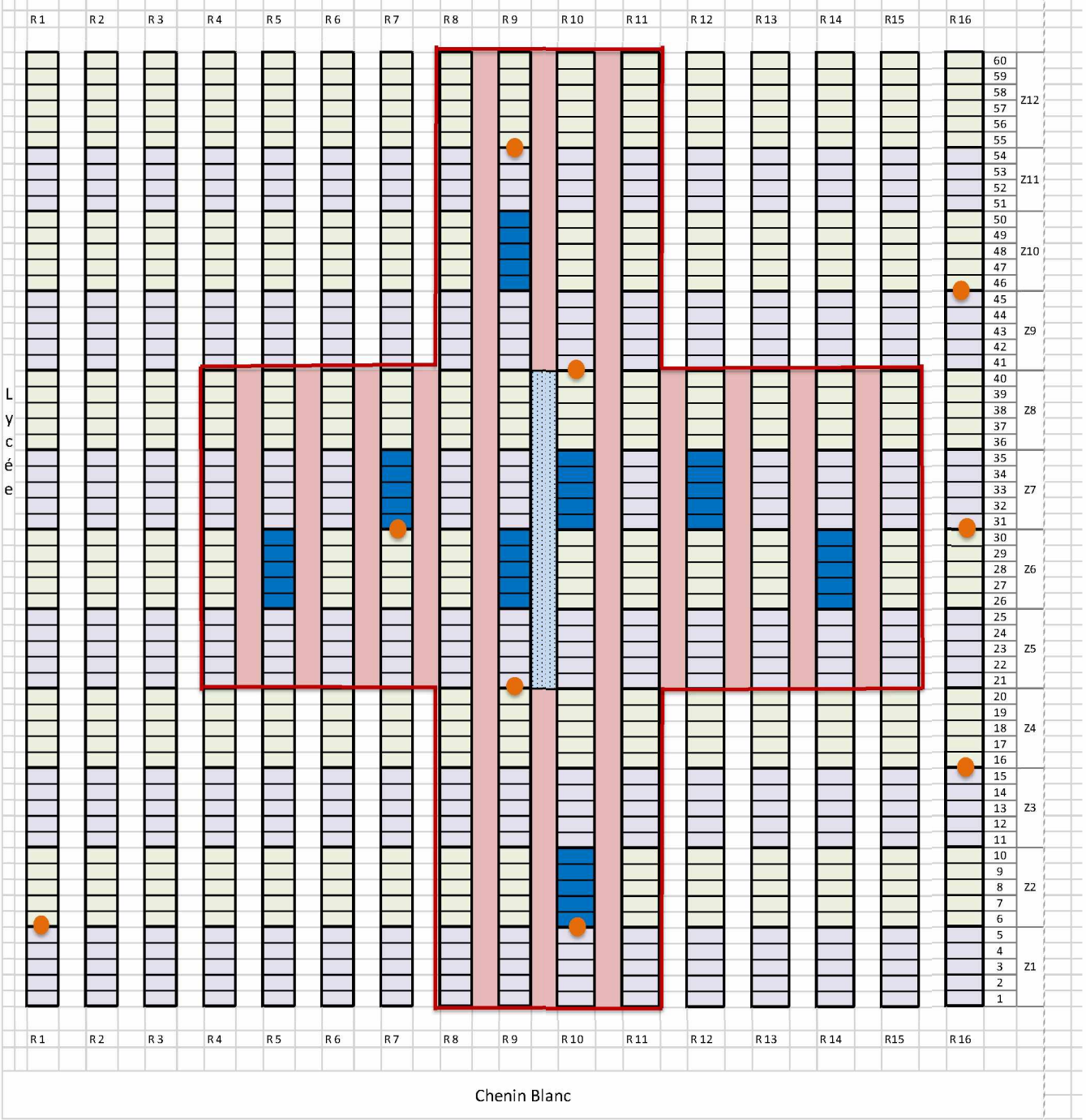
Annex 2 - Active substances compatible with the utilization of AQ10 (continuation)

Active substance	Commercial name	Compatibility with AQ10	Downy mildew	Powdery mildew	Black-rot	Excoriose	Botrytis	Insecticide
DIMETHOMORPHE	FORUM 50 WP	1	Yes					
AZOXYSTROBIN	QUADRIS	0	Yes	Yes	Yes	Yes		
MANCOZEBE	DITHANE DG	0	Yes		Yes	Yes		
MANEBE	Divers	0	Yes		Yes	Yes		
METIRAME-ZINC	POLYRAM	0	Yes		Yes	Yes		
FAMOXADONE	EQUATION PRO	0	Yes			Yes		
FOLPEL	divers	0	Yes			Yes		
KRESOXIM METHYL	STROBY	0	Yes			Yes		
CAPTANE	divers	0	Yes					
CHOROTHALONIL	DACONIL	0	Yes					
PROPINEBE	ANTRACOL	0	Yes					
TRIFLOXISTROBINE	FLINT	0		Yes	Yes	Yes		
DINOCAP	KARATHANE STAR	0		Yes				
SULPHUR	divers	0		Yes				
SULPHUR+TERPENIC ALCOHOL	HELIOSULPHUR	0		Yes				
DITHIANON	DELAN	0				Yes		
CYPRODINIL + FLUDIOXONIL	SWICH	0					Yes	
FENHEXAMIDE	TELDOR	0					Yes	
THIRAME	divers	0					Yes	
DODINE	SYLLIT	0						
ZIRAME	divers	0						

Source: adapted from (Cantin, 2015)

Annex 3 - Scheme of the plot

Name	:	B122
Cultivar	:	Chenin blanc
Year of plantation	:	2004
Number of plants	:	800 (960 calculated)
Number of rows	:	16
Number of zones per row	:	12
Number of plants per zone	:	5
Surface	:	0,26 ha
Distance inter-plants	:	1m10
Distance inter-row	:	2 m
Dominant wind	:	South West
	:	



Annex 4 - Security information, composition and dosage of AQ10® WG

AQ 10® WG

Fungicida antiodico a base di *Ampelomyces quisqualis*.

SCHEDA DI SICUREZZA
ETICHETTA

COMPOSIZIONE:
100 grammi di prodotto contengono:
- *Ampelomyces quisqualis* (isolato M-10) .. g 58*
- Coformulanti q.b. a ..... g 100
**Contiene non meno di 5,0 x 10^9spore/g*

Formulazione:
Granuli idrodispersibili in acqua.

Classificazione CLP:
Non classificato

Tempo di carenza:
0 giorni.

