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LOW-INPUT TREATMENTS TO CONTROL DOWNY MILDEW IN THE VINEYARDS

Mémoire de Fin d'Études

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NOTE BIBLIOGRAPHIQUE	
AUTEUR: LOPEZ MOTA Natalia Signalement du mémoire : TRAITEMENTS A FAIBLE IMPACT ENVIRONNEMENTAL DANS LA LUTTE CONTRE LE MILDIOU AU VIGNOBLE Mots clés: Mildiou, viticulture biologique, biodynamie, lutte chimique, alternatives au cuivre, Mycosin, micro-organismes efficaces.	
	RÉSUMÉ D'AUTEUR
PLAN INDICATIF	L'essai a été réalisé du débourrement jusqu'à la récolte de la saison 2010, dans un vignoble expérimental d'un domaine viticole dans le Trentino-Alto Adige, en Italie. Trois stratégies différentes pour le contrôle du mildiou ont été testées dans cet essai, en plus de la stratégie utilisée par le domaine, en cherchant des solutions pour le remplacement de cuivre.
BUTS DE L'ETUDE	Évaluer l'efficacité de certains traitements à faibles impacts environnementaux présents sur le marché, afin de lutter contre le mildiou dans le vignoble. Produits testées : Mycosin Vin ® et Micro-organismes effectifs (EM)®.
METHODES& TECHNIQUES	Quatre stratégies étaient évaluées dans cette essai : T1 Domaine, T2 EM, T3 EM+Cu, et T4 Mycosin. L'essai s'est déroulé en trois étapes principales : Application des traitements au cours de la saison selon les prévisions météorologiques (total de 11 applications). Monitorage de diffusion et classification de la sévérité des infestations du mildiou au cours de la saison. Analyses complémentaires : Nutrition des feuilles (N (Dumas Méthode), P, K, Ca, Mg, S, Fe, B, Mn, Zn et Cu (Emission Optical Spectromètre), SPAD analyse (coprophile content) et analyse des grappes à la récolte (fertilité, poids moyen des baies, poids moyen de la grappe, productivité, sucre °Bx, pH, acidité).
RESULTATS	Des différences significatives ont été trouvées entre les différentes stratégies évaluées pour le contrôle des infestations tardives du mildiou (après véraison). Les résultats consistant en la perte de qualité des grappes et l'attaque de mildiou dans certaines des stratégies (Témoin, EM, EM+CU).
CONCLUSIONS	Mycosin Vin ® est un produit qui a obtenu les meilleurs résultats de l'essai, lesquels étaient statiquement comparables à ceux obtenus par la stratégie qui a représenté le programme utilisé par le domaine. Les obstacles les plus importants qui empêchent ce produit d'être un remplacement possible au cuivre, sont associés aux dosages élevés nécessaires pour son efficacité, aux coûts de production et à la facilité de son l'application. La stratégie testant avec le produit EM ® , a démontré un contrôle insuffisant de la maladie. Les fortes infestations tardives de mildiou pourraient être attribuées à un manque d'efficacité du produit (à confirmer), une faible persistance du produit dans les feuilles ou une stratégie d'application insuffisante du produit.

BIBLIOGRAPHIC NOTE

AUTHOR: LOPEZ MOTA Natalia

Subject :

LOW-INPUT TREATMENTS TO CONTROL DOWNY MILDEW IN THE VINEYARDS

Downy mildew, organic viticulture, biodynamic viticulture, Copper alternatives, Mycosin, Effective Microorganisms.

	Summary
PLAN	The trial was performed during the growing season of 2010 in an experimental vineyard of a wine estate located in Trentino-Alto Adige, Italy. Three different treatments for downy mildew management were tested in this trial in addition to the strategy used by the Estate for the same growing season.
AIMS	Evaluate the effectiveness of some of the low-input treatments present in the market to control Downy mildew in the vineyards. Products tested: Mycosin Vin ® and Effective Microorganisms (EM)®.
METHODOLOGY & TECHNIQUES	Four treatments were tested in this trial: T1 Estate program, T2 EM, T3 EM+Cu and T4 Mycosin. The trial was performed in three steps: Application of the treatments with preventive criteria in accordance to weather previsions (total of 11 applications). Monitoring downy mildew infestations throughout the season and measuring the % of diffusion and severity. Complementary analyses: Leaf nutrition (N (Dumas Method), P, K, Ca, Mg, S, Fe, B, Mn, Zn and Cu (Optical spectrophotometer Emission), SPAD analyse (chlorophyll content) and grapes analysis in the harvest (fertility, average weight of the berries and clusters, productivity, Sugar °Bx, pH and acidity)
RESULTS	Significant differences were found in between the different strategies evaluated for downy mildew control after late infestations in the leaves occurred (after veraison). The Estate Program presented the best control for downy mildew with no significant differences with Mycosin. Consistent results of grape quality losses were found amongst the strategies EM, EM+Cu and the control in comparison with Mycosin and the Estate Program.
CONCLUSIONS	Mycosin Vin ® is the product that presented the best results for downy mildew control, which were statistically comparable to those obtained by the wine estate strategy used by the wine estate. The biggest limitations that the product presented to be considerable as a possible replacement for copper are associated with the high dosages necessary for its efficiency and thus production costs. The product EM ® demonstrated a poor control for downy mildew. The high amount of leaves damages during the late infestation period could be attributed to lack of efficiency (need to be confirmed in further studies, low persistence in the leaves or inadequate strategy for the product application.

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1. Background

Environmentally friendly farming has existed since the very beginning of agriculture. As time passed by, unsustainable production systems became prominent as a consequence of changes in life style, evolving new technologies and lack of environmental projection. The possibility of cultivating big extensions of land, usually of mono-cultivars, emerged after new technologies had been developed during the First World War. Managing the fields was now done through intensive use of fertilizers, pesticide and herbicides to pare down the disequilibrium caused in these ecosystems.

At the beginning of 20th century organic farming established formally as a reaction movement to agriculture's growing reliance on synthetic fertilizers, although, from the total agricultural output, organic farming has remained tiny since its early beginning.

As environmental awareness and concern increased amongst the population, the originally supply-driven movement, later on became consumer- and governmental-driven. Gradually, environmentally friendly farming has been upraising since the last decades, the last one being the most relevant for organic agriculture and biodynamic agriculture.

In 1999, 11 million hectares of the world's agriculture land were certified according to organic standards, whereas the latest data reported in the 2010 FIBL global-survey indicate to have 35 million hectares of the world's agricultural land certified according to organic standards - that makes an increase of more than 300% over a period of 10 years (Willer, 2010).

Efforts on developing a kind of agriculture that understands and respects nature, provides high quality products, and which regards an economical viability are being made all over the world. Year by year, a remarkable growth of people being involved in this sector can be noticed throughout the whole production chain, while needs for new technologies and bio-agriproducts emerge.

One of the biggest challenges that organic agriculture still confronts is the eradication of some non-environmental friendly substances used as fungicides without compromising its production.

Downy mildew, caused by *Plasmopara viticola*, is a major fungal disease of grapevines originally from America and spread almost all over the world. A severe infection can cause leaves to fall prematurely, reducing yield and berry sugar content, and exposing remaining bunches to sunburn, while a total crop loss may occur if a severe infection is not taken care of, especially around the time of flowering.

The control of the disease is achieved by fungicide applications. In organic viticulture the less hazard active substances are allowed, but at the moment only few active substances with fungicide activity have shown real effects to control downy mildew. Copper-based treatments are the most effective and are used above all in organics. However, since copper is a metal that accumulates in the soil, it can cause an impact on soil diversity if found in too strong concentrations. Thus, the results of these treatments are incompatible with organic farming's aims.

Until new products and more environmental friendly substances can replace the use of copper, the determination taken by The Commission of the European Communities and other certifying bodies, is to fix a ceiling on copper use. The limit established for organic agriculture in Italy is 6kg of Copper/ha/year (Regulation EC n. 473/2002), while the certifying bodies for biodynamics establish a limit of 3 kg of Copper/ha/year. (IFOAM.ORG)

Since long time ago, low input alternative control strategies based on plant extracts, biological control, clay and substances that trigger the vines' natural immune system are under development all over the world, whereas their effectiveness is still an inconclusive issue. (Molot, 2008)

For wine estates and grape growers that work under organic standards and who make efforts to improve their viticulture practices regarding the biodiversity of their soils and plants health, it can be interesting to implement some of the newly developed products that match better with their aims and vision, but it still represents a risk that not everybody is willing to take.

As it is well known, it is always one of the biggest challenges to turn theory into practice. If a winery wants to apply a new treatment which has been developed through a field trial, variables like meteorological conditions, ecological site facts, machinery, wealth of the vineyards and cultural practices have to be considered in order to achieve good results.

Furthermore, in order to get closer to real low input solutions that will help in the future to control downy mildew in organic vineyards, research centres will have to keep their studies and field trials as close as possible to the practical use of how wineries or grape growers will apply these products under 'real life' conditions. Therefore, the collaboration between research centres and grape growers has to be as close as possible. This also helps to enrich the research line and enables wineries to give a feedback and make considerations for further possible developments.

Thus, this project aims to check the effectiveness of some of these low-input alternative treatments under the specific conditions of a biodynamic vineyard from a wine estate in the Trentino region of Italy. The possibility to change the winery's actual treatment for downy mildew was evaluated, and the information about the products and their implementation was contributed to the Università Cattolica del Sacro Cuore di Piacenza.

2. Project details

2.1 Location and Duration

This project was performed in Italy in the Trentino-Alto Adige region, at an experimental vineyard of the wine estate

The duration of this project was of 7 months, from 01/03/2010 to 30/09/2010

2.2 Partners
Wine Estate - Italy

Università Cattolica del Sacro Cuore di Piacenza (UCSC)- Italy

Ecole Supérieure d'Agriculture d'Angers (ESA) - France

2.3 Sector
Organic and biodynamic viticulture

3. Aims
Evaluate the effectiveness of some of the low-input treatments present in the market to control downy mildew in the vineyards.

1. To design an application plan that permits to evaluate the effectiveness of the selected products for the trial.
2. To evaluate the effectiveness of these treatments under the weather conditions of 2010 in a representative scale of the estate vineyards.
3. To project, in relation with the results of 2010, the effectiveness of the products for further years more or less susceptible for downy mildew.
4. To determine if one of the treatments could be interesting for the estate to use further.
5. To contribute with information about the different treatments used in the trial for a better understanding of the products and their interactions with *Plasmopara viticola* for further works of the University in the subject.

4. Introduction

4.1 Organics in Italy
Organic agriculture is a method of cultivation and breeding, to produce raw materials for various uses, which allows the use of natural substances and excludes the use of synthetic chemicals and genetically modified organisms (GMOs) or derivatives thereof. The goals of this method are the conservation of the environment regarding its own natural balance.

Organic farming is an important and growing sector in Italian agriculture. Italy ranks first in Europe in both number of organic farms and organic cultivated land area (Global organic farming Statistics and news).

Rudolf Steiner developed the concepts of biodynamic agriculture in Germany in the 1920s. The term organic agriculture originated in England, based on the theories developed by Albert Howard in his

Agricultural Testament of 1940 (Maison de l'Agriculture Bio-Dynamique). Hans-Peter Rusch and Hans Muller developed biological agriculture in Switzerland. Muller used the term organischbiologischer Landbau (organic- biological farming) for the first time in 1949.

The common feature of these movements, which are the source of the current EU regulations on organic agriculture, is the emphasis on the essential link between farming and nature. The focus is on promoting natural equilibrium rather than maximizing yields through the use of synthetic products.

In Italy the earliest pioneering experiences in organic agriculture date back to the nineteen-sixties, but only took off in the nineteen-seventies, involving more and more farmers and consumers seeking an improved quality of life and consumption (Rosa, 2009).

During the mid eighties, the first local coordination agencies established the "Commissione Nazionale Cos'è Biologico" (National Commission for Organic Agriculture). Made up of representatives of organisations and consumers' associations from each Italian region, the Commission established the first nation-wide self-regulatory standards for organic farming.

Since the 1990s, the development of organic agriculture in the EU has been supported by financial subsidies. The first EU organic regulation, Council Regulation 2092/91, was adopted in 1991, came into force in 1992, the numerous small associations of organic farmers and the producers and consumers committees operating in every region reorganised themselves, joining forces through mergers and a federative network. Today, there are around 20 officially recognised certification agencies operating in Italy (The 16th IFOAM Organic World Congress, 2008).

In 2003 and 2004, the number of organic farms in Italy had decreased. Especially in Southern Italy and on the Islands (Sicily, Sardinia) in the past years many farms converted to organic farming, not because of market or ecological reasons, but mainly because of the state subsidies. In some regions aids are not available any more, and many farms have left the organic control system.

Since 2005, after the period of stagnation, the number of farms and the land under organic management raise up again. Currently more than one million hectares of Italy’s agricultural land is organic. (Pinton & Zanoli, 2004)

Most of the Italian organic farms are in the south and the islands (32%), even though with the recent decrease of the numbers of farms the share of the farms in the south went down.