Registrazione del Ministero della salute:
n. 11786 del 22.01.2004

Confezioni:
- 30g x 40
- 10g x 20

Conservazione:
- in frigorifero (4-5 °C) per oltre 2 anni
- in luogo fresco e asciutto non alla luce diretta del sole
(20-21 °C) per oltre 1 anno

CAMPI E DOSI DI IMPIEGO

COLTURA	DOSAGGIO	NOTE
VITE DA VINO	35-70 g/ha	Trattamenti dalla ripresa vegetattiva fino alla raccolta. Un trattamento di fine estate o pre-raccolta limita la formazione dei casmoteci. Ripetere eventualmente il trattamento dopo 14 gg dal primo.
UVA DA TAVOLA	50-70 g/ha	È consigliabile la miscela con un coadiuvante quale Nu-Film-P (250-400 ml/ha)
CUCURBITACEE: zucca, zucchino, melone, cocomero, cetriolo **e** **SOLANACEE:** pomodoro, peperone, melanzana	35-70 g/ha	Utilizzare il dosaggio minore all'inizio dell'attacco e quello maggiore con sintomi più evidenti. È consigliabile la miscela con un coadiuvante quale Nu-Film-P (250-400 ml/ha)
FRAGOLA		
ROSA	35-70 g/ha	È consigliabile la miscela con un coadiuvante quale Nu-Film-P (250-400 ml/ha)

Source: Biogard

Annex 5 - Testing sheet for triangular test of Chardonnay

Test Triangulaire de Chardonnay du 23/09/2016

Code du dégustateur : _____

Cochez le produit différent :

_____ _____ _____

Cochez l'intensité de la différence :

0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10

Critères de distinction:

Annex 6 - Testing sheet for triangular test of Cabernet Franc

Test Triangulaire de Cabernet Franc du 23/09/2016

Code du dégustateur : _____

Cochez le produit différent :

_____ _____ _____

Cochez l'intensité de la différence :

0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10

Critères de distinction:

Annex 7 - Testing sheet for descriptive test of Chardonnay

Analyse sensorielle de Jus de Raisin de Chardonnay du 23/09/2016

Echantillon :

Cochez par variable une note (0 à 10) pour le produit :

Arômes:

Citron:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Ananas:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Poire:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Banana:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Coco:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Tomate Cuit:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Note Florale:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Feuilles de géranium:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Champignon/Moisi:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Terre:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Poussière:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Confiture:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Qualité olfactive:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Gout:

Sucré:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Acidité:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Amertume:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Viscosité/gras:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Persistance aromatique:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Qualité gustative:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Commentaires:

Annex 8 - Testing sheet for descriptive test of Cabernet Franc

Analyse sensorielle de Jus de Raisin de Cabernet Franc du 23/09/2016

Echantillon :

Cochez par variable une note (0 à 10) pour le produit :

Arômes:

Fruit rouges:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Tomate Cuit:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Note Florale:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Feuilles de géranium:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Champignon/Moisi:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Terre:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Poussière:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Confiture:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Qualité olfactive:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Gout:

Sucré:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10
Acidité:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>
Amertume:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10
Viscosité/gras:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10
Persistance aromatique:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10
Qualité gustative:
<0 - 1 - 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9 - 10>

Commentaires:

Annex 9 - Descripteurs pour la fiche de dégustation du jus de raisin blanc

Descripteurs pour Olfaction	Définitions
Note Fruitée	Famille aromatique (banana, ananas, poire, coco, citron...) 0 = absence 10 = très forte
Ananas	0 = absence 10 = très forte
Poire	0 = absence 10 = très forte
Banana	0 = absence 10 = très forte
Coco	0 = absence 10 = très forte
Tomate Cuit	0 = absence 10 = très forte
Note Florale	Famille aromatique (Géranium, Iris, Lys, Rose, Violet, Jasmin, fleur d'acacia, aubépine, …) 0 = absence 10 = très forte
Feuilles de géranium	0 = absence 10 = très forte
Champignon/moisi	0 = absence 10 = très forte
Terre	0 = absence 10 = très forte
Poussière	0 = absence 10 = très forte
Confiture	0 = absence 10 = très forte
Qualité olfactive	Appréciation **personnelle** de la qualité des odeurs 0 = très désagréable 10 = très agréable

Descripteurs pour le gout	Définitions
Sucré	Sensation de présence de sucre 0 = pas sucré 10 = très sucré
Acidité	Saveur acide perçue sur la langue 0 = manque d'acidité (mou) 10 = trop acide (agressif)
Amertume	Saveur amère perçue sur la langue après avoir recraché le jus (comme lorsque l'on boit du café non sucré, du Schweppes) 0 = pas amer 10 = très amer
Viscosité/gras	Sensation d'onctuosité, de gras 0 = absence de gras (fluide – eau) 10 = beaucoup de gras (épais – huile)
Persistance aromatique	Prolongement de la perception des **arômes en bouche** 0 = très désagréable 10 = très agréable
Qualité gustative	Appréciation **personnelle** de **l'ensemble des sensations** perçues en bouche 0 = très désagréable 10 = très agréable

Annex 10 - Descripteurs pour la fiche de dégustation du jus de raisin rouge

Descripteurs pour Olfaction	Définitions
Fruits Rouges	Famille aromatique (Framboise, cerise, mûres, fraise, prunes, myrtilles…) 0 = absence 10 = très forte
Tomate Cuit	0 = absence 10 = très forte
Note Florale	Famille aromatique (Géranium, Iris, Lys, Rose, Violet, Jasmin, fleur d'acacia, aubépine, …) 0 = absence 10 = très forte
Feuilles de géranium	0 = absence 10 = très forte
Note Epicée	Famille aromatique (poivre, réglisse, cannelle, muscade, encens, eucalyptus, vanille, ail, menthe, thym, garrigue, lavande, camphre, truffe, gingembre…) 0 = absence 10 = très forte
Champignon/ Moisi	0 = absence 10 = très forte
Terre	0 = absence 10 = très forte
Poussière	0 = absence 10 = très forte
Confiture	0 = absence 10 = très forte
Qualité olfactive	Appréciation **personnelle** de la qualité des odeurs 0 = très désagréable 10 = très agréable

Descripteurs pour le gout	Définitions
Sucré	Sensation de présence de sucre 0 = pas sucré 10 = très sucré
Acidité	Saveur acide perçue sur la langue 0 = manque d'acidité (mou) 10 = trop acide (agressif)
Amertume	Saveur amère perçue sur la langue après avoir recraché le jus (comme lorsque l'on boit du café non sucré, du Schweppes) 0 = pas amer 10 = très amer
Viscosité/gras	Sensation d'onctuosité, de gras 0 = absence de gras (fluide – eau) 10 = beaucoup de gras (épais – huile)
Persistance aromatique	Prolongement de la perception des **arômes en bouche** 0 = très désagréable 10 = très agréable
Qualité gustative	Appréciation **personnelle** de **l'ensemble des sensations** perçues en bouche 0 = très désagréable 10 = très agréable

Annex 11 - Binomial probability table for triangular tests

Number of tasters	Significance level		
	5%	**1%**	**0.1%**
5	4	5	-
6	5	6	-
7	5	6	7
8	6	7	8
9	6	7	8
10	7	8	9
11	7	8	10
12	8	9	10
13	8	9	11
14	9	10	11
15	9	10	12
16	9	11	12
17	10	11	13
18	10	12	13
19	11	12	14
20	11	13	14
21	12	13	15
22	12	14	15
23	12	14	16
24	13	15	16
25	13	15	17
26	14	15	17
27	14	16	18
28	15	16	18
29	15	17	19
30	15	17	19
31	16	18	20
32	16	18	20
33	17	18	21
34	17	19	21
35	17	19	22
36	18	20	22
37	18	20	22
38	19	21	23
39	19	21	23
40	19	21	24
41	20	22	24
42	20	22	25
43	20	23	25
44	21	23	26
45	21	24	26
46	22	24	27
47	22	24	27
48	22	25	27
49	23	25	28
50	23	26	28

Source : adapted from Amerine, Pangborn, & Roessler (1965)