In Sicily organic farming developed at a very fast rate, with the number of farms increasing 2.5 times and land area almost doubling between 1993 and 1995. In comparison, the average rate of growth for the whole country was 123% and 126%, respectively, for the same period. The development in Sardinia is more recent, and mainly due to the application of EU Regulation 2078/92. When pastures were admitted to qualify for aid, many sheep-grazing pastures were converted into organic ones (for the most part, Sardinian farmers are sheep breeders and producers of the well known "pecorino" cheese). (Pinton & Zanoli, 2004)

Tuscany and Emilia-Romagna have experienced pioneering organic movements dating back to the early 1980s. Indeed, of the certifying bodies five (ICEA, BioAgriCert, Codex, CCPB, QC&I) are based in Emilia-Romagna or Tuscany. Olive trees are grown by a large part of Tuscany's organic farmers, while cereals and fruit & vegetables prevail in Emilia-Romagna. Both regional governments have approved special laws after EU Regulations 2092/91 and 2078/92 to regulate and promote organic farming. (Rosa, 2009)

In the province of Trento, organic farming has been developing gradually since the late seventies, promoted by various associations, research centres and governmental bodies. It has established itself in areas like the Valley Gresta (Horticulture), Val Rendena (Livestock) , Val di Non (fruit), the Adige Valley and Valsugana (fruit and vine). Up to the moment, organic farming is still growing year by year (about 1% of the surface under vines organics standards) but remains low in proportion. The region is built up mainly with small producers that own in between 1 to 5 ha, and cooperative systems. Therefore, one of the biggest challenges that the region confronts, is the amount of enrolled people that is needed to represent a real increase on surface working under organics standards (Centro di Ricerche, Studi e Valorizzazione per la Viticoltura Montan) (Camera di Commercio Industria Artigianato e Agricoltura di Trento, 2009).

4.2 Trentino - Alto Adige wine region

Trentino - Alto Adige is a wine region located in northeast Italy, bordered by Austria to the north, Switzerland to the north-west and by the Italian regions of Lombardy and Veneto to the west and south, respectively.

This wine region produces around 1, 254, 000 hL of wine per year, which represents 2.6 % of the national wine production. The lands under vines cover in total 14,648 ha, making vines the second cultivar growth in the region after apples (Camera di Commercio Industria Artigianato e Agricoltura di Trento, 2009).

There are five native grapes in this region, one is the white Nosiola and the other four are the red Teroldego, Marzemino, Schiava and Lagrein. Other international grape varieties are also grown in the region, such as Chardonnay, Cabernet, Merlot, Moscato, Pinot Nero, Pinot Grigio, Traminer and Müller-Thurgau.

Trentino - Alto Adige is characterized by its big diversity of forests, rivers and glacier lakes. Its extremely mountainous countryside covers a large part of the Dolomites mountains and the southern Alps. About 90% of its lands are cover by mountainous lands, which make from this region a heterogenic landscape with a big variability in climates, soils and environmental conditions and allow, only, 15% of the land to be cultivated (Sevizio Vigilanza e promozione delle Attività Agricole, 2009).

Politically, the region is conformed by two autonomous provinces: Trento capital of Trentino, located in the south, and Bolzano or South Tyrol, capital of Alto Adige, located in the north. Being the region conformed by two different Provinces with different administrations, laws and cultural backgrounds, significant distinctions in between their way of wine production and their wine industry structures can be noticed.

For the purpose of this work, with the wine estate concerned located in the Trentino part, data and information of the region will be referred only to the Trentino area.

4.3 Trentino overview

4.3.1 Wine industry facts

Trentino produces 73% of the total wine produced in Trentino-Alto Adige. As it is presented in the summary of the region, cooperatives systems represent the main share of the wine production. While, the main part of viticulture is done and depends on small grape producers with less than 5 ha. (Camera di Commercio Industria Artigianato e Agricoltura di Trento, 2009)

Summary of the Trentino wine industry 2009

Total wine production	**916,000 hL**
Wine production under DOC	91 %
Wine production under IGT	9 %
Total Wineries	**185**
Share of the total wine production by Wine Estates	6 %
Share of the total wine production by Comerciants	11 %
Share of the total wine production by the Cooperatives	83 %
Total surface under vines	**10,140 ha**
Share of the regional agriculture surface	47 %
Total grape growers	**7,600**
Grape growers with less than 1 ha	69.5% (2,370 ha)
Grape growers with 1 to 5 ha	27.5% (5,090 ha)
Grape growers with more than 5 ha	3.0% (2,680 ha)

Data collected and calculated from: (Camera di Commercio Industria Artigianato e Agricoltura di Trento, 2009)

4.3.2 Viticulture facts

Ecological and climate information

Latitude	11°02 – 11°26
Longitude	45°53 – 46°53
Altitude of vineyards above sea level	100 – 850 m.s.l
Vineyards with altitude >500 m.s.l	13 %
Average annual temperature	12 °C
Average peak annual temperature	35 °C
Average lowest annual temperature	-10 °C
Start date of 10°C mean daily temperature in spring	5st – 10th April
End date of 10°C mean daily temperature in the autumn	20th – 25th October
Days with mean temperature above 10°C	200 days

Annual rainfall	1000 mm
Rainfall in the growing period	530 mm
Months with the most rainfall	May and November

Data collected and calculated from the last 10 years different sources: (Camera di Commercio Industria Artigianato e Agricoltura di Trento, 2009) (IASMA -Agrometeorologia Data Base).

Vineyard's distribution in these mountainous lands is 39% in valleys, 41% in hills and 20% in mountains. (Camera di Commercio Industria Artigianato e Agricoltura di Trento, 2009)

Grape varieties and cultural practices

In the past, the region used to grow mainly red varieties, with Schiava being the main cultivar. Year by year the presence of white varieties has been increasing due to the demands of the international market and the important production of sparkling wine in the region (40% of the national production of sparkling wine vinified with Traditional Method). Therefore, since the 1990s, the main variety in the region is Chardonnay followed by Pinot Grigio and Merlot.

Table 4.1 Presence of the different grape varieties variety grown from 1980-2008

Grape Variety	1980 (%)	1990 (%)	2000 (%)	2005 (%)	2008 (%)
Merlot	9.4	7.9	9.6	9.1	8.0
Teroldego *	7.0	6.6	6.2	6.7	6.5
Schiava *	34.0	23.6	13.0	5.7	4.9
Cabernet	3.6	3.4	5.1	5.3	4.7
Marzemino *	1.6	2.1	3.4	3.7	3.5
Lagrein *	1,2	1.5	2.0	2.4	2.3
Pinot Noir	0.4	0.9	2.0	2.2	2.2
Enantio	12.6	9.3	4.3	0.9	0.7
Rebo	-	-	0.1	0.5	0.5
Moscato Rosa	-	0.1	0.1	0.1	0.1
Other red varieties	10.2	4.1	2.2	0.7	1.9
Total red varieties	**80 %**	**59.4%**	**48%**	**38.3%**	**35.3%**
Chardonnay	8.7	21.6	26.0	26.2	27.1
Pinot Grigio	1.6	6.4	13.0	19.5	21.3
Müller Thurgau	1.4	3.7	6.0	8.4	8.6
Traminer	0.5	1.2	0.5	2.3	2.7
Moscato Giallo	0.9	1.2	1.1	1.2	1.2
Sauvignon Blanc	-	0.7	0.9	1.1	1.1
Pinot Blanc	0.9	2.0	1.7	1.0	0.9
Nosiola *	1.1	1.3	1.2	1.0	0.8
Riesling (renano)	0.3	0.3	0.2	0.4	0.4

Manzoni bianco	-	-	-	-	0.2
Riesling Italico	0.4	0.4	0.2	0.0	-
Other white varieties	4.2	2.5	1.2	0.6	0.4
Total white varieties	**20%**	**40.6%**	**52.0%**	**61.7%**	**64.7%**

*Native varieties

The main trellising system used in the region is *Pergola Trentina,* being 80% of the vineyards grown in this way.

Downy mildew is recognized as the main problem of the region, followed by powdery mildew and mites. Grey mould is only a problem in extremely wet areas. There is an increasing worry about the possible diffusion of *Flavescence doreé* from the near region (Veneto) and treatments against *Scaphoidaeus titans* are done with pyrethrum, where this vector is present. (Camera di Commercio Industria Artigianato e Agricoltura di Trento, 2009) (Centro di Ricerche, Studi e Valorizzazione per la Viticoltura Montagna)

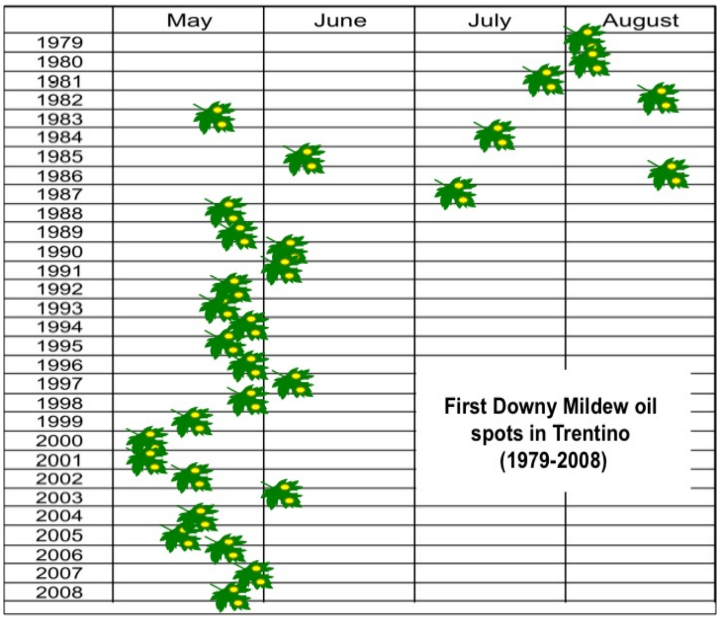


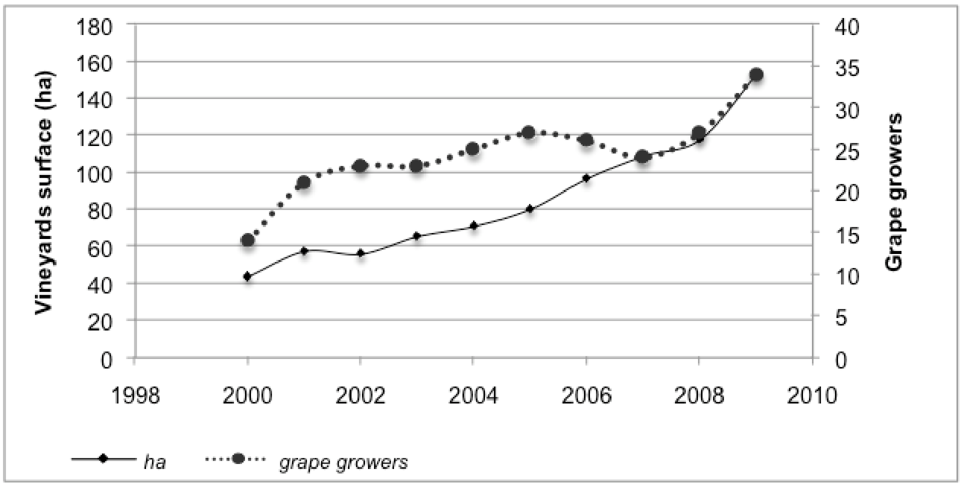
Figure 4.2 First downy mildew oil spots in Trentino. Courtesy of Enzo Mescalchin

4.3.3 Organic viticulture in the region

Subventions and efforts from the province to support more sustainable ways of viticulture exist since the late eighties. The focus of the province has been implementing moderated ways of production to decrease the use of chemicals in the field. Thus, integrated viticulture management stands out in the region with 95% of the grape growers working under the Protocol of 1990 of the Consorzio di Tutela Vini del Trentino, which regulates this kind of viticulture. From what it concerns to organic production, as it is shown in the graphic 4.1 the number of grape growers involved and the surface keep increasing year by year, but still the numbers remain very low.

Vineyards under organic agriculture 1% (153 ha)
Vineyards under conventional agriculture 4%
Vineyards under integrated agriculture 95%

(Camera di Commercio Industria Artigianato e Agricoltura di Trento, 2009)



Graphic 4.1. Evolution of organic viticulture in the Trentino region. Data Source: (Ufficio per le Produzioni biologiche, Provincia Autonoma di Trento, 2010)

After some studies done in organic viticulture by the region, it has been found that disease control is done, mainly, with sulphur, copper, pyrethrum and mating disruption.

For organic viticulturists in Trentino, pest and diseases are not bigger constrains than what they already represent in general for all the grape growers in the region. Although, years like 2008 in which the amount of rainfall reached 1500 mm, downy mildew infections caused about 50%-70% of losses in grape production in the region increase the worries about how to overcome years with big infections of downy mildew among organic farming.

For downy mildew disease management, alternative products to copper are not commonly used – mainly because copper treatments are considered easier to apply, it is cheap, reliable and effective. Usually the number of preventive treatments in a growing season relay in between 10-12 treatments in total (Vechhione, 2005).

Production is currently regulated by the EC Regulation 834/07 and regarding the Provincia Autonoma di Trento, Provincial Law March 28, 2003, No 4. In January 1, 2009 entered into force, EC Regulation 834/07 which replaces the previous Reg (EEC) 2092/91 and introduces new disciplinary bodies for new areas of production non-previous regulated, such as wine processing and aquaculture. (Ufficio per le Produzioni biologiche, Provincia Autonoma di Trento, 2010)

5. Downy mildew

5.1 Introduction

Downy mildew, caused by *Plasmopara viticola*, is a major fungal disease of grapevines. This fungus can infect all green parts of the vine. Symptoms are usually observed on leaves and grape bunches. A severe infection can cause leaves to fall prematurely, reducing yield and berry sugar content, remaining bunches also can be exposed to sunburn. Total crop loss may occur if severe infection is not managed, especially near flowering. While a sever leaf fall also can cause yield loss in the following season due to the inability of the vine to store reserves.