Annex 12 - Observation of phenological stages of purple clover

Date	16/03/2016	06/04/2016	26/04/2016	05/05/2016	20/05/2016	31/0 5/2016	10/06/2016	14/06/2016	27/06/2016	07/07/2016	12/07/2016	25/08/2016
observator	FV, DL	FV	FV	FV	FV	FV	FV	FV	FV	FV	FV	FV
Spreading rate	10%	15%	18%	50%	80%	80%	80%	80%	80%	80%	80%	
Height of the plants	<8cm	<8cm	15cm	20cm	>30cm	>30cm	>30cm	>30cm	>30cm	>30cm	>30cm	
Presence of buds	No	No	No	yes	yes	yes						
Presence of flowers	No	No	No	no	yes	yes	yes	yes	yes			
Phenological stage	Early vegetative	Vegetative	Vegetative	Buds	early flower	early flower	flower	flower	flower	flower	flower	
Other flora	No	Yes	yes	yes	yes	yes	yes	yes	yes			
Inventarium		dandelion (*Taraxacum officinale*); fescue *(Festuca arundinacea*); Geranium (*Geranium pratens*); Vesce (*Vicia villosa*); grass plot (*Grameaceae*); white clover										
Natural presence of Powdey Mildew	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	
Observations					PM inoculation		symptoms of PM	AQ10	AQ10			burned by high temperature conditions

Annex 13 - Meteorological data from Montreuil-Bellay in 2016

Date	Mean T (°C)	T min (°C)	T max (°C)	Rain (mm)	Mean RH (%)	RH min (%)	RH max (%)
01/07/2016	18,6	16,1	22	0,5	81	70	95
02/07/2016	16,8	12,8	22,6	0	64	39	93
03/07/2016	15,9	10,5	19,8	0,5	83	69	95
04/07/2016	16,9	13,5	20,1	0	87	72	99
05/07/2016	17,4	13,6	22,2	0	84	63	100
06/07/2016	19,6	13,2	26,2	0	63	42	88
07/07/2016	21,7	11,1	29,4	0	59	33	94
08/07/2016	22,2	15,3	28,7	0	62	37	93
09/07/2016	21,7	14,4	28,7	0	73	49	98
10/07/2016	21,3	14,6	27,9	0	71	43	98
11/07/2016	18,5	15,1	23,5	0	73	52	95
12/07/2016	16,8	12,6	22,6	7,5	75	47	95
13/07/2016	15,9	11,3	20,4	0	70	47	97
14/07/2016	15,6	10,7	21,4	0	68	42	88
15/07/2016	17,4	9,5	23,9	0	64	36	97
16/07/2016	21,3	12,4	28,2	0	61	42	89
17/07/2016	24,3	15,6	31,2	0	63	40	94
18/07/2016	27,2	18,1	35,6	0	53	24	86
19/07/2016	28,6	17,7	38,3	0	45	14	86
20/07/2016	22	16,6	29	3	75	50	92
21/07/2016	20,8	18	24,3	0	71	58	84
22/07/2016	21,7	14,9	27,1	0	63	42	92
23/07/2016	21,6	14,5	27,6	0	63	38	92
24/07/2016	22,7	14,5	29,2	0	63	39	94
25/07/2016	21,5	15,2	28,4	0	73	49	97
26/07/2016	20,1	14,9	26	0	64	45	84
27/07/2016	19,9	13,1	25,9	0	71	48	92
28/07/2016	21,1	17,6	25,6	0	71	52	91
29/07/2016	20,5	17,7	24,4	0	76	64	87
30/07/2016	19,2	13,5	24,8	0	68	46	95
31/07/2016	20,2	14,5	26	0	62	37	91

Source : Meteorological station of Montreuil-Bellay

Annex 14 - Meteorological data from Montreuil-Bellay in 2016 (continuation)

Date	Mean T (°C)	T min (°C)	T max (°C)	Rain (mm)	Mean RH (%)	RH min (%)	RH max (%)
01/08/2016	19,9	11,6	26,2	0	59	38	88
02/08/2016	20,6	15,8	26,3	0	68	47	86
03/08/2016	21,8	15,6	27,4	0	67	44	88
04/08/2016	17,2	15,5	21,1	5	82	57	94
05/08/2016	17,9	11,1	24,8	0	70	41	98
06/08/2016	19,4	11,4	25,1	0	68	49	94
07/08/2016	22,6	13,3	29,7	0	60	31	93
08/08/2016	20,3	16,9	24,6	0	65	48	86
09/08/2016	17,7	12,8	23,8	0	59	34	89
10/08/2016	15,2	9,6	20,7	0	66	39	92
11/08/2016	17,6	8,7	25,1	0	67	45	93
12/08/2016	19,9	11,5	28,6	0	56	22	94
13/08/2016	21,7	11,6	30,6	0	52	25	87
14/08/2016	23,5	14,3	31,9	0	53	32	83
15/08/2016	23,9	17,1	31,3	0	53	32	79
16/08/2016	24,3	17,1	32,3	0	54	29	80
17/08/2016	23,3	15,5	31,4	0	62	38	83
18/08/2016	20,1	17,4	24,5	0,5	66	46	93
19/08/2016	16,4	11,9	20,1	1	85	65	98
20/08/2016	18,6	13,7	24,8	0	69	33	99
21/08/2016	18,7	13,1	24,7	0	62	35	91
22/08/2016	21,2	11,3	29,5	0	57	30	93
23/08/2016	26,5	15,7	36	0	46	20	79
24/08/2016	28	15,8	36,9	0	47	21	83
25/08/2016	27,7	18,3	35,5	0	57	28	88
26/08/2016	26,9	17,7	34,6	0	55	24	85
27/08/2016	26,9	17,1	34,8	0	61	30	94
28/08/2016	21,8	17,1	27,4	0	66	38	91
29/08/2016	20,5	17,8	24,3	0	69	51	84
30/08/2016	21,2	13,6	27,5	0	65	38	95
31/08/2016							
01/09/2016	22,5	14,3	29,8	0	57	32	87
02/09/2016	22,9	14,2	30,7	0	57	29	90
03/09/2016	22,8	14,6	30,6	0	58	31	91
04/09/2016	21,5	16,6	25,5	0	75	63	91
05/09/2016	21,8	17,1	25,4	0	78	65	95
06/09/2016	23	17,9		0	71	48	93
07/09/2016	24,1	16,5	31,1	0	57	27	95
08/09/2016	21,1	14,9	25,7	0	57	31	85

Source : Meteorological station of Montreuil-Bellay

Annex 15 - Observation of Powdery mildew's disease on leaves of grapevines at the plot

			Leaves													
			Frequence							Intensity						
Row	Zone	Vine	27/07/2016	02/08/2016	09/08/2016	17/08/2016	28/08/2016	01/09/2016	08/09/2016	27/07/2016	02/08/2016	09/08/2016	17/08/2016	25/08/2016	01/09/2016	08/09/2016
14	P6	30	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		29	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		28														
		27	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		26	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
12	P7	35	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		34	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		33	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		32	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		31	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
10	P2	35	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		34	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		33	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		32	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		31	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
10	P7	10	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		9	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		8	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		7	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		6	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
9	P10	50	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		49	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		48	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		47	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		46	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
9	P6	30	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		29	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		28	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		27	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		26	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
7	P7	35	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		34	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		33	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		32	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		31	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
5	P6	30	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		29	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		28	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		27	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0
		26	0	0	0	0	0	0	0	0%	0%	0%	0%	0%	0%	0

Annex 16 - Observation of frequency of attack by Powdery mildew's disease on clusters of grapevines at the plot