Downy mildew originates from North America and has been present spontaneously for indeterminate time in its forests.

Downy mildew was first observed in cultivated grapevines in 1834 in North America. After the outbreak of phylloxera around 1863 and the huge vineyard losses in that time around. European grape growers were eager to use the American root stock on which to graft the vines as a remedy for phylloxera. So it is believed that some time prior to 1878 the fungus was carried to Europe on American stock. In September of 1878 downy mildew was first recorded in France and one year later in Italy. During the next years records of the disease's presence were all around Europe, Turkey, Russia, Africa and Minor Asia. (Mattedi & Varner, 2000)

Plasmopara viticola is specific to grapevines. All varieties of *Vitis vinifera* are susceptible to downy mildew infection. Although, *Vitis vinifera* hybrids, *V. aestivalis* and *V. lambrusca* are less susceptible while *V. rupestris, V. cordifolia* and *V. rotundifolia* are generally resistant. Other species of downy mildew, such as those found on cucurbitis and roses, do not attack grapevines. (Fisher, Gordon, & Magarey, 2007)

Grape downy mildew occurs mainly in regions where it is warm and wet during the vegetative growth of the vine. In Europe the losses of production due to this disease have been enormous.

In order to understand how the disease is managed and how to detect its symptoms, a broad knowledge of its life cycle is necessary.

5.2 Life cycle

Plasmopara viticola is an obligated parasite, meaning that requires a living host to develop. It grows on all green parts of the vine, however, there is one over-wintering stage of the fungus where it is not found on green tissue.

Plasmopara viticola life cycle can be divided in two phases:

1. Overwintering phase (saprophytic), in which, the fungus develops inside of the previous damaged parts of the vines, forming resistant bodies, oospores, which allow it to survive in extreme weather conditions and lack of available nutrients.

2. Infection phase (parasite), in which, *Plasmopara viticola*, after oospores mature requires a living host and grows on all green parts. It is visible to the viticulturist and is the most important phase for control and management of the disease. As it is will be explained further, infection period has two cycles, therefore in a growing season primary infections and later on secondary infections can be noticed. (Oberhofer, 1991) (Burruano, 2000)

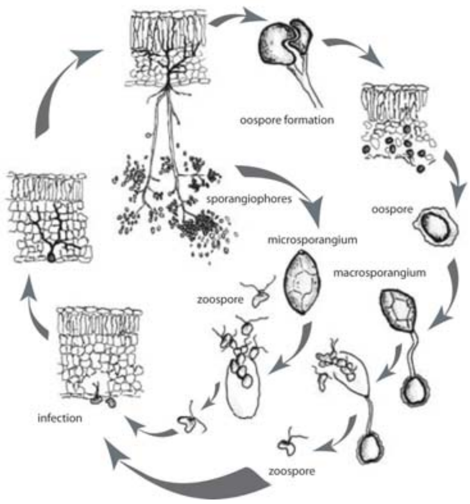


Figure 5.1. Adapted version into English from (Perot & Dagostin, 2007)

Overwintering phase

Plasmopara viticola start to form winter spores (oospores) in the infected leaves once that the nutrition conditions required by the fungus to live decrease. In leaves that are not physiologically active, oospores can start forming during summer (June or July in the North Hemisphere), mainly oospores are formed in late summer and autumn.

These resting bodies fall to the ground when leaves and bunch parts fall in autumn. There they overwinter in infected leaves and litter in the soil for 3 to 5 years (and possibly up to 10 years)(Perot & Dagostin, 2007). Oospores have a thick wall that makes it resistant to fungicides and adverse weather conditions.

From all the oospores present in the soil, with the proper weather conditions, oospores maturation till germination will follow and from this moment the infection phase will start.

It is important to say that studies in the subject have pointed out that the heterogeneity in oospores germination time is still an inconclusive issue. What is known nowadays is that germination of the oospores is not restricted to some year in particular after oospores were formed. Oospores can remain latent in the soil, from what we know, from 1 to 10 years and germination can occur in any moment while maturation conditions are present to allow it (Perot & Dagostin, 2007).

This is to say, that each year the zoospores (responsible of downy mildew infections in the vines), can come from oospores of different years of formation. And even though infection phase is determined by the amount of oospores germinated, it is not possible to do a direct correlation in between the incidence of downy mildew in the past season to assess possible incidence of downy mildew in the next seasons. (Magarey P. A., 2010)

Infection phase

Primary infection

A primary infection occurs when the oospores present in the soil germinate realising zoospores that enter into the stomas of the green parts of the vine. Maturation of the oospores happens in winter and beginning of spring. In presence of water (approximately 10 mm) and with temperatures above 10°C the oospores germinate producing a macrosporangium from which zoospores are released.

For infection to be complete zoospores need to reach the vine in the next 24 hours. Zoospores are able to move though water or by wind, but are mainly spread splash by the rain. This process usually requires another 3-5 mm of rain to ensure vines wetness. For this, the vines must remain wet for at least 2-3 hours at 20°C (or 4-5 hour at 10°C).

The rule of 10:10:24 or "rule of thumb" refers to the conditions required for primary infection to occur. At least 10 mm rainfall is required while the temperature is above 10°C over a 24 hour period. Not all 10:10:24 conditions are suitable for a primary infection. Preparative rains and winter temperatures influence germination time for possible infections to occur.

Once zoospores reach green parts of the vines, infection occurs when the fungus enter through the stomata. Stomata in the vines are localized in the underside of the leaves, the young green parts of the vine and in the berries in the first stages. Therefore, for first primary infection in the growing season to occur shoot must be about 10 cm; it is to say 3 leaves separated or *09 stage* in Lorenz scale. Before this stage, leaves still have the stomata closed, thus it is not possible that vines can be infected. (Magarey P. A., 2010)

Once the fungus is inside the host an incubation period, in which the fungus develops, passes until the symptoms become visible. Incubation period can endure between 5 to 18 days, being the most common from 6 to 10 days. (Fisher, Gordon, & Magarey, 2007)This period is determined by humidity and temperature, above and below 23-24°C incubation time becomes longer. (Goidanich, 1964)

Primary infections levels are usually not very dangerous. However they are very important because this is how the disease starts and from here on it can spread rapidly if secondary infections occur.

Overhead irrigation alone is usually not enough to induce a primary infection. However, rainfall and irrigation combined can be sufficient for this to occur.

Primary infections can continue all along the growing season, whenever mature oospores have the necessary conditions to germinate and when new zoospores from secondary infections are formed. Studies made with molecular markers evidenced that new primary infections coming from germinated oospores can occurred still in the month of August (Gobbin, 2004). Although, as the growing season moves, the number of oospores that germinate decreases (Perot e Zulini, 2003).

Secondary infection (sporulation)

This secondary *Plasmopara viticola* cycle is characterized by the production of sporangiophorus and sporangium seen as white down.

Active infected plant tissue, need to be present for secondary infections to occur. Sporulation requires at least 4 hours of darkness to develop, during which vines should be wet for 2-3 hours, the temperature must be at least 13°C, being the optimal temperatures in between 18-20°C, and humidity above 93%. (Perot & Dagostin, 2007)

Once sporulation occurs, the cycle finished when the new sporangium formed realised new zoospores that can be spread by wind and rain to establish new infection sites.

Secondary infections can occur anytime during the growing season whenever primary infection symptoms are present and conditions are favourable. Secondary infections are the major source of disease spread.

5.3 Symptoms

Downy mildew, as it was explained, grows on all green parts of the vine. Depending to climate conditions and growing period, lesions and symptoms will be seen in so many different ways.

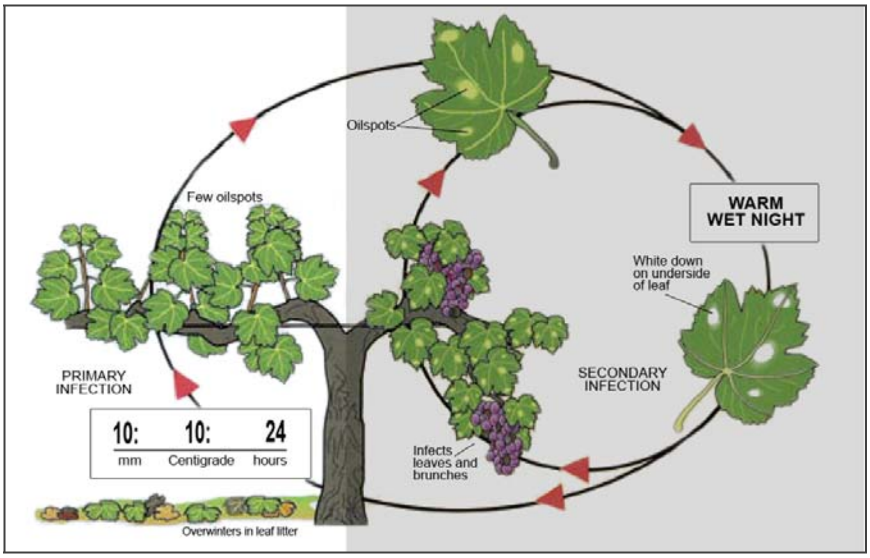


Figure 5.2 Adapted version from (Magarey P. A., 2010)

Leaves

The primary infection symptoms on leaves vary with leaf age. On young leaves, at the beginning of the season, the disease will appear on the upper surface as small yellow spots referred to as oilspots; they are about 10 mm diameter often with a brownish halo. These sports tend to grow to about 50 mm diameter as they mature and the halo fades. As the spots enlarge the may appear to cover most of the leaf, especially if there is more than one spot on the leaf. (Disease Diagnosis). Later in the growing season (late summer and autumn) on mature leaves, leaf infections will appear as small angular, yellow spots that are limited in growth by the veins. These form a tapestry-like (mosaic) pattern of spots that soon turn reddish brown. Defoliation can occur in severely affected vines. (Perot & Dagostin, 2007)

After secondary infection occurs, a dense, raised, white cottony growth develops on the underside of the yellow oilspots. This growth is commonly referred to as “white down”. As the spots age naturally, or after a sporulation event, or after hot weather, their centres dry out and become a reddish brown leaving an outer ring of yellow. The fungus in this yellow ring remains active and given favourable conditions at night, can produce a ring of white down on this outer active edge. (Magarey P. , 2010)

Shoots and other green parts

Primary infection on young shoots, stems and tendrils are seen as oily brown areas. These oily patches may spread into leaf stalks, which turn brown and may die. After suitable warm humid nights and secondary infection occur, these oil patches may also sporulate and be covered with white down. (Fisher, Gordon, & Magarey, 2007)

Inflorescences, bunches and berries

Primary infections on inflorescences are seen as oily brown areas. Infected inflorescences and young bunches rapidly turn brown and wither.

Infected young berries stop growing, harden and may later develop a purple hue. They then turn into a dark brown, shrivel and fall from bunches.

Infected berries of white cultivars may turn dull gray-green, while those of black cultivars turn pinkish red. Infected berries remain firm, compared to ripening healthy berries, and drop easily. Portions of the rachis or the entire cluster also may drop. (Magarey P. A., 2010)

When secondary infection occurs they get covered with white down.

Berries become resistant to infection when they are about pea size (around 5-6 mm diameter). However, they may still be killed if the berry or bunch stems become infected. They may also sunburn and fail to ripen if defoliation occurs from leaf infection. (Burruano, 2000)

5.4 Disease management

A good managing of the disease is determined, besides good cultural practices, by a good spray application. This is to say, by the type of treatment, timing and technique (spray coverage and dose) that the viticulturist uses to control downy mildew. (Magarey P. A., 2010) (Perot & Dagostin, 2007) Fungicides for use against downy mildew can be categorized broadly as either non-systemic or systemic (contact or absorption).

The non-systemic, or also called, contact fungicides provide a protective barrier on the surface of the foliage where they stop the spores germinating. They must be applied as pre-infection fungicides, and must be re-applied prior to an infection event if there was sufficient wash out due to rainfall, or if there was sufficient growth of leaves or of developing berries. New growth following application is not protected. Sprays must be done as close as possible before infection. Access to forecasts of downy mildew events is required to time these sprays the best.

The systemic, are those fungicides that are absorbed by the plant. They can be pre-infection or post-infection fungicides (eradicants). The pre-infection fungicides allow bigger time in between applications and bigger protection due to the interactions with the vine metabolism. The post-infection fungicides kill the pathogen inside the infected tissue when they are applied at the right time. They do not eradicate the disease from the vineyard but they are quickly absorbed into the sprayed foliage and stop the development of the fungus. Some of them are mixed with pre-infection fungicides so the application simultaneously provides a protective shield against new infection. (Magarey P. A., 2010)

The post- infection fungicides are more expensive than their pre-infection counter-parts and dangerous for the environment, but being more effective, requiring much less effort and representing less risk damages for the viticulturists, and thus are usually the most used in conventional agriculture.

In organic agriculture only non-systemic fungicides are authorized, being treatments based in copper compounds the main resource for downy mildew management. Copper until know represents the lowest and safest resource that organic producers have for downy mildew control. (Vechhione, 2005)

6. Trial

6.1 Treatments background

6.1.1 Copper

The use of copper compounds goes back further than the fourth millennium B.C. Records found in the tombs of the early Egyptians suggest that, this ancient civilization employed copper sulphate as a mordant in their dyeing process. The Greek civilization around 400 BC used to prescribe copper sulphate for pulmonary diseases and by the 18^{th} century AC it was already widely employed to treat mental disorders and afflictions of the lungs. (Vechhione, 2005) (Fregoni, 2006)

Copper compounds have their most extensive employment in agriculture where the first recorded use was in 1761, when it was discovered that seed grains soaked in a weak solution of copper sulphate inhibited seed-borne fungi.

By 1807 the steeping of cereal seeds in a copper sulphate solution for a limited time and then drying them with hydrated lime became the standard farming practice for controlling bunt of wheat.

But it is in 1882 when the biggest breakthrough for copper salts occurred, when the professor and scientist Millardet from the Faculty of Science in Bordeaux, while looking for a cure for downy mildew disease, chanced to notice that those vines from Chateau Beaucaillour in Saint Julien, bordering the streets – which had been daubed with a paste of copper sulphate in order to make the grapes unattractive to people passing by – was free of downy mildew. (Fregoni, 2006)

This observation led to experiments with mixtures of copper sulphate, lime and water and in 1885 Millardet announced that he had found a cure for the dreaded mildew. This mixture became known as Bordeaux mixture and marked the beginning of protective crop spraying.

Different copper compounds continued to be developed. In 1910 the tetravalent copper oxycloride activity against downy mildew was demonstrated and in 1930 copper oxides (CuO and Cu_2O) were developed. At the end of the sixties and beginning of seventies copper hydroxide ($CuOH_2$) was introduced in viticulture. And from the nineties on, innovative formulations which allow lower copper dosages, were introduced to the market.

Mode of action and efficacy of copper

The anticryptogamic action of copper is based in the action of the ion Cu^{2+}. Once liberated in water, Cu^{2+} ions enter through the chitin wall and semi-permeable membrane of the fungus provoking concentration of Cu^{2+} ions to arise up to one hundred times inside the spore). The ion Cu^{2+} interact with the chitin wall replacing the H^+, K^+, Ca^{2+}, Mg^{2+} ions. (Perot & Dagostin, 2007)

Copper inside acts by interfering with the respiratory process (Acetyl-coenzyme A synthesis and Krebs cycle), inhibiting the synthesis of proteins, decreasing the cellular membrane activity, slowing down the transfer of ions and blocking the oxido-reductive reactions of the enzymes. (Brunelli & Portillo, 2006)

These mechanisms of Cu^{2+}, consequently, damaged the structures of the fungus before entering through the stomata of the plant and inhibit as well spore germination.

The mechanism of the action of copper is multisite, therefore the probability of selecting resistant strains is close to zero and it has never been observed (even after more than a hundred years of using it as fungicide). (Hoffmann, Heibertshausen, & Baus-Reichel, 2008)

Copper environmental impact

Copper, being a heavy metal, has a high capacity to accumulate in the soil. All the copper sprayed into the vineyards finish accumulated in the soil. During the treatments, it reaches the grapes of vines and vegetation, but as well it is largely dispersed into the ground where it accumulates directly. Later on after rain washouts and leaf fall in autumn the copper that was deposited on vegetation, also reaches the ground. The copper in the soil does not undergo any degradation and it is not metabolized, it does not evaporate and it tends to bound itself to organic matter to soil colloids. (Fregoni, 2006)

The accumulation of copper is more visible in the upper layers, where due to it concentration the biological activity of soil populations like earthworms, fungi and bacteria and nitrogen fixers (Nitrosomonas, Azotobacter, Clostridium, Nitrobacter, Rhizobium-green algae blue, etc..) are significantly compromised because of its toxic effect (Molot, 2008).

As a result of reduced microbial activity there is an accumulation of organic matter and poor availability of nutrients. This is particularly serious for organic farming cannot be used where mineral fertilizers and soil fertility is linked to biological activity of microorganisms present in it. (Perot & Dagostin, 2007) When the ground is reached very high copper content, it can also assist in visible phytotoxicity to the whole plant, consisting of stunted growth and chlorosis.

Copper is easily absorbed by aquatic organisms, over which it has high toxicity, unlike mammals and bees to which it is not particularly harmful. (Hoffmann J. U., 2009)

Copper based fungicides

The mechanism of action is based in the same principles presented for the mechanism of action of copper as a fungicide, in general for all the treatments based on copper it is recommended not to mix it with alkaline substances.
The main difference between the different products available on the market relay in the copper active compound used, copper concentration, resistance to rain wash out and additional substances that can make them more or less complex (i.e. Heliocuivre ® that has pine terpenes).

Heliocuivre ®

Heliocuivre ® is a copper formulation in liquid made principally from copper hydroxide (26.2% gCu^{2+}) and pine terpenes.

The action of pine terpenes in the product (Helioterpen technology) allows to have more regular droplets, increased the retention of the product in the leaves and resistance to leaching.
Depending the growing period, vegetation, and disease diffusion the doses recommended by the producer are: 1,5 L/ha à 2,5 L/ha (150-200 mL/hL considering the use of 1000-1500hL/ha) which can be translated into 393 gCu/ha – 655gCu/ha. (Intrachem, 2010)

Kocide 3000 ®

Kocide 3000 ® is a fungicide in powder made from copper hydroxide (15% gCu). It is one of the most common products used as a fungicide in agriculture due to the costs and simplicity of the formula.

Depending on the growing period, vegetation, and disease diffusion, the dose recommended by the producer is: 2000g/ha to 3000g/ha (133 -300 g/hL considering the use of 1000-1500hL/ha) which can be translated into 300 gCu/ha– 450 gCu/ha. (Dupont)

Heliocuivre ® and Kocide 3000 ® are authorized in Organic Agriculture in accordance to the CEE n° 834/2007.

Other doses tried for based on copper fungicides:
Even though the product producers suggest the previous doses presented and most of the research trials done to evaluate low doses of copper products are around 360 gCu/ha and 400 gCu/ha having satisfactory results, the doses used by the Wine State range in between 150gCu/ha to 300gCu/ha, while biodynamic consultants and practices suggest doses of 50 to 100gCu/ha at the beginning of the season, and later on 250 to 400 gCu/ha when natural nettles or horsetail tisanes are applied with the treatment. (Masson, 2007)

6.1.2 Treatments to test in the trial

Mycosin Vin ®

Mycosin Vin ® is a powder product based on acidified clay with high Aluminum Sulphate content, horsetail extract and diatomaceous earth. (BIOFA-Biologischer Pflazenschutz, 2010)

Mycosin Vin ® is authorized by the standards for Organics and Biodynamics production CEE n° 834/2007, by Demeter International (international certifying body for Biodynamics) and is subscribed in the list of Codex Alimentarius of international authorized products for organics.

It is considered, in the FiBL trials, as a good anticryptogamic when vineyards have middle presion for downy mildew, while in vineyards that have high presion it would be recommended only to use it at the beginning of season. (Hoffmann, Heibertshausen, & Baus-Reichel, 2008)

Active substances of the formulate

Clay — It is well known that clay has a lot of useful properties in agriculture. It has a strong fixing power, fungistatic properties, and it eliminates humidity.

Mixing clay with sulphur allows to decrease the doses, while mixing it with plants tisanes, particularly of nettle or horsetail, increases the fungistatic effect.

Horsetail	It is a natural anti-cryptogamique agent of middle spectrum widely used in biodynamics tisanes preventive for downy Mildew and other fungus diseases.
Diatomaceous earth	it s a naturally occurring, soft, siliceous sedimentary rock, used in agriculture for grain storage as an anticaking agent, as well as an insecticide.

This product requires precautions for its use, particularly if copper applications wanted to be done after its application. Therefore, it is recommended that at least 15 to 20 mm of rain have fallen to avoid burning the leaves (Masson, 2007).

The main countries in which this product is used is Germany, Austria, and Switzerlad, in France it is not authorized because it does not have the AMM (Autorisation de Mise sur le Marché). (Masson, 2007)

Some of its other collateral effects claimed by the producer are:

- Biological restorative for plants with plant health promoting effects in pip fruit, stone fruit and grape.
- Improves uptake of nutrients and increases the biological soil activity.
- Hardens the epidermis and cuticula of the canopy.
- Induces resitance to pathogens.

Doses recommended by the producer

Two to three leaves unfolded	09 Lorenz scale	Mycosin Vin® 5 kg + 3.6 kg of sulphur/ha
Inflorescences fully developed	17 Lorenz scale	Mycosin Vin® 5 kg + 4.8 kg of sulphur/ha
After fruit set	27 Lorenz scale	Mycosin Vin® 5 kg + 2.4 kg of sulphur/ha

(BIOFA-Biologischer Pflazenschutz, 2010)

Previous research

Mycosin Vin ® and its equivalents Ulmasud ®, Mycosan ®, are products that have been under research all over the world since a long time. Its applications in the trials always yield good results in comparison to other alternatives to copper, while its toxicity in the leaves and grapes is one of the problems that are almost always mentioned. (Hoffmann, Heibertshausen, & Baus-Reichel, 2008) (Hoffmann J. U., 2009)
This product is already on the market, although its use amongst wine estates and grape growers still remains low. Mycosin Vin ® represents a solution for copper replacement in organic agriculture, but it is still limitated in its use because it usually is less effective than copper and more expensive.

Effective Microorganisms, EM ®

EM ® contains selected species of microorganisms including predominant populations of lactic acid bacteria and yeasts, and smaller numbers of photosynthetic bacteria, actinomycetes and other types of organisms. All of these are compatible within one another and can coexist in liquid culture. (Bio-nrg)

EM ® consists of mixed cultures of “beneficial” and naturally-occurring microorganisms that can be applied as inoculants to increase the microbial diversity of soils and plants. EM ® is not considered a substitute for other management practices. It is, most of the times, an added dimension for optimizing best soil and crop management practices. It has being claimed that if used properly, EM ® can significantly enhance the beneficial effects of these practices. (Higa & Parr, 1994)

The concept of Effective Microorganisms (EM) was developed in the 1970s by Professor Teruo Higa, from the University of the Ryukyus, Okinawa, Japan.

Active substances of the formulate

Lactic Acid Bacteria	Lactic acid bacteria is, taxonomically, a generic term for bacteria that convert large amounts of sugars into lactic acid through lactic acid fermentation. Through the production of lactic acid, lactic acid bacteria also inhibit the growth of pathogenic microorganisms and other various microorganisms by lowering the pH. Lactic acid bacteria are widely known in the production of fermented foods such as cheese and yogurt that can be naturally preserved for a long period of time. Recent research indicates that besides regulating the intestines, lactic acid bacteria also are known for being involved in immunostimulatory activity having antagonist properties.
Yeast	Known as a fermentation starter, yeast is a microorganism necessary for the brewing of alcohol and the making of bread. Yeast lives in sugar-rich environments such as in nectar and the surface of fruits. Yeast produces many biologically active agents such as amino acids and polysaccharides.

Phototrophic Bacteria	Phototrophic bacteria (also known as photosynthetic bacteria) are an ancient type of bacteria in existence from before the Earth had its present concentration of oxygen. As its name indicates, these bacteria utilize solar energy to metabolize organic and inorganic substances. Phototrophic bacteria exist in rice fields and lakes, and everywhere on Earth. In practical terms, the potential of phototrophic bacteria is particularly seen in the environmental fields. Because it decomposes organic materials well, among these applications is its use in wastewater treatment. Research has also reported on its effectiveness in applied use in agriculture, aquaculture, and animal husbandry. Research is also underway in its use for hydrogen production and its ability to decompose persistent substances. Phototrophic bacteria are involved in various metabolic systems, and play a major role in nitrogen cycling and carbon cycling. Because this role allows the other microorganisms to co-exist, therefore phototrophic bacteria are an essential element the EM formulate.
Clay	See info in Mycosin Vin ® active substances information.

(Higa & Parr, 1994) (Bio-nrg) (EM Research Organization, 2010)

Previous research

The Effective Microorganisms concept may be considered controversial in some parts, because there is not enough scientific studies that to support all of its proponents' claims and consistent results in the studies carried out.

EM technology is supposed to maintain sustainable practices such as farming and sustainable living, and claims to support human health, animal husbandry, compost and waste management. (Filipp, Spornberger, Keppel, & Brunmayer, 2010)

Agriculture studies done mainly in Japan, were mainly directed to soil wealth, starters for composts, leaf photosynthesis activity and fruit yield. In the last years institutes and research centers started trials with the product, in which results in some prove its effect while other give inconsistent results or non significant differences. (Vercesi, Ricerche sperimentali sugli effetti nutrizionali e sanitari dell'impiego di formulati a base di miscele batteriche (EM Bio-nrg s.r.l.) su vite (2007-2009) , 2009) (Higa & Parr, 1994)

The interaction that the microorganisms present in EM ® may have with the natural flora present in the soil can vary amongst others that live in different surroundings. This limits the logic of their effectiveness when applied in different surroundings.

Downy mildew or other disease management

As it has been presented, EM ® does not have a specific function in agriculture. Few studies with the EM ® technology have been carried out to check its effectiveness for disease management, therefore non consistent evidences exist to prove its action to control fungal or other diseases in vines.

On the other side with the logic that the suppliers of the product present and the effectiveness claimed of this technology, they could be taken into account as possible solutions for downy mildew control or other fungal diseases.