			Clusters																				
			Frequency																				
			27/07/2016			02/08/2016			09/08/2016			17/08/2016			25/08/2016			01/09/2016			08/09/2016		
Row	Zone	Vine	NB bunches	Bunches Infected	%	NB bunches	Bunches Infected	%	NB bunches	Bunches Infected	%	NB bunches	Bunches Infected	%	NB bunches	Bunches Infected	%	NB bunches	Bunches Infected	%	NB bunches	Bunches Infected	%
14	P6	30	3	0	0%	3	0	0%	6	0	0%	6	0	0%	6	0	0%	6	0	0%	6	0	0%
		29	15	0	0%	17	0	0%	17	0	0%	17	0	0%	17	0	0%	17	0	0%	17	2	12%
		28																					
		27	10	0	0%	14	0	0%	14	0	0%	14	0	0%	14	0	0%	14	0	0%	14	0	0%
		26	2	0	0%	4	0	0%	4	0	0%	4	0	0%	4	0	0%	4	0	0%	4	0	0%
12	P7	35	8	0	0%	9	0	0%	9	0	0%	9	0	0%	9	0	0%	9	0	0%	9	0	0%
		34	17	0	0%	17	0	0%	17	0	0%	17	0	0%	17	6	35%	17	8	47%	17	8	47%
		33	15	0	0%	17	0	0%	17	0	0%	17	4	24%	17	4	24%	17	4	24%	17	4	24%
		32	19	0	0%	19	0	0%	19	0	0%	19	0	0%	19	1	5%	19	1	5%	19	2	11%
		31	10	0	0%	12	0	0%	12	0	0%	12	0	0%	12	0	0%	12	1	8%	12	1	8%
10	P2	35	4	0	0%	4	0	0%	4	0	0%	4	0	0%	4	0	0%	4	0	0%	4	0	0%
		34	3	0	0%	5	0	0%	5	0	0%	5	0	0%	5	0	0%	5	0	0%	5	0	0%
		33	15	0	0%	15	0	0%	15	0	0%	15	0	0%	15	0	0%	15	0	0%	15	0	0%
		32	2	0	0%	5	0	0%	5	0	0%	5	0	0%	5	0	0%	5	0	0%	5	0	0%
		31	6	0	0%	9	0	0%	9	0	0%	9	0	0%	9	0	0%	9	0	0%	9	0	0%
10	P7	10	11	0	0%	11	0	0%	11	0	0%	11	0	0%	11	0	0%	11	0	0%	11	0	0%
		9	10	0	0%	10	0	0%	10	0	0%	10	0	0%	10	0	0%	10	2	20%	10	5	50%
		8	14	0	0%	14	0	0%	14	0	0%	14	0	0%	14	0	0%	14	1	7%	14	1	7%
		7	14	0	0%	14	0	0%	14	0	0%	14	0	0%	14	0	0%	14	0	0%	14	2	14%
		6	10	0	0%	10	0	0%	10	0	0%	10	0	0%	10	0	0%	10	0	0%	10	1	10%
9	P10	50	15	0	0%	16	0	0%	16	0	0%	16	0	0%	16	0	0%	16	0	0%	16	3	19%
		49	4	0	0%	4	0	0%	4	0	0%	4	0	0%	4	0	0%	4	0	0%	4	0	0%
		48	10	0	0%	13	0	0%	13	0	0%	13	0	0%	13	0	0%	13	0	0%	13	1	8%
		47	11	0	0%	10	0	0%	10	0	0%	10	1	10%	10	1	10%	10	1	10%	10	1	10%
		46	11	0	0%	17	0	0%	17	0	0%	17	0	0%	17	0	0%	17	0	0%	17	0	0%
9	P6	30	10	0	0%	10	0	0%	10	0	0%	10	0	0%	10	1	10%	10	1	10%	10	2	20%
		29	11	0	0%	11	0	0%	11	0	0%	11	0	0%	11	1	9%	11	1	9%	11	2	18%
		28	9	0	0%	10	1	10%	10	1	10%	10	1	10%	10	5	50%	10	4	40%	10	8	80%
		27	2	0	0%	3	1	33%	3	1	33%	5	1	20%	5	1	20%	5	1	20%	5	0	0%
		26	5	0	0%	6	0	0%	6	0	0%	6	0	0%	6	0	0%	6	0	0%	6	0	0%
7	P7	35	5	0	0%	11	0	0%	11	0	0%	11	0	0%	11	0	0%	11	0	0%	11	0	0%
		34	6	0	0%	12	0	0%	12	0	0%	12	0	0%	12	0	0%	12	0	0%	12	0	0%
		33	16	0	0%	17	0	0%	17	0	0%	17	0	0%	17	1	6%	17	1	6%	17	3	18%
		32	5	0	0%	5	0	0%	5	2	40%	5	2	40%	5	2	40%	5	2	40%	5	1	20%
		31	11	0	0%	11	0	0%	11	0	0%	11	0	0%	11	0	0%	11	0	0%	11	0	0%
5	P6	30	15	0	0%	17	0	0%	17	0	0%	17	0	0%	17	0	0%	17	0	0%	17	0	0%
		29	9	0	0%	9	0	0%	9	0	0%	9	0	0%	9	0	0%	9	0	0%	9	0	0%
		28	10	0	0%	10	0	0%	10	0	0%	10	0	0%	10	0	0%	10	0	0%	10	1	10%
		27	2	0	0%	2	0	0%	2	0	0%	5	0	0%	5	0	0%	5	0	0%	5	1	20%
		26	6	0	0%	8	0	0%	8	0	0%	8	0	0%	8	0	0%	8	0	0%	8	0	0%

Annex 17- Observation of intensity of attack by powdery mildew's disease on clusters at the plot

			Clusters						
			Intensity						
			27/07/2016	02/08/2016	09/08/2016	17/08/2016	25/08/2016	01/09/2016	08/09/2016
			%	%	%	%	%	%	%
Row	Zone	Vine							
14	P6	30	0%	0%	0%	0%	0%	0%	0%
		29	0%	0%	0%	0%	0%	0%	51%
		28							
		27	0%	0%	0%	0%	0%	0%	0%
		26	0%	0%	0%	0%	0%	0%	0%
12	P7	35	0%	0%	0%	0%	0%	0%	0%
		34	0%	0%	0%	0%	5%	10%	25%
		33	0%	0%	0%	25%	25%	30%	30%
		32	0%	0%	0%	0%	10%	10%	30%
		31	0%	0%	0%	0%	0%	1%	1%
10	P2	35	0%	0%	0%	0%	0%	0%	0%
		34	0%	0%	0%	0%	0%	0%	0%
		33	0%	0%	0%	0%	0%	0%	0%
		32	0%	0%	0%	0%	0%	0%	0%
		31	0%	0%	0%	0%	0%	0%	0%
10	P7	10	0%	0%	0%	0%	0%	0%	0%
		9	0%	0%	0%	0%	0%	2%	5%
		8	0%	0%	0%	0%	0%	1%	5%
		7	0%	0%	0%	0%	0%	0%	1%
		6	0%	0%	0%	0%	0%	0%	1%
9	P10	50	0%	0%	0%	0%	0%	0%	1%
		49	0%	0%	0%	0%	0%	0%	0%
		48	0%	0%	0%	0%	0%	0%	1%
		47	0%	0%	0%	1%	1%	1%	1%
		46	0%	0%	0%	0%	0%	0%	0%
9	P6	30	0%	0%	0%	0%	1%	1%	5%
		29	0%	0%	0%	0%	1%	1%	1%
		28	0%	1%	1%	1%	25%	35%	30%
		27	0%	1%	1%	1%	2%	2%	0%
		26	0%	0%	0%	0%	0%	0%	0%
7	P7	35	0%	0%	0%	0%	0%	0%	0%
		34	0%	0%	0%	0%	0%	0%	0%
		33	0%	0%	0%	0%	1%	1%	10%
		32	0%	0%	1%	1%	1%	1%	11%
		31	0%	0%	0%	0%	0%	0%	0%
5	P6	30	0%	0%	0%	0%	0%	0%	0%
		29	0%	0%	0%	0%	0%	0%	0%
		28	0%	0%	0%	0%	0%	0%	2%
		27	0%	0%	0%	0%	0%	0%	1%
		26	0%	0%	0%	0%	0%	0%	0%