Recent studies started to be carried out for downy mildew and powdery mildew control preformed by the Università Cattolica del Sacro Cuore di Piacenza to check the products effectiveness and they developed strategies for its application. Results have been released until now for trials only with EM ® and also with EM ® +Cu less diffusion and severity of the disease in comparison with the non-treated controls. (Vercesi, Ricerche circa gli effetti nutrizionali e sanitari su vite di formulati per irrorazione fogliare a base di miscele di microrganismi., 2010) (Vercesi, Ricerche sperimentali sugli effetti nutrizionali e sanitari dell'impiego di formulati a base di miscele batteriche (EM Bio-nrg s.r.l.) su vite (2007-2009) , 2009)

6.2 Methodology and materials

The trial was performed during the growing season of 2010 in an experimental vineyard of the wine estate located in the hills of Cognola (TN). Three different treatments for downy mildew management were tested in this trial in addition to the strategy used by the Estate for the same growing season.

In order to have a representative trial that allows us to evaluate the effectiveness of the products under the real conditions of the wine estate's vineyards, all the cultural activities, biodynamic treatments, and activities performed in the vineyards were carried out as well in the experimental vineyard.

6.2.1 Treatments

Four treatments were tested in this trial:

Treatment	Products
T1 Estate program	Kocide 3000®, Heliocuivre®
T2 EM	Effective Microorganisms (EM)®
T3 EM+Cu	Effective Microorganisms (EM)® + Kocide 3000®
T4 Mycosin	Mycosin Vin ®

The list of the different products used for this trial is presented in the table 6.1. Doses and products used per treatment throughout the season are presented in the following section see table 7.1

Table 6.1 Products information

Product name	Active ingredient	Presentation	Company

Kocide 3000®	Copper hydroxide 46.1%	Powder	DuPont
Heliocuivre®	Copper hydroxide 26.2% + resin (pine terpenes)	Liquid	Intrachem Biogard
Effective Microorganisms ®	Vegetal extracts, clay, and mix of lactic acid bacteria, purple bacteria, and yeast	Liquid	Bio-nrg
Mycosin Vin®	Acidified clay + horsetail extract + diatomaceous earth (kieselgur)	Powder	Biofa

The doses taken for the treatment T1 representing the Estate's own strategy for downy mildew control for 2010 were given by the winery. Doses and characteristics to apply the solutions of EM ® for the treatments T2 EM and T3 EM+Cu was given and monitored conjunctly through the season with the Università Cattolica del Sacro Cuore di Piacenza. The dose used for Mycosin Vin ® was taken directly from what is marked in the product recommendations.

Downy mildew and powdery mildew treatments were done, when it applied, simultaneously like it is done in practice. Thus, each treatment was mixed with the regular product (sulphur) and applied in the same dose used for powdery mildew control by the wine estate.

6.2.2 Experimental vineyard

Plot information

Grape Variety	Teroldego
Clone	Estate own clone selection (10 different clones)
Rootstock	SO4
Trellising system	Guyot
Density of plantation	2.00 x 0.9 m
Year of plantation	2005
Total number of vines	827
Total surface	0.165 ha
Location	Cognola (TN)
Latitude	46°5'N
Longitude	11°9 E
Altitude	340 m
Orientation	East – West
Organic management	Since plantation
Biodynamic management	Since 2007

Plot design

The plot as it is shown in the fig was divided into 14 blocks, of which 12 were used for the 4 different treatments and 2 worked as the control of the trial. Thus, the trial was performed with 3 repetitions per treatment and 2 controls in the same plot.

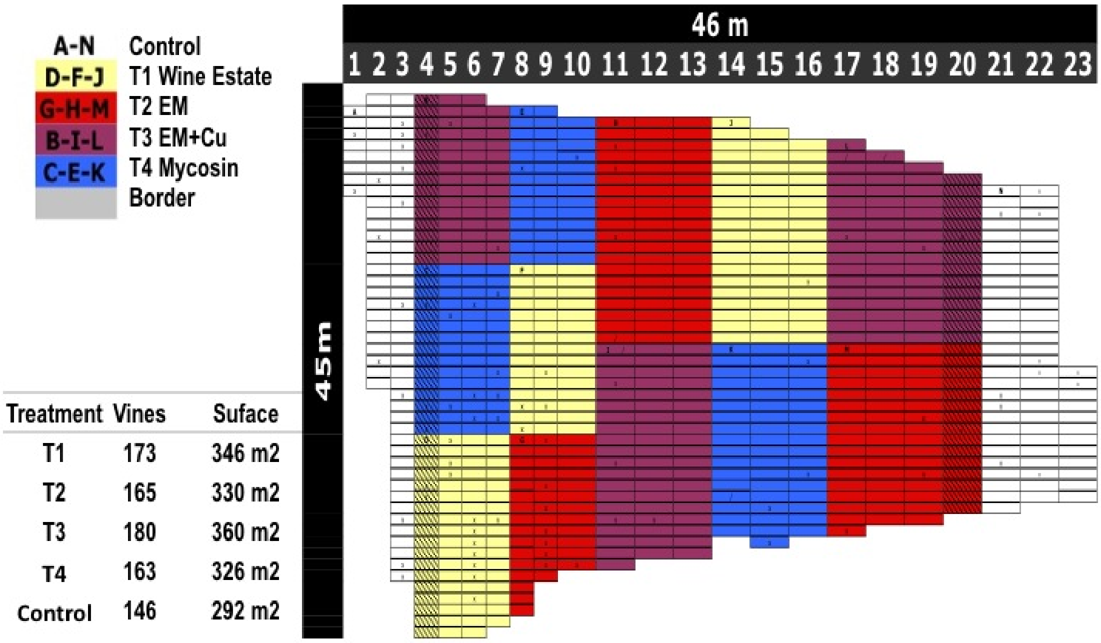


Figure 6.1 Plot map

The three repetitions of each treatment were distributed in the plot in order to cover, where possible, each representative position in the plot (top, bottom, center, borders).

Blocks were made of three rows in order to have the external rows of each treatment as a border and to avoid contamination in between treatments. An extra row used also as border, was left in between each control and the treatments (see plot map, row 4 and 20). These borders were treated as part of the corresponding treatments but not taken into account for the experiment.

6.2.3 Application

The applications for each treatment were done manually to avoid contamination in between treatments. Applications were made with a 20L manual pump sprayer.

To verify that the spraying on the leaves was done properly and was representative of a normal application made by the tractor, 30 water-sensitive papers were set on the leaves (15 in the bottom and 15 in the top part of the leaves).

Applications started when shoots were 10 cm, when average temperature was 10°C, and after 10 mm of rain. Following applications were done either one or two days before precipitations were previewed, according to the disease pressure and the grapevines growth (specially from the phenolgical stages: 17 to 27 Lorenz scale)

6.2.4 Cultural management

The regular cultural management done by the Estate was followed as well for the plot of the trial through the season.

Cultural activities correlated to downy mildew management

Lateral shoots removal	2 times
Leaf removal	1 time
*Shoot re-insertion (Instead of topping)	2 times

**Shoot re-insertion is not directly correlated to downy mildew management, but been correlated to the speed growth of the lateral shoots and therefore number of new leaves in the season, it is mentioned in this section.*

6.2.5 Weather information

The application of the treatments followed preventive program criteria as it is commonly done for contact fungicides and in organic agriculture.

Weather previsions to determine spraying timing were done with the help of the meteorological internet sites *Meteotrentino.it* and *Wetterzentral.de*

Weather collection of data for the previous seasons and current season was done from the Insituto Agrario di San Michele all'Adige Technologic Center. Information was taken using the corresponding meteorological station of Cognola (46) and Mezzolombardo (58).

6.2.6 Monitoring and measuring downy mildew incidence

Monitoring along the whole season was made to determine the first oil spots appearance of the season, primary infestations, and secondary infestations. Monitoring was made at the beginning of the season in the leaves and later on in leaves and grapes.

Predictions to assess probable downy mildew infestations and symptoms manifestations were done after the recovered meteorological information and Goidanich model.

	Downy mildew incubation period (days) (Pertot & Dagostin, 2007)	
Average Temperature (°C)	With %RH < 60%	With %RH > 60%
14°	15	11
15°	13	9.5
16°	11.5	8.5
17°	10	7.5
18°	9	6.5
19°	8	6
20°	7	5
21°	6.5	4.5
22°	6	4.5
23°	5.5	4
24°	5.5	4

25°	6	4.5
26°	6	4.5

Regular controls (each 7-10 days) in accordance to the assessments were carried out scanning as many leaves and grapes as possible during a slow walk through the vines. The position of probable downy mildew spots was asset as well, in order to make more meticulous controls.

Once first oil spots were detected, leaves and grapes downy mildew evolution was followed using a scale to measure the incidence and degree of infection. Records were done when new infection periods occur in throughout the growing season. Even though powdery mildew is not the subject of study in this trial, simultaneous records were made in order to have a wider depiction of the treatments interactions and of the disease incidences in the growing season 2010.

The scale used to classified the severity of the infection (for leaves and berries) into different classes was: 0%, 1-25%, 26-50%, 51-75%, >75%.

In case of grape bunches monitoring a total of 50 random bunches was sample per block, having 150 bunches per treatment. In case of leaf analysis 8 random shoots were taken per block and each leaf of the main shoot was classified, having a total of 24 shoots (150 leaves per treatment). Leaves from the lateral shoots were not taken into account in these controls, although visual classification was done in order to estimate and differentiate the severity of infection.

6.2.7 Leaf analysis

Two analyses were carried out to the leaves blades and the petioles in order to understand if the treatments could interfere physiologically in the vines. First analysis was done at fruit setting and second one at veraison.

For each one of the two analyses carried out, a total of 20 leaves were sampled per block, having a total of three repetitions of 20 leaves blades and petioles per treatment to perform the analysis. Leaves were sampled from a standard position of vine in order to homogenize the age and physiological state of the plant leaves as it is usually done for this kind of analysis.

Thus, leaves were removed from the 4-5 shoot of the guyot, and taken from the leaf next above or below the grape cluster (usually located in between the 3^{rd} and the 6^{th} bud)

Measurements of the following macro and micro elements present in the leaves was carried out at an external laboratory: N (Dumas Method), P, K, Ca, Mg, S, Fe, B, Mn, Zn and Cu (Optical emission spectrometer).

Before the regular protocol of the Laboratory for leaves analysis a pre-washing of the collected leaves was done with Hypochlorite solution at 1% in order to remove possible treatments residues.

6.2.8 Chlorophyll analysis

Leaf chlorophyll content was measured as an indicator of plant health through a SPAD Chlorophyll Meter (Soil Plant Analysis Development).

The instrument measures transmission of red light at 650 nm, at which chlorophyll absorbs light, and transmission of infrared light at 940 nm, at which no absorption occurs. On the basis of these two transmission values the instrument calculates a SPAD value that can be correlated with chlorophyll content.

Analysis was done when veraison started. Two measurements were done per leaf. Three repetitions per block were done. Each repetition was conformed by two leaves of the same plant. Leaves were taken from different positions: bottom (in between the 3th and 6th bud) and middle (in between the 8th and the 10th bud). A total of 18 leaves were analyzed per treatment.

6.2.9 Grapes analysis

During the harvest period, bunch sampling was done in order to calculate plant fertility, productivity, bunch average weigh, berry average weigh, sugar(°Bx), pH and acidity.

Grapes analyses were performed through the partners of this project of the Università Cattolica del Sacro Cuore di Piacenza with the standard methodology used for grape analysis.

6.2.10 Statistic analysis

For data processing, examination, cleaning, transforming, and modelling to determine significant differences, suggesting conclusions, and finally supporting decision making, all raw data were submitted to an analysis of variance through the ANOVA, Fisher test and SNK test of SAS software 6.04 (SAS Institute, Cary, NC, USA).

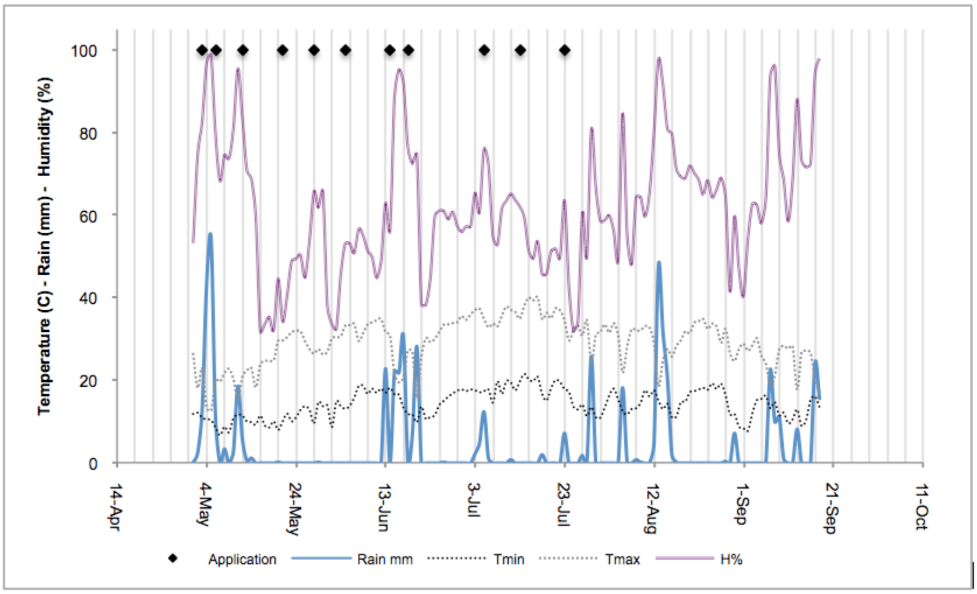
7. Trial log

A total of 11 applications were done for each treatment. Applications were done under preventive criteria always in the same day for all the treatments following the rain period's previsions and the growth rate of the vines.

The main growth stages of the experimental vineyard for the season 2010 are presented as it follows

Lorenz scale	Vine growth stage	Date
4	Budburst	6th April

12	Shoot 10 cm	2nd May
19	Flowering started	4th June
27	Setting	14th June
31	Berries pea size	5th July
35	Veraison started	29th July
38	Harvest	24th September



Graphic 7.1 Meteorological information of the parcel site corresponding to the period from which the treatment started from 1st may until the harvest day on 24th of September. The dates in which applications of the treatments were done is marked as well in the upper part of the graph(IASMA -Agrometeorologia Data Base).

Table 7.1 Date of treatments applications and doses

	03-may	06-may	12-may	21-may	28-may	04-jun	14-jun	18-jun	05-jul	13-jul	23-jul
Hour	02:00 PM	09:00 AM	10:30 AM	10:00 AM	09:00 AM	09:00 AM	09:00 AM	09:00AM	09:00AM	09:00 AM	09:00 AM
Lorenz stage	11-12	12-15	14-15	16	17	19	25-27	27	31-32	33	33
Leaves	3-5	4-6	6-8	9-11	12-13	14-15	15-16	NA	NA	NA	NA
Control	x	x	x	x	x	x	x	x	x	x	x
T1 Estate	150 gCu/ha **Kocide 3000**	300 gCu/ha **Heliocuivre**	250 gCu/ha **Heliocuivre**	250 gCu/ha **Kocide 3000**	250 gCu/ha **Kocide 3000**	150 gCu/ha **Kocide 3000**	150 gCu/ha **Kocide 3000**	204 gCu/ha **Heliocuivre**	150 gCu/ha **Kocide 3000**	150 gCu/ha **Kocide 3000**	150 gCu/ha **Kocide 3000**
	250g/ha **Sulphur**	250 a g/ha **Sulphur**	300g/ha **Sulphur**	250g/ha **Sulphur**	250g/ha **Sulphur**	250g/ha **Sulphur**	250g/ha **Sulphur**	300mL/ha **Sulphur**		250g/ha **Sulphur**	250g/ha **Sulphur**
T2 EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM	EM
	250 g/ha **Sulphur**	250 g/ha **Sulphur**	300 g/ha **Sulphur**	250 g/ha **Sulphur**	250 g/ha **Sulphur**	250 g/ha **Sulphur**	250 g/ha **Sulphur**	300 mL/ha **Sulphur**		250 g/ha **Sulphur**	250 g/ha **Sulphur**
T3 EM + Cu	**EM**+100gCu/ha **Kocide 3000**	EM+100gCu/ha **Kocide 3000**	EM+100gCu/ha **Kocide 3000**	EM+100gCu/ha **Kocide 3000**	EM+100gCu/ha **Kocide 3000**	EM+100gCu/ha **Kocide 3000**	EM+100gCu/ha **Kocide 3000**	EM+100gCu/ha **Kocide 3000**	EM+100gCu/ha **Kocide 3000**	EM+100gCu/ha **Kocide 3000**	EM+100gCu/ha **Kocide 3000**
	250g/ha **Sulphur**	250 g/ha **Sulphur**	300g/ha **Sulphur**	250g/ha **Sulphur**	250g/ha **Sulphur**	250g/ha **Sulphur**	250g/ha **Sulphur**	300mL/ha **Sulphur**		250g/ha **Sulphur**	250g/ha **Sulphur**
T4 MycoSin	5kg/ha **MycoSin VIN**	5kg/ha **MycoSin VIN**	5kg/ha **MycoSin VIN**	5kg/ha **MycoSin VIN**	5kg/ha **MycoSin VIN**	5kg/ha **MycoSin VIN**	5kg/ha **MycoSin VIN**	5kg/ha **MycoSin VIN**	5kg/ha **MycoSin VIN**	5kg/ha **MycoSin VIN**	5kg/ha **MycoSin VIN**
	250 g/ha **Sulphur**	250 g/ha **Sulphur**	300 g/ha **Sulphur**	250 g/ha **Sulphur**	250 g/ha **Sulphur**	250 g/ha **Sulphur**	250 g/ha **Sulphur**	300mL/ha **Sulphur**		250g/ha **Sulphur**	250g/ha **Sulphur**

Table 7.1 presents the details of each application done per treatment, showing the doses and products used through the trial.

8. Results and discussion

8.1 Downy mildew in 2010 growing season

Season 2010 was considered in general all over the region of low risk for downy mildew. Regional reports classified 2010 of low risk and as an easy year for downy mildew, while powdery mildew represented much more problems for grape growers.

Year 2008 was considered one of the hardest years to control downy mildew in the region, in which diffusion and severity of downy mildew at the end of the growing season reported a total of 100% in both parameters in the grapes of untreated controls. In the annexes the comparison in between weather conditions of 2010 and 2008 that can help to dimension season 2010.

In the season 2010 rainy periods were easy to classify to follow downy mildew development throughout the growing season.

Table 8.1 Downy mildew development in the experimental vineyard site.

Rain Period	Date	Rain mm	T°C mean	Growth Stage
1	**2^{nd} to 14^{th} May**	**156 mm**	**13.5°**	**Shoots 10 cm**
Symptoms	1st June	Not important primary infection		
2	**13^{th} to 20^{th} June**	**132 mm**	**18.3°**	**Beginning of fruit set**
Symptoms	1st July	Primary infections in young leaves: less 1% Berries primary infections: less 1%		
3	**3^{rd} to 6^{th} July**	**20.6 mm**	**24.6°**	**Berries pea size**
Symptoms	18th July	Primary infection and secondary infections in young leaves: less 1% Berries primary infections: 25% difussion, 1,77% severity		
4	**23^{rd} July**	**7.2 mm**	**25.3°**	**Berries still hard and green**
Symptoms	28^{th} July	Primary infection and secondary infections in young leaves: less 1% Berries primary infections present, damaged berries started to fell down.		
5	**29^{th} July**	**25.8 mm**	**17.5°**	**Veraison started**
Symptoms	7th August	Primary infection and secondary infections in young leaves: less 1%		
6	**5^{th} - 9^{th} August**	**19.4 mm**	**20.6°**	**50% veraison**
	17^{th} August	Late leaves primary infection (mosaic): less 1%		
7	**12^{th} -17^{th} August**	**107 mm**	**18.3°**	**Veraison finishing**
Symptoms	29^{th} august	Late leaves primary infection and secondary (mosaic): 92% diffusion – 20% severity		

The periods that represent the most important infective periods for Downy mildew development are highlight in the table. Data of diffusion and severity are referred to the controls of the experiment (IASMA -Agrometeorologia Data Base)

8.2 Downy mildew in the grapes

Damages in grapes were very low in this growing season. Important infective periods occur only at the begging of fruit set and when berries had pea size, therefore only some berries already formed were infected, but no important losses of grape bunches occurred.

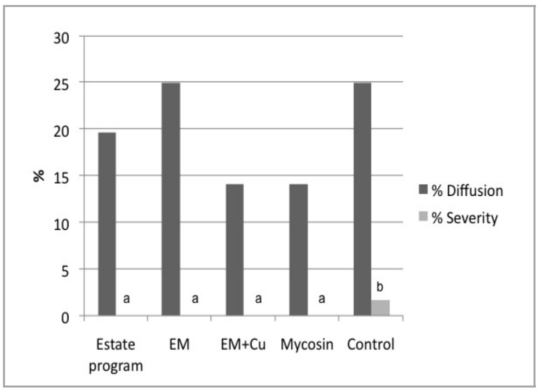
The first infective period occurred after the rainy period of 3rd to 6th june when conditions for downy mildew development were optimum, but not having previous infections the attack remained very low. When the second important infective period occurred, maximum temperatures were above 30°C, what probably helped to slow down the diffusion of the disease.

Controls to measure the diffusion and severity of downy mildew were done after the infective periods (see table 8.2). The proportion of the diffusion amongst the treated vines and the control did not show any significant difference, while the severity was slightly higher in the control.

Table 8.2 – Downy mildew in grapes

Treatment mean	08-July		20-July	
	% Diffusion	%Severity	%Diffusion	%Severity
Estate program	0.00	0.00	19.17	0.21 a
EM	0.00	0.00	25.00	0.30 a
EM + Cu	0.00	0.00	14.17	0.23 a
Mycosin	0.00	0.00	14.17	0.32 a
Control	0.83	0.06	25.00	1.77 b
F	0.884 ns	0.884 ns	0.684 ns	**4.768 ***

*The values in % were transformed into its corresponding angular before the statistical analysis was performed. F values are referred to those values. Values reported in the table correspond to the media of the real % values. F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05.*



Graphic 8.1 Diffusion and Severity of downy mildew late infestation in the main leaves.

20-July -2010

8.3 Downy mildew in the leaves

May to July

Leaves, that are usually the first indicator measured for downy mildew incidence, in this year did not represent a measurable parameter within the period in which treatments were applied (1^{st} May – 23^{rd} July). All the controls done through the season reported less than 1% of damaged leaves for all of the treatments. Main localized areas with oil spots were found in the controls and in EM block.

The main infection in the leaves was seen just after the rainy period from 3rd to 6th June, cultural practices of lateral shoots and leaves removal done in that period and the hot three weeks that followed decreased and stopped the infection points.

August-September

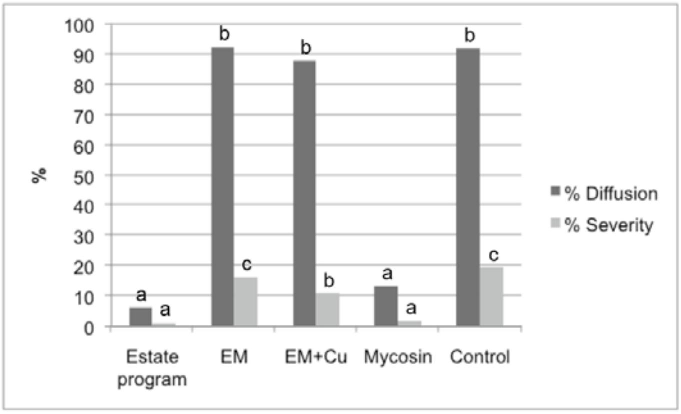
Applications stopped when veraison started on 23th July. After two periods of rain in August a late infestation of downy mildew occurred, damaging leaves from the main and lateral shoots. Leaves symptoms appeared first as small angular yellow and red spots, which turn in one week into reddish brown spots tapestry-like already sporulated.

A control to measure the diffusion and severity in the leaves from the main shoots was carried out. Severity in the lateral shoots leaves was measure through a visual classification see table 8.3.

Table 8.3 – Downy mildew in the leaves

Treatment mean	03-September Leaves from the main shoot				03-September Visual classification lateral leaves
	% Diffusion		% Severity		Severity
Estate program	6.00	a	0.71	a	+
EM	92.67	b	16.04	c	++++
EM + Cu	88.00	b	10.95	b	+++
Mycosin	13.00	a	1.62	a	+
Control	92.00	b	19.48	c	+++++
F	**199.14 ****		**141.29 ****		

*The values in % were transformed into its corresponding angular before the statistical analysis was performed. F values are referred to those values. Values reported in the table correspond to the media of the real % values. F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05.*



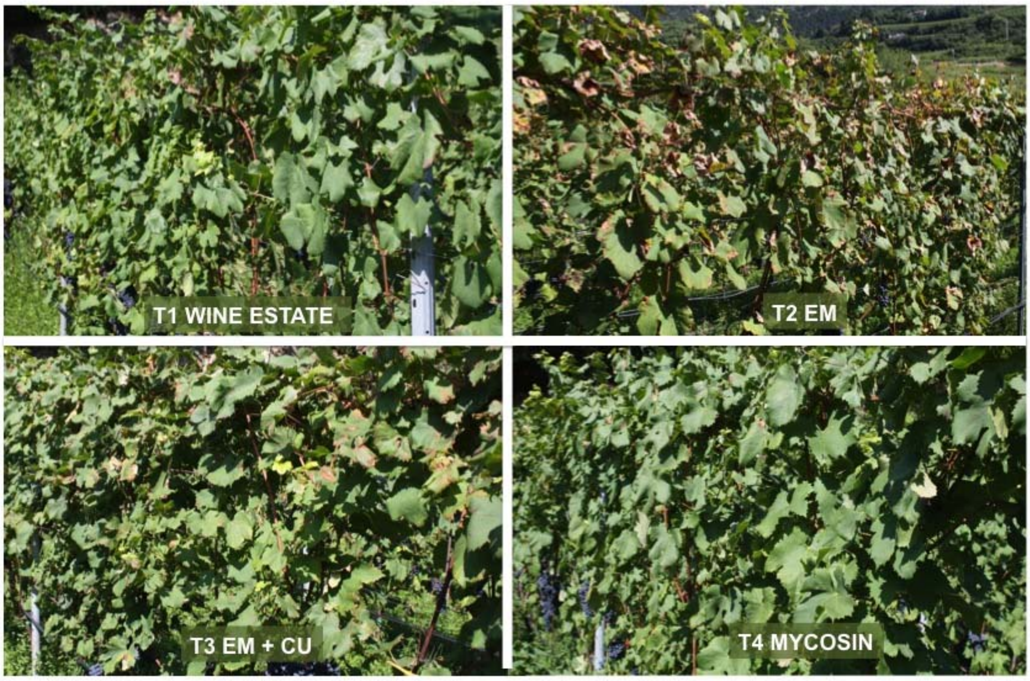
Graphic 8.2 Diffusion and Severity of downy mildew late infestation in the main leaves.