Annex 18 – Results of chemical analysis of 250 healthy grapes of Chardonnay

LABORATOIRE OENOLOGIQUE U.A.P.L
68, Rue Louis Moron 49320 BRISSAC QUINCE
Tél: 02.41.91.23.15 Fax : 02.41.91.20.38
Courriel : labo@uapl.fr

IFV
VAL DE LOIRE
42 RUE GEORGES MOREL BP60057
49 071 BEAUCOUZE CEDEX

N° Adhérent : **454 400**
Echantillon reçu le : **21/09/2016**
Echantillon analysé le : **21/09/2016**
N° Identification : **1 602 062**
Caractéristiques : **FV CHS CHARDONNAY SAIN**

Rapport d'essais N° **1 512**

Désignation de l'analyses	Résultats et Unit	
Acidité totale	3,82	g/1H2SO4
pH	3,30	
Azote assimilable	175	mg /1
Sucres réducteurs par réfracto	203,3	g/1
Alcool probable par réfracto	12,08	% vol

Commentaires :

Edité à Brissac-Quincé, Le 21/09/2016

M.BAHUAU I.MARILLAUD S.MEINEN C.PIERRE

Annex 19 – Results of chemical analysis of 250 infected grapes of Chardonnay

LABORATOIRE OENOLOGIQUE U.A.P.L
68, Rue Louis Moron 49320 BRISSAC QUINCE
Tél: 02.41.91.23.15 Fax : 02.41.91.20.38
Courriel : labo@uapl.fr

IFV
VAL DE LOIRE
42 RUE GEORGES MOREL BP60057
49 071 BEAUCOUZE CEDEX

N° Adhérent	:	**454 400**
Echantillon reçu le	:	**21/09/2016**
Echantillon analysé le	:	**21/09/2016**
N° Identification	:	**1 602 063**
Caractéristiques	:	**FV CHO CHARDONNAY OIDEE**

Rapport d'essais N° **1 513**

Désignation de l'analyses	Résultats et Unit	
Acidité totale	5,19	g/1H2SO4
pH	3,43	
Azote assimilable	248	mg / l
Sucres réducteurs par réfracto	231,1	g/l
Alcool probable par réfracto	13,73	% vol

Commentaires :

Edité à Brissac-Quincé, Le 21/09/2016

M.BAHUAU I.MARILLAUD S.MEINEN C.PIERRE

Annex 20 – Results of chemical analysis of 250 healthy berries of Cabernet Franc

LABORATOIRE OENOLOGIQUE U.A.P.L
68, Rue Louis Moron 49320 BRISSAC QUINCE
Tél: 02.41.91.23.15 Fax : 02.41.91.20.38
Courriel : labo@uapl.fr

IFV
VAL DE LOIRE
42 RUE GEORGES MOREL BP60057
49 071 BEAUCOUZE CEDEX

N° Adhérent : **454 400**
Echantillon reçu le : **22/09/2016**
Echantillon analysé le : **22/09/2016**
N° Identification : **1 602 120**
Caractéristiques : **CABERNET FRANC SAIM 22/09/16**

Rapport d'essais N° **1 534**

Désignation de Ianalyses	Résultats et Unit	
Acidité totale	6,86	g/1H2SO4
pH	3,18	
Azote assimilable	104	mg / 1
Sucres réducteurs par réfracto	167,0	g/1
Alcool probable par réfracto	9,92	% vol
Anthocyanes	741	mg/kg
Indice Polyphénols Totaux	36,4	

Commentaires :

Edité à Brissac-Quincé, Le 22/09/2016

M.BAHUAU I.MARILLAUD S.MEINEN C.PIERRE

Annex 21 - Results of chemical analysis of 250 infected berries of Cabernet Franc

68, Rue Louis Moron **49320 BRISSAC QUINCE**
Tél: 02.41.91.23.15 Fax : 02.41.91.20.38
Courriel : labo@uapl.fr

IFV
VAL DE LOIRE
42 RUE GEORGES MOREL BP60057
49 071 BEAUCOUZE CEDEX

N° Adhérent	:	**454 400**
Echantillon reçu le	:	**22/09/2016**
Echantillon analysé le	:	**22/09/2016**
N° Identification	:	**1 602 119**
Caractéristiques	:	**CABERNET FRANC OIDEE 22/09/16**

Rapport d'essais N° **1 533**

Désignation de Ianalyses	Résultats et Unit	
Anthocyanes	649	mg/kg
Indice Polyphénols Totaux	51,1	

Commentaires :

Edité à Brissac-Quincé, Le 22/09/2016

M.BAHUAU I.MARILLAUD S.MEINEN C.PIERRE

Annex 22- Raw data of weight and yield of clusters from Chardonnay (0% and 5%)

Variety	*Infection level*	*Weight of a cluster (g)*	*Number of berries*	*Weight of stem (g)*	*Weight of berries (g)*	*Weight of a berry (g)*
CH	0	213,6	150	6,4	207,0	1,38
CH	0	293,5	265	12,7	280,8	1,06
CH	0	254,1	187	9,4	244,7	1,31
CH	0	253,6	199	9,8	243,8	1,23
CH	0	197,0	195	7,0	190,0	0,97
CH	0	186,3	133	6,6	179,7	1,35
CH	0	138,2	195	4,6	133,6	0,69
CH	0	72,4	69	2,6	69,8	1,01
CH	0	108,4	89	3,3	105,1	1,18
CH	0	126,2	84	9,9	116,3	1,38
CH	0	75,1	72	2,7	72,4	1,01
CH	0	169,9	152	5,5	164,4	1,08
CH	0	184,4	147	6,8	177,6	1,21
CH	0	201,2	132	7,4	193,8	1,47
CH	0	146,5	103	4,8	141,7	1,38
CH	0	273,5	126	10,6	262,9	2,09
CH	0	93,6	91	3,7	89,9	0,99
CH	0	146,5	139	5,7	140,8	1,01
CH	0	275,2	217	11,8	263,4	1,21
CH	0	125,7	103	4,1	121,6	1,18
CH	0	299,5	206	10,8	288,7	1,40
CH	0	126,6	109	4,8	121,8	1,12
CH	0	106,4	78	3,2	103,2	1,32
CH	0	236,7	163	8,4	228,3	1,40
CH	0	122,6	100	5,2	117,4	1,17
CH	5	113,2	100	5,5	107,7	1,1
CH	5	129,1	187	10,3	118,8	0,6
CH	5	220,5	196	9,4	211,1	1,1
CH	5	213,0	195	9,8	203,2	1,0
CH	5	162,4	195	7,0	155,4	0,8
CH	5	75,0	60	6,6	68,4	1,1
CH	5	147,3	180	4,6	142,7	0,8
CH	5	132,6	130	2,6	130,0	1,0
CH	5	122,6	135	3,3	119,3	0,9
CH	5	207,0	203	10,0	197,0	1,0
CH	5	205,2	187	2,7	202,5	1,1
CH	5	236,6	152	5,5	231,1	1,5
CH	5	100,1	79	6,8	93,3	1,2
CH	5	200,0	123	7,4	192,6	1,6

Annex 23 - Raw data of weight and yield of clusters from Chardonnay (10%, 25%)

Variety	*Infection level*	*Weight of a cluster (g)*	*Number of berries*	*Weight of stem (g)*	*Weight of berries (g)*	*Weight of a berry (g)*
CH	10	125,2	100	6,4	118,8	1,19
CH	10	255,0	125	10,2	244,8	1,96
CH	10	155,0	165	9,1	145,9	0,88
CH	10	166,0	159	10,0	156,0	0,98
CH	10	146,0	126	7,5	138,5	1,10
CH	10	128,3	103	6,6	121,7	1,18
CH	10	235,6	206	10,0	225,6	1,10
CH	10	124,6	110	2,6	122,0	1,11
CH	10	155,0	145	4,0	151,0	1,04
CH	10	201,3	178	10,0	191,3	1,07
CH	10	125,8	99	6,0	119,8	1,21
CH	10	150	152	5,5	144,5	0,95
CH	10	75	100	4,0	71,0	0,71
CH	10	155	198	7,4	147,6	0,75
CH	25	113,5	160	10,0	103,5	1,3
CH	25	196,5	255	12,5	184,0	1,0
CH	25	154,0	185	10,0	144,0	1,1
CH	25	85,0	200	9,8	75,2	1,1
CH	25	186,0	190	7,0	179,0	1,2
CH	25	186,3	123	5,6	180,7	1,0
CH	25	136,0	120	4,6	131,4	1,0
CH	25	73,6	69	2,0	71,6	1,0
CH	25	99,0	89	3,3	95,7	1,1
CH	25	120,0	94	9,6	110,4	1,1
CH	25	78,0	60	3,5	74,5	1,2
CH	25	169,9	145	9,8	160,1	1,2
CH	25	166,9	135	11,6	155,3	1,0
CH	25	202,3	132	7,4	194,9	1,0
CH	25	145,6	111	4,8	140,8	0,9

Annex 24 - Raw data of weight and yield of clusters from Chardonnay (50% and 100%)

Variety	*Infection level*	*Weight of a cluster (g)*	*Number of berries*	*Weight of stem (g)*	*Weight of berries (g)*	*Weight of a berry (g)*
CH	50	146,5	104	12,9	133,6	0,6
CH	50	93,6	87	10,0	83,6	0,7
CH	50	98,9	85	5,5	93,4	0,8
CH	50	153,1	135	9,8	143,3	0,4
CH	50	165,1	133	7,0	158,1	0,9
CH	50	145,0	136	8,0	137,0	1,5
CH	50	138,2	129	9,9	128,3	1,1
CH	50	65,0	64	3,2	61,8	1,0
CH	50	100,0	88	4,5	95,5	1,1
CH	50	123,0	99	10,0	113,0	1,2
CH	50	74,1	56	6,5	67,6	1,2
CH	50	106,3	85	5,5	100,8	1,1
CH	50	105,9	95	6,8	99,1	1,2
CH	50	105,0	100	7,4	97,6	1,5
CH	50	100,0	95	10,0	90,0	1,3
CH	100	112,6	141	5,7	106,9	0,8
CH	100	121,0	132	4,8	116,2	0,9
CH	100	121,0	131	4,9	116,1	0,9
CH	100	48,8	64	2,3	46,5	0,7
CH	100	56,0	100	5,6	50,4	0,5
CH	100	51,6	64	2,0	49,6	0,8
CH	100	43,2	84	3,3	39,9	0,5
CH	100	59,0	62	1,9	57,1	0,9
CH	100	52,5	80	2,2	50,3	0,6
CH	100	55,0	128	5,6	49,4	0,4
CH	100	14,7	58	1,6	13,1	0,2
CH	100	85,1	89	2,4	82,7	0,9
CH	100	37,0	49	2,1	34,9	0,7
CH	100	182,0	170	6,6	175,4	1,0
CH	100	96,4	116	5,6	90,8	0,8
CH	100	106,3	104	3,4	102,9	1,0
CH	100	52,5	73	2,1	50,4	0,7
CH	100	84,2	107	3,4	80,8	0,8
CH	100	99,0	110	4,3	94,7	0,9
CH	100	25,1	41	0,8	24,3	0,6
CH	100	22,9	42	1,2	21,7	0,5
CH	100	50,0	58	1,8	48,2	0,8
CH	100	74,2	77	3,1	71,1	0,9
CH	100	110,3	117	3,1	107,2	0,9
CH	100	144,8	137	4,1	140,7	1,0

Annex 25 - Raw data of weight and yield of clusters from Cabernet Franc (0% and 5%)

Variety	Level of infection	Weight of a cluster (g)	Number of berries	Weight of stem (g)	Weight of berries (g)	weight of a berry (g)
CF	0	144,4	143	5,8	138,6	1,0
CF	0	116,6	124	5,1	111,5	0,9
CF	0	120,2	130	4,5	115,7	0,9
CF	0	164,1	63	7,6	156,5	2,5
CF	0	146,4	143	6,4	140,0	1,0
CF	0	133,9	137	7,8	126,1	0,9
CF	0	141,9	133	9,4	132,5	1,0
CF	0	205,0	167	8,2	196,8	1,2
CF	0	208,5	169	10,4	198,1	1,2
CF	0	99,0	86	4,3	94,7	1,1
CF	0	98,8	113	4,3	94,5	0,8
CF	0	132,7	109	9,0	123,7	1,1
CF	0	104,6	108	4,5	100,1	0,9
CF	0	126,2	127	4,6	121,6	1,0
CF	0	110,8	106	4,9	105,9	1,0
CF	0	120,3	120	5,5	114,8	1,0
CF	0	82,2	92	3,9	78,3	0,9
CF	0	163,7	143	8,3	155,4	1,1
CF	0	121,0	104	4,4	116,6	1,1
CF	0	124,4	106	4,6	119,8	1,1
CF	0	103,0	75	4,6	98,4	1,3
CF	0	163,3	136	7,6	155,7	1,1
CF	0	60,0	61	3,0	57,0	0,9
CF	0	66,8	78	3,0	63,8	0,8
CF	0	67,1	73	2,4	64,7	0,9
CF	5	158,2	150,2	12,9	145,3	1,0
CF	5	77,4	80	10,2	67,2	0,8
CF	5	123,1	100	9,5	113,6	1,1
CF	5	114,0	112	7,6	106,4	1,0
CF	5	63,5	59	3,5	60,0	1,0
CF	5	70,2	65	4,2	66,0	1,0
CF	5	50,9	49	3,7	47,2	1,0
CF	5	105,0	109	7,9	97,1	0,9
CF	5	95,0	85	9,9	85,1	1,0
CF	5	70,9	69	2,9	68,0	1,0
CF	5	99,5	100	12,5	87,0	0,9
CF	5	109,8	116	11,3	98,5	0,8

Annex 26 -Raw data of weight and yield of clusters from Cabernet Franc (10%, 25% and 50%)