02-September-2010

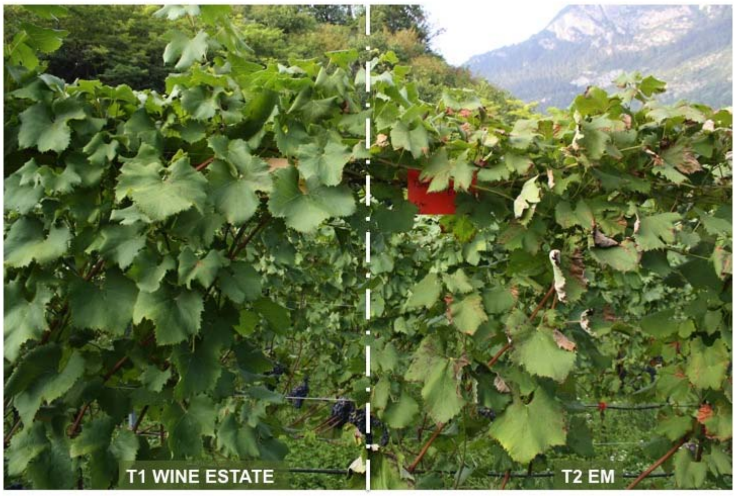
Results show that the biggest diffusion occurred in EM, EM+Cu treatments and the Control with no significant differences in between them, while the lowest diffusion occurred in the Estate Program and Mycosin treatment with no significant differences also in between these two (see picture 8.1).

The values related to the severity of the disease remained relatively low, because infestations of downy mildew in mature leaves are always more localized and tend to spread less. The Control and EM presented the highest disease severity with no significant differences in between them, EM+Cu presented significant differences amongst the treatment with slightly less severity damages than the control and EM. The Estate program and Mycosin, present the lowest severity of the trial with the same significant level.

Damage could be seen clearly from distance amongst the different treatments, seen due to the amount of damaged leaves in the treatments EM, EM+Cu and the control. In picture 8.3 a panoramic view of the experimental vineyard which permits to indentify clearly the damaged blocks from the trial.



Picture 8.1 Downy mildew in the leaves after the late infestation. Picture taken on 02-September-2010



Picture 8.2 Visual comparison of the border in between one block of T1 and T2.
Clear differences in between these treatments could be observed. Picture taken on 02-September-2010

Picture 8.3. Controls can be seen in the extremes of the plot. Control in the left corresponds to block A and Control in the right corresponds to block N. Patches of the damaged block corresponding to EM and EM+Cu could be noticed along the plot. Picture taken on 02-September-2010

Due to the big amount of rainfall during August and September this was a year in which late infestations in the leaves were the most relevant damages in the vines. At the end of the season on the harvest day, all the treatments presented an increase of infestation of downy mildew, but in the treatments in which leaves had been damaged since early September, they were almost dry by the end of September.

8.4 Grapes production

The result of the grape analysis performed are presented in table 8.4 and 8.5.

The weight of the Bunches in the control were the lowest of all the trial with significant differences between them, followed by the bunches from the treatments EM and EM+Cu with no significant differences in between them. The highest values of the experimental vineyard were in the Estate program and Mycosin treatments with no significant differences in between them. From what it is referred to the total production per vine, the only significant difference amongst the treatments was found in the control.

General tendencies related to productivity are consistent amongst the treatments in which the Estate program treatment stays prevalently above in all the parameters, followed by Mycosin, EM+CU and EM, in the corresponding order.

Table 8.4 Grapes production

Treatments	Fertility	Berry average weight (g)		Grape bunch average weight (g)		Production per vine (kg)	
Estate Program	0.914	2.101	a	265	c	2.037	b
EM	1.030	1.790	a	202	b	1.637	b
EM + Cu	0.927	2.148	b	222	bc	1.512	b
Mycosin	0.881	1.861	a	257	c	1.844	b
Control	0.788	1.735	a	130	a	0.752	a
F	1.581 ns	4.918 **		12.365 **		7.697 **	

F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05.

8.5 Grapes quality

From what it concerns to the parameters of grape quality the values of sugar concentration present the same tendency of the productivity results. The only difference found related to acidity were in between the control and the rest of the treatments. Even though there are no significant differences in between the treatments, acidity value in between the Estate program and Mycosin is almost the same, with higher values in the EM+Cu and the highest in EM.

Table 8.5 Grapes quality

Treatments	Sugar (Brix°)	pH	Acidity (g/L)	Sugar/Acidity
Estate Program	21.01 c	3.21	8.05 a	2.635 c
EM	19.30 ab	3.12	9.51 a	2.063 b
EM + Cu	20.34 bc	3.18	8.57 a	2.401 c
Mycosin	21.19 c	3.20	8.04 a	2.667 c
Control	18.82 a	3.15	11.21 b	1.720 a
F	6.739 **	2.101 ns	9.618 **	14.912 **

*F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences found after performed the SNK test with a p<0.05.*

The results of productivity and quality in the grapes from the different treatments confirm the influence that some of the vines suffered due to the late infestations of downy mildew in the leaves.

The biggest indicators of phenological berry ripening are sugar content and acidity, and as it is well known, leaves play a determinate roll for sugar concentration in the berries and ripening of the berries.

Quantitatively, sucrose is the most significant organic compound translocated into the fruit and is also the primary organic compound transported in the phloem. It is considered that one of the major factors in sugar accumulation relates to the redirection of phloem sap from leaves to the fruit.

Next to sugar accumulation, reduction in acidity is quantitatively the most marked chemical change during ripening. Shoot vigor is an influencing factor in fruit acidity (indicated by leaf surface/fruit ratio) and it is correlated with reduced grape acidity and higher pH.

The lower values related to productivity in the case of the Control, EM can be explained through the loss of water that the grapes suffer in the hot days without the proper amount of leaves to regulate the temperatures of the plant. While the lower values in sugar content and higher values in the acidity are indicators of a lower maturity of the grapes.

8.6 Downy mildew treatments effectiveness

Having a low risk year for downy mildew, the results presented from the period in which treatments were applied did not allow us to have a clear vision about the effectiveness of the different treatments. All the treatments up to veraison presented low infestations of downy mildew, but in comparison with the data presented of the Control, none big differences were found also in between the treated vines and the untreated ones. See graphic 8.1

On the other side the period of late infestations in the leaves helps us to see important differences amongst the treatments and to consider not only the effectiveness required from the treatments, but further more the importance of the persistence of the treatments for a grape grower or a wine estate to have healthy leaves that allow the adequate ripening of the grapes up until harvest.

EM

The comparative values in between the treated vines with EM and the Control indicate a bad management for controlling downy mildew. Having low infestations of downy mildew before veraison does not allow us to understand clearly if the treatment had an effect during the applications' period, so we only can conclude from the effectiveness shown when applications stopped.

Therefore, the high infestations of downy mildew in the vines treated with the EM mixture could be attributed to lack of effectiveness of the product, low persistence of the product in the leaves and/or a wrong application strategy (i.e. interactions of EM with Sulphur, frequency and number of the applications required).

EM+Cu

This treatment gave slightly better results than EM alone, most probably due to the effect of the low doses of Cu employed. Its effectiveness did not represent significant advantages, and quality values of the grapes are average. Therefore it does not represent a possible solution for Copper replacement.

Mycosin

This treatment presented good results for downy mildew control. In contrast with most of the research done in the subject none important differences were seen in between the vines treated with Mycosin Vin® and the vines treated with copper. Furthermore, the quality of the grapes did not show significant differences in contrast with the wine Estate program.

8.7 Complementary analyses

8.7.1 Leaves nutrition

Analyses to check the status of leaf nutrition throughout the season were carried out at fruit set and veraison. The results of the analyses are presented in the following tables

Fruit set

Table 8.6 Leaf blade in fruit set

Treatment mean	**N** (%)	**P** (%)	**K** (%)	**Ca** (%)	**Mg** (%)
Estate program	2.550	0.160	1.440	2.890	0.230
EM	2.410	0.150	1.390	2.933	0.220
EM + Cu	2.477	0.167	1.337	3.050	0.243
Mycosin	2.393	0.157	1.520	2.850	0.237
Control	2.385	0.165	1.280	2.770	0.255
F	1.071 ns	2.991 ns	3.189 ns	0.231 ns	0.946 ns

*F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05.*

Tab 8.7 Leaf balde fruit set

Treatment mean	**Fe** (mg/Kg)	**B** (mg/Kg)	**Mn** (mg/Kg)	**Zn** (mg/Kg)	**Cu** (mg/Kg)
Estate program	63.00 a	34.67 ab	133	18	289 c
EM	72.00 b	34.33 ab	161	17	16 a
EM + Cu	75.67 b	36.33 b	163	19	125 b
Mycosin	64.00 a	39.67 b	141	20	24 a
Control	66.50 a	28.50 a	151	17	13 a
F	**14.277 ****	5.244 **	0.827 ns	0.868 ns	79.82 **

*F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05.*

Table 8.8 Petiole fruit set

Treatment mean	**N** (%)	**P** (%)	**K** (%)	**Ca** (%)	**Mg** (%)
Estate program	0.677	0.163	3.103	1.970	0.250
EM	0.600	0.200	3.017	2.093	0.247
EM + Cu	0.630	0.307	3.127	2.253	0.290
Mycosin	0.620	0.193	2.573	2.053	0.253
Control	0.630	0.285	2.650	1.905	0.290
F	0.726 ns	**4.173 ***	2.289 ns	1.319 ns	1.407 ns

*F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05.*

Table 8.9 Petiole in fruit set

Treatment mean	**S** (%)	**Fe** (mg/Kg)	**B** (mg/Kg)	**Mn** (mg/Kg)	**Zn** (mg/Kg)
Estate program	0.187	11	34	57	43
EM	0.180	13	34	69	44
EM + Cu	0.170	16	35	66	46
Mycosin	0.177	13	33	58	37
Control	0.145	21	32	53	52

F	2.409 ns	1.680 ns	0.643 ns	0.650 ns	1.773 ns

*F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05.*

Table 8.10 Leaf blade in veraison

Treatment mean	**N** (%)	**P** (%)	**K** (%)	**Ca** (%)	**Mg** (%)
Estate program	2.063	0.143	1.230	3.100	0.238
EM	1.977	0.150	1.307	3.290	0.240
EM + Cu	2.023	0.147	1.250	3.200	0.243
Mycosin	2.060	0.147	1.357	3.050	0.240
Control	1.990	0.153	1.313	3.187	0.280
F	1.343 ns	0.650 ns	1.469 ns	0.579 ns	3.330 *

*F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05*

Table 8.11 Leaf blade in veraison

Treatment mean	**Fe** (mg/Kg)	**B** (mg/Kg)	**Mn** (mg/Kg)	**Zn** (mg/Kg)	**Cu** (mg/Kg)
Estate program	58.00 a	22.00	156	0.250	204 c
EM	74.67 b	22.33	178	0.253	18 a
EM + Cu	66.33 ab	21.33	170	0.250	122 b
Mycosin	60.00 ab	22.33	147	0.273	19 a
Control	62.67 ab	21.67	157	0.193	11 a
F	**3.386 ***	0.230 ns	0.581 ns	1.221 ns	**25.673 ****

*F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05*

Tab. 8.12 Petiole in veraison

Teshis mean	**N** (%)	**P** (%)	**K** (%)	**Ca** (%)	**Mg** (%)
Estate program	0.637	0.177	4.457	2.227	0.330
EM	0.593	0.223	4.757	2.370	0.307
EM + Cu	0.677	0.217	4.187	2.320	0.340
Mycosin	0.633	0.163	4.150	2.333	0.347
Control	0.600	0.250	3.963	2.253	0.367
F	0.720 ns	1.064 ns	0.663 ns	0.470 ns	0.477 ns

*F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05.*

Tab. 8.13 Petiole in veraison

Treatment mean	**S** (%)		**Fe** (mg/Kg)	**B** (mg/Kg)	**Mn** (mg/Kg)	**Zn** (mg/Kg)
Estate program	0.180	ab	10	34	118	52
EM	0.187	b	12	36	130	58
EM + Cu	0.173	ab	12	34	102	58
Mycosin	0.177	ab	10	35	112	52
Control	0.143	a	13	40	90	70
F	**3.721 ***		0.518 ns	0.513 ns	0.627 ns	1.479 ns

*F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05*

Cu The results of copper content help us to validate the experimental design in which avoiding contamination in between the treatments was important to estimate the effectiveness of the products.

S As it was indicated before, treatments with sulphur for powdery mildew were applied parallel throughout the season. The Controls were the only treatment left without sulphur applications, which can be corroborated being the treatment with less amount of S%.

Fe The treatments with EM ® mixture show higher values in iron content in the leaves, which has already been seen amongst the other research studies with the product. This is probably due to a higher absorption of the element present in the formulation. Further studies in the subject are required to better understand this mechanism.

8.7.2 SPAD analysis

As it is well known chlorophyll is the molecule that absorbs sunlight and uses its energy to synthesise carbohydrates from CO_2 and water, process known as photosynthesis and the basis for sustaining the life processes of all plants.

Nitrogen is a constituent of chlorophyll and several growth regulators thus a close correlation between leaf chlorophyll content and leaf N content can be made.

The analysis of chlorophyll content was carried out at veraison, the results are presented on table 8.14

Table 8.14 SPAD values – Chlorophyll content on 08-August-2010

Thesis mean	**SPAD** bottom	**SPAD** half	
Estate program	34.47	36.90	a
EM	35.08	37.23	a
EM Cu	35.49	36.02	a
MYC	36.44	36.62	a
Control	33.77	32.68	b
F	1.318 ns	**3.952 ****	

*F= Fisher; * = significance with $p<0.05$; ** = significance with $p<0.01$. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a $p<0.05$.*

From what was observed at veraison, only the control shows significant lower values of SPAD which tells us that non of the treatments had differences in the photosynthetic activity of the plant. This also could point that the health of the vine leaves up to veraison was the same.

8.8 Other diseases

Powdery midew

Other diseases as powdery mildew in the grapes represented big problems in some areas of Trentino, while from what was observed in the treated vines from the wine estate, it did not represent any considerable problem, diffusion remain around 4.6% with a severity of 0.1 %.

As it is presented in table 8.15 no significant differences were found in between the treatments, while a significant difference in between the treated vines and the control was evident.

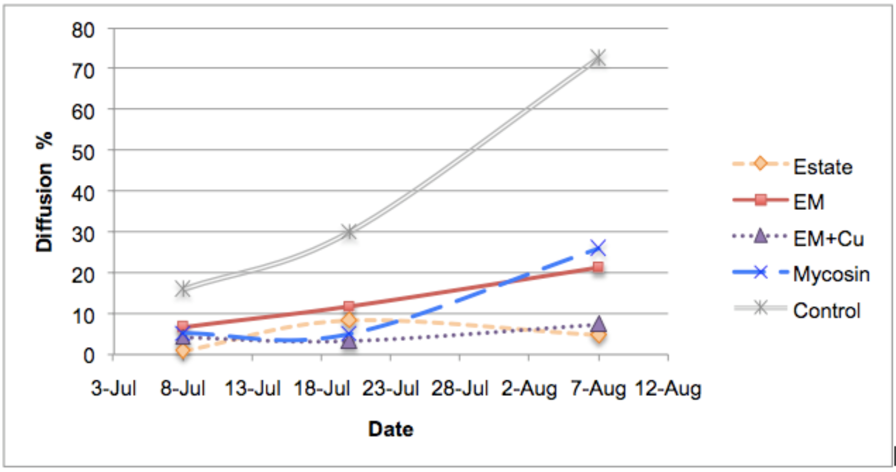
In the last control carried out, the Estate program and EM+Cu represented the lowest values of the disease diffusion with no significant differences, while EM+Cu show slightly higher values of the diffusion comparable to those found in Mycosin and EM.

This confirms that timing and doses employed by the wine estate for Sulphur applications were effective to control possible infestations

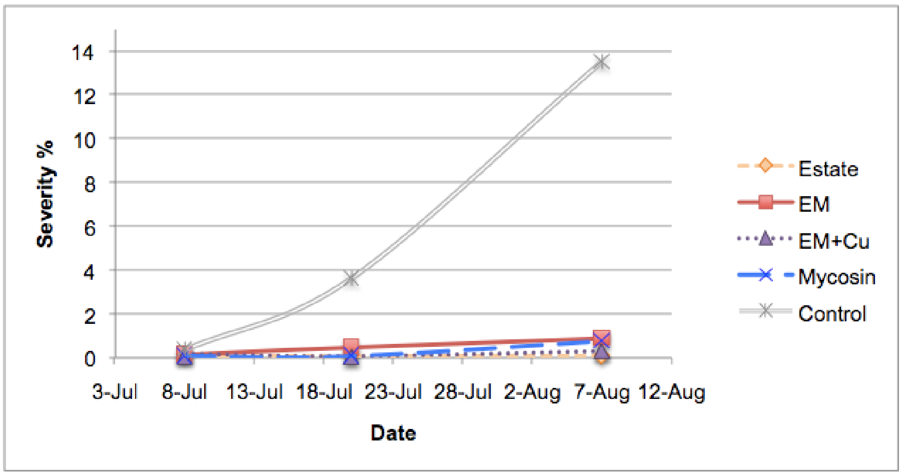
Table 8.15 Powdery mildew in grapes

Treatment mean	08-July Diffusion		08-July Severity	20-July Diffusion	20-July Severity		07-August Diffusion		07-August Severity	
Estate program	0.83	a	0.01	8.33	0.08	a	4.67	a	0.10	a
EM	6.67	a	0.14	11.67	0.46	a	21.33	b	0.89	a
EM + Cu	4.17	a	0.16	3.33	0.05	a	7.33	ab	0.31	a
MYC	5.00	a	0.07	5.00	0.09	a	26.00	b	0.76	a
Control	16.00	b	0.37	30.00	3.62	b	72.67	c	13.51	b
F	**3.732** *		2.444 ns	2.117 ns	**6.241** **		**19.829** **		**8.947** **	

*The values in % were transformed into its corresponding angular before the statistical analysis was performed. F values are referred to those values. Values reported in the table correspond to the media of the real % values. F= Fisher; * = significance with p<0.05; ** = significance with p<0.01. The letters next to the media values correspond to the significant differences founded after performed the SNK test with a p<0.05.*



Graphic 8.3 Difussion of powdery mildew in the grapes



Graphic 8.4 Severity of powdery mildew in the grapes

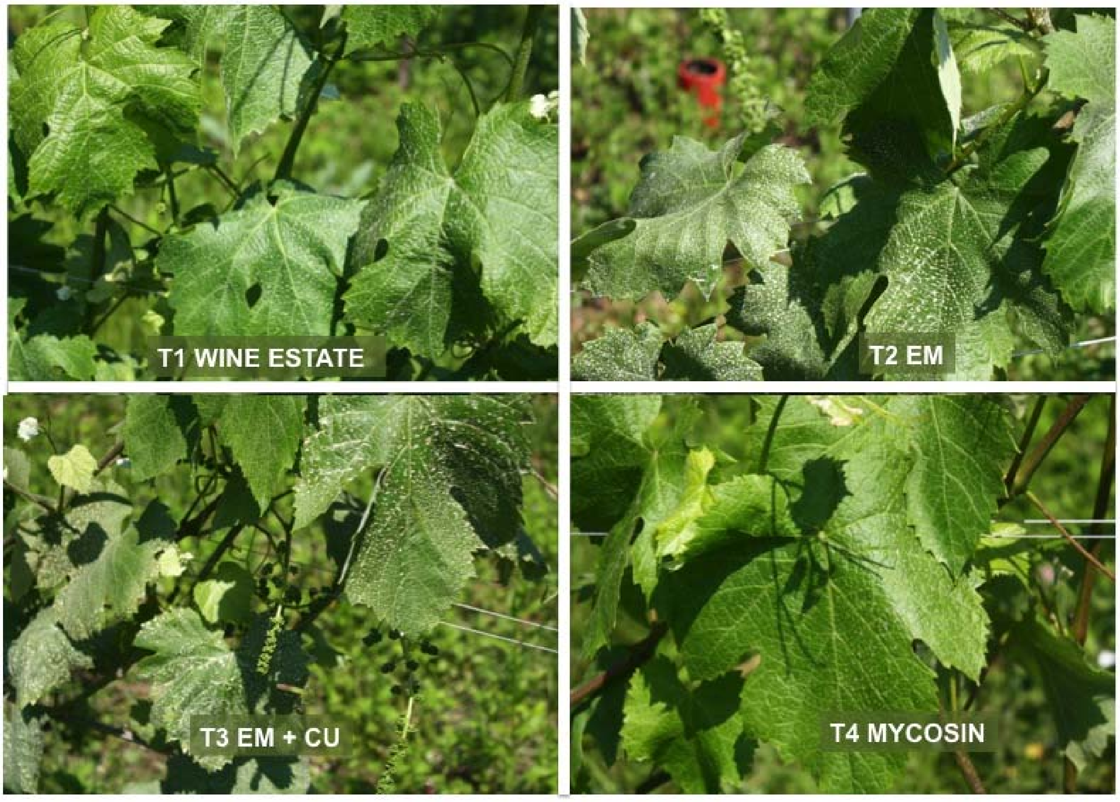
Botrytis

Botrytis, is a disease that usually do not represent a problem in the region, this year due to the strong and continuous period from middle September to harvest 120mm represented a bigger threat than usual. During harvest, the grapes from the Control, EM and EM+Cu had higher botrytis problems than usual.

Probably vines under stress due to the lack of healthy leaves tend to also have more susceptible grapes for other diseases. Botrytis was not a parameter previewed for the experiment therefore none measurements could be done on the harvest day.

9. Products notes

Product	Possitive	Negative	Comment
EM ®		x	Higher residues in the leaves compared with copper fungicides used in the trial.
	x		Low toxicity symptoms were observed in leaves and grapes.
	x		Easy to apply due to its liquid presentation and constant concentration.
		x	Short shelf-life.
Mycosin Vin ®		x	Higher residues in the leaves than the copper fungicides used in the trial after application.
		x	When applied with liquid sulphur products the residues were higher than with sulphur in powder (see picture 9.2)
	x		Low toxicity.
		x	In each application it is required to use sulphur (product indications).
	x		In this trial 7% of the amount of sulphur suggested by the producer was used with good results.
		x	Low solubility.



Picture 9.1 Residual product in the leaves after one day of the application

Picture 9.2 Residual product after mixing the product with liquid sulphur

10. Cost analysis

Product	Total Dose	Cost	Total amount/ha	Total Cost/ha
Kocide 3000 ®	1.400 kgCu/ha	6.20 €/kg	9.30 kg/ha	57.90 €/ha
Heliocuivre ®	0.754 kgCu/ha	15.20 €/L	3.01 L/ha	45.80 €/ha
Wine Estate's downy mildew program	2.154 kgCu/ha			103.70 €/ha
Mycosin Vin ®		9.60 €/kg	55.00 kg/ha	528.00 €/ha

**Costs refered to 2010. Doses for Kocide 3000 ® and Heliocuivre ® are refered to what was used in this trial.*

11. Summary chart

Treatment	EM	EM+Cu	Mycosin
Effectiveness for DM control in the grapes	N/C	N/C	N/C
Effectiveness for DM in the leaves during the period of treatments	N/C	N/C	N/C
Effectiveness for DM in the leaves after treatments stop	-	-	=
Persistence	-	-	=
Quality of the grapes on the harvest day	-	-	=
Grapes Yield	-	-	=
Easy to be applied	+	+	-
Leaves or grapes toxicity, collateral effects	-	-	-
Compatible with the other winery treatments	N/C	N/C	+/-
Costs	N/C	N/C	-

+ Better or positive aspect
= None differences with the Estate program
- Lower or negative aspect
+ / - Aspect to be considered
N / C Not confirmed during the trial

12. Conclusions and Further steps

Mycosin Vin ®

Show in general comparative results to the estate program, which can represents a possible product for copper replacement. The biggest disadvantage that the product presented is high cost due to the high dose required in each application.

Therefore to perform trials by the winery to see its effectiveness in other growing season that can be more susceptible to downy mildew are suggested. Possible trials to try to decrease its doses could be consider in order to evaluate if it is possible to reduced the production costs.

Sulphur seems to be directly related to the effectiveness of the product. In this trial, the doses that were used represent 7% of what the product producer advise, thus, increasing its doses and diminishing the amount of Mycosin Vin ® required could be an interesting aspect to evaluate.

A common practice in biodynamics is the use of natural tisanes of plants that have fungistatic activity (i.e neetles or horsetail). So, trials in which the product is mixed with natural tisanes of neetles or horse tail could be an interesting aspect to explore. As copper no alkaline substances should be mixed with the product, so before mixing Mycosin Vin ® with any other product or tisane checking its pH is highly suggested.

EM ®

From what the results of this trial presented, the strategies treated with EM ® mixture did not represent a possible solution for downy mildew management.

For further studies of the product it is recommended to study the possible interferences that the microorganisms present in the mixture may have with products as sulphur and copper.

Being a product based in an active substance (microorganisms) that depends on factors as Temperature, pH and time (i.e. latent period and reproduction), studies for a better understanding of the survival and the adaptability of the microorganism once that they reach the leaves of the vines are suggested in order to develop its own product strategy for its appliance.

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Annex

Table A.1 Meteorological information of 2008 and 2010.

Year	Month	T°C mean	T°C Min	T°C Max	% RH	Rain (mm)	Total rain (mm)
2010	1	0.5	-2.4	5.2	73	24.2	24.2
2010	2	3	-0.9	8	67	84	108.2
2010	3	7.6	2.7	13.9	60	75.2	183.4
2010	4	12.9	6.3	20.5	58	38.4	221.8
2010	5	16.4	11	22.8	61	156.4	378.2
2010	6	21.6	15.1	29	57	133	511.2
2010	7	24.7	17.3	33.8	57	58.2	569.4
2010	8	21.4	15.3	29	66	134.2	703.6
2010	9	16.9	11.9	23.8	73	182.8	886.4
2008	1	2.7	0.0	6.6	78	116.4	116.4
2008	2	6.0	2.3	11.0	67	36.8	153.2
2008	3	8.1	3.3	13.9	55	21.2	174.4
2008	4	11.1	6.3	16.4	64	142.4	316.8
2008	5	16.8	11.6	22.8	67	160.0	476.8
2008	6	20.4	15.2	27.1	74	115.2	592.0
2008	7	22.0	15.5	29.9	66	119.6	711.6
2008	8	22.5	15.7	30.4	64	112.6	824.2
2008	9	16.7	11.2	23.8	71	80.2	904.4
2008	10	13.1	8.4	19.6	76	173.6	1078.0
2008	11	5.6	2.3	10.6	84	267.8	1345.8
2008	12	1.5	-1.2	6.2	82	209.2	1555.0

(IASMA -Agrometeorologia Data Base) – Meteorological station 46 Cognola