Variety	Level of infection	Weight of a cluster (g)	Number of berries	Weight of stem (g)	Weight of berries (g)	weight of a berry (g)
CF	10	103,7	123	5,5	98,2	0,8
CF	10	104,0	103	5,6	98,4	1,0
CF	10	100,0	98	12,3	87,7	0,9
CF	10	99,6	123	11,6	88,0	0,7
CF	10	75,0	69	4,5	70,5	1,0
CF	10	126,2	130	12,0	114,2	0,9
CF	10	119,2	100	11,0	108,2	1,1
CF	10	69,0	56	4,9	64,1	1,1
CF	10	74,2	60	4,0	70,2	1,2
CF	10	100,1	100	11,0	89,1	0,9
CF	10	87,6	86	10,9	76,7	0,9
CF	10	68,3	69	4,6	63,7	0,9
CF	25	125,2	130	7,2	118,0	0,9
CF	25	146,1	145	9,0	137,1	0,9
CF	25	124,0	134	7,2	116,8	0,9
CF	25	103,2	110	10,5	92,7	0,8
CF	25	56,0	55	3,2	52,8	1,0
CF	25	72,0	60	3,5	68,5	1,1
CF	25	50,2	45	4,3	45,9	1,0
CF	25	60,0	70	10,0	50,0	0,7
CF	25	126,0	127	9,0	117,0	0,9
CF	25	74,6	74	3,1	71,5	1,0
CF	25	59,2	59	3,8	55,4	0,9
CF	25	88,3	90	6,0	82,3	0,9
CF	50	75,6	69	4,5	71,1	1,0
CF	50	96,5	102	8,9	87,6	0,9
CF	50	113,0	110	10,6	102,4	0,9
CF	50	128,7	123	6,9	121,8	1,0
CF	50	70,2	70	5,2	65,0	0,9
CF	50	54,3	63	13,0	41,3	0,7
CF	50	72,6	69	5,2	67,4	1,0
CF	50	69,8	59	7,9	61,9	1,0
CF	50	56,3	98	1,0	55,3	0,6
CF	50	109,8	125	11,0	98,8	0,8
CF	50	105,6	115	11,5	94,1	0,8
CF	50	55,4	60	4,3	51,1	0,9

Annex 27 - Raw data of weight and yield of clusters from Cabernet Franc (100%)

Variety	Level of infection	Weight of a cluster (g)	Number of berries	Weight of stem (g)	Weight of berries (g)	weight of a berry (g)
CF	100	90,0	220	6,0	84,0	0,4
CF	100	82,6	191	4,9	77,7	0,4
CF	100	27,8	97	3,1	24,7	0,3
CF	100	13,5	68	1,8	11,7	0,2
CF	100	19,4	106	1,8	17,6	0,2
CF	100	12,3	67	2,4	9,9	0,1
CF	100	14,7	74	2,4	12,3	0,2
CF	100	12,5	55	1,1	11,4	0,2
CF	100	24,7	91	2,1	22,6	0,2
CF	100	15,8	41	1,5	14,3	0,3
CF	100	17,3	88	2,7	14,6	0,2
CF	100	12,5	55	1,1	30,0	0,4
CF	100	24,7	91	2,1	19,9	0,2
CF	100	15,8	41	1,5	25,5	0,4
CF	100	17,3	88	2,7	57,7	0,5
CF	100	31,7	77	1,7	34,9	0,5
CF	100	22,2	90	2,3	20,3	0,3
CF	100	27,0	68	1,5	22,7	0,5
CF	100	62,0	123	4,3	10,3	0,2
CF	100	37,9	70	3,0	26,5	0,3
CF	100	22,0	81	1,7	20,3	0,3
CF	100	24,1	49	1,4	22,7	0,5
CF	100	12,3	64	2,0	10,3	0,2
CF	100	29,8	100	3,3	26,5	0,3
CF	100	46,4	104	2,8	43,6	0,4

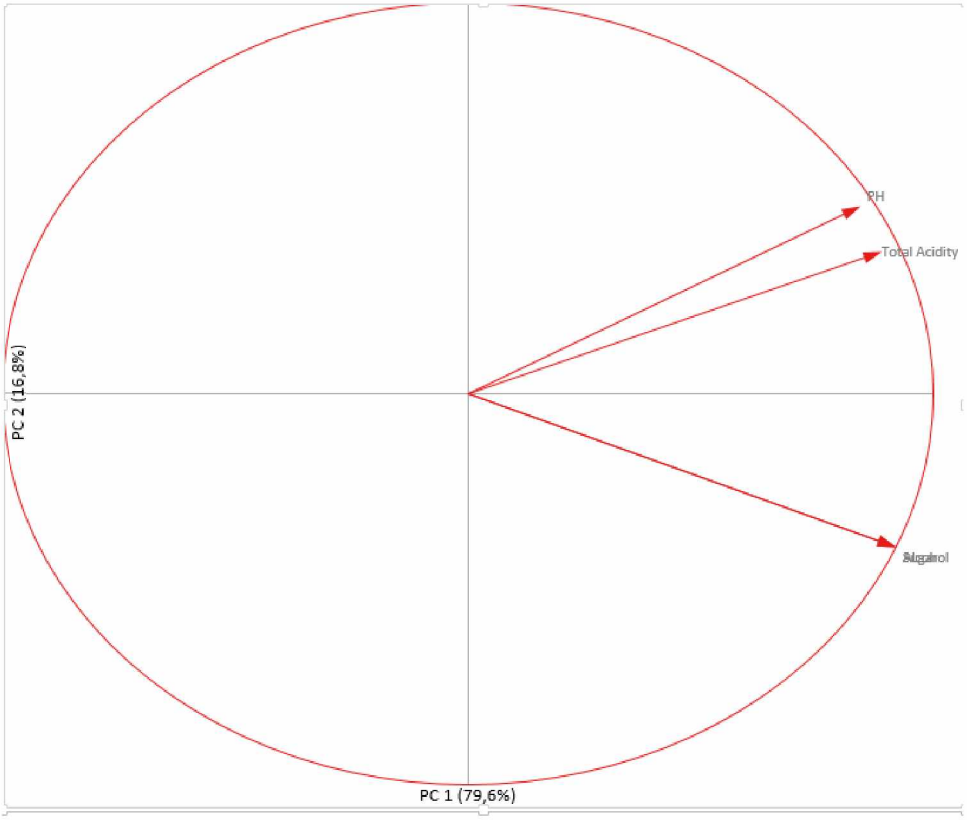
Annex 28 – Results of chemical analysis of musts of Chardonnay

Level of infection	*Sugar (g/l)*	*P. Alcohol (% vol.)*	*Total Acidity (g H_2SO_4/l)*	*PH*
0%	207.0	12.30	3.90	3.34
5%	208.7	12.40	3.90	3.34
10%	207.0	12.30	3.80	3.34
25%	207.0	12.30	3.80	3.35
50%	208.7	12.40	3.90	3.37
100%	208.7	12.40	4.10	3.41

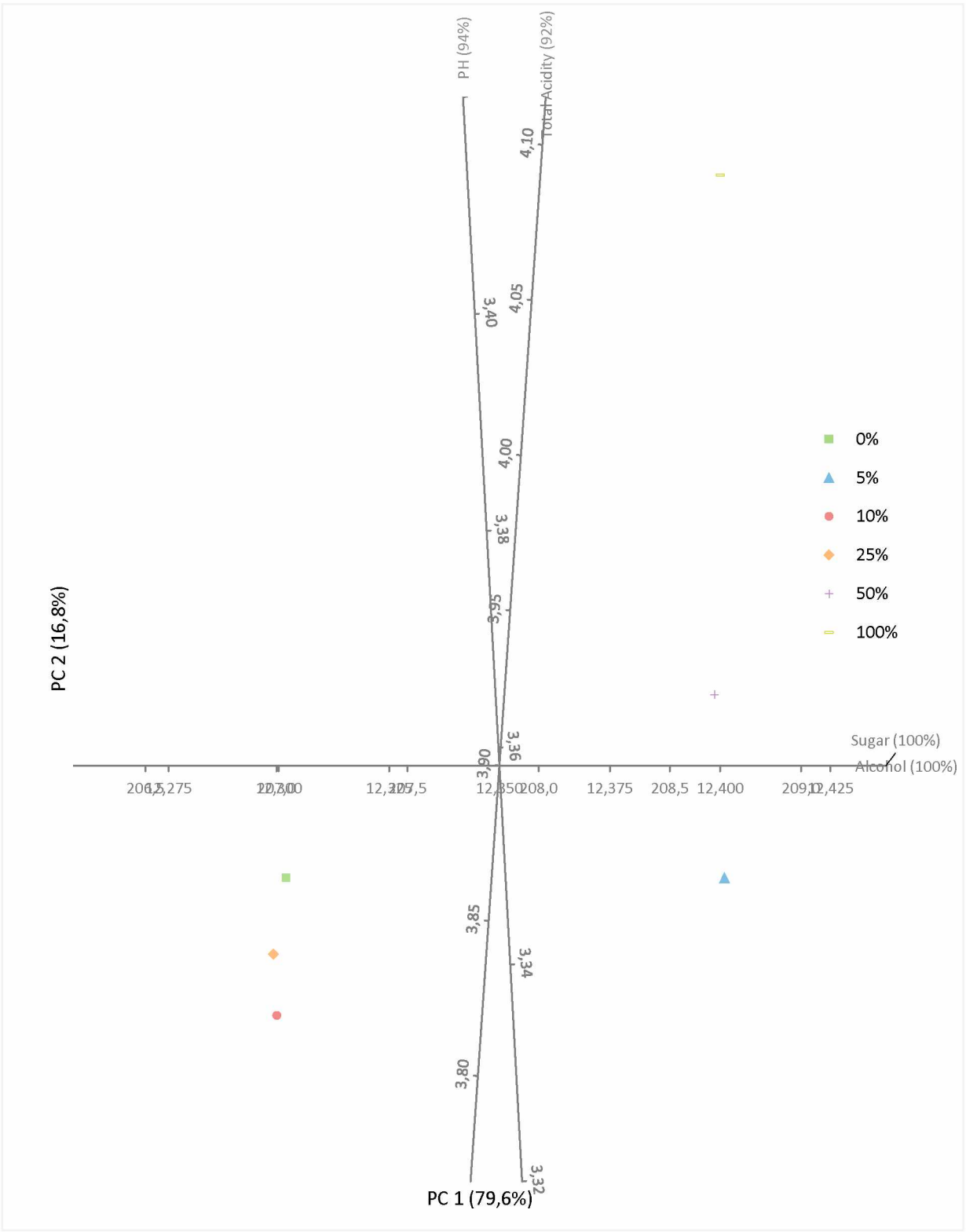
Annex 29 - Results of chemical analysis of musts of Cabernet Franc

Level of infection	*Sugar (g/l)*	*P. Alcohol (% vol.)*	*Total Acidity (g H_2SO_4/l)*	*PH*
0%	166.6	9.9	6.0	2.91
5%	168.3	10.0	6.0	2.94
10%	170.0	10.1	6.1	2.92
25%	171.7	10.2	6.0	2.95
50%	153.2	9.1	6.2	2.99
100%	188.5	11.2	6.9	3.06

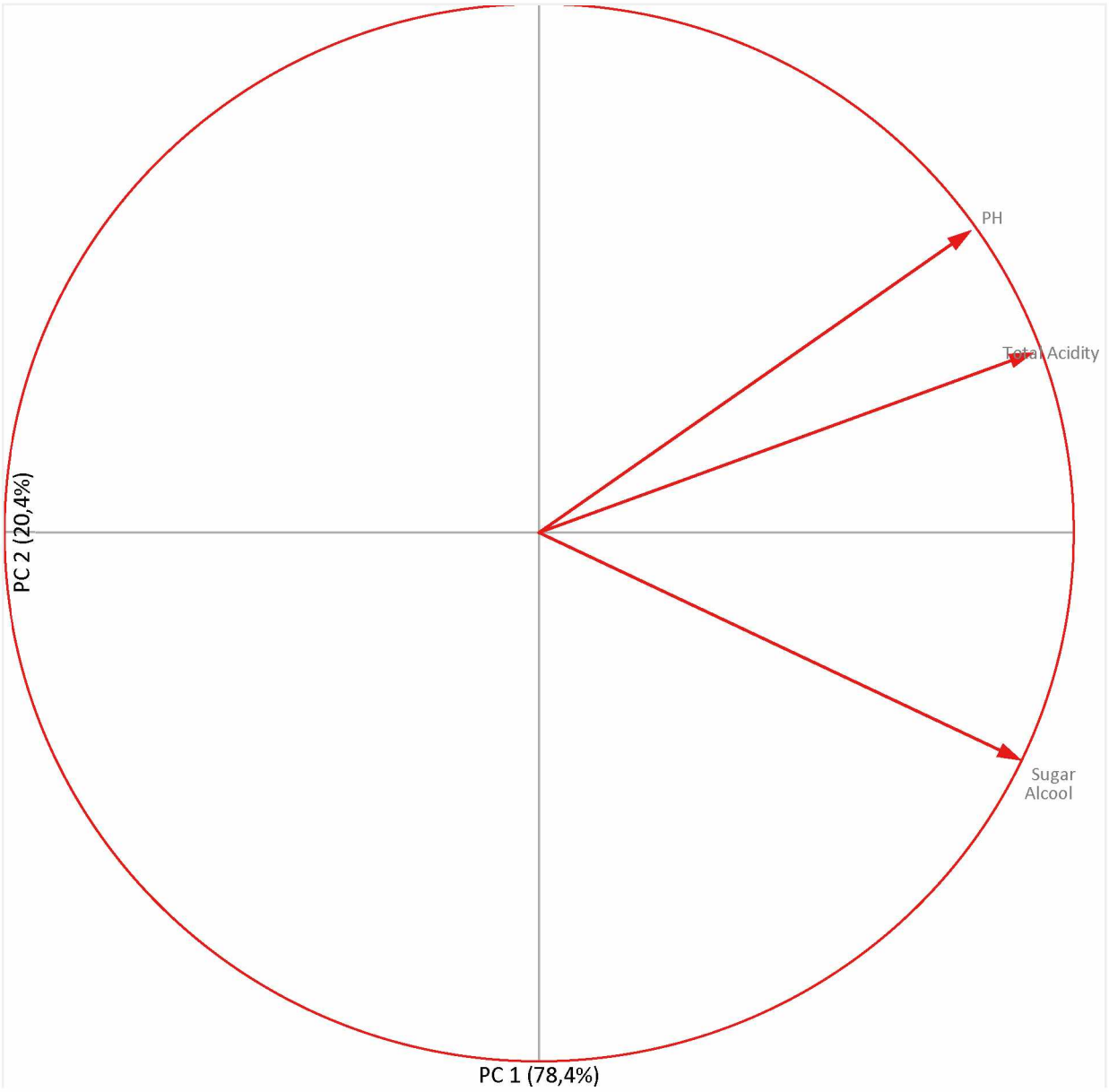
Annex 30- Monoplots for Chardonnay's musts, with a correlation monoplots of 96.5%



Annex 31 - Biplot of Chardonnay's musts, with a correlation bioplots of 96.5%



Annex 32 - Monoplot of Cabernet Franc's musts, with a correlation monoplots of 98.8%



Annex 33 - Biplot of Cabernet Franc's musts, with a correlation bioplots of 98.8%